

CLAIMS

WHAT IS CLAIMED IS:

1. A composition comprising a first RNA linked to a second RNA, wherein the first RNA encodes for a cytokine, and wherein the second RNA encodes for a genetic element that modulates expression of a gene associated with tumor proliferation.
2. The composition of claim 1, wherein the cytokine is interleukin-2 (IL-2), IL-12, IL-15, IL-7, a fragment thereof, or a functional variant thereof.
- ~~3. The composition of claim 1, wherein the cytokine comprises a sequence selected from the group consisting of SEQ ID NOs: 24, 44, 47, 68, and 80.~~
- ~~4. The composition of claim 1, wherein the cytokine comprises a signal peptide.~~
- ~~5. The composition of claim 4, wherein the signal peptide comprises an unmodified signal peptide sequence or a modified signal peptide sequence.~~
- ~~6. The composition of claim 5, wherein the unmodified signal peptide sequence comprises a sequence selected from the group consisting of SEQ ID NOs: 26 and 125-128.~~
- ~~7. The composition of claim 2, wherein the IL-2 comprises a signal peptide.~~
- ~~8. The composition of claim 7, wherein the signal peptide comprises an unmodified IL-2 signal peptide sequence.~~
- ~~9. The composition of claim 8, wherein the unmodified IL-2 signal peptide sequence comprises a sequence listed in SEQ ID NO: 26.~~
- ~~10. The composition of claim 7, wherein the signal peptide comprises an IL-2 signal peptide sequence modified by insertion, deletion, or substitution of at least one amino acid.~~
- ~~11. The composition of claim 10, wherein the IL-2 signal peptide sequence modified by insertion, deletion, or substitution of at least one amino acid comprises a sequence selected from the group consisting of SEQ ID NOs: 27-29.~~
- 12.3. The composition of claim 1, wherein the first RNA is a messenger RNA (mRNA).
- 13.4. The composition of claim 1, wherein the second RNA is a small interfering RNA (siRNA).
- ~~14. The composition of claim 13, wherein the siRNA is capable of binding to an mRNA of the gene associated with tumor proliferation.~~

- ~~15. The composition of claim 13, wherein the second RNA comprises 1, 2, 3, 4, 5, or more species of siRNA, wherein each species of siRNA comprises a different sequence targeting a different region of the same mRNA.~~
- ~~16. The composition of claim 13, wherein the second RNA comprises 1, 2, 3, 4, 5, or more redundant species of siRNA.~~
- ~~17. The composition of claim 15 or 16, wherein each species of the 1, 2, 3, 4, 5, or more species of siRNA is connected by a linker comprising a sequence listed in SEQ ID NO: 22.~~
- ~~18.5. _____~~ The composition of claim 1, wherein the gene associated with tumor proliferation comprises a gene associated with angiogenesis.
- ~~19.6. _____~~ The composition of claim ~~18~~⁵, wherein the gene associated with angiogenesis encodes vascular endothelial growth factor (VEGF), a fragment thereof, or a functional variant thereof.
- ~~20. The composition of claim 19, wherein the VEGF is VEGFA, a fragment thereof, or a functional variant thereof.~~
- ~~21. The composition of claim 20, wherein the VEGFA comprises a sequence listed in SEQ ID NO: 35.~~
- ~~22. The composition of claim 19, wherein the VEGF is an isoform of VEGFA, a fragment thereof, or a functional variant thereof.~~
- ~~23. The composition of claim 19, wherein the VEGF is placental growth factor (PIGF), a fragment thereof, or a functional variant thereof.~~
- ~~24.7. _____~~ The composition of claim 1, wherein the gene associated with tumor proliferation comprises isocitrate dehydrogenase (IDH1), cyclin-dependent kinase 4 (CDK4), CDK6, epidermal growth factor receptor (EGFR), mechanistic target of rapamycin (mTOR), Kirsten rat sarcoma viral oncogene (KRAS), cluster of differentiation (CD155), programmed cell death-ligand 1 (PD-L1), or myc proto-oncogene (c-Myc).
- ~~25. The composition of claim 24, wherein the gene associated with tumor proliferation comprises a sequence selected from the group consisting of SEQ ID NOs: 50, 53, 56, 59, 62, 65, 71, 74, and 77.~~
- ~~26. The composition of claim 1, wherein the first RNA is linked to the second RNA by a linker.~~
- ~~27. The composition of claim 26, wherein the linker comprises a tRNA linker or a linker comprising a sequence listed in SEQ ID NO: 21.~~

~~28. The composition of claim 1, further comprising a poly(A) tail, a 5' cap, or a Kozak sequence.~~

~~29. The composition of any one of claims 1-28, wherein the first RNA and the second RNA are both recombinant.~~

~~30.8.~~ A composition comprising a first RNA linked to a second RNA, wherein the first RNA encodes for a cytokine, and wherein the second RNA encodes for a genetic element that modulates expression of a gene associated with recognition by the immune system.

