[0006] Accordingly, there remains a need in the art for methods for inhibiting tumor cell growth at a second tumor site distinct from a primary cancer and for treating or suppressing tumor metastasis at a site distinct from the primary cancer in an individual having a carcinoma. Fortunately, the present disclosure provides for these and other needs.

SUMMARY

[0007] The present disclosure resolves the still unmet need in the art for inhibiting tumor metastasis at distant sites, by the localized delivery and expression of IL-12 together with a Type I interferon (IFN-1) activator/inducer, *e.g.* a RIG-I agonist, a STING agonist, and/or a TLR 7/9 agonist, at a primary tumor site. As demonstrated herein for the first time, the subject therapy stimulates a robust immune response against the primary cancer including cytotoxic CD8+ T cells as well as CD4+ memory T cells, with the latter cell population in particular supporting the systemic effects of the subject therapy on distant metastases. In some examples, the primary tumor site is a mucosal tissue. In some examples, the primary tumor site is other than a mucosal tissue. In preferred examples, the subject methods and compositions comprise the co-expression of IL-12 with at least one RIG-I agonist.

[0008] The disclosure provides a method for activating a memory T cell response to a cancer antigen. The method comprises contacting a primary cancer with a therapeutically effective amount of a composition comprising a nucleic acid polyplex comprising a cationic polymer and/or lipid, a therapeutic nucleic acid construct encoding interleukin-12 (IL-12), and a therapeutic nucleic acid construct comprising a nucleic acid encoding at least one RIG-I agonist, wherein the therapeutic nucleic acid constructs encoding IL-12 and RIG-I are the same or different nucleic acid constructs.

[0009] In some examples, the method is effective for treating or suppressing a primary cancer. In examples, the primary cancer is selected from a breast cancer, colon cancer, prostate cancer, pancreatic cancer, melanoma, lung cancer, ovarian cancer, kidney cancer, brain cancer, a sarcoma, bladder cancer, vaginal cancer, cervical cancer, stomach cancer, a cancer of the gastrointestinal tract, kidney cancer, liver cancer, thyroid cancer, esophageal cancer, nasal cancer, laryngeal cancer, oral cancer, pharyngeal cancer, retinoblastoma, endometrial cancer, and testicular cancer. In examples, the primary cancer is other than a mucosal cancer.

[0010] In some examples, the method is effective for treating or suppressing metastatic disease at a site distinct from a primary cancer. In examples, the primary cancer is selected from a breast cancer, colon cancer, prostate cancer, pancreatic cancer, melanoma, lung cancer, ovarian cancer, kidney cancer, brain cancer, a sarcoma, bladder cancer, vaginal cancer, cervical cancer, stomach cancer, a cancer of the gastrointestinal tract, kidney cancer, liver cancer, thyroid cancer, esophageal cancer, nasal cancer, laryngeal cancer, oral cancer, pharyngeal cancer, retinoblastoma, endometrial cancer, and testicular cancer. In some examples, the site distinct from the primary cancer is at one or more of: liver, lung, bone, brain, lymph node, peritoneum, skin, prostate, breast, colon, rectum, and cervix. In some examples, the metastatic disease is at two or more sites distinct from the primary cancer.

[0011] In some examples, the RIG-I agonist is selected from the group consisting of eRNA11a, VA RNA1, eRNA41H, MK4621, SLR10, SLR14, and SLR20, and more preferably selected from the group consisting of eRNA41H, eRNA11a.

[0012] In some examples, the cationic polymer is selected from the group consisting of polyethyleneimine (PEI), PAMAM, polylysine (PLL), polyarginine, chitosan, and derivatives thereof. In some examples, the cationic polymer comprises a derivatized chitosan, preferably an amino-functionalized chitosan. In some examples, the amino-functionalized chitosan comprises arginine and further comprises, or is functionalized with, a hydrophilic polyol. In some examples, the hydrophilic polyol is selected from gluconic acid and glucose.

