(12) STANDARD PATENT (11) Application No. **AU 2019227988 B2**
(19) AUSTRALIAN PATENT OFFICE

(54) Title
CpG amphiphiles and uses thereof

(51) International Patent Classification(s)
A61K 31/70 (2006.01) ***A61K 31/7115*** (2006.01)
A61K 31/7088 (2006.01)

(21) Application No: **2019227988** (22) Date of Filing: **2019.03.01**

(87) WIPO No: **WO19/169328**

(30) Priority Data

(31) Number	(32) Date	(33) Country
62/637,824	**2018.03.02**	**US**

(43) Publication Date: **2019.09.06**
(44) Accepted Journal Date: **2024.11.07**

(71) Applicant(s)
Elicio Therapeutics, Inc.

(72) Inventor(s)
DEMUTH, Peter C.;STEINBUCK, Martin

(74) Agent / Attorney
FPA Patent Attorneys Pty Ltd, Level 19, South Tower 80 Collins Street, Melbourne, VIC, 3000, AU

(56) Related Art
HUA LI WANG et al, ARCH GYNECOL OBSTET, vol. 289, 2014, pages 155-162

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

CORRECTED VERSION

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
06 September 2019 (06.09.2019)

WIPO | PCT

(10) International Publication Number
WO 2019/169328 A9

(51) International Patent Classification:
A61K 31/70 (2006.01) *A61K 31/7115* (2006.01)
A61K 31/7088 (2006.01)

(21) International Application Number:
PCT/US2019/020398

(22) International Filing Date:
01 March 2019 (01.03.2019)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/637,824 02 March 2018 (02.03.2018) US

(71) Applicant: **ELICIO THERAPEUTICS INC.** [US/US]; One Kendall Square, Building 1400 West, Suite 14303, Cambridge, MA 02139 (US).

(72) Inventors: **DEMUTH, Peter, C.**; 74A Brookings Street, Medford, MA 02155 (US). **STEINBUCK, Martin**; 1 Emerson Place, Apt 6J, Boston, MA 02114 (US).

(74) Agent: **MICHAUD, Susan, M.** et al.; Clark & Elbing LLP, 101 Federal Street, 15th Floor, Boston, MA 02110 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

(54) Title: CPG AMPHIPHILES AND USES THEREOF

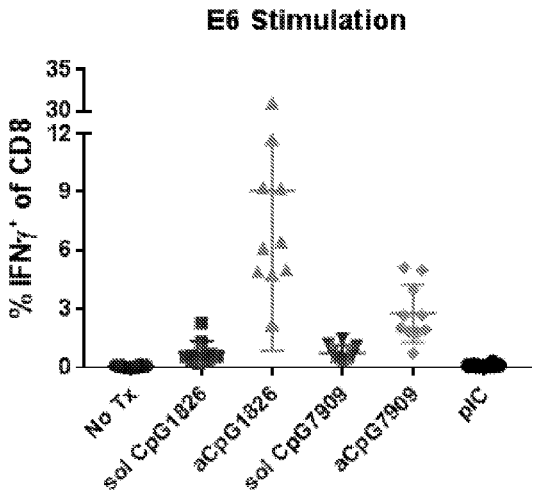


FIG. 1

(57) Abstract: The invention provides compounds including a CpG oligodeoxynucleotide sequence linked to a lipid by a linker and related compositions and methods. The invention features a compound consisting of the nucleotide sequence of SEQ ID NO:1, at its 5' end, bonded or linked by a linker to a lipid. Further, the invention features a method of treating a cancer in a human patient, comprising administering to the patient the compound. Further, the invention features a pharmaceutical composition including the compound and a pharmaceutically acceptable carrier. The invention features a kit including (I) the compound or a composition comprising the compound; and (ii) a protein comprising SEQ ID NO:2 or SEQ ID NO:3.

WO 2019/169328 A9

[Continued on next page]

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

— *with international search report (Art. 21(3))*

(48) Date of publication of this corrected version:
26 December 2019 (26.12.2019)

(15) Information about Correction:
see Notice of 26 December 2019 (26.12.2019)

CPG AMPHIPHILES AND USES THEREOF

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on _____ is named _____ and is _____ bytes in size.

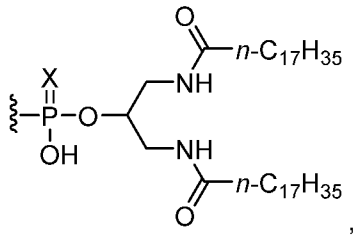
BACKGROUND OF THE INVENTION

Human papillomavirus (HPV)-related cancer is one of the fastest growing cancers in the world. Overall, 5% of all cancers world-wide can be attributed to HPV infections. There continues to be a need for further and more effective cancer treatments.

SUMMARY OF THE INVENTION

The invention provides compounds that can be used in therapeutic methods.

Accordingly, in the first aspect, the invention features a compound consisting of the nucleotide sequence 5'-TCGTCGTTTTGTCGTTTTGTCGTT-3' (SEQ ID NO:1), at its 5' end, bonded or linked by a linker to the following lipid:



,

or a salt thereof,

where X is O or S.

In one embodiment of the first aspect of the invention, the nucleotide sequence is bonded to the lipid.

In another embodiment of the first aspect of the invention, all internucleoside groups connecting the nucleosides in 5'-TCGTCGTTTTGTCGTTTTGTCGTT-3' (SEQ ID NO:1) are phosphorothioates.

In the second aspect, the invention features a method of treating a cancer in a human patient. This method includes administering to the patient the compound of the first aspect of the invention, a protein including the amino acid sequence:

MHQKRTAMFQ DPQERPRKLP QLCTELQTTI HDIILECVYC KQQLLRREVY DFAFRDLCIV YRDGNPYAVG DKCLKFYSKI SEYRHYCYSL YGTTLEQQYN KPLCDLLIRC INGQKPLCPE EKQRHLDKKQ RFHNGRGRWT GRCMSCCRSS RTRRETQL (SEQ ID NO:2), and a protein including the amino acid sequence:

MHGDTPTLHE YMLDLQPETT DLYGYGQLND SSEEEDEIDG PAGQAEPDRA HYNIVTFCCK CDSTLRLCVQ STHVDIRTLE DLLMGTLGIV CPICSQKP (SEQ ID NO:3).

In one embodiment of the second aspect of the invention, the cancer is human papillomavirus (HPV) positive (e.g., HPV type 16 positive).

In another embodiment of the second aspect of the invention, the cancer is a head or neck squamous cell carcinoma.

In an additional embodiment of the second aspect of the invention, the patient is receiving or has received platinum-containing chemotherapy. In a further embodiment, an anti-PD-1 antibody (e.g., pembrolizumab or nivolumab) is administered to the patient.

In another embodiment of the second aspect of the invention, the compound of the first aspect of the invention and the proteins including the amino acid sequences of SEQ ID NO:2 and SEQ ID NO:3 are administered concurrently.

In a further embodiment of the second aspect of the invention, the compound of the first aspect of the invention and the proteins including the amino acid sequences of SEQ ID NO:2 and SEQ ID NO:3 are administered sequentially.

In a further aspect, the invention features another method of treating a cancer in a human patient. This method includes administering to the patient the compound of the first aspect of the invention, a protein including the amino acid sequence:
MHQKRTAMFQ DPQERPRKLP QLCTELQTTI HDIILECVYC KQQLLRREVY DFAFRDLCIV YRDGNPYAVG DKCLKFYSKI SEYRHYCYSL YGTTLEQQYN KPLCDLLIRC INGQKPLCPE EKQRHLDKKQ RFHNGRGRWT GRCMSCCRSS RTRRETQL (SEQ ID NO:2), a protein including the amino acid sequence:
MHGDTPTLHE YMLDLQPETT DLYGYGQLND SSEEEDEIDG PAGQAEPDRA HYNIVTFCCK CDSTLRLCVQ STHVDIRTLE DLLMGTLGIV CPICSQKP (SEQ ID NO:3), and
an anti-PD-1 antibody (e.g., pembrolizumab or nivolumab).

In the third aspect, the invention features a pharmaceutical composition including a compound of the first aspect of the invention and a pharmaceutically acceptable carrier.

In one embodiment of the third aspect, the pharmaceutical composition further includes a protein including the amino acid sequence: MHQKRTAMFQ DPQERPRKLP QLCTELQTTI HDIILECVYC KQQLLRREVY DFAFRDLCIV YRDGNPYAVG DKCLKFYSKI SEYRHYCYSL YGTTLEQQYN KPLCDLLIRC INGQKPLCPE EKQRHLDKKQ RFHNGRGRWT GRCMSCCRSS RTRRETQL (SEQ ID NO:2), and a protein including the amino acid sequence:
MHGDTPTLHE YMLDLQPETT DLYGYGQLND SSEEEDEIDG PAGQAEPDRA HYNIVTFCCK CDSTLRLCVQ STHVDIRTLE DLLMGTLGIV CPICSQKP (SEQ ID NO:3).

In the fourth aspect, the invention features a kit including (i) a compound of the first aspect of the invention or a composition of the second aspect of the invention and (ii) a protein including the amino acid sequence: MHQKRTAMFQ DPQERPRKLP QLCTELQTTI HDIILECVYC KQQLLRREVY DFAFRDLCIV YRDGNPYAVG DKCLKFYSKI SEYRHYCYSL YGTTLEQQYN KPLCDLLIRC INGQKPLCPE EKQRHLDKKQ RFHNGRGRWT GRCMSCCRSS RTRRETQL (SEQ ID NO:2),
and a protein including the amino acid sequence: MHGDTPTLHE YMLDLQPETT DLYGYGQLND SSEEEDEIDG PAGQAEPDRA HYNIVTFCCK CDSTLRLCVQ STHVDIRTLE DLLMGTLGIV CPICSQKP (SEQ ID NO:3).

