

(19) **DANMARK**



Patent- og
Varemærkestyrelsen

(10) **DK/EP 3240801 T3**

(12) Oversættelse af
europæisk patentskrift

-
- (51) Int.Cl.: **A 61 K 31/7125 (2006.01)** **A 61 P 35/00 (2006.01)** **C 07 K 14/56 (2006.01)**
C 07 K 16/28 (2006.01)
- (45) Oversættelsen bekendtgjort den: **2021-02-08**
- (80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2021-01-20**
- (86) Europæisk ansøgning nr.: **15876030.6**
- (86) Europæisk indleveringsdag: **2015-12-22**
- (87) Den europæiske ansøgnings publiceringsdag: **2017-11-08**
- (86) International ansøgning nr.: **US2015067269**
- (87) Internationalt publikationsnr.: **WO2016109310**
- (30) Prioritet: **2014-12-31 US 201462098568 P** **2015-01-22 US 201562106526 P**
2015-02-19 US 201562118165 P
- (84) Designerede stater: **AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR**
- (73) Patenthaver: **Checkmate Pharmaceuticals, Inc., 1 Broadway, 14th Floor, Cambridge MA 02142, USA**
- (72) Opfinder: **KRIEG, Arthur, M., 49 Trowbridge Street Unit 3, Cambridge, MA 02138, USA**
- (74) Fuldmægtig i Danmark: **NORDIC PATENT SERVICE A/S, Bredgade 30, 1260 København K, Danmark**
- (54) Benævnelse: **KOMBINATIONSTUMORIMMUNTERAPI**
- (56) Fremdragne publikationer:
WO-A2-03/024481
WO-A2-2012/006634
US-A1- 2009 082 295
US-A1- 2012 251 494
MANGSBO SARA M ET AL: "Enhanced Tumor Eradication by Combining CTLA-4 or PD-1 Blockade With CpG Therapy", JOURNAL OF IMMUNOTHERAPY, LIPPINCOTT WILLIAMS & WILKINS, US, vol. 33, no. 3, 1 April 2010 (2010-04-01), pages 225-235, XP009144370, ISSN: 1524-9557
STEFAN NIERKENS ET AL: "Route of Administration of the TLR9 Agonist CpG Critically Determines the Efficacy of Cancer Immunotherapy in Mice", PLOS ONE, vol. 4, no. 12, 1 January 2009 (2009-01-01), pages e8368-e8368, XP055042111, ISSN: 1932-6203, DOI: 10.1371/journal.pone.0008368
WONG R M ET AL: "TLR-9 signaling and TCR stimulation co-regulate CD8+ T cell-associated PD-1 expression", IMMUNOLOGY LETTERS, ELSEVIER BV, NL, vol. 127, no. 1, 2 December 2009 (2009-12-02), pages 60-67, XP026745618, ISSN: 0165-2478, DOI: 10.1016/J.IMLET.2009.09.002 [retrieved on 2009-09-12]
LEMKE CAITLIN D ET AL: "Combination Lymphoma Immunotherapy Using Checkpoint Blockade and Intratumoral Virus-like Particles Containing CpG TLR9 Agonist - Abstract 3023", BLOOD, & 58TH ANNUAL MEETING AND EXPOSITION OF THE AMERICAN-SOCIETY-OF-HEMATOLOGY; SAN DIEGO, CA, USA; DECEMBER 03 -06, 2016, vol. 128, no. 22, 2 December 2016 (2016-12-02), XP002771299,

Fortsættes ...

- QI XU-FENG ET AL: "CpG oligodeoxynucleotide induces apoptosis and cell cycle arrest in A20 lymphoma cells via TLR9-mediated pathways", *MOLECULAR IMMUNOLOGY*, PERGAMON, GB, vol. 54, no. 3, 26 January 2013 (2013-01-26), pages 327-337, XP028998721, ISSN: 0161-5890, DOI: 10.1016/J.MOLIMM.2013.01.001
- KIM Y H ET AL: "Phase I trial of a Toll-like receptor 9 agonist, PF-3512676 (CPG 7909), in patients with treatment-refractory, cutaneous T-cell lymphoma", *JOURNAL OF THE AMERICAN ACADEMY OF DERMATOLOGY*, MOSBY, INC, US, vol. 63, no. 6, 1 December 2010 (2010-12-01), pages 975-983, XP027499048, ISSN: 0190-9622, DOI: 10.1016/J.JAAD.2009.12.052 [retrieved on 2010-11-18]
- ASSAF A ET AL: "A threshold level of TLR9 mRNA predicts cellular responsiveness to CpG-ODN in haematological and non-haematological tumour cell lines", *CELLULAR IMMUNOLOGY*, ACADEMIC PRESS, SAN DIEGO, CA, US, vol. 259, no. 1, 1 January 2009 (2009-01-01), pages 90-99, XP026459070, ISSN: 0008-8749 [retrieved on 2009-06-06]
- MASON K A ET AL: "Molecular targeting of toll-like receptor 9 with CpG oligodeoxynucleotides (ODN) for enhancement of tumor radioresponse", *INTERNATIONAL JOURNAL OF RADIATION: ONCOLOGY BIOLOGY PHYS*, PERGAMON PRESS, USA, vol. 60, no. 1, 1 September 2004 (2004-09-01), page S346, XP004557674, ISSN: 0360-3016
- KRUG ET AL.: 'Identification of CpG oligonucleotide sequences with high induction of IFN- α in plasmacytoid dendritic cells'. *EUROPEAN JOURNAL OF IMMUNOLOGY* vol. 31, no. 7, 01 July 2001, pages 2154 - 2163, XP002292085 DOI: 10.1002/1521-4141(200107)31:7<2154::AID-IMMU 2154>3.0.CO;2-U
- BUTT ET AL.: 'Immunosuppressive networks and checkpoints controlling antitumor immunity and their blockade in the development of cancer immunotherapeutics and vaccines'. *ONCOGENE* vol. 33, no. 38, 18 September 2013, pages 4623 - 4631, XP055257733 DOI: 10.1038/ONC.2013.432
- KORTYLEWSKI ET AL.: 'In vivo delivery of siRNA to immune cells by conjugation to a tlr9 agonist enhances antitumor immune responses'. *NATURE BIOTECHNOLOGY* vol. 27, no. 10, 01 October 2009, pages 925 - 932, XP055460942 DOI: 10.1038/NBT.1564
- HARTMANN EVELYN ET AL: "Identification and functional analysis of tumor-infiltrating plasmacytoid dendritic cells in head and neck cancer.", *CANCER RESEARCH* 01 OCT 2003, vol. 63, no. 19, 1 October 2003 (2003-10-01), pages 6478-6487, ISSN: 0008-5472
- LABIDI-GALY SANA INTIDHAR ET AL: "Quantitative and functional alterations of plasmacytoid dendritic cells contribute to immune tolerance in ovarian cancer.", *CANCER RESEARCH* 15 AUG 2011, vol. 71, no. 16, 15 August 2011 (2011-08-15), pages 5423-5434, ISSN: 1538-7445
- SISIRAK VANJA ET AL: "Impaired IFN- α production by plasmacytoid dendritic cells favors regulatory T-cell expansion that may contribute to breast cancer progression.", *CANCER RESEARCH* 15 OCT 2012, vol. 72, no. 20, 15 October 2012 (2012-10-15), pages 5188-5197, ISSN: 1538-7445
- ZOU W ET AL: "Stromal-derived factor-1 in human tumors recruits and alters the function of plasmacytoid precursor dendritic cells.", *NATURE MEDICINE* DEC 2001, vol. 7, no. 12, December 2001 (2001-12), pages 1339-1346, ISSN: 1078-8956
- WATKINS STEPHANIE K ET AL: "FOXO3 programs tumor-associated DCs to become tolerogenic in human and murine prostate cancer.", *THE JOURNAL OF CLINICAL INVESTIGATION* APR 2011, vol. 121, no. 4, April 2011 (2011-04), pages 1361-1372, ISSN: 1558-8238
- CONRAD CURDIN ET AL: "Plasmacytoid dendritic cells promote immunosuppression in ovarian cancer via ICOS costimulation of Foxp3(+) T-regulatory cells.", *CANCER RESEARCH* 15 OCT 2012, vol. 72, no. 20, 15 October 2012 (2012-10-15), pages 5240-5249, ISSN: 1538-7445
- FAGET JULIEN ET AL: "ICOS-ligand expression on plasmacytoid dendritic cells supports breast cancer progression by promoting the accumulation of immunosuppressive CD4+ T cells.", *CANCER RESEARCH* 01 DEC 2012, vol. 72, no. 23, 1 December 2012 (2012-12-01), pages 6130-6141, ISSN: 1538-7445
- KRUG A ET AL: "Identification of CpG oligonucleotide sequences with high induction of IFN- α /beta in plasmacytoid dendritic cells.", *EUROPEAN JOURNAL OF IMMUNOLOGY* JUL 2001, vol. 31, no. 7, July 2001 (2001-07), pages 2154-2163, ISSN: 0014-2980

DESCRIPTION

BACKGROUND OF THE INVENTION

[0001] Many scientists have sought to treat cancer by activating the immune system against the tumor. However, despite occasional successes, durable responses to immune therapy have been rare and limited to just a few tumor types. Current understanding of cancer immunotherapy among those skilled in the art has been summarized in recent review articles, including for example Chen and Mellman, *Immunity* 2013 39(1): 1-10. The cycle for induction of therapeutic immune responses against tumors may be broken down into seven distinct steps (**Figure 1**):

1. 1. Release of cancer cell antigens;
2. 2. Presentation of cancer cell antigens by antigen-presenting cells (APC, usually in draining lymph nodes);
3. 3. T-cell priming and activation;
4. 4. Trafficking of CD8⁺ T cells to tumors;
5. 5. Infiltration of CD8⁺ T cells into tumors;
6. 6. Recognition of cancer cells by the infiltrating CD8⁺ T cells; and
7. 7. Killing of cancer cells.

[0002] The art teaches that there are multiple negative and positive mediators of each step of the anti-tumor response. Recent research interest has focused on understanding and addressing the role that negative mediators play in inhibiting the anti-tumor immune response. For example, interleukin-10 (IL-10) is a factor that can have complicated effects, locally immune suppressive in the tumor, but systemically can actually have anti-tumor activity (reviewed in Vicari and Trinchieri, *Immunol. Rev.*, 2004). Although Toll-like receptor (TLR) agonists such as TLR9-activating CpG oligonucleotides (CpG ODN) have immune stimulatory effects that can promote anti-tumor responses, they are also known in the art to induce immune suppressive factors such as IL-10 (reviewed in Lu, *Frontiers Immunol*, 2014). The combination of the B-class CpG 1668, administered into or adjacent to the tumor, with antibody binding either CTLA-4, PD-1 or both, administered systemically, improved long-term survival in a murine experimental bladder cancer and led to increased levels of tumor-reactive T cells and reduced numbers of Tregs at the tumor site (Mangbo et al., *Journal of Immunotherapy*, 2010). The art does not teach designs of TLR9 agonists that have improved anti-tumor effects as a result of inducing lower amounts of IL-10 production. Nevertheless, this increasing recent understanding of the cycle of tumor immunity has heightened awareness that it may be possible to increase the clinical efficacy of cancer immunotherapy by using combinations of agents that act at different points in this cycle for induction of therapeutic immune responses against tumors, but the art does not provide a deep enough understanding of the

immunobiology of cancer to predict which of the many different possible combinations will be preferred.

[0003] Another possible way to consider the development of the anti-cancer T-cell response is the 3-signal model for the induction of a T-cell response, summarized by Kim and Cantor, *Cancer Immunol Res* 2014 2:929-936) and presented in **Figure 2**. In this model signal 1 to the T cell come from the presentation of antigen by an APC on the appropriate MHC to the T cell receptor. Signal 2 is the requirement for a costimulatory signal through the interaction of CD28 on the T cell by B7-1 or B7-2 on the APC (this signal is antagonized by CTLA-4 present on Treg: the efficacy of anti-CTLA-4 antibodies in cancer immunotherapy results from their inhibition of this "off" signal). Finally, signal 3 is the modulation of T cell function resulting from signals via inflammatory cytokine receptors and PD-1. In particular for the induction of optimal CD8⁺ T cell responses, which are known to be critical for successful cancer immunotherapy, type I IFN signaling is a very positive signal, but when chronic or prolonged also can paradoxically lead to T cell exhaustion and unresponsiveness, which is mediated through upregulation of PD-1 expression. Blocking of PD-1 by antibodies to it, or against its major ligand regulating anti-tumor immunity, PD-L1, therefore restores the ability of the T cell to proliferate and produce cytokines in the tumor microenvironment.

[0004] Recently there have been several early clinical successes with the use of "checkpoint inhibitor" (CPI) compounds, such as antibodies, which block the negative immune effects of the checkpoint molecules such as CTLA-4, PD-1, and its ligand, PD-L1. Systemic administration of anti-CTLA-4 antibodies has led to durable responses in ~10% of patients with melanoma, and some encouraging early results in other tumor types, but at the cost of a high rate of adverse effects, including death in some patients. Anti-PD-1/PD-L1 human clinical trials also have been reporting encouraging results, apparently with a lower rate of severe toxicity. However, analyses of the responding patients have revealed that across multiple different types of cancer, responses to anti-PD-L1 therapy are relatively restricted to patients with tumor-infiltrating lymphocytes (TIL) and a Th1 pattern of gene expression in the tumor (Powles et al., *Nature* 2014 515:558; Herbst et al., *Nature* 2014 515:563; Tumei et al., *Nature* 2014 515:568). That is, responses can be seen in some patients with preexisting immunity to the tumor, but are quite unlikely to occur in patients without this. Aside from melanoma, in which pre-existing anti-tumor immunity is relatively common, TIL are relatively uncommon in most other tumor types, indicating that CPI may be of limited benefit in most types of cancer. Thus, there is a need to improve the efficacy of CPI for cancer therapy.

SUMMARY OF THE INVENTION

[0005] The present invention provides methods for promoting immune activation and reducing immune inhibition, thus metaphorically both "stepping on the gas" and "releasing the brakes" of the immune system, to treat cancer. The invention can be used, for example, to convert "cold" (treatment-resistant or -refractory) cancers or tumors to "hot" ones amenable to treatment,

including treatment with checkpoint inhibition.

[0006] This invention provides specific subtypes of CpG ODN with reduced amounts of phosphorothioate modifications compared to the CpG ODN most widely used in past cancer immunotherapy, as set out in the claims, for the improved immunotherapy of cancer, including cancers that would be unlikely to respond to any of these therapies alone, or in other combinations.

[0007] CpG ODN bind and stimulate TLR9, an innate immune receptor which is constitutively expressed in only two type of human immune cell: B cells, which respond to TLR9 stimulation by proliferating and secreting immunoglobulin; and plasmacytoid dendritic cells (pDC), which respond to TLR9 stimulation by secreting large amounts of type I IFN (IFN- α and IFN- β). The present invention is based, at least in part, on the finding that the IFN- α response to CpG ODN is important for tumor immunotherapy. The present invention is based, at least in part, on the finding that a strong IFN- α response to CpG ODN is important for tumor immunotherapy, including tumor immunotherapy using intratumoral administration of CpG ODN.

[0008] A-class CpG DNA as used in the invention are characterized, at least in part, by their propensity to induce high amounts of type I IFN.

[0009] Type I IFN is believed to play a key role in tumor rejection. For example, Type I IFN augments CD8⁺ T-cell survival, expansion, and effector differentiation; promotes dendritic cell (DC) maturation, cross-presentation of tumor-associated antigens to CD8⁺ T cells; is required for immune surveillance against carcinogen-induced tumors; and is required for rejection of implanted tumors. Additionally, levels of type I IFN-related mRNA correlate with tumor-infiltrating lymphocytes (TILs) in human metastases.

[0010] In addition to inducing higher levels of type I IFN than anything else, TLR9 ligands which are A-class CpG DNA also activate pDC and induce secretion of hundreds of other Th1-promoting genes and factors; and convert pDC from immature/tolerance-promoting phenotype to mature, activated, cytotoxic T lymphocyte (CTL)-inducing phenotype.

[0011] The present invention also is based, at least in part, on the finding that delivery of the A-class CpG DNA into tumors (directly or indirectly) induces the expression of adhesion molecules in the local vasculature in and around the tumor, and promotes the egress of activated T cells (CD4⁺ and CD8⁺) from capillaries into the tumor and surrounding region. Some of these T cells will be specific to the unmutated and mutated tumor-associated antigens (TAA). In the absence of checkpoint inhibitors and/or XRT, these T cells may be inhibited by the tumor, but in combination, this creates a much more powerful anti-tumor effect than can be achieved with CpG or the checkpoint inhibitors or XRT on their own.

[0012] The present invention in certain aspects is based on the use of CpG ODN classes other than those that have historically been used for cancer immunotherapy. In particular, the present invention in certain aspects is based on the use of high IFN- α secreting classes, the A-

class, with reduced amounts of phosphorothioate (PS) modifications compared to B-class CpG ODN that have been widely used in the past. B-class CpG ODN are typically completely phosphorothioate-modified to increase their resistance to nucleases and the magnitude of the B-cell activation. In contrast, since a focus of the present invention is on achieving a high type I IFN response, rather than B-cell activation, the A-class CpG DNA of the present invention have either no phosphorothioate modifications, or only 1 or 2 phosphorothioate modifications at the 5' end and 1 to 4 phosphorothioate modifications at the 3' end.

[0013] Those skilled in the art understand that intra- or peritumoral CpG in human cancer patients will activate APC in the tumor draining lymph nodes, enhancing step 2 of the cancer immunity cycle (see **Figure 3**). However, what is not well understood by those skilled in the art is that this route of administration of A-class CpG DNA will also induce TIL and convert the tumor microenvironment to a more Th1-like state that is more conducive to induction of clinically beneficial anti-tumor immunity. The intratumoral administration of A-class CpG DNA induces T cell infiltration into the tumors, notably including CD8⁺ T cell infiltration. The importance of this is that this CD8⁺ T cell infiltration into tumors is believed to be the best predictor of response to treatment with anti-PD-1 or anti-PD-L1. Because the human clinical trials performed in the past with intratumoral administration of CpG oligonucleotides used B-class ODN, there would have been significant local production of IL-10 in the tumor that would have inhibited the anti-tumor immune response. The present invention features A-class CpG DNA, which induce lower amounts of IL-10 production and higher amounts of type I IFN secretion compared to the B-class ODN used in the past. Such A-class CpG DNA will provide improved synergy in cancer therapy when combined with checkpoint inhibitors using the methods of the invention.

[0014] An aspect of the invention is a TLR9 agonist which is an A-class CpG DNA and a checkpoint inhibitor (CPI) which is an antibody or antigen-binding fragment thereof which binds specifically to PD-1 for use in a method of treating a cancerous tumor in a subject, wherein the A-class CpG DNA is administered into or adjacent to the tumor and the antibody or antigen-binding fragment thereof which binds specifically to PD-1 is administered systemically.

[0015] In certain embodiments, the sequence of the A-class CpG DNA is GGGGGGGGGGACGATCGTCGGGGGGGGGG (SEQ ID NO:82).

[0016] In certain embodiments, the cancerous tumor is a lymphoma or a cancerous tumor of an organ or tissue selected from the group consisting of skin, head and neck, esophagus, stomach, liver, colon, rectum, pancreas, lung, breast, cervix, ovary, kidney, bladder, prostate, thyroid, brain, muscle, and bone.

[0017] In certain embodiments, the cancerous tumor is melanoma.

[0018] In certain embodiments, the cancerous tumor is lymphoma.

[0019] In certain embodiments, the cancerous tumor is a cancer of the bone marrow.

[0020] In certain embodiments, the cancerous tumor is a carcinoid tumor.

[0021] In certain embodiments, the cancerous tumor is neuroblastoma.

[0022] In certain embodiments, the subject is a human.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023]

Figure 1 (prior art) is a schematic representation of the cancer immunity cycle, depicting seven steps. From Chen and Mellman, *Immunity* 2013.

Figure 2 is a schematic representation of the three signals needed for induction of anti-tumor immunity. Each T cell expresses a unique TCR that recognizes a specific antigen in the context of a specific MHC (signal 1). CD4 and CD8 coreceptors increase the sensitivity of antigen recognition by TCR. Optimal T-cell expansion and acquisition of effector function require signals transduced by costimulatory receptors (signal 2). CD28-BB7-1/B7-2 interaction delivers an activation signal, whereas CTLA-4-B71/B7-2 interaction inhibits T-cell activation. Signaling via CD28 and CTLA-4 is also critical for the development and function of CD4 Treg. Inflammatory signals often induce upregulation of surface cytokine receptors and other receptors, including PD-1 (signal 3). Expression of PD-1 is associated with acquisition of an exhausted phenotype in T cells during infection and cancer. PD-1-PD-L1 interaction is involved in the inhibition of T_{FR} activity and has also been implicated in pTreg generation. Preclinical and clinical data with checkpoint blockade using anti-CTLA-4, anti-PD-1, and anti-PD-L1 Abs suggest that increased antitumor immunity may be achieved by the combined effects of enhanced Teff activity and depletion or reduced suppression by CD4 Treg. From Kim and Cantor, *Cancer Immunol. Res.* 2014 2:926-936.

Figure 3 is a schematic representation of the cancer immunity cycle, depicting roles for CpG ODN, CPI, and XRT. Adapted from Chen and Mellman, *Immunity* 2013.

Figure 4 is a graph depicting IFN- α induction for Set 1 CpG-A oligonucleotides. PBS, phosphate buffered saline control.

Figure 5 is a graph depicting IFN- α induction for selected Set 1 CpG-A oligonucleotides. PBS, phosphate buffered saline control.

Figure 6 is a graph depicting IFN- α induction for Set 2 CpG-A oligonucleotides. Y-axis, pg/mL IFN- α . PBS, phosphate buffered saline control; TE, Tris-EDTA.

Figure 7 is a graph depicting interleukin-10 (IL-10) induction for Set 2 CpG-A oligonucleotides. Y-axis, pg/mL IL-10. PBS, phosphate buffered saline control; TE, Tris-EDTA.

Figure 8 is a graph depicting effect of phosphorodithioate backbone modification on IFN- α induction by Set 3 CpG-A oligonucleotides.

Figure 9 is a graph depicting structure-activity relationship of reducing the number of 5' and/or 3' G in CpG-A oligonucleotide G10 or changing the palindrome on induction of IFN- α secretion from normal human peripheral blood mononuclear cells (PBMCs). nAb, new anti-Qb antibody; oAb, old anti-Qb antibody; PBS, phosphate buffered saline control.

Figure 10 is a graph depicting structure-activity relationship of reducing the number of 5' and/or 3' G in CpG-A oligonucleotide G10 or changing the palindrome on induction of IP-10 secretion from normal human peripheral blood mononuclear cells (PBMCs). nAb, new anti-Qb antibody; oAb, old anti-Qb antibody; PBS, phosphate buffered saline control.

Figure 11 is a graph depicting structure-activity relationship of reducing the number of 5' and/or 3' G in CpG-A oligonucleotide G10 or changing the palindrome on induction of IL-10 secretion from normal human peripheral blood mononuclear cells (PBMCs). nAb, new anti-Qb antibody; oAb, old anti-Qb antibody; PBS, phosphate buffered saline control.

Figure 12 is a pair of graphs depicting tumor volumes in A20 lymphoma-bearing mice. All mice were primed with a low dose (20 μ g) of CMP-001 to induce anti-Qb antibodies so that the virus-like particles (VLP) will be opsonized and activate DC once treatment is initiated. Lymphoma cells were inoculated on both flanks of mice on day 0. Beginning on day 7, tumors on one side (treated) of mice were directly injected with CpG (CMP-001) or saline, while tumors on the other side (untreated) were not. Mice then also received intraperitoneal anti-PD-1 or saline twice weekly, as indicated. The graph in Panel A depicts average tumor volumes for "untreated" (distant) tumors. The graph in Panel B depicts average tumor volumes for "treated" tumors. N = 10 for each group.

Figure 13 is a graph depicting survival curves for mice in the experiment in Figure 12.

DETAILED DESCRIPTION OF THE INVENTION

[0024] Toll-like receptor (TLR) ligands in general are known to be potential inducers of the presentation of cancer cell antigens by APC. However, it is not previously known what particular TLR ligands are preferred, and even in the case of TLR9 ligands, it is not previously known which, if any, class of CpG ODN is preferred, nor are their preferred doses and routes of administration previously known. Nearly all human clinical trials of CpG ODN in oncology have used B-class ODN administered via a systemic route, while a few trials have explored intratumoral administration (discussed further below).

[0025] The invention of immune stimulatory CpG oligodeoxynucleotides (ODN) and subsequent inventions of various classes and designs of CpG ODN provided new opportunities for cancer immunotherapy. Based on encouraging preclinical data in rodent models, human

clinical trials of CpG ODN have been performed in oncology patients using systemic and intratumoral administration of several different CpG ODN alone or in combination with various chemotherapy regimens, vaccines, antibodies, and radiotherapy, but again, clinical responses have been uncommon, and despite some encouraging early clinical trial results, phase 3 trials have so far failed (reviewed in Krieg, *Nucleic Acid Ther.* 2012 22(2): 77-89). Therefore, there exists a need to provide improved oligonucleotide therapeutic approaches to increase the success rate of cancer immunotherapy.

