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(71) Applicant: **BOSTON BIOMEDICAL, INC.** [US/US]; 640 Memorial Drive, Cambridge, MA 02139 (US).

(72) Inventor: **LI, Chiang, J.**; 8 Museum Way, Cambridge, MA 02141 (US).

(74) Agents: **UHM, Tony, K.** et al.; Boston Biomedical, Inc., 640 Memorial Drive, Cambridge, MA 02139 (US).

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[Continued on next page]

(54) Title: ASYMMETRIC INTERFERING RNA COMPOSITIONS THAT SILENCE K-RAS AND METHODS OF USES THEREOF

(57) Abstract: The invention provides novel compositions for use in silencing K-Ras gene expression. More particularly, the invention provides novel asymmetrical interfering RNA molecules as inhibitors of K-Ras expression, and to pharmaceutical compositions and uses thereof in the treatment of cancer or a related disorder in a mammal.

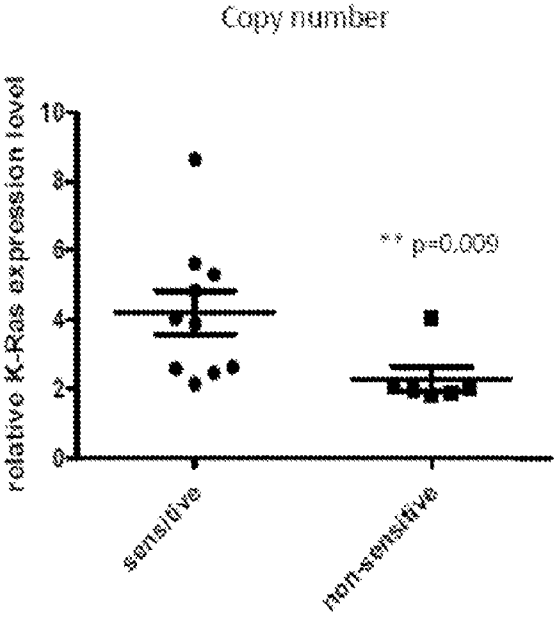

Figure 5(A)

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

ASYMMETRIC INTERFERING RNA COMPOSITIONS THAT SILENCE K-RAS AND METHODS OF USES THEREOF

FIELD OF THE INVENTION

[0001] The invention generally relates to compositions for use in silencing K-Ras gene expression. More particularly, the invention relates to novel asymmetrical interfering RNA molecules as inhibitors of K-Ras expression, and to pharmaceutical compositions and uses thereof in the treatment of cancer or a related disorder in a mammal.

BACKGROUND OF THE INVENTION

[0002] Gene silencing through RNAi (RNA-interference) by use of small or short interfering RNA (siRNA) has emerged as a therapeutic tool. However, other than the prominent delivery issue, the development of RNAi-based drugs faces challenges of limited efficacy of siRNA, non-specific effects of siRNA such as interferon-like responses and sense-strand mediated off-target gene silencing, and the prohibitive or high cost associated with siRNA synthesis. The gene silencing efficacy by siRNA is limited to about 50% or less for majority of genes in mammalian cells. The manufacture of these molecules is expensive (much more expensive than manufacturing anti sense deoxynucleotides), inefficient, and requires chemical modification. Finally, the observation that the extracellular administration of synthetic siRNAs can trigger interferon-like responses has added a significant barrier for RNAi-based research and RNAi-based therapeutic development.

[0003] The protein K-Ras is a molecular switch that under normal conditions regulates cell growth and cell division. Mutations in this protein lead to the formation of tumors through continuous cell growth. About 30% of human cancers have a mutated Ras protein that is constitutively bound to GTP due to decreased GTPase activity and insensitivity to GAP action. Ras is also an important factor in many cancers in which it is not mutated but rather functionally activated through inappropriate activity of other signal transduction elements. Mutated K-Ras proteins are found in a large proportion of all tumour cells. K-Ras protein occupies a central position of interest. The identification of oncogenically mutated K-Ras in many human cancers led to major efforts to target this constitutively activated protein as a rational and selective treatment. Despite decades of active agent research, significant challenges still remain to develop therapeutic inhibitors of K-Ras.

[0004] Hypermalignant cancer cells that are highly tumorigenic and metastatic have been isolated from cancer patients with a variety of tumor types and found to have high stemness properties, termed cancer stem cells (CSCs). These stemness-high cancer cells are hypothesized to be fundamentally responsible for cancer metastasis and relapse. A number of stemness genes, such as β-catenin, Nanog, Sox2, Oct3/4 have been implicated in cancer cell stemness. However, the role of oncogenes, such as K-Ras, in cancer cell stemness is not clear.

[0005] Accordingly, there exists a need to develop novel compositions and methods for selectively silencing K-Ras gene express or K-Ras activity in a subject diagnosed with cancer, with better efficacy and potency, rapid onset of action, better durability, and fewer adverse side effects.

SUMMARY OF THE INVENTION

[0006] To elucidate the role of K-Ras in the maintenance of cancer cell stemness, the present inventors employed asymmetric silencing RNA technology (aiRNA) which is able to silence target genes with high potency and precision. Moreover, aiRNA technology can be readily applied to CSCs. The present inventors made a surprising discovery that CSCs are not only addicted to activating mutations of K-Ras, or activation of the downstream regulators of the Ras pathway, but also that CSCs with amplified mutant K-Ras become highly sensitive to K-Ras silencing. Furthermore, the present inventors made a surprising discovery that the DNA copy numbers of the mutant K-Ras directly predicts sensitivity of cancer stem cells to K-Ras silencing, which suggests that amplified mutated K-Ras is required to the maintenance of the malignancy and cancer cell stemness, which may have significant implication for understanding the connection between oncogene and cancer cell stemness and for developing cancer stem cell inhibitors.

[0007] The present invention provides compositions and methods that use a class of small duplex RNA that can induce potent gene silencing in mammalian cells, which is termed herein asymmetrical interfering RNAs (aiRNA). aiRNA is described, for example, in PCT Publication No. WO 2009/029688, the contents of which are hereby incorporated by reference in their entirety. This class of RNAi-inducers is identified by the length asymmetry of the two RNA strands. This structural design is not only functionally potent in effecting gene silencing, but offers several advantages over the current state-of-art siRNAs. Among the advantages, aiRNA can have RNA duplex structure of much shorter length than the other siRNA, which should reduce the cost of synthesis and abrogate/reduce the length-dependent

triggering of nonspecific interferon-like responses. In addition, the asymmetry of the aiRNA structure abrogates and/or otherwise reduces the sense-strand mediated off-target effects. Furthermore, aiRNA is more efficacious, potent, rapid-onset, and durable than siRNA in inducing gene silencing. AiRNA can be used in all areas that other siRNA or shRNA are being applied or contemplated to be used, including biology research, R&D research in biotechnology and pharmaceutical industry, and RNAi-based therapies.

[0008] The duplex RNA molecule comprises a first strand with a length from 18-23 nucleotides and a second strand with a length from 12-17 nucleotides, wherein the second strand is substantially complementary to the first strand, and forms a double-stranded region with the first strand, wherein the first strand has a 3'-overhang from 1-9 nucleotides, and a 5'-overhang from 0-8 nucleotides, wherein said duplex RNA molecule is capable of effecting selective K-Ras gene silencing in a eukaryotic cell. In some embodiments, the first strand comprises a sequence being substantially complementary to a target K-Ras mRNA sequence. In a further embodiment, the first strand comprises a sequence being at least 70 percent complementary to a target K-Ras mRNA sequence. In another embodiment, the eukaryotic cell is a mammalian cell or an avian cell.