~~31.9.~~ The composition of claim ~~30~~8, wherein the cytokine is interleukin-2 (IL-2), a fragment thereof, or a functional variant thereof.

~~32. The composition of claim 31, wherein the IL-2 comprises a sequence comprising SEQ ID NO: 24.~~

~~33. The composition of claim 31, wherein the IL-2 comprises a signal peptide.~~

~~34. The composition of claim 33, wherein the signal peptide comprises an unmodified IL-2 signal peptide sequence.~~

~~35. The composition of claim 34, wherein the IL-2 signal peptide sequence comprises a sequence listed in SEQ ID NO: 26.~~

~~36. The composition of claim 33, wherein the signal peptide comprises an IL-2 signal peptide sequence modified by insertion, deletion and/or substitution of at least one amino acid.~~

~~37. The composition of claim 36, wherein the IL-2 signal peptide sequence modified by insertion, deletion, or substitution of at least one amino acid comprises a sequence selected from the group consisting of SEQ ID NOs: 27-29.~~

~~38.10.~~ The composition of claim ~~30~~8, wherein the first RNA is a messenger RNA (mRNA).

~~39.11.~~ The composition of claim ~~30~~8, wherein the second RNA is a small interfering RNA (siRNA).

~~40.12.~~ The composition of claim ~~39~~11, wherein the siRNA is capable of binding to an mRNA of the gene associated with recognition by the immune system.

~~41.13.~~ The composition of claim ~~40~~12, wherein the gene associated with recognition by immune system encodes MHC class I chain-related sequence A (MICA), a fragment thereof, or a functional variant thereof or wherein the gene associated with recognition by the immune system encodes MHC class I chain-related sequence B (MICB), a fragment thereof, or a functional variant thereof.

- ~~42. The composition of claim 41, wherein the MICA comprises a sequence listed in SEQ ID NO: 38.~~
- ~~43. The composition of claim 40, wherein the gene associated with recognition by the immune system encodes MHC class I chain related sequence B (MICB), a fragment thereof, or a functional variant thereof.~~
- ~~44. The composition of claim 43, wherein the MICB comprises a sequence listed in SEQ ID NO: 41.~~
- ~~45.14. The composition of claim 4012, wherein the gene associated with recognition by the immune system encodes endoplasmic reticulum protein (ERp5), a disintegrin and metalloproteinase (ADAM), matrix metalloproteinase (MMP), a fragment thereof, or a functional variant thereof.~~
- ~~46. The composition of claim 45, wherein the ADAM is ADAM17.~~
- ~~47. The composition of claim 39, wherein the second RNA comprises 1, 2, 3, 4, 5, or more species of siRNA, wherein each species of siRNA comprises a different sequence targeting a different region of the same mRNA.~~
- ~~48. The composition of claim 39, wherein the second RNA comprises 1, 2, 3, 4, 5, or more redundant species of siRNA.~~
- ~~49. The composition of claim 47 or 48, wherein each species of the 1, 2, 3, 4, 5, or more species of siRNA is connected by a linker comprising a sequence listed in SEQ ID NO: 22.~~
- ~~50. The composition of claim 30, wherein the first RNA is linked to the second RNA by a linker.~~
- ~~51. The composition of claim 50, wherein the linker comprises a tRNA linker or a linker comprising a sequence listed in SEQ ID NO: 21.~~
- ~~52. The composition of claim 30, further comprising a poly(A) tail, a 5' cap, or a Kozak sequence.~~
- ~~53. The composition of any one of claims 30-52, wherein the first RNA and the second RNA are both recombinant.~~
- ~~54.15. A composition comprising:~~
a first RNA encoding for interleukin-2 (IL-2), IL-15, a fragment thereof, or a functional variant thereof linked to a second RNA encoding for a genetic element that modulates expression of vascular endothelial growth factor A (VEGFA), an isoform of VEGFA, placental growth factor (PIGF), cluster of differentiation 155 (CD155),

programmed cell death-ligand 1 (PD-L1), myc proto-oncogene (c-Myc), a fragment thereof, or a functional variant thereof.

~~55.16.~~ The composition of claim ~~54~~15, wherein the first RNA is a messenger RNA (mRNA).

~~56.~~ The composition of claim ~~54~~, wherein the IL-2 comprises a sequence comprising SEQ ID NO: 24.

