Claims

1. A kynureninase homodimer covalently bound to one or more polyethylene glycol (PEG) molecules, wherein the ratio of PEG molecules to homodimer is from about 10:1 to about 40:1

2. The homodimer of claim 1, wherein the ratio of PEG molecules to homodimer is from about 20:1 to about 30:1, optionally wherein the ratio of PEG molecules to homodimer is about 20:1, 21:2, 22:1, 23:1, 24:1, or 25:1.

3. The homodimer of claim 1, wherein the ratio of PEG molecules to homodimer is about 25:1.

4. The homodimer of any one of claims 1-3, wherein the PEG molecule has a molecular weight from about 1 kDa to about 10 kDa.

~~5. The homodimer of claim 4, wherein the PEG molecule has molecular weight from about 3 kDa to about 7 kDa.~~

~~6. The homodimer of claim 5, wherein the PEG molecule has a molecular weight of about 5 kDa.~~

~~7. The homodimer of any one of claims 1-6, wherein the PEG molecule is linear.~~

~~8. The homodimer of any one of claims 1-6, wherein the PEG molecule is branched~~

~~9.~~5. The homodimer of any one of claims 1-~~8~~4, wherein the homodimer is covalently bound to the PEG molecule by way of one or more lysine or cysteine residues.

~~10. The homodimer of claim 9, wherein the homodimer is covalently bound to the PEG molecule by way of one or more lysine residues.~~

~~11.~~6. The homodimer of any one of claims 1-~~10~~5, wherein the homodimer comprises two polypeptide monomers each having an amino acid sequence that is at least 85% identical to SEQ ID NO: 1, and wherein each polypeptide monomer has one or more amino acid substitutions, relative to SEQ ID NO: 1, selected from the group consisting of L8P, K38E, Y47L, I48F, K50Q, I51M, S60N, K64N, D65G, E66K, N67D, N67P, D67S, A68F, A68T, A68V, F71L, F71M, L72N, K84E, E88N, E89K, E89S, E90Q, D92E, K93N, K93T, A95H, A95Q, K96N, I97H, I97L, I97V, A98G, A99G, A99I, A99R, A99S, A99T, A99V, Y100N, Y100S, Y100T, G101A, H102W, E103F, E103H, E103N, E103Q, E103R, E103V, E103W, V104D, V104E, V104F, V104H, V104K, V104L, V104R, G105A, G105H, G105S, G105T, E106D, K106D, K106E, K106H, K106N, R107P, R107S, P108R, I110A, I110F, I110L, I110M, I110T, T111D, T111H, T111N, T111R, G112A, G112C, G112D, G112K, G112L, G112M, G112Q, G112R, G112S, G112T, G112Y, N127K, I131V, A132V, L133V, A136T, L137T, T138S, N140D, H142Q, Q14R, Y156H, K163T, D168E, H169R, Q175L, I183F, I183L, I183P, I183S, E184A, E184D, E184R, E184T, E184V, E185T, M187L, M189I, K191A, K191G, K191H, K191M, K191N, K191R, K191S, K191T, K191W, E197A, E197D, E197F, E197K, E197M, E197Q, E197S, E197T, E197V, I201C, I201E, I201F, 1201H, I201L, I201S, I201T, I201V, H203K, L219M, L219W, F220L, V223I, F225Y, H230F, H230L, H230Y, N232S, Y246F, F249W, D250E, S274A, S274C, S274G, S274N, S274T, L278M, A280G, A280S, A280T, G281S,

A282M, A282P, G284N, I285L, V303L, V303S, F306W, F306Y, S311N, K315E, D317E, D317K, I331C, I331L, I331N, I331S, I331T, I331V, N333T, P334N, P335T, L337T, L338A, L338Q, S341I, K373E, K373N, N375A, N375H, Y376C, Y376F, Y376L, K378G, K378P, K378Q, K378R, K380G, K380S, A382G, A382R, A382T, T383S, K384G, K384N, P386K, P386S, V387L, N389E, I405L, F407Y, S408D, S408N, N411R, D413S, D413V, Q416T, E419A, E419L, K420E, R421N, V424I, K427M, N429E, G432A, and A436T.

~~12. The homodimer of claim 11, wherein each polypeptide monomer has an amino acid sequence that is at least 90% identical to SEQ ID NO: 1.~~

~~13. The homodimer of claim 12, wherein each polypeptide monomer has an amino acid sequence that is at least 95% identical to SEQ ID NO: 1.~~

~~14.~~7. The homodimer of ~~any one of claims 11-13~~claim 6, wherein each polypeptide monomer comprises a substitution at A282, F306, ~~or~~ F249, A99, G112, E103, V104, and/or S408 relative to SEQ ID NO: 1.