[0009] In some embodiments, the target K-Ras mRNA sequence is a human K-Ras target sequence. In some embodiments, the target K-Ras mRNA sequence is a human K-Ras target sequence selected from at least a portion of the sequence shown in GenBank Accession No. NM_004985 shown below as SEQ ID NO: 1:

```
  1 tcctaggcgg cggccgcggc ggcggaggca gcagcggcgg cggcagtggc ggcggcgaag
 61 gtggcggcgg ctcggccagt actcccggcc cccgccattt cggactggga gcgagcgcgg
121 cgcaggcact gaaggcggcg gcggggccag aggctcagcg gctcccaggt gcgggagaga
181 ggcctgctga aaatgactga atataaactt gtggtagttg gagctggtgg cgtaggcaag
241 agtgccttga cgatacagct aattcagaat cattttgtgg acgaatatga tccaacaata
301 gaggattcct acaggaagca agtagtaatt gatggagaaa cctgtctctt ggatattctc
361 gacacagcag gtcaagagga gtacagtgca atgagggacc agtacatgag gactggggag
421 ggctttcttt gtgtatttgc cataaataat actaaatcat ttgaagatat tcaccattat
481 agagaacaaa ttaaaagagt taaggactct gaagatgtac ctatggtcct agtaggaaat
541 aaatgtgatt tgccttctag aacagtagac acaaaacagg ctcaggactt agcaagaagt
601 tatggaattc cttttattga aacatcagca aagacaagac agggtgttga tgatgccttc
661 tatacattag ttcgagaaat tcgaaaacat aaagaaaaga tgagcaaaga tggtaaaaag
721 aagaaaaaga agtcaaagac aaagtgtgta attatgtaaa tacaatttgt actttttct
781 taaggcatac tagtacaagt ggtaattttt gtacattaca ctaaattatt agcatttgtt
841 ttagcattac ctaatttttt tcctgctcca tgcagactgt tagcttttac cttaaatgct
901 tattttaaaa tgacagtgga agtttttttt tcctctaagt gccagtattc ccagagtttt
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961 ggtttttgaa ctagcaatgc ctgtgaaaaa gaaactgaat acctaagatt tctgtcttgg
1021 ggtttttggt gcatgcagtt gattacttct tatttttctt accaattgtg aatgttggtg
1081 tgaaacaaat taatgaagct tttgaatcat ccctattctg tgttttatct agtcacataa
1141 atggattaat tactaatttc agttgagacc ttctaattgg tttttactga aacattgagg
1201 gaacacaaat ttatgggctt cctgatgatg attcttctag gcatcatgtc ctatagtttg
1261 tcatccctga tgaatgtaaa gttacactgt tcacaaaggt tttgtctcct ttccactgct
1321 attagtcatg gtcactctcc ccaaaatatt atatttttc tataaaaaga aaaaaatgga
1381 aaaaaattac aaggcaatgg aaactattat aaggccattt ccttttcaca ttagataaat
1441 tactataaag actcctaata gcttttcctg ttaaggcaga cccagtatga aatggggatt
1501 attatagcaa ccattttggg gctatattta catgctacta aatttttata ataattgaaa
1561 agattttaac aagtataaaa aattctcata ggaattaaat gtagtctccc tgtgtcagac
1621 tgctctttca tagtataact ttaaatcttt tcttcaactt gagtctttga agatagtttt
1681 aattctgctt gtgacattaa aagattattt gggccagtta tagcttatta ggtgttgaag
1741 agaccaaggt tgcaaggcca ggccctgtgt gaacctttga gctttcatag agagtttcac
1801 agcatggact gtgtccccac ggtcatccag tgttgtcatg cattggttag tcaaaatggg
1861 gagggactag ggcagtttgg atagctcaac aagatacaat ctcactctgt ggtggtcctg
1921 ctgacaaatc aagagcattg cttttgtttc ttaagaaaac aaactctttt ttaaaaatta
1981 cttttaaata ttaactcaaa agttgagatt ttggggtggt ggtgtgccaa gacattaatt
2041 tttttttaa acaatgaagt gaaaaagttt tacaatctct aggtttggct agttctctta
2101 acactggtta aattaacatt gcataaacac ttttcaagtc tgatccatat ttaataatgc
2161 tttaaaataa aaataaaaac aatccttttg ataaatttaa aatgttactt attttaaaat
2221 aaatgaagtg agatggcatg gtgaggtgaa agtatcactg gactaggaag aaggtgactt
2281 aggttctaga taggtgtctt ttaggactct gattttgagg acatcactta ctatccattt
2341 cttcatgtta aaagaagtca tctcaaactc ttagtttttt ttttttacaa ctatgtaatt
2401 tatattccat ttacataagg atacacttat ttgtcaagct cagcacaatc tgtaaatttt
2461 taacctatgt tacaccatct tcagtgccag tcttgggcaa aattgtgcaa gaggtgaagt
2521 ttatatttga atatccattc tcgttttagg actcttcttc catattagtg tcatcttgcc
2581 tccctacctt ccacatgccc catgacttga tgcagtttta atacttgtaa ttcccctaac
2641 cataagattt actgctgctg tggatatctc catgaagttt tcccactgag tcacatcaga
2701 aatgccctac atcttatttc ctcagggctc aagagaatct gacagatacc ataaagggat
2761 ttgacctaat cactaatttt caggtggtgg ctgatgcttt gaacatctct ttgctgccca
2821 atccattagc gacagtagga tttttcaaac ctggtatgaa tagacagaac cctatccagt
2881 ggaaggagaa tttaataaag atagtgctga aagaattcct taggtaatct ataactagga
2941 ctactcctgg taacagtaat acattccatt gttttagtaa ccagaaatct tcatgcaatg
3001 aaaaatactt taattcatga agcttacttt ttttttttgg tgtcagagtc tcgctcttgt
3061 cacccaggct ggaatgcagt ggcgccatct cagctcactg caacctccat ctcccaggtt
3121 caagcgattc tcgtgcctcg gcctcctgag tagctgggat tacaggcgtg tgccactaca
3181 ctcaactaat ttttgtattt ttaggagaga cggggtttca ccctgttggc caggctggtc
3241 tcgaactcct gacctcaagt gattcaccca ccttggcctc ataaacctgt tttgcagaac
3301 tcatttattc agcaaatatt tattgagtgc ctaccagatg ccagtcaccg cacaaggcac
3361 tgggtatatg gtatccccaa acaagagaca taatcccggt ccttaggtag tgctagtgtg

3421 gtctgtaata tcttactaag gcctttggta tacgacccag agataacacg atgcgtattt
3481 tagttttgca aagaaggggt ttggtctctg tgccagctct ataattgttt tgctacgatt
3541 ccactgaaac tcttcgatca agctacttta tgtaaatcac ttcattgttt taaaggaata
3601 aacttgatta tattgttttt ttatttggca taactgtgat tcttttagga caattactgt
3661 acacattaag gtgtatgtca gatattcata ttgacccaaa tgtgtaatat tccagttttc
3721 tctgcataag taattaaaat atacttaaaa attaatagtt ttatctgggt acaaataaac
3781 aggtgcctga actagttcac agacaaggaa acttctatgt aaaaatcact atgatttctg
3841 aattgctatg tgaaactaca gatctttgga acactgttta ggtagggtgt taagacttac
3901 acagtacctc gtttctacac agagaaagaa atggccatac ttcaggaact gcagtgctta
3961 tgaggggata tttaggcctc ttgaattttt gatgtagatg ggcatttttt taaggtagtg
4021 gttaattacc tttatgtgaa ctttgaatgg tttaacaaaa gatttgtttt tgtagagatt
4081 ttaaaggggg agaattctag aaataaatgt tacctaatta ttacagcctt aaagacaaaa
4141 atccttgttg aagttttttt aaaaaaagct aaattacata gacttaggca ttaacatgtt
4201 tgtggaagaa tatagcagac gtatattgta tcatttgagt gaatgttccc aagtaggcat
4261 tctaggctct atttaactga gtcacactgc ataggaattt agaacctaac ttttataggt
4321 tatcaaaact gttgtcacca ttgcacaatt ttgtcctaat atatacatag aaactttgtg
4381 gggcatgtta agttacagtt tgcacaagtt catctcattt gtattccatt gatttttttt
4441 ttcttctaaa catttttttct tcaaacagta tataactttt tttaggggat tttttttttag
4501 acagcaaaaa ctatctgaag atttccattt gtcaaaaagt aatgatttct tgataattgt
4561 gtagtaatgt tttttagaac ccagcagtta ccttaaagct gaatttatat ttagtaactt
4621 ctgtgttaat actggatagc atgaattctg cattgagaaa ctgaatagct gtcataaaat
4681 gaaactttct ttctaaagaa agatactcac atgagttctt gaagaatagt cataactaga
4741 ttaagatctg tgttttagtt taatagtttg aagtgcctgt ttgggataat gataggtaat
4801 ttagatgaat ttaggggaaa aaaaagttat ctgcagatat gttgagggcc catctctccc
4861 cccacacccc cacagagcta actgggttac agtgttttat ccgaaagttt ccaattccac
4921 tgtcttgtgt tttcatgttg aaaatacttt tgcatttttc ctttgagtgc caatttctta
4981 ctagtactat ttcttaatgt aacatgttta cctggaatgt attttaacta tttttgtata
5041 gtgtaaactg aaacatgcac attttgtaca ttgtgctttc ttttgtggga catatgcagt
5101 gtgatccagt tgttttccat catttggttg cgctgaccta ggaatgttgg tcatatcaaa
5161 cattaaaaat gaccactctt ttaattgaaa ttaacttta aatgtttata ggagtatgtg
5221 ctgtgaagtg atctaaaatt tgtaatattt ttgtcatgaa ctgtactact cctaattatt
5281 gtaatgtaat aaaaatagtt acagtgacta tgagtgtgta tttattcatg aaatttgaac
5341 tgtttgcccc gaaatggata tggaatactt tataagccat agacactata gtataccagt
5401 gaatctttta tgcagcttgt tagaagtatc ctttatttct aaaaggtgct gtggatatta
5461 tgtaaaggcg tgtttgctta aacttaaaac catatttaga agtagatgca aaacaaatct
5521 gcctttatga caaaaaaata ggataacatt atttatttat ttccttttat caaagaaggt
5581 aattgataca caacaggtga cttggtttta ggcccaaagg tagcagcagc aacattaata
5641 atggaaataa ttgaatagtt agttatgtat gttaatgcca gtcaccagca ggctatttca
5701 aggtcagaag taatgactcc atacatatta tttatttcta taactacatt taaatcatta
5761 ccagg (SEQ ID NO: 1)

[0010] In some embodiments, the target K-Ras mRNA sequence is a target sequence shown in Table 1 below.

Table 1. Target K-Ras Sequences

Target SEQ ID NO:	Position in NM_004985 Sequence	K-Ras Target Sequence	Targeted by aiRNA ID NO:
2	1701	GGCCAGTTATAGCTTATTA	1
3	514	GGTCCTAGTAGGAAATAAA	2
4	1464	GGCAGACCCAGTATGAAAT	3
5	2010	GGTGTGCCAAGACATTAAT	4
6	2538	GGACTCTTCTTCCATATTA	5
7	1382	GGCAATGGAAACTATTATA	6
8	1024	GCAGTTGATTACTTCTTAT	7
9	574	GGACTTAGCAAGAAGTTAT	8
10	2427	GCTCAGCACAATCTGTAAA	9
11	1295	CTCCTTTCCACTGCTATTA	10
12	1063	GTTGGTGTGAAACAAATTA	11
13	240	CGAUACAGCUAAUUCAGAA	12
14	245	CAGCUAAUUCAGAAUCAUU	13
15	247	GCUAAUUCAGAAUCAUUUU	14
16	271	CGAAUAUGAUCCAACAAUA	15
17	2935	CCTGGTAACAGTAATACAT	16
18	569	GCTCAGGACTTAGCAAGAA	17
19	3495	CTCTGTGCCAGCTCTATAA	18
20	1508	GGGCTATATTTACATGCTA	19
21	330	CCTGTCTCTTGGATATTCT	20
22	406	GGAGGGCTTTCTTTGTGTA	21
23	2649	GTGGATATCTCCATGAAGT	22
24	461	CACCATTATAGAGAACAAA	23
25	3409	GGTCTGTAATATCTTACTA	24
26	234	CCTTGACGATACAGCTAAT	25
27	2779	GCTGATGCTTTGAACATCT	26
28	1251	CATCCCTGATGAATGTAAA	27
29	420	GUGUAUUUGCCAUAAAUAA	28
30	430	CAUAAAUAAUACUAAAUCA	29
31	441	CUAAAUCAUUUGAAGAUAU	30
32	452	GAAGAUAUUCACCAUUAUA	31
33	4055	TGGTTTAACAAAAGATTTG	W32
34	4359	TGTCCTAATATATACATAG	W33
35	991	TGAAAAAGAAACTGAATAC	W34
36	2428	CTCAGCACAATCTGTAAAT	35
37	1611	GCTCTTTCATAGTATAACT	36
38	3399	GTGCTAGTGTGGTCTGTAA	37
39	3402	CTAGTGTGGTCTGTAATAT	38
40	4204	GCAGACGTATATTGTATCA	39
41	4234	GTTCCCAAGTAGGCATTCT	40
42	268	GGACGAATATGATCCAACA	41

Target SEQ ID NO:	Position in NM_004985 Sequence	K-Ras Target Sequence	Targeted by aiRNA ID NO:
43	304	GAAGCAAGTAGTAATTGAT	42
44	1206	GCTTCCTGATGATGATTCT	43
45	3237	CCTGACCTCAAGTGATTCA	44
46	2567	GCCTCCCTACCTTCCACAT	W45
47	1403	GCCATTTCCTTTTCACATT	W46
48	4207	GACGTATATTGTATCATTT	W47
49	1402	GGCCATTTCCTTTTCACAT	W48
50	4075	GGGGGAGAATTCTAGAAAT	W49
51	4234	GTTCCCAAGTAGGCATTCT	50
52	268	GGACGAATATGATCCAACA	51
53	304	GAAGCAAGTAGTAATTGAT	52
54	1206	GCTTCCTGATGATGATTCT	53
55	5247	GAACTGTACTACTCCTAAT	54
56	3237	CCTGACCTCAAGTGATTCA	55
57	3386	GTCCTTAGGTAGTGCTAGT	56
58	1601	GTGTCAGACTGCTCTTTCA	57
59	1607	GACTGCTCTTTCATAGTAT	58
60	1255	CCTGATGAATGTAAAGTTA	59
61	2124	CAAGTCTGATCCATATTTA	60
62	688	GATGAGCAAAGATGGTAAA	61
63	2497	CAAGAGGTGAAGTTTATAT	62
64	3870	GGTAGGGTGTTAAGACTTA	63
65	1226	CTAGGCATCATGTCCTATA	64
66	4226	GAGTGAATGTTCCCAAGTA	65
67	517	CCTAGTAGGAAATAAATGT	66
68	3774	GCCTGAACTAGTTCACAGA	67
69	2970	CCAGAAATCTTCATGCAAT	68
70	2646	GCTGTGGATATCTCCATGA	69
71	303	GGAAGCAAGTAGTAATTGA	70
72	4203	CAGACGTATATTGTATCAT	71
73	233	GCCTTGACGATACAGCTAA	72
74	2259	GAAGGTGACTTAGGTTCTA	73
75	2076	GGCTAGTTCTCTTAACACT	74
76	3660	GTGTATGTCAGATATTCAT	75
77	1760	GAACCTTTGAGCTTTCATA	76
78	3789	CAGACAAGGAAACTTCTAT	77
79	3541	CTTCGATCAAGCTACTTTA	78
80	4954	GAGTGCCAATTTCTTACTA	79
81	1909	GCTGACAAATCAAGAGCAT	80
82	2346	GTCATCTCAAACTCTTAGT	81
83	638	GATGATGCCTTCTATACAT	82
84	2840	CTGGTATGAATAGACAGAA	83
85	2673	CACTGAGTCACATCAGAAA	84
86	4320	GTTGTCACCATTGCACAAT	85
87	2422	GTCAAGCTCAGCACAATCT	86
88	1484	GGGATTATTATAGCAACCA	87

Target SEQ ID NO:	Position in NM_004985 Sequence	K-Ras Target Sequence	Targeted by aiRNA ID NO:
89	2252	CTAGGAAGAAGGTGACTTA	88
90	493	GGACTCTGAAGATGTACCT	89
91	3135	CTGAGTAGCTGGGATTACA	90
92	4921	CATGAGTTCTTGAAGAATA	91
93	266	GTGGACGAATATGATCCAA	92
94	2647	CTGTGGATATCTCCATGAA	93
95	3791	GACAAGGAAACTTCTATGT	94
96	4197	GAATATAGCAGACGTATAT	95
97	3544	CGATCAAGCTACTTTATGT	96
98	2839	CCTGGTATGAATAGACAGA	97
99	2943	CAGTAATACATTCCATTGT	98
100	1758	GTGAACCTTTGAGCTTTCA	99
101	175	GCTGAAAATGACTGAATAT	101
102	176	CTGAAAATGACTGAATATA	102
103	178	GAAAATGACTGAATATAAA	103
104	240	CGATACAGCTAATTCAGAA	104
105	245	CAGCTAATTCAGAATCATT	105
106	247	GCTAATTCAGAATCATTTT	106
107	256	GAATCATTTTGTGGACGAA	107
108	271	CGAATATGATCCAACAATA	108
109	278	GATCCAACAATAGAGGATT	109
110	282	CAACAATAGAGGATTCCTA	110
111	292	GGATTCCTACAGGAAGCAA	111
112	297	CCTACAGGAAGCAAGTAGT	112
113	298	CTACAGGAAGCAAGTAGTA	113
114	301	CAGGAAGCAAGTAGTAATT	114
115	307	GCAAGTAGTAATTGATGGA	115
116	311	GTAGTAATTGATGGAGAAA	116
117	320	GATGGAGAAACCTGTCTCT	117
118	324	GAGAAACCTGTCTCTTGGA	118
119	326	GAAACCTGTCTCTTGGATA	119
120	333	GTCTCTTGGATATTCTCGA	120
121	335	CTCTTGGATATTCTCGACA	121
122	337	CTTGGATATTCTCGACACA	122
123	340	GGATATTCTCGACACAGCA	123
124	347	CTCGACACAGCAGGTCAAG	124
125	356	GCAGGTCAAGAGGAGTACA	125
126	362	CAAGAGGAGTACAGTGCAA	126
127	365	GAGGAGTACAGTGCAATGA	127
128	377	CAATGAGGGACCAGTACA	128
129	385	GGACCAGTACATGAGGACT	129
130	405	GGGAGGGCTTTCTTTGTGT	130
131	407	GAGGGCTTTCTTTGTGTAT	131
132	409	GGGCTTTCTTTGTGTATTT	132
133	416	CTTTGTGTATTTGCCATAA	133
134	422	GTATTTGCCATAAATAAT	134

Target SEQ ID NO:	Position in NM_004985 Sequence	K-Ras Target Sequence	Targeted by aiRNA ID NO:
135	441	CTAAATCATTTGAAGATAT	135
136	452	GAAGATATTCACCATTATA	136
137	463	CCATTATAGAGAACAAATT	137
138	464	CATTATAGAGAACAAATTA	138
139	471	GAGAACAAATTAAAAGAGT	139
140	473	GAACAAATTAAAAGAGTTA	140
141	486	GAGTTAAGGACTCTGAAGA	141
142	488	GTTAAGGACTCTGAAGATG	142
143	493	GGACTCTGAAGATGTACCT	143
144	494	GACTCTGAAGATGTACCTA	144
145	498	CTGAAGATGTACCTATGGT	145
146	509	CCTATGGTCCTAGTAGGAA	146
147	510	CTATGGTCCTAGTAGGAAA	147
148	515	GTCCTAGTAGGAAATAAAT	148
149	521	GTAGGAAATAAATGTGATT	149
150	542	CCTTCTAGAACAGTAGACA	150
151	546	CTAGAACAGTAGACACAAA	151
152	549	GAACAGTAGACACAAAACA	152
153	561	CAAAACAGGCTCAGGACTT	153
154	566	CAGGCTCAGGACTTAGCAA	154
155	568	GGCTCAGGACTTAGCAAGA	155
156	572	CAGGACTTAGCAAGAAGTT	156
157	577	CTTAGCAAGAAGTTATGGA	157
158	581	GCAAGAAGTTATGGAATTC	158
159	585	GAAGTTATGGAATTCCTTT	159
160	588	GTTATGGAATTCCTTTTAT	160
161	593	GGAATTCCTTTTATTGAAA	161
162	608	GAAACATCAGCAAAGACAA	162
163	612	CATCAGCAAAGACAAGACA	163
164	618	GCAAAGACAAGACAGGGTG	164
165	619	CAAAGACAAGACAGGGTGT	165
166	622	GACAAGACAGGGTGTTGAT	166
167	624	CAAGACAGGGTGTTGATGA	167
168	629	CAGGGTGTTGATGATGCCT	168
169	632	GGTGTTGATGATGCCTTCT	169
170	633	GTGTTGATGATGCCTTCTA	170
171	635	GTTGATGATGCCTTCTATA	171
172	639	ATGATGCCTTCTATACATT	172
173	641	GATGCCTTCTATACATTAG	173
174	644	GCCTTCTATACATTAGTTC	174
175	646	CTTCTATACATTAGTTCGA	175
176	649	CTATACATTAGTTCGAGAA	176
177	662	CGAGAAATTCGAAAACATA	177
178	663	GAGAAATTCGAAAACATAA	178
179	671	CGAAAACATAAAGAAAAGA	179
180	672	GAAAACATAAAGAAAAGAT	180

Target SEQ ID NO:	Position in NM_004985 Sequence	K-Ras Target Sequence	Targeted by aiRNA ID NO:
181	677	CATAAAGAAAAGATGAGCA	181
182	693	GCAAAGATGGTAAAAAGAA	182
183	694	CAAAGATGGTAAAAAGAAG	183
184	698	GATGGTAAAAAGAAGAAAA	184
185	701	GGTAAAAAGAAGAAAAAGA	185
186	702	GTAAAAAGAAGAAAAAGAA	186
187	709	GAAGAAAAAGAAGTCAAAG	187
188	712	GAAAAAGAAGTCAAAGACA	188
189	718	GAAGTCAAAGACAAAGTGT	189
190	721	GTCAAAGACAAAGTGTGTA	190
191	723	CAAAGACAAAGTGTGTAAT	191
192	727	GACAAAGTGTGTAATTATG	192
193	729	CAAAGTGTGTAATTATGTA	193
194	752	CAATTTGTACTTTTTTCTT	194
195	758	GTACTTTTTTCTTAAGGCA	195
196	761	CTTTTTTCTTAAGGCATAC	196
197	768	CTTAAGGCATACTAGTACA	197
198	775	CATACTAGTACAAGTGGTA	198
199	779	CTAGTACAAGTGGTAATTT	199
200	782	GTACAAGTGGTAATTTTTG	200
201	788	GTGGTAATTTTTGTACATT	201
202	791	GTAATTTTTGTACATTACA	202
203	800	GUACAUUACACUAAAUUAU	203
204	808	CATTACACTAAATTATTAG	204
205	810	CTAAATTATTAGCATTTGT	205
206	821	GCATTTGTTTTAGCATTAC	206
207	827	GTTTTAGCATTACCTAATT	207
208	851	CCTGCTCCATGCAGACTGT	208
209	852	CTGCTCCATGCAGACTGTT	209
210	854	GCTCCATGCAGACTGTTAG	210
211	857	CCATGCAGACTGTTAGCTT	211
212	862	GACTGTTAGCTTTTACCTTA	212
213	868	GUUAGCUUUUACCUUAAAU	213
214	872	GCUUUUACCUUAAAUGCUU	214
215	873	CUUUUACCUUAAAUGCUUA	215
216	911	GUUUUUUUUUCCUCUAAGU	216
217	931	CCAGUAUUCCCAGAGUUUU	217
218	941	CAGAGUUUUGGUUUUUGAA	218
219	943	GAGUUUUGGUUUUUGAACU	219
220	960	CUAGCAAUGCCUGUGAAAA	220
221	970	CUGUGAAAAAGAAACUGAA	221
222	972	GUGAAAAAGAAACUGAAUA	222
223	984	CUGAAUACCUAAGAUUUCU	223
224	986	GAAUACCUAAGAUUUCUGU	224
225	1025	CAGUUGAUUACUUCUUAUU	225
226	1027	GUUGAUUACUUCUUAUUUU	226

Target SEQ ID NO:	Position in NM_004985 Sequence	K-Ras Target Sequence	Targeted by aiRNA ID NO:
227	1030	GAUUACUUCUUAUUUUUCU	227
228	1038	CUUAUUUUUCUUACCAAUU	228
229	1047	CUUACCAAUUGUGAAUGUU	229
230	1059	GAAUGUUGGUGUGAAACAA	230
231	1067	GUGUGAAACAAAUUAAUGA	231
232	1101	CCUAUUCUGUGUUUUAUCU	232
233	1102	CUAUUCUGUGUUUUAUCUA	233
234	1125	CAUAAAUGGAUUAAUUACU	234
235	1159	CUUCUAAUUGGUUUUUACU	235
236	1162	CUAAUUGGUUUUUACUGAA	236
237	1169	GUUUUUACUGAAACAUUGA	237
238	1230	GCAUCAUGUCCUAUAGUUU	238
239	1278	GUUCACAAAGGUUUUGUCU	239
240	1403	GCCAUUUCCUUUUCACAUU	240
241	1404	CCAUUUCCUUUUCACAUUA	241
242	855	CTCCATGCAGACTGTTAGC	242
243	858	CATGCAGACTGTTAGCTTT	243
244	861	GCAGACTGTTAGCTTTTAC	244
245	866	CTGTTAGCTTTTACCTTAA	245
246	879	CCTTAAATGCTTATTTTAA	246