[0026] Tumor vaccines in which a cancer patient is vaccinated with a conserved unmutated self antigen together with an adjuvant have been a goal of immuno-oncologists for many years, yet despite successfully inducing immunity against the selected antigen, have almost uniformly failed to deliver clear clinical benefits. B-class CpG ODN have enhanced the induction of anti-tumor CD8⁺ T cell responses in multiple cancer vaccine clinical trials (for example, Kruit et al., *J Clin Oncol* 2013; Tarhini et al., *J Immunother* 2013; Lovgren et al., *Cancer Immunol Immunother* 2012; Karbach et al., *Clin Cancer Res* 2011; Karbach et al., *Int J Cancer* 2010; Speiser et al., *JCI* 2005, and in a single trial an unmodified A-class CpG ODN was used as a vaccine adjuvant (Speiser et al., *J. Immunother* 2010), yet these have seldom been associated with clinical responses, and a phase 3 clinical trial of this approach conducted by GSK (GlaxoSmithKline) using the MAGE-3 tumor antigen so far appears to have been a failure. In particular it is noteworthy that the vaccine clinical trial using an A-class CpG ODN showed relatively weak induction of a CTL response that increased approximately two-fold from baseline in only about half of the patients, compared to an approximate average 10-fold increased CTL response in those melanoma patients previously vaccinated using B-class CpG ODN, indicating the state of the art. It is possible that the immune system will not easily overcome self-tolerance to unmutated self antigens to a degree sufficient to reject a tumor, leading many of those skilled in the art to search for ways to induce tumor immunity against alternative, mutated tumor antigens. Recent studies using deep sequencing of tumor transcriptomes have revealed that all cancers contain variable numbers of unique mutated antigens, referred to as tumor-specific neoantigens (Rajasagi et al., *Blood* 2014 124(3): 453-462), and those skilled in the art have sought ways to direct the anti-tumor immune response against such antigens. One approach being pursued is to synthesize some or all of these neoantigens as peptides, and to vaccinate a cancer patient with the appropriate antigenic peptides to be presented on Class II MHC in a formulation such as viral-like particle and using a very strong adjuvant, such as a CpG B-class ODN. Such an approach would be extremely complex and expensive to develop. Therefore, there is a need for improved methods to induce anti-tumor immune responses against tumor-specific neoantigens.

[0027] The present invention provides a superior approach by turning the tumor itself into a vaccine, due to altering the tumor microenvironment in such a way as to disengage the "brakes" of the checkpoint inhibitors, while inducing strong cell-mediated immunity, using TLR9 agonists.

[0028] Radiotherapy has long been used in the treatment of cancer, and it is currently employed in the treatment of approximately 60% of patients with solid tumors (reviewed in

Prasanna et al., J Thoracic Dis. 2014 6(4):287-302). Although radiotherapy often can shrink tumors, this effect is most commonly palliative, and durable responses are extremely uncommon. Moreover, radiotherapy is generally only suitable for treating one or a small number of tumor lesions, and thus is not generally used in the treatment of metastatic cancer.

[0029] In some unusual cases, XRT can lead to regression of distant tumor masses as a result of the induction of a specific immune response against tumor antigens present not only in the irradiated lesion, but also in distant metastases. This has been termed an "abscopal effect", and particularly since a recent case report by Postow et al. (N. Engl. J. Med. 2012 366(10): 925-31), this term has come to be used to include other forms of localized tumor therapy besides just radiotherapy.

[0030] Abscopal effects can be seen when XRT is given either before or after anti-CTLA-4 therapy: for example, more than half of 21 melanoma patients treated with XRT following anti-CTLA-4 therapy showed evidence for distal tumor regressions (Grimaldi et al., Oncoimmunol. 2014 3: e28780).

I. DEFINITIONS

[0031] Unless otherwise defined herein, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well known and commonly used in the art.

[0032] The methods and techniques of the present invention are generally performed according to methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. Such references include, e.g., Sambrook and Russell, Molecular Cloning, A Laboratory Approach, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (2001), Ausubel et al., Current Protocols in Molecular Biology, John Wiley & Sons, NY (2002), and Harlow and Lane Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1990). Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. The nomenclatures used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

[0033] As used herein, each of the following terms has the meaning associated with it in this

section.

[0034] The articles "a" and "an" are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

[0035] As used herein, the twenty conventional amino acids and their abbreviations follow conventional usage. See Immunology--A Synthesis (2nd Edition, E. S. Golub and D. R. Gren, Eds., Sinauer Associates, Sunderland, Mass. (1991)).

[0036] Conventional notation is used herein to portray polypeptide sequences: the left-hand end of a polypeptide sequence is the amino-terminus; the right-hand end of a polypeptide sequence is the carboxyl-terminus.

[0037] A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain R group with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent sequence identity or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well-known to those of skill in the art. See, e.g., Pearson, Methods Mol. Biol. 243:307-31 (1994).

[0038] Examples of groups of amino acids that have side chains with similar chemical properties include 1) aliphatic side chains: glycine, alanine, valine, leucine, and isoleucine; 2) aliphatic-hydroxyl side chains: serine and threonine; 3) amide-containing side chains: asparagine and glutamine; 4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; 5) basic side chains: lysine, arginine, and histidine; 6) acidic side chains: aspartic acid and glutamic acid; and 7) sulfur-containing side chains: cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine.

[0039] Alternatively, a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet et al., Science 256:1443-45 (1992). A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

[0040] Preferred amino acid substitutions are those which: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming protein complexes, and (4) confer or modify other physicochemical or functional properties of such analogs. Analogs comprising substitutions, deletions, and/or insertions can include various muteins of a sequence other than the naturally-occurring peptide sequence. For example, single or multiple amino acid substitutions (preferably conservative amino acid substitutions) may be made in the naturally-occurring sequence (preferably in the portion of the polypeptide

outside the domain(s) forming intermolecular contacts). A conservative amino acid substitution should not substantially change the structural characteristics of the parent sequence (e.g., a replacement amino acid should not tend to break a helix that occurs in the parent sequence, or disrupt other types of secondary structure that characterizes the parent sequence). Examples of art-recognized polypeptide secondary and tertiary structures are described in *Proteins, Structures and Molecular Principles* (Creighton, Ed., W. H. Freeman and Company, New York (1984)); *Introduction to Protein Structure* (C. Branden and J. Tooze, eds., Garland Publishing, New York, N.Y. (1991)); and Thornton et al., *Nature* 354:105 (1991).

[0041] Sequence similarity for polypeptides, and similarly sequence identity for polypeptides, is typically measured using sequence analysis software. Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, GCG contains programs such as "Gap" and "Bestfit" which can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutin thereof. See, e.g., GCG Version 6.1. Polypeptide sequences also can be compared using FASTA using default or recommended parameters, a program in GCG Version 6.1. FASTA (e.g., FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson, *Methods Enzymol.* 183:63-98 (1990); Pearson, *Methods Mol. Biol.* 132:185-219 (2000)). Another preferred algorithm when comparing a sequence of the invention to a database containing a large number of sequences from different organisms is the computer program BLAST, especially blastp or tblastn, using default parameters. See, e.g., Altschul et al., *J. Mol. Biol.* 215:403-410 (1990); Altschul et al., *Nucleic Acids Res.* 25:3389-402 (1997).

[0042] An intact "antibody" comprises at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. See generally, *Fundamental Immunology*, Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)). Each heavy chain is comprised of a heavy chain variable region (HCVR or V_H) and a heavy chain constant region (C_H). The heavy chain constant region is comprised of three domains, $CH1$, $CH2$ and $CH3$. Each light chain is comprised of a light chain variable region (LCVR or V_L) and a light chain constant region. The light chain constant region is comprised of one domain, C_L . The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxyl-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The assignment of amino acids to each domain is in accordance with the definitions of Kabat, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md. (1987 and 1991)), or Chothia & Lesk, *J. Mol. Biol.* 196:901-917 (1987); Chothia et al., *Nature* 342:878-883 (1989).

[0043] The variable regions of the heavy and light chains contain a binding domain that

interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component (C1q) of the classical complement system.

[0044] The term "antibody" can include antigen-binding portions of an intact antibody that retain capacity to specifically bind the antigen of the intact antibody, e.g., PD-1. Antigen-binding portions may be produced by recombinant DNA techniques or by enzymatic or chemical cleavage of intact antibodies.

[0045] Examples of antigen-binding portions include (i) a Fab fragment, a monovalent fragment consisting of the V_L , V_H , C_L and $CH1$ domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the V_H and $CH1$ domains; (iv) a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody, (v) a single domain antibody ("dAb"), which consists of a V_H domain as described in Ward et al., Nature 341:544-546 (1989); and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, V_H and V_L , are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_H and V_L regions pair to form monovalent molecules (known as single chain Fv (scFv); See, e.g., Bird et al. Science 242:423-426 (1988); and Huston et al. Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988)). Such single chain antibodies are included by reference to the term "antibody".

[0046] A "bispecific antibody" has two different binding specificities, see, e.g., U.S. Pat. No. 5,922,845 and U.S. Pat. No. 5,837,243; Zeilder J. Immunol. 163:1246-1252 (1999); Somasundaram Hum. Antibodies 9:47-54 (1999); Keler Cancer Res. 57:4008-4014 (1997). For example, the invention provides bispecific antibodies having one binding site for a cell surface antigen, such as human PD-1, and a second binding site for an Fc receptor on the surface of an effector cell. The invention also provides multispecific antibodies, which have at least three binding sites.

[0047] Contemplated by the present invention are bispecific antibodies which bind any PD-1 and a different checkpoint inhibitor. For example, the different CPI may be selected from the group consisting of PD-L1, CTLA-4, TIM3, and LAG3. Thus, for example, bispecific antibodies may bind PD-1 and PD-L1, PD-1 and CTLA-4, PD-1 and TIM3, PD-1 and LAG3. In certain embodiments, the bispecific antibodies may bind PD-1 and PD-L1, PD-1 and CTLA-4, PD-1 and TIM3, or PD-1 and LAG3. In certain embodiments, the bispecific antibodies may bind PD-1 and PD-L1, or PD-1 and CTLA-4. In certain embodiments, the bispecific antibodies may bind PD-1 and PD-L1.

[0048] The term "bispecific antibodies" further includes "diabodies." Diabodies are bivalent, bispecific antibodies in which the V_H and V_L domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the

same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (See, e.g., Holliger et al., Proc. Natl. Acad. Sci. USA 90:6444-6448 (1993); Pollak et al., Structure 2:1121-1123 (1994)).

[0049] The terms "human antibody" or "human sequence antibody", as used interchangeably herein, include antibodies having variable and constant regions (if present) derived from human germline immunoglobulin sequences. The human sequence antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). However, the term "human antibody", as used herein, is not intended to include "chimeric" antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences (i.e., "humanized" or PRIMATIZED™ antibodies).

[0050] The term "chimeric antibody" as used herein means an antibody that comprises regions from two or more different antibodies. For example, in one embodiment, one or more of the CDRs are derived from a human anti-PD-1 antibody. In another embodiment, all of the CDRs are derived from a human anti-PD-1 antibody. In another embodiment, the CDRs from more than one human anti-PD-1 antibody are combined in a chimeric human antibody. For instance, a chimeric antibody may comprise a CDR1 from the light chain of a first human anti-PD-1 antibody, a CDR2 from the light chain of a second human anti-PD-1 antibody, and a CDR3 from the light chain of a third human anti-PD-1 antibody; and similarly the CDRs from the heavy chain may be derived from one or more other anti-PD-1 antibodies. Further, the framework regions may be derived from one of the same anti-PD-1 antibodies or from one or more different human(s).

[0051] Moreover, as discussed previously herein, chimeric antibody includes an antibody comprising a portion derived from the germline sequences of more than one species.

[0052] By the term "compete", as used herein with regard to an antibody, is meant that a first antibody, or an antigen-binding portion thereof, competes for binding with a second antibody, or an antigen-binding portion thereof, where binding of the first antibody with its cognate epitope is detectably decreased in the presence of the second antibody compared to the binding of the first antibody in the absence of the second antibody. The alternative, where the binding of the second antibody to its epitope is also detectably decreased in the presence of the first antibody, can, but need not be the case. That is, a first antibody can inhibit the binding of a second antibody to its epitope without that second antibody inhibiting the binding of the first antibody to its respective epitope. However, where each antibody detectably inhibits the binding of the other antibody with its cognate epitope or ligand, whether to the same, greater, or lesser extent, the antibodies are said to "cross-compete" with each other for binding of their respective epitope(s). For instance, cross-competing antibodies can bind to the epitope, or portion of the epitope, to which antibodies of the invention bind. Both competing and cross-competing antibodies are encompassed by the present invention. Regardless of the mechanism by which such competition or cross-competition occurs (e.g., steric hindrance,

conformational change, or binding to a common epitope, or portion thereof, and the like), the skilled artisan would appreciate, based upon the teachings provided herein, that such competing and/or cross-competing antibodies are encompassed and can be useful for the methods disclosed herein.

[0053] The term "epitope" includes any protein determinant capable of specific binding to an immunoglobulin or T-cell receptor. Epitopic determinants usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three-dimensional structural characteristics, as well as specific charge characteristics. Conformational and nonconformational epitopes are distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents.

[0054] By the phrase "specifically binds," as used herein, is meant a compound, e.g., a protein, a nucleic acid, an antibody, and the like, which recognizes and binds a specific molecule, but does not substantially recognize or bind other molecules in a sample. For instance, the phrase "specifically binds" may characterize an antibody or a peptide inhibitor which recognizes and binds a cognate ligand (e.g., an anti-PD-1 antibody that binds with its cognate antigen, PD-1) in a sample, but does not substantially recognize or bind other molecules in the sample. Thus, under designated assay conditions, the specified binding moiety (e.g., an antibody or an antigen-binding portion thereof) binds preferentially to a particular target molecule and does not bind in a significant amount to other components present in a test sample. A variety of assay formats may be used to select an antibody that specifically binds a molecule of interest. For example, solid-phase ELISA immunoassay, immunoprecipitation, BIAcore and Western blot analysis are used to identify an antibody that specifically reacts with PD-1. Typically a specific or selective reaction will be at least twice background signal or noise and more typically more than 10 times background, even more specifically, an antibody is said to "specifically bind" an antigen when the equilibrium dissociation constant (K_D) is $\leq 1 \mu\text{M}$, preferably $\leq 100 \text{ nM}$, and most preferably $\leq 10 \text{ nM}$.

[0055] Preferably, an "antibody which binds specifically to a CPI" is an antibody or antigen-binding fragment thereof, which, in addition to binding its target CPI, interferes with reciprocal interaction between the bound target CPI and its cognate ligand. For example, an antibody which binds specifically to PD-1 preferably is an antibody or antigen-binding fragment thereof, which, in addition to binding PD-1, interferes with reciprocal interaction between PD-1 and its cognate ligand, PD-L1.

[0056] The term " K_D " refers to the equilibrium dissociation constant of a particular antibody-antigen interaction.

[0057] As used herein, "substantially pure" means an object species is the predominant species present (i.e., on a molar basis it is more abundant than any other individual species in the composition), and preferably a substantially purified fraction is a composition wherein the object species (e.g., an anti-PD-1 antibody) comprises at least about 50 percent (on a molar basis) of all macromolecular species present. Generally, a substantially pure composition will

comprise more than about 80 percent of all macromolecular species present in the composition, more preferably more than about 85%, 90%, 95%, and 99%. Most preferably, the object species is purified to essential homogeneity (contaminant species cannot be detected in the composition by conventional detection methods) wherein the composition consists essentially of a single macromolecular species.

[0058] By the term "therapeutically effective amount," as used herein, is meant an amount that when administered to a mammal, preferably a human, mediates a detectable therapeutic response compared to the response detected in the absence of the compound. A therapeutic response, such as, but not limited to, inhibition of and/or decreased tumor growth (including tumor size stasis), tumor size, metastasis, and the like, can be readily assessed by a plethora of art-recognized methods, including, e.g., such methods as disclosed herein.

[0059] The skilled artisan would understand that the effective amount of the compound or composition administered herein varies and can be readily determined based on a number of factors such as the disease or condition being treated, the stage of the disease, the age and health and physical condition of the mammal being treated, the severity of the disease, the particular compound being administered, and the like.

[0060] A "therapeutically effective amount" is intended to qualify the amount of an agent required to detectably reduce to some extent one or more of the symptoms of a neoplastic disorder, including, but not limited to: 1) reduction in the number of cancer cells; 2) reduction in tumor size; 3) inhibition (i.e., slowing to some extent, preferably stopping) of cancer cell infiltration into peripheral organs; 4) inhibition (i.e., slowing to some extent, preferably stopping) of tumor metastasis; 5) inhibition, to some extent, of tumor growth; 6) relieving or reducing to some extent one or more of the symptoms associated with the disorder; and/or 7) relieving or reducing the side effects associated with the administration of anticancer agents.

[0061] A "therapeutically effective amount" of a TLR9 agonist can also be defined based on a biomarker response using any of the well-defined blood or tissue markers for TLR9 activation that are well known to those skilled in the art. The A-class CpG DNA of the present invention are broadly similar to other CpG ODN (e.g., B-class) in their induction of a Th1-like cytokine and chemokine response in the serum, plasma, PBMC, and/or tissues or biopsies, which can be measured as described by Krieg et al., J. Immunother., 2004 27:460-471 using for example cytokine assays for IP-10, I-TAC, MIG, MIP-1 β , MIP-3 β , IL-6, IL-12p40, or IFN- α from serum or plasma collected approximately 24 hr after the treatment, or can also be assessed by RT-PCR assays of PBMC. A therapeutically effective amount of the A-class CpG DNA that is injected intratumorally into a cancer patient will increase serum IP-10 levels by 24 hours to at least 100 pg/ml, and preferably to between 100-100,000 pg/ml, and most preferably to between 1,000 to 10,000 pg/mL.

[0062] In contrast to chemotherapy drugs, for which the dose is generally escalated to the maximal tolerated dose (MTD), immune stimulatory drugs such as the A-class CpG DNA of the present invention function best at an optimal biologic dose (OBD), which is generally below the

MTD. The serum cytokines and chemokines provide one simple measure to estimate the optimal biologic dose. The intended biologic effect of the A-class CpG DNA of the present invention is to convert the tumor microenvironment (and that of the draining lymph nodes) from immunosuppressive --with a low level of IFN production and lacking in activated TIL -- to an immune activated microenvironment that shows increased production of IFN, especially type I IFN, and which now has increased TIL that display activation markers such as PD-L1, as reflected for example in the tumor biopsy characteristics of patients responding to treatment with anti-PD-1 or anti-PD-L1 reported by Tumeh et. al., Nature 2014 515:568-571; and by Herbst et al., Nature 2014 515:563-567, respectively, or additionally by Taube et al., Clin Cancer Res. 2014. Expressed another way, recent studies have demonstrated that anti-PD-1 or anti-PD-L1 therapy is generally only effective in patients who already have TIL, and already have a tumor microenvironment that reflects IFN effects (such as expression of PD-L1, which is induced by IFN). Patients who lack these characteristics on a pre-treatment tumor biopsy are unlikely to respond to therapy with anti-PD-1 or anti-PD-L1 unless they also receive treatment with an agent that induces TIL and high production of type I IFN: the A-class CpG DNA of the present invention are the perfect agent for this purpose.

[0063] The major endogenous source of type I IFN in humans and other animals is the plasmacytoid dendritic cell (pDC). pDC produce more than 99% of the type I IFN that is made in response to pathogen infection (Siegal et al., Science 1999). Yet very few molecularly-defined stimuli have been shown to activate the pDC to secrete high levels of type I IFN. In fact, to date A-class CpG ODN are by far the strongest stimulus for pDC production of type I IFN that have been reported in the scientific literature, and, surprisingly, the A-class CpG DNA of the present invention are even more effective than those previously known in the art.

[0064] Certain preferred A-class CpG DNA induce high or large amounts of type I IFN. Assays for measuring type I IFN are well known in the art and include *in vitro* enzyme-linked immunosorbent assay (ELISA) and cell-based assays, such as are described herein. Without meaning to be limiting, large or high amounts of type I IFN can refer to greater than or equal to about 1000 pg/mL IFN- α as measured according to such *in vitro* assays. In certain embodiments, large or high amounts of type I IFN can refer to greater than or equal to about 2000 pg/mL IFN- α as measured according to such *in vitro* assays. In certain embodiments, large or high amounts of type I IFN can refer to greater than or equal to about 3000 pg/mL IFN- α as measured according to such *in vitro* assays. In certain embodiments, large or high amounts of type I IFN can refer to greater than or equal to about 4000 pg/mL IFN- α as measured according to such *in vitro* assays. In certain embodiments, large or high amounts of type I IFN can refer to greater than or equal to about 5,000 pg/mL IFN- α as measured according to such *in vitro* assays.

[0065] Combined with the teachings provided herein, by choosing among the various active compounds and weighing factors such as potency, relative bioavailability, patient body weight, severity of adverse side-effects and preferred mode of administration, an effective prophylactic or therapeutic treatment regimen can be planned which does not cause substantial toxicity and yet is effective to treat the particular subject. The effective amount for any particular application

can vary depending on such factors as the disease or condition being treated, the severity of the disease or condition, and the health and size of the subject. One of ordinary skill in the art can empirically determine the effective amount of TLR9 agonist (i.e., A-class CpG DNA), CPI (i.e., anti-PD-1 antibodies or antigen-binding fragments thereof), and/or other therapeutic agent(s) without necessitating undue experimentation.

[0066] For example, a human clinical trial of a B-class CpG ODN together with an anti-CTLA-4 antibody was reported by Millward et al., 2013. The clinical trial demonstrated a way to combine a TLR9 agonist given by subcutaneous injection with an anti-CTLA-4 antibody given systemically that could be used in future clinical trials of other CpG ODN and other checkpoint inhibitors, but the trial failed to demonstrate significant clear clinical benefit from the combination. This failure demonstrates the non-obviousness of the present invention. Even though there have been publications of A-class CpG ODN with high IFN- α secretion, it was not obvious to the investigators running the clinical trial to use such a CpG ODN instead of the B-class CpG ODN. It was not obvious to give the CpG ODN or anti-CTLA-4 antibody locally into the tumor instead of by the systemic route. As a result, the approach was abandoned following the completion of the trial. Likewise, Mangsbo et al. (J. Immunother 2010 33:225) reported the combination of an intratumoral B-class CpG ODN with anti-CTLA-4 or anti-PD-1 in mouse tumor models. Positive results were seen with the combinations, but again, there was no guidance to perform such therapy using a high IFN-inducing type of CpG ODN, such as the A-class or other ODN of the present invention.

[0067] To date, there appears to be no realization among those skilled in the field of the desirability and advantage to combine a high-IFN-inducing class of CpG ODN together with checkpoint inhibitor therapy. For a combination of agents to have optimal synergy in cancer immunotherapy, the immune suppressive effects of one agent should be reversed by another. For example, IFN induce the expression of PD-L1 on tumors, which suppresses the immune response. High IFN-inducing CpG ODN of the invention induce the expression of PD-L1, but when they are used in combination with an anti-PD-1 antibody or an anti-PD-L1 antibody, the potential immune suppressive effects of the PD-L1 are overcome by the antibody. On the other hand, the present invention is based, at least in part, on the discovery that the combination of an intratumoral B-class CpG ODN with a systemic checkpoint inhibitor will be less than optimally synergistic (or not synergistic at all) because the induction of IL-10 results in pleiotropic immune suppressive effects that are not reversed by checkpoint inhibitor therapy. Thus, the present invention provides combinations of agents that together provide unexpected, e.g., synergistic, benefits in cancer immunotherapy.

[0068] The therapeutically effective amount of A-class CpG DNA and anti-PD-1 antibody or antigen-binding fragment thereof alone or together can be initially determined from *in vitro* and/or animal models. A therapeutically effective dose can also be determined from human data for the specific CpG ODN and/or specific antibodies or for other compounds which are known to exhibit similar pharmacological activities. The applied dose can be adjusted based on the relative bioavailability and potency of the administered compound. Adjusting the dose to achieve maximal efficacy based on the methods described above and other methods as are

well-known in the art is well within the capabilities of the ordinarily skilled artisan.

[0069] "Instructional material", as that term is used herein, includes a publication, a recording, a diagram, or any other medium of expression which can be used to communicate the usefulness of the compound, combination, and/or composition of the invention in the kit for affecting, alleviating or treating the various diseases or disorders recited herein. Optionally, or alternately, the instructional material can describe one or more methods of alleviating the diseases or disorders in a cell, a tissue, or a mammal, including as disclosed elsewhere herein.

[0070] The instructional material of the kit may, for example, be affixed to a container that contains the compound and/or composition of the invention or be shipped together with a container which contains the compound and/or composition. Alternatively, the instructional material may be shipped separately from the container with the intention that the recipient uses the instructional material and the compound cooperatively.

