35(1), pp 567-572). More generally, about 12% of all human cancers worldwide are caused by oncogenic virus infection (see e.g., Mesri et al., 2014, Cell Host & Microbe 15:266-282; Bouvard, V., et al. (2009). Lancet Oncol. 10, 321–322; Boyle, P., and Levin, B. (2008). World Cancer Report 2008. (Lyon: International Agency for Research on Cancer and World Health Organization Press); de Martel, C., et al., (2012). Lancet Oncol. 13, 607–615).

[0011] The present invention provides an immunotherapeutic treatment of cancers, including virus-induced cancers. These and other advantages are provided in the present disclosure.

BRIEF SUMMARY

[0012] The present disclosure provides compositions and methods for enhancing immunotherapy of cancer, such as virus-induced cancer, through combination of local and systemic immune stimulation. In one embodiment of the present disclosure, a method of treating cancer is provided comprising a) inducing an immune response specific for an immunogen in a subject, comprising administering to the subject a first composition comprising a vector particle, the vector particle comprising a recombinant expression vector wherein the recombinant expression vector comprises a polynucleotide encoding the immunogen, and wherein the polynucleotide is operatively linked to at least one regulatory expression sequence; and b) administering locally to the subject a second composition comprising a pharmaceutically suitable adjuvant, wherein the second composition does not comprise or is substantially devoid of immunogen, wherein the first composition and the second composition each further comprise a pharmaceutically suitable excipient, and wherein the first composition and second composition are administered concurrently or sequentially. In certain embodiments, administration of the second composition induces expression of one or more cytokines capable of recruiting CD8 T-cells to the local site of administration.

[0012a] In one aspect of the present disclosure, there is provided a use of a composition comprising

a) a first composition comprising a vector particle, the vector particle comprising a recombinant expression vector wherein the recombinant expression vector comprises a polynucleotide encoding a cancer-associated antigen, and wherein the polynucleotide is operatively linked to at least one regulatory expression sequence, thereby inducing an immune response against the cancer-associated antigen in the subject; and

b) a second composition comprising a TLR4 agonist; wherein the second composition does not comprise antigen;

wherein the first composition and the second composition each further comprise a pharmaceutically suitable excipient,

in the manufacture of a medicament for treating a cancer and increasing T cells in a tumor environment in a subject;

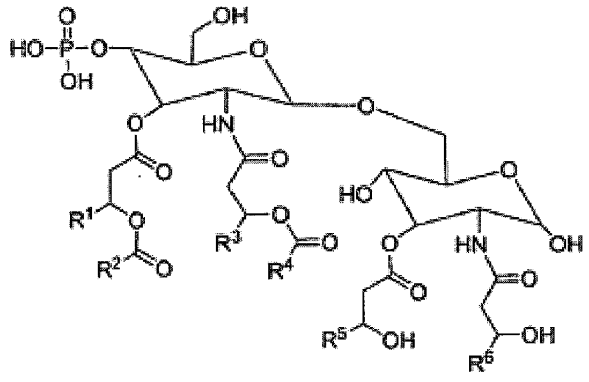
wherein the first composition is to be administered systemically to the subject; and wherein the second composition is to be administered intratumorally, peritumorally, intranodally or intravesically to the subject; and wherein the first composition and second composition are to be administered concurrently or sequentially, thereby increasing T cells in the thumor environment; and.

wherein the TLR4 agonist is a non-toxic lipid A-related adjuvant glucopyranosyl lipid A (GLA).

[0012b] In a further aspect of the present disclosure, there is provided a use of a composition comprising:

a) a first composition comprising a lentiviral vector particle, the lentiviral vector particle comprising a recombinant expression vector, wherein the recombinant expression vector comprises a polynucleotide encoding an immunogen associated with cancer, wherein the polynucleotide is operatively linked to at least one regulatory expression sequence, wherein the lentiviral vector particle further comprises an envelope comprising a Sindbis virus E2 glycoprotein having at least one amino acid change compared to SEQ ID NO:1, wherein residue 160 is either absent or an amino acid other than glutamic acid, and wherein E2 glycoprotein is not part of a fusion protein with Sindbis virus E3 protein; and

