CLAIMS

What is claimed is:

~~1. A method for treating cancer in an individual comprising administering to the individual an effective amount of an O_2-carrier polypeptide.~~

1. Use of an effective amount of a PEGylated H-NOX protein in the manufacture of a medicament for treating cancer in an individual, wherein the PEGylated H-NOX protein is to be administered in combination with immunotherapy, wherein the PEGylated H-NOX protein is a trimeric H-NOX protein comprising three monomers, wherein the monomers comprise an H-NOX domain and a trimerization domain, wherein the H-NOX domain is a *Thermoanaaerobacter tengcongensis* H-NOX domain set forth in SEQ ID NO:2 comprising an L144F suibstitution, and wherein the immunotherapy is an anti-CTLA-4 therapy, an anti-PD-1 therapy, an anti-PD-L1 therapy, a dual PD-1/CTLA-4 blockade therapy, or a dual PD-1/PD-L1 blockade therapy.

2. The ~~method~~use of claim 1, wherein the cancer is brain cancer, glioblastoma, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, melanoma, lung cancer, uterine cancer, ovarian cancer, colorectal cancer, anal cancer, liver cancer, hepatocellular carcinoma, stomach cancer, testicular cancer, endometrial cancer, cervical cancer, Hodgkin's Disease, non-Hodgkin's lymphoma, esophageal cancer, intestinal cancer, thyroid cancer, adrenal cancer, bladder cancer, kidney cancer, breast cancer, multiple myeloma, sarcoma, or squamous cell cancer.

3. ~~A method for modulating tumor immunity in an individual with a tumor comprising administering to the individual an effective amount of an O_2-carrier polypeptide.~~

4. The ~~method of claim 3, wherein the modulating tumor immunity comprises enhancing an immune response to the tumor.~~

~~5. A method for increasing lymphocyte infiltration to a tumor in an individual comprising administering to the individual an effective amount of an O_2-carrier polypeptide.~~

~~6. The method of claim 5, wherein the increase in lymphocyte infiltration to the tumor comprises an increase in infiltration of one or more of CD4 cells, CD8 cells, or NK cells.~~

~~7. The method of claim 5 or 6, wherein the increase in lymphocyte infiltration to the tumor is accompanied by inhibition of one or more of Treg cells, tumor associated macrophages or myeloid derived suppressor cells in the tumor.~~

~~8. The method~~use of any ~~one~~ of claims ~~5-7, wherein the increase in lymphocyte infiltration to the tumor is accompanied by an increase in MHC1 expression on the tumor cells.~~

9. A method for decreasing expression of hypoxia inducible factor 1α (HIF-1α) and/or 2α (HIF-2α) in a tumor in an individual comprising administering to the individual an effective amount of an O_2 carrier polypeptide.

10. A method for decreasing expression of programmed death ligand-1 (PD-L1) in a tumor in an individual comprising administering to the individual an effective amount of an O_2 carrier polypeptideor 2, wherein the individual is a human.

4. The use11. A method for decreasing expression of A2A adenosine receptor (A2AR) in a tumor in an individual comprising administering to the individual an effective amount of an O_2 carrier polypeptide.

12. The method of any one of claims 3-11, wherein the tumor is a brain tumor, a glioblastoma, a bone tumor, a pancreatic tumor, a skin tumor, a tumor of the head or neck, a melanoma, a lung tumor, a uterine tumor, an ovarian tumor, a colorectal tumor, an anal tumor, a liver tumor, a hepatocellular carcinoma, a stomach tumor, a testicular tumor, an endometrial tumor, a cervical tumor, a vaginal tumor, a Hodgkin's lymphoma, a non-Hodgkin's lymphoma, an esophageal tumor, an intestinal tumor, a thyroid tumor, an adrenal tumor, a bladder tumor, a kidney tumor, breast tumor, a multiple myeloma tumor, a sarcoma, or a squamous cell tumor.

13. The method of any one of claims 1-123, wherein the individualH-NOX protein is a mammal.

14. The method of claim 13, wherein the mammal is a human.

15. The method of claim 14, wherein the mammal is a pet, a laboratory research animal, or a farm animal.

16. The method of claim 15, wherein the pet, research animal or farm animal is a dog, a cat, a horse, a monkey, a rabbit, a rat, a mouse, a guinea pig, a hamster, a pig, or a cow.