[0071] The A-class CpG DNA and anti-PD-1 antibody or antigen-binding fragment thereof of the invention may be provided in a medicinal dispenser. A medical dispenser is a package defining a plurality of medicinal storage compartments, each compartment for housing an individual unit of medicament. In an embodiment, an entire medicinal course of treatment is housed in a plurality of medicinal storage compartments.

[0072] A package defining a plurality of medicinal storage compartments may be any type of disposable pharmaceutical package or card which holds medicaments in individual compartments. For example, the package is a blister package constructed from a card, which may be made from stiff paper material, a blister sheet and backing sheet. Such cards are well known to those of ordinary skill in the art.

[0073] As an example, a medicinal dispenser may house an entire medicinal course of treatment. The dispenser may include the day indicia to indicate which day the individual units of medicament are to be taken. These may be marked along a first side of the medicinal package. The dose indicia may also be marked, for example along a second side of the medicinal package perpendicular to the first side of the medicinal package, thereby indicating the time which the individual unit of medicament should be taken. The unit doses may be contained in the dispenser which is a blister pack.

[0074] Except when noted, the terms "patient" or "subject" are used interchangeably and refer to mammals such as human patients and non-human primates, as well as veterinary subjects such as rabbits, rats, and mice, and other animals. Preferably, "patient" or "subject" refers to a human.

[0075] In certain embodiments, a subject is an adult human.

[0076] In certain embodiments, a subject is a child. In certain embodiments, a subject is less than about 18 years of age. In certain embodiments, a subject is less than about 12 years of

age.

[0077] As used herein, to "treat" means reducing the frequency with which symptoms of a disease (i.e., tumor growth and/or metastasis, or other effect mediated by the numbers and/or activity of immune cells, and the like) are experienced by a patient. Treatment may be prophylactic (to prevent or delay the onset of the disease, or to prevent the manifestation of clinical or subclinical symptoms thereof) or therapeutic suppression or alleviation of symptoms after the manifestation of the disease. The term "treat" includes the administration of the compounds or agents of the present invention to (i) prevent or delay the onset of the symptoms, complications, or biochemical indicia of, (ii) alleviate the symptoms of, and/or (iii) inhibit or arrest the further development of, the disease, condition, or disorder.

[0078] "Combination therapy" embraces the administration of an A-class CpG DNA and anti-PD-1 antibody or antigen-binding fragment thereof as part of a specific treatment regimen intended to provide a beneficial effect from the co-action of these therapeutic agents. In some embodiments, the anti-PD-1 antibody or antigen-binding fragment thereof is a bispecific antibody or bispecific antigen-binding fragment thereof. The beneficial effect of the combination includes, but is not limited to, pharmacokinetic or pharmacodynamic co-action resulting from the combination of therapeutic agents. Administration of these therapeutic agents in combination typically is carried out over a defined time period (usually minutes, hours, days, or weeks depending upon the combination selected). "Combination therapy" generally is not intended to encompass the administration of two or more of these therapeutic agents as part of separate monotherapy regimens that incidentally and arbitrarily result in the combinations of the present invention.

[0079] "Combination therapy" embraces administration of these therapeutic agents in a sequential manner, that is, wherein each therapeutic agent is administered at a different time, as well as administration of these therapeutic agents, or at least two of the therapeutic agents, in a substantially simultaneous manner.

[0080] "Combination therapy" also can embrace the administration of the A-class CpG DNA and anti-PD-1 antibody or antigen-binding fragment thereof as described above in further combination with non-drug therapies (such as, but not limited to, radiotherapy (XRT) or surgery). In some embodiments, the anti-PD-1 antibody or antigen-binding fragment thereof is a bispecific antibody or bispecific antigen-binding fragment thereof. Where the combination therapy further comprises radiation treatment, the radiation treatment may be conducted at any suitable time so long as a beneficial effect from the co-action of the combination of the therapeutic agents and radiation treatment is achieved. For example, in appropriate cases, the beneficial effect is still achieved when the radiation treatment is temporally removed from the administration of the therapeutic agents, by days or even weeks.

[0081] "Combination therapy" also can embrace the administration of the A-class CpG DNA and anti-PD-1 antibody or antigen-binding fragment thereof as described above in further combination with other biologically active ingredients (such as, but not limited to, a further and

different antineoplastic agent, a dendritic vaccine or other tumor vaccine). In some embodiments, the anti-PD-1 antibody or antigen-binding fragment thereof is a bispecific antibody or bispecific antigen-binding fragment thereof. However, in certain embodiments, "combination therapy" specifically excludes the administration of a dendritic cell or tumor vaccine.

II. CpG DNA

[0082] CpG oligonucleotides (CpG DNA; CpG ODN) contain specific sequences found to elicit an immune response. These specific sequences are referred to as "immunostimulatory motifs", and the oligonucleotides that contain immunostimulatory motifs are referred to as "immunostimulatory oligonucleotide molecules" and equivalently, "immunostimulatory oligonucleotides". Immunostimulatory oligonucleotides include at least one immunostimulatory motif, and preferably that motif is an internal motif. The term "internal immunostimulatory motif" refers to the position of the motif sequence within an oligonucleotide sequence which is at least one nucleotide longer (at both the 5' and 3' ends) than the motif sequence.

[0083] CpG oligonucleotides include at least one unmethylated CpG dinucleotide. An oligonucleotide containing at least one unmethylated CpG dinucleotide is an oligonucleotide molecule which contains a cytosine-guanine dinucleotide sequence (i.e., "CpG DNA" or DNA containing a 5' cytosine linked by a phosphate bond to a 3' guanine) and activates the immune system. The entire CpG oligonucleotide can be unmethylated or portions may be unmethylated, but at least the C of the 5' CG 3' must be unmethylated.

[0084] CpG ODN are generally about 8-100 nucleotides long. In certain embodiments, CpG ODN are about 8-50 nucleotides long, about 8-40 nucleotides long, about 8-30 nucleotides long, about 8-24 nucleotides long, about 8-20 nucleotides long, or about 8-16 nucleotides long.

[0085] By 2004, structure-activity relationship studies of CpG ODN had defined three families with distinct structural and biological characteristics (Hartmann et al., Eur. J. Immunol. 2003, 33:1633-1641; Marshall et al., J. Leukocyte Biol. 2003 73: 781-792; Vollmer et al., Eur. J. Immunol. 2004 34:251-262). Typical B-class ODN have a completely phosphorothioate backbone, do not form higher-ordered structures, and are strong B cell stimulators, inducing relatively high levels of IL-10 secretion, but induce relatively little NK activity or IFN- α secretion (Krieg, 2002, and Krieg, unpublished observations). B-class CpG ODN induce immune-suppressive counter-regulatory effects including not only the secretion of IL-10, but also the expression of IDO, which can promote the development of Treg cells *in vitro* (Moseman et al., J. Immunol. 2004 173(7): 4433-4442; Chen et al., J. Immunol. 2008 181(8): 5396-5404). The relevance of these *in vitro* data to *in vivo* tumor immunotherapy has been uncertain, and has not delayed the clinical development of B-class ODN, but the present invention is based in part on a new discovery that these effects of B-class ODN will suppress anti-tumor immune responses, which can be avoided using other classes of CpG ODN that are structurally designed not to activate the NF-KB pathway leading to IL-10 secretion.

[0086] The phosphorothioate backbone used in B-class CpG ODN has multiple complex effects on the resulting immune response compared to that seen with a CpG ODN with the same sequence but without a phosphorothioate backbone. One very important effect of the phosphorothioate (PS) backbone is protection against nuclease degradation. Completely PS-modified ODN are nearly completely stable in serum and tissues for at least 24 hr, whereas unmodified and unprotected ODN are degraded within a few minutes. In serum the major nuclease activity is a 3' exonuclease against which CpG ODN can be protected with just 1 or a few PS linkages at the 3' end of the ODN. But in tissues there also are 5' exonucleases as well as endonucleases, and these can degrade native DNA that is not otherwise protected. Native DNA can be protected against exonucleases by circularization using techniques well described in the literature. See, for example, U.S. Patent Nos. 8,017,591; 7,635,468; 7,074,772; 6,849,725; 6,451,593; and 6,451,563; and U.S. Published Patent Application No. 2003/0125279. Alternatively or in addition, the native (i.e., otherwise unmodified and unprotected) ODN can be formulated in nanoparticles or other formulations well known in the art to block nuclease access to the ODN.

[0087] In general, native CpG DNA (phosphodiester) activates TLR9 in both B cells and pDC. B cells produce cytokine and start to proliferate (this is predominantly driven through NF-KB activation), but unless the TLR9 stimulation is sustained, the proliferation is usually modest, and relatively little stimulation of Ig secretion and class switching occurs. pDC are activated by native CpG DNA to secrete type I IFN and to express costimulatory receptors, but the magnitude of the stimulation depends critically on the form of the DNA. In contrast to these effects of native CpG DNA, B-class phosphorothioate CpG DNA provides a far more powerful and sustained TLR9 signal for B cells, inducing them to proliferate strongly and leading to Ig secretion and class switching as reported in the literature. But the phosphorothioate backbone has a very different effect on the TLR9-mediated pDC response, reducing substantially the IFN secretion (apparently through suppressing IRF7-mediated signaling), but usually still providing strong induction of costimulatory molecule expression. Thus, for the present invention, the use of native DNA usually will provide higher type I IFN responses and will be therapeutically effective as long as the native DNA is protected from degradation. From 1 to 3 phosphorothioate modifications can be added onto the 5' and 3' termini of native DNA to protect it from nuclease degradation without diminishing the type I IFN response.

[0088] Early on in the development of CpG ODN for cancer immunotherapy, those skilled in the art generally believed that B-cell activation was desirable, and therefore focused development efforts on the B-class ODN. Indeed, perhaps B-cell activation is desirable for a tumor vaccine, in order to drive the production of anti-tumor Ab, which are well known in the field to be able to contribute to the anti-tumor response. Some early human clinical trials employing intratumoral administration of B-class CpG gave encouraging evidence of dendritic cell activation in the tumor draining lymph nodes (e.g., Molenkamp BG et al., Clin Cancer Res. 2007 13(10): 2961-2969). However, clinical responses to this local intratumoral therapy were quite limited, and studies of the total lymphocyte population in the draining lymph nodes showed an approximate two-fold increase in the release of IL-10 in CpG-treated patients

(Table 2 in Molenkamp et al.). Considering the negative effects of IL-10 for tumor immunotherapy, and the need for improved CpG ODN that do not induce its production, or which induce a lower level of this production, the present invention further provides improved CpG ODN with reduced induction of IL-10.

[0089] Nevertheless, it has now been discovered, in accordance with the present invention, that for intratumoral administration in particular, B cell activation with the concomitant IL-10 and IDO induction, is undesirable, and perhaps deleterious. This is difficult or impossible to demonstrate using mouse models because of the species-specific differences in the TLR9 expression and differences in the cytokine responses. The present invention is based on a new analysis of previously published and unpublished data on the human immune cell responses to various CpG ODN, together with a new analysis of the immune effects and deficiencies of other cancer immunotherapies and XRT.

[0090] For cancer immunotherapy IL-10 can sometimes have positive effects (especially with systemic therapy, see for example Mumm and Oft, Bioessays 2013 35(7): 623-631), but IL-10 is generally considered to have negative immune effects in the local tumor microenvironment, inhibiting immune rejection (reviewed in Sato et al., Immunol Res. 2011 51(2-3): 170-182). Thus, the present invention is based in part on the discovery that B-class CpG ODN, which induce high levels of IL-10, are not preferred for intra-tumoral therapy.

[0091] The B-class of CpG oligonucleotides is represented by the formula:



wherein X_1 and X_2 are nucleotides. In some embodiments, X_1 may be adenine, guanine, or thymine and/or X_2 may be cytosine, adenine, or thymine.

[0092] The B-class of CpG oligonucleotides is also represented by the formula:



wherein X_1 , X_2 , X_3 , and X_4 are nucleotides. X_2 may be adenine, guanine, or thymine. X_3 may be cytosine, adenine, or thymine.

[0093] The B-class of CpG oligonucleotides also includes oligonucleotides represented by at least the formula:



wherein X_1 , X_2 , X_3 , and X_4 are nucleotides and N is any nucleotide and N_1 and N_2 are oligonucleotide sequences composed of from about 0-25 N 's each. $X_1 X_2$ may be a dinucleotide selected from the group consisting of: GpT, GpG, GpA, ApA, ApT, ApG, CpT, CpA, CpG, TpA, TpT, and TpG; and $X_3 X_4$ may be a dinucleotide selected from the group consisting of: TpT, ApT, TpG, ApG, CpG, TpC, ApC, CpC, TpA, ApA, and CpA.

[0094] The B-class of CpG oligonucleotides is disclosed in PCT Published Patent Applications WO 96/02555 and WO 98/18810, and U.S. Pat. No. 6,194,388 B1 and U.S. Pat. No. 6,239,116 B1, issued Feb. 27, 2001 and May 29, 2001 respectively.

[0095] In contrast to the B-class CpG ODN, A-class CpG ODN are potent activators of natural killer cells and IFN- α secretion from plasmacytoid dendritic cells (pDC), but only weakly stimulate B cells, and induce very little IL-10 secretion. Canonical A-class ODN contain polyG motifs at the 5' and/or 3' ends which are capable of forming complex higher-ordered structures known as G-tetrads and a central phosphodiester region containing one or more CpG motifs within a self-complementary palindrome (reviewed in (Krieg, 2006). For example, U.S. Patent Nos. 6,949,520 and 7,776,344 show that in certain preferred embodiments the A-class CpG ODN has a sequence corresponding to any of the following:

ggGGTCAACGTTGAggggggG	(SEQ ID NO:43);
tcgtcgttttgtcgttttgtcgtt	(SEQ ID NO:44);
ggggtcgtcgttttgggggg	(SEQ ID NO:45);
tcgtcgttttgtcgttttgggggg	(SEQ ID NO:46);
ggggtcgacgtcgagggggg	(SEQ ID NO:47);
ggggtcacgatgagggggg	(SEQ ID NO:48);
ggGGGACGATCGTCggggggG	(SEQ ID NO:49);
gggggtcgtacgacgggggg	(SEQ ID NO:50);
ggGGGACGATATCGTCggggggG	(SEQ ID NO:51);
ggGGGACGACGTCGTCggggggG	(SEQ ID NO:52);
ggGGGACGAGCTGCTCggggggG	(SEQ ID NO:53);
ggGGGACGTACGTCggggggG	(SEQ ID NO:54);
ggGGGACGATCGTTGggggggG	(SEQ ID NO:55);
ggGGAACGATCGTCgggggG	(SEQ ID NO:56);
ggGGGGACGATCGTCggggggG	(SEQ ID NO:57);
ggGGGACGATCGTCGggggggG	(SEQ ID NO:58);
ggGGGTCATCGATGAggggggG	(SEQ ID NO:59);
ggGGTCGTCGACGAggggggG	(SEQ ID NO:60);
ggGGTCGTTCTGAACGAggggggG	(SEQ ID NO:61);
ggGGACGTTCTGAACGTggggggG	(SEQ ID NO:62);
ggGGAACGACGTCGTTggggggG	(SEQ ID NO:63);
ggGGAACGTACGTCggggggG	(SEQ ID NO:64);
ggGGAACGTACGTACGTTggggggG	(SEQ ID NO:65);
ggGGTCACCGGTGAggggggG	(SEQ ID NO:66);
ggGGTCGACGTACGTCGAggggggG	(SEQ ID NO:67);
ggGGACCGGTACCGGTggggggG	(SEQ ID NO:68);
ggGTCGACGTCTGAggggggG	(SEQ ID NO:69);
ggGGTCGACGTCGaggggg	(SEQ ID NO:70);

ggGGAACGTTAACGTTgggggG	(SEQ ID NO:71);
ggGGACGTCGACGTgggggG	(SEQ ID NO:72);
ggGGGTCGTTTCGTTgggggG	(SEQ ID NO:73);
ggGACGATCGTCGgggggG	(SEQ ID NO:74);
ggGTCGTCGACGAggggggG	(SEQ ID NO:75);
ggTCGTCGACGAGgggggG	(SEQ ID NO:76);
ggGGACGATCGTCGgggggG	(SEQ ID NO:77);
ggGGTCGACGTCGACGTCGAGgggggG	(SEQ ID NO:78); and
ggGGACGACGTCGTGgggggG	(SEQ ID NO:79),

wherein each lower case letter represents a nucleotide linked to its 3'-adjacent nucleotide by a phosphorothioate (PS) linkage; and each upper case letter represents a nucleotide linked to its 3'-adjacent nucleotide (if present) by a phosphodiester (PO) linkage, except that the 3'-terminal nucleotide is represented by an upper case letter since it has no 3'-adjacent nucleotide.

[0096] In certain more preferred embodiments the immunostimulatory nucleic acid has a sequence corresponding to

ggGGGACGAGCTCGTCgggggG	(SEQ ID NO:80);
ggGGGACGATCGTCGgggggG	(SEQ ID NO:58);
ggGGACGATCGAACGTgggggG	(SEQ ID NO:81);
ggGGTCGACGTCGACGTCGAGgggggG	(SEQ ID NO:78); or
ggGGACGACGTCGTGgggggG	(SEQ ID NO:79);

wherein each lower case letter represents a nucleotide linked to its 3'-adjacent nucleotide by a phosphorothioate (PS) linkage; and each upper case letter represents a nucleotide linked to its 3'-adjacent nucleotide (if present) by a phosphodiester (PO) linkage, except that the 3'-terminal nucleotide is represented by an upper case letter since it has no 3'-adjacent nucleotide.

[0097] In certain embodiments, an A-class CpG ODN for use in accordance with the methods of the instant invention has a sequence provided as: 5'-GGGGGGGGGGGACGATCGTCGGGGGGGGGGG-3' (SEQ ID NO:82; also referred to herein as "G10"). Such oligonucleotide and formulations thereof useful in accordance with the present invention are described in WO 2003/024481; US 2003/0099668; US 2012/0301499; WO 2004/084940; US 7,517,520; US 2010/0098722; WO 2007/068747; US 2007/0184068; US 8,574,564; WO 2007/144150; US 8,541,559; WO 2008/073960; and US 8,586,728.

[0098] Methods to reduce the amount of B cell activation with CpG ODN and increase or maintain the amount of IFN- α induction are not well known to those skilled in the art, but without committing to a particular mechanism of action underlying the invention, it has now been discovered in accordance with the invention that B cell proliferation and IL-10 secretion appear to require a more sustained TLR9 signal compared to that required to induce plasmacytoid dendritic cells (pDC) to secrete IFN- α . Such a sustained TLR9 signal is provided by the B-class CpG ODN to a greater degree than the A-class CpG ODN mentioned above. In

addition, the duration of the TLR9 signal can be shortened by positioning phosphodiester (PO) linkages at the CpG ("semi-soft" designs) and or at other positions within the ODN. The "softest" CpG ODN with the least sustained B cell activation are those with completely phosphodiester backbones, but these are so rapidly degraded *in vivo* that the IFN- α response is also compromised, unless the ODN is circular (to protect against exonucleases), or is delivered in a formulation such as virus-like particles (VLP), nanoparticles (NP), immune stimulating complexes (ISCOMs), or the like, which also protects against nucleases.

[0099] The immunostimulatory oligonucleotide molecules may have a homogeneous backbone (e.g., entirely phosphodiester (PO) or entirely phosphorothioate (PS)) or a chimeric backbone. An exception to this is the A-class CpG design (and A/E-class) in which the central portion of the ODN including at least 8 nucleotides and preferably 10 or more nucleotides must be phosphodiester for optimal activity. For purposes of the instant invention, a chimeric backbone refers to a partially stabilized backbone, wherein at least one internucleotide linkage is phosphodiester or phosphodiester-like, and wherein at least one other internucleotide linkage is a stabilized internucleotide linkage, wherein the at least one phosphodiester or phosphodiester-like linkage and the at least one stabilized linkage are different. The stabilized linkage(s) is/are preferentially placed at the 5' and 3' ends of the oligonucleotide in order to protect the ends from exonucleases: the phosphodiester linkages are placed in the middle and contribute to inducing a stronger IFN- α response than can easily be achieved with PS alone.

[0100] Since boranophosphonate linkages have been reported to be stabilized relative to phosphodiester linkages, for purposes of the chimeric nature of the backbone, boranophosphonate linkages can be classified either as phosphodiester-like or as stabilized, depending on the context. For example, a chimeric backbone according to the instant invention could, in one embodiment, include at least one phosphodiester (phosphodiester or phosphodiester-like) linkage and at least one boranophosphonate (stabilized) linkage. In another embodiment, a chimeric backbone according to the instant invention could include boranophosphonate (phosphodiester or phosphodiester-like) and phosphorothioate (stabilized) linkages. A "stabilized internucleotide linkage" shall mean an internucleotide linkage that is relatively resistant to *in vivo* degradation (e.g., via an exo- or endo-nuclease), compared to a phosphodiester internucleotide linkage. Preferred stabilized internucleotide linkages include, without limitation, phosphorothioate, phosphorodithioate, methylphosphonate and methylphosphorothioate. Other stabilized internucleotide linkages include, without limitation, peptide, alkyl, dephospho type linkages, and others as described above.

[0101] Modified backbones such as phosphorothioates may be synthesized using automated techniques employing either phosphoramidate or H-phosphonate chemistries. Aryl- and alkyl-phosphonates can be made, e.g., as described in U.S. Pat. No. 4,469,863; and alkylphosphotriesters (in which the charged oxygen moiety is alkylated), e.g., as described in U.S. Pat. No. 5,023,243 and European Patent No. 092,574, can be prepared by automated solid phase synthesis using commercially available reagents. Methods for making other DNA backbone modifications and substitutions have been described. Uhlmann E et al. (1990) Chem Rev 90:544; Goodchild J (1990) Bioconjugate Chem 1:165. Methods for preparing chimeric

oligonucleotides are also known. For instance patents issued to Uhlmann et al. have described such techniques, including, for example, US Patent Nos. 7,566,703, 7,795,235, 8,283,328, and 8,304,396.

[0102] Mixed backbone modified ODN may be synthesized using a commercially available DNA synthesizer and standard phosphoramidite chemistry. F. E. Eckstein, "Oligonucleotides and Analogues--A Practical Approach", IRL Press, Oxford, UK, 1991 ; and M. D. Matteucci and M. H. Caruthers, Tetrahedron Lett. 21, 719 (1980). After coupling, phosphorothioate (PS) linkages are introduced by sulfurization using the Beaucage reagent (R. P. Iyer, W. Egan, J. B. Regan and S. L. Beaucage, J. Am. Chem. Soc. 112, 1253 (1990)) (0.075 M in acetonitrile) or phenyl acetyl disulfide (PADS) followed by capping with acetic anhydride, 2,6-lutidine in tetrahydrofuran (1:1:8; v:v:v) and N-methylimidazole (16% in tetrahydrofuran). This capping step is performed after the sulfurization reaction to minimize formation of undesired phosphodiester (PO) linkages at positions where a phosphorothioate linkage should be located. In the case of the introduction of a phosphodiester linkage, e.g. at a CpG dinucleotide, the intermediate phosphorous-III is oxidized by treatment with a solution of iodine in water/pyridine. After cleavage from the solid support and final deprotection by treatment with concentrated ammonia (15 hrs at 50 °C), the ODN are analyzed by HPLC on a Gen-Pak Fax column (Millipore-Waters) using a NaCl-gradient (e.g. buffer A: 10 mM NaH₂PO₄ in acetonitrile/water=1:4/v:v pH 6.8; buffer B: 10 mM NaH₂PO₄, 1.5 M NaCl in acetonitrile/water=1:4/v:v; 5 to 60% B in 30 minutes at 1 ml/min) or by capillary gel electrophoresis. The ODN can be purified by HPLC or by FPLC on a Source High Performance column (Amersham Pharmacia). HPLC-homogeneous fractions are combined and desalted via a C18 column or by ultrafiltration. The ODN was analyzed by MALDI-TOF mass spectrometry to confirm the calculated mass.

[0103] The oligonucleotides of the invention can also include other modifications. These include nonionic DNA analogs, such as alkyl- and aryl-phosphates (in which the charged phosphonate oxygen is replaced by an alkyl or aryl group), phosphodiester and alkylphosphotriesters, in which the charged oxygen moiety is alkylated. Oligonucleotides which contain diol, such as tetraethyleneglycol or hexaethyleneglycol, at either or both termini have also been shown to be substantially resistant to nuclease degradation.

[0104] In some embodiments the oligonucleotides may be "soft" or "semi-soft" oligonucleotides. A soft oligonucleotide is an immunostimulatory oligonucleotide having a partially stabilized backbone, in which phosphodiester or phosphodiester-like internucleotide linkages occur only within and immediately adjacent to at least one internal pyrimidine-purine dinucleotide (YZ). Preferably YZ is YG, a pyrimidine-guanosine (YG) dinucleotide. The at least one internal YZ dinucleotide itself has a phosphodiester or phosphodiester-like internucleotide linkage. A phosphodiester or phosphodiester-like internucleotide linkage occurring immediately adjacent to the at least one internal YZ dinucleotide can be 5', 3', or both 5' and 3' to the at least one internal YZ dinucleotide.