~~57.~~ The composition of claim ~~54~~, wherein the IL-2 comprises a signal peptide.

~~58.~~ The composition of claim ~~57~~, wherein the signal peptide comprises an unmodified IL-2 signal peptide sequence.

~~59.~~ The composition of claim ~~58~~, wherein the IL-2 signal peptide sequence comprises a sequence listed in SEQ ID NO: 26.

~~60.~~ The composition of claim ~~57~~, wherein the signal peptide comprises an IL-2 signal peptide sequence modified by insertion, deletion and/or substitution of at least one amino acid.

~~61.~~ The composition of claim ~~60~~, wherein the IL-2 signal peptide sequence modified by insertion, deletion, or substitution of at least one amino acid comprises a sequence selected from the group consisting of SEQ ID NOs: 27-29.

~~62.~~ The composition of claim ~~54~~, wherein the IL-15 comprises a sequence comprising SEQ ID NO: 68.

~~63.~~ The composition of claim ~~54~~, wherein the IL-15 comprises a signal peptide.

~~64.~~ The composition of claim ~~63~~, wherein the signal peptide comprises an unmodified IL-15 signal peptide sequence.

~~65.~~ The composition of claim ~~64~~, wherein the unmodified IL-15 signal peptide sequence comprises a sequence listed in SEQ ID NO: 144.

~~66.17.~~ The composition of claim ~~54~~15, wherein the second RNA is a small interfering RNA (siRNA).

~~67.18.~~ The composition of claim ~~66~~17, wherein the siRNA is capable of binding to an mRNA of VEGFA, an isoform of VEGFA, PIGF, CD155, PD-L1, or c-Myc.

~~68.~~ The composition of claim ~~67~~, wherein the VEGFA comprises a sequence comprising SEQ ID NO: 35.

~~69.~~ The composition of claim ~~67~~, wherein the CD155 comprises a sequence comprising SEQ ID NO: 71.

~~70.~~ The composition of claim ~~67~~, wherein the PD-L1 comprises a sequence comprising SEQ ID NO: 74.

- ~~71. The composition of claim 67, wherein the c-Myc comprises a sequence comprising SEQ ID NO: 77.~~
- ~~72. The composition of claim 66, wherein the second RNA comprises 1, 2, 3, 4, 5, or more species of siRNA, wherein each species of siRNA comprises a different sequence targeting a different region of the same mRNA.~~
- ~~73. The composition of claim 66, wherein the second RNA comprises 1, 2, 3, 4, 5, or more redundant species of siRNA.~~
- ~~74. The composition of claim 72 or 73, wherein each species of the 1, 2, 3, 4, 5, or more species of siRNA is connected by a linker comprising a sequence listed in SEQ ID NO: 22.~~
- ~~75. The composition of claim 54, wherein the first RNA is linked to the second RNA by a linker.~~
- ~~76. The composition of claim 75, wherein the linker comprises a tRNA linker or a linker comprising a sequence comprising SEQ ID NO: 21.~~
- ~~77. The composition of claim 54, further comprising a poly(A) tail, a 5' cap, or a Kozak sequence.~~
- ~~78. The composition of any one of claims 54-77, wherein the first RNA and the second RNA are both recombinant.~~
- ~~79.~~19. A composition comprising:
a first RNA encoding for interleukin-2 (IL-2), a fragment thereof, or a functional variant thereof linked to a second RNA encoding for a genetic element that modulates expression of MHC class I chain-related sequence A (MICA), MHC class I chain-related sequence B (MICB), endoplasmic reticulum protein (ERp5), a disintegrin and metalloproteinase (ADAM), matrix metalloproteinase (MMP), a fragment thereof, or a functional variant thereof.
- ~~80. The composition of claim 79, wherein the ADAM is ADAM17.~~
- ~~81.~~20. The composition of claim 7919, wherein the first RNA is a messenger RNA (mRNA).
- ~~82. The composition of claim 79, wherein the IL-2 comprises a sequence comprising SEQ ID NO: 24.~~
- ~~83. The composition of claim 79, wherein the IL-2 comprises a signal peptide.~~
- ~~84. The composition of claim 83, wherein the signal peptide comprises an unmodified IL-2 signal peptide sequence.~~