b) a second composition comprising a TLR4 agonist, wherein the second composition does not comprise an immunogen; wherein the TLR4 agonist is glucopyranosyl lipid A (GLA) formulated in a stable oil-in-water emulsion, wherein the GLA has the formula:



where: R^1, R^3, R^5 and R^6 equal to undecyl, and R^2 and R^4 equal to tridecyl;

wherein the first composition and the second composition each further comprise a pharmaceutically suitable excipient,

in the manufacture of a medicament for treating a cancer and increasing T cells in a tumor environment in a subject,

wherein the first composition is to be administered intramuscularly, intradermally or subcutaneously and induces an immune response specific for the immunogen associated with the cancer in the subject; and wherein the second composition is to be administered intratumorally or peritumorally; and wherein the first composition or second composition are to be administered concurrently or sequentially; thereby increasing T cells in the tumor environment.

[0012c] In another aspect of the present disclosure, there is provided a use of a composition comprising:

a) a first composition comprising

i. antigen-specific T cells specific for a cancer-associated antigen, said antigen-specific T cells having been expanded *ex vivo*,

ii. T cells that have been genetically modified to express a chimeric antigen receptor (CAR) that recognizes the cancer-associated antigen, or

iii. T cells that have been genetically modified to express a specific chimeric T cell receptor (TCR) that recognizes the cancer-associated antigen in the context of MHC; and

b) a second composition comprising a pharmaceutically suitable adjuvant; wherein the second composition does not comprise an immunogen;

wherein the first composition and the second composition each further comprise a pharmaceutically suitable excipient;

in the manufacture of a medicament for treating a cancer and increasing T cells in a tumor environment in a subject;

wherein the first composition is to be administered systemically to the subject; and the second composition is to be administered intratumorally and wherein the first composition and second composition are to be administered concurrently or sequentially; thereby increasing T cells in the tumor environment; and.

wherein the adjuvant is a non-toxic lipid A-related adjuvant glucopyranosyl lipid A (GLA).

[0012d] In a further aspect of the present disclosure, there is provided a use of a composition comprising:

a) a first composition comprising a vector particle, the vector particle comprising a recombinant expression vector wherein the recombinant expression vector comprises a polynucleotide encoding a cancer-associated antigen, and wherein the polynucleotide is operatively linked to at least one regulatory expression sequence, and

b) a second composition comprising a TLR4 agonist wherein the composition does not comprise antigen;

wherein the first composition and the second composition each further comprise a pharmaceutically suitable excipient,

in the manufacture of a medicament for increasing T cells in a tumor microenvironment in a subject,

where the first composition is to be administered systemically, thereby inducing an immune response against the cancer-associated antigen in the subject; and where the second composition is to be administered intratumorally or peritumorally to the subject; and where the first and second composition are to be administered concurrently or sequentially; thereby increasing T cells in the tumor microenvironment; and,

wherein the TLR4 agonist is a non-toxic lipid A-related adjuvant flucopyranosyl lipid A (GLA).

[0013] In another embodiment, an aforementioned method is provided wherein the first composition and the second composition are administered sequentially, and wherein the first composition is administered to the subject at a first site and the second composition is administered to the subject at a second site, wherein the first site and the second site are different. In still another embodiment, an aforementioned method is provided wherein the first composition is administered at the first site via a first route and the second composition is administered at the second site via a second route.

CLAIMS

We claim the following:

1. Use of a composition comprising

a) a first composition comprising a vector particle, the vector particle comprising a recombinant expression vector wherein the recombinant expression vector comprises a polynucleotide encoding a cancer-associated antigen, and wherein the polynucleotide is operatively linked to at least one regulatory expression sequence, thereby inducing an immune response against the cancer-associated antigen in the subject; and

b) a second composition comprising a TLR4 agonist; wherein the second composition does not comprise antigen;

wherein the first composition and the second composition each further comprise a pharmaceutically suitable excipient,

in the manufacture of a medicament for treating a cancer and increasing T cells in a tumor environment in a subject;

wherein the first composition is to be administered systemically to the subject; and wherein the second composition is to be administered intratumorally, peritumorally, intranodally or intravesically to the subject; and wherein the first composition and second composition are to be administered concurrently or sequentially, thereby increasing T cells in the tumor environment; and,

wherein the TLR4 agonist is a non-toxic lipid A-related adjuvant glucopyranosyl lipid A (GLA).