[0105] In particular, phosphodiester or phosphodiester-like internucleotide linkages involve

"internal dinucleotides". An internal dinucleotide in general shall mean any pair of adjacent nucleotides connected by an internucleotide linkage, in which neither nucleotide in the pair of nucleotides is a terminal nucleotide, i.e., neither nucleotide in the pair of nucleotides is a nucleotide defining the 5' or 3' end of the oligonucleotide. Thus a linear oligonucleotide that is n nucleotides long has a total of $n-1$ dinucleotides and only $n-3$ internal dinucleotides. Each internucleotide linkage in an internal dinucleotide is an internal internucleotide linkage. Thus a linear oligonucleotide that is n nucleotides long has a total of $n-1$ internucleotide linkages and only $n-3$ internal internucleotide linkages. The strategically placed phosphodiester or phosphodiester-like internucleotide linkages, therefore, refer to phosphodiester or phosphodiester-like internucleotide linkages positioned between any pair of nucleotides in the oligonucleotide sequence. In some embodiments the phosphodiester or phosphodiester-like internucleotide linkages are not positioned between either pair of nucleotides closest to the 5' or 3' end.

[0106] Preferably a phosphodiester or phosphodiester-like internucleotide linkage occurring immediately adjacent to the at least one internal YZ dinucleotide is itself an internal internucleotide linkage. Thus for a sequence N_1 YZ N_2 , wherein N_1 and N_2 are each, independent of the other, any single nucleotide, the YZ dinucleotide has a phosphodiester or phosphodiester-like internucleotide linkage, and in addition (a) N_1 and Y are linked by a phosphodiester or phosphodiester-like internucleotide linkage when N_1 is an internal nucleotide, (b) Z and N_2 are linked by a phosphodiester or phosphodiester-like internucleotide linkage when N_2 is an internal nucleotide, or (c) N_1 and Y are linked by a phosphodiester or phosphodiester-like internucleotide linkage when N_1 is an internal nucleotide and Z and N_2 are linked by a phosphodiester or phosphodiester-like internucleotide linkage when N_2 is an internal nucleotide.

[0107] Soft oligonucleotides according to the instant invention are believed to be relatively susceptible to nuclease cleavage compared to completely stabilized oligonucleotides. Without intending to be bound to a particular theory or mechanism, it is believed that soft oligonucleotides of the invention are susceptible to cleavage resulting in fragments with reduced or no immunostimulatory activity relative to full-length soft oligonucleotides. Incorporation of at least one nuclease-sensitive internucleotide linkage, particularly near the middle of the oligonucleotide, is believed to provide an "off switch" which alters the pharmacokinetics and pharmacodynamics of the oligonucleotide so as to reduce the duration of maximal immunostimulatory activity of the oligonucleotide. In particular, the nuclease-sensitive linkage may reduce the magnitude of NF- κ B induction while increasing the magnitude of the IRF3 and/or IRF7 induction. TLR9 activation can lead to strong activation of either or both of the NF- κ B pathway (leading to expression of cytokines such as IL-6 and expression of costimulatory molecules) and the IRF3/7 pathways leading to IFN- α secretion. There generally seems to be some antagonism between these pathways. For example, B-class CpG ODN predominantly activate the former, whereas the A-class CpG ODN activate the latter. Strong NF- κ B induction is associated with B-class CpG oligos and may lead to increased IL-10 secretion. While this may be useful for systemic CpG oligo therapy, it is not desirable for

intratumoral therapy. The increased IRF3/7 induction provided by the nuclease-sensitive internucleotide linkage leads to great production of IFN- α in the tumor microenvironment, which improves the chances for a productive and therapeutic anti-tumor immune response following intratumoral therapy without increasing the production of undesirable IL-10. This reduced half-life of CpG oligos containing nuclease-sensitive linkages can be of particular value in tissues and in clinical applications in which it is desirable to avoid injury related to chronic local inflammation or immunostimulation, e.g., the kidney, since the oligos are less likely to accumulate in the tissue to high concentrations.

[0108] A semi-soft oligonucleotide is an immunostimulatory oligonucleotide having a partially stabilized backbone, in which phosphodiester or phosphodiester-like internucleotide linkages occur only within at least one internal pyrimidine-purine (YZ) dinucleotide. Semi-soft oligonucleotides generally possess increased immunostimulatory potency relative to corresponding fully stabilized immunostimulatory oligonucleotides. Due to the greater potency of semi-soft oligonucleotides, semi-soft oligonucleotides may be used, in some instances, at lower effective concentrations and have lower effective doses than conventional fully stabilized immunostimulatory oligonucleotides in order to achieve a desired biological effect.

[0109] It is believed that the foregoing properties of semi-soft oligonucleotides generally increase with increasing "dose" of phosphodiester or phosphodiester-like internucleotide linkages involving internal YZ dinucleotides. Thus it is believed, for example, that generally for a given oligonucleotide sequence with four internal YZ dinucleotides, an oligonucleotide with four internal phosphodiester or phosphodiester-like YZ internucleotide linkages is more immunostimulatory than an oligonucleotide with three internal phosphodiester or phosphodiester-like YZ internucleotide linkages, which in turn is more immunostimulatory than an oligonucleotide with two internal phosphodiester or phosphodiester-like YZ internucleotide linkages, which in turn is more immunostimulatory than an oligonucleotide with one internal phosphodiester or phosphodiester-like YZ internucleotide linkage. Importantly, inclusion of even one internal phosphodiester or phosphodiester-like YZ internucleotide linkage often can be advantageous over no internal phosphodiester or phosphodiester-like YZ internucleotide linkage. In addition to the number of phosphodiester or phosphodiester-like internucleotide linkages, the position along the length of the oligonucleotide can also affect potency.

[0110] The soft and semi-soft oligonucleotides will generally include, in addition to the phosphodiester or phosphodiester-like internucleotide linkages at preferred internal positions, 5' and 3' ends that are resistant to degradation. Such degradation-resistant ends can involve any suitable modification that results in an increased resistance against exonuclease digestion over corresponding unmodified ends. For instance, the 5' and 3' ends can be stabilized by the inclusion there of at least one phosphate modification of the backbone. In a preferred embodiment, the at least one phosphate modification of the backbone at each end is independently a phosphorothioate, phosphorodithioate, methylphosphonate, or methylphosphorothioate internucleotide linkage. In another embodiment, the degradation-resistant end includes one or more nucleotide units connected by peptide or amide linkages at the 3' end.

[0111] A phosphodiester internucleotide linkage is the type of linkage characteristic of oligonucleotides found in nature. The phosphodiester internucleotide linkage includes a phosphorus atom flanked by two bridging oxygen atoms and bound also by two additional oxygen atoms, one charged and the other uncharged. Phosphodiester internucleotide linkage is particularly preferred when it is important to reduce the tissue half-life of the oligonucleotide or to get the strongest possible induction of type I IFN secretion from pDC.

[0112] A phosphodiester-like internucleotide linkage is a phosphorus-containing bridging group that is chemically and/or diastereomerically similar to phosphodiester. Measures of similarity to phosphodiester include susceptibility to nuclease digestion and ability to activate RNase H. Thus, for example phosphodiester, but not phosphorothioate, oligonucleotides are susceptible to nuclease digestion, while both phosphodiester and phosphorothioate oligonucleotides activate RNase H. In a preferred embodiment the phosphodiester-like internucleotide linkage is boranophosphate (or equivalently, boranophosphonate) linkage. U.S. Pat. No. 5,177,198; U.S. Pat. No. 5,859,231; U.S. Pat. No. 6,160,109; U.S. Pat. No. 6,207,819; Sergueev et al., (1998) JAm Chem Soc 120:9417-27. In another preferred embodiment the phosphodiester-like internucleotide linkage is diastereomerically pure Rp phosphorothioate. It is believed that diastereomerically pure Rp phosphorothioate is more susceptible to nuclease digestion and is better at activating RNase H than mixed or diastereomerically pure Sp phosphorothioate. Stereoisomers of CpG oligonucleotides are the subject of published PCT application WO 00/06588. It is to be noted that for purposes of the instant invention, the term "phosphodiester-like internucleotide linkage" specifically excludes phosphorodithioate and methylphosphonate internucleotide linkages.

[0113] As described above the soft and semi-soft oligonucleotides of the invention may have phosphodiester like linkages between C and G. One example of a phosphodiester-like linkage is a phosphorothioate linkage in an Rp conformation. Oligonucleotide p-chirality can have apparently opposite effects on the immune activity of a CpG oligonucleotide, depending upon the time point at which activity is measured. Krieg et al., Oligonucleotides 2003 13(6):491-499. At an early time point of 40 minutes, the Rp but not the Sp stereoisomer of phosphorothioate CpG oligonucleotide induces JNK phosphorylation in mouse spleen cells. In contrast, when assayed at a late time point of 44 hr, the Sp but not the Rp stereoisomer is active in stimulating spleen cell proliferation. This difference in the kinetics and bioactivity of the Rp and Sp stereoisomers does not result from any difference in cell uptake, but rather most likely is due to two opposing biologic roles of the p-chirality. First, the enhanced activity of the Rp stereoisomer compared to the Sp for stimulating immune cells at early time points indicates that the Rp may be more effective at interacting with the CpG receptor, TLR9, or inducing the downstream signaling pathways. On the other hand, the faster degradation of the Rp PS-oligonucleotides compared to the Sp results in a much shorter duration of signaling, so that the Sp PS-oligonucleotides appear to be more biologically active when tested at later time points probably because of the greater nuclease-resistance of the Sp linkage, which provided a more sustained signal through TLR9 for B cell proliferation.

[0114] Thus the oligonucleotides may be heterogeneous in backbone composition thereby containing any possible combination of polymer units linked together.

[0115] The term "oligonucleotide" also encompasses oligonucleotides with substitutions or modifications, such as in the sugars. For example, they include oligonucleotides having backbone sugars that are covalently attached to low molecular weight organic groups other than a hydroxyl group at the 2' position and other than a phosphate group or hydroxy group at the 5' position. Thus modified oligonucleotides may include a 2'-O-alkylated ribose group. In addition, modified oligonucleotides may include sugars such as arabinose or 2'-fluoroarabinose instead of ribose.

[0116] The immunostimulatory oligonucleotides of the instant invention can encompass various chemical modifications and substitutions, in comparison to natural RNA and DNA, involving a phosphodiester internucleotide bridge, or a 13-D-ribose unit. Examples of chemical modifications are known to the skilled person and are described, for example, in Uhlmann E et al. (1990) Chem Rev 90:543; "Protocols for Oligonucleotides and Analogs" Synthesis and Properties & Synthesis and Analytical Techniques, S. Agrawal, Ed, Humana Press, Totowa, USA 1993; Crooke S T et al. (1996) Annu Rev Pharmacol Toxicol 36:107-129; and Hunziker J et al. (1995) Mod Synth Methods 7:331-417. An oligonucleotide according to the invention may have one or more modifications, wherein each modification is located at a particular phosphodiester internucleotide bridge and/or at a particular β -D-ribose unit in comparison to an oligonucleotide of the same sequence which is composed of natural DNA or RNA.

[0117] For example, the invention relates to an oligonucleotide which may comprise one or more modifications and wherein each modification is independently selected from: a) the replacement of a phosphodiester internucleotide bridge located at the 3' and/or the 5' end of a nucleotide by a modified internucleotide bridge; b) the replacement of phosphodiester bridge located at the 3' and/or the 5' end of a nucleotide by a dephospho bridge; c) the replacement of a sugar phosphate unit from the sugar phosphate backbone by another unit; and d) the replacement of a β -D-ribose unit by a modified sugar unit.

[0118] More detailed examples for the chemical modification of an oligonucleotide are as follows:

A phosphodiester internucleotide bridge located at the 3' and/or the 5' end of a nucleotide can be replaced by a modified internucleotide bridge, wherein the modified internucleotide bridge is for example selected from phosphorothioate, phosphorodithioate, NR^1R^2 -phosphoramidate, boranophosphate, α -hydroxybenzyl phosphonate, phosphate-(Ci-C₂₁)-O-alkyl ester, phosphate-[(C₆-C₁₂)aryl-(C₁-C₂₁)-O-alkyl]ester, (C₁-C₈)alkylphosphonate and/or (C₆-C₁₂)arylphosphonate bridges, (C₇-C₁₂)- α -hydroxymethyl-aryl (e.g., disclosed in WO 95/01363), wherein (C₆-C₁₂)aryl, (C₆-C₂₀)aryl and (C₆-C₁₄)aryl are optionally substituted by halogen, alkyl, alkoxy, nitro, cyano, and where R¹ and R² are, independently of each other, hydrogen, (C₁-C₁₈)-alkyl, (C₆-C₂₀)-aryl, (C₆-C₁₄)-aryl-(C₁-C₈)-alkyl, preferably hydrogen, (C₁-C₈)-alkyl,

preferably (C₁-C₄)-alkyl and/or methoxyethyl, or R¹ and R² form, together with the nitrogen atom carrying them, a 5-6-membered heterocyclic ring which can additionally contain a further heteroatom from the group O, S and N.

[0119] The replacement of a phosphodiester bridge located at the 3' and/or the 5' end of a nucleotide by a dephospho bridge (dephospho bridges are described, for example, in Uhlmann E and Peyman A in "Methods in Molecular Biology", Vol. 20, "Protocols for Oligonucleotides and Analogs", S. Agrawal, Ed., Humana Press, Totowa 1993, Chapter 16, pp. 355 ff), wherein a dephospho bridge is for example selected from the dephospho bridges formacetal, 3'-thioformacetal, methylhydroxylamine, oxime, methylenedimethyl-hydrazo, dimethylenesulfone and/or silyl groups.

[0120] A sugar phosphate unit (i.e., a β-D-ribose and phosphodiester internucleotide bridge together forming a sugar phosphate unit) from the sugar phosphate backbone (i.e., a sugar phosphate backbone is composed of sugar phosphate units) can be replaced by another unit, wherein the other unit is for example suitable to build up a "morpholino-derivative" oligomer (as described, for example, in Stirchak E P et al. (1989) Oligonucleotides Res 17:6129-41), that is, e.g., the replacement by a morpholino-derivative unit; or to build up a polyamide oligonucleotide ("PNA"; as described for example, in Nielsen P E et al. (1994) Bioconjug Chem 5:3-7), that is, e.g., the replacement by a PNA backbone unit, e.g., by 2-aminoethylglycine.

[0121] A 3-ribose unit or a β-D-2'-deoxyribose unit can be replaced by a modified sugar unit, wherein the modified sugar unit is for example selected from β-D-ribose, α-D-2'-deoxyribose, L-2'-deoxyribose, 2'-F-2'-deoxyribose, 2'-F-arabinose, 2'-O-(C₁-C₆)alkyl-ribose, preferably 2'-O-(C₁-C₆)alkyl-ribose is 2'-O-methylribose, 2'-O-(C₂-C₆)alkenyl-ribose, 2'-[O-(C₁-C₆)alkyl-O-(C₁-C₆)alkyl]-ribose, 2'-NH₂-2'-deoxyribose, β-D-xylo-furanose, α-arabinofuranose, 2,4-dideoxy-β-D-erythro-hexo-pyranose, and carbocyclic (described, for example, in Froehler J (1992) J Am Chem Soc 114:8320) and/or open-chain sugar analogs (described, for example, in Vandendriessche et al. (1993) Tetrahedron 49:7223) and/or bicyclosugar analogs (described, for example, in Tarkov M et al. (1993) Helv Chim Acta 76:481).

[0122] In some embodiments the sugar is 2'-O-methylribose, particularly for one or both nucleotides linked by a phosphodiester or phosphodiester-like internucleotide linkage.

[0123] In particular sequences described herein a set of modified bases is defined. For instance the letter Y is used to refer to a nucleotide containing a cytosine or a modified cytosine. A modified cytosine as used herein is a naturally occurring or non-naturally occurring pyrimidine base analog of cytosine which can replace this base without impairing the immunostimulatory activity of the oligonucleotide. Modified cytosines include but are not limited to 5-substituted cytosines (e.g., 5-methyl-cytosine, 5-fluoro-cytosine, 5-chloro-cytosine, 5-bromo-cytosine, 5-iodo-cytosine, 5-hydroxy-cytosine, 5-hydroxymethyl-cytosine, 5-difluoromethyl-cytosine, and unsubstituted or substituted 5-alkynyl-cytosine), 6-substituted cytosines, N4-substituted cytosines (e.g., N4-ethyl-cytosine), 5-aza-cytosine, 2-mercapto-

cytosine, isocytosine, pseudo-isocytosine, cytosine analogs with condensed ring systems (e.g., N,N'-propylene cytosine or phenoxazine), and uracil and its derivatives (e.g., 5-fluorouracil, 5-bromo-uracil, 5-bromovinyl-uracil, 4-thio-uracil, 5-hydroxy-uracil, 5-propynyl-uracil). Some of the preferred cytosines include 5-methyl-cytosine, 5-fluoro-cytosine, 5-hydroxy-cytosine, 5-hydroxymethyl-cytosine, and N4-ethyl-cytosine. In another embodiment of the invention, the cytosine base is substituted by a universal base (e.g., 3-nitropyrrole, P-base), an aromatic ring system (e.g., fluorobenzene or difluorobenzene) or a hydrogen atom (dSpacer).

[0124] The letter Z is used to refer to guanine or a modified guanine base. A modified guanine as used herein is a naturally occurring or non-naturally occurring purine base analog of guanine which can replace this base without impairing the immunostimulatory activity of the oligonucleotide. Modified guanines include but are not limited to 7-deazaguanine, 7-deaza-7-substituted guanine (such as 7-deaza-7-(C2-C6)alkynylguanine), 7-deaza-8-substituted guanine, hypoxanthine, N2-substituted guanines (e.g., N2-methyl-guanine), 5-amino-3-methyl-3H,6H-thiazolo[4,5-d]pyrimidine-2,7-dione, 2,6-diaminopurine, 2-aminopurine, purine, indole, adenine, substituted adenines (e.g., N6-methyl-adenine, 8-oxo-adenine) 8-substituted guanine (e.g., 8-hydroxyguanine and 8-bromoguanine), and 6-thioguanine. In another embodiment of the invention, the guanine base is substituted by a universal base (e.g., 4-methyl-indole, 5-nitro-indole, and K-base), an aromatic ring system (e.g. benzimidazole or dichloro-benzimidazole, 1-methyl-1H-[1,2,4]triazole-3-carboxylic acid amide) or a hydrogen atom (dSpacer).

[0125] The oligonucleotides may have one or more accessible 5' ends. It is possible to create modified oligonucleotides having two such 5' ends. This may be achieved, for instance by attaching two oligonucleotides through a 3'-3' linkage to generate an oligonucleotide having one or two accessible 5' ends. The 3'3'-linkage may be a phosphodiester, phosphorothioate or any other modified internucleotide bridge. Methods for accomplishing such linkages are known in the art. For instance, such linkages have been described in Seliger, H. et al., Oligonucleotide analogs with terminal 3'-3'- and 5'-5'-internucleotidic linkages as antisense inhibitors of viral gene expression, *Nucleosides & Nucleotides* (1991), 10(1-3), 469-77; and Jiang, et al., Pseudo-cyclic oligonucleotides: in vitro and in vivo properties, *Bioorganic & Medicinal Chemistry* (1999), 7(12), 2727-2735.

[0126] Additionally, 3'3'-linked oligonucleotides where the linkage between the 3'-terminal nucleotides is not a phosphodiester, phosphorothioate or other modified bridge, can be prepared using an additional spacer, such as tri- or tetra-ethyleneglycol phosphate moiety (Durand, M. et al, Triple-helix formation by an oligonucleotide containing one (dA)₁₂ and two (dT)₁₂ sequences bridged by two hexaethylene glycol chains, *Biochemistry* (1992), 31(38), 9197-204, U.S. Pat. No. 5,658,738, and U.S. Pat. No. 5,668,265). Alternatively, the non-nucleotidic linker may be derived from ethanediol, propanediol, or from an abasic deoxyribose (dSpacer) unit (Fontanel, Marie Laurence et al., Sterical recognition by T4 polynucleotide kinase of non-nucleosidic moieties 5'-attached to oligonucleotides; *Oligonucleotides Research* (1994), 22(11), 2022-7) using standard phosphoramidite chemistry. The non-nucleotidic linkers can be incorporated once or multiple times, or combined with each other allowing for any

desirable distance between the 3'-ends of the two ODNs to be linked.

[0127] The oligonucleotides may be partially resistant to degradation (e.g., are stabilized). A "stabilized oligonucleotide molecule" shall mean an oligonucleotide that is relatively resistant to *in vivo* degradation (e.g. via an exo- or endo-nuclease). Oligonucleotide stabilization can be accomplished via backbone modifications. Oligonucleotides having phosphorothioate linkages provide maximal protection for the oligonucleotide from degradation by intracellular exo- and endo-nucleases. Other modified oligonucleotides include phosphodiester modified oligonucleotides, combinations of phosphodiester and phosphorothioate oligonucleotide, methylphosphonate, methylphosphorothioate, phosphorodithioate, p-ethoxy, and combinations thereof. Oligonucleotides which contain diol, such as tetraethyleneglycol or hexaethyleneglycol, at either or both termini have also been shown to be substantially resistant to nuclease degradation. Circular ODN are protected against exonuclease degradation. For example, the Mologen double stem-loop immunomodulator MGN1703 (formerly dSLIM-30L1) is a covalently closed 116-nucleotide dumbbell-shaped CpG-containing phosphodiester backbone oligonucleotide having the sequence 5'-AGGTGGTAACCCCTAGGGGTTACCACCTTCATTGGAAAACGTTCTTCGGGGCGTTCTTAGGTGGTAACCCCTAGGGGTTACCACCTTCATTGGAAAACGTTCTTCGGGGCGTTCTT-3' (SEQ ID NO:501). Schmidt M et al., Allergy 2006 61: 56-63; Kapp, K et al., Mol Ther Nucleic Acids 2014 3: e170.

[0128] The immunostimulatory oligonucleotides may also contain one or more unusual linkages between the nucleotide or nucleotide-analogous moieties. The usual internucleoside linkage is a 3'5'-linkage. All other linkages are considered to be unusual internucleoside linkages, such as 2'5'-, 5'5'-, 3'3'-, 2'2'-, 2'3'-linkages. The nomenclature 2' to 5' is chosen according to the carbon atom of ribose. However, if unnatural sugar moieties are employed, such as ring-expanded sugar analogs (e.g. hexanose, cyclohexene or pyranose) or bi- or tricyclic sugar analogs, then this nomenclature changes according to the nomenclature of the monomer. In 3'-deoxy- β -D-ribose analogs (also called p-DNA), the mononucleotides are e.g. connected via a 4'2'-linkage.

[0129] If the oligonucleotide contains one 3'3'-linkage, then this oligonucleotide may have two unlinked 5'-ends. Similarly, if the oligonucleotide contains one 5'5'-linkage, then this oligonucleotide may have two unlinked 3'-ends. The accessibility of unlinked ends of nucleotides may be better accessible by their receptors. Both types of unusual linkages (3'3'- and 5'5') were described by Ramalho Ortigao et al. (Antisense Research and Development (1992) 2, 129-46), whereby oligonucleotides having a 3'3'-linkage were reported to show enhanced stability towards cleavage by nucleases.

[0130] Different types of linkages can also be combined in one molecule which may lead to branching of the oligomer. If one part of the oligonucleotide is connected at the 3'-end via a 3'3'-linkage to a second oligonucleotide part and at the 2'-end via a 2'3'-linkage to a third part of the molecule, this results e.g. in a branched oligonucleotide with three 5'-ends (3'3'-, 2'3'- branched).

III. CHECKPOINT INHIBITORS

A. PD-1

[0131] Programmed death-1 receptor (PD-1), also known as CD279, is a type 1 membrane protein expressed on activated T cells (including CD8⁺ T cells), B cells, and macrophages. Its cognate ligands are PD-L1 and PD-L2, and binding of PD-1 particularly by PD-L1 blocks "Signal 3" in T cells and potently inhibits the effector arm of an adaptive immune response, for example by leading to the death of T cells expressing PD-1.

[0132] In humans, PD-1 is a 268-amino acid polypeptide having an amino acid sequence published as GenBank Accession No. NP_005009. The protein includes an extracellular IgV domain, transmembrane domain, and intracellular domain having two phosphorylation sites.

[0133] The K_D for interaction between PD-1 and PD-L1 is 770 nM.

[0134] In preferred embodiments of the invention, the antibody inhibits binding between PD-1 and PD-L1. Preferably, the antibody can inhibit binding with PD-L1 with an IC_{50} of about 100 nM or lower; more preferably, about 10 nM or lower, for example about 5 nM or lower; yet more preferably, about 2 nM or lower; or even more preferably, for example, about 1 nM or lower.