- ~~85. The composition of claim 84, wherein the IL-2 signal peptide sequence comprises a sequence listed in SEQ ID NO: 26.~~
- ~~86. The composition of claim 83, wherein the signal peptide comprises an IL-2 signal peptide sequence modified by insertion, deletion and/or substitution of at least one amino acid.~~
- ~~87. The composition of claim 86, wherein the IL-2 signal peptide sequence modified by insertion, deletion, or substitution of at least one amino acid comprises a sequence selected from the group consisting of SEQ ID NOs: 27-29.~~
- ~~88.21. The composition of claim 79~~19~~, wherein the second RNA is a small interfering RNA (siRNA).~~
- ~~89.22. The composition of claim 88~~21~~, wherein the siRNA is capable of binding to an mRNA of MICA, MICB, ERp5, ADAM, or MMP.~~
- ~~90. The composition of claim 89, wherein the MICA comprises a sequence comprising SEQ ID NO: 38.~~
- ~~91. The composition of claim 89, wherein the MICB comprises a sequence comprising SEQ ID NO: 41.~~
- ~~92. The composition of claim 89, wherein the ADAM is ADAM17.~~
- ~~93. The composition of claim 88, wherein the second RNA comprises 1, 2, 3, 4, 5, or more species of siRNA, wherein each species of siRNA comprises a different sequence targeting a different region of the same mRNA.~~
- ~~94. The composition of claim 88, wherein the second RNA comprises 1, 2, 3, 4, 5, or more redundant species of siRNA.~~
- ~~95. The composition of claim 93 or 94, wherein each species of the 1, 2, 3, 4, 5, or more species of siRNA is connected by a linker comprising a sequence listed in SEQ ID NO: 22.~~
- ~~96. The composition of claim 79, wherein the first RNA is linked to the second RNA by a linker.~~
- ~~97. The composition of claim 96, wherein the linker comprises a tRNA linker or a linker comprising a sequence listed in SEQ ID NO: 21.~~
- ~~98. The composition of claim 79, further comprising a poly(A) tail, a 5' cap, or a Kozak sequence.~~
- ~~99. The composition of any one of claims 79-98, wherein the first RNA and the second RNA are both recombinant.~~
- ~~100.23. A composition comprising:~~

a first RNA encoding for interleukin-12 (IL-12), IL-7, a fragment thereof, or a functional variant thereof linked to a second RNA encoding for a genetic element that modulates expression of isocitrate dehydrogenase (IDH1), cyclin-dependent kinase 4 (CDK4), CDK6, epidermal growth factor receptor (EGFR), mechanistic target of rapamycin (mTOR), Kirsten rat sarcoma viral oncogene (KRAS), programmed cell death-ligand 1 (PD-L1), a fragment thereof, or a functional variant thereof.

~~101.~~24. The composition of claim ~~100~~23, wherein the first RNA is a messenger RNA (mRNA).

~~102.~~ The composition of claim 100, wherein the IL-12 comprises a sequence comprising SEQ ID NO: 44 or SEQ ID NO: 47.

~~103.~~ The composition of claim 100, wherein the IL-12 comprises a signal peptide.

~~104.~~ The composition of claim 103, wherein the signal peptide comprises an unmodified IL-12 signal peptide.

~~105.~~ The composition of claim 104, wherein the unmodified IL-12 signal peptide comprises a sequence listed in SEQ ID NO: 142 or SEQ ID NO: 143.

~~106.~~ The composition of claim 100, wherein the IL-7 comprises a sequence comprising SEQ ID NO: 80.

~~107.~~ The composition of claim 100, wherein the IL-7 comprises a signal peptide.

~~108.~~ The composition of claim 107, wherein the signal peptide comprises an unmodified IL-7 signal peptide.

~~109.~~ The composition of claim 108, wherein the unmodified IL-7 signal peptide comprises a sequence listed in SEQ ID NO: 128.

~~110.~~25. The composition of claim ~~100~~23, wherein the second RNA is a small interfering RNA (siRNA).

~~111.~~26. The composition of claim ~~110~~25, wherein the siRNA is capable of binding to an mRNA of IDH1, CDK4, CDK6, EGFR, mTOR, KRAS, or PD-L1.

~~112.~~ The composition of claim 111, wherein IDH1 comprises a sequence comprising SEQ ID NO: 50.

~~113.~~ The composition of claim 111, wherein CDK4 comprises a sequence comprising SEQ ID NO: 53.

~~114.~~ The composition of claim 111, wherein CDK6 comprises a sequence comprising SEQ ID NO: 56.

~~115.~~ The composition of claim 111, wherein mTOR comprises a sequence comprising SEQ ID NO: 62.