17. The method of any one of claims 1-16, wherein the O_2 carrier polypeptide isto be administered by intravenous, intra-arterial, intratumoral, intravesicular, inhalation, intraperitoneal, intrapulmonary, intramuscular, subcutaneous, intra-tracheal, transmucosal, intraocular, intrathecal, or transdermal administration.

185. The methoduse of any one of claims 1-174, wherein administration of the O_2 carrier polypeptideH-NOX protein is to be repeated.

196. The method of claim 18, wherein administration of the O_2 carrier polypeptide is repeated daily, twice a day or about 1-4 times a week from about 4 weeks to about 8 weeks.

20. The method of claim 18 or 19 wherein the O_2 carrier polypeptide is administered every four, every 8, every 12 or every 24 hours for a period of about one to about 10 days.

~~21. The method~~use of any one of claims 1-~~20~~5, wherein the ~~O_2 carrier polypeptide~~H-NOX protein is to be administered as a bolus and/or by infusion.

~~22~~7. The ~~method~~use of any one of claims 1-~~20~~6, wherein the ~~O_2 carrier polypeptide~~H-NOX protein is ~~administered by infusion.~~

~~23. The method of claim 22, wherein the O_2 carrier polypeptide is infused in the individual for about 15 minutes, about 30 minutes, about 1 hour, about 2 hours, about 3 hours, about 6 hours, about 12 hours or about 24 hours.~~

~~24. The method of any one of claims 1-23, wherein the O_2 carrier polypeptide is~~to be administered in combination with radiation therapy.

~~25~~8. The ~~method~~use of claim ~~24~~7, wherein the radiation therapy is ~~administered to the individual 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 20 or 24 hours after the O_2 carrier polypeptide is administered.~~

~~26. The method of claim 24 or 25, wherein the radiation is X-radiation.~~

~~27. The method of claim 26, wherein the X-radiation is administered at about 0.5 gray~~ to ~~about 75 gray.~~

~~28. The method of any one of claims 24-27, wherein the administration of the O_2 carrier polypeptide and/or the administration of the radiation is repeated.~~

~~29. The method of claim 28, wherein the administration is repeated any number of times between about two times to about forty times or more.~~

~~30. The method of claim 28 or 29, wherein the administration is repeated after one week, two weeks, three weeks, or four weeks or more.~~

~~31. The method of any one of claims 1-23, wherein the O_2 carrier polypeptide is administered in combination with chemotherapy or immunotherapy.~~

~~32. The method of claim 31, wherein the chemotherapy comprises a cytotoxin.~~

~~33. The method of claim 32, wherein the administration of the O_2 carrier polypeptide and/or the administration of the chemotherapy is repeated.~~

~~34. The method of claim 31, wherein the immunotherapy is one or more of an anticancer vaccine, an adoptive immune cell therapy or an agent that targets an immune checkpoint regulator.~~

~~35. The method of claim 31 or 34, wherein the immunotherapy targets one or more of CTLA-4, PD1, PD-L1, or an immune checkpoint regulator.~~

~~36. The method of claim 31 or 34, wherein the adoptive immude therapy is a chimeric antigen receptor expressing T cell or an engineered TCR T cell.~~

37. The method of any one of claims 31 or 34-36, wherein the administration of the O_2 carrier polypeptide and/or the administration of the immunotherapy is repeated.

38. The method of any one of claims 1-37, wherein the O_2 carrier polypeptide is in a pharmaceutical composition.

39. The method of claim 38, wherein the pharmaceutical composition further comprises a pharmaceutically acceptable carrier.

40. The method of any one of claims 1-39 wherein the O_2 carrier polypeptide is an H-NOX protein.

41. A method for treating cancer in an individual comprising administering to the individual an effective amount of an H-NOX protein.

42. The method of claim 41, wherein the cancer is brain cancer, glioblastoma, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, melanoma, lung cancer, uterine cancer, ovarian cancer, colorectal cancer, anal cancer, liver cancer, hepatocellular carcinoma, stomach cancer, testicular cancer, endometrial cancer, cervical cancer, Hodgkin's Disease, non-Hodgkin's lymphoma, esophageal cancer, intestinal cancer, thyroid cancer, adrenal cancer, bladder cancer, kidney cancer, breast cancer, multiple myeloma, sarcoma, or squamous cell cancer.

