

WHAT IS CLAIMED IS:

1. An IL10 agonist comprising, from amino to carboxy terminus:
 - (a) an IgG Fc domain;
 - (b) a linker moiety; and
 - (c) an IL10 moiety.
2. The IL10 agonist of claim 1, wherein the IgG Fc domain comprises CH2 and CH3 domains from an IgG1, IgG2, IgG3, IgG4 or a combination thereof.
3. The IL10 agonist of claim 1 or claim 2, wherein the CH2 and CH3 domains are from an IgG1.
4. The IL10 agonist of claim 3, wherein the Fc domain comprises or consists of an amino acid sequence having at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO:32 or to SEQ ID NO: 33.
5. IL10 agonist of claim 1 or claim 2, wherein the CH2 and CH3 domains are from an IgG4.
6. The IL10 agonist of claim 5, wherein the Fc domain comprises or consists of an amino acid sequence having at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 31 or to amino acids 18 to 228 of SEQ ID NO: 31.
7. The IL10 agonist of any one of claims 1 to 6, wherein the Fc domain has reduced effector function.
8. The IL10 agonist of any one of claims 1 to 7, comprising a hinge domain at the N-terminus of the Fc domain.
9. The IL10 agonist of claim 8, wherein the hinge domain is derived from the same IgG as the CH2 and/or CH3 domains.

10. The IL10 agonist of claim 8, wherein the hinge domain is derived from a different IgG than the CH2 and/or CH3 domains.

11. The IL10 agonist of any one of claims 8 to 10, wherein the hinge domain comprises a chimeric hinge sequence, optionally wherein the chimeric hinge sequence comprises or consists of an amino acid sequence of SEQ ID NO:12 or SEQ ID NO:13.

12. The IL10 agonist of any one of claims 1 to 11, wherein the linker moiety comprises or consists of a monomer or multimer of G_nS or SG_n , wherein n is an integer from 1 to 7.

13. The IL10 agonist of any one of claims 1 to 12, wherein the linker comprises a monomer or a multimer of amino acid sequence G_4S (SEQ NO: 51).

14. The IL10 agonist of any one of claims 1 to 13, wherein the linker comprises 1, 2, or 3 repeats of amino sequence G_4S (SEQ NO: 51).

15. The IL10 agonist of any one of claims 1 to 14, wherein the linker connects the Fc domain and the IL10 moiety.

16. The IL10 agonist of any one of claims 1 to 15, wherein the IL10 moiety comprises or consists of an amino acid sequence having at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to mature human IL10 (SEQ NO: 1).

17. The IL10 agonist of any one of claims 1 to 15, wherein the IL10 moiety comprises or consists of an amino acid sequence having at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to mature murine IL10 (SEQ NO: 30).

18. The IL10 agonist of any one of claim 1 to 17, which is a homodimer.

19. The IL10 agonist of any one of claims 1 to 17, which is a heterodimer.

20. The IL10 agonist of claim 18 or claim 19, which comprises two Fc domains, optionally wherein the IL10 agonist is dimerized through the Fc domains.

21. The IL10 agonist of any one of claims 1 to 20, wherein the IL10 moiety lacks an amino acid spacer positioned between helices D and E of an IL10 moiety interdomain region.
22. The IL10 agonist of any one of claims 1 to 21, which is not conjugated to polyethylene glycol.
23. The IL10 agonist of any one of claims 1 to 22, which is not conjugated to albumin.
24. The IL10 agonist of any one of claims 1 to 23, which is not conjugated to a human serum albumin binder.
25. The IL10 agonist of any one of claims 1 to 24, which is not conjugated to an XTEN, a PAS, a hydrophilic polymer, a polysialic acid, a fatty acid, or a hydroxyethyl starch.
26. The IL10 agonist of any one of claims 1 to 25, which is not conjugated to any stabilizing moiety.
27. The IL10 agonist of any one of claims 1 to 26, wherein the IL10 moiety does not include an N-linked glycan, an O-linked glycan, or neither an N-linked glycan nor an O-linked glycan.
28. The IL10 agonist of any one of claims 1 to 27, wherein the IL10 agonist lacks a targeting moiety that:
- (a) binds to a tumor associated antigen;
 - (b) binds to a tumor microenvironment antigen;
 - (c) binds to a cell surface molecule of tumor reactive lymphocytes; or
 - (d) binds to a checkpoint inhibitor.
29. The IL10 agonist of any one of claims 1 to 28, wherein the IL10 agonist lacks a targeting moiety that binds PD-1.
30. The IL10 agonist of any one of claims 1 to 29 which does not contain any cytokine other than IL10.

31. An IL10 agonist which comprises or consists of an amino acid sequence having at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the sequence of IL10M1 (SEQ ID NO: 34).

32. The IL10 agonist of claim 31, which comprises or consists of the amino acid sequence of IL10M1.

33. An IL10 agonist which comprises or consists of an amino acid sequence having at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the sequence of IL10M4 (SEQ ID NO: 37).

34. The IL10 agonist of claim 33, which comprises or consists of the amino acid sequence of IL10M4.

35. An IL10 agonist which comprises or consists of an amino acid sequence having at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the sequence of IL10M5 (SEQ ID NO: 38).

36. The IL10 agonist of claim 35, which comprises or consists of the amino acid sequence of IL10M5.

37. An IL10 agonist which comprises or consists of an amino acid sequence having at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the sequence of IL10M11 (SEQ ID NO: 44).

38. The IL10 agonist of claim 37, which comprises or consists of the amino acid sequence of IL10M11.

39. The IL10 agonist of any one of claims 1 to 37, which is the product of recombinant expression in a mammalian cell.

40. A nucleic acid or plurality of nucleic acids encoding the IL10 agonist of any one of claims 1 to 39.

41. A host cell engineered to express the IL10 agonist of any one of claims 1 to 38 or the nucleic acid or plurality of nucleic acids of claim 40.

