Claims

1. An immunostimulatory bacterium, comprising a plasmid encoding a therapeutic product under control of a eukaryotic promoter, wherein:

the genome of the immunostimulatory bacterium is modified, whereby the bacterium is flagella deficient; and

the wild-type bacterium comprises flagella.

2. The immunostimulatory bacterium of claim 1, wherein the bacterium comprises genome modifications whereby TLR2 and TLR4 signaling is reduced.

3. The immunostimulatory bacterium of claim 1 or claim 2 that is *pagP*$^{-}$and is flagellin^{-}.

4. The immunostimulatory bacterium of any of claims 1-3 that has penta-acylated lipopolysaccaride (LPS).

5. The immunostimulatory bacterium of any of claims 1-3 that comprises genome modifications whereby the bacterium is *pagP*$^{-}$/*msbB*$^{-}$.

6. The immunostimulatory bacterium of claim 1 that comprises genome modifications whereby the bacterium is *pagP*$^{-}$.

7. The immunostimulatory bacterium any of claims 1-6, wherein the immunostimulatory bacterium comprises genome modifications whereby the bacterium is one or more of *purI*$^{-}$ (*purM*$^{-}$), *msbB*$^{-}$, *purD*$^{-}$, *pagP*$^{-}$, *adrA*$^{-}$, *csgD*$^{-}$, *qseC*$^{-}$, and *hilA*$^{-}$.

8. The immunostimulatory bacterium of any of claims 1-7, wherein the bacterium is auxotrophic for adenosine, or is auxotrophic for adenosine and adenine.

9. The immunostimulatory bacterium any of claims 1-8, wherein the bacterium does not express endogenous aspartate-semialdehyde dehydrogenase (*asd*) by virtue of disruption or deletion of all or a portion of the endogenous gene encoding aspartate-semialdehyde dehydrogenase (*asd*).

10. The immunostimulatory bacterium of claim 9 that encodes aspartate-semialdehyde dehydrogenase (*asd*) on the plasmid under control of a bacterial promoter.

11. The immunostimulatory bacterium of any of claims 1-10 that comprises genome modifications whereby the bacterium is *asd*$^-$, *purI*$^-$, *msbB*$^-$, flagella deficient, and *pagP*$^-$.

12. The immunostimulatory bacterium of any of claims 1-11 that comprises genome modifications whereby the bacterium is *csgD*$^-$.

13. The immunostimulatory bacterium of any one of claims 1-12 that has a modification in the type 3 secretion system (T3SS) or in the type 4 secretion system (T4SS), whereby infection of epithelial and/or other non-immune cells is reduced relative to infection by the bacterium without the modification.

14. The immunostimulatory bacterium of any of claims 1-13, wherein:

the immunostimulatory bacterium is a *Salmonella* species; and

the immunostimulatory bacterium is an adenosine auxotroph.

15. The immunostimulatory bacterium of any of claims 1-14, wherein the plasmid is present in a copy number of less than 150.

16. The immunostimulatory bacterium of any of claims 1-15, wherein expression of the therapeutic product is under control of eukaryotic regulatory sequences.

17. The immunostimulatory bacterium of any of claims 1-16, wherein the plasmid that encodes the therapeutic product comprises a construct that includes an enhancer, a promoter, the open reading frame encoding the therapeutic product, and a polyA tail.

18. The immunostimulatory bacterium of claim 17, wherein the nucleic acid encoding the therapeutic product comprises terminators selected from among an SV40 terminator, a terminator from the gene encoding human growth hormone (hGH), a terminator from the gene encoding bovine growth hormone (bGH), and a terminator from the gene encoding rabbit beta-globulin (rbGlob).

19. The immunostimulatory bacterium of any of claims 1-18, wherein the nucleic acid encoding the therapeutic product is operatively linked for expression to a nucleic acid encoding a secretory signal, whereby, upon expression in a host, the therapeutic product is secreted.

20. The immunostimulatory bacterium of any of claims 1-19, wherein the eukaryotic promoter is an RNA polymerase II promoter or an RNA polymerase III promoter.

21. The immunostimulatory bacterium of claim 20, wherein the promoter is a promoter selected from among a cytomegalovirus (CMV) promoter, an SV40 promoter, an Epstein-Barr virus (EBV) promoter, a herpes virus promoter, an adenovirus promoter, an EF-1 alpha promoter, an EIF4A1 promoter, a CAG promoter, an MND promoter, a GAPDH promoter, a CBA (chicken beta actin) promoter, a PGK promoter, a UBC promoter, and a CD68 promoter.

22. The immunostimulatory bacterium of claim 20 or claim 21, wherein the promoter is active in a tumor microenvironment (TME) of a eukaryotic subject.

23. The immunostimulatory bacterium of claim 22, wherein the promoter is active in hypoxic conditions, or in conditions where the pH is less than 7.

24. The immunostimulatory bacterium of any of claims 1-23, wherein the therapeutic product is an anti-cancer therapeutic product.