43. A method for modulating tumor immunity in an individual with a tumor comprising administering to the individual an effective amount of an H-NOX protein.

44. The method of claim 43, wherein the modulating tumor immunity comprises enhancing an immune response to the tumor.

45. A method for increasing lymphocyte infiltration to a tumor in an individual comprising administering to the individual an effective amount of an H-NOX protein.

46. The method of claim 45, wherein the increase in lymphocyte infiltration to the tumor comprises an increase in infiltration of one or more of CD4 cells, CD8 cells, or NK cells.

47. The method of claim 45 or 46, wherein the increase in lymphocyte infiltration to the tumor is accompanied by inhibition of one or more of Treg cells, tumor associated macrophages or myeloid derived suppressor cells in the tumor.

48. The method of any one of claims 45-47, wherein the increase in lymphocyte infiltration to the tumor is accompanied by an increase in MHC1 expression on the tumor cells.

49. A method for decreasing expression of HIF-1α and/or HIF-2α in a tumor in an individual comprising administering to the individual an effective amount of an H-NOX protein.

50. A method for decreasing expression of PD-L1 in a tumor in an individual comprising administering to the individual an effective amount of an H-NOX protein.

51. A method for decreasing expression of A2AR in a tumor in an individual comprising administering to the individual an effective amount of an H-NOX protein.

52. The method of any one of claims 41-51, wherein the tumor is a brain tumor, a glioblastoma, a bone tumor, a pancreatic tumor, a skin tumor, a tumor of the head or neck, a melanoma, a lung tumor, a uterine tumor, an ovarian tumor, a colorectal tumor, an anal tumor, a liver tumor, a hepatocellular carcinoma, a stomach tumor, a testicular tumor, an endometrial tumor, a cervical tumor, a vaginal tumor, a Hodgkin's lymphoma, a non-Hodgkin's lymphoma, an esophageal tumor, an intestinal tumor, a thyroid tumor, an adrenal tumor, a bladder tumor, a kidney tumor, a breast tumor, a multiple myeloma tumor, a sarcoma, or a squamous cell tumor.

53. The method of any one of claims 41-52, wherein the individual is a mammal.

54. The method of claim 53, wherein the mammal is a human.

55. The method of claim 52, wherein the mammal is a pet, a laboratory research animal, or a farm animal.

56. The method of claim 55, wherein the pet, research animal or farm animal is a dog, a cat, a horse, a monkey, a rabbit, a rat, a mouse, a guinea pig, a hamster, a pig, or a cow.

57. The method of any one of claims 41-56, wherein the H-NOX protein is administered by intravenous, intra-arterial, intratumoral, intravesicular, inhalation, intraperitoneal, intrapulmonary, intramuscular, subcutaneous, intra-tracheal, transmucosal, intraocular, intrathecal, or transdermal administration.

58. The method of any one of claims 41-57, wherein administration of the H-NOX protein is repeated.

59. The method of claim 58, wherein administration of the H-NOX protein is repeated daily or twice a day from about 4 weeks to about 8 weeks.

60. The method of claim 58 or 59 wherein the H-NOX protein is administered every four, every 8, every 12 or every 24 hours for a period of about one to about 10 days.

61. The method of any one of claims 41-60, wherein the H-NOX protein is administered as a bolus.

62. The method of any one of claims 41-60, wherein the H-NOX protein is administered by infusion.

63. The method of claim 62, wherein the H-NOX protein is infused in the individual for about 15 minutes, about 30 minutes, about 1 hour, about 1 hour, about 2 hours, about 3 hours, about 6 hours, about 12 hours or about 24 hours.

~~64. The method of any one of claims 41-63, wherein the H-NOX protein is administered in combination with radiation therapy.~~

~~65. The method of claim 64, wherein the radiation therapy is~~be administered to the individual 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 20, 22 or 24 hours after the H-NOX protein is administered.

~~66~~9. The ~~method of claim 64 or 65, wherein the radiation is X-radiation.~~

~~67. The method of claim 66, wherein the X-radiation is administered at about 0.5 gray to about 75 gray.~~

~~68. The method~~use of any one of claims ~~64-67~~1-6, wherein the H-NOX protein is to be administered in combination with chemotherapy or immunotherapy.