~~15. The homodimer of any one of claims 11-14, wherein each polypeptide monomer comprises a substitution at A99 relative to SEQ ID NO: 1.~~

~~16. The homodimer of any one of claims 11-15, wherein each polypeptide monomer comprises a substitution at G112 relative to SEQ ID NO: 1.~~

~~17. The homodimer of any one of claims 11-16, wherein each polypeptide monomer comprises a substitution at E103 relative to SEQ ID NO: 1.~~

~~18. The homodimer of any one of claims 11-17, wherein each polypeptide monomer comprises a substitution at V104 relative to SEQ ID NO: 1.~~

~~19. The homodimer of any one of claims 11-18, wherein each polypeptide monomer comprises a substitution at S408 relative to SEQ ID NO: 1.~~

~~20.~~8. The homodimer of ~~any one of claims 11-19~~claim 6 or 7, wherein each polypeptide monomer comprises (i) the F306W substitution, (ii) the L72N substitution, (iii) the H102W and N333T substitutions, (iv) the I183P substitution, (v) the R107P substitution, and/or (vi) the A436T substitution.

~~21. The homodimer of any one of claims 11-20, wherein each polypeptide monomer comprises the L72N substitution.~~

~~22. The homodimer of any one of claims 11-21, wherein each polypeptide monomer comprises the H102W and N333T substitutions.~~

~~23. The homodimer of any one of claims 11-22, wherein each polypeptide monomer comprises the I183P substitution.~~

~~24. The homodimer of any one of claims 11-23, wherein each polypeptide monomer comprises the R107P substitution.~~

~~25. The homodimer of any one of claims 11-24, wherein each polypeptide monomer comprises the A436T substitution.~~

~~26. The homodimer of any one of claims 11-25, wherein each polypeptide monomer comprises at least one substitution selected from L72N, H102W, A282P, F306W, I331S and N333T relative to SEQ ID NO: 1.~~

~~27. The homodimer of claim 26, wherein each polypeptide monomer comprises at least two, three, four or five substitutions selected from L72N, H102W, A282P, F306W, I331S and N333T.~~

~~28.~~9. The homodimer of ~~claim 27~~any one of claims 6-8, wherein each polypeptide monomer comprises the substitutions L72N, H102W, A282P, F306W, I331S and N333T relative to SEQ ID NO: 1.

~~29.~~10. The homodimer of any one of claims 1-~~28~~9, wherein the homodimer comprises two polypeptide monomers each having an amino acid sequence that is at least 85% identical to SEQ ID NO: 3.

~~30. The homodimer of claim 29, wherein each polypeptide monomer has an amino acid sequence that is at least 90% identical to SEQ ID NO: 3.~~

~~31. The homodimer of claim 30, wherein each polypeptide monomer has an amino acid sequence that is at least 95% identical to SEQ ID NO: 3.~~

~~32.~~11. The homodimer of claim ~~31~~10, wherein each polypeptide monomer has the amino acid sequence of SEQ ID NO: 3.

~~33.~~12. The homodimer of any one of claims 1-~~28~~9, wherein the homodimer comprises two polypeptide monomers each having an amino acid sequence that is at least 85% identical to SEQ ID NO: 2.

~~34. The homodimer of claim 33, wherein each polypeptide monomer has an amino acid sequence that is at least 90% identical to SEQ ID NO: 2.~~

~~35. The homodimer of claim 34, wherein each polypeptide monomer has an amino acid sequence that is at least 95% identical to SEQ ID NO: 2.~~

~~36.~~13. The homodimer of claim ~~35~~12, wherein each polypeptide monomer has the amino acid sequence of SEQ ID NO: 2.

~~37.~~14. The homodimer of any of claims 1-~~36~~13, wherein the homodimer has a catalytic activity ($kcat/K_M$) of at least $8000 M^{-1} s^{-1}$.

~~38. The homodimer of claim 37, wherein the homodimer has a catalytic activity ($kcat/K_M$) of between about $8000 M^{-1}s^{-1}$ and $40000 M^{-1}s^{-1}$; $10000 M^{-1}s^{-1}$ and $40000 M^{-1}s^{-1}$; $20000 M^{-1}s^{-1}$ and $40000 M^{-1}s^{-1}$; or $25000 M^{-1}s^{-1}$ and $35000 M^{-1}s^{-1}$.~~

~~39.~~15. The homodimer of any one of claims 1-~~38~~14, wherein the ratio of PEG molecules to homodimer is about 25:1, the PEG molecule has a molecular weight of about 5 kDa and is bound to the homodimer by way of an N-hydroxysuccinimide ester carbonate linking group.

