Claims

We claim:

1. A method of manufacturing a phosphorylated form of a fusion polypeptide, wherein the fusion polypeptide comprises:

(a) an immunomodulatory polypeptide that comprises an immune agonist moiety; and

(b) a metal-hydroxide binding polypeptide,

wherein the metal-hydroxide binding polypeptide comprises an amino acid sequence that includes a plurality of phosphorylation sites, so that the fusion polypeptide can adopt phosphorylated and unphosphorylated forms,

the method comprising:

co-expressing, in a host cell, the fusion polypeptide and a kinase, wherein the kinase phosphorylates the fusion polypeptide, and wherein the fusion polypeptide and kinase are expressed in a ratio within a range of about 4:1 to about 10:1.

2. The method of claim 1, wherein the fusion polypeptide comprises 6-10 phosphates, or wherein the fusion polypeptide comprises 7-9 phosphates.

3. The method of claim 1, wherein the step of co-expressing comprises expressing from a bicistronic construct, or expressing from a transposon plasmid,

optionally wherein the transposon plasmid is a DNA plasmid, and/or

optionally wherein the cell is further transfected with a nucleotide sequence encoding a transposase enzyme, and further optionally wherein the transposase enzyme is an integrase or DDE/D integrase, and wherein the nucleotide sequence encoding the transposase enzyme is RNA.

4. The method of claim 1, wherein nucleotide sequences encoding the fusion polypeptide and the kinase are integrated into the genome of the host cell, optionally wherein the integration results in a single copy integration per genomic loci.

5. The method of claim 1, wherein the ratio is 8:1.

6. The method of claim 1, wherein the kinase is Fam20C kinase, optionally wherein the Fam20C kinase has an amino acid sequence that is at least 80% identical to that of SEQ ID NO: 5.

7. The method of claim 1, wherein the moiety comprises an IL12 moiety or a functional fragment thereof or wherein the immune agonist moiety comprises a first and a second moiety or a functional fragment thereof;

optionally wherein the first moiety comprises an IL12 moiety or a functional fragment thereof and the second moiety comprises an IL12 moiety or a functional fragment thereof; and

further optionally wherein the first moiety comprising an IL12 moiety has an amino acid sequence that is at least 80% identical to that of SEQ ID NO: 2 and the second moiety comprising an IL12 moiety has an amino acid sequence that is at least 80% identical to that of SEQ ID NO: 3.

8. The method of claim 1, further comprising manufacturing a high purity preparation of a phosphorylated form of the fusion polypeptide from a cell extract of the host cell that comprises the phosphorylated form wherein the method does not comprise an affinity chromatography step.

9. The method of claim 8, wherein the method includes one or more purification steps selected from:

an anion chromatography step; and a hydrophobic interaction chromatography step; and

optionally wherein the one or more purification steps comprises:

at least one anionic chromatography capture step and at least one hydrophobic interaction chromatography step,

at least two anionic chromatography capture steps and at least one hydrophobic interaction chromatography step, or

a first anionic chromatography step, a hydrophobic interaction chromatography step, and a second anionic chromatography step, optionally wherein a first step of filtration is performed after the second hydrophobic interaction chromatography step and/or wherein

the first step of filtration is an ultrafiltration/diafiltration step, and optionally wherein a second step of filtration is performed after the second anionic chromatography capture step and/or wherein the second step of filtration is an ultrafiltration/diafiltration step; and

further optionally wherein:

the first anionic chromatography capture step utilizes low salt concentrations for binding phosphorylated fusion polypeptides to a chromatography column and higher salt concentrations to elute phosphorylated fusion polypeptides;

the hydrophobic interaction chromatography step utilizes high ammonium sulfate concentrations for binding phosphorylated fusion polypeptides to a chromatography column and lower ammonium sulfate concentrations to elute phosphorylated fusion polypeptides; and/or

the second anionic chromatography step utilizes low salt concentrations for binding phosphorylated fusion polypeptides to a chromatography column and higher salt concentrations to elute phosphorylated fusion polypeptides.

10. The method of claim 9, wherein the purifying step comprises an initial step of viral inactivation, optionally wherein the viral inactivation step is performed by contacting the fusion polypeptide with a detergent.

11. The method of claim 1, further comprising the steps of:

I) culturing the host cell, wherein the host cell is an engineered mammalian cell expressing:

i) a first nucleotide sequence encoding the fusion polypeptide; and

ii) a second nucleotide sequence encoding the kinase;

wherein the first and second nucleotide sequences being in cis with one another and each under control of regulatory nucleotide sequences that control expression so that the fusion polypeptide is expressed at a ratio of about 4:1 to about 10:1 of that at which the kinase is expressed; and

II) purifying the phosphorylated fusion polypeptide without an affinity chromatography step.

12. A mammalian cell engineered to contain:

i) a first nucleotide sequence encoding a fusion polypeptide, wherein the fusion polypeptide comprises:

a) an immunomodulatory polypeptide that comprises an immune agonist moiety; and

b) a metal-hydroxide binding polypeptide, wherein the metal-hydroxide binding polypeptide comprises an amino acid sequence that includes a plurality of phosphorylation sites, so that the fusion polypeptide can adopt phosphorylated and unphosphorylated forms; and

ii) a second nucleotide sequence encoding a kinase;

wherein the first and second nucleotide sequences are in cis with one another and each is under the control of a regulatory nucleotide sequence that controls expression so that the fusion polypeptide is expressed at a ratio of about 4:1 to about 10:1 of that at which the kinase is expressed.

13. The cell of claim 12, wherein the regulatory nucleotide sequence is a promoter.

14. The cell of claim 13, wherein promoters of different strength are used to produce the desired ratio of the fusion polypeptide and the kinase,

optionally wherein the first nucleic acid sequence encoding the fusion polypeptide is under the control of a CMV promoter or EF1a promoter, and

optionally wherein the second nucleic acid sequence encoding the kinase is under the control of a SV40 promoter or Ubc promoter.

15. A preparation of cultured mammalian cells of claim 12.