42. The host cell of claim 41, which is a mammalian cell.

43. A method of producing the IL10 agonist of any one of claims 1 to 38, comprising culturing the host cell of claim 41 or claim 42 and recovering the IL10 agonist expressed thereby.

44. The method of claim 42, further comprising:

- (a) contacting the recovered IL10 agonist with an affinity column configured to trap the IL10 agonist, optionally wherein the affinity column is a Protein A affinity column;
- (b) eluting the affinity column to produce an elute; and
- (c) performing size-exclusion chromatography on the elute.

45. An IL10 agonist produced by a method comprising (a) culturing the host cell of claim 41 or claim 42 and (b) recovering the IL10 agonist expressed thereby.

46. The IL10 agonist of claim 45, wherein the method further comprises:

- (a) contacting the recovered IL10 agonist with an affinity column configured to trap the IL10 agonist;
- (b) eluting the affinity column to produce an elute; and
- (c) performing size-exclusion chromatography on the elute.

47. A method of preparing a pharmaceutical composition, comprising formulating (a) an IL10 agonist prepared by the methods of claim 43 or claim 44, or (b) the IL10 agonist of claim 45 or claim 46, with an excipient.

48. A pharmaceutical composition comprising (a) the IL10 agonist of any one of claims 1 to 39, 45, and 46 and an excipient, (b) an IL10 agonist obtained by the method of claim 43 or claim 44, or (c) the IL10 agonist which is obtained by the method of claim 47.

49. The pharmaceutical composition of claim 48 which comprises at least 10 mg, at least 20 mg or at least 50 mg of the IL10 agonist.

50. The pharmaceutical composition of claim 48 or claim 49 in which less than 30%, less than 25%, less than 20%, less than 15%, less than 10%, or less than 5% of the IL10 agonist is present in an aggregate form, as determined by size exclusion ultra performance liquid chromatography (SE-UPLC).

51. The pharmaceutical composition of claim 48 or claim 49 which does not contain a detectable amount of aggregates of the IL10 agonist as determined by size exclusion ultra performance liquid chromatography (SE-UPLC).

52. The pharmaceutical composition of any one of claims 48 to 51 which does not contain (a) a detectable amount of C-terminal truncated variants of the IL10 agonist and/or (b) a detectable amount of N-terminal truncated variants of the IL10 agonist.

53. An IL10 agonist of any one of claims 1 to 39, 45, or 46 or a pharmaceutical composition of any one of claims 48 to 52 for use in a method of treating cancer, the method comprising administering to a subject in need thereof the IL10 agonist of any one of claims 1 to 39, 45, or 46 or a pharmaceutical composition of any one of claims 48 to 52.

54. The IL10 agonist or pharmaceutical composition of claim 53, wherein the cancer is a solid tumor.

55. The IL10 agonist or pharmaceutical composition of claim 54, wherein the solid tumor is colon cancer, melanoma, squamous cell carcinoma, lymphoma, a tumor of the pancreas, or a tumor of the lung.

56. The IL10 agonist or pharmaceutical composition of claim 54 or claim 55, wherein the solid tumor is resistant to treatment with an anti-PD1 antibody.

57. The IL10 agonist or pharmaceutical composition of any one of claims 54 to 56, wherein the solid tumor is resistant to treatment with an anti-PD1 antibody monotherapy.

58. The IL10 agonist or pharmaceutical composition of any one of claims 53 to 57, which further comprises administering an anti-PD1 antibody to the subject.

59. The IL10 agonist or pharmaceutical composition of any one of claims 53 to 58, wherein the anti-PD1 antibody is MDX-1106 (nivolumab), MK-3475 (pembrolizumab), MEDI-0680 (AMP-514), PDR001, REGN2810, or BGB-108.

60. The IL10 agonist or pharmaceutical composition of any one of claims 54 to 59, which results in an increase in CD8 T cell density in the solid tumor, optionally wherein the increase is at least a 2-fold increase, at least a 3-fold increase, or at least a 4-fold increase..

61. The IL10 agonist or pharmaceutical composition of claims 54 to 60, which results in an increase in CD45+ immune cell infiltration in the solid tumor, optionally wherein the increase is at least a 10% increase.

62. The IL10 agonist or pharmaceutical composition of claim 61, wherein CD45+ immune cells comprise CD4 T cells, myeloid cells, or both CD4 T cells and myeloid cells.

63. The IL10 agonist or pharmaceutical composition of claims 54 to 62, wherein the method upregulates serum IL12 relative to a suitable control not treated with the IL10 agonist or pharmaceutical composition or relative to the subject's own serum IL12 levels prior to treatment with the IL10 agonist or pharmaceutical composition, optionally wherein the method upregulates serum IL12 at least 2-fold, at least 5-fold, at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, or at least 30-fold relative to the suitable control or relative to the subject's own serum IL12 levels prior to treatment with the IL10 agonist or pharmaceutical composition.

64. The IL10 agonist or pharmaceutical composition of any one of claims 54 to 63, wherein the method upregulates serum IL4 relative to a suitable control not treated with the IL10 agonist or pharmaceutical composition or relative to the subject's own serum IL4 levels prior to treatment with the IL10 agonist or pharmaceutical composition, optionally wherein the method upregulates serum IL4 at least 2-fold, at least 5-fold, at least 10-fold, at least 15-fold, or at least 20-fold relative to the suitable control or relative to the subject's own serum IL4 levels prior to treatment with the IL10 agonist or pharmaceutical composition..