

Claims

We claim:

1. A fusion polypeptide comprising:
 - (a) an immunomodulatory polypeptide that comprises a IL12B moiety or functional fragment thereof and a IL12A moiety or a functional fragment thereof; and
 - (b) a metal-hydroxide binding polypeptide whose amino acid sequence includes a plurality of phosphorylation sites, so that it can adopt phosphorylated and unphosphorylated forms, wherein the phosphorylation site is or comprises an S-X-E motif, and wherein X is any amino acid.
2. The fusion polypeptide of claim 1, wherein the fusion polypeptide, when exposed to a metal-hydroxide forms a complex therewith.
3. The fusion polypeptide of claim 2, wherein the metal hydroxide is aluminum hydroxide.
4. The fusion polypeptide of claim 2, wherein the complex forms more readily when the metal-hydroxide-binding polypeptide is in a phosphorylated form than when it is in an unphosphorylated form.
5. The fusion polypeptide of claim 4, wherein one or more of the phosphorylation sites is targeted by a Fam20C kinase.
6. The fusion polypeptide of claim 1, wherein the plurality of phosphorylation sites comprises more than 4 S-X-E motifs.
7. The fusion polypeptide of claim 1, wherein the plurality of phosphorylation sites comprises 8 S-X-E motifs.
8. The fusion polypeptide of claim 7, wherein at least two adjacent S-X-E motifs are separated by a spacer.

9. The fusion polypeptide of claim 8, wherein the spacer comprises at least four glycine residues.
10. The fusion polypeptide of claim 7, wherein each SXE motif is separated from each adjacent S-X-E motif by a spacer.
11. The fusion polypeptide of claim 1, wherein the metal binding polypeptide comprises an amino acid sequence of SEQ ID NO: 6.
12. Use of a complex comprising:
- (a) a fusion polypeptide comprising:
 - (i) an immunomodulatory polypeptide that comprises a IL12B moiety or functional fragment thereof and a IL12A moiety or a functional fragment thereof; and
 - (ii) a metal-hydroxide binding peptide whose amino acid sequence includes a plurality of phosphorylation sites, so that it can adopt phosphorylated and unphosphorylated forms, wherein the phosphorylation site is or comprises an S-X-E motif, and wherein X is any amino acid; and
 - (b) a metal hydroxide,
- in the manufacture of a medicament for treating a subject with a tumor, and wherein the complex is to be administered to the subject.
13. The use of claim 12, wherein (a) and (b) are:
- (i) formulated together; or
 - (ii) mixed prior to administration.
14. The use of claim 12, wherein the complex is to be administered to the subject
- (i) by intratumoral injection;
 - (ii) by peritumoral injection;
 - (iii) to a tumor-draining lymph node or lymph nodes; or

- (iv) in combination with a second therapeutic.
15. The use of claim 14, wherein the second therapeutic is:
- (i) radiation;
 - (ii) surgical tumor resection;
 - (iii) a chemotherapy or targeted therapy;
 - (iv) an anti-tumor antibody; or
 - (v) an immune modulator.
16. Use of a fusion polypeptide comprising:
- (a) an immunomodulatory polypeptide that comprises a IL12B moiety or functional fragment thereof and a IL12A moiety or a functional fragment thereof; and
 - (b) a metal-hydroxide binding peptide whose amino acid sequence includes a plurality of phosphorylation sites, so that it can adopt phosphorylated and unphosphorylated forms, wherein the phosphorylation site is or comprises an S-X-E motif, and wherein X is any amino acid,
 - in the manufacture of a medicament for treating a subject with a tumor, wherein the fusion polypeptide is to be administered to the subject;
 - wherein the fusion polypeptide is formulated with a metal hydroxide; and
 - wherein the subject is to receive therapy with at least one additional therapeutic.
17. The use of claim 16, wherein the fusion polypeptide and metal-hydroxide are:
- (i) formulated together, forming a complex, or
 - (ii) mixed prior to administration.
18. The use of claim 16, wherein the fusion polypeptide is to be administered to the subject:
- (i) by intratumoral injection;
 - (ii) by peritumoral injection; or
 - (iii) to a tumor-draining lymph node or lymph nodes.

19. The method of claim 16, wherein the at least one additional therapeutic is
 - (i) radiation;
 - (ii) surgical tumor resection;
 - (iii) a chemotherapy or targeted therapy;
 - (iv) an anti-tumor antibody; or
 - (v) an immune modulator.
20. The fusion polypeptide of claim 1, wherein the IL12B and IL12A moieties or functional fragments thereof are linked via a first linker.
21. The fusion polypeptide of claim 20, wherein the first linker comprises a polypeptide.
22. The fusion polypeptide of claim 21, wherein the polypeptide comprises a (G₄S)₃ linker.
23. The fusion polypeptide of claim 1, wherein the immunomodulatory polypeptide and the metal-hydroxide binding polypeptide are linked via a second linker.
24. The fusion polypeptide of claim 23, wherein the second linker comprises a polypeptide.
25. The fusion polypeptide of claim 24, wherein the polypeptide comprises the amino acid sequence, GGGGEGGGG.
26. The fusion polypeptide of claim 1, wherein the metal-hydroxide binding polypeptide is linked via a second linker to the c-terminus of the immunomodulatory polypeptide.
27. The fusion polypeptide of claim 1, wherein the polypeptide comprises an amino acid sequence having at least 90 % identity to SEQ ID NO: 2.
28. The fusion polypeptide of claim 27, wherein the polypeptide comprises an amino acid sequence according to SEQ ID NO: 2.

29. A method of manufacturing a phosphorylated form of the fusion polypeptide of claim 1, by contacting the fusion polypeptide with a kinase.
30. The method of claim 29, wherein the contacting comprises co-expressing the fusion polypeptide and a kinase.
31. The method of claim 30, wherein the fusion polypeptide and kinase are co-expressed at a ratio of 2:1 to 100:1.
32. The method of claim 31, wherein the ratio is 8:1.
33. The method of claim 32, wherein the 8:1 ratio is achieved using a single vector with two promoters to express the fusion polypeptide and the kinase.
34. The method of claim 30, wherein the kinase is Fam20C.
35. The method of claim 30, further comprising a step of purifying the phosphorylated form.
36. The method of claim 35, wherein the step of purifying comprises chromatography.
37. The method of claim 30, wherein the fusion polypeptide is exposed to a metal-hydroxide to form a complex therewith.
38. A method of manufacturing a complex by contacting a phosphorylated form of a fusion polypeptide of claim 1 with a metal hydroxide.
39. A complex comprising the fusion polypeptide of claim 1 and a metal hydroxide.
40. The complex of claim 39, wherein the complex comprises an average of 2-8 phosphates per fusion polypeptide.

41. The complex of claim 39, wherein the complex is characterized as having greater than 95% metal hydroxide retention.
42. The complex of claim 39, wherein the complex comprises a ratio of 1:1 to 1:20 by mass of fusion polypeptide to metal hydroxide as defined by metal mass.
43. A pharmaceutical composition comprising the fusion polypeptide of claim 1.
44. The pharmaceutical composition of claim 43, formulated as a fusion polypeptide metal-hydroxide complex.