[0135] Further, in another embodiment, the anti-PD-1 antibody has a binding affinity for PD-1 that is at least as strong as that of PD-L1. In certain embodiments, the anti-PD-1 antibody has a binding affinity for PD-1 that is at least 10 times as strong as that of PD-L1. In certain embodiments, the anti-PD-1 antibody has a binding affinity for PD-1 that is at least 100 times as strong as that of PD-L1. In certain embodiments, the anti-PD-1 antibody has a binding affinity for PD-1 that is at least 1000 times as strong as that of PD-L1.

[0136] Anti-PD-1 antibodies are known in the art and include, for example, those disclosed in U.S. Pat. No. 6,808,710 to Wood et al., U.S. Pat. No. 7,488,802 to Collins et al., and U.S. Pat. No. 8,728,474 to Honjo et al. Anti-PD-1 antibodies are commercially available as pembrolizumab (formerly known as lambrolizumab and MK-3475, KEYTRUDA®, Merck, K_D 29 pM) and nivolumab (OPDIVO®, Bristol-Myers Squibb, K_D 2.6 nM). Additional anti-PD-1 antibodies currently under development include pidilizumab (CT-011, Cure Tech).

E. Origin of Antibodies

[0137] While the anti-PD-1 antibodies discussed previously herein may be preferred, the

skilled artisan, based upon the disclosure provided herein, would appreciate that the invention encompasses a wide variety of anti-PD-1 antibodies and is not limited to these particular antibodies. More particularly, while human antibodies are preferred for use in humans, the invention is in no way limited to human antibodies; rather, the invention encompasses useful antibodies regardless of species origin, and includes, among others, chimeric humanized and/or primatized antibodies. Also, although certain of the antibodies exemplified herein were obtained using a transgenic mammal, e.g., a mouse comprising a human immune repertoire, the skilled artisan, based upon the disclosure provided herein, would understand that the present invention is not limited to an antibody produced by this or by any other particular method. Instead, the invention includes an anti-PD-1 antibody produced by any method, including, but not limited to, a method known in the art (e.g., screening phage display libraries, and the like) or to be developed in the future for producing an anti-PD-1 antibody of the invention. Based upon the extensive disclosure provided herein and in, e.g., U.S. Pat. No. 6,682,736 to Bedian et al., and U.S. Pat. App. Pub. No. 2002/0088014, one skilled in the art can readily produce and identify an anti-PD-1 antibody useful for treatment of cancer in combination with an A-class CpG ODN using the novel methods disclosed herein.

[0138] The present invention encompasses human antibodies produced using a transgenic non-human mammal, i.e., XenoMouse™ (Abgenix, Inc., Fremont, Calif.) as disclosed in the U.S. Pat. No. 6,682,736, to Hanson et al.

[0139] Another transgenic mouse system for production of "human" antibodies is referred to as "HuMAb-Mouse™" (Medarex, Princeton, N.J.), which contain human immunoglobulin gene miniloci that encode unrearranged human heavy (mu and gamma) and kappa light chain immunoglobulin sequences, together with targeted mutations that inactivate the endogenous mu and kappa chain loci (Lonberg et al. Nature 368:856-859 (1994), and U.S. Pat. No. 5,770,429).

[0140] However, the invention uses human anti-PD-1 antibodies produced using any transgenic mammal such as, but not limited to, the Kirin TC Mouse™ (Kirin Beer Kabushiki Kaisha, Tokyo, Japan) as described in, e.g., Tomizuka et al., Proc Natl Acad Sci USA 97:722 (2000); Kuroiwa et al., Nature Biotechnol 18:1086 (2000); U.S. Patent Application Publication No. 2004/0120948, to Mikayama et al.; and the HuMAb-Mouse™ (Medarex, Princeton, N.J.) and XenoMouse™ (Abgenix, Inc., Fremont, Calif.), *supra*. Thus, the invention encompasses using an anti-PD-1 antibody produced using any transgenic or other non-human animal.

[0141] Moreover, while the preferred method of producing a human anti-PD-1 antibody comprises generation of the antibodies using a non-human transgenic mammal comprising a human immune repertoire, the present invention is in no way limited to this approach. Rather, as would be appreciated by one skilled in the art once armed with the disclosure provided herein, the invention encompasses using any method for production of a human, or any other antibody specific for PD-1 produced according to any method known in the art or to be developed in the future for production of antibodies that specifically bind an antigen of interest

[0142] Human antibodies can be developed by methods that include, but are not limited to, use of phage display antibody libraries.

[0143] In another embodiment, the antibodies employed in methods of the invention are not fully human, but "humanized". In particular, murine antibodies or antibodies from other species can be "humanized" or "primatized" using techniques well known in the art. See, e.g., Winter and Harris *Immunol. Today* 14:43-46 (1993), Wright et al. *Crit. Reviews in Immunol.* 12:125-168 (1992), and U.S. Pat. No. 4,816,567, to Cabilly et al., and Mage and Lamoyi in *Monoclonal Antibody Production Techniques and Applications* pp. 79-97, Marcel Dekker, Inc., New York, N.Y. (1987).

[0144] As will be appreciated based upon the disclosure provided herein, antibodies for use in the invention can be obtained from a transgenic non-human mammal, and hybridomas derived therefrom, but can also be expressed in cell lines other than hybridomas.

[0145] Mammalian cell lines available as hosts for expression are well known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC), including but not limited to Chinese hamster ovary (CHO) cells, NS0, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), and human hepatocellular carcinoma cells (e.g., Hep G2). Non-mammalian prokaryotic and eukaryotic cells can also be employed, including bacterial, yeast, insect, and plant cells.

[0146] Various expression systems can be used as well known in the art, such as, but not limited to, those described in e.g., Sambrook and Russell, *Molecular Cloning, A Laboratory Approach*, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (2001), and Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley & Sons, NY (2002). These expression systems include dihydrofolate reductase (DHFR)-based systems, among many others. The glutamine synthetase system of expression is discussed in whole or part in connection with European Patents Nos. EP 0 216 846, EP 0 256 055, EP 0 323 997 and EP 0 338 841. In one embodiment, the antibody used is made in NS0 cells using a glutamine synthetase system (GS-NS0). In another embodiment, the antibody is made in CHO cells using a DHFR system. Both systems are well-known in the art and are described in, among others, Barnes et al. *Biotech & Bioengineering* 73:261-270 (2001), and references cited therein.

[0147] Site-directed mutagenesis of the antibody CH2 domain to eliminate glycosylation may be preferred in order to prevent changes in either the immunogenicity, pharmacokinetic, and/or effector functions resulting from non-human glycosylation. Further, the antibody can be deglycosylated by enzymatic (see, e.g., Thotakura et al. *Meth. Enzymol.* 138:350 (1987)) and/or chemical methods (see, e.g., Hakimuddin et al., *Arch. Biochem. Biophys.* 259:52 (1987)).

[0148] Further, the invention encompasses using an anti-PD-1 antibody comprising an altered glycosylation pattern. The skilled artisan would appreciate, based upon the disclosure provided herein, that an anti-PD-1 antibody can be modified to comprise additional, fewer, or different

glycosylation sites compared with the corresponding unaltered antibody. Such modifications are described in, e.g., U.S. Patent Application Publication Nos. 2003/0207336, and 2003/0157108, and International Patent Publication Nos. WO 01/81405 and 00/24893.

[0149] Additionally, the invention comprises using an anti-PD-1 antibody regardless of the glycoform, if any, present on the antibody. Moreover, methods for extensively remodeling the glycoform present on a glycoprotein are well-known in the art and include, e.g., those described in International Patent Publication Nos. WO 03/031464, WO 98/58964, and WO 99/22764, and US Patent Application Publication Nos. 2004/0063911, 2004/0132640, 2004/0142856, 2004/0072290, and U.S. Pat. No. 6,602,684 to Umana et al.

[0150] Further, the invention encompasses using an anti-PD-1 antibody with any art-known covalent and non-covalent modification, including, but not limited to, linking the polypeptide to one of a variety of nonproteinaceous polymers, e.g., polyethylene glycol, polypropylene glycol, or polyoxyalkylenes, in the manner set forth in, for example, U.S. Patent Application Publication Nos. 2003/0207346 and 2004/0132640, and U.S. Pat. Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192; and 4,179,337.

[0151] Additionally, the invention encompasses using an anti-PD-1 antibody, or antigen-binding portion thereof, chimeric protein comprising, e.g., a human serum albumin polypeptide, or fragment thereof. Whether the chimeric protein is produced using recombinant methods by, e.g., cloning of a chimeric nucleic acid encoding the chimeric protein, or by chemical linkage of the two peptide portions, the skilled artisan would understand once armed with the teachings provided herein that such chimeric proteins are well-known in the art and can confer desirable biological properties such as, but not limited to, increased stability and serum half-life to the antibody of the invention and such molecules are therefore included herein.

[0152] Antibodies that are generated for use in the invention need not initially possess a particular desired isotype. Rather, the antibody as generated can possess any isotype and can be isotype switched thereafter using conventional techniques. These include direct recombinant techniques (see, e.g., U.S. Pat. No. 4,816,397), and cell-cell fusion techniques (see e.g., U.S. Pat. No. 5,916,771).

[0153] The effector function of the antibodies of the invention may be changed by isotype switching to an IgG1, IgG2, IgG3, IgG4, IgD, IgA, IgE, or IgM for various therapeutic uses. Furthermore, dependence on complement for cell killing can be avoided through the use of bispecifics, immunotoxins, or radiolabels, for example.

IV. CpG DNA AND CHECKPOINT INHIBITOR COMBINATION IMMUNOTHERAPY

[0154] The present invention relates to combination tumor immunotherapy comprising locally administering A-class CpG ODN into or in proximity to a cancerous tumor, and systemically administering a checkpoint inhibitor, such as an anti-PD-1 antibody, to treat cancer. A single

human clinical trial has been reported in which patients were treated with a combination of a CpG ODN (B-class, dosed subcutaneously up to 0.15 mg/kg/wk) and an anti-CTLA-4 antibody (Millward M et al., Br. J. Cancer 2013 108(10): 1998-2004). This study established an MTD for a weekly combination of IV anti-CTLA-4 and subcutaneous CpG over 12 weeks of therapy in 21 patients with stage IV melanoma. Although the results of the study were not considered encouraging enough to warrant continued development of TLR9 agonists in oncology (all immune-oncology drug development by the sponsor was terminated), several interesting findings from the study support the utility of the present invention. First, the combination of a TLR9 agonist and a checkpoint inhibitor is relatively well tolerated - there was no observed systemic autoimmune disease, and only three patients developed dose-limiting toxicities during the prespecified initial 6 week period, two of whom were in the highest dose group of the anti-CTLA-4 antibody. Second, there was no induction of antibody response against the anti-CTLA-4 antibody from the combination regimen. Third, two patients achieved partial responses to the treatment, and several others had unusually prolonged stable disease.

[0155] Combination of high IFN-inducing A-class CpG ODN and anti-PD-1 is useful for treatment of primary and secondary (i.e., metastatic) cancers. More specifically, among many potential treatment options, A-class CpG DNA and anti-PD-1 antibody or antigen-binding fragment thereof combination therapy can be used to treat cancer. In certain embodiments, the cancer to be treated is or includes a cancerous tumor. A "cancerous tumor" as used herein refers to an abnormal swelling or macroscopic collection of cells comprising abnormal cells characterized by their growth or proliferation without regulation by normal external signals. In certain embodiments, a cancerous tumor is a carcinoma, sarcoma, or adenocarcinoma; these are sometimes referred to as solid tumors. In certain embodiments, a cancerous tumor excludes hematologic malignancies. In certain embodiments, a cancerous tumor includes certain hematologic malignancies, e.g., lymphomas.

[0156] Representative cancers treatable by the methods of the invention specifically include, without limitation, cancers of skin, head and neck, esophagus, stomach, liver, colon, rectum, pancreas, lung, breast, cervix, ovary, kidney, bladder, prostate, thyroid, brain, muscle, and bone. Also specifically included among cancers treatable by the methods of the invention are melanoma, renal cell carcinoma, and non-small cell lung cancer (NSCLC). Also specifically included among cancers treatable by the methods of the invention are lymphoma, cancer of the bone marrow, carcinoid tumor, and neuroblastoma.

[0157] While in some embodiments the foregoing cancers are preferred, the present invention relates to treatment of a wide variety of malignant cell proliferative disorders, including, but not limited to Kaposi's sarcoma, synovial sarcoma, mesothelioma, hepatobiliary (hepatic and biliary duct), a primary or secondary brain tumor, lung cancer (NSCLC and SCLC), bone cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular melanoma, cancer of the anal region, stomach (gastric) cancer, gastrointestinal (gastric, colorectal, and duodenal) cancer, colon cancers, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the

thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, prostate cancer, cancer of the penis, testicular cancer, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, pancreatic cancers, neoplasms of the central nervous system (CNS) including primary or secondary CNS tumor, spinal axis tumors, brain stem glioma, glioblastoma, meningioma, myoblastoma, astrocytoma, pituitary adenoma, adrenocortical cancer, gall bladder cancer, cholangiocarcinoma, fibrosarcoma, neuroblastoma, and retinoblastoma; as well as, in some embodiments, non-Hodgkin's lymphoma (NHL, including indolent and aggressive), Hodgkin's lymphoma, cutaneous T-cell lymphoma, lymphocytic lymphomas, primary CNS lymphoma, chronic or acute myeloid leukemia, chronic or acute lymphocytic leukemia, erythroblastoma, and multiple myeloma; or a combination of two or more of the foregoing cancers.

[0158] The cancers to be treated may be refractory cancers. A refractory cancer as used herein is a cancer that is resistant to the ordinary standard of care prescribed. These cancers may appear initially responsive to a treatment (and then recur), or they may be completely non-responsive to the treatment. The ordinary standard of care will vary depending upon the cancer type, and the degree of progression in the subject. It may be a chemotherapy, an immunotherapy, surgery, radiation, or a combination thereof. Those of ordinary skill in the art are aware of such standards of care. Subjects being treated according to the invention for a refractory cancer therefore may have already been exposed to another treatment for their cancer. Alternatively, if the cancer is likely to be refractory (e.g., given an analysis of the cancer cells or history of the subject), then the subject may not have already been exposed to another treatment.

[0159] In certain embodiments, refractory cancers include cancers which are refractory to treatment with a checkpoint inhibitor. Cancers of this type are sometimes referred to as "cold". Methods of the instant invention can be used to treat such "cold" cancers or tumors to convert them into "hot" ones, i.e., cancers or tumors which respond to treatment, including treatment with a checkpoint inhibitor, even the same checkpoint inhibitor.

[0160] Examples of refractory cancers include but are not limited to melanomas, renal cell carcinomas, colon cancer, liver (hepatic) cancers, pancreatic cancer, non-Hodgkin's lymphoma, lung cancer, and leukemias.

[0161] The A-class CpG DNA and antibody or antigen-binding fragment thereof which binds specifically to PD-1 for use in a method of treating a cancerous tumor of the invention in certain instances may be useful for replacing existing surgical procedures or drug therapies, although in other instances the present invention is useful in improving the efficacy of existing therapies for treating such conditions. Accordingly combination therapy may be used to treat subjects that are undergoing or that will undergo a treatment for cancer. For example, the agents may be administered to a subject in combination with another antiproliferative (e.g., an anti-cancer) therapy. Suitable anti-cancer therapies include surgical procedures to remove the tumor mass, chemotherapy, or localized radiation. The other antiproliferative therapy may be administered before, concurrent with, or after treatment with the CpG ODN/CPI combination of the invention.

There may also be a delay of several hours, days, and in some instances weeks between the administration of the different treatments, such that the CpG ODN/CPI combination may be administered before or after the other treatment. The invention further contemplates the use of the CpG ODN/CPI combination in cancer subjects prior to and following surgery, radiation or chemotherapy.

[0162] In one embodiment, the invention provides an A-class CpG DNA and an antibody or antigen-binding fragment thereof which binds specifically to PD-1, producing or increasing an anti-tumor response, wherein the A-class CpG ODN enhances an anti-tumor response by an amount of anti-PD-1 antibody or antigen-binding fragment thereof which is otherwise sub-optimal for inducing the same level of anti-tumor response when used alone. In certain embodiments, the A-class CpG ODN is not used in conjunction with an anti-PD-1 antibody to elicit an anti-tumor response, administering the A-class CpG ODN alone does not produce or increase the anti-tumor response. In alternate embodiments, both the A-class CpG ODN and the anti-PD-1 antibody can elicit an anti-tumor response alone and/or when administered in combination.

[0163] In one embodiment, the invention provides an A-class CpG DNA and an antibody or antigen-binding fragment thereof which binds specifically to PD-1, producing or increasing an anti-tumor, wherein the A-class CpG ODN enhances an anti-tumor response by an amount of antibody which is otherwise sub-optimal for inducing the same level of anti-tumor response when used alone. In certain embodiments, when the A-class CpG ODN is not used in conjunction with an anti-PD-1 antibody to elicit an anti-tumor response, administering A-class CpG ODN alone does not produce or increase the anti-tumor response. In alternate embodiments, both the A-class CpG ODN and the anti-PD-1 antibody can elicit an anti-tumor response alone and/or when administered in combination.

[0164] In certain embodiments, the A-class CpG ODN may enhance the effects of the Anti-PD-1 antibody (or vice-versa) in an additive manner. In a preferred embodiment, the A-class CpG ODN enhances the effects of the anti-PD-1 antibody (or vice versa) in a synergistic manner. In another embodiment, the anti-PD-1 antibody enhances the effect of an A-class CpG ODN in an additive manner. Preferably, the effects are enhanced in a synergistic manner. Thus, in certain embodiments, the invention encompasses A-class CpG DNA and an antibody or antigen-binding fragment thereof which binds specifically to PD-1 for use in methods of disease treatment or prevention that provide better therapeutic profiles than expected based on administration of the A-class CpG ODN alone and the anti-PD-1 antibody alone.

[0165] In certain embodiments, the A-class CpG ODN may enhance the effects of the anti-PD-1 antibody (or vice-versa) in an additive manner. In a preferred embodiment, the A-class CpG ODN enhances the effects of the anti-PD-1 antibody (or vice versa) in a synergistic manner. In another embodiment, the anti-PD-1 antibody enhances the effect of the A-class CpG ODN in an additive manner. Preferably, the effects are enhanced in a synergistic manner. Thus, in certain embodiments, the invention encompasses an A-class CpG DNA and an antibody or antigen-binding fragment thereof which binds specifically to PD-1 for use in methods of disease

treatment or prevention that provide better therapeutic profiles than expected based on administration of an A-class CpG ODN alone and anti-PD-1 antibody alone.

[0166] In certain embodiments, the A-class CpG ODN is administered with an anti-PD-1 antibody (with or without other modalities such as radiotherapy) as a part of a neoadjuvant therapeutic regimen to achieve an anti-tumor effect that will make possible curative surgery.

[0167] In certain embodiments, the A-class CpG ODN is administered together with an anti-PD-1 antibody (with or without other modalities such as radiotherapy) following surgical resection of a primary or metastatic tumor or in the setting of minimal residual disease in order to prevent tumor recurrence.

[0168] Also encompassed by the invention are combination therapies that have additive potency or an additive therapeutic effect while reducing or avoiding unwanted or adverse effects. The invention also encompasses synergistic combinations where the therapeutic efficacy is greater than additive, while unwanted or adverse effects are reduced or avoided. In certain embodiments, the A-class CpG DNA and antibody or antigen-binding fragment thereof which binds specifically to PD-1 for use in methods of the invention permit treatment or prevention of diseases and disorders wherein treatment is improved by an enhanced anti-tumor response using lower and/or less frequent doses of A-class CpG ODN and/or anti-PD-1 antibody to reduce the incidence of unwanted or adverse effects caused by the administration of A-class CpG ODN alone and/or anti-PD-1 antibody alone, while maintaining or enhancing efficacy of treatment, preferably increasing patient compliance, improving therapy, and/or reducing unwanted or adverse effects.

[0169] The invention provides a TLR9 agonist which is an A-class CpG DNA and an antibody or antigen-binding fragment thereof which binds specifically to PD-1 for use in a method of treating a cancerous tumor, wherein the A-class CpG DNA is administered into or adjacent to the tumor; and the antibody or antigen-binding fragment thereof which binds specifically to PD-1 is administered systemically.

[0170] In certain embodiments, the A-class CpG DNA has a sequence provided as: 5'-GGGGGGGGGGGACGATCGTCGGGGGGGGGG-3' (SEQ ID NO:82).

[0171] In certain embodiments the A-class CpG DNA is a circular CpG DNA with a native backbone, e.g., MGN1703.

[0172] In certain embodiments the A-class CpG DNA is an unmodified native CpG DNA administered in a formulation comprising a nanoparticle, VLP, ISCOM or other nuclease-resistant delivery vehicle.

[0173] In certain embodiments, the anti-PD-1 antibody or antigen-binding fragment thereof comprises (i) a first antibody or antigen-binding fragment thereof which binds specifically to PD-1, and (ii) a second antibody or antigen-binding fragment thereof which binds specifically to

PD-L1.

[0174] In certain embodiments, the anti-PD-1 antibody or antigen-binding fragment thereof comprises (i) a first antibody or antigen-binding fragment thereof which binds specifically to PD-1, and (ii) a second antibody or antigen-binding fragment thereof which binds specifically to TIM3.

[0175] In certain embodiments, the anti-PD-1 antibody or antigen-binding fragment thereof comprises (i) a first antibody or antigen-binding fragment thereof which binds specifically to PD-1, and (ii) a second antibody or antigen-binding fragment thereof which binds specifically to LAG3.

[0176] In certain embodiments, the anti-PD-1 antibody or antigen-binding fragment thereof comprises a bispecific antibody or bispecific antigen-binding fragment thereof which binds specifically to PD-1 and to PD-L1.

[0177] In certain embodiments, the anti-PD-1 antibody or antigen-binding fragment thereof comprises a bispecific antibody or bispecific antigen-binding fragment thereof which binds specifically to PD-1 and to TIM3.

[0178] In certain embodiments, the anti-PD-1 antibody or antigen-binding fragment thereof comprises a bispecific antibody or bispecific antigen-binding fragment thereof which binds specifically to PD-1 and to LAG3.

[0179] In certain embodiments, the A-class CpG DNA is administered prior to administration of the anti-PD-1 antibody or antigen-binding fragment thereof.

[0180] In certain embodiments, the A-class CpG DNA and the anti-PD-1 antibody or antigen-binding fragment thereof are administered substantially at the same time.

[0181] In certain embodiments, the cancerous tumor is a lymphoma or a cancerous tumor of a tissue selected from the group consisting of skin, head and neck, esophagus, stomach, liver, colon, rectum, pancreas, lung, breast, cervix, ovary, kidney, bladder, prostate, thyroid, brain, muscle, and bone.

[0182] In certain embodiments, the cancerous tumor is melanoma.

[0183] In certain embodiments, the cancerous tumor is lymphoma.

[0184] In certain embodiments, the cancerous tumor is a cancer of the bone marrow.

[0185] In certain embodiments, the cancerous tumor is a carcinoid tumor.

[0186] In certain embodiments, the cancerous tumor is neuroblastoma.

[0187] In certain embodiments, the subject is a human.

[0188] In certain embodiments, the A-class CpG DNA and the antibody or antigen-binding fragment thereof which binds specifically to PD-1 are for use in a method that further comprises administration of radiotherapy to the subject, said radiotherapy initiated prior to the administration of the A-class CpG DNA.

[0189] In certain embodiments, the radiotherapy is hypofractionated radiotherapy.

[0190] In certain embodiments, the A-class CpG DNA has a sequence provided as: 5'-GGGGGGGGGGGACGATCGTCGGGGGGGGGGG-3' (SEQ ID NO:82).

[0191] In certain embodiments the A-class CpG DNA is a circular CpG DNA with a native backbone, e.g., MGN1703.

[0192] In certain embodiments the A-class CpG DNA is an unmodified native CpG DNA administered in a formulation comprising a nanoparticle, VLP, ISCOM or other nuclease-resistant delivery vehicle.

[0193] In certain embodiments, the anti-PD-1 antibody comprises (i) a first antibody or antigen-binding fragment thereof which binds specifically to PD-1, and (ii) a second antibody or antigen-binding fragment thereof which binds specifically to PD-L1.

[0194] In certain embodiments, the anti-PD-1 antibody comprises (i) a first antibody or antigen-binding fragment thereof which binds specifically to PD-1, and (ii) a second antibody or antigen-binding fragment thereof which binds specifically to TIM3.

[0195] In certain embodiments, the anti-PD-1 antibody comprises (i) a first antibody or antigen-binding fragment thereof which binds specifically to PD-1, and (ii) a second antibody or antigen-binding fragment thereof which binds specifically to LAG3.

[0196] In certain embodiments, the anti-PD-1 antibody comprises a bispecific antibody or bispecific antigen-binding fragment thereof which binds specifically to PD-1 and to PD-L1.

[0197] In certain embodiments, the anti-PD-1 antibody comprises a bispecific antibody or bispecific antigen-binding fragment thereof which binds specifically to PD-1 and to TIM3.

[0198] In certain embodiments, the anti-PD-1 antibody comprises a bispecific antibody or bispecific antigen-binding fragment thereof which binds specifically to PD-1 and to LAG3.