10. The use of claim 9, wherein the administration of the H-NOX protein and/or the administration of the ~~radiation is repeated.~~

~~69. The method of claim 68, wherein the administration is repeated any number of times between about two times to about forty times or more.~~

~~70. The method of claim 68 or 69, wherein the administration is repeated after one week, two weeks, three weeks, or four weeks or more.~~

~~71. The method of any one of claims 41-63, wherein the H-NOX protein is administered in combination with chemotherapy or immunotherapy.~~

~~72. The method of claim 71, wherein the chemotherapy comprises a cytotoxin.~~

~~73. The method of claim 72, wherein the administration of the H-NOX protein and/or the administration of the chemotherapy is repeated.~~

~~74. The method of claim 71, wherein the~~ immunotherapy is ~~one or more of an anticancer vaccine, an adoptive immune cell therapy or an agent that targets an immune checkpoint regulator~~to be repeated.

11. ~~75. The method of claim 71 or 74, wherein the immunotherapy targets one or more of CTLA-4, PD1, PD-L1, or an immune checkpoint regulator.~~

~~76. The method of claim 71, 74 or 75, wherein the adoptive immude therapy is a chimeric antigen receptor expressing T cell or an engineered TCR T cell.~~

~~77. The method of any one of claims 71, or 74-76, wherein the administration of the H-NOX protein and/or the administration of the immunotherapy is repeated.~~

~~78. The method of any one of claims 41-77, wherein the H-NOX protein is a *T. tengcongensis* H-NOX, a *L. pneumophilia* 2 H-NOX, a *H. sapiens* β1, a *R. norvegicus* β1, a *C. lupus* H-NOX, a *D. melangaster* β1, a *D. melangaster* CG14885-PA, a *C. elegans* GCY-35, a *N.*~~

~~*punctiforme* H-NOX, *C. crescentus* H-NOX, a *S. oneidensis* H-NOX, or *C. acetobutylicum* H-NOX.~~

~~79. The method of any one of claims 41-77, wherein the H-NOX protein comprises a H-NOX domain corresponding to the H-NOX domain of *T. tengcongensis* set forth in SEQ ID NO:2.~~

~~80. The method of any one of claims 41-78, wherein the H-NOX comprises one or more distal pocket mutations.~~

~~81. The method of claim 80, wherein the distal pocket mutation is an amino acid substitution at a site corresponding to L144 of *T. tengcongensis* H-NOX.~~

~~82. The method of claim 80 or 81, wherein the H-NOX is a *T. tengcongensis* H-NOX comprising an amino acid substitution at position 144.~~

~~83. The method of claim 82, wherein the amino acid substitution at position 144 is an L144F substitution.~~

~~84. The method of any one of claims 41-83, wherein the H-NOX protein is a polymeric H-NOX protein.~~

~~85. The method of claim 84, wherein the polymeric H-NOX protein comprises monomers, wherein the monomers comprise an H-NOX domain and a polymerization domain.~~

~~86. The method of claim 85~~The use of claim 1-10, wherein the H-NOX domain is covalently linked to the polymerization domain.

~~87~~12. The ~~method of any one of claims 84-86, wherein the polymeric H-NOX protein is a trimeric H-NOX protein.~~

~~88. The method~~use of claim ~~87~~1, wherein the trimeric H-NOX protein comprises one or more trimerization domains.

~~89. The method of claim 88, wherein the trimeric H-NOX protein comprises three monomers, wherein the monomers comprise an H-NOX domain and a trimerization domain, wherein the trimerization domain is a bacteriophage T4 trimerization domain.~~

~~90. The method of claim 88 or 89~~13. The use of claim 12, wherein the trimerization domain is a foldon domain.

~~91~~14. The ~~method~~use of claim ~~90~~13, wherein the foldon domain comprises the amino acid sequence of SEQ ID NO:4.