Definitions

A "linker," as used herein, refers to a monovalent or divalent group, in which one valency is covalently bonded to one biologically functional group, and the other valency is covalently bonded to another biologically functional group. In one example, a linker connects a nucleotide sequence of, e.g., a CpG oligonucleotide, to a lipid (e.g., -P(X)(OH)-O-CH(CH_2NHCO-(CH_2)$_{16}$-CH_3)$_2$, or a salt thereof, where X is O or

S, as described herein). Such linkers can optionally include one or more nucleotides, for example, a dinucleotide (e.g., GG).

A "pharmaceutically acceptable carrier," as used herein, refers to a vehicle capable of suspending or dissolving the active compound, and having the properties of being nontoxic and non-inflammatory in a patient. Moreover, a pharmaceutically acceptable carrier may include a pharmaceutically acceptable additive, such as a preservative, antioxidant, fragrance, emulsifier, dye, or excipient known or used in the field of drug formulation and that does not significantly interfere with the therapeutic effectiveness of the biological activity of the active agent, and that is non-toxic to the patient.

The terms "treat," "treatment," and "treating" refer to therapeutic approaches in which the goal is to reverse, alleviate, ameliorate, inhibit, slow down, or stop the progression or severity of a condition associated with a disease or disorder, e.g., cancer. These terms include reducing or alleviating at least one adverse effect or symptom of a condition, disease, or disorder. Treatment is generally "effective" if one or more symptoms or clinical markers are reduced, or if a desired response (e.g., a specific immune response) is induced. Alternatively, treatment is "effective" if the progression of a disease is reduced or halted.

The invention provides several advantages. For example, in including lipid moieties and, optionally, a linker, certain compounds of the invention bind to endogenous albumin in subjects to whom they are administered, which enhances delivery of the compounds to the lymph nodes of the subjects. This facilitates the induction of a therapeutic immune response against, for example, HPV proteins administered to the subject, leading to effective cancer treatment.

Other features and advantages of the invention will be apparent from the following detailed description, the drawings, and the claims.

DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph showing the immune response against HPV16 E6.

Figure 2 is a graph showing the immune response against HPV16 E7.

Figure 3 is a graph showing tetramer stain analysis for HPV16 E7.

Figure 4 is a series of graphs showing that administration of HPV16 E6 and HPV16 E7 with an amphiphile-CpG (aCpG) decreased tumor size compared to aCpG alone or no treatment (No Tx).

Figure 5 is a graph showing superior HPV tetramer response with aCpG vaccine compared with soluble CpG vaccine at different time points.

Figure 6 is a graph showing sustained HPV tetramer responses over time with aCpG administered once weekly or once every two weeks.

Figure 7 is a graph showing tumor size response to E7 vaccine treatment.

Figure 8 is a graph showing improved survival in E7-vaccine treated C57BL6 mice implanted with TC-1 tumor cells.

Figure 9 is a series of graphs showing serum cytokine level changes after dosing.

Figure 10 is a graph showing tetramer responses over time for an HPV16 E7/aCpG vaccine.

Figure 11 is a graph showing tumor growth response to HPV16 E7/aCpG vaccination plus or minus administration of an anti-PD-1 antibody.

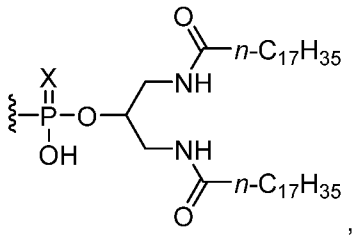
Figure 12 is a graph showing the effects of HPV16 E7/aCpG vaccination plus or minus administration of an anti-PD-1 antibody on survival in TC-1 tumor bearing mice.

Figure 13 is a graph showing tetramer analysis for aCpG dose escalation.

Figure 14 shows the structure of amphiphile-CpG-7909; 5'-(Diacyl lipid) TCG TCG TTT TGT CGT TTT GTC GTT-3' (SEQ ID NO:1). All bases are DNA. All linkages are phosphoramidite, including the link between the diacyl lipid and the oligodeoxynucleotide.

DETAILED DESCRIPTION OF THE INVENTION

The invention provides compounds that can be used in therapeutic methods. The compounds include CpG oligodeoxynucleotides (ODNs) (e.g., a CpG ODN having the sequence 5'-TCGTCGTTTTGTCGTTTTGTCGTT-3' (SEQ ID NO:1)). The CpG ODN is linked, at its 5' end, to a lipid, such as the following:



,

or a salt thereof,

where X is O or S. Preferably, X is S. The CpG oligonucleotide may be directly bonded to the lipid. Alternatively, the CpG may be linked to the lipid through a linker, such as GG. In the CpG oligonucleotide, all internucleoside groups are phosphorothioates (e.g., all internucleoside groups in the compound may be phosphorothioates).

The CpG ODN can function as an adjuvant to elicit an immune response in a subject, such as an immune response against a cancer antigen (e.g., a HPV antigen). As such, the compounds and compositions of the invention can be used in therapeutic methods. In particular, if the CpG ODN containing compound is administered in combination with one or more HPV proteins the compound can induce an immune response to HPV positive cancer cells. Accordingly, the invention provides methods of treating cancer in a subject (e.g., a human patient) by administering one or more compounds or compositions of the invention to the subject. In various examples, the cancer is a HPV positive (e.g., a HPV type 16 positive) cancer.

The HPV positive cancer may be a head or neck squamous cell carcinoma, a cervical cancer, anal cancer, vulvar cancer, head and neck cancer, oropharyngeal cancer, penile cancer, vaginal cancer, virally induced cancer, bladder cancer, pancreatic cancer, lung cancer, liver cancer, ovarian cancer, colon cancer, stomach cancer, neuroblastoma, breast cancer, prostate cancer, renal cancer, leukemia, sarcoma, carcinoma, basal cell carcinoma, non-small cell lung carcinoma, non-Hodgkin's lymphoma, acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), B-cells chronic lymphocytic leukemia (B-CLL), multiple myeloma (MM), erythroleukemia, renal cell carcinoma, sarcoma, melanoma, astrocytoma, oligoastrocytoma, biliary tract cancer, choriocarcinoma, CNS cancer, larynx cancer, small cell lung cancer, non-small cell lung cancer (NSCLC), adenocarcinoma, giant (or oat) cell carcinoma, squamous cell carcinoma, oral cavity cancer, skin cancer, basal cell cancer, squamous cell cancer, testicular cancer, thyroid cancer, uterine cancer, rectal cancer, a cancer of the respiratory system, or a cancer of the urinary system.

Optionally, the methods of the invention can further include administering a compound or composition of the invention in combination with a second (or further) different approach to treatment.

The invention also provides kits that each contain, for example, a first vessel that includes one or more compounds of the invention, optionally together with a second vessel that includes a cancer antigen, such as an HPV protein described herein.

CpG

CpG ODNs are short synthetic single-stranded DNA molecules containing unmethylated CpG dinucleotides in particular sequence contexts. CpG ODNs possess a partially or completely phosphorothioated (PS) backbone, as opposed to the natural phosphodiester (PO) backbone in DNA molecules. Three major classes of stimulatory CpG ODNs have been identified based on structural characteristics and activity on human peripheral blood mononuclear cells (PBMCs), in particular B cells and plasmacytoid dendritic cells (pDCs). These three classes are Class A (Type D), Class B (Type K), and Class C.

CpG1826 and CpG7909 both are in CpG class B. Class B CpG ODNs contain a full PS backbone with one or more CpG dinucleotides. They strongly activate B cells and TLR9-dependent NF-κB signaling but weakly stimulate IFN-α secretion.

Mutated HPV

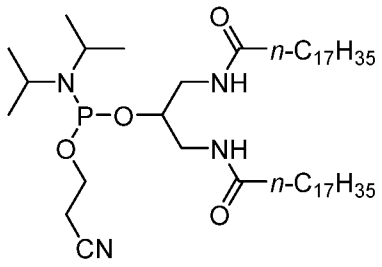
Point mutations at C70G, C113G, and I135G (underlined below) can be introduced into the wild-type HPV16 E6 viral protein to prevent stereochemical interaction with human p53. The performance of this component as an antigen is dictated by the sequence of the protein, with the structure of the protein being inconsequential to that intended function.

mHPV 16 E6 (158 aa; SEQ ID NO:2)
MHQKRTAMFQ DPQERPRKLP QLCTELQTTI HDIILECVYC KQQLLRREVY DFAFRDLCIV YRDGNPYAVG
DKCLKFYSKI SEYRHYCYSL YGTTLEQQYN KPLCDLLIRC INGQKPLCPE EKQRHLDKKQ
RFHNGRGRWT GRCMSCCRSS RTRRETQL

Point mutations at C24G and E26G (underlined below) can be introduced into the wild-type HPV16 E7 viral protein to prevent stereochemical interaction with human Rb1. Again, the performance of this component as an antigen is dictated by the sequence of the protein, with the structure of the protein being inconsequential to that intended function.

mHPV 16 E7 (98 aa; SEQ ID NO:3)
MHGDTPTLHE YMLDLQPETT DLYGYGQLND SSEEEDEIDG PAGQAEPDRA HYNIVTFCCK
CDSTLRLCVQ STHVDIRTLE DLLMGTLGIV CPICSQKP.