247	901	GACAGTGGAAGTTTTTTTT	247
248	902	ACAGTGGAAGTTTTTTTTT	248
249	921	CCTCTAAGTGCCAGTATTC	249
250	924	CTAAGTGCCAGTATTCCCA	250
251	928	GTGCCAGTATTCCCAGAGT	251
252	930	GCCAGTATTCCCAGAGTTT	252
253	931	CCAGTATTCCCAGAGTTTT	253
254	932	CAGTATTCCCAGAGTTTTG	254
255	934	GTATTCCCAGAGTTTTGGT	255
256	939	CCCAGAGTTTTGGTTTTTG	256
257	940	CCAGAGTTTTGGTTTTTGA	257
258	942	AGAGTTTTGGTTTTTGAAC	258
259	945	GTTTTGGTTTTTGAACTAG	259
260	950	GGTTTTTGAACTAGCAATG	260
261	957	GAACTAGCAATGCCTGTGA	261
262	963	GCAATGCCTGTGAAAAAGA	262
263	964	CAATGCCTGTGAAAAAGAA	263
264	968	GCCTGTGAAAAAGAAACTG	264
265	969	CCTGTGAAAAAGAAACTGA	265
266	973	TGAAAAAGAAACTGAATAC	266
267	980	GAAACTGAATACCTAAGAT	267
268	1001	CTGTCTTGGGGTTTTTGGT	268
269	1003	GTCTTGGGGTTTTTGGTGC	269
270	1005	CTTGGGGTTTTTGGTGCAT	270
271	1010	GCATGCAGTGTTTTTGGTG	271
272	1011	GTTTTTGGTGCATGCAGTT	272

Target SEQ ID NO:	Position in NM_004985 Sequence	K-Ras Target Sequence	Targeted by aiRNA ID NO:
273	1410	CCTTTTCACATTAGATAAA	273
274	1411	CTTTTCACATTAGATAAAT	274
275	1474	GTATGAAATGGGGATTATT	275
276	1450	CCATTTTGGGGCTATATTT	276
277	1451	CATTTTGGGGCTATATTTA	277
278	1546	GAAAAGATTTTAACAAGTA	278
279	1559	CAAGTATAAAAAATTCTCA	279
280	1576	CATAGGAATTAAATGTAGT	280
281	1611	GCTCTTTCATAGTATAACT	281
282	1612	CTCTTTCATAGTATAACTT	282
283	1614	CTTTCATAGTATAACTTTA	283
284	1628	CTTTAAATCTTTTCTTCAA	284
285	1641	CTTCAACTTGAGTCTTTGA	285
286	1644	CAACTTGAGTCTTTGAAGA	286
287	1650	GAGTCTTTGAAGATAGTTT	287
288	1652	GTCTTTGAAGATAGTTTTA	288
289	1654	CTTTGAAGATAGTTTTAAT	289
290	1704	CAGTTATAGCTTATTAGGT	290
291	1712	GCTTATTAGGTGTTGAAGA	291
292	1770	GCTTTCATAGAGAGTTTCA	292
293	1826	CATGCATTGGTTAGTCAAA	293
294	1925	CATTGCTTTTGTTTCTTAA	294
295	1929	GCTTTTGTTTCTTAAGAAA	295
296	1930	CTTTTGTTTCTTAAGAAAA	296
297	1939	CTTAAGAAAACAAACTCTT	297
298	1944	GAAAACAAACTCTTTTTTA	298
299	2041	CAATGAAGTGAAAAAGTTT	299
300	2045	GAAGTGAAAAAGTTTTACA	300
301	2084	CTCTTAACACTGGTTAAAT	301
302	2086	CTTAACACTGGTTAAATTA	302
303	2096	GTTAAATTAACATTGCATA	303
304	2110	GCATAAACACTTTTCAAGT	304
305	2169	CAATCCTTTTGATAAATTT	305
306	2263	GTGACTTAGGTTCTAGATA	306
307	2287	CTTTTAGGACTCTGATTTT	307
308	2311	CATCACTTACTATCCATTT	308
309	2314	CACTTACTATCCATTTCTT	309
310	2316	CTTACTATCCATTTCTTCA	310
311	2320	CTATCCATTTCTTCATGTT	311
312	2324	CCATTTCTTCATGTTAAAA	312
313	2343	GAAGTCATCTCAAACTCTT	313
314	2348	CATCTCAAACTCTTAGTTT	314
315	2351	CTCAAACTCTTAGTTTTTT	315
316	2380	CTATGTAATTTATATTCCA	316
317	2403	CATAAGGATACACTTATTT	317
318	2432	GCACAATCTGTAAATTTTT	318

Target SEQ ID NO:	Position in NM_004985 Sequence	K-Ras Target Sequence	Targeted by aiRNA ID NO:
319	2454	CTATGTTACACCATCTTCA	319

[0011] In some embodiments, the RNA duplex molecule, also referred to herein as an asymmetrical interfering RNA molecule or aiRNA molecule, comprises a sense strand sequence, an antisense strand sequence or a combination of a sense strand sequence and antisense strand sequence selected from those shown in Table 2 below.

Table 2.

aiRNA ID NO:	Sense Strand Sequence	Sense Strand SEQ ID NO:	Antisense Strand Sequence	Antisense Strand SEQ ID NO:
1	CAGUUAUAGCUUAUU	320	AAUAAUAAGCUAUAACUGGCC	638
2	CCUAGUAGGAAAUAA	321	AAUUUAUUUCCUACUAGGACC	639
3	AGACCCAGUAUGAAA	322	AAAUUUCAUACUGGGUCUGCC	640
4	GUGCCAAGACAUUAA	323	AAAUUAAUGUCUUGGCACACC	641
5	CUCUUCUUCCAUAUU	324	AAUAAUAUGGAAGAAGAGUCC	642
6	AAUGGAAACUAUUAU	325	AAUAUAAUAGUUUCCAUUGCC	643
7	GUUGAUUACUUCUUA	326	AAAUAAGAAGUAAUCAACUGC	644
8	CUUAGCAAGAAGUUA	327	AAAUAACUUCUUGCUAAGUCC	645
9	CAGCACAAUCUGUAA	328	AAUUUACAGAUUGUGCUGAGC	646
10	CUUUCCACUGCUAUU	329	AAUAAUAGCAGUGGAAAGGAG	647
11	GGUGUGAAACAAAUU	330	AAUAAUUUGUUUCACACCAAC	648
12	UACAGCUAAUUCAGA	331	AAUUCUGAAUUAGCUGUAUCG	649
13	CUAAUUCAGAAUCAU	332	AAAAUGAUUCUGAAUUAGCUG	650
14	AAUUCAGAAUCAUUU	333	AAAAAAUGAUUCUGAAUUAGC	651
15	AUAUGAUCCAACAAU	334	AAUAUUGUUGGAUCAUAUUCG	652
16	GGUAACAGUAAUACA	335	AAAUGUAUUACUGUUACCAGG	653
17	CAGGACUUAGCAAGA	336	AAUUCUUGCUAAGUCCUGAGC	654
18	UGUGCCAGCUCUAUA	337	AAUUAUAGAGCUGGCACAGAG	655
19	CUAUAUUUACAUGCU	338	AAUAGCAUGUAAAUAUAGCCC	656
20	GUCUCUUGGAUAUUC	339	AAAGAAUAUCCAAGAGACAGG	657
21	GGGCUUUCUUUGUGU	340	AAUACACAAAGAAAGCCCUCC	658
22	GAUAUCUCCAUGAAG	341	AAACUUCAUGGAGAUAUCCAC	659
23	CAUUAUAGAGAACAA	342	AAUUUGUUCUCUAUAAUGGUG	660
24	CUGUAAUAUCUUACU	343	AAUAGUAAGAUAUUACAGACC	661
25	UGACGAUACAGCUAA	344	AAAUUAGCUGUAUCGUCAAGG	662
26	GAUGCUUUGAACAUC	345	AAAGAUGUUCAAAGCAUCAGC	663
27	CCCUGAUGAAUGUAA	346	AAUUUACAUUCAUCAGGGAUG	664
28	UAUUUGCCAUAAAUA	347	AAUUAUUUAUGGCAAAUACAC	665
29	AAAUAAUACUAAAUC	348	AAUGAUUUAGUAUUAUUUAUG	666
30	AAUCAUUUGAAGAUA	349	AAAUAUCUUCAAAUGAUUUAG	667
31	GAUAUUCACCAUUAU	350	AAUAUAAUGGUGAAUAUCUUC	668
W32	UUUAACAAAAGAUUU	351	AACAAAUCUUUUGUUAAACCA	669
W33	CCUAAUAUAUACAUA	352	AACUAUGUAUAUAUUAGGACA	670

aiRNA ID NO:	Sense Strand Sequence	Sense Strand SEQ ID NO:	Antisense Strand Sequence	Antisense Strand SEQ ID NO:
W34	AAAAGAAACUGAAUA	353	AAGUAUUCAGUUUCUUUUUCA	671
35	AGCACAAUCUGUAAA	354	AAAUUUACAGAUUGUGCUGAG	672
36	CUUUCAUAGUAUAAC	355	AAAGUUAUACUAUGAAAGAGC	673
37	CUAGUGUGGUCUGUA	356	AAUUACAGACCACACUAGCAC	674
38	GUGUGGUCUGUAAUA	357	AAAUAUUACAGACCACACUAG	675
39	GACGUAUAUUGUAUC	358	AAUGAUACAAUAUACGUCUGC	676
40	CCCAAGUAGGCAUUC	359	AAAGAAUGCCUACUUGGGAAC	677
41	CGAAUAUGAUCCAAC	360	AAUGUUGGAUCAUAUUCGUCC	678
42	GCAAGUAGUAAUUGA	361	AAAUCAAUUACUACUUGCUUC	679
43	UCCUGAUGAUGAUUC	362	AAAGAAUCAUCAUCAGGAAGC	680
44	GACCUCAAGUGAUUC	363	AAUGAAUCACUUGAGGUCAGG	681
W45	UCCCUACCUUCCACA	364	AAAUGUGGAAGGUAGGGAGGC	682
W46	AUUUCCUUUUCACAU	365	AAAAUGUGAAAAGGAAAUGGC	683
W47	GUAUAUUGUAUCAUU	366	AAAAAUGAUACAAUAUACGUC	684
W48	CAUUUCCUUUUCACA	367	AAAUGUGAAAAGGAAAUGGCC	685
W49	GGAGAAUUCUAGAAA	368	AAAUUUCUAGAAUUCUCCCCC	686
50	CCCAAGUAGGCAUUC	369	AAAGAAUGCCUACUUGGGAAC	687
51	CGAAUAUGAUCCAAC	370	AAUGUUGGAUCAUAUUCGUCC	688
52	GCAAGUAGUAAUUGA	371	AAAUCAAUUACUACUUGCUUC	689
53	UCCUGAUGAUGAUUC	372	AAAGAAUCAUCAUCAGGAAGC	690
54	CUGUACUACUCCUAA	373	AAAUUAGGAGUAGUACAGUUC	691
55	GACCUCAAGUGAUUC	374	AAUGAAUCACUUGAGGUCAGG	692
56	CUUAGGUAGUGCUAG	375	AAACUAGCACUACCUAAGGAC	693
57	UCAGACUGCUCUUUC	376	AAUGAAAGAGCAGUCUGACAC	694
58	UGCUCUUUCAUAGUA	377	AAAUACUAUGAAAGAGCAGUC	695
59	GAUGAAUGUAAAGUU	378	AAUAACUUUACAUUCAUCAGG	696
60	GUCUGAUCCAUAUUU	379	AAUAAAUAUGGAUCAGACUUG	697
61	GAGCAAAGAUGGUAA	380	AAUUUACCAUCUUUGCUCAUC	698
62	GAGGUGAAGUUUAUA	381	AAAUAUAAACUUCACCUCUUG	699
63	AGGGUGUUAAGACUU	382	AAUAAGUCUUAACACCCUACC	700
64	GGCAUCAUGUCCUAU	383	AAUAUAGGACAUGAUGCCUAG	701
65	UGAAUGUUCCCAAGU	384	AAUACUUGGGAACAUUCACUC	702
66	AGUAGGAAAUAAAUG	385	AAACAUUUAUUUCCUACUAGG	703
67	UGAACUAGUUCACAG	386	AAUCUGUGAACUAGUUCAGGC	704
68	GAAAUCUUCAUGCAA	387	AAAUUGCAUGAAGAUUUCUGG	705
69	GUGGAUAUCUCCAUG	388	AAUCAUGGAGAUAUCCACAGC	706
70	AGCAAGUAGUAAUUG	389	AAUCAAUUACUACUUGCUUCC	707
71	ACGUAUAUUGUAUCA	390	AAAUGAUACAAUAUACGUCUG	708
72	UUGACGAUACAGCUA	391	AAUUAGCUGUAUCGUCAAGGC	709
73	GGUGACUUAGGUUCU	392	AAUAGAACCUAAGUCACCUUC	710
74	UAGUUCUCUUAACAC	393	AAAGUGUUAAGAGAACUAGCC	711
75	UAUGUCAGAUAUUCA	394	AAAUGAAUAUCUGACAUACAC	712
76	CCUUUGAGCUUUCAU	395	AAUAUGAAAGCUCAAAGGUUC	713
77	ACAAGGAAACUUCUA	396	AAAUAGAAGUUUCCUUGUCUG	714
78	CGAUCAAGCUACUUU	397	AAUAAAGUAGCUUGAUCGAAG	715
79	UGCCAAUUUCUUACU	398	AAUAGUAAGAAAUUGGCACUC	716

aiRNA ID NO:	Sense Strand Sequence	Sense Strand SEQ ID NO:	Antisense Strand Sequence	Antisense Strand SEQ ID NO:
80	GACAAAUCAAGAGCA	399	AAAUGCUCUUGAUUUGUCAGC	717
81	AUCUCAAACUCUUAG	400	AAACUAAGAGUUUGAGAUGAC	718
82	GAUGCCUUCUAUACA	401	AAAUGUAUAGAAGGCAUCAUC	719
83	GUAUGAAUAGACAGA	402	AAUUCUGUCUAUUCAUACCAG	720
84	UGAGUCACAUCAGAA	403	AAUUUCUGAUGUGACUCAGUG	721
85	GUCACCAUUGCACAA	404	AAAUUGUGCAAUGGUGACAAC	722
86	AAGCUCAGCACAAUC	405	AAAGAUUGUGCUGAGCUUGAC	723
87	AUUAUUAUAGCAACC	406	AAUGGUUGCUAUAAUAAUCCC	724
88	GGAAGAAGGUGACUU	407	AAUAAGUCACCUUCUUCCUAG	725
89	CUCUGAAGAUGUACC	408	AAAGGUACAUCUUCAGAGUCC	726
90	AGUAGCUGGGAUUAC	409	AAUGUAAUCCCAGCUACUCAG	727
91	GAGUUCUUGAAGAAU	410	AAUAUUCUUCAAGAACUCAUG	728
92	GACGAAUAUGAUCCA	411	AAUUGGAUCAUAUUCGUCCAC	729
93	UGGAUAUCUCCAUGA	412	AAUUCAUGGAGAUAUCCACAG	730
94	AAGGAAACUUCUAUG	413	AAACAUAGAAGUUUCCUUGUC	731
95	UAUAGCAGACGUAUA	414	AAAUAUACGUCUGCUAUAUUC	732
96	UCAAGCUACUUUAUG	415	AAACAUAAAGUAGCUUGAUCG	733
97	GGUAUGAAUAGACAG	416	AAUCUGUCUAUUCAUACCAGG	734
98	UAAUACAUUCCAUUG	417	AAACAAUGGAAUGUAUUACUG	735
99	AACCUUUGAGCUUUC	418	AAUGAAAGCUCAAAGGUUCAC	736
101	GAAAAUGACUGAAUA	419	AAAUAUUCAGUCAUUUUCAGC	737
102	AAAAUGACUGAAUAU	420	AAUAUAUUCAGUCAUUUUCAG	738
103	AAUGACUGAAUAUAA	421	AAUUUAUAUUCAGUCAUUUUC	739
104	UACAGCUAAUUCAGA	422	AAUUCUGAAUUAGCUGUAUCG	740
105	CUAAUUCAGAAUCAU	423	AAAAUGAUUCUGAAUUAGCUG	741
106	AAUUCAGAAUCAUUU	424	AAAAAAUGAUUCUGAAUUAGC	742
107	UCAUUUUGUGGACGA	425	AAUUCGUCCACAAAAUGAUUC	743
108	AUAUGAUCCAACAAU	426	AAUAUUGUUGGAUCAUAUUCG	744
109	CCAACAAUAGAGGAU	427	AAAAUCCUCUAUUGUUGGAUC	745
110	CAAUAGAGGAUUCCU	428	AAUAGGAAUCCUCUAUUGUUG	746
111	UUCCUACAGGAAGCA	429	AAUUGCUUCCUGUAGGAAUCC	747
112	ACAGGAAGCAAGUAG	430	AAACUACUUGCUUCCUGUAGG	748
113	CAGGAAGCAAGUAGU	431	AAUACUACUUGCUUCCUGUAG	749
114	GAAGCAAGUAGUAAU	432	AAAAUUACUACUUGCUUCCUG	750
115	AGUAGUAAUUGAUGG	433	AAUCCAUCAAUUACUACUUGC	751
116	GUAAUUGAUGGAGAA	434	AAUUUCUCCAUCAAUUACUAC	752
117	GGAGAAACCUGUCUC	435	AAAGAGACAGGUUUCUCCAUC	753
118	AAACCUGUCUCUUGG	436	AAUCCAAGAGACAGGUUUCUC	754
119	ACCUGUCUCUUGGAU	437	AAUAUCCAAGAGACAGGUUUC	755
120	UCUUGGAUAUUCUCG	438	AAUCGAGAAUAUCCAAGAGAC	756
121	UUGGAUAUUCUCGAC	439	AAUGUCGAGAAUAUCCAAGAG	757
122	GGAUAUUCUCGACAC	440	AAUGUGUCGAGAAUAUCCAAG	758
123	UAUUCUCGACACAGC	441	AAUGCUGUGUCGAGAAUAUCC	759
124	GACACAGCAGGUCAA	442	AACUUGACCUGCUGUGUCGAG	760
125	GGUCAAGAGGAGUAC	443	AAUGUACUCCUCUUGACCUGC	761
126	GAGGAGUACAGUGCA	444	AAUUGCACUGUACUCCUCUUG	762

aiRNA ID NO:	Sense Strand Sequence	Sense Strand SEQ ID NO:	Antisense Strand Sequence	Antisense Strand SEQ ID NO:
127	GAGUACAGUGCAAUG	445	AAUCAUUGCACUGUACUCCUC	763
128	UGAGGGACCAGUAC	446	AAUGUACUGGUCCCUCAUUG	764
129	CCAGUACAUGAGGAC	447	AAAGUCCUCAUGUACUGGUCC	765
130	AGGGCUUUCUUUGUG	448	AAACACAAAGAAAGCCCUCCC	766
131	GGCUUUCUUUGUGUA	449	AAAUACACAAAGAAAGCCCUC	767
132	CUUUCUUUGUGUAUU	450	AAAAAUACACAAAGAAAGCCC	768
133	UGUGUAUUUGCCAUA	451	AAUUAUGGCAAAUACACAAAG	769
134	UUUGCCAUAAAAUAA	452	AAAUUAUUUAUGGCAAAUAC	770
135	AAUCAUUUGAAGAUA	453	AAAUAUCUUCAAAUGAUUUAG	771
136	GAUAUUCACCAUUAU	454	AAUAUAAUGGUGAAUAUCUUC	772
137	UUAUAGAGAACAAAU	455	AAAAUUUGUUCUCUAUAAUGG	773
138	UAUAGAGAACAAAUU	456	AAUAAUUUGUUCUCUAUAAUG	774
139	AACAAAUUAAAAGAG	457	AAACUCUUUUAAUUUGUUCUC	775
140	CAAAUUAAAAGAGUU	458	AAUAACUCUUUUAAUUUGUUC	776
141	UUAAGGACUCUGAAG	459	AAUCUUCAGAGUCCUUAACUC	777
142	AAGGACUCUGAAGAU	460	AACAUCUUCAGAGUCCUUAAC	778
143	CUCUGAAGAUGUACC	461	AAAGGUACAUCUUCAGAGUCC	779
144	UCUGAAGAUGUACCU	462	AAUAGGUACAUCUUCAGAGUC	780
145	AAGAUGUACCUAUGG	463	AAACCAUAGGUACAUCUUCAG	781
146	AUGGUCCUAGUAGGA	464	AAUUCCUACUAGGACCAUAGG	782
147	UGGUCCUAGUAGGAA	465	AAUUUCCUACUAGGACCAUAG	783
148	CUAGUAGGAAAUAAA	466	AAAUUUAUUUCCUACUAGGAC	784
149	GGAAAUAAAUGUGAU	467	AAAAUCACAUUUAUUUCCUAC	785
150	UCUAGAACAGUAGAC	468	AAUGUCUACUGUUCUAGAAGG	786
151	GAACAGUAGACACAA	469	AAUUUGUGUCUACUGUUCUAG	787
152	CAGUAGACACAAAAC	470	AAUGUUUUGUGUCUACUGUUC	788
153	AACAGGCUCAGGACU	471	AAAAGUCCUGAGCCUGUUUUG	789
154	GCUCAGGACUUAGCA	472	AAUUGCUAAGUCCUGAGCCUG	790
155	UCAGGACUUAGCAAG	473	AAUCUUGCUAAGUCCUGAGCC	791
156	GACUUAGCAAGAAGU	474	AAAACUUCUUGCUAAGUCCUG	792
157	AGCAAGAAGUUAUGG	475	AAUCCAUAACUUCUUGCUAAG	793
158	AGAAGUUAUGGAAUU	476	AAGAAUUCCAUAACUUCUUGC	794
159	GUUAUGGAAUUCCUU	477	AAAAAGGAAUUCCAUAACUUC	795
160	AUGGAAUUCCUUUUA	478	AAAUAAAAGGAAUUCCAUAAC	796
161	AUUCCUUUUAUUGAA	479	AAUUUCAAUAAAAGGAAUUCC	797
162	ACAUCAGCAAAGACA	480	AAUUGUCUUUGCUGAUGUUUC	798
163	CAGCAAAGACAAGAC	481	AAUGUCUUGUCUUUGCUGAUG	799
164	AAGACAAGACAGGGU	482	AACACCCUGUCUUGUCUUUGC	800
165	AGACAAGACAGGGUG	483	AAACACCCUGUCUUGUCUUUG	801
166	AAGACAGGGUGUUGA	484	AAAUCAACACCCUGUCUUGUC	802
167	GACAGGGUGUUGAUG	485	AAUCAUCAACACCCUGUCUUG	803
168	GGUGUUGAUGAUGCC	486	AAAGGCAUCAUCAACACCCUG	804
169	GUUGAUGAUGCCUUC	487	AAAGAAGGCAUCAUCAACACC	805
170	UUGAUGAUGCCUUCU	488	AAUAGAAGGCAUCAUCAACAC	806
171	GAUGAUGCCUUCUAU	489	AAUAUAGAAGGCAUCAUCAAC	807
172	AUGCCUUCUAUACAU	490	AAAAUGUAUAGAAGGCAUCAU	808

aiRNA ID NO:	Sense Strand Sequence	Sense Strand SEQ ID NO:	Antisense Strand Sequence	Antisense Strand SEQ ID NO:
173	GCCUUCUAUACAUUA	491	AACUAAUGUAUAGAAGGCAUC	809
174	UUCUAUACAUUAGUU	492	AAGAACUAAUGUAUAGAAGGC	810
175	CUAUACAUUAGUUCG	493	AAUCGAACUAAUGUAUAGAAG	811
176	UACAUUAGUUCGAGA	494	AAUUCUCGAACUAAUGUAUAG	812
177	GAAAUUCGAAAACAU	495	AAUAUGUUUUCGAAUUUCUCG	813
178	AAAUUCGAAAACAUA	496	AAUUAUGUUUUCGAAUUUCUC	814
179	AAACAUAAAGAAAAG	497	AAUCUUUUCUUUAUGUUUUCG	815
180	AACAUAAAGAAAAGA	498	AAAUCUUUUCUUUAUGUUUUC	816
181	AAAGAAAAGAUGAGC	499	AAUGCUCAUCUUUUCUUUAUG	817
182	AAGAUGGUAAAAAGA	500	AAUUCUUUUUACCAUCUUUGC	818
183	AGAUGGUAAAAAGAA	501	AACUUCUUUUUACCAUCUUUG	819
184	GGUAAAAAGAAGAAA	502	AAUUUUCUUCUUUUUACCAUC	820
185	AAAAAGAAGAAAAAG	503	AAUCUUUUUCUUCUUUUUACC	821
186	AAAAGAAGAAAAAGA	504	AAUUCUUUUUCUUCUUUUUAC	822
187	GAAAAAGAAGUCAAA	505	AACUUUGACUUCUUUUUCUUC	823
188	AAAGAAGUCAAAGAC	506	AAUGUCUUUGACUUCUUUUUC	824
189	GUCAAAGACAAAGUG	507	AAACACUUUGUCUUUGACUUC	825
190	AAAGACAAAGUGUGU	508	AAUACACACUUUGUCUUUGAC	826
191	AGACAAAGUGUGUAA	509	AAAUUACACACUUUGUCUUUG	827
192	AAAGUGUGUAAUUAU	510	AACAUAAUUACACACUUUGUC	828
193	AGUGUGUAAUUAUGU	511	AAUACAUAAUUACACACUUUG	829
194	UUUGUACUUUUUUCU	512	AAAAGAAAAAAGUACAAAUUG	830
195	CUUUUUUCUUAAGGC	513	AAUGCCUUAAGAAAAAAGUAC	831
196	UUUUCUUAAGGCAUA	514	AAGUAUGCCUUAAGAAAAAAG	832
197	AAGGCAUACUAGUAC	515	AAUGUACUAGUAUGCCUUAAG	833
198	ACUAGUACAAGUGGU	516	AAUACCACUUGUACUAGUAUG	834
199	GUACAAGUGGUAAUU	517	AAAAAUUACCACUUGUACUAG	835
200	CAAGUGGUAAUUUUU	518	AACAAAAAUUACCACUUGUAC	836
201	GUAAUUUUUGUACAU	519	AAAAUGUACAAAAAUUACCAC	837
202	AUUUUUGUACAUUAC	520	AAUGUAAUGUACAAAAAUUAC	838
203	CAUUACACUAAAUUA	521	AAAUAAUUUAGUGUAAUGUAC	839
204	UACACUAAAUUAUUA	522	AACUAAUAAUUUAGUGUAAUG	840
205	AAUUAUUAGCAUUUG	523	AAACAAAUGCUAAUAAUUUAG	841
206	UUUGUUUUAGCAUUA	524	AAGUAAUGCUAAAACAAAUGC	842
207	UUAGCAUUACCUAAU	525	AAAAUUAGGUAAUGCUAAAAC	843
208	GCUCCAUGCAGACUG	526	AAACAGUCUGCAUGGAGCAGG	844
209	CUCCAUGCAGACUGU	527	AAAACAGUCUGCAUGGAGCAG	845
210	CCAUGCAGACUGUUA	528	AACUAACAGUCUGCAUGGAGC	846
211	UGCAGACUGUUAGCU	529	AAAAGCUAACAGUCUGCAUGG	847
212	UGUUAGCUUUUACCUU	530	AAUAAGGUAAAAGCUAACAGUC	848
213	AGCUUUUACCUUAAA	531	AAAUUUAAGGUAAAAGCUAAC	849
214	UUUACCUUAAAUGCU	532	AAAAGCAUUUAAGGUAAAAGC	850
215	UUACCUUAAAUGCUU	533	AAUAAGCAUUUAAGGUAAAAG	851
216	UUUUUUUCCUCUAAG	534	AAACUUAGAGGAAAAAAAAAC	852
217	GUAUUCCCAGAGUUU	535	AAAAAACUCUGGGAAUACUGG	853
218	AGUUUUGGUUUUUGA	536	AAUUCAAAAACCAAAACUCUG	854

aiRNA ID NO:	Sense Strand Sequence	Sense Strand SEQ ID NO:	Antisense Strand Sequence	Antisense Strand SEQ ID NO:
219	UUUUGGUUUUUGAAC	537	AAAGUUCAAAAACCAAAACUC	855
220	GCAAUGCCUGUGAAA	538	AAUUUUCACAGGCAUUGCUAG	856
221	UGAAAAAGAAACUGA	539	AAUUCAGUUUCUUUUUCACAG	857
222	AAAAAGAAACUGAAU	540	AAUAUUCAGUUUCUUUUUCAC	858
223	AAUACCUAAGAUUUC	541	AAAGAAAUCUUAGGUAUUCAG	859
224	UACCUAAGAUUUCUG	542	AAACAGAAAUCUUAGGUAUUC	860
225	UUGAUUACUUCUUAU	543	AAAAUAAGAAGUAAUCAACUG	861
226	GAUUACUUCUUAUUU	544	AAAAAAUAAGAAGUAAUCAAC	862
227	UACUUCUUAUUUUUC	545	AAAGAAAAAUAAGAAGUAAUC	863
228	AUUUUUCUUACCAAU	546	AAAAUUGGUAAGAAAAAUAAG	864
229	ACCAAUUGUGAAUGU	547	AAAACAUUCACAAUUGGUAAG	865
230	UGUUGGUGUGAAACA	548	AAUUGUUUCACACCAACAUUC	866
231	UGAAACAAAUUAAUG	549	AAUCAUUAAUUUGUUUCACAC	867
232	AUUCUGUGUUUUAUC	550	AAAGAUAAAACACAGAAUAGG	868
233	UUCUGUGUUUUAUCU	551	AAUAGAUAAAACACAGAAUAG	869
234	AAAUGGAUUAAUUAC	552	AAAGUAAUUAAUCCAUUUAUG	870
235	CUAAUUGGUUUUUAC	553	AAAGUAAAAACCAAUUAGAAG	871
236	AUUGGUUUUUACUGA	554	AAUUCAGUAAAAACCAAUUAG	872
237	UUUACUGAAACAUUG	555	AAUCAAUGUUUCAGUAAAAAC	873
238	UCAUGUCCUAUAGUU	556	AAAAACUAUAGGACAUGAUGC	874
239	CACAAAGGUUUUGUC	557	AAAGACAAAACCUUUGUGAAC	875
240	AUUUCCUUUUCACAU	558	AAAAUGUGAAAAGGAAAUGGC	876
241	UUUCCUUUUCACAUU	559	AAUAAUGUGAAAAGGAAAUGG	877
242	CAUGCAGACUGUUAG	560	AAGCUAACAGUCUGCAUGGAG	878
243	GCAGACUGUUAGCUU	561	AAAAAGCUAACAGUCUGCAUG	879
244	GACUGUUAGCUUUUA	562	AAGUAAAAGCUAACAGUCUGC	880