[0013] In some examples, the nucleic acid polyplex further comprises a reversible coating comprising one or more polyanion-containing block co-polymers having at least one polyanionic anchor region and at least one hydrophilic tail region, preferably wherein the polyanion-containing block co-polymer is a linear diblock and/or triblock co-polymer.

[0014] In some examples, the therapeutic nucleic acid construct encoding IL-12, comprises SEQ ID NO: 8.

[0014a] In one aspect, there is provided use of a composition in the manufacture of a medicament for activating a memory T cell response to a primary cancer in a patient in need thereof, wherein the composition comprises a nucleic acid polyplex comprising a cationic polymer and/or lipid, a therapeutic nucleic acid construct encoding interleukin-12 (IL-12), and a

therapeutic nucleic acid construct comprising a nucleic acid encoding at least one RIG-I agonist, wherein the therapeutic nucleic acid constructs encoding IL-12 and at least one RIG-I agonist are the same or different nucleic acid constructs.

[0015] I disclosure also provides a method for treating or suppressing tumor metastasis at a site distinct from a primary cancer in an individual having a primary cancer such as, *e.g.*, bladder cancer, wherein the method comprises contacting to the primary cancer with a therapeutically effective amount of a composition comprising a nucleic acid polyplex comprising a cationic polymer and/or lipid, a therapeutic nucleic acid construct encoding interleukin-12 (IL-12), and a therapeutic nucleic acid construct comprising a nucleic acid encoding at least one

RIG-I agonist, wherein the therapeutic nucleic acid constructs encoding IL-12 and RIG-I are the same or different nucleic acid constructs.

[0016] In examples, the primary cancer is a cancer selected from a breast cancer, colon cancer, prostate cancer, pancreatic cancer, melanoma, lung cancer, ovarian cancer, kidney cancer, brain cancer, a sarcoma, bladder cancer, vaginal cancer, cervical cancer, stomach cancer, a cancer of the gastrointestinal tract, kidney cancer, thyroid cancer, esophageal cancer, nasal cancer, laryngeal cancer, oral cancer, pharyngeal cancer, retinoblastoma, endometrial cancer, and testicular cancer. In one example, the primary cancer is a mucosal cancer selected from the group consisting of a gastrointestinal cancer, a nasal or pulmonary cancer, and a genitourinary cancer. In some examples, the primary mucosal cancer is a gastrointestinal cancer, selected from the group consisting of an oral cancer, an esophageal cancer, a stomach cancer, a pancreatic cancer, a liver cancer, a colorectal cancer, and a rectal cancer. In some examples, the primary mucosal cancer is a nasal or pulmonary cancer selected from the group consisting of a paranasal sinus cancer, an oropharyngeal cancer, a tracheal cancer, and a lung cancer. In some examples, the primary mucosal cancer is a genitourinary cancer selected from the group consisting of a bladder cancer, a urothelial cancer, a urethral cancer, a testicular cancer, a kidney cancer, a prostate cancer, a penile cancer, an adrenal cancer, a uterine cancer, a cervical cancer, and an ovarian cancer. In some examples, the genitourinary cancer is bladder cancer.

[0017] In some examples, the tumor metastatic site is at one or more of: liver, lung, bone, brain, lymph node, peritoneum, skin, prostate, breast, colon, rectum, and cervix. In some examples, the tumor metastasis is at two or more different sites.

[0018] In some examples, the RIG-I agonist is selected from the group consisting of eRNA11a, VA RNA1, eRNA41H, MK4621, SLR10, SLR14, and SLR20, and more preferably selected from the group consisting of eRNA41H, eRNA11a.

[0019] In other examples, the cationic polymer is selected from the group consisting of polyethyleneimine (PEI), PAMAM, polylysine (PLL), polyarginine, chitosan, and derivatives thereof. In another example, the cationic polymer comprises a derivatized chitosan, preferably an amino-functionalized chitosan.

[0020] In some examples, the cationic polymer is an amino-functionalized chitosan that comprises arginine and further comprises, or is functionalized with, a hydrophilic polyol. In some examples, the hydrophilic polyol is selected from gluconic acid and glucose.

