CLAIMS

What is claimed is:

1) A method of inducing an immune response against a disease in a subject, the method comprising the steps of :

a) Obtaining a biological sample from said subject,

b) Evaluating the expression of a predetermined number of biomarkers in said biological sample,

c) Administering to said subject a composition comprising a recombinant Listeria strain, said strain comprising a nucleic acid sequence encoding at least one fusion protein,

i) wherein said fusion protein comprises a biomarker identified in said biological sample,

ii) wherein said biomarker is associated with said disease,

iii) wherein said biomarker is fused to a PEST-containing polypeptide,

iv) wherein each fusion protein within said Listeria comprises a different biomarker, thereby inducing a multi-target anti-disease immune response in said subject; OR,

d) Administering to said subject a predetermined number of compositions each comprising a recombinant Listeria strain, said strain comprising a nucleic acid sequence encoding fusion protein,

i) wherein said fusion protein comprises a biomarker identified in said biological sample,

ii) wherein said biomarker is associated with said disease,

iii) wherein said biomarker is fused to a PEST-containing polypeptide; and,

iv) wherein each Listeria strain within each composition comprises a different biomarker in said fusion protein,

v) thereby inducing a multi-target immune response against said disease in said subject.

2) The method of claim 1, wherein said disease is a congenital disease, and infectious disease, a cancer or a tumor growth.

3) The method of claims 1-2, wherein said biomarker is detected in a biological sample obtained from said subject prior to administering said Listeria-based vaccine.

4) The method of claim 3, wherein said biological sample is blood, tissue, DNA, RNA, sperm, cerebro-spinal fluid, sputum or serum.

5) The method of claims 1-4, further comprising the step of administering an alternate composition or alternate form of a vaccine prior to, concurrently with, or following administration of said Listeria-based vaccine.

6) The method of claim 6, wherein said alternate composition or alternate form of a vaccine is a DNA vaccine encoding a fusion protein comprising a biomarker expressed in said biological sample, a viral vector comprising said fusion protein, a virus-like particle comprising said fusion protein, or a live recombinant non-Listeria bacterial vector expressing said fusion protein.

7) The method of claims 1-6, further comprising the step of administering an additional active agent.

8) The method of claim 7, wherein said additional active agent is an immune checkpoint inhibitor, an antibody or fragment thereof, a chimeric antigen receptor (CAR) T cell, or a combination thereof.

9) The method of claims 1-8, further comprising the step administering a booster of said composition or mixture of compositions.

10) The method of claims 1-9, further comprising the step administering a booster dose of said alternate composition or alternate form of a vaccine.

11) The method of claims 1-12, wherein said predetermined number of biomarkers are one to ten biomarkers, each different from the rest.

12) The method of claims 1-10, wherein said recombinant Listeria in said composition in 1.b) comprises one to ten fusion proteins each comprising a different biomarker from the rest.

13) The method of claims 1-8, wherein said mixture of compositions comprises one to ten compositions comprising said recombinant Listeria strain in 1.d).

14) The method of claims 1-13, wherein said PEST-containing peptide is an N-terminal Listeriolysin O (LLO), a PEST-sequence, or an N-terminal ActA sequence.

15) The method of claims 1-14, wherein said Listeria strains comprise a genomic mutation or deletion of the chromosomal dal/dat genes.

16) The method of claims 1-14, wherein said Listeria strains comprise a genomic mutation or a deletion of the chromosomal actA gene.

17) The method of claim 1-14, wherein said Listeria strains comprise a genomic mutation or a deletion of the chromosomal prfA gene.

18) The method of claims 1-17, wherein said nucleic acid sequence in said Listeria strains is present in a stable extrachromosomal plasmid within said Listeria.

19) The method of claim 18, wherein said plasmid comprises sequences that encode for integration of the plasmid into the Listeria chromosome.

20) The method of claims 16-18, wherein said plasmid comprising a nucleic acid sequence that encodes a protein that complements said genomic mutation or deletion in said dal/dat genes, actA gene or said prfA gene in said Listeria.

21) The method of claims 1-20, wherein said biomarker is a tumor antigen.

22) The method of claim 21, wherein said tumor antigen is associated with the formation of or proliferation of said tumor.

23) The method of claims 21, wherein said tumor antigen is expressed by a tumor or its vasculature.

24) The method of any one of claims 1-23, wherein said method results in an increase of a CD8 T-cell / T-regulatory cell suppressor ratio.

25) The method of any one of claims 1-24, wherein said method results in preventing the recurrence of said disease.

26) The method of any one of claims 1-24, wherein said method results in treatment of said disease.

27) The method of any one of claims 1-24, wherein said disease is a cancer or tumor growth.

CLAIMS

1. A method of providing a kit for inducing a multi-target immune response against a disease in a subject, comprising:

(a) obtaining an expression profile of a predetermined number of biomarker peptides associated with the disease in a biological sample from the subject; and

(b) providing the kit, wherein the kit comprises a composition comprising a recombinant *Listeria* strain comprising a nucleic acid encoding a fusion protein, wherein the fusion protein comprises two or more different antigenic biomarker peptides identified in the biological sample fused to a PEST-containing peptide.