245	UUAGCUUUUACCUUA	563	AAUUAAGGUAAAAGCUAACAG	881
246	UAAAUGCUUAUUUUA	564	AAUUAAAAUAAGCAUUUAAGG	882
247	AGUGGAAGUUUUUUU	565	AAAAAAAAAACUUCCACUGUC	883
248	GUGGAAGUUUUUUUU	566	AAAAAAAAAAACUUCCACUGU	884
249	CUAAGUGCCAGUAUU	567	AAGAAUACUGGCACUUAGAGG	885
250	AGUGCCAGUAUUCCC	568	AAUGGGAAUACUGGCACUUAG	886
251	CCAGUAUUCCCAGAG	569	AAACUCUGGGAAUACUGGCAC	887
252	AGUAUUCCCAGAGUU	570	AAAAACUCUGGGAAUACUGGC	888
253	GUAUUCCCAGAGUUU	571	AAAAAACUCUGGGAAUACUGG	889
254	UAUUCCCAGAGUUUU	572	AACAAAACUCUGGGAAUACUG	890
255	UUCCCAGAGUUUUGG	573	AAACCAAAACUCUGGGAAUAC	891
256	AGAGUUUUGGUUUUU	574	AACAAAAACCAAAACUCUGGG	892
257	GAGUUUUGGUUUUUG	575	AAUCAAAAACCAAAACUCUGG	893
258	GUUUUGGUUUUUGAA	576	AAGUUCAAAAACCAAAACUCU	894
259	UUGGUUUUUGAACUA	577	AACUAGUUCAAAAACCAAAAC	895
260	UUUUGAACUAGCAAU	578	AACAUUGCUAGUUCAAAAACC	896
261	CUAGCAAUGCCUGUG	579	AAUCACAGGCAUUGCUAGUUC	897
262	AUGCCUGUGAAAAAG	580	AAUCUUUUUCACAGGCAUUGC	898
263	UGCCUGUGAAAAAGA	581	AAUUCUUUUUCACAGGCAUUG	899
264	UGUGAAAAAGAAACU	582	AACAGUUUCUUUUUCACAGGC	900

aiRNA ID NO:	Sense Strand Sequence	Sense Strand SEQ ID NO:	Antisense Strand Sequence	Antisense Strand SEQ ID NO:
265	GUGAAAAAGAAACUG	583	AAUCAGUUUCUUUUUCACAGG	901
266	AAAAGAAACUGAAUA	584	AAGUAUUCAGUUUCUUUUUCA	902
267	ACUGAAUACCUAAGA	585	AAAUCUUAGGUAUUCAGUUUC	903
268	UCUUGGGGUUUUUGG	586	AAACCAAAAACCCCAAGACAG	904
269	UUGGGGUUUUUGGUG	587	AAGCACCAAAAACCCCAAGAC	905
270	GGGGUUUUUGGUGCA	588	AAAUGCACCAAAAACCCCAAG	906
271	UGCAGUGUUUUUGGU	589	AACACCAAAAACACUGCAUGC	907
272	UUUGGUGCAUGCAGU	590	AAAACUGCAUGCACCAAAAAC	908
273	UUUCACAUUAGAUAA	591	AAUUUAUCUAAUGUGAAAAGG	909
274	UUCACAUUAGAUAAA	592	AAAUUUAUCUAAUGUGAAAAG	910
275	UGAAAUGGGGAUUAU	593	AAAAUAAUCCCCAUUUCAUAC	911
276	UUUUGGGGCUAUAUU	594	AAAAAUAUAGCCCCAAAAUGG	912
277	UUUGGGGCUAUAUUU	595	AAUAAAUAUAGCCCCAAAAUG	913
278	AAGAUUUUAACAAGU	596	AAUACUUGUUAAAAUCUUUUC	914
279	GUAUAAAAAAUUCUC	597	AAUGAGAAUUUUUUAUACUUG	915
280	AGGAAUUAAAUGUAG	598	AAACUACAUUUAAUUCCUAUG	916
281	CUUUCAUAGUAUAAC	599	AAAGUUAUACUAUGAAAGAGC	917
282	UUUCAUAGUAUAACU	600	AAAAGUUAUACUAUGAAAGAG	918
283	UCAUAGUAUAACUUU	601	AAUAAAGUUAUACUAUGAAAG	919
284	UAAAUCUUUUCUUCA	602	AAUUGAAGAAAAGAUUUAAAG	920
285	CAACUUGAGUCUUUG	603	AAUCAAAGACUCAAGUUGAAG	921
286	CUUGAGUCUUUGAAG	604	AAUCUUCAAAGACUCAAGUUG	922
287	UCUUUGAAGAUAGUU	605	AAAAACUAUCUUCAAAGACUC	923
288	UUUGAAGAUAGUUUU	606	AAUAAAACUAUCUUCAAAGAC	924
289	UGAAGAUAGUUUUAA	607	AAAUUAAAACUAUCUUCAAAG	925
290	UUAUAGCUUAUUAGG	608	AAACCUAAUAAGCUAUAACUG	926
291	UAUUAGGUGUUGAAG	609	AAUCUUCAACACCUAAUAAGC	927
292	UUCAUAGAGAGUUUC	610	AAUGAAACUCUCUAUGAAAGC	928
293	GCAUUGGUUAGUCAA	611	AAUUUGACUAACCAAUGCAUG	929
294	UGCUUUUGUUUCUUA	612	AAUUAAGAAACAAAAGCAAUG	930
295	UUUGUUUCUUAAGAA	613	AAUUUCUUAAGAAACAAAAGC	931
296	UUGUUUCUUAAGAAA	614	AAUUUUCUUAAGAAACAAAAG	932
297	AAGAAAACAAACUCU	615	AAAAGAGUUUGUUUUCUUAAG	933
298	AACAAACUCUUUUUU	616	AAUAAAAAAAGAGUUUGUUUUC	934
299	UGAAGUGAAAAAGUU	617	AAAAACUUUUUCACUUCAUUG	935
300	GUGAAAAAGUUUUAC	618	AAUGUAAAACUUUUUCACUUC	936
301	UUAACACUGGUUAAA	619	AAAUUUAACCAGUGUUAAGAG	937
302	AACACUGGUUAAAUU	620	AAUAAUUUAACCAGUGUUAAG	938
303	AAAUUAACAUUGCAU	621	AAUAUGCAAUGUUAAUUUAAC	939
304	UAAACACUUUUCAAG	622	AAACUUGAAAAGUGUUUAUGC	940
305	UCCUUUUGAUAAAUU	623	AAAAAUUUAUCAAAAGGAUUG	941
306	ACUUAGGUUCUAGAU	624	AAUAUCUAGAACCUAAGUCAC	942
307	UUAGGACUCUGAUUU	625	AAAAAAUCAGAGUCCUAAAAG	943
308	CACUUACUAUCCAUU	626	AAAAAUGGAUAGUAAGUGAUG	944
309	UUACUAUCCAUUUCU	627	AAAAGAAAUGGAUAGUAAGUG	945
310	ACUAUCCAUUUCUUC	628	AAUGAAGAAAUGGAUAGUAAG	946

aiRNA ID NO:	Sense Strand Sequence	Sense Strand SEQ ID NO:	Antisense Strand Sequence	Antisense Strand SEQ ID NO:
311	UCCAUUUCUUCAUGU	629	AAAACAUGAAGAAAUGGAUAG	947
312	UUUCUUCAUGUUAAA	630	AAUUUUAACAUGAAGAAAUGG	948
313	GUCAUCUCAAACUCU	631	AAAAGAGUUUGAGAUGACUUC	949
314	CUCAAACUCUUAGUU	632	AAAAACUAAGAGUUUGAGAUG	950
315	AAACUCUUAGUUUUU	633	AAAAAAAACUAAGAGUUUGAG	951
316	UGUAAUUUAUAUUCC	634	AAUGGAAUAUAAAUUACAUAG	952
317	AAGGAUACACUUAUU	635	AAAAAUAAGUGUAUCCUUAUG	953
318	CAAUCUGUAAAUUUU	636	AAAAAAAUUUACAGAUUGUGC	954
319	UGUUACACCAUCUUC	637	AAUGAAGAUGGUGUAACAUAG	955

[0012] In some embodiments, the RNA duplex molecule (aiRNA) comprises a sense strand sequence selected from the group consisting of SEQ ID NOs: 320-637. In some embodiments, the RNA duplex molecule (aiRNA) comprises an antisense strand sequence selected from the group consisting of SEQ ID NOs: 638-955. In some embodiments, the RNA duplex molecule (aiRNA) comprises a sense strand sequence selected from the group consisting of SEQ ID NOs: 320-637 and an antisense strand sequence selected from the group consisting of SEQ ID NOs: 638-955.

[0013] In some embodiments, the RNA duplex molecule (aiRNA) comprises a sense strand sequence that is at least, e.g., 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95% or more identical to a sequence selected from the group consisting of SEQ ID NOs: 320-637. In some embodiments, the RNA duplex molecule (aiRNA) comprises an antisense strand sequence that is at least, e.g., 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95% or more identical to a sequence selected from the group consisting of SEQ ID NOs: 638-955. In some embodiments, the RNA duplex molecule (aiRNA) comprises a sense strand sequence that is at least, e.g., 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95% or more identical to a sequence selected from the group consisting of SEQ ID NOs: 320-637 and an antisense strand sequence that is at least, e.g., 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95% or more identical to a sequence selected from the group consisting of SEQ ID NOs: 638-955.

[0014] In some embodiments, at least one nucleotide of the sequence of 5' overhang is selected from the group consisting of A, U, and dT.

[0015] In some embodiments, the GC content of the double stranded region is 20%-70%.

[0016] In some embodiments, the first strand has a length from 19-22 nucleotides.

[0017] In some embodiments, the first strand has a length of 21 nucleotides. In a further embodiment, the second strand has a length of 14-16 nucleotides.

[0018] In some embodiments, the first strand has a length of 21 nucleotides, and the second strand has a length of 15 nucleotides. In a further embodiment, the first strand has a 3'-overhang of 2-4 nucleotides. In an even further embodiment, the first strand has a 3'-overhang of 3 nucleotides.

[0019] In some embodiments, the duplex RNA molecule contains at least one modified nucleotide or its analogue. In a further embodiment, the at least one modified nucleotide or its analogue is sugar-, backbone-, and/or base- modified ribonucleotide. In an even further embodiment, the backbone-modified ribonucleotide has a modification in a phosphodiester linkage with another ribonucleotide. In some embodiments, the phosphodiester linkage is modified to include at least one of a nitrogen or a sulphur heteroatom. In another embodiment, the nucleotide analogue is a backbone-modified ribonucleotide containing a phosphothioate group.

[0020] In some embodiments, the at least one modified nucleotide or its analogue is an unusual base or a modified base. In another embodiment, the at least one modified nucleotide or its analogue comprises inosine, or a tritylated base.

[0021] In a further embodiment, the nucleotide analogue is a sugar-modified ribonucleotide, wherein the 2'-OH group is replaced by a group selected from H, OR, R, halo, SH, SR, NH_2, NHR, NR_2, or CN, wherein each R is independently C1-C6 alkyl, alkenyl or alkynyl, and halo is F, Cl, Br or I.

[0022] In some embodiments, the first strand comprises at least one deoxynucleotide. In a further embodiment, the at least one deoxynucleotides are in one or more regions selected from the group consisting of 3'-overhang, 5'-overhang, and double-stranded region. In another embodiment, the second strand comprises at least one deoxynucleotide.

[0023] The present invention also provides a method of modulating K-Ras expression, e.g., silencing K-Ras expression or otherwise reducing K-Ras expression, in a cell or an organism comprising the steps of contacting said cell or organism with an asymmetrical duplex RNA molecule of the disclosure under conditions wherein selective K-Ras gene silencing can occur, and mediating a selective K-Ras gene silencing effected by the duplex RNA molecule towards K-Ras or nucleic acid having a sequence portion substantially corresponding to the double-stranded RNA. In a further embodiment, said contacting step comprises the step of introducing said duplex RNA molecule into a target

cell in culture or in an organism in which the selective K-Ras silencing can occur. In an even further embodiment, the introducing step is selected from the group consisting of transfection, lipofection, electroporation, infection, injection, oral administration, inhalation, topical and regional administration. In another embodiment, the introducing step comprises using a pharmaceutically acceptable excipient, carrier, or diluent selected from the group consisting of a pharmaceutical carrier, a positive-charge carrier, a liposome, a protein carrier, a polymer, a nanoparticle, a nanoemulsion, a lipid, and a lipoid.

[0024] In some embodiments, the modulating method is used for determining the function or utility of a gene in a cell or an organism.

[0025] In some embodiments, the modulating method is used for treating or preventing a disease or an undesirable condition. In some embodiments, the disease or undesirable condition is a cancer, for example, gastric cancer.

[0026] The disclosure provides compositions and methods for targeting K-Ras in the treatment, prevention, delaying the progression of, or otherwise ameliorating a symptom of gastric cancer. In some embodiments, the method comprises administering to subject in need thereof a therapeutically effective amount of a duplex RNA molecule of the disclosure. In some embodiments, the subject is human. In some embodiments, the subject is suffering from gastric cancer. In some embodiments, the subject is diagnosed with gastric cancer. In some embodiments, the subject is predisposed to gastric cancer.

[0027] The disclosure also provides compositions and methods for targeting K-Ras to inhibit the survival and/or proliferation of cancer stem cells. In some embodiments, the method comprises administering to subject in need thereof a therapeutically effective amount of a duplex RNA molecule of the disclosure. In some embodiments, the subject is human. In some embodiments, the subject is suffering from gastric cancer. In some embodiments, the subject is diagnosed with gastric cancer. In some embodiments, the subject is predisposed to gastric cancer.

[0028] The disclosure also provides compositions and methods for targeting K-Ras in the inhibition of to inhibit the survival and/or proliferation of CSCs in the treatment, prevention, delaying the progression of, or otherwise ameliorating a symptom of gastric cancer. In some embodiments, the method comprises administering to subject in need thereof a therapeutically effective amount of a duplex RNA molecule of the disclosure. In some embodiments, the subject is human. In some embodiments, the subject is suffering from gastric cancer. In some embodiments, the subject is diagnosed with gastric cancer. In some embodiments, the subject is predisposed to gastric cancer.

[0029] The disclosure also provides a method for treating cancer in a selected patient population, the method comprising the steps of: (a) measuring a level of mutant K-Ras gene amplification in a biological sample obtained from a patient candidate diagnosed of a cancer; (b) confirming that the patient candidate's mutant K-Ras gene amplification level is above a benchmark level; and (c) administering to the patient candidate a duplex RNA molecule comprising a first strand comprising a nucleotide sequence with a length from 18-23 nucleotides, wherein the nucleotide sequence of the first strand is substantially complementary to a target K-Ras mRNA sequence, and a second strand comprising a nucleotide sequence with a length from 12-17 nucleotides, wherein the second strand is substantially complementary to the first strand, and forms a double-stranded region with the first strand, wherein the first strand has a 3'-overhang from 1-9 nucleotides, and a 5'-overhang from 0-8 nucleotides, and wherein said duplex RNA molecule is capable of effecting selective K-Ras gene silencing.

[0030] In some embodiments, the steps (a), (b), and (c) may be performed by one actor or several actors.

[0031] In some embodiments, a patient candidate's mutant K-Ras gene amplification level is considered to be above a benchmark level if it is at least, e.g., 2-fold greater relative to that of a control patient who would not respond favorably to the claimed treatment method according to the present invention. Likewise, a skilled physician may determine that the optimal benchmark level of the DNA copy number is, e.g., about 3-fold or 4-fold greater relative to that of a non-responsive patient, based on the data presented in the present disclosure.

[0032] The disclosure also provides a method for treating cancer in a selected patient population, the method comprising the steps of: (a) measuring an expression level of mutant K-Ras protein in a biological sample obtained from a patient candidate diagnosed of a cancer; (b) confirming that the patient candidate's mutant K-Ras protein expression level is above a benchmark level; and (c) administering to the patient candidate a duplex RNA molecule comprising a first strand comprising a nucleotide sequence with a length from 18-23 nucleotides, wherein the nucleotide sequence of the first strand is substantially complementary to a target K-Ras mRNA sequence, and a second strand comprising a nucleotide sequence with a length from 12-17 nucleotides, wherein the second strand is substantially complementary to the first strand, and forms a double-stranded region with the first strand, wherein the first strand has a 3'-overhang from 1-9 nucleotides, and a 5'-overhang

from 0-8 nucleotides, and wherein said duplex RNA molecule is capable of effecting selective K-Ras gene silencing.

[0033] In some embodiments, the steps (a), (b), and (c) may be performed by one actor or several actors.

[0034] In some embodiments, a patient candidate's mutant K-Ras protein expression level is considered to be above a benchmark level if it is at least, e.g., 2-fold greater relative to that of a control patient who would not respond favorably to the claimed treatment method according to the present invention. Likewise, a skilled physician may determine that the optimal benchmark level of the mutant K-Ras protein expression is, e.g., about 3-fold or 4-fold greater relative to that of a non-responsive patient, based on the data presented in the present disclosure.

[0035] The present invention further provides a kit. The kit comprises a first RNA strand with a length from 18-23 nucleotides and a second RNA strand with a length from 12-17 nucleotides, wherein the second strand is substantially complementary to the first strand, and capable of forming a duplex RNA molecule with the first strand, wherein the duplex RNA molecule has a 3'-overhang from 1-9 nucleotides, and a 5'-overhang from 0-8 nucleotides, wherein said duplex RNA molecule is capable of effecting K-Ras specific gene silencing.