[0021] In some examples, the nucleic acid polyplex further comprises a reversible coating comprising one or more polyanion-containing block co-polymers having at least one polyanionic anchor region and at least one hydrophilic tail region, preferably wherein the polyanion-containing block co-polymer is a linear 5eblock and/or triblock co-polymer.

[0022] In some examples, therapeutic nucleic acid construct encoding IL-12 comprises SEQ ID NO: 8.

[0023] In some examples, the contacting comprises intravesical instillation. In another example, the administration is oral dosage or intrarectal/intracolonic to gastrointestinal tract (GIT). In some examples, the administration is intrarectal/intracolonic administration to the gastrointestinal tract (GIT). In still other examples, the contacting is by intratumoral injection. In still other examples, the contacting is intranasal or intratracheal administration to the lungs.

[0024a] In another aspect, there is provided use of a composition in the manufacture of a medicament for treating or suppressing tumor metastasis at a site distinct from the site of a primary cancer in a patient in need thereof, wherein the composition comprises a nucleic acid polyplex comprising a cationic polymer and/or lipid, a therapeutic nucleic acid construct encoding interleukin-12 (IL-12), and a therapeutic nucleic acid construct comprising a nucleic acid encoding at least one RIG-I agonist, wherein the therapeutic nucleic acid constructs encoding IL-12 and at least one RIG-I agonist are the same or different nucleic acid constructs.

[0024] Other features, objects and advantages will be apparent from the disclosure that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] The present disclosure is disclosed with reference to the accompanying drawings, wherein:

[0026] **FIG. 1 (A)** Experimental treatment timeline of female C57BL/6J mice with mEG-70 constructs in an orthotopic model of bladder cancer. Mice bladders were instilled with MB49-Luciferase cells (MB49-Luc; 1 x 10^5 cells) at Day 1. Implantation was confirmed by in vivo imaging of luciferase signal at Day 9 post instillation. Mice were distributed equally to treatment groups (n = 22) based on the level of bioluminescence and received an intravesical instillation (IVI) of mEG-70 (1 mg DNA/mL; equivalent to 80 µg DNA) on Day 10 (Tx1) and Day 17

(Tx2), with control animals receiving an instillation of 1% mannitol (sham). A cohort of tumor-bearing animals was untreated. Survival was monitored for 85 days. **(B)** mEG-70-treated animals exhibited long-term survival compared to control mice, of which approximately 70% succumbed to disease. The survival curve for mEG-70 is significantly different from the survival of sham-treated (1% mannitol) or untreated mice (*$p<0.05$ and **$p<0.01$, respectively). **(C)** Mice treated with mEG-70 that demonstrated complete disease regression and did not relapse during the 76-day observation period (referred to as 'mEG-70 cured'), were re-

CLAIMS

1. Use of a composition in the manufacture of a medicament for activating a memory T cell response to a primary cancer in a patient in need thereof, wherein the composition comprises a nucleic acid polyplex comprising a cationic polymer and/or lipid, a therapeutic nucleic acid construct encoding interleukin-12 (IL-12), and a therapeutic nucleic acid construct comprising a nucleic acid encoding at least one RIG-I agonist, wherein the therapeutic nucleic acid constructs encoding IL-12 and at least one RIG-I agonist are the same or different nucleic acid constructs.

2. The use of claim 1, wherein the medicament is effective for treating or suppressing a primary cancer in said patient other than a mucosal tumor.

3. The use of claim 1, wherein the medicament is effective for treating or suppressing metastatic disease in said patient at a site distinct from the primary cancer.

4. The use of claim 1, wherein the primary cancer is selected from breast cancer, colon cancer, prostate cancer, pancreatic cancer, melanoma, lung cancer, pulmonary cancer, ovarian cancer, kidney cancer, brain cancer, a sarcoma, bladder cancer, vaginal cancer, cervical cancer, stomach cancer, gastrointestinal cancer, liver cancer, thyroid cancer, esophageal cancer, nasal cancer, laryngeal cancer, oral cancer, pharyngeal cancer, retinoblastoma, endometrial cancer, and testicular cancer.