CpG ODNs may be bonded directly or linked by a linker to the lipid. These compounds may be produced using the ordinary phosphoramidite chemistry known in the art. In some examples, the CpG ODN or CpG ODN-GG may be reacted with the following compound:



to produce an intermediate, which upon oxidation with (e.g., phosphite oxidation methods known in the art, e.g., a sulfurizing agent, such as 3-((N,N-dimethylaminomethylidene)amino)-3H-1,2,4-dithiazole-5-thione) and hydrolysis of the cyanoethyl group may produce a compound of the invention.

In order that this invention be more fully understood, the following examples are set forth. These examples are for the purpose of illustration only and are not to be construed as limiting the scope of the invention in any way.

EXAMPLES

Example 1: HPV16 E6 and HPV16 E7 proteins in combination with a CpG amphiphile adjuvant generates an immune response

Mice were immunized prophylactically and the immune response generated was recorded via E7-Tetramer stain and IFNγ-intracellular cytokine staining (ICS) upon HPV16 E6 and HPV16 E7 (E6/E7) stimulation.

The experimental design included the following 6 groups of mice (n=10 for each group)

1. No immunization
2. E6/E7 + soluble CpG1826
3. E6/E7 + amphiphile CpG1826 (aCpG1826)
4. E6/E7 + soluble CpG7909
5. E6/E7 + amphiphile CpG7909 (aCpG7909; Figure 14)
6. E6/E7 + polyIC (pIC)

pIC was used as a benchmark adjuvant control.

Protein stock solutions were dissolved in 8M urea. Adjuvant stock solutions are dissolved in H_2O. Final injections are diluted with 1X phosphate buffered saline (PBS) (C_F of urea <1M).

For aCpG1826, the sequence used was the soluble CpG1826 sequence (5'-tccatgacgttcctgacgtt-3'; SEQ ID NO:4) with two guanines added at the 5' end (5'-gg tccatgacgttcctgacgtt-3'; SEQ ID NO:5). A concentration of 5nmol for each 100µl injection was used for both soluble CpG1826 and aCpG1826. CpG1826 is an optimal mouse sequence while CpG7909 is optimal for humans and poorly active in mice. CpG1826 and CpG7909 are in the same CpG class (class B) and generally have similar activity profiles in their respective species.

For both aCpG7909 and soluble CpG7909, the sequence used was 5'-tcgtcgttttgtcgttttgtcgtt-3' (SEQ ID NO:6) at a concentration of 5nmol for each 100µl injection.

Mutated HPV16 E6 with point mutations at C70G, C113G, and I135G (underlined below) was used for immunization. The amino acid sequence used is provided below.

mHPV 16 E6 (158 aa; SEQ ID NO:2)

MHQKRTAMFQ DPQERPRKLP QLCTELQTTI HDIILECVYC KQQLLRREVY DFAFRDLCIV YRDGNPYAVG DKCLKFYSKI SEYRHYCYSL YGTTLEQQYN KPLCDLLIRC INGQKPLCPE EKQRHLDKKQ RFHNGRGRWT GRCMSCCRSS RTRRETQL

Mutated HPV16 E7 with point mutations at C24G and E26G (underlined below) was used for immunization. The amino acid sequence used is provided below.

mHPV 16 E7 (98 aa; SEQ ID NO:3)
MHGDTPTLHE YMLDLQPETT DLYGYGQLND SSEEEDEIDG PAGQAEPDRA HYNIVTFCCK CDSTLRLCVQ STHVDIRTLE DLLMGTLGIV CPICSQKP

For E6/E7, 10µg each of mutated HPV16 E6 and mutated HPV16 E7 was used per 100µl injection.

Female C57BL/6J mice (B6) were immunized subcutaneously (s.c.) with the primer dose (E6/7+aCpG) and one booster dose after 2 weeks.

Tetramer analysis for H-2Db HPV16 E7 (RAHYNIVTF; SEQ ID NO:7) was performed 7 days after the booster dose (Figure 3).

Intracellular cytokine staining (ICS) for IFNγ was performed on peripheral blood 7 days after the booster dose to analyze immune responses to E6/E7.

The E6 stimulation used the following peptides: (E6-10: EVYDFAFRDL (SEQ ID NO:8); E6 49-57: VYDFAFRDL (SEQ ID NO:9); E6 37-45: CVYCKQQLL; (SEQ ID NO:10); E6 72-80: KCLKFYSKI (SEQ ID NO:11); and E6 100-108: NKPLCDLLI (SEQ ID NO:12) to generate the data shown in Figure 1.

Deconvolution of the E6 stimuli revealed that E49-57 was the only peptide that resulted in stimulation.

For E7 stimulation the following peptide was used: RAHYNIVTF (SEQ ID NO:13). This peptide was used to generate the data shown in Figure 2.

As shown in Figures 1 and 2, use of aCpG1826 generated a strong immune response against both mutant HPV16 E6 and mutant HPV16 E7.

As also shown in Figures 1 and 2, use of aCpG7909, which is optimal for humans and generally performs poorly in mice, surprisingly generated a strong immune response against both mutant HPV16 E6 and mutant HPV16 E7.

E6/E7 + aCpG decreased tumor growth compared to either aCpG alone or no treatment with a corresponding increase in percent survival (Figure 4).

Example 2: Determination of a dosing schedule for HPV 16 E7 and aCPG

To determine an optimal dosing schedule for E7 + aCpG with respect to anti-tumor efficacy in female C57BL/6U (B6) mice implanted with TC-1 tumors, weekly dosing was compared to dosing every 2 weeks and to baseline (prime only). E7 + aCpG was compared to E7 + soluble CpG. All vaccines were administered 3 times (prime and 2 boosts).

Female C57BL/6J mice (B6) were inoculated with 50,000 TC-1 cells subcutaneously in the flank on Day 0 and 12 days later; mice were separated into treatment groups and treated as indicated in Table 1.

Table 1

Test Article	Dose[a]	Dosing Interval	Injection Volume (ROA)	N	Endpoints
NA	0	Untreated Control	NA	10	Serum cytokines Anti-E7 serum antibodies Tetramer analysis for H-2Db HPV16 E7 Tumor size Survival
E7 + aCpG	10 µg E7 1.24 nmol aCpG-1826[b]	Single Dose	100 µL, divided (SC)	10	Serum cytokines Anti-E7 serum antibodies Tetramer analysis for H-2Db HPV16 E7 Tumor size Survival
		Weekly	100 µL, divided (SC)	20	
		Every 2 Weeks	100 µL, divided (SC)	20	
E7 + CpG	10 µg E7 1.24 nmol CpG-1826	Single Dose	100 µL, divided (SC)	10	Anti-E7 serum antibodies Tetramer analysis for H-2Db HPV16 E7 Tumor size Survival
		Weekly	100 µL, divided (SC)	20	
		Every 2 Weeks	100 µL, divided (SC)	20	

[a] Protein stock solutions were dissolved in 8M urea. Adjuvant stock solutions were dissolved in H_2O. Final injections were diluted with 1X PBS (C_F of urea <1M).

[b] 8 µg equivalent

NA=not applicable; PBS=phosphate-buffered saline; ROA=route of administration; SC=subcutaneous

- Throughout the study tumor sizes were measured every other day up to Day 40 post inoculation and animal survival was monitored. Tetramer analysis for H-2Db HPV16 E7 (RAHYNIVTF) was performed 7 days after each vaccine administration.
- Serum samples were taken 1 hour and 4 hours after each vaccine administration for the aCpG groups and analyzed via cytometric bead array for cytokine expression (IFNγ, TNFα, IL-6, IL-10, IL-12p70, MCP-1).
- Anti-E7 serum antibody titers were analyzed 14 days after initial vaccination. ELISA plates were coated with whole protein E7, upon which serum antibodies were captured and detected with anti-Fc antibody.

The HPV-tetramer specific T cell response to the protein/amphiphilic CpG vaccine was superior to that of the protein/soluble CpG vaccine after both a single dose and repeated doses (Figure 5). The HPV-tetramer response to protein/aCpG was increased further after administration of boost vaccinations, and the increases were sustained out to Days 28 and 35 (Figure 6) for the once weekly and once every 2 weeks regimens, respectively. The strong HPV- tetramer response in the aCpG groups correlated to reductions in tumor size compared to animals vaccinated with soluble CpG (Figure 7) and improved survival (Figure 8).

Treatment-related increases in systemic cytokines were comparable between soluble and aCpG groups except for IL-10, which was lower for aCpG compared to soluble CpG, and IFNγ which was higher for aCpG compared to soluble CpG (Figure 9).

Example 3: Antitumor efficacy of E7 protein in combination with either soluble or amphiphilic CpG and with or without the addition of an anti-PD-1 antibody

To evaluate the antitumor efficacy of E7 protein in combination with either soluble or amphiphilic CpG and with or without the addition of an anti-PD-1 antibody, female C57BL/6J mice (B6) were inoculated in the flank at baseline with 50,000 TC-1 cells. Eleven days post- inoculation the mice were divided into 5 groups as shown in Table 2. The comparison group was untreated.