~~40.~~16. A method of producing a PEGylated kynureninase homodimer, wherein the homodimer comprises two polypeptide monomers, the method comprising contacting the homodimer with a

PEGylation agent, and wherein the molar input ratio of PEGylation agent to homodimer is from about 10:1 to about 50:1.

~~41.~~17. The method of claim ~~40~~16, wherein the molar input ratio of PEGylation agent to homodimer is from about 20:1 to about 40:1, optionally wherein the molar input ratio of PEGylation agent to homodimer is from about 20:1 to about 30:1, optionally wherein the molar input ratio of PEGylation agent to homodimer is about 20:1.

~~42.~~18. The method of claim ~~40~~16, wherein the molar input ratio of PEGylation agent to homodimer is from about 25:1 to about 35:1.

~~43.~~19. The method of claim ~~40~~18, wherein the molar input ratio of PEGylation agent to homodimer is about 30:1.

~~44.~~20. The method of any one of claims ~~40-43~~16-19, wherein the PEG molecule has a molecular weight from about 1 kDa to about 10 kDa.

~~45. The method of claim 44, wherein the PEG molecule has molecular weight from about 3 kDa to about 7 kDa.~~

~~46. The method of claim 45, wherein the PEG molecule has a molecular weight of about 5 kDa.~~

~~47. The method of any one of claims 40-46, wherein the PEG molecule is linear.~~

~~48. The method of any one of claims 40-46, wherein the PEG molecule is branched~~

~~49.~~21. The method of any one of claims ~~40-48~~16-20, wherein the homodimer is covalently bound to the PEG molecule by way of one or more lysine or cysteine residues.

~~50. The method of claim 49, wherein the homodimer is covalently bound to the PEG molecule by way of one or more lysine residues.~~

~~51.~~22. The method of any one of claims ~~40-50~~16-21, wherein each polypeptide monomer has an amino acid sequence that is at least 85% identical to SEQ ID NO: 1, and wherein each polypeptide monomer has one or more amino acid substitutions, relative to SEQ ID NO: 1, selected from the group consisting of L8P, K38E, Y47L, I48F, K50Q, I51M, S60N, K64N, D65G, E66K, N67D, N67P, D67S, A68F, A68T, A68V, F71L, F71M, L72N, K84E, E88N, E89K, E89S, E90Q, D92E, K93N, K93T, A95H, A95Q, K96N, I97H, I97L, I97V, A98G, A99G, A99I, A99R, A99S, A99T, A99V, Y100N, Y100S, Y100T, G101A, H102W, E103F, E103H, E103N, E103Q, E103R, E103V, E103W, V104D, V104E, V104F, V104H, V104K, V104L, V104R, G105A, G105H, G105S, G105T, E106D, K106D, K106E, K106H, K106N, R107P, R107S, P108R, I110A, I110F, I110L, I110M, I110T, T111D, T111H, T111N, T111R, G112A, G112C, G112D, G112K, G112L, G112M, G112Q, G112R, G112S, G112T, G112Y, N127K, I131V, A132V, L133V, A136T, L137T, T138S, N140D, H142Q, Q14R, Y156H, K163T, D168E, H169R, Q175L, I183F, I183L, I183P, I183S, E184A, E184D, E184R, E184T, E184V, E185T, M187L, M189I, K191A, K191G, K191H, K191M, K191N, K191R, K191S, K191T, K191W, E197A, E197D, E197F, E197K, E197M, E197Q, E197S, E197T, E197V, I201C, I201E, I201F, 1201H, I201L, I201S, I201T, I201V, H203K, L219M, L219W, F220L, V223I, F225Y, H230F, H230L, H230Y, N232S, Y246F, F249W, D250E, S274A, S274C, S274G, S274N,

S274T, L278M, A280G, A280S, A280T, G281S, A282M, A282P, G284N, I285L, V303L, V303S, F306W, F306Y, S311N, K315E, D317E, D317K, I331C, I331L, I331N, I331S, I331T, I331V, N333T, P334N, P335T, L337T, L338A, L338Q, S341I, K373E, K373N, N375A, N375H, Y376C, Y376F, Y376L, K378G, K378P, K378Q, K378R, K380G, K380S, A382G, A382R, A382T, T383S, K384G, K384N, P386K, P386S, V387L, N389E, I405L, F407Y, S408D, S408N, N411R, D413S, D413V, Q416T, E419A, E419L, K420E, R421N, V424I, K427M, N429E, G432A, and A436T.