[0199] In certain embodiments, the A-class CpG DNA is administered prior to administration of the anti-PD-1 antibody.

[0200] In certain embodiments, the A-class CpG DNA and the anti-PD-1 antibody are administered substantially at the same time.

[0201] In certain embodiments, the cancerous tumor is a lymphoma or a cancerous tumor of a tissue selected from the group consisting of skin, head and neck, esophagus, stomach, liver, colon, rectum, pancreas, lung, breast, cervix, ovary, kidney, bladder, prostate, thyroid, brain, muscle, and bone.

[0202] In certain embodiments, the cancerous tumor is melanoma.

[0203] In certain embodiments, the cancerous tumor is lymphoma.

[0204] In certain embodiments, the cancerous tumor is a cancer of the bone marrow.

[0205] In certain embodiments, the cancerous tumor is a carcinoid tumor.

[0206] In certain embodiments, the cancerous tumor is neuroblastoma.

[0207] In certain embodiments, the subject is a human.

[0208] In certain embodiments, the A-class CpG DNA and antibody or antigen-binding fragment thereof which binds specifically to PD-1 are for use in a method further using a second antibody or antigen-binding fragment thereof which binds specifically to PD-L1..

[0209] In certain embodiments, the A-class CpG DNA has a sequence provided as: 5'-GGGGGGGGGGGACGATCGTCGGGGGGGGGGG-3' (SEQ ID NO:82).

[0210] In certain embodiments the A-class CpG DNA is a circular CpG DNA with a native backbone, e.g., MGN1703.

[0211] In certain embodiments the A-class CpG DNA is an unmodified native CpG DNA administered in a formulation comprising a nanoparticle, VLP, ISCOM or other nuclease-resistant delivery vehicle.

[0212] In certain embodiments, the A-class CpG DNA is administered prior to administration of the first CPI.

[0213] In certain embodiments, the A-class CpG DNA and the anti-PD-1 antibody are administered substantially at the same time.

[0214] In certain embodiments, the A-class CpG DNA is administered after administration of the anti-PD-1 antibody.

[0215] In certain embodiments, the cancerous tumor is a lymphoma or a cancerous tumor of a

tissue selected from the group consisting of skin, head and neck, esophagus, stomach, liver, colon, rectum, pancreas, lung, breast, cervix, ovary, kidney, bladder, prostate, thyroid, brain, muscle, and bone.

[0216] In certain embodiments, the cancerous tumor is melanoma.

[0217] In certain embodiments, the cancerous tumor is lymphoma.

[0218] In certain embodiments, the cancerous tumor is a cancer of the bone marrow.

[0219] In certain embodiments, the cancerous tumor is a carcinoid tumor.

[0220] In certain embodiments, the cancerous tumor is neuroblastoma.

[0221] In certain embodiments, the subject is a human.

[0222] In certain embodiments, the method includes administering to a subject in need thereof an effective amount of radiotherapy (XRT). Standard XRT doses are in the range of 1.8 to 2.2 Gy/day, but recent studies indicate that the immune effects of XRT on tumors may be increased through the use of XRT at doses of 3-20 Gy/d for 1-3 days. Those expert in the art will recognize that different tumors have differing levels of radio-sensitivity, and will adjust the amount and intensity of the XRT accordingly.

[0223] In certain embodiments, the radiotherapy is hypofractionated radiotherapy.

V. ADDITIONAL COMBINATION THERAPY

[0224] Methods of the invention can be used in conjunction with other anti-cancer therapies, including chemotherapy, other immunotherapy, radiotherapy, hormone therapy, and the like. Conventional chemotherapeutics and targeted antineoplastic agents have been developed based on the simplistic notion that cancer constitutes a cell-autonomous genetic or epigenetic disease. However, it is becoming clear that many of the available anticancer drugs that have collectively saved millions of life-years mediate therapeutic effects by eliciting *de novo* or reactivating pre-existing tumor-specific immune responses. Accumulating evidence indicates that the therapeutic efficacy of several antineoplastic agents relies on their capacity to influence the tumor-host interaction, tipping the balance toward the activation of an immune response specific for malignant cells.

[0225] For example, Table 1 lists certain FDA-approved anticancer agents whose efficacy is reduced by immune deficiencies (Zitvogel L. et al., Immunity 2013 39(1):74-88).

Table 1.

Agent	Tumor	Immune Defects
5-fluorouracil	EL4 lymphomas	<i>Nu/Nu</i> genotype
anthracyclines	CT26 colorectal carcinomas, MCA205 fibrosarcomas, MCA-induced tumors	<i>Nu/Nu</i> genotype, depletion of CD8 ⁺ or γ/δ T cells, blockade of CD11b, neutralization of IL-1, IL-17, or IFN- γ
ATRA $\pm$ arsenic trioxide	murine APLs	SCID phenotype
arsenic trioxide	CT26 colorectal cancers	<i>Nu/Nu</i> genotype
cisplatin + digoxin	MCA205 fibrosarcomas	<i>Nu/Nu</i> genotype
cyclophosphamide	AB1-HA mesotheliomas	<i>Ifngr</i> ^{-/-} , <i>Tnfsf10</i> ^{-/-} , depletion of CD8 ⁺ T cells or NK cells
dasatinib	P815 mastocytomas	depletion of CD4 ⁺ or CD8 ⁺ T cells
gemcitabine	AB12 mesotheliomas, EJ-6-2 fibrosarcomas, EL4 lymphomas, TC1 insulinomas	<i>Nu/Nu</i> genotype
imatinib	AK7 mesotheliomas, B16 melanomas, RMA-S lymphomas GISTs developing in <i>Kit</i> ^{V558/+} mice	depletion of NK cells <i>Rag1</i> ^{-/-} , depletion of CD8 ⁺ T cells
mitomycin C + digoxin	MCA205 fibrosarcomas	<i>Nu/Nu</i> genotype
oxaliplatin	CT26 colorectal carcinomas, MCA205 fibrosarcomas	<i>Nu/Nu</i> genotype
paclitaxel	Ret-driven melanomas	depletion of CD8 ⁺ T cells
PLX4720 (BRAF inhibitor)	SM1WT1 melanomas	<i>Ccr2</i> ^{-/-} , <i>Ifng</i> ^{-/-} , <i>Prf1</i> ^{-/-} , depletion of CD8 ⁺ T cells
Table 1 Abbreviations: APL, acute promyelocytic leukemia; ATRA, all- <i>trans</i> retinoic acid; BRAF, B-Raf; GIST, gastrointestinal stromal tumor; IFN, interferon; IL, interleukin; MCA, 3-methylcholanthrene; NK, natural killer; SCID, severe combined immunodeficient		

VI. DOSAGE REGIMENS

[0226] Dosage regimens can be adjusted to provide the optimum desired response. For example, a single bolus can be administered, several divided doses can be administered over time, or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in

dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic or prophylactic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

[0227] Thus, the skilled artisan would appreciate, based upon the disclosure provided herein, that the dose and dosing regimen is adjusted in accordance with methods well-known in the therapeutic arts. That is, the maximum tolerable dose can be readily established, and the effective amount providing a detectable therapeutic benefit to a patient can also be determined, as can the temporal requirements for administering each agent to provide a detectable therapeutic benefit to the patient. Accordingly, while certain dose and administration regimens are exemplified herein, these examples in no way limit the dose and administration regimen that can be provided to a patient in practicing the present invention. Further, one skilled in the art would understand, once armed with the teachings provided herein, that a therapeutic benefit, such as, but not limited to, detectable decrease in tumor size and/or metastasis, and increased time to recurrence, among many other parameters, can be assessed by a wide variety of methods known in the art for assessing the efficacy of treatment of cancer, and these methods are encompassed herein, as well as methods to be developed in the future.

[0228] It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated, and may include single or multiple doses. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition. For example, doses may be adjusted based on pharmacokinetic or pharmacodynamic parameters, which may include clinical effects such as toxic effects and/or laboratory values. Thus, the present invention encompasses intra-patient dose-escalation as determined by the skilled artisan. Determining appropriate dosages and regimens for administration of the active compound or compounds are well-known in the relevant art and would be understood to be encompassed by the skilled artisan once provided the teachings disclosed herein.

CpG ODN Dosing

[0229] In accordance with the methods of the present invention, A-class CpG ODN is administered locally to the cancerous tumor, i.e., by intratumoral or peritumoral administration. Alternatively or in addition, in certain embodiments A-class CpG ODN is administered locally to the cancerous tumor by, for example, intraperitoneal injection or infusion or intravesicular

instillation.

[0230] Most of the prior art with CpG used subcutaneous administration, not intratumoral or peritumoral. Intratumoral therapy in oncology is generally preferred only for the treatment of primary lesions, not in the situation of metastatic disease. The reason for this is that most intratumoral therapies have only a local effect. In some unusual cases, intratumoral therapies can lead to regression of distant tumor masses as a result of the induction of a specific immune response against tumor antigens present not only in the injected lesion, but also in distant metastases. In the case of radiotherapy (XRT), this has been termed an "abscopal effect" as described above. Some authors have noted cases in which abscopal effects have been induced by TLR agonists, including intratumoral TLR9 (Brody et al, J. Clin. Oncol. 2010 28(28): 4324-4332; Kim et al., Blood 2012 119(2): 355-363), but these responses have been uncommon and generally of brief duration.

[0231] The immune effects of XRT given prior to CpG ODN administration will disrupt the inhibitory mechanisms that normally limit the efficacy of the CpG-induced response, increasing the potential for clinical response. In addition, the production of IFN- α in the tumor has been associated with and is required for an improved response to XRT (Burnette et al, Cancer Res. 2011 71: 2488-2496), providing further evidence for benefit from the use of intratumoral high IFN CpG following XRT.

[0232] In one form, the present invention improves the induction of abscopal responses from XRT by administering XRT, preferably hypofractionated XRT (as described in Prasanna et al.), to a cancer patient and then administering an intratumoral or peritumoral high IFN-inducing A-class CpG ODN in the same region or lymphatic drainage. Preferred peritumoral injections are in the same lymphatic drainage as the tumor, in order to facilitate that the same APC are exposed both to the tumor Ag released following XRT to the tumor, and to the TLR ligand.

[0233] Methods of intratumoral or peritumoral delivery of A-class CpG ODN include not only direct injection, but also can include topical delivery intraperitoneal delivery for abdominal tumors such as ovarian, pancreatic, colon, or gastric), intraocular for eye malignancies, oral for gastric and intestinal cancer, and intravesicular administration for bladder cancer. Also contemplated for intratumoral administration of A-class CpG ODN is systemic delivery using tumor delivery vehicles such as tumor-targeted aptamers, antibody conjugates, nanoparticles, ISCOMS, VLP, multilaminar vesicles, pH-sensitive peptides, and cationic peptides.

[0234] For intratumoral or peritumoral administration, the A-class CpG ODN dose typically is fixed. Doses of A-class CpG ODN for parenteral (including intratumoral and peritumoral) delivery for inducing an immune response when A-class CpG ODN is administered in combination with the antibody or antigen-binding fragment thereof which binds specifically to PD-1 typically range from about 1 μ g to 100 mg per administration, which depending on the application could be given daily, weekly, or monthly and any other amount of time therebetween.

[0235] In certain embodiments, subject doses of A-class CpG ODN for intratumoral and peritumoral delivery typically range from about 10 µg to about 100 mg per administration, which depending on the application could be given daily, weekly, or monthly and any other amount of time therebetween. In certain embodiments, subject doses of A-class CpG ODN for intratumoral and peritumoral delivery typically range from about 100 µg to about 100 mg per administration, which depending on the application could be given daily, weekly, or monthly and any other amount of time therebetween. In certain embodiments, subject doses of A-class CpG ODN for intratumoral and peritumoral delivery typically range from about 1 mg to about 100 mg per administration, which depending on the application could be given daily, weekly, or monthly and any other amount of time therebetween. In certain embodiments, subject doses of A-class CpG ODN for intratumoral and peritumoral delivery typically range from about 10 mg to about 100 mg per administration, which depending on the application could be given daily, weekly, or monthly and any other amount of time therebetween.

[0236] In yet other embodiments, doses of A-class CpG ODN for parenteral (including intratumoral and peritumoral) delivery for inducing an immune response when A-class CpG ODN is administered in combination with the antibody or antigen-binding fragment thereof which binds specifically to PD-1 typically range from about 1 µg to about 50 mg per administration, which depending on the application could be given daily, weekly, or monthly and any other amount of time therebetween. In certain embodiments, subject doses of A-class CpG ODN for intratumoral and peritumoral delivery typically range from about 10 µg to about 50 mg per administration, which depending on the application could be given daily, weekly, or monthly and any other amount of time therebetween. In certain embodiments, subject doses of A-class CpG ODN for intratumoral and peritumoral delivery typically range from about 100 µg to about 50 mg per administration, which depending on the application could be given daily, weekly, or monthly and any other amount of time therebetween. In certain embodiments, subject doses of A-class CpG ODN for intratumoral and peritumoral delivery typically range from about 1 mg to about 50 mg per administration, which depending on the application could be given daily, weekly, or monthly and any other amount of time therebetween. In certain embodiments, subject doses of A-class CpG ODN for intratumoral and peritumoral delivery typically range from about 10 mg to about 50 mg per administration, which depending on the application could be given daily, weekly, or monthly and any other amount of time therebetween.

[0237] In yet other embodiments, doses of A-class CpG ODN for parenteral (including intratumoral and peritumoral) delivery for inducing an immune response when A-class CpG ODN is administered in combination with the antibody or antigen-binding fragment thereof which binds specifically to PD-1, typically range from about 1 µg to about 10 mg per administration, which depending on the application could be given daily, weekly, or monthly and any other amount of time therebetween. In certain embodiments, subject doses of A-class CpG ODN for intratumoral and peritumoral delivery typically range from about 10 µg to about 10 mg per administration, which depending on the application could be given daily, weekly, or monthly and any other amount of time therebetween. In certain embodiments, subject doses of A-class CpG ODN for intratumoral and peritumoral delivery typically range from about 100 µg to about 10 mg per administration, which depending on the application could be given daily, weekly, or

monthly and any other amount of time therebetween. In certain embodiments, subject doses of A-class CpG ODN for intratumoral and peritumoral delivery typically range from about 1 mg to about 10 mg per administration, which depending on the application could be given daily, weekly, or monthly and any other amount of time therebetween.

[0238] In yet other embodiments, doses of A-class CpG ODN for parenteral (including intratumoral and peritumoral) delivery for inducing an immune response when A-class CpG ODN is administered in combination with the antibody or antigen-binding fragment thereof which binds specifically to PD-1, typically range from about 1 µg to about 1 mg per administration, which depending on the application could be given daily, weekly, or monthly and any other amount of time therebetween. In certain embodiments, subject doses of A-class CpG ODN for intratumoral and peritumoral delivery typically range from about 10 µg to about 1 mg per administration, which depending on the application could be given daily, weekly, or monthly and any other amount of time therebetween. In certain embodiments, subject doses of A-class CpG ODN for intratumoral and peritumoral delivery typically range from about 100 µg to about 1 mg per administration, which depending on the application could be given daily, weekly, or monthly and any other amount of time therebetween.

[0239] For each of the fixed doses described above, in certain embodiments the dose will be administered in a volume of less than or equal to about 1 mL. In certain embodiments, the dose will be administered in a volume of about 0.1 mL up to about 1 mL. In other embodiments, the dose volume will be up to 4 mL, which is commonly used for intratumoral injection of certain oncolytic viruses, such as talimogene laherparepvec (T-vec).

[0240] In certain embodiments of the invention, a sustained release delivery system, including for example nanoparticles, ISCOMS, VLP, and dendrimers (reviewed in, for example, Gomes Dos Santos AL et al., *Curr Pharm Biotechnol.* 2005 6(1): 7-15; Joshi VB et al., *AAPS J.* 2013 15(1): 85-94; and Arima H et al., *Curr Top Med Chem.* 2014 14(4): 465-77), may be used to administer a single intratumoral or peritumoral therapeutic dose of the A-class CpG ODN. In certain embodiments of the invention, a sustained release delivery system, including for example nanoparticles, ISCOMS, VLP, and dendrimers, may be used to administer a single intratumoral or peritumoral therapeutic dose of the A-class CpG ODN, with no further A-class CpG ODN required.

[0241] As is well known in the art, individual doses are increased when using a sustained delivery system of any of the types well described in the literature.

[0242] In certain embodiments using a single administration of a sustained release formulation of A-class CpG ODN, the subject dose of A-class CpG ODN for intratumoral and peritumoral delivery typically ranges from about 0.1 mg to about 500 mg per administration, and it is given within one week of XRT; within one week of an antibody or antigen-binding fragment thereof which binds specifically to PD-1r; or within one week of both XRT and an antibody or antigen-binding fragment thereof which binds specifically to PD-1. In certain embodiments using a single administration of a sustained release formulation of A-class CpG ODN, the subject dose

of A-class CpG ODN for intratumoral and peritumoral delivery typically ranges from about 1 mg to about 500 mg per administration, and it is given within one week of XRT; within one week of an antibody or antigen-binding fragment thereof which binds specifically to PD-1; or within one week of both XRT and an antibody or antigen-binding fragment thereof which binds specifically to PD-1. In certain embodiments using a single administration of a sustained release formulation of A-class CpG ODN, the subject dose of A-class CpG ODN for intratumoral and peritumoral delivery typically ranges from about 10 mg to about 500 mg per administration, and it is given within one week of XRT; within one week of an antibody or antigen-binding fragment thereof which binds specifically to PD-1; or within one week of both XRT and an antibody or antigen-binding fragment thereof which binds specifically to PD-1. In certain embodiments using a single administration of a sustained release formulation of A-class CpG ODN, the subject dose of A-class CpG ODN for intratumoral and peritumoral delivery typically ranges from about 100 mg to about 500 mg per administration, and it is given within one week of XRT; within one week of an antibody or antigen-binding fragment thereof which binds specifically to PD-1; or within one week of both XRT and an antibody or antigen-binding fragment thereof which binds specifically to PD-1.

[0243] In certain embodiments using a single administration of a sustained release formulation of A-class CpG ODN, the subject doses of A-class CpG ODN for intratumoral and peritumoral delivery typically range from about 0.1 mg to about 250 mg per administration, and it is given within one week of XRT; within one week of an antibody or antigen-binding fragment thereof which binds specifically to PD-1; or within one week of both XRT and an antibody or antigen-binding fragment thereof which binds specifically to PD-1. In certain embodiments using a single administration of a sustained release formulation of A-class CpG ODN, the subject dose of A-class CpG ODN for intratumoral and peritumoral delivery typically ranges from about 1 mg to about 250 mg per administration, and it is given within one week of XRT; within one week of an antibody or antigen-binding fragment thereof which binds specifically to PD-1; or within one week of both XRT and an antibody or antigen-binding fragment thereof which binds specifically to PD-1. In certain embodiments using a single administration of a sustained release formulation of A-class CpG ODN, the subject dose of A-class CpG ODN for intratumoral and peritumoral delivery typically ranges from about 10 mg to about 250 mg per administration, and it is given within one week of XRT; within one week of an antibody or antigen-binding fragment thereof which binds specifically to PD-1; or within one week of both XRT and an antibody or antigen-binding fragment thereof which binds specifically to PD-1. In certain embodiments using a single administration of a sustained release formulation of A-class CpG ODN, the subject dose of A-class CpG ODN for intratumoral and peritumoral delivery typically ranges from about 100 mg to about 250 mg per administration, and it is given within one week of XRT; within one week of an antibody or antigen-binding fragment thereof which binds specifically to PD-1; or within one week of both XRT and an antibody or antigen-binding fragment thereof which binds specifically to PD-1.

[0244] In certain embodiments using a single administration of a sustained release formulation of A-class CpG ODN, the subject dose of A-class CpG ODN for intratumoral and peritumoral delivery typically ranges from about 0.1 mg to about 100 mg per administration, and it is given

within one week of XRT; within one week of an antibody or antigen-binding fragment thereof which binds specifically to PD-1; or within one week of both XRT and an antibody or antigen-binding fragment thereof which binds specifically to PD-1. In certain embodiments using a single administration of a sustained release formulation of A-class CpG ODN, the subject dose of A-class CpG ODN for intratumoral and peritumoral delivery typically ranges from about 1 mg to about 100 mg per administration, and it is given within one week of XRT; within one week of an antibody or antigen-binding fragment thereof which binds specifically to PD-1; or within one week of both XRT and an antibody or antigen-binding fragment thereof which binds specifically to PD-1. In certain embodiments using a single administration of a sustained release formulation of A-class CpG ODN, the subject dose of A-class CpG ODN for intratumoral and peritumoral delivery typically ranges from about 10 mg to about 100 mg per administration, and it is given within one week of XRT; within one week of an antibody or antigen-binding fragment thereof which binds specifically to PD-1; or within one week of both XRT and an antibody or antigen-binding fragment thereof which binds specifically to PD-1.

[0245] The desired clinical effect of the administered dose of A-class CpG ODN can readily be followed using standard assays and methods well known to those skilled in the art. For example, biomarker responses to TLR9 stimulation can be measured as described elsewhere herein.

CPI Antibody Dosing

[0246] Certain commercially available anti-PD-1 antibodies are currently approved in the United States for intravenous infusion dosing at 2 mg/kg body weight once every three weeks. Other commercially available anti-PD-1 antibodies are currently approved in the United States for intravenous infusion dosing at 3 mg/kg body weight once every two weeks.

[0247] In accordance with the present invention, antibody or antigen-binding fragment thereof which binds specifically to PD-1 is administered, at least in part, systemically, e.g., intravenously.

[0248] Exemplary, non-limiting doses for a therapeutically effective amount of an antibody or antigen-binding fragment thereof which binds specifically to PD-1 systemically administered according to the invention are: at least about 0.1 mg/kg body weight, at least about 0.3 mg/kg body weight, at least about 0.5 mg/kg body weight, at least about 1 mg/kg body weight, at least about 2 mg/kg body weight, at least about 3 mg/kg body weight, at least about 4 mg/kg body weight, at least about 5 mg/kg body weight, and at least about 6 mg/kg body weight.

[0249] In certain embodiments, a therapeutically effective amount of systemically administered antibody or antigen-binding fragment thereof which binds specifically to PD-1 can range from about 0.1 to about 30 mg/kg body weight, about 0.3 to about 25 mg/kg body weight, about 1 to about 20 mg/kg body weight, about 2 to about 20 mg/kg body weight, about 3 to about 20 mg/kg body weight, about 5 to about 20 mg/kg body weight, about 10 to about 20 mg/kg body

weight, about 1 to about 15 mg/kg body weight, about 2 to about 15 mg/kg body weight, about 3 to about 15 mg/kg body weight, about 5 to about 15 mg/kg body weight, about 10 to about 15 mg/kg body weight, about 1 to about 10 mg/kg body weight, about 2 to about 10 mg/kg body weight, about 3 to about 10 mg/kg body weight, or about 5 to about 10 mg/kg body weight.

[0250] In certain embodiments, the antibody or antigen-binding fragment thereof which binds specifically to PD-lis systemically administered at a dose of at least about 0.3 mg/kg body weight, at least about 1 mg/kg body weight, at least about 2 mg/kg body weight, at least about 3 mg/kg body weight, at least about 5 mg/kg body weight, at least about 6 mg/kg body weight, at least 10 mg/kg body weight, at least about 15 mg/kg body weight, or at least about 20 mg/kg body weight.

[0251] In certain embodiments, the antibody or antigen-binding fragment thereof which binds specifically to PD-lis administered by intravenous (i.v.) infusion at a dose ranging from about 0.1 to about 50 mg/kg body weight, from about 0.3 to about 20 mg/kg body weight, from about 1 to about 15 mg/kg body weight, from about 2 to about 15 mg/kg body weight, from about 3 to about 15 mg/kg body weight, or from about 6 to about 15 mg/kg body weight.

[0252] In certain embodiments, the antibody or antigen-binding fragment thereof which binds specifically to PD-lis administered in an intravenous formulation as a sterile aqueous solution containing about 5 to about 20 mg/mL of antibody or antigen-binding fragment thereof which binds specifically to PD-1, in an appropriate buffer system.

[0253] In accordance with certain embodiments of the present invention the antibody or antigen-binding fragment thereof which binds specifically to PD-1 is administered, in part, locally to the cancerous tumor, i.e., by intratumoral or peritumoral administration. In certain embodiments, such local administration is by direct injection, while in other embodiments, such administration can be topical delivery, intraperitoneal delivery for abdominal tumors such as ovarian, pancreatic, intraocular delivery for eye malignancies, oral delivery for gastric and intestinal cancer, and intravesicular administration for bladder cancer. Also contemplated for intratumoral administration of antibody or antigen-binding fragment thereof which binds specifically to PD-lis systemic delivery using tumor delivery vehicles such as tumor-targeted aptamers, nanoparticles, ISCOMS, VLP, and cationic peptides.

[0254] For local, i.e., intratumoral or peritumoral, administration, the antibody or antigen-binding fragment thereof which binds specifically to PD-1 advantageously can be administered at a dose about 10-fold less to about 20-fold less than the systemic doses just listed above.

