the tumor microenvironment. The current knowledge base regarding IL-12 combination therapies is quite limited, however, and has focused primarily on the co-administration and/or co-expression of IL-12 with an additional immunomodulatory cytokine such as IL-2, IL-7, IL-15, IL-18 and IL-21. See, e.g. Weiss et al. , Expert Opin Biol Ther. 7: 1705-1721 (2007). Notably, the potential synergistic interaction between IL-12 and alternative innate and/or adaptive immune stimulation strategies has been largely unexplored.

[0009] Pattern recognition receptors (PRR) comprise another element of the innate immune system, recognizing conserved pathogen-associated nucleic acid (NA) sequences. PRR activation by their nucleotide ligand induces production of type I interferons (IFN) and proinflammatory cytokines, serving as the first line of defense for viral and microbial infections. Iwasaki and Medzhitov, Science 327:291-95 (2010). These NA-sensing PRRs include the endosomal toll-like receptor (TLR) family (Majer et al. , Curr. Opin. Immunol. 44:26-33 (2017)), the cytosolic DNA sensors AIM 2 and cyclic GMP-AMP synthetase (cGAS), part of the cGAS-stimulator of interferon (STING) pathway (Chen et al ., Nat. Immunol. 17: 1142-49 (2016)), and the cytosolic RNA sensors retinoic acid-inducible gene I (RIG-I)-like receptor family (Schlee, Immunobiology , 218: 1322-35 (2013)). Interestingly, there are remarkably few references evaluating both IL-12 and RIG-I, and the few that do demonstrate no meaningful interaction between the two. Jiang et al. (J. Exp. Med. 216:2854-68 (2019)) showed that eliminating endogenous IL-12 in vivo by way of a neutralizing antibody had no effect on an induced RIG-I response, while the earlier studies by Kong et al. (Cell Host & Microbe 6: 150-61 (2009)) showed that altering levels of RIG-I had no effect upon IL-12 expression in cell-based assays. As such, the combination of IL-12 and RIG-I in particular would appear to be unavailing.

SUMMARY OF THE INVENTION

[0009a] In one aspect, provided herein is 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 encoding at least one RIG-I agonist, wherein the therapeutic nucleic acid constructs encoding IL-12 and the at least one RIG-I are the same or different nucleic acid constructs.

[0009b] In another aspect, provided herein is use of the composition as described herein in the manufacture of a medicament for treating mucosal cancer, wherein the medicament locally expresses IL-12 in a mucosal tissue in a patient in need thereof.

[0009c] In another aspect, provided herein is use of the composition as described herein in the manufacture of a medicament for treating bladder cancer in a patient in need thereof.

[0009d] In another aspect, provided herein is the composition as described herein for use in therapy.

[0010] The present invention resolves the still unmet need in the art for the effective localized expression of IL-12, employing derivatized-chitosan polyplexes reversibly coated with a polyanion-containing block co-polymer for robust transfection of mucosal tissues present in or proximal to a tumor. Moreover, as presented herein for the first time, the effective localized expression of IL-12 obtained with the subject compositions and methods can be advantageously combined with one or more additional innate and/or adaptive immune stimulation strategies to further enhance the cytotoxic immune response against the tumor. In preferred embodiments, IL-

CLAIMS:

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

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

3. The composition according to claim 2, wherein said RIG-I agonist is eRNA41H and/or eRNA11a.

4. The composition according to any one of claims 1-3, wherein said cationic polymer is selected from the group consisting of polyethyleneimine (PEI), PAMAM, polylysine (PLL), polyarginine, chitosan, and chitosan derivatives.

5. The composition according to claim 4, wherein the cationic polymer comprises, or is, a derivatized chitosan.

6. The composition according to claim 5, wherein the derivatized chitosan is an amino-functionalized chitosan.

7. The composition according to claim 6, wherein said amino-functionalized chitosan comprises arginine.

8. The composition according to claim 7, wherein said chitosan further comprises, or is functionalized with, a hydrophilic polyol.

9. The composition according to claim 8, wherein said hydrophilic polyol is glucose or gluconic acid.

10. The composition according to any one of claims 1-9, 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.

11. The composition according to claim 10, wherein the polyanion-containing block co-polymer is a linear diblock and/or triblock co-polymer.

12. The composition according to any one of claims 1-11, wherein said therapeutic nucleic acid construct encoding IL-12 comprises SEQ ID NO: 7.

13. The composition according to any one of claims 1-12, 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 plasmid selected from the group consisting of: gWIZ, pVAX, NTC8685 or NTC9385R.

14. The composition according to claim 13, 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.

15. The composition according to any one of claims 1-14, wherein the 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, EFla, CMV/EFla, CAG, and CMV/EFla/HTLV promoters.

16. The composition according to claim 15, wherein the expression control element comprises a CMV promoter.

17. The composition according to any one of claims 1-16, wherein the 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 synthetic Beta-globin-based intron.

18. The composition according to any one of claims 1-17, wherein the 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 HTLV-IR.

19. The composition according to any one of claims 1-18, wherein the therapeutic nucleic acid construct encoding IL-12 and/or the therapeutic nucleic acid construct encoding the at least one RIG-I agonist further comprises kanamycin selection or sucrose-based selection element.

20. The composition according to claim 19, wherein the 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 sucrose-based selection element.

21. The composition according to any one of claims 1-20, wherein the therapeutic nucleic acid construct encoding IL-12 and/or the therapeutic nucleic acid construct encoding the at least one RIG-I agonist further comprises a pUC or R6K origin of replication.

22. The composition according to claim 21, wherein the 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 R6K origin of replication.

23. The composition according to any one of claims 1-22, wherein the cationic polymer is a derivatized chitosan functionalized with arginine and glucose, the polyanion-containing block co-polymer is PEG-b-PLE, and the therapeutic nucleic acid construct encoding IL-12 and the at least one therapeutic nucleic acid construct encoding the at least one RIG-I agonist are the same and comprise SEQ ID NO: 7.

24. Use of the composition according to any one of claims 1-23 in the manufacture of a medicament for treating mucosal cancer, wherein the medicament locally expresses IL-12 in a mucosal tissue in a patient in need thereof.

25. Use of the composition according to any one of claims 1-23 in the manufacture of a medicament for treating bladder cancer in a patient in need thereof.

26. The use according to claim 24 or 25, further comprising that an additional immunostimulatory molecule selected from the group consisting of IFN-1 activators and immune checkpoint inhibitors is to be administered simultaneously or sequentially.

27. The use according to claim 26, wherein said IFN-1 agonist and/or said immune checkpoint inhibitor is to be administered separately.

28. The use according to claim 26 or 27, wherein said immune checkpoint inhibitor is selected from the group consisting of ipilimumab, tremelimumab, nivolumab, atezolizumab and/or pembrolizumab.

29. The composition according to any one of claims 1-23 for use in therapy.