~~52. The method of claim 51, wherein each polypeptide monomer has an amino acid sequence that is at least 90% identical to SEQ ID NO: 1.~~

~~53. The method of claim 52, wherein each polypeptide monomer has an amino acid sequence that is at least 95% identical to SEQ ID NO: 1.~~

~~54.~~23. The method of any one of claims ~~40-53~~16-22, wherein each polypeptide monomer comprises a substitution at A282, F306, ~~or~~ F249, A99, G112, E103, V104, and/or S408 relative to SEQ ID NO: 1.

~~55. The method of any one of claims 40-54, wherein each polypeptide monomer comprises a substitution at A99 relative to SEQ ID NO: 1.~~

~~56. The method of any one of claims 40-55, wherein each polypeptide monomer comprises a substitution at G112 relative to SEQ ID NO: 1.~~

~~57. The method of any one of claims 40-56, wherein each polypeptide monomer comprises a substitution at E103 relative to SEQ ID NO: 1.~~

~~58. The method of any one of claims 40-57, wherein each polypeptide monomer comprises a substitution at V104 relative to SEQ ID NO: 1.~~

~~59. The method of any one of claims 40-58, wherein each polypeptide monomer comprises a substitution at S408 relative to SEQ ID NO: 1.~~

~~60.~~24. The method of ~~any one of claims 51-59~~claim 22 or 23, wherein each polypeptide monomer comprises the (i) F306W substitution, (ii) the L72N substitution, (iii) the H102W and N333T substitutions, (iv) the I183P substitution, (v) the R107P substitution, and/or (vi) the A436T substitution.

~~61. The method of any one of claims 51-60, wherein each polypeptide monomer comprises the L72N substitution.~~

~~62. The method of any one of claims 51-61, wherein each polypeptide monomer comprises the H102W and N333T substitutions.~~

~~63. The method of any one of claims 51-62, wherein each polypeptide monomer comprises the I183P substitution.~~

~~64. The method of any one of claims 51-63, wherein each polypeptide monomer comprises the R107P substitution.~~

~~65. The method of any one of claims 51-64, wherein each polypeptide monomer comprises the A436T substitution.~~

~~66. The method of any one of claims 40-65, wherein each polypeptide monomer comprises at least one substitution selected from L72N, H102W, A282P, F306W, I331S and N333T relative to SEQ ID NO: 1.~~

~~67. The method of claim 66, wherein each polypeptide monomer comprises at least two, three, four or five substitutions selected from L72N, H102W, A282P, F306W, I331S and N333T relative to SEQ ID NO: 1.~~

~~68.~~25. The method of ~~claim 67~~any one of claims 22-24, wherein each polypeptide monomer comprises the substitutions L72N, H102W, A282P, F306W, I331S and N333T relative to SEQ ID NO: 1.

~~69.~~26. The method of any one of claims ~~40-68~~16-25, wherein each polypeptide monomer has an amino acid sequence that is at least 85% identical to SEQ ID NO: 3.

~~70. The method of claim 69, wherein each polypeptide monomer has an amino acid sequence that is at least 90% identical to SEQ ID NO: 3.~~

~~71. The method of claim 70, wherein each polypeptide monomer has an amino acid sequence that is at least 95% identical to SEQ ID NO: 3.~~

~~72.~~27. The method of claim ~~71~~26, wherein each polypeptide monomer has the amino acid sequence of SEQ ID NO: 3.

~~73.~~28. The method of any one of claims ~~40-68~~16-25, wherein each polypeptide monomer has an amino acid sequence that is at least 85% identical to SEQ ID NO: 2.

~~74. The method of claim 73, wherein each polypeptide monomer has an amino acid sequence that is at least 90% identical to SEQ ID NO: 2.~~

~~75. The method of claim 74, wherein each polypeptide monomer has an amino acid sequence that is at least 95% identical to SEQ ID NO: 2.~~

~~76.~~29. The method of claim ~~75~~28, wherein each polypeptide monomer has the amino acid sequence of SEQ ID NO: 2.

~~77.~~30. The method of any of claims ~~40-76~~16-29, wherein the homodimer has a catalytic activity ($kcat/K_M$) of at least $8000 M^{-1} s^{-1}$.