2. The method of claim 1, wherein the fusion protein in the recombinant *Listeria* strain in step (b) comprises two to ten antigenic biomarker peptides.

3. A method of providing a kit for inducing a multi-target immune response against a disease in a subject, comprising:

(a) obtaining an expression profile of a predetermined number of biomarker peptides associated with the disease in a biological sample from the subject; and

(b) providing the kit, wherein the kit comprises a composition comprising a recombinant *Listeria* strain comprising a nucleic acid encoding two or more fusion proteins, wherein each of the two or more fusion proteins comprises a different antigenic biomarker peptide identified in the biological sample fused to a PEST-containing peptide.

4. The method of claim 3, wherein the recombinant *Listeria* strain in step (b) comprises two to ten fusion proteins.

5. A method of providing a kit for inducing a multi-target immune response against a disease in a subject, comprising:

(a) obtaining an expression profile of a predetermined number of biomarker peptides associated with the disease in a biological sample from the subject; and

(b) providing the kit, wherein the kit comprises a mixture of two or more compositions, each comprising a recombinant *Listeria* strain comprising a nucleic acid encoding a fusion protein, wherein the fusion protein in each recombinant *Listeria* strain comprises a

different antigenic biomarker peptide identified in the biological sample fused to a PEST-containing peptide.

6. The method of claim 5, wherein the mixture of two or more compositions in step (b) comprises two to ten compositions.

7. The method of any preceding claim, wherein step (a) comprises evaluating expression of the predetermined number of biomarker peptides associated with the disease in the biological sample.

8. The method of any preceding claim, wherein the antigenic biomarker peptides are overexpressed in subjects having the disease as compared to normal levels of expression of the antigenic biomarker peptides in healthy subjects.

9. The method of any preceding claim, wherein the disease is a congenital disease, and infectious disease, a cancer, or a tumor growth.

10. The method of any preceding claim, wherein the biological sample is blood, tissue, DNA, RNA, sperm, cerebrospinal fluid, sputum, or serum.

11. The method of any preceding claim, wherein the kit in step (b) further comprises an additional composition.

12. The method of claim 11, wherein the additional composition is a DNA vaccine encoding an additional fusion protein comprising an additional antigenic biomarker peptide expressed in the biological sample, a viral vector comprising the additional fusion protein, a virus-like particle comprising the additional fusion protein, or a live, recombinant, non-*Listeria* bacterial vector expressing the additional fusion protein.

13. The method of any preceding claim, wherein the kit in step (b) further comprises an active agent.

14. The method of claim 12, wherein the active agent is an immune checkpoint inhibitor, an antibody or fragment thereof, a chimeric antigen receptor engineered T cell, or a combination thereof.

15. The method of any preceding claim, wherein the PEST-containing peptide is an N-terminal listeriolysin O (LLO) peptide or an N-terminal ActA peptide.

16. The method of any preceding claim, wherein the recombinant *Listeria* strain comprises a genomic mutation or deletion of a *dal* gene and a *dat* gene.

17. The method of claim 16, wherein the nucleic acid in the recombinant *Listeria* strain comprises an open reading frame encoding an alanine racemase enzyme or a D-amino acid transferase enzyme.

18. The method of any preceding claim, wherein the recombinant *Listeria* strain comprises a genomic mutation or a deletion of an *actA* gene.

19. The method of any preceding claim, wherein the recombinant *Listeria* strain comprises a genomic mutation or a deletion of a *prfA* gene.

20. The method of any preceding claim, wherein the nucleic acid in the recombinant *Listeria* strain is present in a stable extrachromosomal plasmid.

21. The method of any one of claims 1-19, wherein the nucleic acid in the recombinant *Listeria* strain is present in a plasmid comprising sequences that encode for integration of the plasmid into a *Listeria* chromosome.

22. The method of any preceding claim, wherein the *Listeria* is *Listeria monocytogenes*.

23. The method of any preceding claim, wherein the disease is a tumor, and the biomarker is a tumor antigen expressed by the tumor or its vasculature.

24. The method of claim 23, wherein the tumor antigen is associated with the formation of or proliferation of the tumor.

25. The method of any preceding claim, wherein the recombinant *Listeria* strain is a recombinant, attenuated, auxotrophic *Listeria monocytogenes* strain comprising a genomic mutation or deletion of a *dal* gene, a *dat* gene, and an *actA* gene, and wherein the nucleic acid in the recombinant *Listeria* strain is present in a stable extrachromosomal plasmid and further

comprises an open reading frame encoding an alanine racemase enzyme or a D-amino acid transferase enzyme.

26. The method of claim 25, wherein the disease is a tumor, and the biomarker is a tumor antigen expressed by the tumor or its vasculature.