5. The use of claim 3, wherein the site distinct from the primary cancer is one or more of: liver, lung, bone, brain, lymph node, peritoneum, skin, prostate, breast, colon, rectum, and cervix.

6. The use of claim 3, wherein the metastatic disease is at two or more sites distinct from the primary cancer.

7. The use of claim 1, wherein said at least one RIG-I agonist is selected from the group consisting of eRNA11a, VA RNA1, eRNA41H, MK4621, SLR10, SLR14, and SLR20.

8. The use of claim 7, wherein said at least one RIG-I agonist is eRNA41H and/or eRNA11a.

9. The use of claim 1, wherein said cationic polymer is selected from the group consisting of polyethyleneimine (PEI), PAMAM, polylysine (PLL), polyarginine, chitosan, and derivatives thereof.

10. The use of claim 9, wherein the cationic polymer comprises a derivatized chitosan.

11. The use of claim 10, wherein the derivatized chitosan is amino-functionalized chitosan.

12. The use of claim 11, wherein said amino-functionalized chitosan comprises arginine and further comprises, or is functionalized with, a hydrophilic polyol.

13. The use of claim 12, wherein the hydrophilic polyol is selected from gluconic acid and glucose.

14. The use of claim 1, wherein the nucleic acid polyplex further comprises a reversible coating comprising one or more polyanion-containing block co-polymers having at least one polyanionic anchor region and at least one hydrophilic tail region.

15. The use of claim 14, wherein the polyanion-containing block co-polymer is a linear diblock and/or triblock co-polymer.

16. The use of claim 1, wherein the therapeutic nucleic acid construct encoding IL-12 and/or the therapeutic nucleic acid construct encoding the at least one RIG-I agonist is comprised within a plasmid selected from the group consisting of: gWIZ, pVAX, NTC8685 and NTC9385R.

17. The use of claim 16, wherein said therapeutic nucleic acid construct encoding IL-12 and/or the therapeutic nucleic acid construct encoding the at least one RIG-I agonist is comprised within a NTC9385R plasmid.

18. The use of claim 1, wherein said therapeutic nucleic acid construct encoding IL-12 and/or the therapeutic nucleic acid construct encoding the at least one RIG-I agonist further comprises an expression control element selected from the group consisting of CMV, EF1a, CMV/EF1a, CAG, and CMV/EF1a/HTLV promoters.

19. The use of claim 18, wherein said therapeutic nucleic acid construct encoding IL-12 and/or the therapeutic nucleic acid construct encoding the at least one RIG-I agonist further comprises a CMV promoter.

20. Use of a composition in the manufacture of a medicament for treating or suppressing tumor metastasis at a site distinct from the site of a primary cancer in a patient in need thereof, wherein the composition comprises a nucleic acid polyplex comprising a cationic polymer and/or lipid, a therapeutic nucleic acid construct encoding interleukin-12 (IL-12), and a therapeutic nucleic acid construct comprising a nucleic acid encoding at least one RIG-I agonist, wherein the therapeutic nucleic acid constructs encoding IL-12 and at least one RIG-I agonist are the same or different nucleic acid constructs.

21. The use of claim 20, wherein the primary cancer is selected from a breast cancer, colon cancer, prostate cancer, pancreatic cancer, melanoma, lung cancer, pulmonary cancer, ovarian cancer, kidney cancer, brain cancer, a sarcoma, bladder cancer, vaginal cancer, cervical cancer, stomach cancer, gastrointestinal cancer, liver cancer, thyroid cancer, esophageal cancer, nasal cancer, laryngeal cancer, oral cancer, pharyngeal cancer, retinoblastoma, endometrial cancer, and testicular cancer.