Table 2

Test Article	Dose[a]	Dosing Interval	Injection Volume (ROA)	N	Endpoints
NA	0	Untreated Control	NA	10	
E7 + CpG	10 µg E7 1.24 nmol CpG-1826	Every 2 Weeks	100 µL, divided (SC)	10	
E7 + aCpG	10 µg E7 1.24 nmol aCpG- 1826	Every 2 Weeks	100 µL, divided (SC)	10	Tumor size every other day up to Day 40 Survival
E7 + CpG + PD-1 antibody	10 µg E7 1.24 nmol CpG-1826 230 µL anti-PD-1	Every 2 Weeks for both vaccine and antibody	100 µL, divided (SC) Antibody IP (100 µL divided)	10	Tetramer analysis for H- 2Db HPV16 E7 (RAHYNIVTF peptide; SEQ ID NO:13) 7 days after each vaccine administration
E7 + aCpG + PD-1 antibody	10 µg E7 1.24 nmol aCpG- 1826 230 µL anti-PD-1	Every 2 Weeks For both vaccine and antibody	100 µL, divided (SC) Antibody IP (100 µL divided)	10	

[a] Protein stock solutions were dissolved in 8M urea. Adjuvant stock solutions were dissolved in H_2O. Final injections were diluted with 1X PBS (C_F of urea <1M).
IP=intraperitoneal; NA=not applicable; PBS=phosphate-buffered saline; ROA=route of administration; SC=subcutaneous

Throughout the study tumor sizes were measured every other day up to day 40 post inoculation and animal survival was monitored. Tetramer analysis for H-2Db HPV16 E7 (RAHYNIVTF) was performed 7 days after each vaccine administration.

Administration of E7/Amph-CpG vaccine, without or without anti-PD-1 antibody, caused a robust increase in HPV Tetramer+ CD8-cells specific to the HPV16 E7 (RAHYNIVTF; SEQ ID NO:13) peptide (Figure 10). These responses were clearly visible as early as after the first dose, peaked on the 2nd dose, and were sustained out to the 3rd dose (in contrast to the lower responses observed with E7/CpG, which though increased by concomitant administration of anti-PD-1 were not sustained.

Corresponding to these strong HPV Tetramer+ CD8 responses, tumor growth was halted around Day 24 and reversed after the first dose of E7/Amph-CpG (with or without anti-PD-1) and tumor size remained small and stable out to the end of the study, in contrast to the other groups where growth progressed (Figure 11).

Also, corresponding to the effects on tumor size, treatment with E7/aCpG vaccine (with or without the anti-PD-1 antibody) had a significant effect on survival and resulted in 6/7 (85%) cures for E7/aCpG without antibody and 8/10 (80%) cures for E7/aCpG plus anti- PD-1 antibody (Figure 12).

Example 4: aCpG dose escalation study

To determine a dose of aCpG that produces the highest antigen-specific Tetramer+ CD8 response over the course of 6 doses, a dose escalation study was conducted using a fixed dose of 10 µg ovalbumin (OVA) as the antigen. Soluble CpG was used as a comparator. Tolerability (based on body weight and general observations) was also assessed. The study design is outlined in Table 3.

Table 3

Antigen/Dose	Adjuvant	Adjuvant Dose (nmol)	ROA (Dose Volume)
OVA 10 µg	Amph-CpG-1826	0.12	SC (100 µL, divided)
		0.60	
		1.2	
		6	
		12	
	Soluble CpG-1826	1.2	
		6	
		12	
		60	

ROA=route of administration; SC=subcutaneous

Up to 6 doses of vaccine were administered at 2-week intervals for a total study length of 11 weeks.

Peripheral blood samples were collected 7 days after each injection and flow cytometric analyses of tetramer on CD8+ cells were performed using H-2KbOVA (SIINFEKL; SEQ ID NO:14).

Significant increases in tetramer+ CD8+ cells were observed only in the groups treated with aCpG + OVA, with 6 nmol producing the greatest pharmacological effect (Figure 13). No weight loss, loss of interest/appetite, or wounds/lesions were observed.

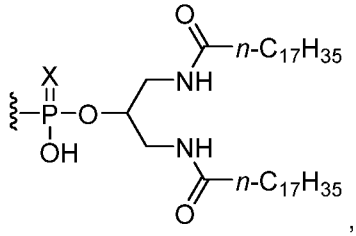
Other Embodiments

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure come within known or customary practice within the art to which the invention pertains and may be applied to the essential features hereinbefore set forth.

All publications, patents, and patent applications are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

Some embodiments of the invention are within the following numbered paragraphs.

1. A compound consisting of the nucleotide sequence 5'-TCGTCGTTTTGTCGTTTTGTCGTT-3' (SEQ ID NO:1), at its 5' end, bonded or linked by a linker to the following lipid:



,

or a salt thereof,

wherein X is O or S.

2. The compound of paragraph 1, wherein the nucleotide sequence is bonded to the lipid.

3. The compound of paragraph 1 or 2, wherein all internucleoside groups connecting the nucleosides in 5'-TCGTCGTTTTGTCGTTTTGTCGTT-3' (SEQ ID NO:1) are phosphorothioates.

4. A method of treating a cancer in a human patient comprising administering to the patient the compound of any one of paragraphs 1 to 3, a protein comprising the amino acid sequence:

MHQKRTAMFQ DPQERPRKLP QLCTELQTTI HDIILECVYC KQQLLRREVY DFAFRDLCIV YRDGNPYAVG DKCLKFYSKI SEYRHYCYSL YGTTLEQQYN KPLCDLLIRC INGQKPLCPE EKQRHLDKKQ RFHNGRGRWT GRCMSCCRSS RTRRETQL (SEQ ID NO:2),

and a protein comprising the amino acid sequence:

MHGDTPTLHE YMLDLQPETT DLYGYGQLND SSEEEDEIDG PAGQAEPDRA HYNIVTFCCK CDSTLRLCVQ STHVDIRTLE DLLMGTLGIV CPICSQKP (SEQ ID NO:3).

5. The method of paragraph 4, wherein the cancer is human papillomavirus (HPV) positive.

6. The method of paragraph 5, wherein the cancer is HPV type 16 positive.

7. The method of any one of paragraphs 4 to 6, wherein the cancer is a head or neck squamous cell carcinoma.

8. The method of any one of paragraphs 4 to 7, wherein the patient is receiving or has received platinum-containing chemotherapy.

9. The method of paragraph 4, wherein the compound of paragraph 1 and the proteins comprising the amino acid sequences of SEQ ID NO:2 and SEQ ID NO:3 are administered concurrently.

10. The method of paragraph 4, wherein the compound of paragraph 1 and the proteins comprising the amino acid sequences of SEQ ID NO:2 and SEQ ID NO:3 are administered sequentially.

11. A pharmaceutical composition comprising a compound of any one of paragraphs 1 to 3 and a pharmaceutically acceptable carrier.

12. The pharmaceutical composition of paragraph 11, wherein the composition further comprises a protein comprising the amino acid sequence:
MHQKRTAMFQ DPQERPRKLP QLCTELQTTI HDIILECVYC KQQLLRREVY DFAFRDLCIV YRDGNPYAVG DKCLKFYSKI SEYRHYCYSL YGTTLEQQYN KPLCDLLIRC INGQKPLCPE EKQRHLDKKQ RFHNGRGRWT GRCMSCCRSS RTRRETQL (SEQ ID NO:2),
and a protein comprising the amino acid sequence:
MHGDTPTLHE YMLDLQPETT DLYGYGQLND SSEEEDEIDG PAGQAEPDRA HYNIVTFCCK CDSTLRLCVQ STHVDIRTLE DLLMGTLGIV CPICSQKP (SEQ ID NO:3).

13. A kit comprising (i) a compound of any one of paragraphs 1 to 3 or a composition of paragraph 11 and (ii) a protein comprising the amino acid sequence:
MHQKRTAMFQ DPQERPRKLP QLCTELQTTI HDIILECVYC KQQLLRREVY DFAFRDLCIV YRDGNPYAVG DKCLKFYSKI SEYRHYCYSL YGTTLEQQYN KPLCDLLIRC INGQKPLCPE EKQRHLDKKQ RFHNGRGRWT GRCMSCCRSS RTRRETQL (SEQ ID NO:2),
and a protein comprising the amino acid sequence:
MHGDTPTLHE YMLDLQPETT DLYGYGQLND SSEEEDEIDG PAGQAEPDRA HYNIVTFCCK CDSTLRLCVQ STHVDIRTLE DLLMGTLGIV CPICSQKP (SEQ ID NO:3).

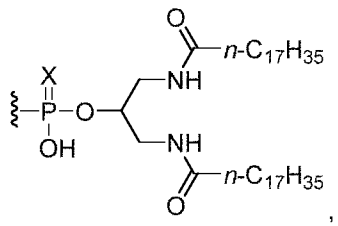
Other embodiments are within the following claims.

What is claimed is:

2019227988 02 Oct 2024

CLAIMS

1. A compound consisting of the nucleotide sequence 5'-TCGTCGTTTTGTCGTTTTGTCGTT-3' (SEQ ID NO:1), at its 5' end, bonded or linked by a bivalent linker to the following lipid:



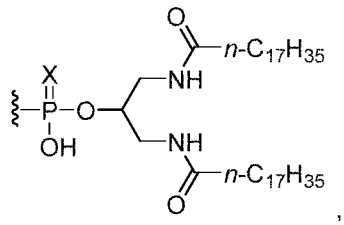
or a salt thereof,
wherein X is O or S.

2. The compound of claim 1, wherein the nucleotide sequence is bonded to the lipid.

3. The compound of claim 1 or 2, wherein all internucleoside groups connecting the nucleosides in 5'-TCGTCGTTTTGTCGTTTTGTCGTT-3' (SEQ ID NO:1) are phosphorothioates.

