BRIEF SUMMARY

In one aspect, the present disclosure provides a use of a composition comprising non-viable cells of *Streptococcus pyogenes* in the manufacture of a medicament for treating non-muscle invasive bladder cancer in a subject, wherein the subject is also to be administered an immune checkpoint inhibitor.

In another aspect, the present disclosure provides a use of a composition comprising non-viable cells of *Streptococcus pyogenes* in the manufacture of a medicament for combination therapy to treat non-muscle invasive bladder cancer in a subject, wherein the medicament is to be administered in combination with an immune checkpoint inhibitor.

In another aspect, the present disclosure provides a use of a composition comprising non-viable cells of *Streptococcus pyogenes* in the manufacture of a first medicament and use of an immune checkpoint inhibitor in the manufacture of a second medicament, wherein the first and second medicaments are to be used in combination therapy to treat non-muscle invasive bladder cancer in a subject.

In another aspect, the present disclosure provides a use of a pharmaceutical composition comprising non-viable cells of *Streptococcus pyogenes* in combination with an immune checkpoint inhibitor for treating non-muscle invasive bladder cancer in a subject.

In another aspect, the present disclosure provides a use of a medicament comprising a composition comprising non-viable cells of *Streptococcus pyogenes* in combination with an immune checkpoint inhibitor for treating non-muscle invasive bladder cancer in a subject.

In another aspect, the present disclosure provides a use of a composition comprising non-viable cells of *Streptococcus pyogenes* in the manufacture of a medicament for combination therapy to treat non-muscle invasive bladder cancer in a subject, wherein the medicament is to be administered in combination with an anti-PD-1 antibody, and wherein the medicament is formulated for intravesical administration to the subject.

In another aspect, the present disclosure provides a use of a composition comprising non-viable cells of *Streptococcus pyogenes* in the manufacture of a medicament for a combination therapy to treat high-grade non-muscle invasive bladder cancer in a human adult subject, wherein: (1) the medicament is formulated to be administered in combination with pembrolizumab (2) the medicament is formulated for intravesical administration to the subject; and (2) the medicament is formulated for intravesical administration to the subject; and (3) the pembrolizumab is to be administered intravenously to the subject; wherein the high grade non-

muscle invasive bladder cancer is Bacillus Calmette-Guerin (BCG) failed, BCG-unresponsive, BCG-refractory, BCG-relapsing, BCG inadequately treated, the subject has received adequate BCG treatment, or any combination thereof.

In another aspect, the present disclosure provides a use of a composition comprising non-viable cells of *Streptococcus pyogenes* in the manufacture of a medicament for a combination therapy to treat high-grade non-muscle invasive bladder cancer in a human adult subject, wherein: (1) the medicament is formulated to be administered in combination with pembrolizumab (2) the medicament is formulated for intravesical administration to the subject; and (3) the pembrolizumab is to be administered intravenously to the subject; wherein: the high grade non-muscle invasive bladder cancer is Bacillus Calmette-Guerin (BCG) failed, BCG-unresponsive, BCG-refractory, BCG-relapsing, BCG inadequately treated, the subject has received adequate BCG treatment, or any combination thereof; and the medicament is formulated to be administered in at least six weekly 40 KE doses of the composition comprising non-viable cells of *Streptococcus pyogenes*, and at least two 400 mg doses of pembrolizumab are to be administered to the subject six weeks apart.

In one example, the present disclosure provides methods of treating triple negative breast cancer in a subject, comprising administering to the subject (i) a composition comprising non-viable cells of *Streptococcus pyogenes*; and (ii) an immune checkpoint inhibitor.

In another example, the present disclosure provides a pharmaceutical composition comprising non-viable cells of *Streptococcus pyogenes* for use in combination with an immune checkpoint inhibitor for treating triple negative breast cancer.

In another example, the present disclosure provides a medicament comprising non-viable cells of *Streptococcus pyogenes* for use in combination with an immune checkpoint inhibitor for treating triple negative breast cancer.

In some examples, the immune checkpoint inhibitor is an inhibitor of PD 1/PD-L1/PD-L2 axis, CD80, CD86, B7-H3, B7 H4, HVEM, adenosine, GAL9, VISTA, CEACAM-1, PVRL2, CTLA 4, BTLA, KIR, LAG3, TIM3, A2aR, CD244/2B4, CD160, TIGIT, LAIR-1, PVRIG/CD112R, arginase, indoleamine 2,3 dioxygenase (IDO), IL-10, IL-4, IL-1RA, IL-35, or any combination thereof.

In some examples, the immune checkpoint inhibitor is an inhibitor of the PD-1/PD-L1/PD-L2 axis.