22. The use of claim 21, wherein the primary cancer is

a gastrointestinal cancer selected from the group consisting of an oral cancer, an esophageal cancer, a stomach cancer, a pancreatic cancer, a liver cancer, a colorectal cancer, and a rectal cancer;

a nasal or pulmonary cancer selected from the group consisting of a paranasal sinus cancer, an oropharyngeal cancer, a tracheal cancer, and a lung cancer; or

a genitourinary cancer selected from the group consisting of a bladder cancer, a urothelial cancer, a urethral cancer, a testicular cancer, a kidney cancer, a prostate cancer, a penile cancer, an adrenal cancer, a uterine cancer, a cervical cancer, and an ovarian cancer.

23. The use of claim 22, wherein the genitourinary cancer is bladder cancer.

24. The use of claim 20, wherein the tumor metastatic site is at one or more of: liver, lung, bone, brain, lymph node, peritoneum, skin, prostate, breast, colon, rectum, and cervix.

25. The use of claim 20, wherein the tumor metastasis is at two or more different sites.

26. The use of claim 20, wherein said at least one RIG-I agonist is selected from the group consisting of eRNA11a, VA RNA1, eRNA41H, MK4621, SLR10, SLR14, and SLR20, and more preferably selected from the group consisting of eRNA41H, eRNA11a.

27. The use of claim 26, wherein said at least one RIG-I agonist is eRNA41H or eRNA11a.

28. The use of claim 20, wherein said cationic polymer is selected from the group consisting of polyethyleneimine (PEI), PAMAM, polylysine (PLL), polyarginine, chitosan, and derivatives thereof.

29. The use of claim 28, wherein the cationic polymer comprises a derivatized chitosan.

30. The use of claim 29, wherein the derivatized chitosan is an amino-functionalized chitosan.

31. The use of claim 30, wherein said amino-functionalized chitosan comprises arginine and further comprises, or is functionalized with, a hydrophilic polyol.

32. The use of claim 31, wherein the hydrophilic polyol is selected from gluconic acid and glucose.

33. The use of claim 20, wherein the nucleic acid polyplex further comprises a reversible coating comprising one or more polyanion-containing block co-polymers having at least one polyanionic anchor region and at least one hydrophilic tail region.

34. The use of claim 33, wherein the polyanion-containing block co-polymer is a linear diblock and/or triblock co-polymer.

35. The use of claim 20, wherein the therapeutic nucleic acid construct encoding IL-12 and/or the therapeutic nucleic acid construct encoding the at least one RIG-I agonist is comprised within a plasmid selected from the group consisting of: gWIZ, pVAX, NTC8685 and NTC9385R.

36. The use of claim 35, wherein said therapeutic nucleic acid construct encoding IL-12 and/or the therapeutic nucleic acid construct encoding the at least one RIG-I agonist is comprised within a NTC9385R plasmid.

37. The use of claim 20, wherein said therapeutic nucleic acid construct encoding IL-12 and/or the therapeutic nucleic acid construct encoding the at least one RIG-I agonist

further comprises an expression control element selected from the group consisting of CMV, EF1a, CMV/EF1a, CAG, and CMV/EF1a/HTLV promoters.

38. The use of claim 37, wherein said therapeutic nucleic acid construct encoding IL-12 and/or the therapeutic nucleic acid construct encoding the at least one RIG-I agonist further comprises a CMV promoter.

39. The use of claim 20, wherein the medicament is a dosage form for intravesical instillation.

40. The use of claim 20, wherein the medicament is a dosage form for oral administration or for intrarectal/intracolonic administration to the gastrointestinal tract (GIT).

41. The use of claim 20, wherein the medicament is a dosage form for intratumoral injection.

42. The use of claim 20, wherein the medicament is a dosage form for intranasal or intratracheal administration to the lungs.

43. The use according to claim 15 or 34, wherein the cationic polymer is a derivatized chitosan functionalized with arginine and glucose; the the polyanion-containing block co-polymer is PEG-b-PLE; and the therapeutic nucleic acid construct encoding IL-12 and the therapeutic nucleic acid construct encoding at least one RIG-I agonist are the same and comprise SEQ ID NO: 8.