4. A method of treating a human papillomavirus (HPV) positive cancer in a human patient comprising administering to the patient the compound of claim 1 or 2, a protein comprising the amino acid sequence:
MHQKRTAMFQ DPQERPRKLP QLCTELQTTI HDIILECVYC KQQLLRREVY DFAFRDLCIV YRDGNPYAVG DKCLKFYSKI SEYRHYCYSL YGTTLEQQYN KPLCDLLIRC INGQKPLCPE EKQRHLDKKQ RFHNGRGRWT GRCMSCCRSS RTRRETQL (SEQ ID NO:2),
and a protein comprising the amino acid sequence:
MHGDTPTLHE YMLDLQPETT DLYGYGQLND SSEEEDEIDG PAGQAEPDRA HYNIVTFCCK CDSTLRLCVQ STHVDIRTLE DLLMGTLGIV CPICSQKP (SEQ ID NO:3).

5. Use of a compound consisting of the nucleotide sequence 5'-TCGTCGTTTTGTCGTTTTGTCGTT-3' (SEQ ID NO:1), at its 5' end, bonded or linked by a bivalent linker to the following lipid:



or a salt thereof,
wherein X is O or S;
in the manufacture of a medicament for treating a human papillomavirus (HPV) positive cancer, wherein the medicament is for administration with a protein comprising the amino acid sequence:

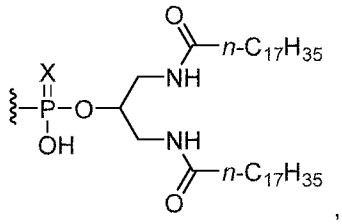
2019227988 02 Oct 2024

MHQKRTAMFQ DPQERPRKLP QLCTELQTTI HDIILECVYC KQQLLRREVY DFAFRDLCIV YRDGNPYAVG DKCLKFYSKI SEYRHYCYSL YGTTLEQQYN KPLCDLLIRC INGQKPLCPE EKQRHLDKKQ RFHNGRGRWT GRCMSCCRSS RTRRETQL (SEQ ID NO:2),

and a protein comprising the amino acid sequence:

MHGDTPTLHE YMLDLQPETT DLYGYGQLND SSEEEDEIDG PAGQAEPDRA HYNIVTFCCK CDSTLRLCVQ STHVDIRTLE DLLMGTLGIV CPICSQKP (SEQ ID NO:3).

6. Use of a compound consisting of the nucleotide sequence 5'-TCGTCGTTTTGTCGTTTTGTCGTT-3' (SEQ ID NO:1), at its 5' end, bonded or linked by a bivalent linker to the following lipid:



,

or a salt thereof,

wherein X is O or S;

a protein comprising the amino acid sequence:

MHQKRTAMFQ DPQERPRKLP QLCTELQTTI HDIILECVYC KQQLLRREVY DFAFRDLCIV YRDGNPYAVG DKCLKFYSKI SEYRHYCYSL YGTTLEQQYN KPLCDLLIRC INGQKPLCPE EKQRHLDKKQ RFHNGRGRWT GRCMSCCRSS RTRRETQL (SEQ ID NO:2),

and a protein comprising the amino acid sequence:

MHGDTPTLHE YMLDLQPETT DLYGYGQLND SSEEEDEIDG PAGQAEPDRA HYNIVTFCCK CDSTLRLCVQ STHVDIRTLE DLLMGTLGIV CPICSQKP (SEQ ID NO:3); in the manufacture of a medicament for treating a human papillomavirus (HPV) positive cancer.

7. The method of claim 4, or the use of claim 5 or claim 6, wherein the cancer is HPV type 16 positive.

8. The method of claim 4 or claim 7, or the use of any one of claims 5-7 wherein the cancer is a head or neck squamous cell carcinoma.

9. The method of any one of claims 4, 7 or 8, , wherein the patient is receiving or has received platinum-containing chemotherapy; or the use of any one of claims 5-8 wherein the patient is to receive or has received platinum-containing chemotherapy.

10. The method of any one of claims 4 or 7-9, wherein the compound and the proteins comprising the amino acid sequences of SEQ ID NO:2 and SEQ ID NO:3 are administered concurrently; or the use of any one of claims 5 or 7-9 wherein the medicament and the proteins comprising the amino acid sequences of SEQ ID NO:2 and SEQ ID NO:3 are for administration concurrently.

2019227988 02 Oct 2024

11. The method of any one of claims 4 or 7-9, wherein the compound and the proteins comprising the amino acid sequences of SEQ ID NO:2 and SEQ ID NO:3 are administered sequentially; or the use of any one of claims 5 or 7-8 wherein the medicament and the proteins comprising the amino acid sequences of SEQ ID NO:2 and SEQ ID NO:3 are for administration sequentially.

12. A pharmaceutical composition comprising a compound of any one of claims 1-3 and a pharmaceutically acceptable carrier.

13. The pharmaceutical composition of claim 12, wherein the composition further comprises a protein comprising the amino acid sequence:
MHQKRTAMFQ DPQERPRKLP QLCTELQTTI HDIILECVYC KQQLLRREVY DFAFRDLCIV YRDGNPYAVG DKCLKFYSKI SEYRHYCYSL YGTTLEQQYN KPLCDLLIRC INGQKPLCPE EKQRHLDKKQ RFHNGRGRWT GRCMSCCRSS RTRRETQL (SEQ ID NO:2),
and a protein comprising the amino acid sequence:
MHGDTPTLHE YMLDLQPETT DLYGYGQLND SSEEEDEIDG PAGQAEPDRA HYNIVTFCCK CDSTLRLCVQ STHVDIRTLE DLLMGTLGIV CPICSQKP (SEQ ID NO:3).

14. A kit comprising (i) a compound of any one of claims 1-3, and (ii) a protein comprising the amino acid sequence:
MHQKRTAMFQ DPQERPRKLP QLCTELQTTI HDIILECVYC KQQLLRREVY DFAFRDLCIV YRDGNPYAVG DKCLKFYSKI SEYRHYCYSL YGTTLEQQYN KPLCDLLIRC INGQKPLCPE EKQRHLDKKQ RFHNGRGRWT GRCMSCCRSS RTRRETQL (SEQ ID NO:2),
and a protein comprising the amino acid sequence:
MHGDTPTLHE YMLDLQPETT DLYGYGQLND SSEEEDEIDG PAGQAEPDRA HYNIVTFCCK CDSTLRLCVQ STHVDIRTLE DLLMGTLGIV CPICSQKP (SEQ ID NO:3).

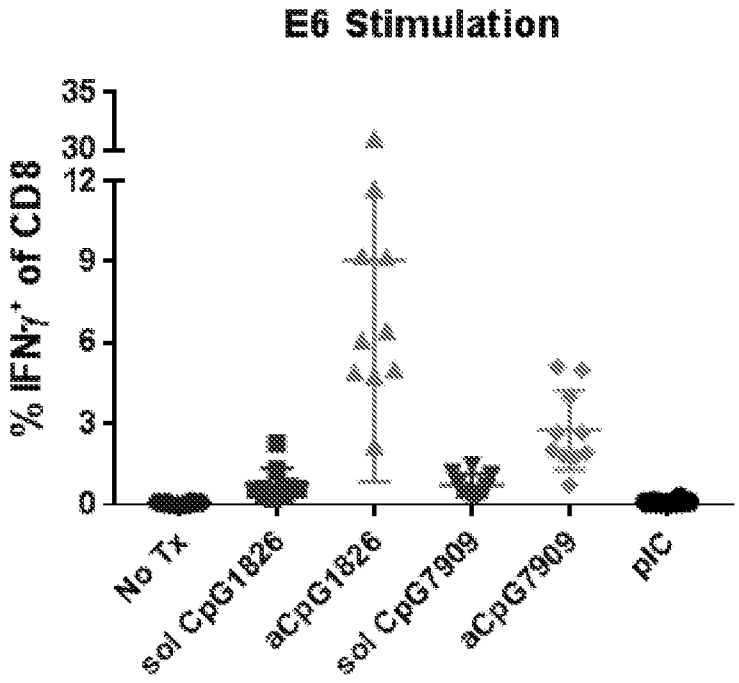

E6 Stimulation
% IFNγ+ of CD8
35
30
12
9
6
3
0
No Tx
sol CpG1826
aCpG1826
sol CpG7909
aCpG7909
pIC

FIG. 1

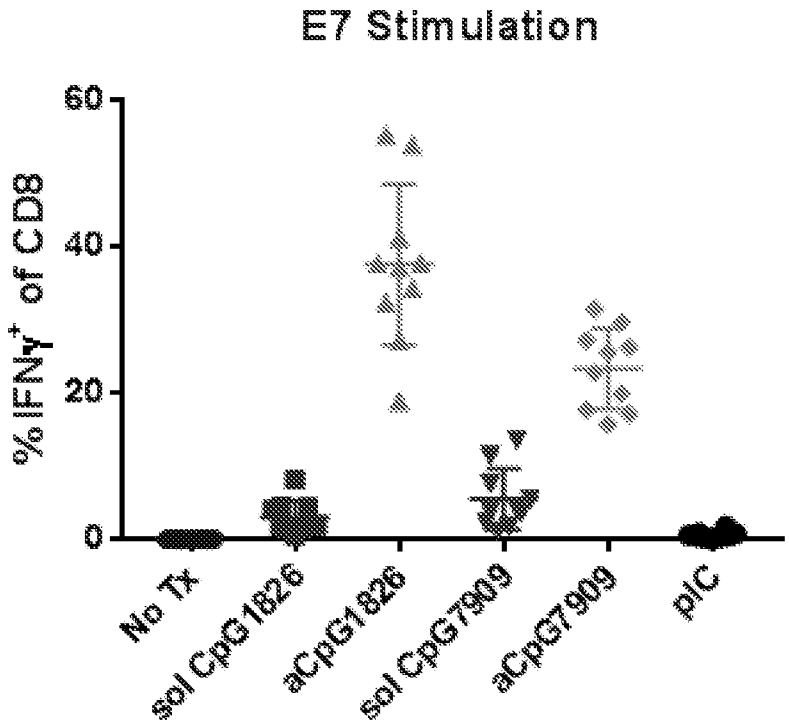

E7 Stimulation
% IFNγ+ of CD8
60
40
20
0
No Tx
sol CpG1826
aCpG1826
sol CpG7909
aCpG7909
pIC