116. — The composition of claim 111, wherein EGFR comprises a sequence comprising SEQ ID NO: 59.
117. — The composition of claim 111, wherein KRAS comprises a sequence comprising SEQ ID NO: 65.
118. — The composition of claim 111, wherein PD-L1 comprises a sequence comprising SEQ ID NO: 74.
119. — The composition of claim 110, wherein the second RNA comprises 1, 2, 3, 4, 5, or more species of siRNA, wherein each species of siRNA comprises a different sequence targeting a different region of the same mRNA.
120. — The composition of claim 110, wherein the second RNA comprises 1, 2, 3, 4, 5, or more redundant species of siRNA.
121. — The composition of claim 119 or 120, wherein each species of the 1, 2, 3, 4, 5, or more species of siRNA is connected by a linker comprising a sequence listed in SEQ ID NO: 22.
122. — The composition of claim 100, wherein the first RNA is linked to the second RNA by a linker.
123. — The composition of claim 122, wherein the linker comprises a tRNA linker or a linker comprising a sequence comprising SEQ ID NO: 21.
124. — The composition of claim 100, further comprising a poly(A) tail, a 5' cap, or a Kozak sequence.
125. — The composition of any one of claims 100-124, wherein the first RNA and the second RNA are both recombinant.
126. 27. A pharmaceutical composition comprising a therapeutically effective amount of the composition of any one of claims 1-125 26 and a pharmaceutically acceptable excipient.
127. 28. A method of treating a cancer, comprising administering Use of the composition of any one of claims 1-125 26 or of the pharmaceutical composition of claim 126 27 in the manufacture of a medicament for the treatment of cancer to a subject having a cancer.
128. — 29. The method use of claim 127 28, wherein the cancer is selected from the group consisting of a solid tumor, ;
129. — The method of claim 127, wherein the cancer is melanoma, ;
130. — The method of claim 127, wherein the cancer is renal cell carcinoma, and ;
131. The method of claim 127, wherein the cancer is a head and neck cancer.

132. ~~The method of claim 131, wherein the head and neck cancer is head and neck squamous cell carcinoma.~~
133. ~~— The method of claim 131, wherein the head and neck cancer is laryngeal cancer, hypopharyngeal cancer, nasal cavity cancer, paranasal sinus cancer, nasopharyngeal cancer, oral cancer, oropharyngeal cancer, salivary gland cancer, brain tumors, esophageal cancer, eye cancer, parathyroid cancer, sarcoma of the head and neck, or thyroid cancer.~~
134. ~~— The method of claim 127, wherein the cancer is located at an upper aerodigestive tract.~~
135. ~~— The method of claim 134, wherein the upper aerodigestive tract comprises a paranasal sinus, a nasal cavity, an oral cavity, a salivary gland, a tongue, a nasopharynx, an oropharynx, a hypopharynx, or a larynx.~~
136. ~~— The method of claim 127, wherein the subject has a head and neck cancer.~~
137. ~~— The method of claim 136, wherein the subject having the head and neck cancer has a history of tobacco usage.~~
138. ~~— The method of claim 136, wherein the subject having the head and neck cancer has a human papillomavirus (HPV) DNA.~~
139. ~~— The method of any one of claims 127-138, wherein the subject is a human.~~
140. 30. A composition comprising a recombinant polynucleic acid construct comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1-17 and 125-141.
141. 31. A composition according to any one of claims 1-~~125~~26 for use in modulating the expression of two or more genes in a cell.
142. 32. A cell comprising the composition of any one of claims 1-~~125~~26.
143. 33. A vector comprising a recombinant polynucleic acid construct encoding the composition of any one of claims 1-~~125~~26.
144. 34. A method of producing an siRNA and an mRNA from a single RNA transcript in a cell, comprising introducing into the cell the composition of any one of claims 1-~~125~~26 or the vector of claim ~~143~~33.
145. 35. A method of modulating protein expression comprising introducing the composition of any one of claims 1-~~125~~26 or the vector of claim ~~143~~33 into a cell, wherein the expression of a protein encoded by the second RNA is decreased compared to a cell without the composition of any one of claims 1-~~125~~26 or the vector of claim ~~143~~33.

~~146.~~ 36. A method of modulating protein expression comprising introducing the composition of any one of claims ~~1-125-26~~ or the vector of claim ~~143-33~~ into a cell, wherein the expression of a protein encoded by the first RNA is increased compared to a cell without the composition of any one of claims ~~1-125-26~~ or the vector of claim ~~143-33~~.

~~147.~~ 37. A method of modulating protein expression comprising introducing the composition of any one of claims ~~1-125-26~~ or the vector of claim ~~143-33~~ into a cell, wherein the expression of a protein encoded by the second RNA is decreased compared to a cell without the composition of any one of claims ~~1-125-26~~ or the vector of claim ~~143-33~~, and wherein the expression of a protein encoded by the first RNA is increased compared to a cell without the composition of any one of claims ~~1-125-26~~ or the vector of claim ~~143-33~~.