In some examples, the immune checkpoint inhibitor is a PD-1 inhibitor.

In some examples, the composition comprising non-viable cells of *Streptococcus pyogenes* comprises *Streptococcus pyogenes* [A Group, Type 3] Su strain.

In some examples, the composition comprising non-viable cells of *Streptococcus pyogenes* comprises benzylpenicillin-treated *Streptococcus pyogenes.*

In another example, the present disclosure provides methods of treating non-muscle invasive bladder cancer in a subject, comprising administering to the subject (i) a composition comprising non-viable cells of *Streptococcus pyogenes*; and (ii) an immune checkpoint inhibitor.

In another example, the present disclosure provides a pharmaceutical composition comprising non-viable cells of *Streptococcus pyogenes* for use in combination with an immune checkpoint inhibitor for treating non-muscle invasive bladder cancer.

In another example, the present disclosure provides a medicament comprising non-viable cells of *Streptococcus pyogenes* for use in combination with an immune checkpoint inhibitor for treating non-muscle invasive bladder cancer.

In some examples, the immune checkpoint inhibitor is an inhibitor of PD 1/PD-L1/PD-L2 axis, CD80, CD86, B7-H3, B7 H4, HVEM, adenosine, GAL9, VISTA, CEACAM-1, PVRL2, CTLA 4, BTLA, KIR, LAG3, TIM3, A2aR, CD244/2B4, CD160, TIGIT, LAIR-1,

PVRIG/CD112R, arginase, indoleamine 2,3 dioxygenase (IDO), IL-10, IL-4, IL-1RA, IL-35, or any combination thereof.

In some examples, the immune checkpoint inhibitor is an inhibitor of the PD-1/PD-L1/PD-L2 axis.

In some examples, the immune checkpoint inhibitor is a PD-1 inhibitor.

In some examples, the composition comprising non-viable cells of *Streptococcus pyogenes* comprises *Streptococcus pyogenes* [A Group, Type 3] Su strain.

In some examples, the composition comprising non-viable cells of *Streptococcus pyogenes* comprises benzylpenicillin-treated *Streptococcus pyogenes.*

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

FIG. 1: Evaluation of EMT6 triple negative breast cancer tumor growth (pre-randomization) in the 54 mice enrolled in the study.

FIG. 2: In vivo efficacy results with an exemplary composition comprising non-viable cells of *Streptococcus pyogenes* ("Composition 002" or "Comp. 002") in the EMT6 Model.

FIGS. 3A-3B: In vivo efficacy results with intratumor (**FIG. 3A**) or intravenous (**FIG. 3B**) delivery of Composition 002 as a single agent and in combination with anti-PD1 antibody.

FIGS. 4A-4B: Body weigh measure data of primary tumors (**FIG. 4A**) and spleens (**FIG. 4B**) of mice dosed with Composition 002 intratumorally as a single agent and in combination with anti-PD1 antibody.

FIGS. 5A-5B: Weigh measure data of primary tumors (**FIG. 5A**) and spleens (**FIG. 5B**) of mice dosed with Composition 002 intravenously as a single agent and in combination with anti-PD1 antibody.

FIGS. 6A-6B: Measure of mouse body weights average (**FIG. 6A**) and percent body weight change (FIG. 6B) in Balb/c mice bearing EMT6 tumors (pre-randomization).

FIGS. 7A-7B: Measure of mouse body weights average (**FIG. 7A**) and percent body weight change (**FIG. 7B**) in Balb/c mice bearing EMT6 tumors (post-randomization, all groups).

FIGS. 8A-8B: Measure of percent body weight change (post-randomization) in Balb/c mice bearing EMT6 tumors in the intratumor delivery groups (**FIG. 8A**) and intravenous delivery groups (**FIG. 8B**).

FIGS. 9A-9B: Analysis of % $CD3^+$ T cells (gated on $CD45^+$ $CD3^+$) in spleen (Left) and tumors-TILs (Right) of the intravenous treatment groups (**FIG. 9A**) and intratumor groups (**FIG. 9B**).

CLAIMS

1. Use of a composition comprising non-viable cells of *Streptococcus pyogenes* in the manufacture of a medicament for treating non-muscle invasive bladder cancer in a subject, wherein the subject is also to be administered an immune checkpoint inhibitor.

2. Use of a composition comprising non-viable cells of *Streptococcus pyogenes* in the manufacture of a medicament for combination therapy to treat non-muscle invasive bladder cancer in a subject, wherein the medicament is to be administered in combination with an immune checkpoint inhibitor.

3. Use of a composition comprising non-viable cells of *Streptococcus pyogenes* in the manufacture of a first medicament and use of an immune checkpoint inhibitor in the manufacture of a second medicament, wherein the first and second medicaments are to be used in combination therapy to treat non-muscle invasive bladder cancer in a subject.