~~92~~15. The ~~method~~use of any one of claims ~~41-91, wherein the H-NOX protein is fused to an Fc domain of an immunoglobulin.~~

~~93. The method of any one of claims 41-92, wherein the H-NOX protein is covalently bound to polyethylene glycol.~~

~~94. The method of any one of claims 41-93~~1-14, wherein the O_2 dissociation constant of the H-NOX protein is within 2 orders of magnitude of that of hemoglobin, and wherein ~~the NO reactivity of the H-NOX protein is at least 10-fold lower than that of hemoglobin.~~;

~~95. The method of any one of claims 41-94, wherein~~a) the NO reactivity of the H-NOX protein is at least 10-fold lower than that of hemoglobin.

b) the O_2 dissociation constant of the polymeric H-NOX protein is between about 1 nM and about 1000 nM at 20 °C~~.~~;

~~96. The method of any one of claims 41-95, wherein~~c) the O_2 dissociation constant of the H-NOX protein is between about 1 μM and about 10 μM at 20 °C~~.~~;

~~97. The method of any one of claims 41-96, wherein~~d) the O_2 dissociation constant of the H-NOX protein is between about 10 μM and about 50 μM at 20 °C~~.~~;

~~98. The method of any one of claims 41-97, wherein~~c) the NO reactivity of the H-NOX protein is less than about 700 s^{-1} at 20 °C~~.~~;

~~99. The method of any one of claims 41-98, wherein~~f) the NO reactivity of the H-NOX protein is at least 100-fold lower or at least 1000-fold lower than that of hemoglobin~~.~~;

~~100. The method of claim 99, wherein the NO reactivity of the H-NOX protein is at least 1,000-fold lower than that of hemoglobin.~~

~~101. The method of any one of claims 41-100, wherein~~g) the k_{off} for oxygen of the H-NOX protein is less than or equal to about 0.65 s^{-1}at 20 °C~~.~~

~~102. The method of any one of claims 41-101, wherein the k_{off} for oxygen of the H-NOX protein is~~ , or between about 0.21 $s^{-1}$and about 0.65 s^{-1} at 20 °C~~.~~

~~103. The method of any one of claims 41-102, wherein the k_{off} for oxygen of the H-NOX protein is~~, or between about 1.35 $s^{-1}$and about 2.9 s^{-1} at 20 °C. or;

~~104. The method of any one of claims 41-103, wherein~~h) the rate of heme autoxidation of the H-NOX protein is less than about 1 h^{-1}at 37 °C.

~~105~~16. The ~~method~~use of any one of claims ~~41-104~~1-15, wherein the H-NOX protein is in a pharmaceutical composition.

~~106~~17. The ~~method~~use of claim ~~105~~16, wherein the pharmaceutical composition further comprises a pharmaceutically acceptable carrier.

~~107. Use of an O_2 carrier protein for treating cancer in an individual.~~

~~108. The use of claim 107, wherein the cancer is brain cancer, glioblastoma, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, melanoma, lung cancer, uterine cancer, ovarian cancer, colorectal cancer, anal cancer, liver cancer, hepatocellular carcinoma, stomach cancer, testicular cancer, endometrial cancer, cervical cancer, Hodgkin's Disease, non-~~

Hodgkin's lymphoma, esophageal cancer, intestinal cancer, thyroid cancer, adrenal cancer, bladder cancer, kidney cancer, breast cancer, multiple myeloma, sarcoma, or squamous cell cancer.

109. Use of an O_2 carrier protein for modulating tumor immunity in an individual.

110. The use of claim 109, wherein the modulating tumor immunity comprises enhancing an immune response to the tumor.

111. Use of an O_2 carrier polypeptide for increasing lymphocyte infiltration to a tumor in an individual.

112. The use of claim 111, wherein the increase in lymphocyte infiltration to the tumor comprises an increase in infiltration of one or more of CD4 cells, CD8 cells, or NK cells.

113. The use of claim 111 or 112, wherein the increase in lymphocyte infiltration to the tumor is accompanied by inhibition of one or more of Treg cells, tumor associated macrophages or myeloid derived suppressor cells in the tumor.

114. The use of any one of claims 111-113, wherein the increase in lymphocyte infiltration to the tumor is accompanied by an increase in MHC1 expression on the tumor cells.