FIG. 2

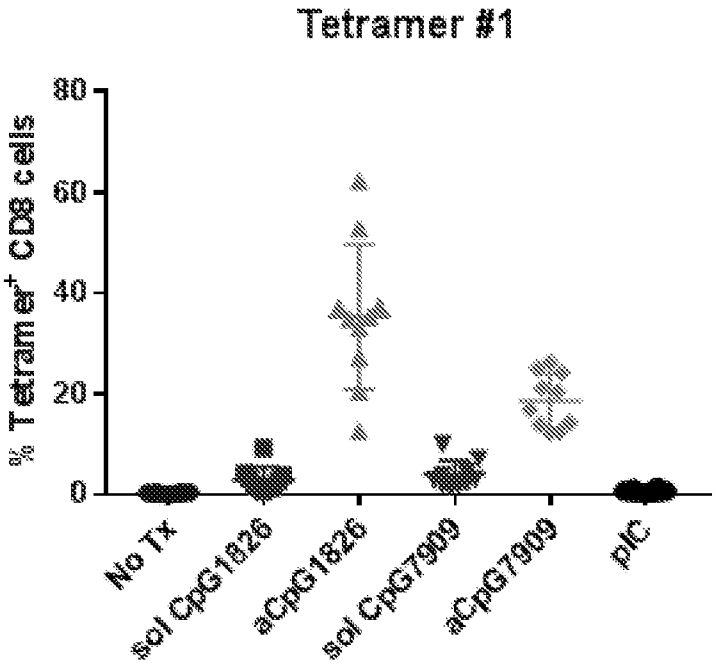

Tetramer #1
% Tetramer+ CD8 cells
80
60
40
20
0
No Tx
sol CpG1826
aCpG1826
sol CpG7909
aCpG7909
pIC

FIG. 3

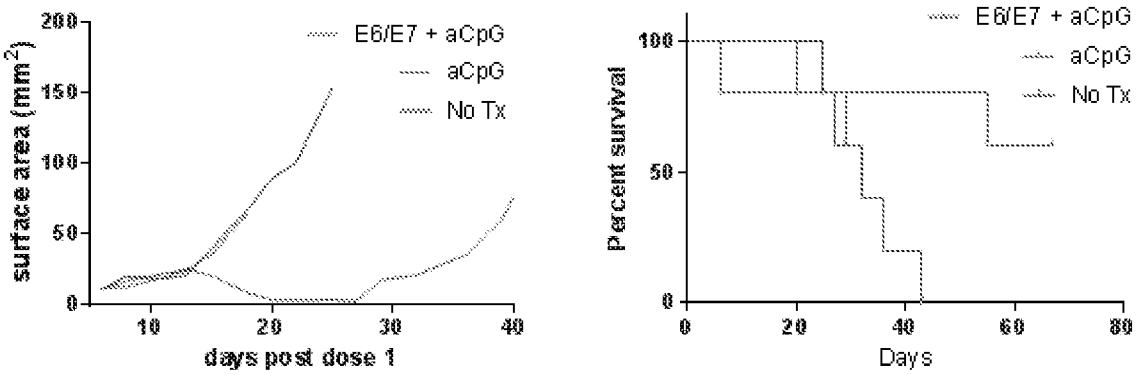

surface area (mm2)
200
150
100
50
0
10
20
30
40
days post dose 1
E6/E7 + aCpG
aCpG
No Tx
Percent survival
100
50
0
0
20
40
60
80
Days
E6/E7 + aCpG
aCpG
No Tx

FIG. 4

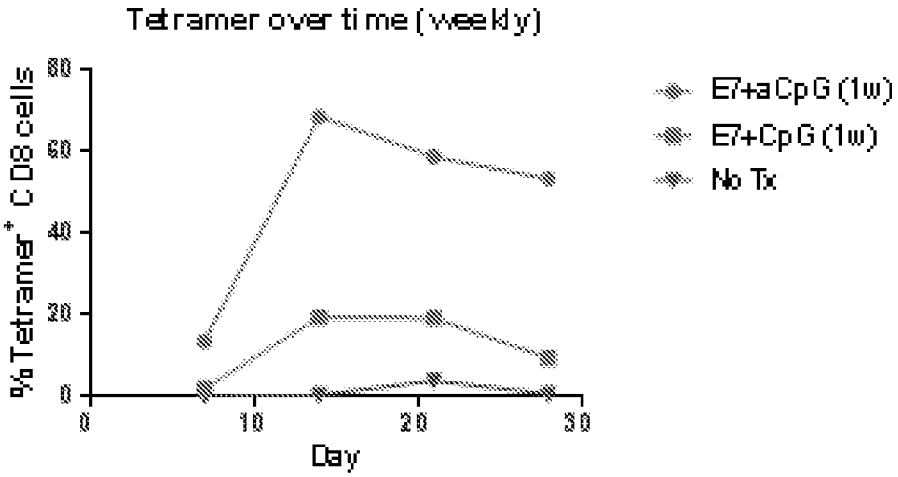

Tetramer over time (weekly)
% Tetramer+ CD8 cells
80
60
40
20
0
0
10
20
30
Day
E7+aCpG (1w)
E7+CpG (1w)
No Tx

FIG. 5

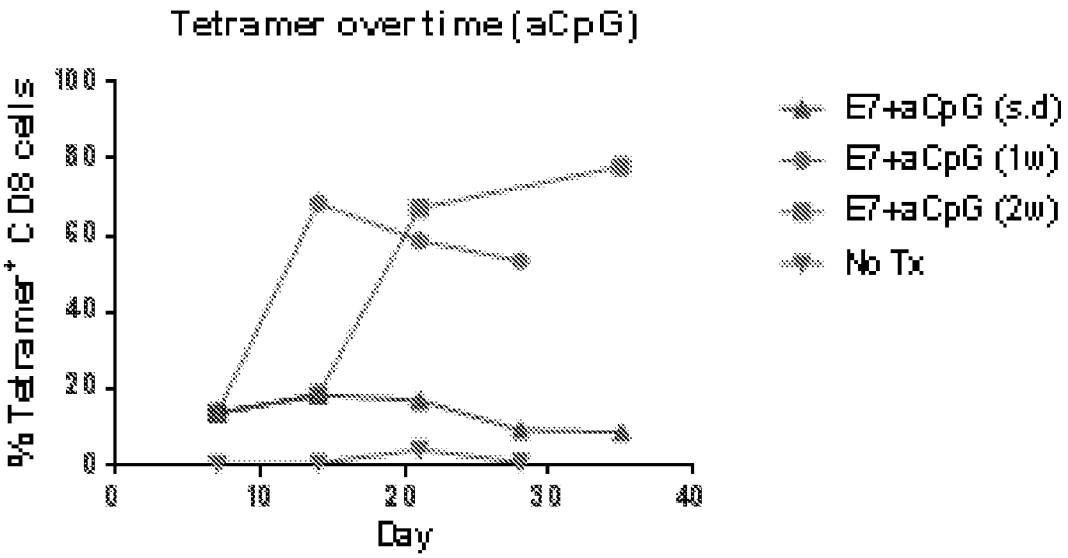

Tetramer overtime (aCpG)
% Tetramer+ CD8 cells
100
80
60
40
20
0
0
10
20
30
40
Day
E7+aCpG (s.d)
E7+aCpG (1w)
E7+aCpG (2w)
No Tx

FIG. 6

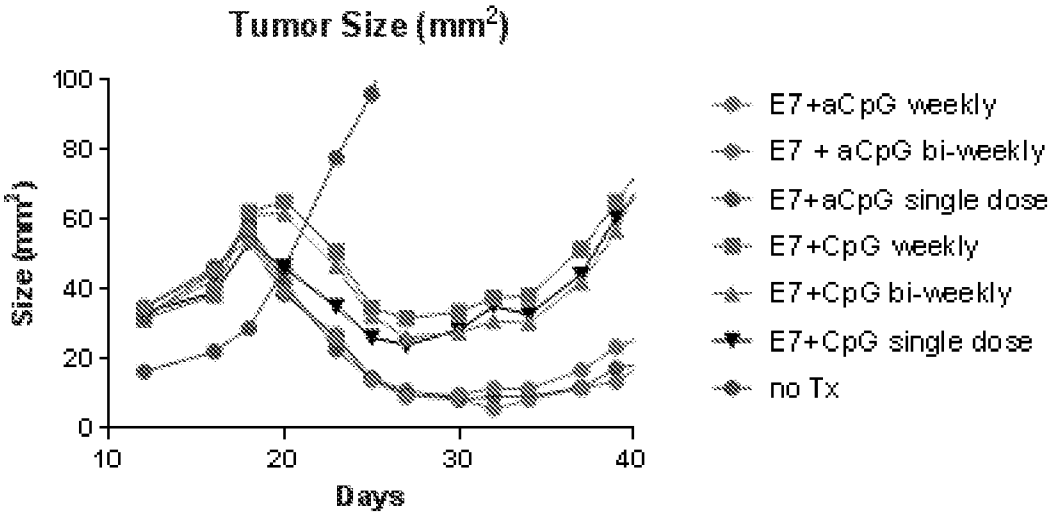

Tumor Size (mm2)
Size (mm2)
100
80
60
40
20
0
10
20
30
40
Days
E7+aCpG weekly
E7 + aCpG bi-weekly
E7+aCpG single dose
E7+CpG weekly
E7+CpG bi-weekly
E7+CpG single dose
no Tx