4. Use of a pharmaceutical composition comprising non-viable cells of *Streptococcus pyogenes* in combination with an immune checkpoint inhibitor for treating non-muscle invasive bladder cancer in a subject.

5. Use of a medicament comprising a composition comprising non-viable cells of *Streptococcus pyogenes* in combination with an immune checkpoint inhibitor for treating non-muscle invasive bladder cancer in a subject.

6. The use of any one of claims 1-5, wherein the composition comprising non-viable cells of *Streptococcus pyogenes* is to be administered intravesically, intratumorally, intravenously, intramuscularly, subcutaneously, or intraperitoneally.

7. The use of any one of claims 1-6, wherein the non-viable cells of *Streptococcus pyogenes* are present in the composition in an amount of 10 KE or greater.

8. The use of any one of claims 1-6, wherein the non-viable cells of *Streptococcus pyogenes* are present in the composition in an amount of at least 20 KE.

9. The use of any one of claims 1-6, wherein the non-viable cells of *Streptococcus pyogenes* are present in the composition in an amount of at least 40 KE.

10. The use of any one of claims 1-6, wherein the non-viable cells of *Streptococcus pyogenes* are to be administered to the subject at a dose of 1 KE to 100 KE, 5 KE to 50 KE, or about 0.1 KE, 0.5 KE, 1 KE, 2.5 KE, 5 KE, 10 KE, 15 KE, 20 KE, 30 KE, 40 KE, 50 KE, 60 KE, 70 KE, 80 KE, 90 KE, or 100 KE.

11. The use of any one of claims 1-10, wherein the non-viable cells of *Streptococcus pyogenes* comprise cells of the Su strain of *Streptococcus pyogenes.*

12. The use of any one of claims 1-11, wherein the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof, a nucleic acid molecule, a gene editing system, or a small molecule.

13. The use of any one of claims 1-12, wherein the immune checkpoint inhibitor is an inhibitor of PD-1/PD-L1/PD-L2 axis, CD80, CD86, B7-H3, B7 H4, HVEM, adenosine, GAL9, VISTA, CEACAM-1, CTLA 4, BTLA, KIR, LAG3, TIM3, A2aR, CD244/2B4, CD160, TIGIT, LAIR-1, PVRIG/CD112R, arginase, indoleamine 2,3 dioxygenase (IDO), IL-10, IL-4, IL-1RA, IL-35, or any combination thereof.

14. The use of claim 13, wherein the inhibitor of the PD-1/PD-L1/PD-L2 axis comprises a PD-1 inhibitor, optionally wherein the PD-1 inhibitor is an antibody.

15. The use of claim 14, wherein the PD-1 antibody comprises pembrolizumab, nivolumab, cetrelimab, cemiplimab, sasanlimab, tislelizumab, or dostarlimab.

16. The use of claim 13, wherein the inhibitor of the PD-1/PD-L1/PD-L2 axis comprises a PD-L1 inhibitor, optionally wherein the PD-L1 inhibitor is an antibody.

17. The use of claim 16, wherein the PD-L1 antibody comprises atezolizumab, durvalumab, envafolimab, or avelumab.

18. The use of claim 13, wherein the inhibitor of the PD-1/PD-L1/PD-L2 axis comprises a PD-L2 inhibitor, optionally wherein the PD-L2 inhibitor is an antibody.

19. The use of claim 13, wherein the inhibitor of the PD-1/PD-L1/PD-L2 axis comprises a PD-1 inhibitor and a PD-L1 inhibitor, optionally wherein the PD-1 inhibitor and/or PD-L1 inhibitor is an antibody.

20. The use of any one of claims 1-19, wherein the non-muscle invasive bladder cancer is completely or partially resistant to the PD-1 inhibitor and/or PD-L1 inhibitor.

21. The use of claim 13, wherein the immune checkpoint inhibitor comprises a CTLA-4 inhibitor, optionally wherein the CTLA-4 inhibitor is an antibody.

22. The use of claim 21, wherein the CTLA-4 antibody comprises ipilimumab, tremelimumab, tuvonralimab.

23. The use of any one of claims 1-22, wherein the composition comprising non-viable cells of *Streptococcus pyogenes* is to be administered at least one day prior to the immune checkpoint inhibitor.

24. The use of any one of claims 1-23, wherein the subject is to be administered an additional anti-cancer agent.

25. The use of any one of claims 1-24, wherein the composition comprising non-viable cells of *Streptococcus pyogenes* is to be administered prior to, concurrently with, or subsequent to the immune checkpoint inhibitor.