[0036] The present invention also provides a method of preparing the duplex RNA molecule. The method comprises the steps of synthesizing the first strand and the second strand, and combining the synthesized strands under conditions, wherein the duplex RNA molecule is formed, which is capable of effecting sequence-specific gene silencing. In some embodiments, the method further comprises a step of introducing at least one modified nucleotide or its analogue into the duplex RNA molecule during the synthesizing step, after the synthesizing and before the combining step, or after the combining step. In another embodiment, the RNA strands are chemically synthesized, or biologically synthesized.

[0037] The present invention provides an expression vector. The vector comprises a nucleic acid or nucleic acids encoding the duplex RNA molecule operably linked to at least one expression-control sequence. In some embodiments, the vector comprises a first nucleic acid encoding the first strand operably linked to a first expression-control sequence, and a second nucleic acid encoding the second strand operably linked to a second expression-control sequence. In another embodiment, the vector is a viral, eukaryotic, or bacterial expression vector.

[0038] The present invention also provides a cell. In some embodiments, the cell comprises the vector. In another embodiment, the cell comprises the duplex RNA molecule. In a further embodiment, the cell is a mammalian, avian, or bacterial cell.

[0039] The modulating method can also be used for studying drug target in vitro or in vivo. The present invention provides a reagent comprising the duplex RNA molecule.

[0040] The present invention also provides a method of preparing a duplex RNA molecule of the disclosure comprising the steps of synthesizing the first strand and the second strand, and combining the synthesized strands under conditions, wherein the duplex RNA molecule is formed, which is capable of effecting K-Ras sequence-specific gene silencing. In some embodiments, the RNA strands are chemically synthesized, or biologically synthesized. In another embodiment, the first strand and the second strand are synthesized separately or simultaneously.

[0041] In some embodiments, the method further comprises a step of introducing at least one modified nucleotide or its analogue into the duplex RNA molecule during the synthesizing step, after the synthesizing and before the combining step, or after the combining step.

[0042] The present invention further provides a pharmaceutical composition. The pharmaceutical composition comprises as an active agent at least one duplex RNA molecule and one or more carriers selected from the group consisting of a pharmaceutical carrier, a positive-charge carrier, a liposome, a protein carrier, a polymer, a nanoparticle, a cholesterol, a lipid, and a lipoid.

[0043] Other features and advantages of the present invention are apparent from the additional descriptions provided herein including the different examples. The provided examples illustrate different components and methodology useful in practicing the present invention. The examples do not limit the claimed invention. Based on the present disclosure the skilled artisan can identify and employ other components and methodology useful for practicing the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0044] Figure 1(A) shows an *in vitro* study in which aiRNA ID NO: 21 ("aiK-Ras #1") was used to target K-Ras Target SEQ ID NO: 22 to determine the IC_{50} for aiK-Ras #1.

[0045] Figure 1(B) shows an *in vitro* study in which aiRNA ID NO: 142 ("aiK-Ras #2") was used to target K-Ras Target SEQ ID NO: 142 to determine the IC_{50} for aiK-Ras #2.

[0046] Figure 2(A) shows detection of siRNA and aiRNA loading to RISC by northern blot analysis.

[0047] Figure 2(B) shows detection of TLR3/aiRNA or siRNA binding.

[0048] Figure 2(C) shows that TLR3/RNA complexes were immunoprecipitated with anti-HA antibody.

[0049] Figure 3(A) shows colony formation assay in AGS and DLD1 transfected with aiK-Ras #1 or aiK-Ras #2.

[0050] Figure 3(B) shows western blot analysis of lysate from AGS and DLD1.

[0051] Figure 3(C) shows colony formation assay results in large cell panel.

[0052] Figure 4 shows western blot analysis of K-Ras and EGFR-RAS pathway molecules.

[0053] Figure 5(A) shows that aiK-Ras sensitivity was correlated with K-Ras amplification in K-Ras mutant large cell panel.

[0054] Figure 5(B) shows that aiK-Ras sensitivity was correlated with K-Ras amplification in K-Ras mutant large cell panel.

[0055] Figure 6(A) shows stemness gene expression in CSC culture.

[0056] Figure 6(B) shows the results of sphere formation assay in various cell lines.

[0057] Figure 6(C) shows depletion of CD44-high population in AGS and DLD1 cells with aiK-Ras #1 and aiK-Ras #2.

[0058] Figure 7(A) shows heat map of CSC-related genes in cancer cells transfected with aiK-Ras.

[0059] Figure 7(B) shows confirmation of down-regulated Notch signaling by western blot.

DETAILED DESCRIPTION OF THE INVENTION

[0060] The present invention relates to asymmetric duplex RNA molecules that are capable of effecting selective K-Ras gene silencing in a eukaryotic cell. In some embodiments, the duplex RNA molecule comprises a first strand and a second strand. The first strand is longer than the second strand. The second strand is substantially complementary to the first strand, and forms a double-stranded region with the first strand.

[0061] The protein K-Ras is a molecular switch that under normal conditions regulates cell growth and cell division. Mutations in this protein lead to the formation of tumors through continuous cell growth. About 30% of human cancers have a mutated Ras protein that is constitutively bound to GTP due to decreased GTPase activity and insensitivity

to GAP action. Ras is also an important factor in many cancers in which it is not mutated but rather functionally activated through inappropriate activity of other signal transduction elements. Mutated K-Ras proteins are found in a large proportion of all tumor cells. K-Ras protein occupies a central position of interest. The identification of oncogenically mutated K-Ras in many human cancers led to major efforts to target this constitutively activated protein as a rational and selective treatment. Despite decades of active agent research, significant challenges still remain to develop therapeutic inhibitors of K-Ras.

[0062] The compositions and methods provided herein are useful in elucidating the function of K-Ras in the cancer development and maintenance. The compositions and methods use asymmetric interfering RNAs (aiRNAs) that are able to silence target genes with high potency leading to long-lasting knockdown, and reducing off-target effects, and investigated the dependency of K-Ras on cell survival in several types of human cancer cell lines. Much to our surprise, we found K-Ras plays a more significant role for gastric cancer maintenance compared to other types of cancer. aiRNA-induced silencing of K-Ras was found to inhibit the cell proliferation of gastric cancer cells and the ability of gastric cancer cells to form colonies compared to other cancer types. Accumulating evidence has revealed that cancer stem cells (CSCs) are highly associated with prognosis, metastasis, and recurrence. To investigate the effect of K-Ras on CSCs, we tested the K-Ras gene silencing effects on an *in vitro* CSC culturing system. As a result, K-Ras inhibition decreased the colonies derived from gastric CSCs and altered the gene expression patterns of several genes involved in "stemness" compared to other cancer types. The results of these studies suggest that gastric cancer and gastric CSCs are affected by the K-Ras oncogene and that Kras aiRNAs are promising therapeutic candidates for the treatment of gastric cancer. Accordingly, the disclosure provides compositions and methods for targeting K-Ras in the treatment, prevention, delaying the progression of, or otherwise ameliorating a symptom of gastric cancer. The disclosure also provides compositions and methods for targeting K-Ras to inhibit the survival and/or proliferation of CSCs, as well as compositions and methods for targeting K-Ras in the inhibition of to inhibit the survival and/or proliferation of CSCs in the treatment, prevention, delaying the progression of, or otherwise ameliorating a symptom of gastric cancer. In some embodiments, the method comprises administering to subject in need thereof a therapeutically effective amount of a duplex RNA molecule of the disclosure. In some embodiments, the subject is human. In some embodiments, the subject is suffering from gastric cancer. In some embodiments, the subject is diagnosed with gastric cancer. In some embodiments, the subject is predisposed to gastric cancer.

[0063] In some embodiments, the duplex RNA molecule used in the compositions and methods of the disclosure has a 3'-overhang from 1-8 nucleotides and a 5'-overhang from 1-8 nucleotides, a 3'-overhang from 1-10 nucleotides and a blunt end, or a 5'-overhang from 1-10 nucleotides and a blunt end. In another embodiment, the duplex RNA molecule has two 5'-overhangs from 1-8 nucleotides or two 3'-overhangs from 1-10 nucleotides. In a further embodiment, the first strand has a 3'-overhang from 1-8 nucleotides and a 5'-overhang from 1-8 nucleotides. In an even further embodiment, the duplex RNA molecule is an isolated duplex RNA molecule.

[0064] In some embodiments, the first strand has a 3'-overhang from 1-10 nucleotides, and a 5'-overhang from 1-10 nucleotides or a 5'-blunt end. In another embodiment, the first strand has a 3^1-overhang from 1-10 nucleotides, and a 5^1-overhang from 1-10 nucleotides. In an alternative embodiment, the first strand has a 3'-overhang from 1-10 nucleotides, and a 5'-blunt end.

[0065] In some embodiments, the first strand has a length from 5-100 nucleotides, from 12-30 nucleotides, from 15-28 nucleotides, from 18-27 nucleotides, from 19-23 nucleotides, from 20-22 nucleotides, or 21 nucleotides.

[0066] In another embodiment, the second strand has a length from 3-30 nucleotides, from 12-26 nucleotides, from 13-20 nucleotides, from 14-23 nucleotides, 14 or 15 nucleotides.

[0067] In some embodiments, the first strand has a length from 5-100 nucleotides, and the second strand has a length from 3-30 nucleotides; or the first strand has a length from 10-30 nucleotides, and the second strand has a length from 3-29 nucleotides; or the first strand has a length from 12-30 nucleotides and the second strand has a length from 10-26 nucleotides; or the first strand has a length from 15-28 nucleotides and the second strand has a length from 12-26 nucleotides; or the first strand has a length from 19-27 nucleotides and the second strand has a length from 14-23 nucleotides; or the first strand has a length from 20-22 nucleotides and the second strand has a length from 14-15 nucleotides. In a further embodiment, the first strand has a length of 21 nucleotides and the second strand has a length of 13-20 nucleotides, 14-19 nucleotides, 14-17 nucleotides, 14 or 15 nucleotides.

[0068] In some embodiments, the first strand is at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides longer than the second strand.

[0069] In some embodiments, the duplex RNA molecule further comprises 1-10 unmatched or mismatched nucleotides. In a further embodiment, the unmatched or

mismatched nucleotides are at or near the 3' recessed end. In an alternative embodiment, the unmatched or mismatched nucleotides are at or near the 5' recessed end. In an alternative embodiment, the unmatched or mismatched nucleotides are at the double-stranded region. In another embodiment, the unmatched or mismatched nucleotide sequence has a length from 1-5 nucleotides. In an even further embodiment, the unmatched or mismatched nucleotides form a loop structure.

[0070] In some embodiments, the first strand or the second strand contains at least one nick, or formed by two nucleotide fragments.

[0071] In some embodiments, the gene silencing is achieved through one or two, or all of RNA interference, modulation of translation, and DNA epigenetic modulations.

[0072] In some embodiments, the target K-Ras mRNA sequence to be silenced is a target sequence shown in Table 1.

[0073] In some embodiments, the RNA duplex molecule, also referred to herein as an asymmetrical interfering RNA molecule or aiRNA molecule, comprises a sense strand sequence, an antisense strand sequence or a combination of a sense strand sequence and antisense strand sequence selected from those shown in Table 2.

[0074] In some embodiments, the RNA duplex molecule (aiRNA) comprises a sense strand sequence selected from the group consisting of SEQ ID NOs: 320-637. In some embodiments, the RNA duplex molecule (aiRNA) comprises an antisense strand sequence selected from the group consisting of SEQ ID NOs: 638-955. In some embodiments, the RNA duplex molecule (aiRNA) comprises a sense strand sequence selected from the group consisting of SEQ ID NOs: 320-637 and an antisense strand sequence selected from the group consisting of SEQ ID NOs: 638-955.

[0075] In some embodiments, the RNA duplex molecule (aiRNA) comprises a sense strand sequence that is at least, e.g, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95% or more identical to a sequence selected from the group consisting of SEQ ID NOs: 320-637. In some embodiments, the RNA duplex molecule (aiRNA) comprises an antisense strand sequence that is at least, e.g., 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95% or more identical to a sequence selected from the group consisting of SEQ ID NOs: 638-955. In some embodiments, the RNA duplex molecule (aiRNA) comprises a sense strand sequence that is at least, e.g., 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95% or more identical to a sequence selected from the group consisting of SEQ ID NOs: 320-637 and an antisense strand sequence that is at least, e.g., 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95% or more identical to a sequence selected from the group consisting of SEQ ID NOs: 638-955.

[0076] As used in the specification and claims, the singular form "a", "an", and "the" include plural references unless the context clearly dictate otherwise. For example, the term "a cell" includes a plurality of cells including mixtures thereof.

[0077] As used herein, a "double stranded RNA," a "duplex RNA" or a "RNA duplex" refers to an RNA of two strands and with at least one double-stranded region, and includes RNA molecules that have at least one gap, nick, bulge, and/or bubble either within a double-stranded region or between two neighboring double-stranded regions. If one strand has a gap or a single-stranded region of unmatched nucleotides between two double-stranded regions, that strand is considered as having multiple fragments. A double-stranded RNA as used here can have terminal overhangs on either end or both ends.. In some embodiments, the two strands of the duplex RNA can be linked through certain chemical linker.

[0078] As used herein, an "antisense strand" refers to an RNA strand that has substantial sequence complementarity against a target messenger RNA.

[0079] The term "isolated" or "purified" as used herein refers to a material that is substantially or essentially free from components that normally accompany it in its native state. Purity and homogeneity are typically determined using analytical chemistry techniques such as polyacrylamide gel electrophoresis or high performance liquid chromatography.

[0080] As used herein, "modulating" and its grammatical equivalents refer to either increasing or decreasing (e.g., silencing), in other words, either up-regulating or down-regulating. As used herein, "gene silencing" refers to reduction of gene expression, and may refer to a reduction of gene expression about, e.g., 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 95% of the targeted gene.