[0255] In accordance with the present invention, antibody or antigen-binding fragment thereof which binds specifically to PD-1 dosing will typically be less frequent than A-class CpG ODN dosing. For example, anti-PD-1 antibody may be administered about once every three weeks to about once every three months. The invention further specifically contemplates antibody or antigen-binding fragment thereof which binds specifically to PD-1 dosing that is more frequent

than about once every three weeks and less frequent than about once every three months.

[0256] Intratumoral or peritumoral A-class CpG and systemic antibody or antigen-binding fragment thereof which binds specifically to PD-1 can be given on the same or different days.

[0257] Further, an exemplary dose escalation protocol with respect to A-class CpG ODN, antibody or antigen-binding fragment thereof which binds specifically to PD-1, or both can be used to determine the maximum tolerated dose (MTD), to assess dose-limiting toxicity (DLT), if any, associated with administration of the combination therapy. For example, with respect to Anti-PD-1 antibody dose escalation at a given dose of A-class CpG ODN, such protocol can comprise administering increasing doses, such as, but not limited to about 0.1 mg/kg, 0.3 mg/kg, 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg, 5 mg/kg, 6 mg/kg, 7 mg/kg, 10 mg/kg, 12 mg/kg, 15 mg/kg, or more than 15 mg/kg, or any combination thereof, more preferably, successive doses of 0.1 mg/kg, 0.3 mg/kg, 1 mg/kg, 2 mg/kg, 3 mg/kg, 6 mg/kg, 10 mg/kg, 15 mg/kg or 20 mg/kg are administered and the patient is assessed for toxicity, if any, as well as for efficacy of treatment, among other parameters. Such studies to determine toxicity and efficacy of dose regimens are well-known in the art. See, for example, Millward M. et al., Br. J. Cancer 2013 108(10):1998-2004.

VII. PHARMACEUTICAL COMPOSITIONS

[0258] In certain embodiments, the A-class CpG ODN is formulated with a marker, e.g., a radio-opaque marker or dye, that facilitates visualization of the A-class CpG ODN administration into and/or adjacent to the tumor to be treated. Alternatively the A-class CpG ODN is covalently conjugated to or otherwise labeled with a compound that enables the detection of the area of administration. Examples of such labels are well known in the art, and include fluorescent dyes, aptamers, fluorescent RNAs such as spinach and derivatives thereof, quantum dots, gold and other nanoparticles, antibodies, etc.

[0259] A-class CpG ODN may be directly administered to the subject or may be administered in conjunction with a nucleic acid delivery complex. A nucleic acid delivery complex shall mean a nucleic acid molecule associated with (e.g., ionically or covalently bound to; or encapsulated within) a targeting means (e.g., a molecule that results in higher affinity binding to target cell. Examples of nucleic acid delivery complexes include oligonucleotides associated with a sterol (e.g. cholesterol), a lipid (e.g., a cationic lipid, virosome, or liposome), or a target cell-specific binding agent (e.g., a ligand recognized by target cell specific receptor). Preferred complexes may be sufficiently stable *in vivo* to prevent significant uncoupling prior to internalization by the target cell. However, the complex can be cleavable under appropriate conditions within the cell so that the nucleic acid is released in a functional form.

[0260] Delivery vehicles or delivery devices for delivering oligonucleotides and/or antigens to surfaces have been described. The A-class CpG ODN and/or the antigen and/or other therapeutics may be administered alone (e.g., in saline or buffer) or using any delivery vehicles

known in the art. For instance the following delivery vehicles have been described: Cochleates; Emulsomes, ISCOMs; Liposomes; Live bacterial vectors (e.g., *Salmonella*, *Escherichia coli*, *Bacillus Calmette-Guerin*, *Shigella*, *Lactobacillus*); Live viral vectors (e.g., Vaccinia, adenovirus, Herpes Simplex); Microspheres; Oligonucleotide vaccines; Polymers; Polymer rings; Proteosomes; Sodium Fluoride; Transgenic plants; Virosomes; Virus-like particles, and cationic lipids, peptides, or other carriers that have a charge interaction with the polyanionic oligonucleotide. Other delivery vehicles are known in the art and some additional examples are provided below in the discussion of vectors.

[0261] In one embodiment, the antibody or antigen-binding fragment thereof which binds specifically to PD-1 is administered parenterally (e.g., intravenously) in an aqueous solution while the A-class CpG ODN is administered by intratumoral or peritumoral injection. Preferred formulations and dosage forms of the A-class CpG ODN are described in U.S. Patent Application Publication No. US 2004/0198680. However, the skilled artisan would understand, based upon the disclosure provided herein, that the invention is not limited to these, or any other, formulations, doses, routes of administration, and the like. Thus, the following discussion describes various formulations for practicing the methods of the invention comprising administration of any antibody or antigen-binding fragment thereof which binds specifically to PD-1 in combination with an A-class CpG ODN, but the invention is not limited to these formulations, but comprises any formulation as can be readily determined by one skilled in the art once armed with the teachings provided herein for use in the methods of the invention.

[0262] The antibodies employed in the invention can be incorporated into pharmaceutical compositions suitable for administration to a subject. Typically, the pharmaceutical composition comprises the antibody and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Examples of pharmaceutically acceptable carriers include one or more of water, saline, phosphate buffered saline, dextrose, trehalose, glycerol, ethanol and the like, as well as combinations thereof. In many cases, it will be preferable to include isotonic agents, for example, sugars, poly alcohols such as mannitol, sorbitol, or sodium chloride in the composition. Pharmaceutically acceptable substances such as wetting or minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the antibody or antibody portion.

[0263] The antibodies may be in a variety of forms. These include, for example, liquid, semi solid and solid dosage forms, such as liquid solutions (e.g., injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. The preferred form depends on the intended mode of administration and therapeutic application. Typical preferred compositions are in the form of injectable or infusible solutions, such as compositions similar to those used for passive immunization of humans with other antibodies. The preferred mode of administration is parenteral (e.g., intravenous, subcutaneous, intraperitoneal, intramuscular). In a preferred embodiment, the antibody is administered by intravenous infusion or injection. In another preferred embodiment, the antibody is administered by

intramuscular or subcutaneous injection.

[0264] Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high drug concentration. Sterile injectable solutions can be prepared by incorporating the antibody in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filter sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

[0265] The A-class CpG ODN can be administered by a variety of methods known in the art, including, without limitation, local injection or infusion into and/or adjacent to a tumor. As used herein, "into a tumor" or "intratumoral" means anywhere generally within the margins of a tumor. As used herein, "adjacent to a tumor" or "peritumoral" means anywhere generally within about a 2.5 cm thick zone surrounding the margins of a tumor. The invention also contemplates local injection or infusion of the A-class CpG ODN into and/or adjacent to a tumor bed following surgical resection of a tumor. Non-needle injection may be employed, if desired. In certain embodiments the A-class CpG ODN can be administered locally to lung by inhalation or bronchoalveolar lavage. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results.

[0266] The antibody or antigen-binding fragment thereof which binds specifically to PD-1 can be administered by a variety of methods known in the art, including, without limitation, oral, parenteral, mucosal, by-inhalation, topical, buccal, nasal, and rectal. For certain therapeutic applications, the preferred route/mode of administration is subcutaneous, intramuscular, intravenous or infusion. Non-needle injection may be employed, if desired. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results.

[0267] Dosage regimens may be adjusted to provide the optimum desired response. For example, a single bolus may be administered, several divided doses may be administered over time, or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound

calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the antibody and the particular therapeutic or prophylactic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

[0268] It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated, and may include single or multiple doses. It is to be further understood that for any particular subject, specific dosage regimens may be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

[0269] In one embodiment, the antibody is administered in an intravenous formulation as a sterile aqueous solution containing 5 or 10 mg/mL of antibody, with sodium acetate, polysorbate 80, and sodium chloride at a pH ranging from about 5 to 6. Preferably, the intravenous formulation is a sterile aqueous solution containing 5 or 10 mg/mL of antibody, with 20 mM sodium acetate, 0.2 mg/ml polysorbate 80, and 140 mM sodium chloride at pH 5.5.

[0270] In one embodiment, part of the dose is administered by an intravenous bolus and the rest by infusion of the antibody formulation. For example, a 0.01 mg/kg intravenous injection of the antibody may be given as a bolus, and the rest of a predetermined antibody dose may be administered by intravenous injection. A predetermined dose of the antibody may be administered, for example, over a period of an hour and a half to two hours to five hours.

[0271] The formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient into association with a carrier or one or more other accessory ingredients, and then, if necessary or desirable, shaping or packaging the product into a desired single- or multi-dose unit.

[0272] A pharmaceutical composition of the invention may be prepared, packaged, or sold in bulk, as a single unit dose, or as a plurality of single unit doses. As used herein, a "unit dose" is discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient is generally equal to the dosage of the active ingredient which would be administered to a subject or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage.

[0273] The relative amounts of the active ingredient, the pharmaceutically acceptable carrier, and any additional ingredients in a pharmaceutical composition of the invention will vary, depending upon the identity, size, and condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of example, the composition may comprise between 0.1% and 100% (w/w) active ingredient.

[0274] In addition to the active ingredient, a pharmaceutical composition of the invention may further comprise one or more additional pharmaceutically active agents. Particularly contemplated additional agents include anti-emetics, anti-diarrheals, chemotherapeutic agents, cytokines, and the like.

[0275] Controlled- or sustained-release formulations of a pharmaceutical composition of the invention may be made using conventional technology.

[0276] As used herein, "parenteral administration" of a pharmaceutical composition includes any route of administration characterized by physical breaching of a tissue of a subject and administration of the pharmaceutical composition through the breach in the tissue. Parenteral administration thus includes, but is not limited to, administration of a pharmaceutical composition by injection of the composition, by application of the composition through a surgical incision, by application of the composition through a tissue-penetrating non-surgical wound, and the like. In particular, parenteral administration is contemplated to include, but is not limited to, intravenous, intraperitoneal, intramuscular, subcutaneous, intracisternal, and kidney dialytic infusion techniques.

[0277] Formulations of a pharmaceutical composition suitable for parenteral administration comprise the active ingredient combined with a pharmaceutically acceptable carrier, such as sterile water or sterile isotonic saline. Such formulations may be prepared, packaged, or sold in a form suitable for bolus administration or for continuous administration. Injectable formulations may be prepared, packaged, or sold in unit dosage form, such as in ampules or in multi-dose containers containing a preservative. Formulations for parenteral administration include, but are not limited to, suspensions, solutions, emulsions in oily or aqueous vehicles, pastes, and implantable sustained-release or biodegradable formulations as discussed below. Such formulations may further comprise one or more additional ingredients including, but not limited to, suspending, stabilizing, or dispersing agents. In one embodiment of a formulation for parenteral administration, the active ingredient is provided in dry (e.g., powder or granular) form for reconstitution with a suitable vehicle (e.g., sterile pyrogen-free water) prior to parenteral administration of the reconstituted composition.

[0278] A composition of the present invention can be administered by a variety of methods known in the art. The route and/or mode of administration vary depending upon the desired results. The active compounds can be prepared with carriers that protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are described by e.g., Sustained and Controlled Release Drug Delivery Systems, J. R. Robinson, ed., Marcel Dekker, Inc., New York, (1978). Pharmaceutical compositions are preferably manufactured under GMP conditions.

[0279] The pharmaceutical compositions may be prepared, packaged, or sold in the form of a

sterile injectable aqueous or oily suspension or solution. This suspension or solution may be formulated according to the known art, and may comprise, in addition to the active ingredient, additional ingredients such as the dispersing agents, wetting agents, or suspending agents described herein. Such sterile injectable formulations may be prepared using a nontoxic parenterally-acceptable diluent or solvent, such as water or 1,3-butane diol, for example. Other acceptable diluents and solvents include, but are not limited to, Ringer's solution, isotonic sodium chloride solution, and fixed oils such as synthetic mono- or diglycerides. Other parentally-administrable formulations which are useful include those which comprise the active ingredient in microcrystalline form, in a liposomal preparation, or as a component of a biodegradable polymer system. Compositions for sustained release or implantation may comprise pharmaceutically acceptable polymeric or hydrophobic materials such as an emulsion, an ion exchange resin, a sparingly soluble polymer, or a sparingly soluble salt.

[0280] The A-class CpG ODN and anti-PD-1 antibody active ingredient components of the invention can be administered to an animal, preferably a mammal, more preferably a human. The precise dosage administered of each active ingredient will vary depending upon any number of factors, including but not limited to, the type of animal and type of disease state being treated, the age of the animal and the route(s) of administration.

[0281] The A-class CpG ODN and anti-PD-1 antibody active ingredient components of the invention may be co-administered with any of numerous other compounds (antihormonal therapy agents, cytokines, anti-cytokine antibodies, or anti-cytokine receptor antibodies, inhibitors of indoleamine 2,3-dioxygenase (IDO) or tryptophan 2,3-dioxygenase (TDO), chemotherapeutic, antibiotic and/or antiviral drugs, among many others). Alternatively, such other compound(s) may be administered an hour, a day, a week, a month, or even more, in advance of the A-class CpG ODN - anti-PD-1 antibody combination, or any permutation thereof. Further, such other compound(s) may be administered an hour, a day, a week, or even more, after administration of radiation, stem cell transplant, or administration of any therapeutic agent (e.g., cytokine, chemotherapeutic compound, and the like), or any permutation thereof. The frequency and administration regimen will be readily apparent to the skilled artisan and will depend upon any number of factors such as, but not limited to, the type and severity of the disease being treated, the age and health status of the animal, the identity of the compound or compounds being administered, the route of administration of the various compounds, and the like. Several instructive examples demonstrating methods of coadministering A-class CpG ODN - anti-PD-1 antibody combination to treat cancer are provided, but the invention is not limited in any way to these examples, which merely serve to illustrate methods encompassed by the invention.

VIII. KITS

[0282] A therapeutically effective amount of A-class CpG ODN and a therapeutically effective amount of an antibody or antigen-binding fragment thereof which binds specifically to PD-1, may be provided as a kit, along with instructional materials which describe use of the

combination to perform the methods of the invention. In certain embodiments, the kits comprise a therapeutically effective amount of A-class CpG ODN and a therapeutically effective amount of an antibody or antigen-binding fragment thereof which binds specifically to PD-1, along with instructional materials which describe use of the combination to perform the methods of the invention. Although exemplary kits are described below, the contents of other useful kits will be apparent to the skilled artisan in light of the present disclosure. Each of these kits is included within the invention.

[0283] Further, the kit can comprise a wide plethora of additional agents for treatment of cancer. Such agents are set forth previously and include chemotherapeutic compounds, cancer vaccines, TLR agonists other than a CpG ODN, other CpG ODNs, receptor tyrosine kinase inhibitors (such as, but not limited to, SU11248), agents useful in treating abnormal cell growth or cancer, antibodies or other ligands that inhibit tumor growth by binding to IGF-1R, a chemotherapeutic agent (taxane, vinca alkaloid, platinum compound, intercalating antibiotics, among many others), and cytokines, among many others, as well as palliative agents to treat, e.g., any toxicities that arise during treatment such as, but not limited to, an anti-diarrheal, an anti-emetic, and the like.

[0284] Having now described the present invention in detail, the same will be more clearly understood by reference to the following examples, which are included herewith for purposes of illustration only and are not intended to be limiting of the invention.

EXAMPLES

Example 1

[0285] In order to achieve optimal synergy for a combination of a CpG ODN and checkpoint inhibitor (+/- XRT), the CpG ODN should be designed to induce the maximal level of type I IFN possible, with the lowest level of IL-10 possible. Of the CpG ODN classes described above, the closest to this ideal is the A-class. In order to improve the A-class ODN, they can be understood in terms of two semi-independent components: (i) the 5' and 3' termini of the A-class CpG ODN, and (ii) the core palindrome. The purpose of the polyG domains in the 5' and 3' termini is to form G tetrads that self-assemble into nanoparticles, positioning the palindromes in a favorable way to activate TLR9, and providing a very strong multimerization of TLR9 in the early endosomes, leading to strong IRF3/7 activation (and downstream IFN- α secretion) without triggering a more sustained signal that would lead to B cell activation and strong IL-10 production. The G tetrads formed by the polyG domains may also help to stabilize the ODN extracellularly and improve ODN uptake into dendritic cells (DC) and other APC by interacting with scavenger receptors and other cell surface receptors that bind G tetrads. The polyG domains often have one or a few PS linkages at the 5' and 3' ends, but this is not required for high level stimulation of pDC IFN- α secretion, especially if the dosage is increased,

or the ODN is delivered using a stabilizing formulation, such as a nanoparticle, VLP, ISCOM, or the like. The purpose of the palindrome is to form a duplex outside the cell, stabilizing a structure that will be taken up effectively by the target DC into endosomes and then will activate TLR9 in a transient manner to induce IRF3/7 without strong NF- κ B activation.

Optimization of the 5' and 3' termini of A-class ODN

[0286]

1. *1. Number of Gs.* A-class ODN described in the prior art nearly always contain 5 or more consecutive Gs at both ends, or at least at one end. However this is not required for the ODN activity, and in fact including fewer Gs makes the ODN much easier to synthesize, and does not necessarily dramatically impact the amount of IFN- α induced. In accordance with the instant invention, certain preferred A-class ODN have 4 Gs at one or both ends, while other preferred A-class ODN have more than 6 Gs, 10 Gs, or more than 10 Gs, at the 5' and 3' ends, or at least at the 3' end of the ODN.
2. *2. Number of Phosphorothioate (PS) Linkages.* Some A-class ODN described in the prior art contain no phosphorothioate linkages at all, but usually they have two phosphorothioate internucleotide linkages at the 5' end of the ODN and five at the 3' end. While these phosphorothioate linkages do stabilize the ODN against nucleases and increase protein binding and cell surface uptake to some degree, they also introduce chiral centers and increase the complexity of manufacturing. Certain preferred A-class ODN of the invention contain 0, 1, or 2 PS linkages at the 5' end, and 2, 3, or 4 PS linkages at the 3' end. In certain embodiments, preferred A-class ODN of the invention contain 1 or 2 PS linkages at the 5' end, and 2, 3, or 4 PS linkages at the 3' end.
3. *3. Chirality of the Phosphorothioate (PS) Linkages.* When A-class ODN disclosed in the prior art have PS linkages, they have always been stereo-random. However, the two stereoisomers have quite different immune effects on the TLR9 signaling, as published previously (Krieg AM et al., Oligonucleotide 2003 13(6): 491-9). Improved A-class CpG may have all R, all S, or specified R and S chirality at each position within the polyG domains. When the CpG ODN contains any PS linkage, preferably at least the 3' end of the CpG ODN has a Sp linkage because of its greater resistance to nuclease degradation.

Optimization of the Palindrome of A-class CpG ODN

[0287]

1. *1. Positioning of Deoxyadenosine Nucleotides.* Preferred palindromes contain at least one, and preferably two or more deoxyadenosines. These are preferably located in the

- 5' half of the palindrome, with the consequence that the complementary thymidines are located in the 3' half of the preferred palindromes (except that when the thymidines are modified by a halogen, as described in point 3 below, the preferred palindromes may have deoxyadenosine or thymidine in the 5' or 3' or both regions of the palindrome).
2. 2. *Position of CpG Dinucleotides.* Preferred palindromes contain at least one CpG dinucleotide that is preceded by a 5'T and/or at least one CpG dinucleotide preceded by a 5'A.
3. 3. *Modifications of Thymidine Nucleosides.* We have defined in the current invention a new type of A-class CpG ODN, which we now call A/E-class CpG ODN, that contains not only the novel design features listed above, but also the modifications to one or more thymidine nucleosides previously described as E-class CpG ODN, as described in U.S. Patent No. 8,580,268 and U.S. Published Application 2014/0163213. Specifically, preferred A/E-class CpG ODN of the invention contain a halogen-modified uracil in place of one or more of the thymidines in the palindrome. The halogen-modified uracil is most preferably 5-iodo-2'-deoxyuridine ("I"), but also may be 5-bromo-2'-deoxyuridine, or 5-chloro-2'-deoxyuridine.

[0288] Examples of preferred A-class CpG ODN are:

ggGGGACGAGCTCGTCgggggG (SEQ ID NO:80);

ggGGGACGATCGTCGgggggG (SEQ ID NO:58);

ggGGACGATCGAACGTgggggG (SEQ ID NO:81);

ggGGTCGACGTCGACGTCGAGgggggG (SEQ ID NO:78); and

ggGGACGACGTCGTGgggggG (SEQ ID NO:79),

where each lower case letter represents a nucleotide linked to its 3'-adjacent nucleotide by a phosphorothioate (PS) linkage; and each upper case letter represents a nucleotide linked to its 3'-adjacent nucleotide (if present) by a phosphodiester (PO) linkage, except that the 3'-terminal nucleotide is represented by an upper case letter since it has no 3'-adjacent nucleotide.

[0289] Examples of preferred novel A-class CpG ODN sequences are:

gGGGACGATCGTCGgggG	(SEQ ID NO:502);
ggGGTCGACGTACGTCGAggggG	(SEQ ID NO:503);
gGGGTCGTCGACGAggggG	(SEQ ID NO:504);
ggGGACGAGCTCGTCgggggG	(SEQ ID NO:505);
ggGGGACGAGCTCGTCggggG	(SEQ ID NO:506);
gGGGACGAGCTCGTCggggG	(SEQ ID NO:507);
gGGGACGAGCTCGTCgggG	(SEQ ID NO:508);
ggGGACGA TCGTCGgggggG	(SEQ ID NO:77);

ggGGGACGA TCGTCGgggggG	(SEQ ID NO:49);
gGGGACGA TCGTCGgggggG	(SEQ ID NO:509);
gGGGACGA TCGTCGggggG	(SEQ ID NO:502);
gGGGACGA TCGAACGTgggggG	(SEQ ID NO:81);
ggGGACGA TCGAACGTgggggG	(SEQ ID NO:510);
gGGGACGA TCGAACGTgggggG	(SEQ ID NO:510);
gGGGACGA TCGAACGTgggG	(SEQ ID NO:511);
gGGGTCGACGTCGACGTCGAGgggggG	(SEQ ID NO:78);
ggGGTCGACGTCGACGTCGAGgggggG	(SEQ ID NO:512);
gGGGTCGACGTCGACGTCGAGgggggG	(SEQ ID NO:512);
gGGGTCGACGTCGACGTCGAGgggG	(SEQ ID NO:513);
gGGGACGACGTCGTGggggGG	(SEQ ID NO:514);
gGGGACGACGTCGTGggggggG	(SEQ ID NO:79);
ggGGACGACGTCGTGgggggG	(SEQ ID NO:514);
gGGGACGACGTCGTGgggggG	(SEQ ID NO:514);
gGGGACGACGTCGTGgggG	(SEQ ID NO:515); and
ggGTCGTCGACGAggggG	(SEQ ID NO:516),

where again each lower case letter represents a nucleotide linked to its 3'-adjacent nucleotide by a phosphorothioate (PS) linkage; and each upper case letter represents a nucleotide linked to its 3'-adjacent nucleotide (if present) by a phosphodiester (PO) linkage, except that the 3'-terminal nucleotide is represented by an upper case letter since it has no 3'-adjacent nucleotide.