115. Use of an O_2 carrier polypeptide for decreasing expression of HIF-1α and/or HIF-2α in a tumor in an individual.

116. Use of an O_2 carrier polypeptide for decreasing expression of PD-L1 in a tumor in an individual.

117. Use of an O_2 carrier polypeptide for decreasing expression of A2AR in a tumor in an individual.

118. The use of any one of claims 109-117, wherein the tumor is a brain tumor, a glioblastoma, a bone tumor, a pancreatic tumor, a skin tumor, a tumor of the head or neck, a melanoma, a lung tumor, a uterine tumor, an ovarian tumor, a colorectal tumor, an anal tumor, a liver tumor, a hepatocellular carcinoma, a stomach tumor, a testicular tumor, an endometrial tumor, a cervical tumor, a vaginal tumor, a Hodgkin's lymphoma, a non-Hodgkin's lymphoma, an esophageal tumor, an intestinal tumor, a thyroid tumor, an adrenal tumor, a bladder tumor, a kidney tumor, a breast tumor, a multiple myeloma tumor, a sarcoma, or a squamous cell tumor.

119. The use of any one of claims 107-118, wherein the individual is a mammal.

120. The use of claim 119, wherein the mammal is a human.

121. The use of any one of claims 107-120, wherein the O_2 carrier polypeptide is an H-NOX protein.

122. The use of claim 121, wherein the H-NOX protein is a *T. tengcongensis* H-NOX, a *L. pneumophilia* 2 H-NOX, a *H. sapiens* β1, a *R. norvegicus* β1, a *C. lupus* H-NOX domain, a *D.*

~~*melangaster* β1, a *D. melangaster* CG14885-PA, a *C. elegans* GCY-35, a *N. punctiforme* H-NOX, *C. crescentus* H-NOX, a *S. oneidensis* H-NOX, or *C. acetobutylicum* H-NOX.~~

~~123. The use of any one of claims 121-122, wherein the H-NOX protein comprises a H-NOX domain corresponding to the H-NOX domain of *T. tengcongensis* set forth in SEQ ID NO:2.~~

~~124. The use of any one of claims 121-123, wherein the H-NOX comprises one or more distal pocket mutations.~~

~~125. The use of claim 124, wherein the distal pocket mutation is an amino acid substitution at a site corresponding to L144 of *T. tengcongensis* H-NOX.~~

~~126. The use of claim 124 or 125, wherein the H-NOX is a *T. tengcongensis* H-NOX comprising an amino acid substitution at position 144.~~

~~127. The use of claim 126, wherein the amino acid substitution at position 144 is an L144F substitution.~~

~~128. The use of any one of claims 121-127, wherein the H-NOX protein is a polymeric H-NOX protein.~~

~~129. The use of claim 128, wherein the polymeric H-NOX protein comprises monomers, wherein the monomers comprise an H-NOX domain and a polymerization domain.~~

~~130. The use of claim 129, wherein the H-NOX domain is covalently linked to the polymerization domain.~~

~~131. The use of any one of claims 128-130, wherein the polymeric H-NOX protein is a trimeric H-NOX protein.~~

~~132. The use of claim 131, wherein the trimeric H-NOX protein comprises one or more trimerization domains.~~

~~133. The use of claim 132, wherein the trimeric H-NOX protein comprises three monomers, wherein the monomers comprise an H-NOX domain and a trimerization domain, wherein the trimerization domain is a bacteriophage T4 trimerization domain.~~

~~134. The use of claim 132 or 133, wherein the trimerization domain is a foldon domain.~~

~~135. The use of claim 134, wherein the foldon domain comprises the amino acid sequence of SEQ ID NO:4.~~

~~136. The use of any one of claims 131-135, wherein the H-NOX protein is fused to an Fc domain of an immunoglobulin.~~

~~137. The use of any one of claims 121-136, wherein the H-NOX protein is covalently bound to polyethylene glycol.~~

~~138~~18. A kit for modulating tumor immunity in an individual comprising an O_2 carrier protein for the use ~~in the method~~ of any one of claims 1-~~106~~17.

~~139~~19. The kit of claim ~~138~~18, wherein the kit further comprises one or more of a vial, a vessel, an ampule, a bottle, a jars, or flexible packaging.

~~140. The kit of claim 138 or 139, wherein the kit further comprises one or more buffers.~~

~~141. The kit of any one of claims 138-140, wherein the kit further comprises instructions for use.~~