[0081] As used herein, the term "subject" refers to any animal (e.g., a mammal), including, but not limited to humans, non-human primates, rodents, and the like, which is to be the recipient of a particular treatment. Under some circumstances, the terms "subject" and "patient" are used interchangeably herein in reference to a human subject.

[0082] Terms such as "treating" or "treatment" or "to treat" or "alleviating" or "to alleviate" as used herein refer to both (1) therapeutic measures that cure, slow down, lessen symptoms of, and/or halt progression of a diagnosed pathologic condition or disorder and (2) prophylactic or preventative measures that prevent or slow the development of a targeted pathologic condition or disorder. Thus those in need of treatment include those already with the disorder; those prone to have the disorder; and those in whom the disorder is to be prevented. A subject is successfully "treated" according to the methods of the

present invention if the patient shows one or more of the following: a reduction in the number of or complete absence of cancer cells; a reduction in the tumor size; inhibition of or an absence of cancer cell infiltration into peripheral organs including the spread of cancer into soft tissue and bone; inhibition of or an absence of tumor metastasis; inhibition or an absence of tumor growth; relief of one or more symptoms associated with the specific cancer; reduced morbidity and mortality; and improvement in quality of life.

[0083] As used herein, the terms "inhibiting", "to inhibit" and their grammatical equivalents, when used in the context of a bioactivity, refer to a down-regulation of the bioactivity, which may reduce or eliminate the targeted function, such as the production of a protein or the phosphorylation of a molecule. When used in the context of an organism (including a cell), the terms refer to a down-regulation of a bioactivity of the organism, which may reduce or eliminate a targeted function, such as the production of a protein or the phosphorylation of a molecule. In particular embodiments, inhibition may refer to a reduction of about, e.g., 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 95% of the targeted activity. When used in the context of a disorder or disease, the terms refer to success at preventing the onset of symptoms, alleviating symptoms, or eliminating the disease, condition or disorder.

[0084] As used herein, the term "substantially complementary" refers to complementarity in a base-paired, double-stranded region between two nucleic acids and not any single-stranded region such as a terminal overhang or a gap region between two double-stranded regions. The complementarity does not need to be perfect; there may be any number of base pair mismatches, for example, between the two nucleic acids. However, if the number of mismatches is so great that no hybridization can occur under even the least stringent hybridization conditions, the sequence is not a substantially complementary sequence. When two sequences are referred to as "substantially complementary" herein, it means that the sequences are sufficiently complementary to each other to hybridize under the selected reaction conditions. The relationship of nucleic acid complementarity and stringency of hybridization sufficient to achieve specificity is well known in the art. Two substantially complementary strands can be, for example, perfectly complementary or can contain from 1 to many mismatches so long as the hybridization conditions are sufficient to allow, for example discrimination between a pairing sequence and a non-pairing sequence. Accordingly, substantially complementary sequences can refer to sequences with base-pair complementarity of, e.g., 100%, 95%, 90%, 80%, 75%, 70%, 60%, 50% or less, or any number in between, in a double-stranded region.

[0085] RNA interference (abbreviated as RNAi) is a cellular process for the targeted destruction of single-stranded RNA (ssRNA) induced by double-stranded RNA (dsRNA). The ssRNA is gene transcript such as a messenger RNA (mRNA). RNAi is a form of post-transcriptional gene silencing in which the dsRNA can specifically interfere with the expression of genes with sequences that are complementary to the dsRNA. The antisense RNA strand of the dsRNA targets a complementary gene transcript such as a messenger RNA (mRNA) for cleavage by a ribonuclease.

[0086] In RNAi process, long dsRNA is processed by a ribonuclease protein Dicer to short forms called small interfering RNA (siRNA). The siRNA is separated into guide (or antisense) strand and passenger (or sense) strand. The guide strand is integrated into RNA-induced-silencing-complex (RISC), which is a ribonuclease-containing multi-protein complex. The complex then specifically targets complementary gene transcripts for destruction.

[0087] RNAi has been shown to be a common cellular process in many eukaryotes. RISC, as well as Dicer, is conserved across the eukaryotic domain. RNAi is believed to play a role in the immune response to virus and other foreign genetic material.

[0088] Small interfering RNAs (siRNAs) are a class of short double-stranded RNA (dsRNA) molecules that play a variety of roles in biology. Most notably, it is involved in the RNA interference (RNAi) pathway where the siRNA interferes with the expression of a specific gene. In addition, siRNAs also play roles in the processes such as an antiviral mechanism or shaping the chromatin structure of a genome. In some embodiments, siRNA has a short (19-21 nt) double- strand RNA (dsRNA) region with 2-3 nucleotide 3' overhangs with 5'-phosphate and 3'-hydroxyl termini.

[0089] Dicer is a member of RNase III ribonuclease family. Dicer cleaves long, double-stranded RNA (dsRNA), pre-microRNA (miRNA), and short hairpin RNA (shRNA) into short double-stranded RNA fragments called small interfering RNA (siRNA) about 20-25 nucleotides long, usually with a two-base overhang on the 3' end. Dicer catalyzes the first step in the RNA interference pathway and initiates formation of the RNA-induced silencing complex (RISC), whose catalytic component argonaute is an endonuclease capable of degrading messenger RNA (mRNA) whose sequence is complementary to that of the siRNA guide strand.

[0090] As used herein, an effective siRNA sequence is a siRNA that is effective in triggering RNAi to degrade the transcripts of a target gene. Not every siRNA complementary to the target gene is effective in triggering RNAi to degrade the transcripts

of the gene. Indeed, time-consuming screening is usually necessary to identify an effective siRNA sequence. In some embodiments, the effective siRNA sequence is capable of reducing the expression of the target gene by more than 90%, more than 80%, more than 70%, more than 60%, more than 50%, more than 40%, or more than 30%.

[0091] The present invention uses a structural scaffold called asymmetric interfering RNA (aiRNA) that can be used to effect siRNA-like results, and also to modulate miRNA pathway activities, initially described in detail PCT Publications WO 2009/029688 and WO 2009/029690, the contents of which are hereby incorporated by reference in their entirety.

[0092] The structural design of aiRNA is not only functionally potent in effecting gene regulation, but also offers several advantages over the current state-of-art, RNAi regulators (mainly antisense, siRNA). Among the advantages, aiRNA can have RNA duplex structure of much shorter length than the current siRNA constructs, which should reduce the cost of synthesis and abrogate or reduce length-dependent triggering of nonspecific interferon-like immune responses from host cells. The shorter length of the passenger strand in aiRNA should also eliminate or reduce the passenger strand's unintended incorporation in RISC, and in turn, reduce off-target effects observed in miRNA-mediated gene silencing. AiRNA can be used in all areas that current miRNA-based technologies are being applied or contemplated to be applied, including biology research, R&D in biotechnology and pharmaceutical industries, and miRNA-based diagnostics and therapies.

[0093] In some embodiments, the first strand comprises a sequence being substantially complimentary to a target K-Ras mRNA sequence. In another embodiment, the second strand comprises a sequence being substantially complimentary to a target K-Ras mRNA sequence.

[0094] The present invention is pertinent to asymmetrical double stranded RNA molecules that are capable of effecting K-Ras gene silencing. In some embodiments, an RNA molecule of the present invention comprises a first strand and a second strand, wherein the second strand is substantially complementary, or partially complementary to the first strand, and the first strand and the second strand form at least one double-stranded region, wherein the first strand is longer than the second strand (length asymmetry). The RNA molecule of the present invention has at least one double-stranded region, and two ends independently selected from the group consisting of a 5'-overhang, a 3'-overhang, and a blunt.

[0095] Any single-stranded region of the RNA molecule of the invention, including any terminal overhangs and gaps in between two double-stranded regions, can be stabilized against degradation, either through chemical modification or secondary

structure. The RNA strands can have unmatched or imperfectly matched nucleotides. Each strand may have one or more nicks (a cut in the nucleic acid backbone), gaps (a fragmented strand with one or more missing nucleotides), and modified nucleotides or nucleotide analogues. Not only can any or all of the nucleotides in the RNA molecule chemically modified, each strand may be conjugated with one or more moieties to enhance its functionality, for example, with moieties such as one or more peptides, antibodies, antibody fragments, aptamers, polymers and so on.

[0096] In some embodiments, the first strand is at least 1 nt longer than the second strand. In a further embodiment, the first strand is at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nt longer than the second strand. In another embodiment, the first strand is 20-100 nt longer than the second strand. In a further embodiment, the first strand is 2-12 nt longer than the second strand. In an even further embodiment, the first strand is 3-10 nt longer than the second strand.

[0097] In some embodiments, the first strand, or the long strand, has a length of 5-100 nt, or preferably 10-30 or 12-30 nt, or more preferably 15-28 nt. In one embodiment, the first strand is 21 nucleotides in length. In some embodiments, the second strand, or the short strand, has a length of 3-30 nt, or preferably 3-29 nt or 10-26 nt, or more preferably 12-26 nt. In some embodiments, the second strand has a length of 15 nucleotides.

[0098] In some embodiments, the double-stranded region has a length of 3-98 basepairs (bp). In a further embodiment, the double-stranded region has a length of 5-28 bp. In an even further embodiment, the double-stranded region has a length of 10-19 bp. The length of the double-stranded region can be 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 bp.

[0099] In some embodiments, the double-stranded region of the RNA molecule does not contain any mismatch or bulge, and the two strands are perfectly complementary to each other in the double-stranded region. In another embodiment, the double-stranded region of the RNA molecule contains mismatch and/or bulge.

[00100] In some embodiments, the terminal overhang is 1-10 nucleotides. In a further embodiment, the terminal overhang is 1-8 nucleotides. In another embodiment, the terminal overhang is 3 nt.

[00101] The present invention also provides a method of modulating K-Ras gene expression in a cell or an organism (silencing method). The method comprises the steps of contacting said cell or organism with the duplex RNA molecule under conditions wherein selective K-Ras gene silencing can occur, and mediating a selective K-Ras gene silencing

effected by the said duplex RNA molecule towards a target K-Ras nucleic acid having a sequence portion substantially corresponding to the double-stranded RNA.

[00102] In some embodiments, the contacting step comprises the step of introducing said duplex RNA molecule into a target cell in culture or in an organism in which the selective gene silencing can occur. In a further embodiment, the introducing step comprises transfection, lipofection, infection, electroporation, or other delivery technologies.

[00103] In some embodiments, the silencing method is used for determining the function or utility of a gene in a cell or an organism.

[00104] The silencing method can be used for modulating the expression of a gene in a cell or an organism. In some embodiments, the gene is associated with a disease, e.g., a human disease or an animal disease, a pathological condition, or an undesirable condition. In some embodiments, the disease is gastric cancer.

[00105] The RNA molecules of the present invention can be used for the treatment and or prevention of various diseases or undesirable conditions, including gastric cancer. In some embodiments, the present invention can be used as a cancer therapy or to prevent or to delay the progression of cancer. The RNA molecules of the present invention can he used to silence or knock down *k-Ras*, which is involved with cell proliferation or other cancer phenotypes.

[00106] The present invention provides a method to treat a disease or undesirable condition. The method comprises using the asymmetrical duplex RNA molecule to effect gene silencing of a gene associated with the disease or undesirable condition.

[00107] The present invention further provided a pharmaceutical composition. The pharmaceutical comprises (as an active agent) at least one asymmetrical duplex RNA molecule. In some embodiments, the pharmaceutical comprises one or more carriers selected from the group consisting of a pharmaceutical carrier, a positive-charge carrier, a liposome, a protein carrier, a polymer, a nanoparticle, a nanoemulsion, a lipid, and a lipoid. In some embodiments, the composition is for diagnostic applications. In some embodiments, the composition is for therapeutic applications.

[00108] The pharmaceutical compositions and formulations of the present invention can be the same or similar to the pharmaceutical compositions and formulations developed for siRNA, miRNA, and antisense RNA (see e.g., de Fougerolles et al., 2007, "Interfering with disease: a progress report on siRNA-based therapeutics." Nat Rev Drug Discov *6,* 443453; Kim and Rossi, 2007, "Strategies for silencing human disease using RNA interference." Nature reviews *8,* 173-184), except for the RNA ingredient. The siRNA, miRNA, and antisense RNA in the pharmaceutical compositions and formulations can be

replaced by the duplex RNA molecules of the present disclosure. The pharmaceutical compositions and formulations can also be further modified to accommodate the duplex RNA molecules of the present disclosure.