FIG. 7

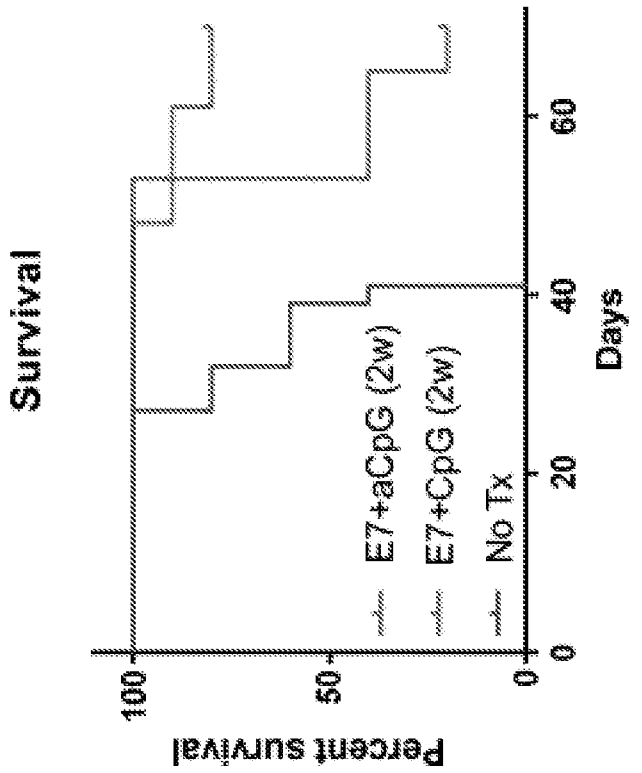

Survival
Percent survival
100
50
0
0
20
40
60
Days
E7+aCpG (2w)
E7+CpG (2w)
No Tx

FIG. 8

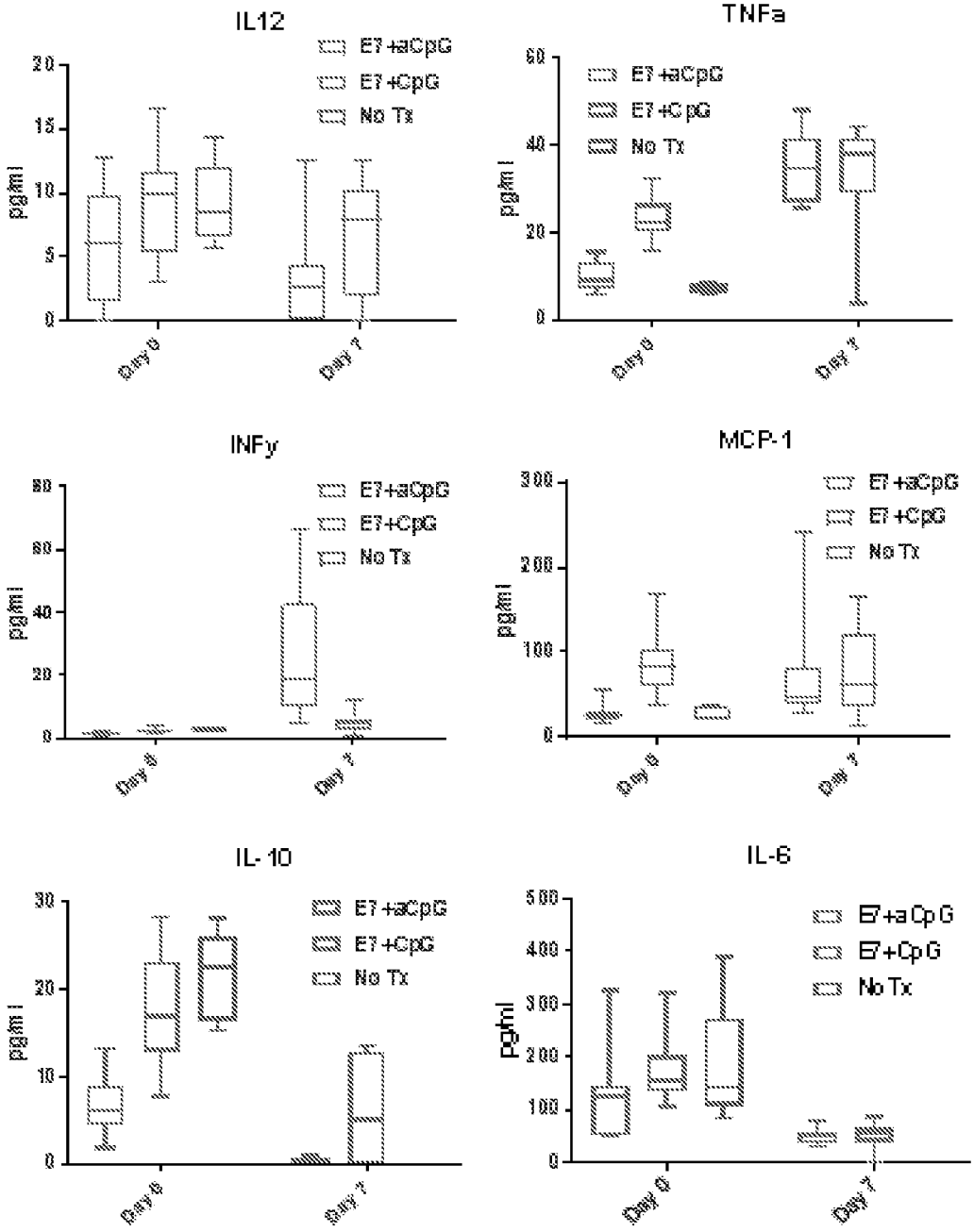

IL12
E7+aCpG
E7+CpG
No Tx
pg/ml
20
15
10
5
0
Day 0
Day 7
TNFa
60
40
20
0
INFy
80
60
40
20
MCP-1
300
200
100
IL-10
30
IL-6
500
400
300
200
100
pg/ml

FIG. 9

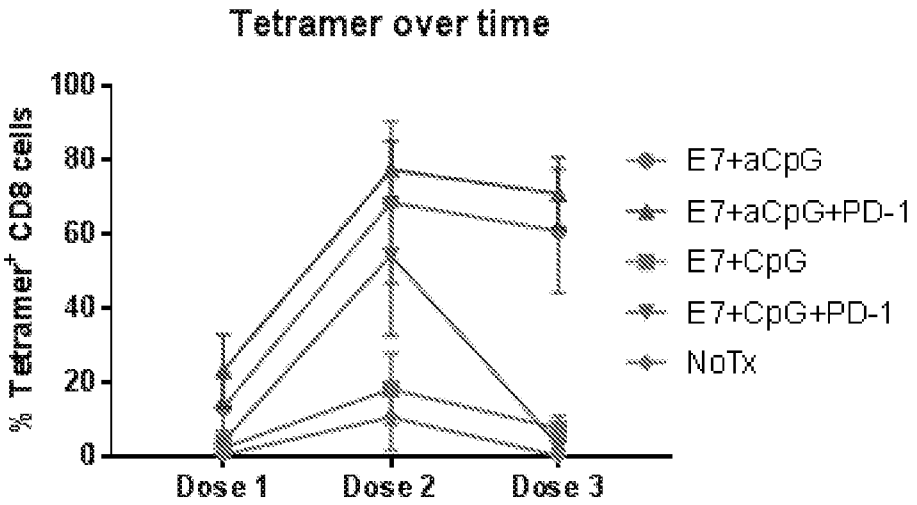

Tetramer over time
% Tetramer+ CD8 cells
100
80
60
40
20
0
Dose 1
Dose 2
Dose 3
E7+aCpG
E7+aCpG+PD-1
E7+CpG
E7+CpG+PD-1
NoTx

FIG. 10

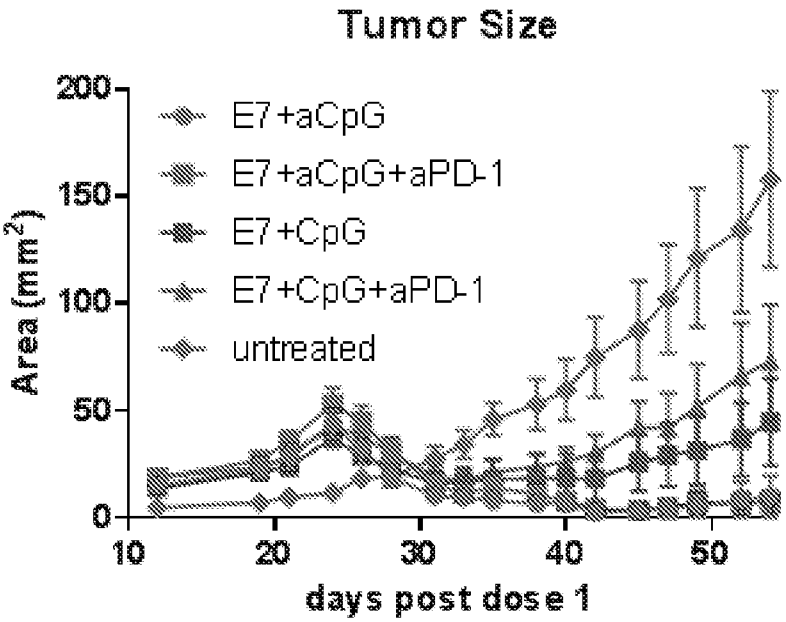

Tumor Size
Area (mm2)
200
150
100
50
0
10
20
30
40
50
days post dose 1
E7+aCpG
E7+aCpG+aPD-1
E7+CpG
E7+CpG+aPD-1
untreated