25. The immunostimulatory bacterium of claim 24, wherein the anti-cancer therapeutic product stimulates the immune system of the host.

26. The immunostimulatory bacterium of claim 24 or claim 25, wherein the anti-cancer therapeutic product is an immunostimulatory protein that confers or contributes to an anti-tumor immune response in the tumor microenvironment.

27. The immunostimulatory bacterium of claim 26, wherein the immunostimulatory protein that confers or contributes to an anti-tumor immune response in the tumor microenvironment is a cytokine or is a chemokine.

28. The immunostimulatory bacterium of any of claims 1-18 and 20-27, wherein the therapeutic product is an immunostimulatory protein that confers or contributes to an anti-tumor immune response in the tumor microenvironment, and is encoded on the plasmid so that, when expressed, it is membrane-anchored.

29. The immunostimulatory bacterium of any of claims 1-28, wherein the therapeutic product is an immunostimulatory protein that confers or contributes to an anti-tumor immune response in the tumor microenvironment, and is selected from among one or more of: IL-2, IL-7, IL-12p70 (IL-12p40 + IL-12p35), IL-15, IL-36 gamma, IL-2 that has attenuated binding to IL-2Ra, IL-15/IL-15R alpha chain complex, IL-18, IL-21, IL-23, IL-2 that is modified so that it has attenuated binding or does not bind to IL-2Ra, CXCL9, CXCL10, CXCL11, interferon-α, interferon-β, interferon-γ, CCL3, CCL4, CCL5, proteins

that are involved in or that effect or potentiate the recruitment and/or persistence of T cells, CD40, CD40 ligand (CD40L), CD28, OX40, OX40 ligand (OX40L), 4-1BB, 4-1BB ligand (4-1BBL), members of the B7-CD28 family, CD47 antagonists, TGF-beta antagonist polypeptides, and members of the tumor necrosis factor receptor (TNFR) superfamily.

30. The immunostimulatory bacterium of any of claims 1-29, wherein the therapeutic product is an IL-15/IL-15R alpha chain complex.

31. The immunostimulatory bacterium of any of claims 1-25, wherein the therapeutic product is a TGF-beta antagonist polypeptide.

32. The immunostimulatory bacterium of any of claims 1-25, wherein the therapeutic product is an anti-cancer antibody or an antigen-binding fragment thereof.

33. The immunostimulatory bacterium of claim 32, wherein the antibody or antigen-binding fragment thereof is an antigen-binding fragment that is selected from among a Fab, Fab', $F(ab')_2$, single-chain Fv (scFv), Fv, dsFv, nanobody, diabody fragment, and a single-chain antibody.

34. The immunostimulatory bacterium of claim 32, wherein the anti-cancer therapeutic product is a bi-specific antibody, an intrabody, a chimeric antibody, or an antigen-binding portion thereof.

35. The immunostimulatory bacterium of claim 34, wherein the anti-cancer therapeutic product is an antibody or a portion thereof that is a DART antibody or that is a tandem scFv (BiTE).

36. The immunostimulatory bacterium of any of claims 24-26, 28 and 32-35, wherein the anti-cancer therapeutic product is an anti-tumor antibody or antigen-binding fragment thereof that is an immune checkpoint inhibitor.

37. The immunostimulatory bacterium of any of claims 32-36, wherein:

the antibody or antigen-binding fragment thereof is an antagonist of PD-1, PD-L1, CTLA-4, VEGF, VEGFR2, or IL-6; or

the antibody is an anti-PD-1, anti-PD-L1, anti-CTLA-4, anti-VEGF, anti-VEGFR2, or anti-IL-6 antibody, or an antigen-binding fragment thereof.

38. The immunostimulatory bacterium of any of claims 24-26 and 28, wherein the anti-cancer therapeutic product comprises a cytotoxin selected from among ricin, saporin, and shiga toxin.

39. The immunostimulatory bacterium of any of claims 1-38, wherein the bacterium encodes and expresses a tumor antigen.

40. The immunostimulatory bacterium of any of claims 1-39, wherein the plasmid encodes inhibitory RNA (RNAi) or a CRISPR cassette, wherein:

the RNAi or CRISPR cassette is encoded on a plasmid in the bacterium under control of a eukaryotic promoter; and

the RNAi or CRISPR cassette inhibits or disrupts or suppresses an immune checkpoint.

41. The immunostimulatory bacterium of any of claims 1-13 and 15-40, wherein the bacterium is a strain of *Salmonella*, *Shigella*, *E. coli*, *Bifidobacteriae*, *Rickettsia*, *Vibrio*, *Listeria*, *Klebsiella*, *Bordetella*, *Neisseria*, *Aeromonas*, *Francisella*, *Cholera*, *Corynebacterium*, *Citrobacter*, *Chlamydia*, *Haemophilus*, *Brucella*, *Mycobacterium*, *Mycoplasma*, *Legionella*, *Rhodococcus*, *Pseudomonas*, *Helicobacter*, *Bacillus*, or *Erysipelothrix*, or is an attenuated strain thereof, or is a modified strain thereof, of any of the preceding list of bacterial strains.