[00109] A "pharmaceutically acceptable salt" or "salt" of the disclosed duplex RNA molecule is a product of the disclosed duplex RNA molecule that contains an ionic bond, and is typically produced by reacting the disclosed duplex RNA molecule with either an acid or a base, suitable for administering to a subject. Pharmaceutically acceptable salt can include, but is not limited to, acid addition salts including hydrochlorides, hydrobromides, phosphates, sulphates, hydrogen sulphates, alkylsulphonates, arylsulphonates, acetates, benzoates, citrates, maleates, fumarates, succinates, lactates, and tartrates; alkali metal cations such as Na, K, Li, alkali earth metal salts such as Mg or Ca, or organic amine salts.

[00110] A "pharmaceutical composition" is a formulation containing the disclosed duplex RNA molecules in a form suitable for administration to a subject. In one embodiment, the pharmaceutical composition is in bulk or in unit dosage form. The unit dosage form is any of a variety of forms, including, for example, a capsule, an IV bag, a tablet, a single pump on an aerosol inhaler, or a vial. The quantity of active ingredient *(e.g.,* a formulation of the disclosed duplex RNA molecule or salts thereof) in a unit dose of composition is an effective amount and is varied according to the particular treatment involved. One skilled in the art will appreciate that it is sometimes necessary to make routine variations to the dosage depending on the age and condition of the patient. The dosage will also depend on the route of administration. A variety of routes are contemplated, including oral, pulmonary, rectal, parenteral, transdermal, subcutaneous, intravenous, intramuscular, intraperitoneal, intranasal, and the like. Dosage forms for the topical or transdermal administration of a duplex RNA molecule of this invention include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. In one embodiment, the active duplex RNA molecule is mixed under sterile conditions with a pharmaceutically acceptable carrier, and with any preservatives, buffers, or propellants that are required.

[00111] The present invention provides a method of treatment comprising administering an effective amount of the pharmaceutical composition to a subject in need. In some embodiments, the pharmaceutical composition is administered via a route selected from the group consisting of iv, sc, topical, po, and ip. In another embodiment, the effective amount is 1 ng to 1 g per day, 100 ng to 1 g per day, or 1 ug to 1 mg per day.

[00112] The present invention also provides pharmaceutical formulations comprising a duplex RNA molecule of the present invention in combination with at least one pharmaceutically acceptable excipient or carrier. As used herein, "pharmaceutically acceptable excipient" or "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Suitable carriers are described in "Remington: The Science and Practice of Pharmacy, Twentieth Edition," Lippincott Williams & Wilkins, Philadelphia, PA., which is incorporated herein by reference. Examples of such carriers or diluents include, but are not limited to, water, saline, Ringer's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active duplex RNA molecule, use thereof in the compositions is contemplated. Supplementary active duplex RNA molecules can also be incorporated into the compositions.

[00113] A duplex RNA molecule of the present invention is administered in a suitable dosage form prepared by combining a therapeutically effective amount *(e.g.,* an efficacious level sufficient to achieve the desired therapeutic effect through inhibition of tumor growth, killing of tumor cells, treatment or prevention of cell proliferative disorders, etc.) of a duplex RNA molecule of the present invention (as an active ingredient) with standard pharmaceutical carriers or diluents according to conventional procedures (i.e., by producing a pharmaceutical composition of the invention). These procedures may involve mixing, granulating, compressing, or dissolving the ingredients as appropriate to attain the desired preparation. In another embodiment, a therapeutically effective amount of a duplex RNA molecule of the present invention is administered in a suitable dosage form without standard pharmaceutical carriers or diluents.

[00114] Pharmaceutically acceptable carriers include solid carriers such as lactose, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, stearic acid and the like. Exemplary liquid carriers include syrup, peanut oil, olive oil, water and the like. Similarly, the carrier or diluent may include time-delay material known in the art, such as glyceryl monostearate or glyceryl distearate, alone or with a wax, ethylcellulose, hydroxypropylmethylcellulose, methylmethacrylate or the like. Other fillers, excipients, flavorants, and other additives such as are known in the art may also be included in a pharmaceutical composition according to this invention.

[00115] The pharmaceutical compositions containing active duplex RNA molecules of the present invention may be manufactured in a manner that is generally known, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes. Pharmaceutical compositions may be formulated in a conventional manner using one or more physiologically acceptable carriers comprising excipients and/or auxiliaries which facilitate processing of the active duplex RNA molecules into preparations that can be used pharmaceutically. Of course, the appropriate formulation is dependent upon the route of administration chosen.

[00116] A duplex RNA molecule or pharmaceutical composition of the invention can be administered to a subject in many of the well-known methods currently used for chemotherapeutic treatment. For example, for treatment of cancers, a duplex RNA molecule of the invention may be injected directly into tumors, injected into the blood stream or body cavities or taken orally or applied through the skin with patches. For treatment of psoriatic conditions, systemic administration (e.g., oral administration), or topical administration to affected areas of the skin, are preferred routes of administration. The dose chosen should be sufficient to constitute effective treatment but not as high as to cause unacceptable side effects. The state of the disease condition (e.g., gastric cancer) and the health of the patient should be closely monitored during and for a reasonable period after treatment.

EXAMPLES

[00117] Examples are provided below to further illustrate different features of the present invention. The examples also illustrate useful methodology for practicing the invention. These examples do not limit the claimed invention.

EXAMPLE 1: *In vitro* potency of aiK-Ras

[00118] Figure 1(A) shows an *in vitro* study in which aiRNA ID NO: 21 ("aiK-Ras #1") was used to target K-Ras Target SEQ ID NO: 22 to determine the IC_{50} for aiK-Ras #1. DLD1 cells (ATCC) were transfected with aiK-Ras #1. 48 hours after transfection, cells were collected and RNA was isolated. The IC_{50} of aiK-Ras #1 was determined by qPCR. Remaining mRNA was standardized to the GAPDH expression level. The IC_{50} of 3.1 pM indicates that aiK-Ras #1 silences K-Ras gene expression with high potency.

[00119] Figure 1(B) shows an *in vitro* study in which aiRNA ID NO: 142 ("aiK-Ras #2") was used to target K-Ras Target SEQ ID NO: 142 to determine the IC_{50} for aiK-Ras #2. DLD1 cells were transfected with aiK-Ras #2. 48 hours after transfection, cells were collected and RNA was isolated. The IC_{50} of aiK-Ras #2 was determined by qPCR. Remaining mRNA was standardized to the GAPDH expression level. The IC_{50} of 3.5 pM indicates that aiK-Ras #1 silences K-Ras gene expression with high potency.

EXAMPLE 2: Reduced off-target effect of aiK-Ras

[00120] Figure 2(A) shows detection of siRNA and aiRNA loading to RISC by northern blot analysis. To analyze small RNA RISC loading, HEK293 Flag-Ago2 stable cells were transfected with aiRNA or siRNA duplexes. Cells were lysed at the indicated time points and immunoprecipitated with Flag antibody (Sigma, Catalog # F1804). Immunoprecipitates were washed, RNA isolated from the complex by TRIZOL (Life Technologies, 15596-018) extraction, and loaded on 15% TBE-Urea PAGE or 15% TBE non-denaturing PAGE gels. Following electrophoreses, RNA was transferred to Hybonad-XL Nylon membrane. Then hybridizing the r-P32 labeled detect sense strand or anti-sense strand probe to RNA on the membrane. HEK293 cells (Invivogen, Catalog # 293-null) expressing Flag-Ago2 were transfected with siRNA or aiRNA, after which an immunoprecipitation assay was conducted. FLAG-Ago2 HEK 293 cells stably expressing FLAG-Ago2 cells were generated through transient transfection of FLAG-Ago2 neomycin plasmid DNA vectors. After selective neomycin containing medium culture, the monoclonal populations were selected by western blot. Non-denatured gel was used to detect dsRNA.

[00121] Figure 2(B) shows reduced off-target of aiRNA. HeLa cells were transfected with luciferase reporter genes fused with antisense or sense strand-based aiRNA or siRNA target sequences and aiK-Ras#2 or siK-Ras#2 (5 nM). Figure 2(C) shows that TLR3/RNA complexes were immunoprecipitated with anti-HA antibody (Invivogen, Catalog # ab-hatag). RNA was extracted from the pellet, and northern blot analysis was performed to determine the interaction between aiRNA/siRNA and the TLR3 receptor.

[00122] Figures 2(A)-(C) show that the asymmetric structure of aiK-Ras #1 and aiK-Ras #2 reduced sense strand mediated off-target effect and LTR3 binding.

EXAMPLE 3: aiK-Ras sensitivity in K-Ras mutant cells

[00123] Figure 3(A) shows colony formation assay in AGS (ATCC) and DLD1 cells transfected with aiK-Ras #1 or aiK-Ras #2. Cells were transfected with 1 nM GFP aiRNA

(control; GGTTATGTACAGGAACGCA (SEQ ID NO: 956)) or 1 nM aiK-Ras #1 or aiK-Ras #2 for 24 hours. Cells were then trypsinized and re-plated on 6-well plates at 500-2000 cells/well to determine the colony formation ability of the cells. After 11-14 days, colonies were stained with Giemsa stain and were counted. For the western blot analysis, cells were washed with ice-cold PBS and lysed in lysis buffer [50 mM Hepes (pH 7.5), 1% Nonidet P-40, 150 mM NaCl, 1 mM EDTA, and 1× Halt Protease Inhibitor Cocktail (Thermo Scientefic, Catalog # 87786)]. Soluble protein (10 µg) was separated by SDS/PAGE and transferred to PVDF membrane. Primary antibodies against were used in this study. The antigen–antibody complexes were visualized by enhanced chemiluminescence (BioRad, Catalog # 170-5060).

[00124] Figure 3(B) shows western blot analysis of lysate from AGS and DLD1, and the transfection effects of aiK-Ras #1 and aiK-Ras #2 on K-Ras expression, cleaved caspase 3, and cleaved PARP.

[00125] Figure 3(C) shows colony formation assay results in a large cell panel. All cell lines in the panel were obtained from ATCC. Cells harboring K-Ras mutant are highlighted.

EXAMPLE 4: Correlation between aiK-Ras sensitivity and K-Ras amplification

[00126] Figure 4 shows western blot analysis of K-Ras and EGFR-RAS pathway molecules. Lysate (10 µg/lane) was loaded and total and phosphorylated forms of EGFR, cRaf, MEK, and ERK were detected. Activated form of K-Ras (K-Ras GTP) was affinity-purified from cell lysate using GST-Raf-RBD and analyzed by western blotting with K-Ras antibody. The following antibodies were used for western blot: Actin (Sigma, Catalog # A5316) K-RAS (Santa Cruz, sc30 and Cell signaling, Catalog # 8955), Cleaved PARP (Cell Signaling, Catalog # 5625), Cleaved Caspase-3 (Cell Signaling, Catalog # 9664), Phospho-EGF Receptor (Cell Signaling, Catalog # 3777), EGF Receptor (Cell Signaling, Catalog # 4267), Phospho-c-Raf (Cell signaling, Catalog # 9427), c-Raf (Cell Signaling, Catalog # 9422), Phospho-MEK1/2 (Cell Signaling, Catalog # 9154), MEK1/2 (Cell Signaling, Catalog # 8727), Phospho-p44/42 MAPK (Erk1/2) (Cell Signaling, Catalog # 4370), p44/42 MAPK (Erk1/2) (Cell Signaling, Catalog # 4695), Jagged1 (Cell Signaling, Catalog # 2620), Notch1 (Cell Signaling, Catalog # 3608), c-Myc (Cell Signaling, Catalog # 5605). RBD pulldown was performed using a Ras Activation Kit (Abcam, Catalog # ab128504) according to the manufacturer's protocol. Precipitations were blotted for K-Ras (Santa Cruz, Catalog # sc30). Actin (Sigma, Catalog # A5316) was blotted as loading control. Figure 4 shows that aiK-Ras

sensitivity correlates with K-Ras amplification, and not with the activation state of the Ras pathway molecules.

[00127] Figure 5(A) shows that aiK-Ras sensitivity was correlated with K-Ras amplification in K-Ras mutant large cell panel. All cell lines in the panel were obtained from ATCC. Copy number of K-Ras was analyzed by qPCR. Statistical difference was determined by two-sided Mann-Whitney's U test. Difference with $p<0.05$ was considered statistically significant.

[00128] Figure 5(B) shows that aiK-Ras sensitivity was correlated with K-Ras amplification in K-Ras mutant large cell panel. K-Ras protein expression level was measured by western blot. Band of western blot was quantified by Image Lab (Biorad). Statistical difference was determined by two-sided Mann-Whitney's U test. Difference with $p<0.05$ was considered statistically significant.

[00129] Figures 3(A)-(C) and 5(A)-(B) show that aiK-Ras sensitivity varies in K-Ras mutant cells and it correlates with K-Ras copy number.

EXAMPLE 6: Effect of aiK-Ras on CSC-like phenotype in sensitive cell lines

[00130] Figure 6(A) shows stemness gene expression in CSC culture. AGS cells were cultured in CSC medium [DMEM nutrient mixture F-12 (DMEM/F-12, Life technologies, Catalog # 11320-033) containing B-27 supplement (Life Technologies, Catalog # 17504-044), 20 ng/mL EGF (R&D Systems, Catalog # 236-EG), 10 ng/mL FGF (R&D Systems, Catalog # 233-FB), and 1% penicillin/streptomycin] for 2 weeks. Nanog, Oct4, and Sox2 gene expression of CSC spheres was quantified by qPCR.

[00131] Figure 6(B) shows the results of sphere formation assay in various cell lines. For the sphere formation assay, agarose coated plates were prepared to dispense autoclaved 0.5% agar and aspirated immediately. Transfected cells were trypsinized and counted, then diluted to 2000 cells/100 uL of 1 x CSC medium. 1.9 mL of warmed CSC medium including 0.33% agarose (Sigma type VII, Catalog # A-4018) was added to the cells in CSC medium for final agarose concentration of 0.3%. The plate was placed at 4°C for 10 minutes to cool. The plate was placed 10 minutes at room temperature and 1 mL of CSC medium was added to the top layer. The plate was incubated in a 37°C /5% CO_2 incubator for 18-25 days. To count spheres, CSC medium was aspirated and Crystal violet (EMD, Catalog # 192-12) solution in PBS were added and incubated for 1 hour at room temperature to stain spheres.

[00132] Cells were trypsinized and re-plated in CSC medium/3% soft agar onto agar coated 6-well plates at 2000 cells/well to determine the sphere formation ability of the cells.

After 18-25 days, spheres were stained with crystal violet, and the number of spheres was counted.

[00133] Figure 6(C) shows depletion of CD44-high population in AGS and DLD1 cells with aiK-Ras #1 and aiK-Ras #2. CD44 expression was detected by flow cytometry, wherein AGS and DLD1 cells were stained with PE conjugated anti-CD44 (BD Pharmingen, Catalog # 555479) in Stain Buffer (BD Pharmingen, Catalog # 554657) on ice for 45 minutes and washed once with Stain Buffer. CD44 positive population was detected with flow cytometry (Attune Acoustic Focusing Cytometer, Life technologies).

[00134] Figures 6(A)-(C) show that aiK-Ras according to the present invention modulate CSC-like phenotype in sensitive cell lines.

EXAMPLE 7: Effect of K-Ras knockdown on CSC-related gene expression patterns.