26. The use of any one of claims 1-25, wherein the composition comprising non-viable cells of *Streptococcus pyogenes* comprises *Streptococcus pyogenes* [A Group, Type 3] Su strain.

27. The use of any one of claims 1-26, wherein the composition comprising non-viable cells of *Streptococcus pyogenes* comprises benzylpenicillin-treated *Streptococcus pyogenes.*

28. The use of any one of claims 1-27, wherein the composition comprising non-viable cells of *Streptococcus pyogenes* further comprises maltose, magnesium sulfate, potassium dihydrogen phosphate, sodium chloride, methionine, and benzylpenicillin.

29. The use of any one of claims 1-28, wherein the composition comprising non-viable cells of *Streptococcus pyogenes* is a lyophilized composition, optionally wherein the lyophilized composition is reconstituted prior to administration.

30. The use of any one of claims 1-29, wherein the subject has low grade non-muscle invasive bladder cancer.

31. The use of any one of claims 1-29, wherein the subject has high-grade non-muscle invasive bladder cancer.

32. The use of any one of claims 1-29, wherein the non-muscle invasive bladder cancer has been identified as low risk.

33. The use of any one of claims 1-29, wherein the non-muscle invasive bladder cancer has been identified as intermediate risk.

34. The use of any one of claims 1-29, wherein the non-muscle invasive bladder cancer has been identified as high risk.

35. The use of any one of claims 1-29, wherein the non-muscle invasive bladder cancer has been identified as high-grade Ta or T1.

36. The use of any one of claims 1-29, wherein the non-muscle invasive bladder cancer has been identified as cancer in situ (CIS) with or without Ta and/or T1.

37. The use of any one of claims 1-36, wherein the non-muscle invasive bladder cancer is recurrent.

38. The use of any one of claims 1-37, wherein the subject has not received prior BCG treatment.

39. The use of any one of claims 1-37, wherein the subject has received adequate BCG treatment.

40. The use of any one of claims 1-37, wherein the subject is unresponsive to BCG treatment.

41. The use of any one of claims 1-37, wherein the non-muscle invasive bladder cancer is BCG failed, BCG refractory, or BCG relapsing.

42. Use of a composition comprising non-viable cells of *Streptococcus pyogenes* in the manufacture of a medicament for combination therapy to treat non-muscle invasive bladder cancer in a subject, wherein the medicament is to be administered in combination with an anti-PD-1 antibody, and wherein the medicament is formulated for intravesical administration to the subject.

43. The use of claim 42, wherein the anti-PD-1 antibody comprises pembrolizumab.

44. The use of claim 42 or 43, wherein the anti-PD-1 antibody is to be administered intravenously.

45. The use of any one of claims 42-44, wherein the anti-PD-1 antibody is to be administered at a dose of 5 mg/kg to 10 mg/kg.

46. The use of any one of claims 42-44, wherein the anti-PD-1 antibody is to be administered at a dose of about 400 mg.

47. The use of any one of claims 1-46, wherein the medicament is formulated to be administered once a week.

48. The use of any one of claims 42-47, wherein the anti-PD-1 antibody is to be administered every 6 weeks.

49. Use of a composition comprising non-viable cells of *Streptococcus pyogenes* in the manufacture of a medicament for a combination therapy to treat high-grade non-muscle invasive bladder cancer in a human adult subject, wherein:

(1) the medicament is formulated to be administered in combination with pembrolizumab

(2) the medicament is formulated for intravesical administration to the subject; and

(3) the pembrolizumab is to be administered intravenously to the subject;

wherein the high grade non-muscle invasive bladder cancer is Bacillus Calmette-Guerin (BCG) failed, BCG-unresponsive, BCG-refractory, BCG-relapsing, BCG inadequately treated, the subject has received adequate BCG treatment, or any combination thereof.

50. Use of a composition comprising non-viable cells of *Streptococcus pyogenes* in the manufacture of a medicament for a combination therapy to treat high-grade non-muscle invasive bladder cancer in a human adult subject, wherein:

(1) the medicament is formulated to be administered in combination with pembrolizumab

(2) the medicament is formulated for intravesical administration to the subject; and

(3) the pembrolizumab is to be administered intravenously to the subject;

wherein:

the high grade non-muscle invasive bladder cancer is Bacillus Calmette-Guerin (BCG) failed, BCG-unresponsive, BCG-refractory, BCG-relapsing, BCG inadequately treated, the subject has received adequate BCG treatment, or any combination thereof; and

the medicament is formulated to be administered in at least six weekly 40 KE doses of the composition comprising non-viable cells of *Streptococcus pyogenes*, and

at least two 400 mg doses of pembrolizumab are to be administered to the subject six weeks apart.