~~78. The method of claim 77, wherein the homodimer has a catalytic activity ($kcat/K_M$) of between about $8000 M^{-1}s^{-1}$ and $40000 M^{-1}s^{-1}$; $10000 M^{-1}s^{-1}$ and $40000 M^{-1}s^{-1}$; $20000 M^{-1}s^{-1}$ and $40000 M^{-1}s^{-1}$; or $25000 M^{-1}s^{-1}$ and $35000 M^{-1}s^{-1}$.~~

~~79.~~31. The method of ~~claim 78~~any one of claims 16-30, wherein the ratio of PEG molecules to homodimer is about 25:1, the PEG molecule has a molecular weight of about 5 kDa and is bound to the homodimer by way of an N-hydroxysuccinimide ester carbonate linking group.

80.32. The method of any of claims 40-7916-31, wherein the homodimer is contacted with the PEGylation agent in an aqueous buffer.

81.33. The method of claim 8032, wherein the aqueous buffer comprises disodium phosphate and/or sodium acetate.

82. The method of claim 80, wherein the aqueous buffer comprises sodium acetate.

83.34. The method of any one of claims 80-82claim 32 or 33, wherein the aqueous buffer ranges in pH from about 7.5 to about 9.5.

84.35. The method of any one claims 80-8332-34, wherein the aqueous buffer ranges in ionic strength from about 250 mM to about 350 mM.

85.36. The method of any one of claims 40-8416-35, wherein size exclusion chromatography is used to isolate the homodimer.

86.37. A kynureninase homodimer produced by the method of any one of claims 40-8516-36.

87.38. A pharmaceutical formulation comprising the kynureninase homodimer of any one of claims 1-3915 and 8637 in a pharmaceutically acceptable carrier.

88.39. A method of treating a subject having a tumor comprising administering to a subject an effective amount of the The formulation of claim 8738 or the enzymekynureninase homodimer of any one of claims 1-3915 and 8637 for use in treating a subject having a tumor.

89.40. The method of formulation or kynureninase homodimer for use in accordance with claim 8839, wherein the subject has been identified as having an IDO1, IDO2, or TDO expressing tumor.

90.41. The method offormulation or kynureninase homodimer for use in accordance with claim 88 or 8939 or 40, wherein the tumor is a solid tumor or a hematological tumor.

91. The method of claim 88 or 89, wherein the tumor is a hematological tumor.

92.42. The method offormulation or kynureninase homodimer for use in accordance with any of one of claims 88-9139-41, wherein the subject is a human patient.

93.43. The method offormulation or kynureninase homodimer for use in accordance with any one of claims 88-9239-42, wherein the formulation is to be administered intratumorally, intravenously, intradermally, intraarterially, intraperitoneally, intralesionally, intracranially, intraarticularly, intraprostaticaly, intrapleurally, intratracheally, intraocularly, intranasally, intravitreally, intravaginally, intrarectally, intramuscularly, subcutaneously, subconjunctival, intravesicularlly, mucosally, intrapericardially, intraumbilically, orally, by inhalation, by injection, by infusion, by continuous infusion, by localized perfusion bathing target cells directly, via a catheter, or via a lavage.

94.44. The method offormulation or kynureninase homodimer for use in accordance with any one of claims 88-9339-43, further comprising administeringwherein the formulation or kynureninase homodimer is to be administered to the subject with a second anti-cancer therapy.

95.45. The method offormulation or kynureninase homodimer for use in accordance with claim 9444, wherein the second anticancer therapy is radiation therapy, surgical therapy, an immunotherapy or a second anticancer compound.

96.46. The method offormulation or kynureninase homodimer for use in accordance with claim 9545, wherein the second anticancer compound is an immune checkpoint inhibitor.

97.47. The method offormulation or kynureninase homodimer for use in accordance with claim 9545, wherein the second anticancer compound comprises an anti- PDI, anti-CTLA-4, or anti-PD-LI antibody.

98. The method of claim 95, wherein the second anticancer compound is an antibody.

99. The method of claim 95, wherein the second anticancer compound is an antibody- drug conjugate.

100. The method of claim 95, wherein the immunotherapy comprises administering immune effector cells or an immunogenic composition.

101. The method of claim 100, wherein the immunogenic composition comprises cancer cell antigens.

102. The method of claim 100, wherein the immune effector cells comprise NK cells or T cells.

103. The method of claim 100, wherein the immune effector cells comprise CAR T cells.

104.48. AThe formulation of claim 38 or the kynureninase homodimer of any one of claims 1-15 and 37 for use in a method of providing a T-cell response in a human subject having a tumor, the method comprising administering to the subject a kynureninase homodimer in accordance with the method of any one of claims 88-103.