2. The use of claim 1, wherein the cancer associated antigen is selected from CTA, NY-ESO-1, LAGE-1,MAGE-A1, MAGE-A3, MAGE-A4, MAGE-A10, CT7, CT10, GAGE, PRAME; BAGE; RAGE, SAGE, HAGE, MPHOSPH1, DEPDC1, IMP3, MAGE-A, BK T-antigen, p53, Ras, c-Myc, A-Raf, B-Raf, C-Raf, cyclin-dependent kinases, MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A6, MAGE-A10, MAGE-A12, MART-1, BAGE, DAM-6, -10, GAGE-1, -2, -8, GAGE-3, -4, -5, -6, -7B, NA88-A, MART-1, MC1R, Gp100, PSA, PSM, Tyrosinase, TRP-1, TRP-2, ART-4, CAMEL, CEA, Cyp-B, hTERT, hTRT, iCE, MUC1, MUC2, Phosphoinositide 3-kinases (PI3Ks), TRK receptors, PRAME, P15, RU1, RU2, SART-1, SART-3, Wilms' tumor antigen (WT1), AFP, β-catenin/m, Caspase-8/m, CEA, CDK-4/m, ELF2M, GnT-V, G250, HSP70-2M, HST-2, KIAA0205,

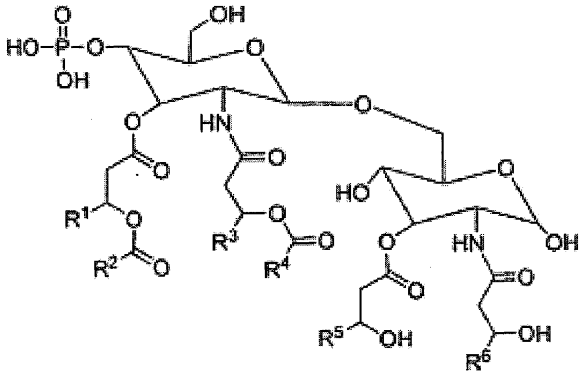
MUM-1, MUM-2, MUM-3, Myosin/m, RAGE, SART-2, TRP-2/INT2, 707-AP, Annexin II, CDC27/m, TPI/mbcr-abl, BCR-ABL, interferon regulatory factor 4 (IRF4), ETV6/AML, LDLR/FUT, Pml/RARα, Tumor-associated calcium signal transducer 1 (TACSTD1) TACSTD2, receptor tyrosine kinases, Epidermal Growth Factor receptor (EGFR), EGFRvIII, platelet derived growth factor receptor (PDGFR), vascular endothelial growth factor receptor (VEGFR), cytoplasmic tyrosine kinases, src-family, syk-ZAP70, integrin-linked kinase (ILK), signal transducers and activators of transcription STAT3, STAT5, and STAT6, hypoxia inducible factors, HIF-1α and HIF-2α, Nuclear Factor-Kappa B (NF-κB), Notch receptors, Notch1-4, c-Met, mammalian targets of rapamycin (mTOR), WNT, extracellular signal-regulated kinases (ERKs), PMSA, PR-3, MDM2, Mesothelin, renal cell carcinoma – 5T4, SM22-alpha, carbonic anhydrases I (CAI) and IX (CAIX), STEAD, TEL/AML1, GD2, proteinase3, hTERT, sarcoma translocation breakpoints, EphA2, ML-IAP, EpCAM, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, ALK, androgen receptor, cyclin B1, polysialic acid, MYCN, RhoC, GD3, fucosyl GM1, mesothelian, PSCA, sLe, PLAC1, GM3, BORIS, Tn, GLoboH, NY-BR-1, RGs5, SART3, STn, PAX5, OY-TES1, sperm protein 17, LCK, HMWMAA, AKAP-4, SSX2, XAGE 1, B7H3, legumain, TIE2, Page4, MAD-CT-1, FAP, MAD-CT-2, and fos related antigen 1.

3. The use of claim 1 or 2, wherein the first composition is to be administered systemically via a first route selected from the group consisting of intramuscular, intradermal or subcutaneous.