[00135] Figure 7(A) shows heat map of CSC-related genes in cancer cells transfected with aiK-Ras. Cells were transfected with 1 nM control aiRNA or aiK-Ras #1 for 48 hours. Real-time PCR was performed on total RNA using specific validated primers for 84 CSC-related genes with RT2 Profiler PCR array. The fold change in gene expression was calculated as the ratio between aiK-Ras #1 and the control aiRNA samples. Figure 7(B) shows confirmation of down-regulated Notch signaling by western blot. Table 3 below summarizes the genes down-regulated >3 fold with aiK-Ras #1 corresponding to the heat map as shown in Figure 7(A)

Table 3.

	AGS	MKN28
Gene symbol	Fold change	Fold change
NOTCH1	-7.87	-4.07
SOX2	-5.49	-3.97
PTCH1	-4.94	-7.04
FOXA2	-4.85	-7.35
FGFR2	-4.29	-3.67
JAG1	-4.16	-3.51
ALCAM	-3.64	-3.11
MYC	-3.51	-3.15
ITGA2	-3.36	-7.98

[00136] The embodiments illustrated and discussed in this specification are intended only to teach those skilled in the art the best way known to the inventors to make and use the invention. Nothing in this specification should be considered as limiting the scope of the present invention. All examples presented are representative and non-limiting. The above-described embodiments of the invention may be modified or varied, without departing from the invention, as appreciated by those skilled in the art in light of the above teachings. It is therefore to be understood that, within the scope of the claims and their equivalents, the invention may be practiced otherwise than as specifically described.

What is claimed is:

1. A method of treating cancer in a subject in need thereof, the method comprising administering to a subject in need thereof a duplex RNA molecule comprising (i) a first strand comprising a nucleotide sequence with a length from 18-23 nucleotides, wherein the nucleotide sequence of the first strand is substantially complementary to a target K-Ras mRNA sequence, and (ii) a second strand comprising a nucleotide sequence with a length from 12-17 nucleotides, wherein the second strand is substantially complementary to the first strand, and forms a double-stranded region with the first strand, wherein the first strand has a 3'-overhang from 1-9 nucleotides, and a 5'-overhang from 0-8 nucleotides, and wherein said duplex RNA molecule is capable of effecting selective K-Ras gene silencing.

2. A method of treating cancer in a selected patient population, the method comprising the steps of:

(a) measuring a level of mutant K-Ras gene amplification in a biological sample obtained from a patient candidate diagnosed of a cancer;

(b) confirming that the patient candidate's mutant K-Ras gene amplification level is above a benchmark level; and

(c) administering to the patient candidate a duplex RNA molecule comprising (i) a first strand comprising a nucleotide sequence with a length from 18-23 nucleotides, wherein the nucleotide sequence of the first strand is substantially complementary to a target K-Ras mRNA sequence, and (ii) a second strand comprising a nucleotide sequence with a length from 12-17 nucleotides, wherein the second strand is substantially complementary to the first strand, and forms a double-stranded region with the first strand, wherein the first strand has a 3'-overhang from 1-9 nucleotides, and a 5'-overhang from 0-8 nucleotides, and wherein said duplex RNA molecule is capable of effecting selective K-Ras gene silencing.

3. A method of treating cancer in a selected patient population, the method comprising the steps of:

(a) measuring an expression level of mutant K-Ras protein in a biological sample obtained from a patient candidate diagnosed of a cancer;

(b) confirming that the patient candidate's mutant K-Ras protein expression level is above a benchmark level; and

(c) administering to the patient candidate a duplex RNA molecule comprising (i) a first strand comprising a nucleotide sequence with a length from 18-23 nucleotides, wherein the nucleotide sequence of the first strand is substantially complementary to a target K-Ras mRNA sequence, and (ii) a second strand comprising a nucleotide sequence with a length from 12-17 nucleotides, wherein the second strand is substantially complementary to the first strand, and forms a double-stranded region with the first strand, wherein the first strand has a 3'-overhang from 1-9 nucleotides, and a 5'-overhang from 0-8 nucleotides, and wherein said duplex RNA molecule is capable of effecting selective K-Ras gene silencing.

4. The method of any one of the preceding claims, wherein the cancer is gastric cancer, or the subject is suffering from or predisposed to gastric cancer.

5. The method of any one of the preceding claims, wherein the nucleotide sequence of the first strand comprises a sequence that is at least 70% complementary to the target K-Ras mRNA sequence.

6. The method of any one of the preceding claims, wherein the first strand has a length from 19-23 nucleotides.

7. The method of any one of the preceding claims, wherein the first strand has a length of 21 nucleotides.

8. The method of claim 7, wherein the second strand has a length of 14-16 nucleotides.

9. The method of claim 8, wherein the second strand has a length of 15 nucleotides.

10. The method of claim 9, wherein the first strand has a 3'-overhang of 2-4 nucleotides.

11. The method of claim 10, wherein the first strand has a 3'-overhang of 3 nucleotides.

12. The method of any one of the preceding claims, wherein the duplex RNA molecule contains at least one modified nucleotide or its analogue.

13. The method of claim 12, wherein the at least one modified nucleotide or its analogue is sugar-, backbone-, and/or base- modified ribonucleotide.

14. The method of claim 13, wherein the backbone-modified ribonucleotide has a modification in a phosphodiester linkage with another ribonucleotide.

15. The method of any one of the preceding claims, wherein the first strand comprises an antisense strand sequence selected from the group consisting of SEQ ID NOs: 638-955.

16. The method of any one of claims 1-14, wherein the second strand comprises a sense strand sequence selected from the group consisting of SEQ ID NOs: 320-637.

17. The method of any one of claims 1-14, wherein the first strand comprises an antisense strand sequence selected from the group consisting of SEQ ID NOs: 638-955 and the second strand comprises a sense strand sequence selected from the group consisting of SEQ ID NOs: 320-637.

18. The method of any one of the preceding claims, wherein the subject is human.

19. A duplex RNA molecule comprising (i) a first strand comprising a nucleotide sequence with a length from 18-23 nucleotides, wherein the nucleotide sequence of the first strand is substantially complementary to a target K-Ras mRNA sequence, and (ii) a second strand comprising a nucleotide sequence with a length from 12-17 nucleotides, wherein the second strand is substantially complementary to the first strand, and forms a double-stranded region with the first strand, wherein the first strand has a 3'-overhang from 1-9 nucleotides, and a 5'-overhang from 0-8 nucleotides, and wherein said duplex RNA molecule is capable of effecting selective K-Ras gene silencing.

20. The duplex RNA molecule of claim 19, wherein the nucleotide sequence of the first strand comprises a sequence that is at least 70% complementary to the target K-Ras mRNA sequence.

21. The duplex RNA molecule of claim 19 or claim 20, wherein the first strand has a length from 19-23 nucleotides.

22. The duplex RNA molecule of any one of claims 19-21, wherein the first strand has a length of 21 nucleotides.

23. The duplex RNA molecule of claim 22, wherein the second strand has a length of 14-16 nucleotides.

24. The duplex RNA molecule of claim 23, wherein the second strand has a length of 15 nucleotides.

25. The duplex RNA molecule of claim 24, wherein the first strand has a 3'-overhang of 2-4 nucleotides.

26. The duplex RNA molecule of claim 25, wherein the first strand has a 3'-overhang of 3 nucleotides.

27. The duplex RNA molecule of any one of claims 19-26, wherein the duplex RNA molecule contains at least one modified nucleotide or its analogue.

28. The duplex RNA molecule of claim 27, wherein the at least one modified nucleotide or its analogue is sugar-, backbone-, and/or base- modified ribonucleotide.

29. The duplex RNA molecule of claim 28, wherein the backbone-modified ribonucleotide has a modification in a phosphodiester linkage with another ribonucleotide.

30. The duplex RNA molecule of any one of claims 19-29, wherein the first strand comprises an antisense strand sequence selected from the group consisting of SEQ ID NOs: 638-955.

31. The duplex RNA molecule of any one of claims 19-29, wherein the second strand comprises a sense strand sequence selected from the group consisting of SEQ ID NOs: 320-637.

32. The duplex RNA molecule of any one of claims 19-29, wherein the first strand comprises an antisense strand sequence selected from the group consisting of SEQ ID NOs: 638-955 and the second strand comprises a sense strand sequence selected from the group consisting of SEQ ID NOs: 320-637.

33. A method of treating cancer in a subject in need thereof, comprising inhibiting K-Ras gene expression or K-Ras activity in said subject.

34. A method of inhibiting the survival and/or proliferation of cancer stem cells (CSCs) in a subject in need thereof, comprising inhibiting K-Ras gene expression or K-Ras activity in said subject.

35. The method of claim 34, wherein inhibiting K-Ras gene expression or K-Ras activity comprises administering to a subject in need thereof a duplex RNA molecule comprising (i) a first strand comprising a nucleotide sequence with a length from 18-23 nucleotides, wherein the nucleotide sequence of the first strand is substantially complementary to a target K-Ras mRNA sequence, and (ii) a second strand comprising a nucleotide sequence with a length from 12-17 nucleotides, wherein the second strand is substantially complementary to the first strand, and forms a double-stranded region with the first strand, wherein the first strand has a 3'-overhang from 1-9 nucleotides, and a 5'-overhang from 0-8 nucleotides, and wherein said duplex RNA molecule is capable of effecting selective K-Ras gene silencing.

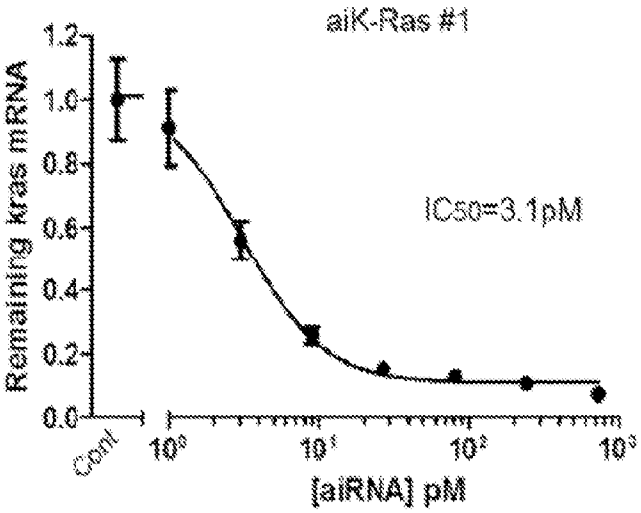


Figure 1(A)

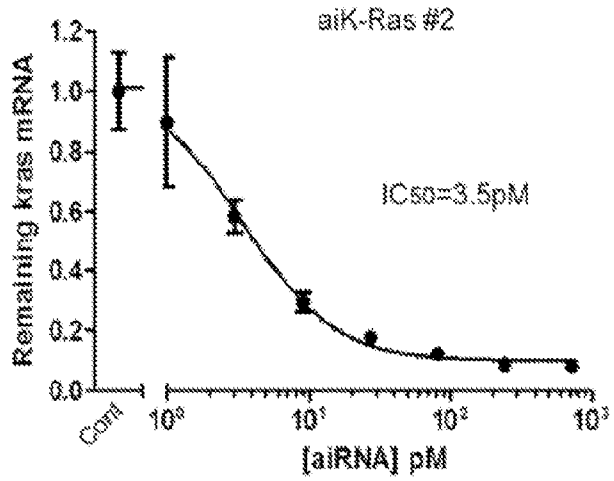
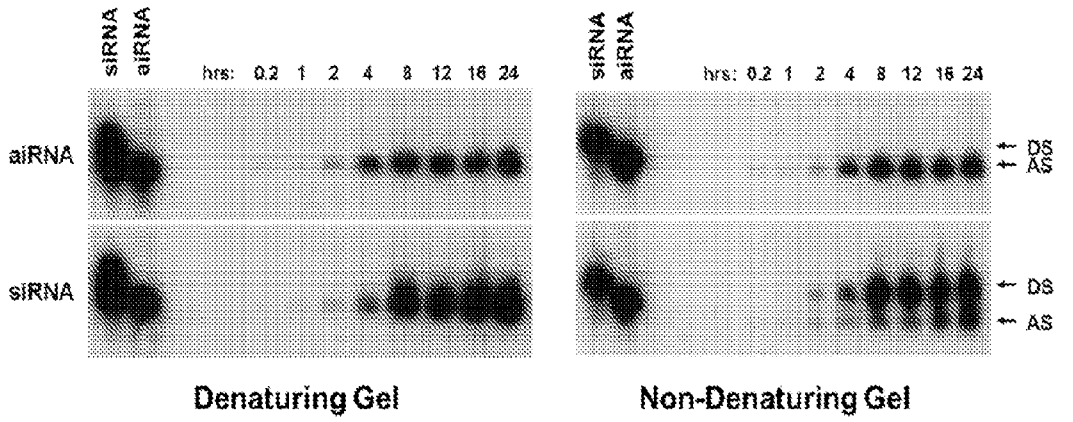


Figure 1(B)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aiRNA
hrs: 0.2 1 2 4 8 12 16 24
aiRNA
siRNA
DS
AS
Denaturing Gel
Non-Denaturing Gel
Antisense Strand Detection

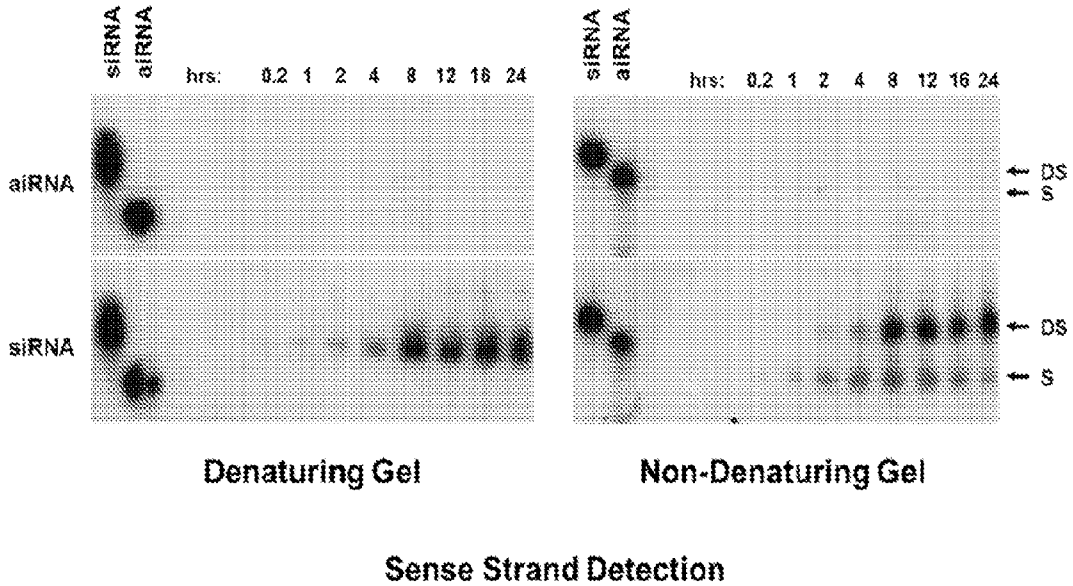

siRNA
aiRNA
hrs: 0.2 1 2 4 8 12 16 24
aiRNA
siRNA
DS
S
Denaturing Gel
Non-Denaturing Gel
Sense Strand Detection

Figure 2(A)

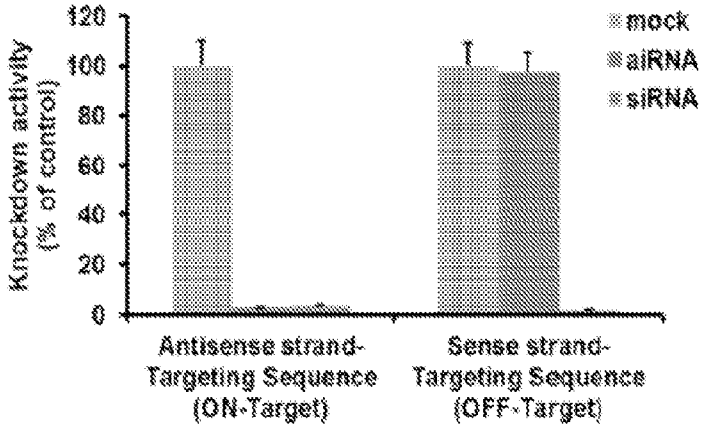


Figure 2(B)

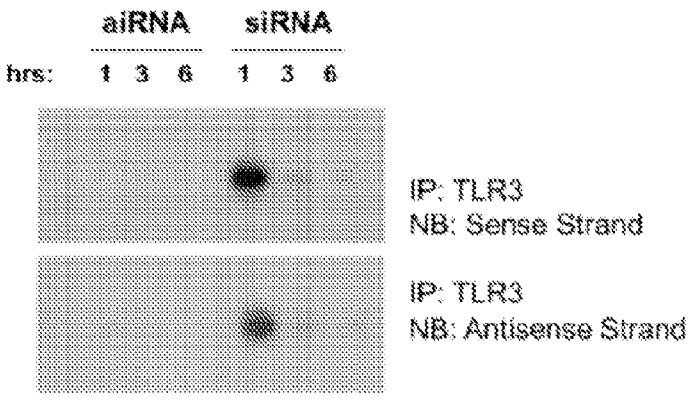


Figure 2(C)

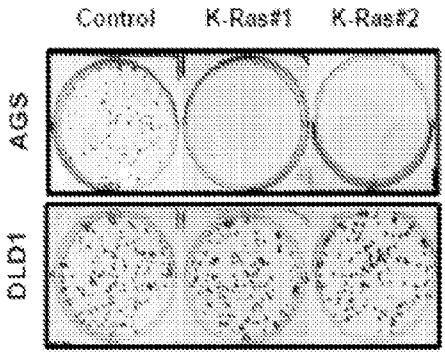


Figure 3(A)

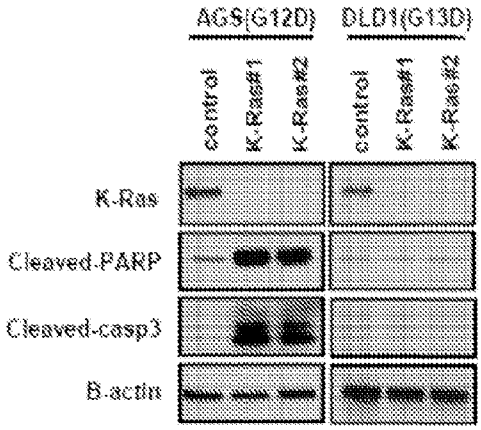
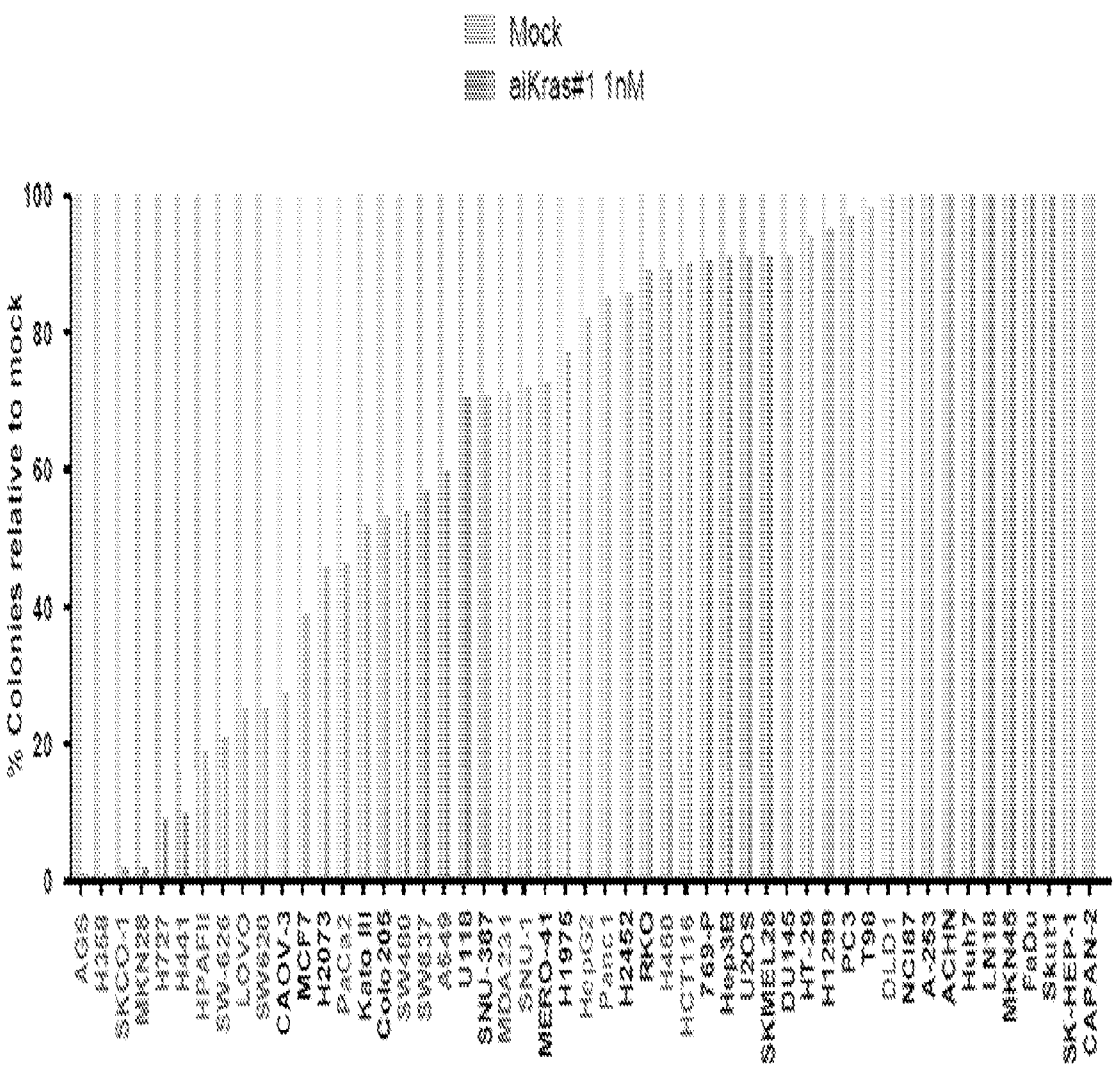


Figure 3(B)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20
40
60
80
100
AGS
H358
SKCO-1
MKN28
H727
H441
HPAFII
SW626
LOVO
SW620
CAOV-3
MCF7
H2073
PaCa2
Kato III
Colo205
SW480
SW837
A549
U118
SNU-387
MDA231
SNU-1
MERO-41
H1975
HepG2
Panc1
H2452
RKO
H460
HCT116
769-P
Hep3B
U2OS
SKMEL28
DU145
HT-29
H1299
PC3
T98
DLD1
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FaDu
SKUT1
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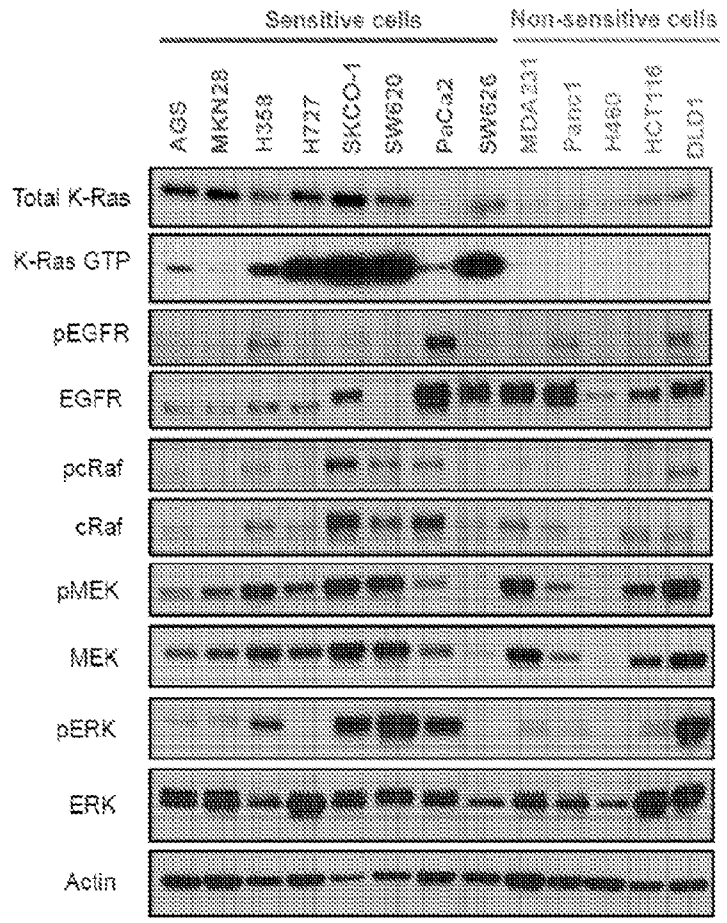
CAPAN-2

Figure 3(C)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Non-sensitive cells
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MKN28
H358
H727
SKCO-1
SW620
PaCa2
SW626
MDA231
Panc1
H460
HCT116
BL01
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K-Ras GTP
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EGFR
pcRaf
cRaf
pMEK
MEK
pERK
ERK
Actin

Figure 4

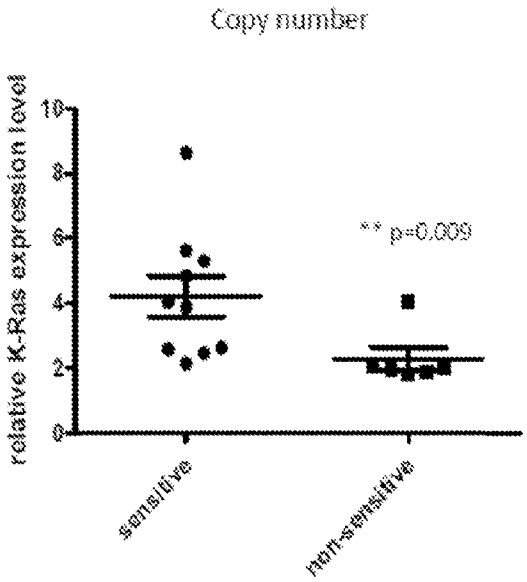


Figure 5(A)

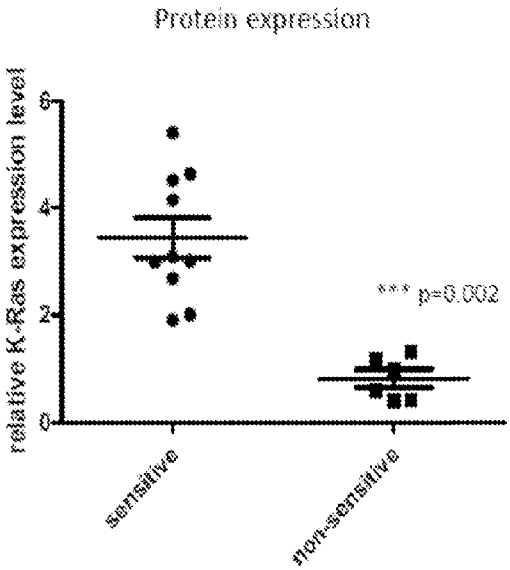


Figure 5(B)

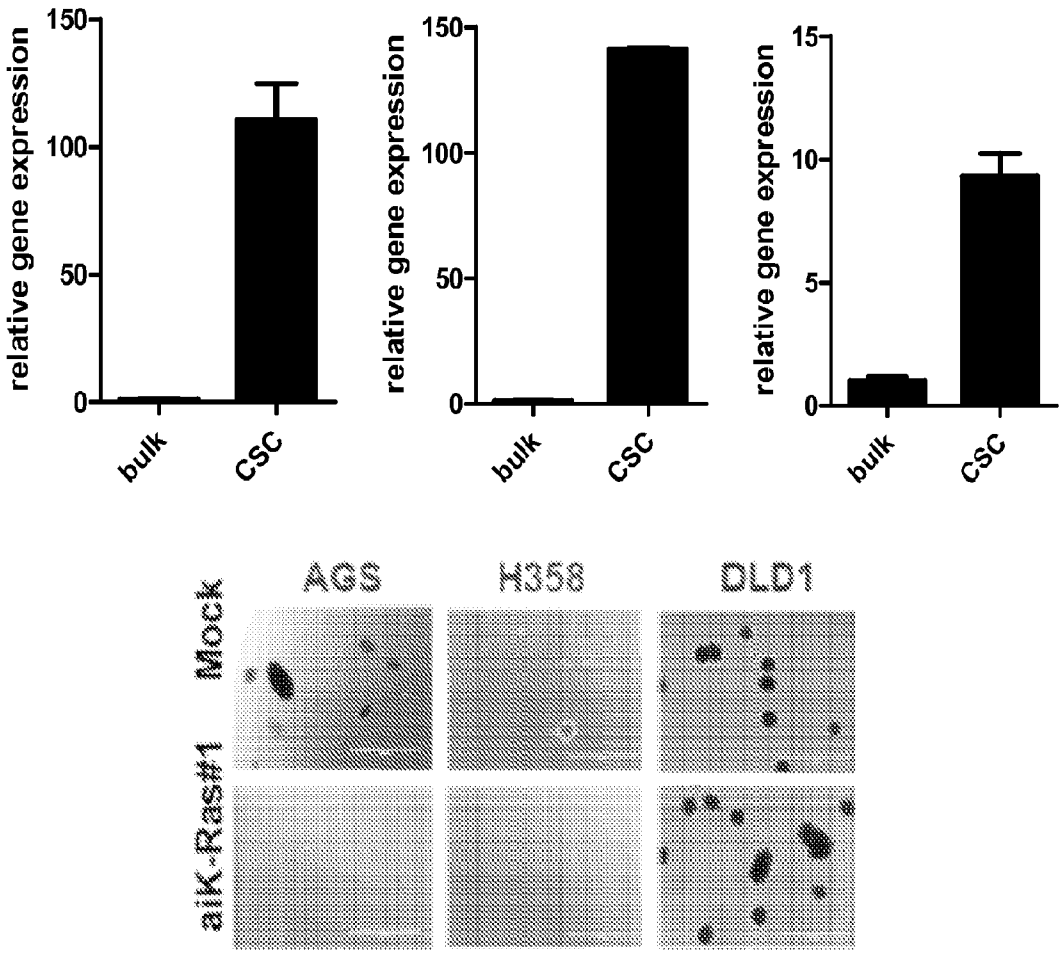
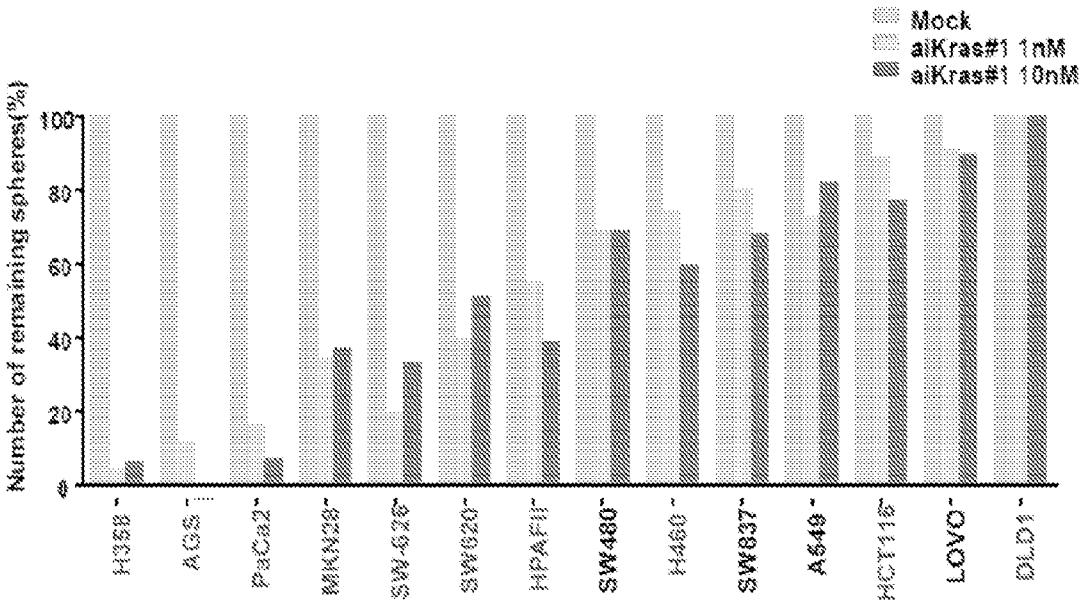

relative gene expression
150
100
50
0
bulk
CSC
relative gene expression
150
100
50
0
bulk
CSC
relative gene expression
15
10
5
0
bulk
CSC
AGS
H358
DLD1
Mock
aiK-Ras#1

Figure 6(A)


Mock
siKras#1 1nM
siKras#1 10nM
Number of remaining spheres(%)
100
80
60
40
20
0
H358
AGS
PaCa2
MKN28
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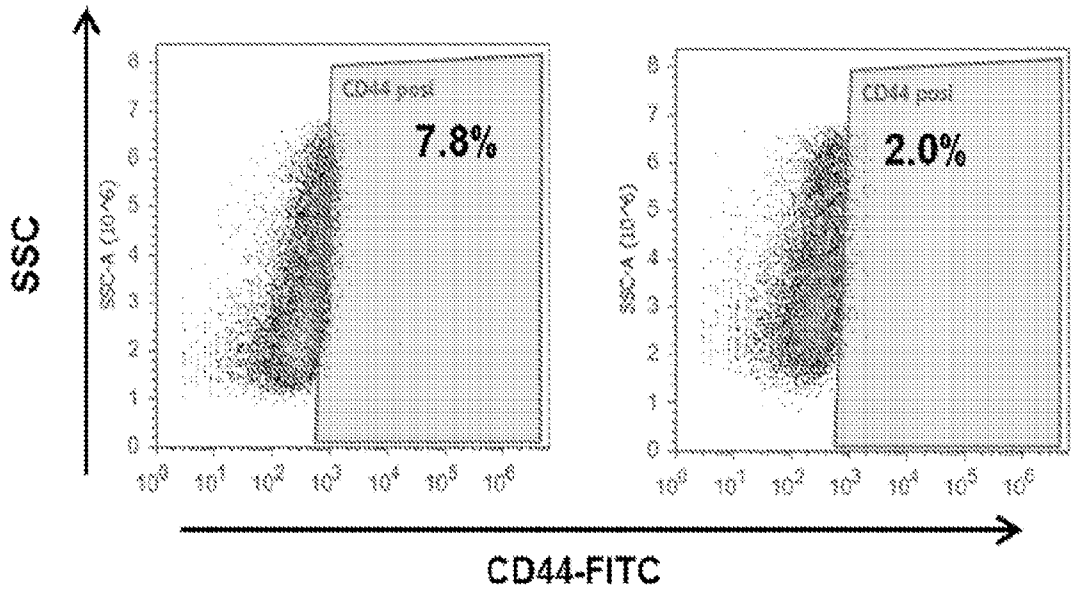
SW620
HPAFII
SW480
H460
SW837
A549
HCT116
LOVO
DLD1

Figure 6(B)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CD44 post
7.8%
CD44 post
2.0%
CD44-FITC

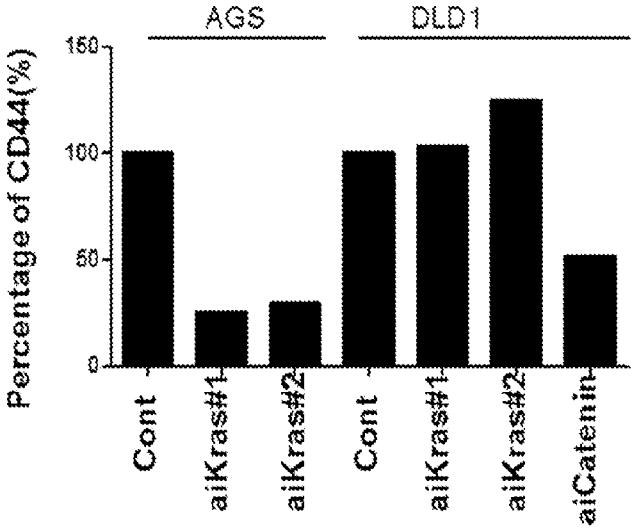

AGS
DLD1
Percentage of CD44(%)
150
100
50
0
Cont
aiKras#1
aiKras#2
Cont
aiKras#1
aiKras#2
aiCatenin

Figure 6(C)

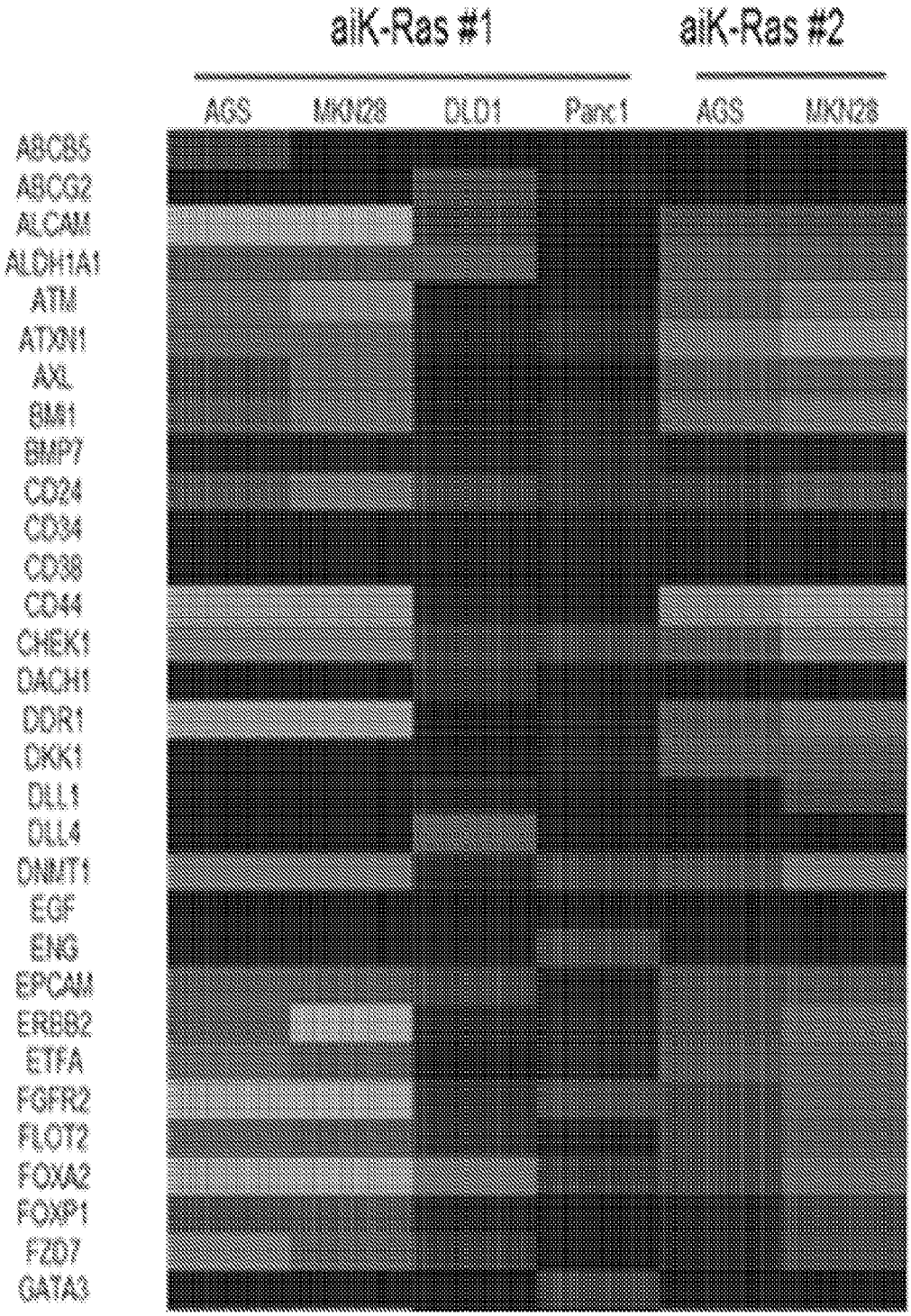
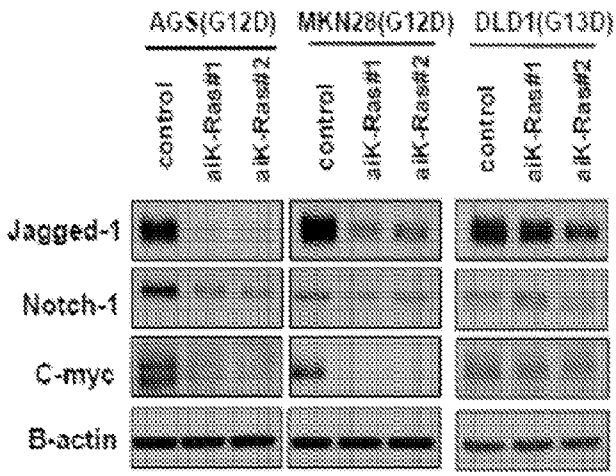

aiK-Ras #1
aiK-Ras #2
AGS
MKN28
DLD1
Panc1
AGS
MKN28
ABCB5
ABCG2
ALCAM
ALDH1A1
ATM
ATXN1
AXL
BMI1
BMP7
CD24
CD34
CD38
CD44
CHEK1
DACH1
DDR1
DKK1
DLL1
DLL4
DNMT1
EGF
ENG
EPCAM
ERBB2
ETFA
FGFR2
FLOT2
FOXA2
FOXP1
FZD7
GATA3

Figure 7(A)


AGS(G12D)
MKN28(G12D)
DLD1(G13D)
control
siK-Ras#1
siK-Ras#2
control
siK-Ras#1
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control
siK-Ras#1
siK-Ras#2
Jagged-1
Notch-1
C-myc
B-actin

Figure 7(B)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 15/20776

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C07H 21/02, C07H 21/04, C12N 15/00 (2015.01)
CPC - A61K 38/00, A61K 48/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC (8): C07H 21/02, C07H 21/04, C12N 15/00 (2015.01)
CPC: A61K 38/00, A61K 48/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 536/23.1, 536/23.5, 435/455 (text search, terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Google patents, Google scholar, Google web, PatBase, Proquest Dialog
gene; silence/interference; K-Ras/KRAS/ V-Ki-ras2/ Kirsten rat sarcoma viral oncogene; expression/activity; asymmetrical/aiRNA RNA interfering/RNAi/RNA-interference/siRNA; overhang; strand; inhibit/reduce/low/decrease; treatment/therapy; cancer/tumor

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2009/0208564 A1 (LI et al.) 20 August 2009 (20.08.2009) para [0233]; para [0010]; para [0070]; Fig. 14; para [0304]claim 2; claim 5; para [0020]	1, 19-21
Y		2-4, 34-35
X	US 2004/0121348 A1 (KREUTZER et al.) 24 June 2004 (24.06.2004) para [0002]; para [0039]; para [0008]	33
Y	SONG et al. Transduction effect of antisense K-ras on malignant phenotypes in gastric cancer cells. Cancer Lett. 31 August 2000. Vol.157. No.1. pp 1-7, especially, Abstract; Col. 2, para 3; page 4, Col. 1, para 1; page 4, Col. 2, para 1	2-4
Y	MOON et al. Role of oncogenic K-Ras in cancer stem cell activation by aberrant Wnt/beta-catenin signaling. J Natl Cancer Inst. February 2014. Vol.106. No. 2. pp 1-10, especially, Abstract; page 8, Col. 1, para 1; page 8, Col. 2, para 1; page 9, Col. 1, para 1	34-35

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 May 2015 (14.05.2015)	19 JUN 2015
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 15/20776

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

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

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

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

3. ☒ Claims Nos.: 5-18, 22-32
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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

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

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

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

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.