FIG. 11

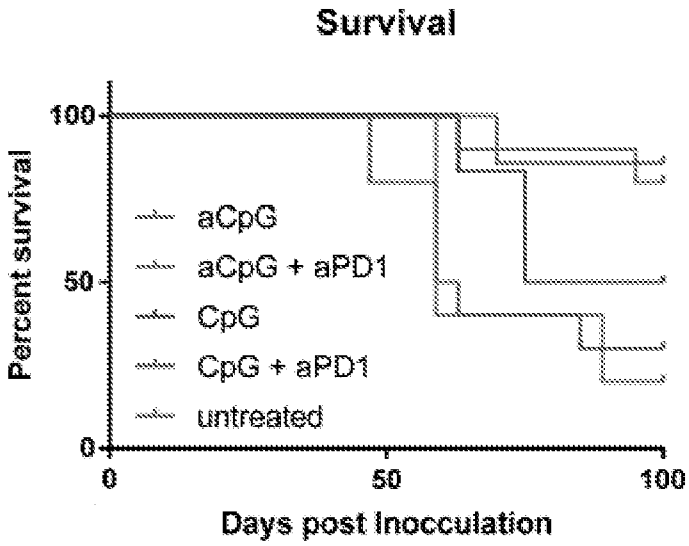

Survival
Percent survival
100
50
0
aCpG
aCpG + aPD1
CpG
CpG + aPD1
untreated
0
50
100
Days post inocculation

FIG. 12

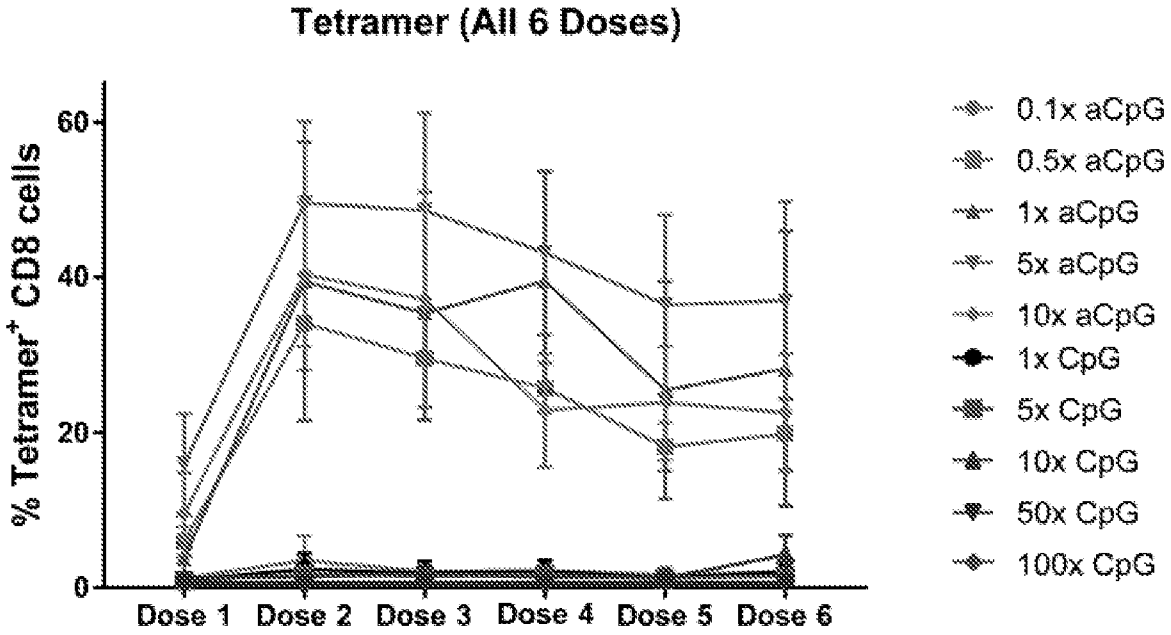

Tetramer (All 6 Doses)
% Tetramer+ CD8 cells
60
40
20
0
Dose 1
Dose 2
Dose 3
Dose 4
Dose 5
Dose 6
0.1x aCpG
0.5x aCpG
1x aCpG
5x aCpG
10x aCpG
1x CpG
5x CpG
10x CpG
50x CpG
100x CpG

FIG. 13

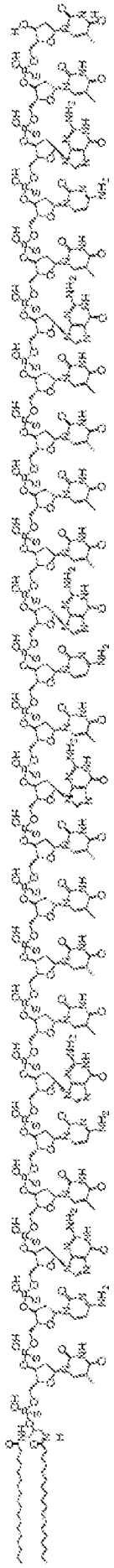


FIG. 14

SEQUENCE LISTING

<110> VEDANTRA PHARMACEUTICALS, INC.

<120> CPG AMPHIPHILES AND USES THEREOF

<130> 51026-027WO2

<140> PCT/US19/20398
<141> 2019-03-01

<150> US 62/637,824
<151> 2018-03-02

<160> 14

<170> PatentIn version 3.5

<210> 1
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 1
tcgtcgtttt gtcgttttgt cgtt 24

<210> 2
<211> 158
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 2

Met His Gln Lys Arg Thr Ala Met Phe Gln Asp Pro Gln Glu Arg Pro
1 5 10 15

Arg Lys Leu Pro Gln Leu Cys Thr Glu Leu Gln Thr Thr Ile His Asp
20 25 30

Ile Ile Leu Glu Cys Val Tyr Cys Lys Gln Gln Leu Leu Arg Arg Glu
35 40 45

```
Val Tyr Asp Phe Ala Phe Arg Asp Leu Cys Ile Val Tyr Arg Asp Gly
    50                  55                  60

Asn Pro Tyr Ala Val Gly Asp Lys Cys Leu Lys Phe Tyr Ser Lys Ile
65                  70                  75                  80

Ser Glu Tyr Arg His Tyr Cys Tyr Ser Leu Tyr Gly Thr Thr Leu Glu
                85                  90                  95

Gln Gln Tyr Asn Lys Pro Leu Cys Asp Leu Leu Ile Arg Cys Ile Asn
            100                 105                 110

Gly Gln Lys Pro Leu Cys Pro Glu Glu Lys Gln Arg His Leu Asp Lys
        115                 120                 125

Lys Gln Arg Phe His Asn Gly Arg Gly Arg Trp Thr Gly Arg Cys Met
    130                 135                 140

Ser Cys Cys Arg Ser Ser Arg Thr Arg Arg Glu Thr Gln Leu
145                 150                 155
```

<210> 3
<211> 98
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 3

```
Met His Gly Asp Thr Pro Thr Leu His Glu Tyr Met Leu Asp Leu Gln
1               5                   10                  15

Pro Glu Thr Thr Asp Leu Tyr Gly Tyr Gly Gln Leu Asn Asp Ser Ser
            20                  25                  30

Glu Glu Glu Asp Glu Ile Asp Gly Pro Ala Gly Gln Ala Glu Pro Asp
        35                  40                  45
```

Arg Ala His Tyr Asn Ile Val Thr Phe Cys Cys Lys Cys Asp Ser Thr
50 55 60

Leu Arg Leu Cys Val Gln Ser Thr His Val Asp Ile Arg Thr Leu Glu
65 70 75 80

Asp Leu Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys Ser Gln
85 90 95

Lys Pro

<210> 4
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 4
tccatgacgt tcctgacgtt 20

<210> 5
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 5
ggtccatgac gttcctgacg tt 22

<210> 6
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 6
tcgtcgtttt gtcgttttgt cgtt 24

<210> 7
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 7

Arg Ala His Tyr Asn Ile Val Thr Phe
1 5

<210> 8
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 8

Glu Val Tyr Asp Phe Ala Phe Arg Asp Leu
1 5 10

<210> 9
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 9

Val Tyr Asp Phe Ala Phe Arg Asp Leu
1 5

<210> 10
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 10

Cys Val Tyr Cys Lys Gln Gln Leu Leu
1   5

<210> 11
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 11

Lys Cys Leu Lys Phe Tyr Ser Lys Ile
1   5

<210> 12
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 12

Asn Lys Pro Leu Cys Asp Leu Leu Ile
1   5

<210> 13
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 13

Arg Ala His Tyr Asn Ile Val Thr Phe
1   5

<210> 14
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 14

Ser Ile Ile Asn Phe Glu Lys Leu
1 5