4. The use of any one of claims 1-3, wherein GLA is formulated in a stable oil-in-water emulsion.

5. The use of any one of claims 1-4 wherein the GLA has the formula:

(I)

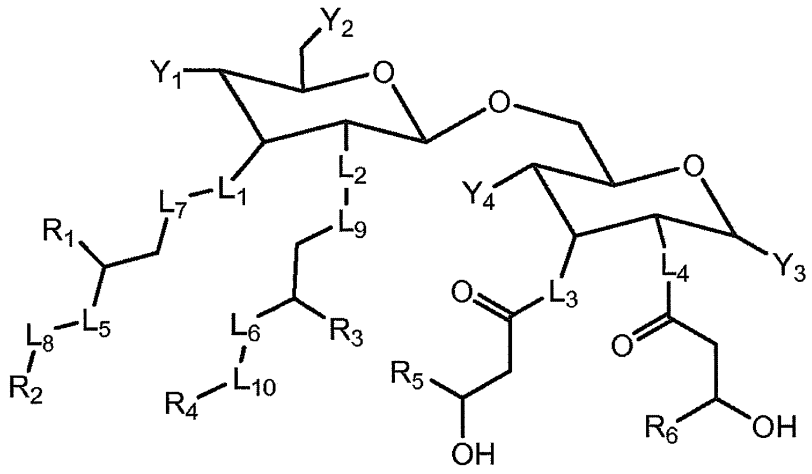


where: R^1, R^3, R^5 and R^6 are C11-C20 alkyl; and R^2 and R^4 are C12-C20 alkyl.

6. The use of claim 5 wherein R^1, R^3, R^5 and R^6 equal to undecyl, and R^2 and R^4 equal to tridecyl.

7. The use of any one of claims 1 to 4 wherein the GLA has the formula:

(II)



or a pharmaceutically acceptable salt thereof, wherein: L1, L2, L3, L4, L5 and L6 are the same or different and are independently selected from –O-, -NH-, and –(CH2)-; L7, L8, L9 and L10 are the same or different, and at any occurrence may be either absent or –C(=O)-; Y1 is an acid functional group; Y2 and Y3 are the same or different and are each independently selected from –OH, -SH, and an acid functional group; Y4 is –OH or –SH; R1, R3, R5 and R6 are the same or different and are each independently selected from the group of C8-C13 alkyl; and R2 and R4 are the same or different and are each independently selected from the group of C6-C11 alkyl.

8. The use of any one of claims 1-7, wherein the first composition is to be administered prior to administration of the second composition.

9. The use of any one of claims 1-7 wherein the first composition and/or the second composition are to be administered 2, 3, 4, 5, 6, 7, 8, 9 or 10 times.

10. The use of any one of claims 1-7, wherein the recombinant expression vector is selected from a retroviral vector, a lentiviral vector, poxvirus vector, vaccinia virus vector, adenovirus vector, adenovirus-associated virus vector, herpes virus vector, alpha virus vector, plasmid DNA and RNA.

11. The use of claim 1, wherein the vector particle is a lentiviral vector particle that comprises the lentiviral vector; a poxvirus vector particle that comprises the poxvirus vector; a vaccinia virus vector particle that comprises the vaccinia virus vector; an adenovirus vector particle that comprises the adenovirus vector; an adenovirus-associated virus vector particle that comprises the adenovirus-associated virus vector; a herpes virus vector particle that comprises the herpes virus vector; or an alpha virus vector particle that comprises the alpha virus vector.

12. The use of claim 1, wherein the vector particle is the lentiviral vector particle and comprises the lentiviral vector.

13. The use of claim 11 or 12, wherein the lentiviral vector particle further comprises an envelope comprising a Sindbis virus E2 glycoprotein having at least one amino acid change compared to SEQ ID NO:1, wherein residue 160 is either absent or an amino acid other than glutamic acid, and wherein E2 glycoprotein is not part of a fusion protein with Sindbis virus E3 protein.

14. The use of claim 13, wherein the lentiviral vector particle delivers the recombinant expression vector to a dendritic cell.

15. The use of any one of claims 1-14, wherein the cancer is an oncogenic virus-induced cancer.

16. The use of claim 15, wherein the cancer-associated antigen is an antigen derived from the oncogenic virus.

17. The use of any one of claims 1-16, wherein the second composition is to be administered in combination with a composition comprising a therapeutic agent selected from the group consisting of a checkpoint inhibitor, a cytokine, chloroquine, an antibody that increase ADCC, an antibody that promotes a co-stimulatory signal, an anti-CD40 antibody.