42. The immunostimulatory bacterium of any of claims 1-41, wherein the immunostimulatory bacterium is derived from an attenuated bacterium, or a wild-type strain.

43. The immunostimulatory bacterium of any of claims 1-13 and 15-42 that is a strain of *Salmonella*.

44. The immunostimulatory bacterium of claim 14 or claim 43 that is a *Salmonella typhimurium* strain.

45. The immunostimulatory bacterium of claim 44, wherein the immunostimulatory bacterium is derived from a *Salmonella typhimurium* strain selected from among strains designated as AST-100, VNP20009, YS1646 (ATCC #202165), RE88, SL7207, χ 8429, χ 8431, χ 8468, and wild-type strain ATCC 14028.

46. The immunostimulatory bacterium of any of claims 1-45 that comprises nucleic acid encoding two therapeutic products.

47. The immunostimulatory bacterium of claim 46, wherein:

different promoters and terminators control expression of each therapeutic product; or

the nucleic acid encoding the therapeutic products is expressed from a single promoter and comprises a single terminator, and the sequence encoding each product comprises an Internal Ribosome Entry Site (IRES), whereby the encoding nucleic acid is polycistronic.

48. The immunostimulatory bacterium of any of claims 1-47, wherein the bacterium has been treated with polymyxin to reduce pyrogenicity.

49. A pharmaceutical composition, comprising the immunostimulatory bacterium of any of claims 1-48 in a pharmaceutically acceptable vehicle.

50. The pharmaceutical composition of claim 49, for use in treating cancer, wherein the therapeutic product is an anti-cancer therapeutic product.

51. The pharmaceutical composition for use of claim 50, wherein the cancer comprises a solid tumor or a hematological malignancy.

52. A combination for use in treating cancer, comprising:

the pharmaceutical composition of claim 49, wherein the therapeutic product is an anti-cancer therapeutic product; and

a second anti-cancer agent or treatment.

53. The combination for use of claim 52, wherein the second anti-cancer agent or treatment comprises an immunotherapy, oncolytic virus therapy, or chemotherapy.

54. The combination for use of claim 53, wherein the second anti-cancer agent or treatment comprises an anti-PD-1, or anti-PD-L1, or anti-CTLA-4, or anti-IL-6, or anti-VEGF, or anti-VEGFR2 antibody, or antigen-binding fragments thereof.

55. The pharmaceutical composition or combination for use of any of claims 50-54, wherein the cancer is selected from among leukemia, lymphoma, gastric cancer, and cancer of the breast, heart, lung, small intestine, colon, spleen, kidney, bladder, head and neck, colorectum, ovary, prostate, brain, pancreas, skin, bone, bone marrow, blood, thymus, uterus, testicles, cervix, and liver.

56. An isolated cell or cultured cells, comprising the immunostimulatory bacterium of any of claims 1-48.

57. The isolated cell or cultured cells of claim 56, wherein the cell or cells is/are or comprise an immune cell, a stem cell, a tumor cell, a primary cell line, or a T-cell.

58. The isolated cell or cultured cells of claim 56 or claim 57, for use in the treatment of a cancer, wherein the therapeutic product is an anti-cancer therapeutic product.

59. The isolated cell or cultured cells for use of claim 58, wherein the cancer is selected from among lymphoma, leukemia, gastric cancer, and cancer of the breast, heart, lung, small intestine, colon, spleen, kidney, bladder, head and neck, colorectum, ovary, prostate, brain, pancreas, skin, bone, bone marrow, blood, thymus, uterus, testicles, cervix, and liver.

60. Use of the pharmaceutical composition of claim 49 in the manufacture of a medicament for treating cancer in a subject, wherein the pharmaceutical composition is to be administered to the subject, wherein the therapeutic product is an anti-cancer therapeutic product.

61. The use of claim 60, wherein the cancer comprises a solid tumor or a hematological malignancy.

62. The use of claim 60, wherein a second anti-cancer agent or treatment is to be further administered.

63. The use of claim 62, wherein the second anti-cancer agent or treatment comprises an immunotherapy, oncolytic virus therapy, or chemotherapy.

64. The use of claim 63, wherein the second anti-cancer agent or treatment comprises an anti-PD-1, or anti-PD-L1, or anti-CTLA-4, or anti-IL-6, or anti-VEGF, or anti-VEGFR2 antibody, or antigen-binding fragments thereof.

65. Use of the isolated cell or cultured cells of claim 56 or claim 57 in the manufacture of a medicament for the treatment of cancer in a subject, wherein the isolated cell or cultured cells is to be administered to the subject, wherein the therapeutic product is an anti-cancer therapeutic product.

66. The use of any of claims 60-65, wherein the cancer is selected from among lymphoma, leukemia, gastric cancer, and cancer of the breast, heart, lung, small intestine, colon, spleen, kidney, bladder, head and neck, colorectum, ovary, prostate, brain, pancreas, skin, bone, bone marrow, blood, thymus, uterus, testicles, cervix, and liver.