18. The use of any one of claims 1-17, wherein the first composition further comprises an adjuvant.

19. The use of any one of claims 1-18, wherein a boosting composition comprising an adjuvant is to be administered in combination with a polypeptide, wherein the polypeptide comprises the cancer-associated antigen, or an immunogenic fragment thereof.

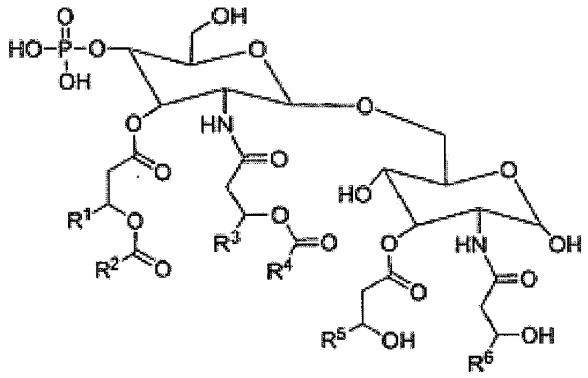
20. The use of any one of claims 1-19, wherein the induced immune response comprises a cytotoxic T lymphocyte immune response.

21. The use of any one of claims 1-20, wherein administering the second composition induces an increase in CD8+ T cells at the site of administration.

22. Use of a composition comprising:

a) a first composition comprising a lentiviral vector particle, the lentiviral vector particle comprising a recombinant expression vector, wherein the recombinant expression vector comprises a polynucleotide encoding an immunogen associated with cancer, wherein the polynucleotide is operatively linked to at least one regulatory expression sequence, wherein the lentiviral vector particle further comprises an envelope comprising a Sindbis virus E2 glycoprotein having at least one amino acid change compared to SEQ ID NO:1, wherein residue 160 is either absent or an amino acid other than glutamic acid, and wherein E2 glycoprotein is not part of a fusion protein with Sindbis virus E3 protein; and

b) a second composition comprising a TLR4 agonist, wherein the second composition does not comprise an immunogen; wherein the TLR4 agonist is glucopyranosyl lipid A (GLA) formulated in a stable oil-in-water emulsion, wherein the GLA has the formula:



where: R^1, R^3, R^5 and R^6 equal to undecyl, and R^2 and R^4 equal to tridecyl;

wherein the first composition and the second composition each further comprise a pharmaceutically suitable excipient,

in the manufacture of a medicament for treating a cancer and increasing T cells in a tumor environment in a subject,

wherein the first composition is to be administered intramuscularly, intradermally or subcutaneously and induces an immune response specific for the immunogen associated with the cancer in the subject; and wherein the second composition is to be administered intratumorally or peritumorally; and wherein the first composition or second composition are to be administered concurrently or sequentially; thereby increasing T cells in the tumor environment.

23. Use of a composition comprising:

a) a first composition comprising

i. antigen-specific T cells specific for a cancer-associated antigen, said antigen-specific T cells having been expanded *ex vivo*,

ii. T cells that have been genetically modified to express a chimeric antigen receptor (CAR) that recognizes the cancer-associated antigen, or

iii. T cells that have been genetically modified to express a specific chimeric T cell receptor (TCR) that recognizes the cancer-associated antigen in the context of MHC; and

b) a second composition comprising a pharmaceutically suitable adjuvant; wherein the second composition does not comprise an immunogen;

wherein the first composition and the second composition each further comprise a pharmaceutically suitable excipient;

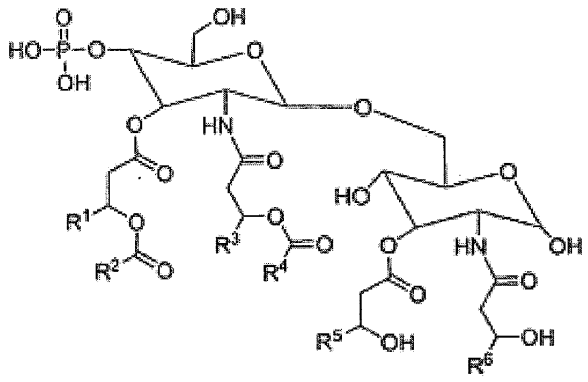
in the manufacture of a medicament for treating a cancer and increasing T cells in a tumor environment in a subject;

wherein the first composition is to be administered systemically to the subject; and the second composition is to be administered intratumorally and wherein the first composition and second composition are to be administered concurrently or sequentially; thereby increasing T cells in the tumor environment; and,

wherein the adjuvant is a non-toxic lipid A-related adjuvant glucopyranosyl lipid A (GLA).

24. The use of claim 23, wherein GLA is formulated in a stable oil-in-water emulsion.

25. The use of claim 23 or claim 24 wherein the GLA has the formula:



where: R^1, R^3, R^5 and R^6 are C_{11}-C_{20} alkyl; and R^2 and R^4 are C_{12}-C_{20} alkyl.

26. The use of claim 25 wherein R^1, R^3, R^5 and R^6 equal to undecyl, and R^2 and R^4 equal to tridecyl.

27. Use of a composition comprising:

a) a first composition comprising a vector particle, the vector particle comprising a recombinant expression vector wherein the recombinant expression vector comprises a polynucleotide encoding a cancer-associated antigen, and wherein the polynucleotide is operatively linked to at least one regulatory expression sequence, and

b) a second composition comprising a TLR4 agonist wherein the composition does not comprise antigen;

wherein the first composition and the second composition each further comprise a pharmaceutically suitable excipient,

in the manufacture of a medicament for increasing T cells in a tumor microenvironment in a subject,

where the first composition is to be administered systemically, thereby inducing an immune response against the cancer-associated antigen in the subject; and where the second composition is to be administered intratumorally or peritumorally to the subject; and where the first and second composition are to be administered concurrently or sequentially; thereby increasing T cells in the tumor microenvironment; and,

wherein the TLR4 agonist is a non-toxic lipid A-related adjuvant glucopyranosyl lipid A (GLA).

28. The use of claim 27, wherein the vector particle is a lentiviral vector particle that comprises the lentiviral vector; a poxvirus vector particle that comprises the poxvirus

vector; a vaccinia virus vector particle that comprises the vaccinia virus vector; an adenovirus vector particle that comprises the adenovirus vector; an adenovirus-associated virus vector particle that comprises the adenovirus-associated virus vector; a herpes virus vector particle that comprises the herpes virus vector; or an alpha virus vector particle that comprises the alpha virus vector.

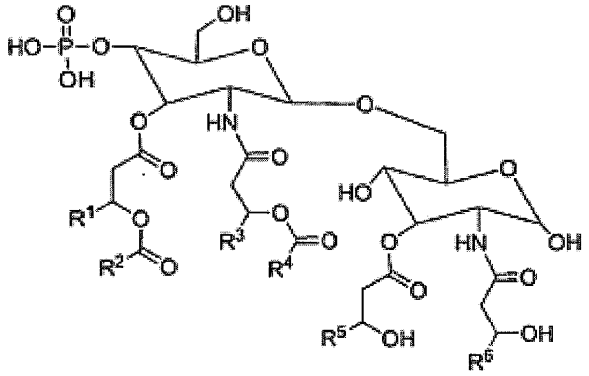
29. The use of claim 28, wherein the vector particle is the lentiviral vector particle and comprises the lentiviral vector.

30. The use of claim 28 or 29, wherein the lentiviral vector particle further comprises an envelope comprising a Sindbis virus E2 glycoprotein having at least one amino acid change compared to SEQ ID NO:1, wherein residue 160 is either absent or an amino acid other than glutamic acid, and wherein E2 glycoprotein is not part of a fusion protein with Sindbis virus E3 protein.

31. The use of claim 30, wherein the lentiviral vector particle delivers the recombinant expression vector to a dendritic cell.

32. The use of any one of claims 27-31, wherein the GLA is formulated in a stable oil-in-water emulsion.

33. The use of any one of claims 27-32 wherein the GLA has the formula:



where: R^1, R^3, R^5 and R^6 are C_{11}-C_{20} alkyl; and R^2 and R^4 are C_{12}-C_{20} alkyl.

34. The use of claim 33 wherein R^1, R^3, R^5 and R^6 equal to undecyl, and R^2 and R^4 equal to tridecyl.