[0290] Examples of preferred novel A/E-class CpG ODN sequences are:

gGGGACGAICGTCGggggG	(SEQ ID NO:1);
gGGGACGAIATCGTCgggggG	(SEQ ID NO:2);
gGGGACGAGCIGCTCgggggG	(SEQ ID NO:3);
ggGGICACCGGTGAgggggG	(SEQ ID NO:4);
ggGGICGACGTACGTCGAgggggG	(SEQ ID NO:5);
ggGGICGACGIACGTCGAgggggG	(SEQ ID NO:6);
ggGGICGACGT ACGICGAgggggG	(SEQ ID NO:7);
ggGGICGACGIACGICGAgggggG	(SEQ ID NO:8);
ggGGACGICGACGTgggG	(SEQ ID NO:9);
ggGGICGACGTCGACGTCGAGgggggG	(SEQ ID NO:10);
ggGGICGACGICGACGTCGAGgggggG	(SEQ ID NO:11);
ggGGICGACGTCGACGICGAGgggggG	(SEQ ID NO:12);
ggGGICGACGICGACGICGAGgggggG	(SEQ ID NO:13);

gGGGACGACGICGIGgggGG	(SEQ ID NO:14);
gGGGICGTCGACGAggggG	(SEQ ID NO:15);
gGGGTCGICGACGAggggG	(SEQ ID NO:16);
gGGGICGICGACGAggggG	(SEQ ID NO:17);
ggGGACGAGCICGTCgggggG	(SEQ ID NO:18);
ggGGGACGAGCICGTCgggggG	(SEQ ID NO:19);
gGGGACGAGCICGTCgggggG	(SEQ ID NO:20);
gGGGACGAGCICGTCgggG	(SEQ ID NO:21);
ggGGACGAICGTCGgggggG	(SEQ ID NO:22);
ggGGGACGAICGTCGgggggG	(SEQ ID NO:23);
gGGGACGAICGTCGggggG	(SEQ ID NO:24);
gGGGACGAICGTCGgggG	(SEQ ID NO:1);
ggGGACGAICGICGgggggG	(SEQ ID NO:25);
ggGGGACGAICGICGgggggG	(SEQ ID NO:26);
gGGGACGAICGICGgggggG	(SEQ ID NO:27);
gGGGACGAICGICGgggG	(SEQ ID NO:28);
gGGGACGAICGAACGTgggggG	(SEQ ID NO:29);
ggGGACGAICGAACGTgggggG	(SEQ ID NO:30);
gGGGACGAICGAACGTggggG	(SEQ ID NO:30);
gGGGACGAICGAACGTgggG	(SEQ ID NO:31);
gGGGACGAICGAACGlgggggG	(SEQ ID NO:32);
ggGGACGAICGAACGlggggG	(SEQ ID NO:33);
gGGGACGAICGAACGlggggG	(SEQ ID NO:33);
gGGGACGAICGAACGlgggG	(SEQ ID NO:34);
gGGGICGACGTCGACGTCGAGgggggG	(SEQ ID NO:35);
ggGGICGACGTCGACGTCGAGgggggG	(SEQ ID NO:10);
gGGGICGACGTCGACGTCGAGggggG	(SEQ ID NO:10);
gGGGICGACGTCGACGTCGAGgggG	(SEQ ID NO:36);
gGGGICGACGICGACGTCGAGgggggG	(SEQ ID NO:37);
ggGGICGACGICGACGTCGAGgggggG	(SEQ ID NO:11);
gGGGICGACGICGACGTCGAGggggG	(SEQ ID NO:11);
gGGGICGACGICGACGTCGAGgggG	(SEQ ID NO:38);
gGGGICGACGTCGACGICGAGgggggG	(SEQ ID NO:39);
ggGGICGACGTCGACGICGAGgggggG	(SEQ ID NO:12);
gGGGICGACGTCGACGICGAGggggG	(SEQ ID NO:12);

gGGGICGACGTCGACGICGAGgggG	(SEQ ID NO:40);
gGGGICGACGICGACGICGAGgggggG	(SEQ ID NO:41);
ggGGICGACGICGACGICGAGggggG	(SEQ ID NO:13);
gGGGICGACGICGACGICGAGggggG	(SEQ ID NO:13); and
gGGGICGACGICGACGICGAGgggG	(SEQ ID NO:42),

where "I" represents 5-iodo-2'-deoxyuridine; each lower case letter represents a nucleotide linked to its 3'-adjacent nucleotide by a phosphorothioate (PS) linkage; and each upper case letter represents a nucleotide linked to its 3'-adjacent nucleotide (if present) by a phosphodiester (PO) linkage, except that the 3'-terminal nucleotide is represented by an upper case letter since it has no 3'-adjacent nucleotide.

[0291] The preferred A-class CpG ODN of the present invention will be synthesized using standard methods well known in the art and described above. The activity of the ODN will be evaluated using *in vitro* dose-response assays on human peripheral blood mononuclear cells (PBMC) for IFN- α and IL-10 secretion as described in the A-class and E-class patents (for example, U.S. Patent No. 8,580,268, Fig. 27 for IFN- α , and U.S. Patent No. 7,795,235, Fig 27 for IL-10). Because humans show inter-individual variation in the magnitude of the IFN- α response to TLR9 stimulation, PBMC from a minimum of 3 different individuals will be tested for all cytokine, chemokine, and IFN assays. Freshly collected PBMC are strongly preferred for maximal responsiveness - after 24 hr the magnitude of the *in vitro* responses to TLR9 ligation will be significantly lower. A-class CpG ODN are typically tested on human PBMC at concentrations from approximately 0.1 μ M to approximately 10 μ M. Supernatants are collected after approximately 24, 48, or 72 hr and tested by enzyme-linked immunosorbent assay (ELISA) or other standard assay for amount of IFN- α (usually the assay just measures one or more of the many isoforms of IFN- α) and/or other IFN-induced chemokines and cytokines.

[0292] Preferred A-class and A/E-class CpG ODN of the invention will induce an average of greater than 1000 pg/ml of IFN- α at the most effective concentration in the assay (potency is less important in this regard than peak efficacy), or more preferably greater than 3,000 pg/ml of IFN- α and most preferably greater than 10,000 pg/ml of IFN- α ; in any case preferred ODN induce the production of at least greater than 10 times the IFN- α induced by a positive control B-class CpG ODN. Supernatants from the same experiments are also tested for IL-10 secretion using similar ELISA assays. Preferred A or A/E-class ODN of the present invention will induce less than 1000 pg/ml, preferably less than 300 pg/ml, and most preferably less than 100 pg/ml of IL-10 secretion under these assay conditions.

[0293] The most preferred CpG ODN selected from these *in vitro* assays will then be evaluated in mouse tumor models, using standard systems well known in the art. The mouse assays are not used to select the most active ODN to be taken into human clinical trials, since the rank-order of the ODN will differ, as a result of structural differences between mouse and human TLR9 and species-specific differences in the cell types expressing TLR9. For these reasons the primary selection for a lead candidate CpG ODN to take into human clinical trials will be based on the results from the *in vitro* assays using human cells.

Example 2

[0294] *In vitro* experiments were performed to examine the effects of changes in palindrome sequence, number of 5' and 3' G, number of 5' and 3' phosphorothioate internucleotide linkages, and substitution of 5-iodo-2'-deoxyuridine within the palindromes on IFN- α secretion by human peripheral blood mononuclear cells (PBMCs).

[0295] PBMCs from a normal human donor were cultured in the presence or absence of the indicated ODN in triplicate and results plotted as mean $\pm$ standard deviation (SD) in Figures 4 and 5, for two different human donors. PBMCs were isolated over histopaque-1077 (Sigma) and plated at 1.25×10^6 /mL, 220 μ L/well in RPMI 1640 (10% FBS, glutamine, Pen/Strep) in a 96-well U-bottom tissue culture plate. ODN were added to a final concentration of 5, 1 or 0.2 μ g/mL (**Fig. 4**) or at a lowest concentration of 0.5 μ g/mL (**Fig. 5**) and cells were incubated for 48 hours. Cells were then spun down and supernatants transferred to new plates and frozen at -20 °C until use. Supernatants were subsequently thawed and used for an IFN- α ELISA (PBL Verikine human IFN- α) following the manufacturer's instructions.

Table 2. Set 1 CpG-A oligos made and tested

#	Sequence	ODN	IFN- α	SEQ ID NO:
1	tcgtcgttttgcgttttgcgtT	2006	low	44
2	ggGGGACGATCGTCgggggG	2216	2+	49
3	gGGGACGATCGTCGgggG	2216b	2+	502
4	ggGGTCGACGTACGTCGAggggG	2301a	+/-	503
5	gGGGTCGTCGACGAggggG	2329a	3+	504
6	ggGGACGAGCTCGTCgggggG	2247a	2+	505
7	ggGGGACGAGCTCGTCgggggG	2247b	2+	517
8	gGGGACGAGCTCGTCggggG	2247c	2+	507
9	gGGGACGAGCTCGTCgggG	2247d	+/-	508
10	ggGGACGATCGTCGgggggG	2255a	2+	77
11	ggGGGACGATCGTCGgggggG	2255b	2+	49
12	gGGGACGATCGTCGgggggG	2255c	2+	509
13	gGGGACGATCGTCGgggG	2255d	2+	502
14	gGGGTCGACGTCGACGTCGAGgggggG	2334a	2+	78
15	ggGGTCGACGTCGACGTCGAGgggggG	2334b	2+	512
16	gGGGTCGACGTCGACGTCGAGgggggG	2334c	2+	512
17	gGGGTCGACGTCGACGTCGAGgggG	2334d	1+	513
18	gGGGACGACGTCGTGgggGG	2336a	3+	514

#	Sequence	ODN	IFN-a	SEQ ID NO:
19	gGGGACGACGTCGTGgggggG	2336b	3+	79
20	ggGGACGACGTCGTGggggG	2336c	2+	514
21	gGGGACGACGTCGTGggggG	2336d	3+	514
22	gGGGACGACGTCGTGgggG	2336e	3+	515
23	ggGTCGTCGACGAggggG	2329e	2+	516
24	gGGGACGAICGTCGgggG	2216a	2+	1
25	ggGGICGACGTACGTCGAggggG	2301b	+/-	5
26	ggGGICGACGIACGTCGAggggG	2301c	low	6
27	ggGGICGACGTACGICGAggggG	2301d	low	7
28	ggGGICGACGIACGICGAggggG	2301e	low	8
29	gGGGTCGTCGACGAggggG	2329a	3+	504
30	gGGGICGTCGACGAggggG	2329b	3+	15
31	gGGGTCGICGACGAggggG	2329c	3+	16
32	gGGGICGICGACGAggggG	2329d	3+	17
33	gGGGACGACGICGIGgggGG	2336b	3+	518
34	tcgaacgttcgaacgttcgaacgttcgaat	SD-101	1+	519
#1 (ODN 2006) is control CpG-B #2 (ODN 2216) is control CpG-A #34 (ODN SD-101) is control CpG-C ##3-23 are novel A-class oligos ##24-33 are CpG-A oligos containing 5-iodo-2'-deoxyuridine ("I") lower case = PS linkage (others are PO)				

[0296] Data from this set of experiments suggests:

Greater than four G on the 3' end of oligo confer good activity: compare, e.g., ODN 2247a-c (five or more 3'G) to ODN 2247d (with four 4G on 3' end).

[0297] Five G on 3' end may be inferior to six G (compare ODN 2334d with 5G to ODN 2334a-c with six or more 3' G).

[0298] It doesn't matter whether there are one or two PS linkages on 5' end: compare ODN 2334b (2 PS) to ODN 2334c (1 PS).

[0299] One PS linkage on 5' end appears to be superior to two PS on 5' end in at least some cases: ODN 2336c is the only version of 2336 that has two 5' PS linkages, and appears to be weaker for IFN-a induction than the other versions, which have one PS.

[0300] As long as there are at least five G at the 3' end, three PS on 3' end appears to be just

as strong as four PS: compare ODN 2336a and 2336e (three PS at 3' end) to ODN 2336b and 2336d with five or four PS, respectively.

[0301] The palindrome present in ODN 2301 is weak (but still stronger than the CpG-B) regardless of other elements: therefore not all palindromes work well.

[0302] One or two halogen substitutions within the palindrome are tolerated well in CpG-A, but do not increase IFN- α -inducing activity (e.g., compare ODN 2329a to 2329b, c, d; or ODN 2336a to 2336b; or ODN 2216 to 2216a).

Example 3

[0303] *In vitro* experiments were performed to examine the effects of changes in palindrome sequence, number of 5' and 3' phosphorothioate internucleotide linkages, formulation of a native DNA CpG-A ODN in a virus-like particle (VLP), and substitution of 2-O-methyl sugars within the 3' end of the CpG-A ODN on potency and peak IFN- α secretion by human PBMC.

[0304] Experimental conditions were generally as in Example 2, except that in this case the indicated ODN were cultured with the PBMC in triplicate at the concentrations of 5 $\mu\text{g/mL}$ (concentration or "conc A" in Figures 6 and 7); 1 $\mu\text{g/mL}$ ("conc B" in Figures 6 and 7) and 0.5 $\mu\text{g/mL}$ ("conc C") for all of the ODN except for two samples:

- 1. 1. The completely PO ODN G10 (labeled as "CYT003" in Figures 6 and 7) was cultured at ODN concentrations of 50 $\mu\text{g/mL}$ ("conc A" in Figures 6 and 7), 10 $\mu\text{g/mL}$ ("conc B") and 2 $\mu\text{g/mL}$ ("conc C"); and
- 2. 2. Samples labeled as "CytQbAb" in Figures 6 and 7 contained the G10 ODN packaged within a virus-like particle comprising the bacteriophage protein Qb as previously described by and in clinical development sponsored by Cytos under the name CYT003 or QbG10 (Beeh et al., J Allergy Clin Immunol 2013;131:866-74) together with an anti-Qb antibody to facilitate uptake of the VLP into immune cells. The VLP in these samples was cultured like G10 at 50 $\mu\text{g/mL}$ ("conc A" in Figures 6 and 7), 10 $\mu\text{g/mL}$ ("conc B"), and 2 $\mu\text{g/mL}$ ("conc C"), but since the dose was based on the whole VLP, yet only 20% of the mass of the VLP comprises G10, the actual mass of G10 in each well was closer to 10 $\mu\text{g/mL}$ ("conc A" in Figures 6 and 7), 2 $\mu\text{g/mL}$ ("conc B") and 0.5 $\mu\text{g/mL}$ ("conc C").

Table 3. Set 2 CpG-A oligos made and tested

#	Sequence	ODN	IFN- α	SEQ ID NO:
1	ggGGGACGA TCGTCgggggG	2216	2+	49
2	gGGGACGACGTCGTGgggGG	2336a	3+	514
3	gGGGACGACGTCGTGggggG	2336al	3+	514
4	GGGGACGACGTCGTGGGggG	2336a2	1+	514

#	Sequence	ODN	IFN- α	SEQ ID NO:
5	GGGGACGACGTCGTGGGGGGG	2336aPO	1+	514
6	mGmGmGmGACGACGTCGTGmGmGmGmGmG	2336m1	weak	520
7	GGGGACGACGTCGTGGGGGGmG	2336m2	neg	521
8	GGGGACGACGTCGTGGGGGGgtT	2336ST	1+	522
9	ggGGACGACGTCGTGgggggG	2336c	2+	514
10	gGGGACGACGTCGTGgggG	2336e	2+	515
11	gGGGTCGTCGACGAGgggG	2329a	2+	504
12	GGGGTCGTCGACGAGGggG	2329a1	weak	504
13	GGGGACGACGTCGTGGGGGGmUmU	2336mU	neg	523
14	GGGGGGGGGGGACGATCGTCGGGGGGGGGGG	G10	3+	82
#1 (ODN 2216) is control CpG-A ##2-13 are novel A-class oligos lower case = PS linkage (others are PO) mG = 2'-O-methyl G mT= 2'-O-methyl T				

[0305] Data from this set of experiments suggests:

Fewer than three PS linkages at the 3' end and no PS linkages on the 5' end leads to a severe reduction in the potency of the CpG-A, but no apparent reduction in the peak achievable IFN- α induction at the highest ODN concentration (compare the very strong IFN- α induction by ODN 2336a and 2336a1 (with three or four PS linkages at 3' end, respectively) which was detectable even at only 0.5 $\mu\text{g/mL}$ to the very similar peak level of IFN- α induction by ODN 2336a2, 2336PO, and 2336ST with 2, 0, or 1 PS linkage, respectively).

[0306] There is no apparent potency advantage to having more than one PS at the 5' end and three PS at the 3' end (compare the similar levels of activity between ODN 2336c and 2336e, with a difference of one PS linkage at both ends).

[0307] The palindrome in ODN 2329 (TCGTCGACGA) (SEQ ID NO:524) appears to be less potent for IFN- α induction than the palindromes in either ODN 2216 (GACGATCGTC) (SEQ ID NO:525) or the ODN 2336 series (ACGACGTCGT) (SEQ ID NO:526).

[0308] CpG-A ODN based on a less potent palindrome like that in ODN 2329 may suffer a correspondingly greater reduction in potency if the number of PS linkages is reduced at the 5' and 3' ends (compare ODN 2329a to 2329a1, with reduced PS linkages).

[0309] Substitution of one or more 2'-O-methyl bases at the 5' and/or 3' ends of the CpG-A ODN leads to a severe reduction in the potency and peak achievable IFN- α induction (compare the 2-O-methyl-substituted ODN 2336m1, 2336m2, and 2336mU to the original unmethylated

versions of ODN 2336).

[0310] The highest peak IFN-a induction seen with any of the ODN was from the G10 ("CYT003" in Fig. 6) and from the VLP containing the G10 ("CytQbAb" in Fig. 6). Since G10 is native DNA with no PS modifications at all, this indicates that PS modification is not required for IFN-a induction, as long as either higher concentrations of the CpG-A ODN are used, or the ODN is packaged or delivered in such a way as to protect it against nucleases, such as in a VLP as used in this experiment. The VLP packaging appears to greatly increase the CpG-A ODN potency, since the dose-response of the "naked" G10 ("CYT003") is very similar to the G10 packaged within the VLP, although the latter contains only -20% of the ODN mass.

[0311] In accordance with this invention, the IL-10 induction by the CpG-B ("2006") and CpG-C ("SD-101") control ODN is significantly higher than that of any of the CpG-A ODN (Fig. 7). This supports the use of intratumorally injected CpG-A ODN of the invention for cancer immunotherapy, where local induction of IL-10 (for example, by CpG-B ODN) would be undesirable.

Example 4

[0312] *In vitro* experiments were performed to examine the effects of changes in CpG-A ODN backbone with either phosphorodithioate (PS2) or phosphorothioate (PS) compared to native DNA (PO) on potency and peak IFN-a secretion by normal human PBMC.

[0313] Experimental conditions were generally as in Example 2, except that in this case the indicated ODN were cultured with the PBMC in triplicate for 72 hr at the concentrations of 0.5 µg/mL or 5 µg/mL.

Table 4. Set 3 CpG-A oligos made and tested

#	Sequence	ODN	IFN-a	SEQ ID NO:
A	G#G#GGGACGATCGTCGGGG#G#G	AF185A	strong	49
B	G#G#GGGAGCATGCCTGGGG#G#G	AF185B	negative	527
C	G#G#GGGAC#GATC#GTCGGGG#G#G	AF185C	weak	49
D	G#G#GGGA#C#GAT#C#GTCGGGG#G#G	AF185D	neg	49
E	G#G#GGG#ACGA#TCGTCGGGG#G#G	AF185E	weak	49
F	G#G#GGGACGAT#CGT#CGGGG#G#G	AF185F	weak	49
G	GGGGGACGATCGTCGGGGGG	AF185G	weak	49
H	G#GGGGACGATCGTCGGGG#G#G	AF185H	strong	49
I	GGGGGAC#GATC#GTCGGGGGG	AF185I	weak	49
# = phosphorodithioate (PS2) internucleotide linkage				

[0314] Data from this set of experiments suggests (Fig. 8):
CpG-A ODN containing one or two PS2 modifications on the 5' and 3' ends (e.g., ODN AF185A and H) are approximately as effective as PO (G10) or PS ends (ODN 2216 has PS linkages at 5' and 3' ends).

[0315] PS2 within palindrome severely reduces activity compared to either no PS2 or PS2 on the ends within the polyG.

[0316] It is possible that the PS2 ends may prove superior to PO or PS *in vivo* due to increased protein binding and nuclease resistance.

Example 5

[0317] *In vitro* experiments were performed to examine the effects of reducing the number of G at the 5' and/or 3' end of the G10 CpG-A ODN, or changing the palindrome while keeping the backbone native DNA.

[0318] Experimental conditions were generally as in Example 2, except that in this case the indicated ODN (Table 5) were cultured with PBMC in duplicate for 48 hr at the concentration of 2.5 µg/mL.

Table 5. Set 4 CpG-A oligos made and tested

#	Sequence	IFN-a	SEQ ID NO:
1	GGGGGGGGGACGATCGTGGGGGGGGGG	++	528
2	GGGGGGGGGGGACGATCGTGGGGGGGG	++	529
3	GGGGGGGGGACGATCGTGGGGGGGG	++	530
4	GGGGGGGGGGGTCGTCGACGAGGGGGGGGG	-	531
5	GGGGGGGGGGGACGAGCTCGTGGGGGGGGGG	+	532
6	GGGGGGGGGGGACGATCGTGGGGGGGGGG	+	533
7	GGGGGGGGGGGTCGACGTCGACGTCGAGGGGGGGG GGG	-	534
8	GGGGGGGGGGGACGACGTCGTGGGGGGGGGG	-	535
9	GGGGGGGGGGGAACGACGTCGTTGGGGGGGGGG	-	536
10	GGGGGGGGGGGACGACGACGATCGTCGTCGTGGGG GGGGGG	-	537
11	GGGGGGGGGGGCACGACGTCGTGGGGGGGGGG	-	538
12		+	539

#	Sequence	IFN-a	SEQ ID NO:
	GGGGGGGGGGGAACGTTTCGAACGTTGGGGGGGGGG G		
13	GGGGGGGGGGGAACGTTTCGAACGTTTCGAACGTTTCG AACGTTGGGGGGGGGGG	-	540
14	GGGGGGGGGGGGTTCGAACGTTTCGAAGGGGGGGGGG G	+	541
15	GGGGGGGGGGGGACGTCGACGTCGGGGGGGGGGGG	+	542
16	GGGGGGGGGGGGGTCGACGTCGACGGGGGGGGGGGG	++	543
17	GGGGGGGGGGGGACGTCGACGTACGTCGACGTGGGG GGGGGG	+	544
18	GGGGGGGGGGGGTACGATATCGTAGGGGGGGGGGG	-	545
19	GGGGGGGGGGGGTACGTATACGTAGGGGGGGGGGG	-	546
20	GGGGGGGGGGGGACGTCGACGTCGGGGGGGGGGGG	-	542
21	GGGGGGGGGGGGCAGCATGCTGGGGGGGGGGGGG	-	547
#1 G10 variant: 5' end reduced G #2 G10 variant: 3' end reduced G #3 G10 variant: both ends reduced G #4 TCGGTC palindrome #5 GACGAG palindrome #6 GACGA palindrome #7 TCGACGTC #8 ACGAC #9 AACGAC #10 ACGACGACGA (SEQ ID NO:548) #11 CACGAC #12 SD-101 palindrome #13 SD-101b #14 TTCGAAC #15 GACGTC #16 GTCGAC #17 ACGTCGACGT (SEQ ID NO:549) #18 TACGAT low CG #19 TACGCT #20 GACGTC #21 GC control			

[0319] Data from this set of experiments suggests:

Reducing the number of G at the 5' and/or 3' ends of G10 (as in 1-3, **Table 5**) reduces the induction of IFN- α expression (**Fig. 9**) without reducing the IP-10 induction (**Fig. 10**).

[0320] Nearly all of the new palindromes when flanked by 10 G at the 5' and 3' ends induced IFN- α and IP-10 secretion that is superior to CpG-B (ODN 2006) but not necessarily superior to the control CpG-C (ODN SD-101).

[0321] CpG-B and CpG-C have the undesirable property of inducing higher IL-10 secretion than any of the new CpG-A ODN (**Fig. 11**).

Example 6

[0322] *In vivo* experiments were performed to evaluate the efficacy of treatment of lymphoma with combination tumor immunotherapy involving intratumoral administration of A-class CpG oligonucleotide and systemic administration of anti-PD-1 checkpoint inhibitor.

[0323] Forty female BALB/c mice were primed with CMP001 (CpG-A G10 formulated in VLP) 12.5 μ g on day -14. This priming step was included with the aim of inducing an anti-Qb antibody response to the Qb VLP so that with subsequent injections, the VLP would be opsonized and quickly taken up by pDC. Primed mice were then inoculated on each flank with 5×10^6 A20 lymphoma cells on day 0. Mice were then divided into four treatment groups, N = 10 per group. Mice in Group 1 (negative control) received saline injection directly into lymphoma tumor on one flank on days 7, 12, and 15; and saline injection i.p. twice weekly beginning on day 7. Mice in Group 2 (CpG alone) received CMP001 100 μ g injection directly into lymphoma tumor on one flank on days 7, 12, and 15; and saline injection i.p. twice weekly beginning on day 7. Mice in Group 3 (CPI alone) received saline injection directly into lymphoma tumor on one flank on days 7, 12, and 15; and anti-PD-1 antibody 175 μ g injection i.p. twice weekly beginning on day 7. Mice in Group 4 (CpG + CPI) received CMP001 100 μ g injection directly into lymphoma tumor on one flank on days 7, 12, and 15; and anti-PD-1 antibody 175 μ g injection i.p. twice weekly beginning on day 7. All mice were monitored for tumor size (treated and untreated (i.e, distant)) and survival. Results are shown in **Table 6** and **Fig. 12** and **Fig. 13**.

Table 6

Treatment	Disease- Free at day 62	Cause of Death		
		Treated Tumor	Untreated Tumor	Metastatic Tumor*
saline/saline	0	5	5	0
CMP001/saline	0	2	7	1
saline/anti-PD-1	0	3	4	3
CMP001/anti-PD-1	3	0	3	4

*Metastatic tumors developed in peripheral lymph nodes and/or in a different location on the back.

[0324] As shown in **Fig. 12**, both treated and untreated (distant) tumors grew more slowly in Group 2 (CpG alone) and Group 3 (CPI alone) than in the negative controls (Group 1). Significantly, both treated and untreated (distant) tumors grew much more slowly, and in several instances even disappeared, in Group 4 (CpG + CPI), and this effect was clearly synergistic.

[0325] As shown in **Fig. 13**, mice in Group 1 (negative control) had a median survival of 15 days following tumor inoculation ("tumor challenge"); mice in Group 2 (CpG alone) had a median survival of 19 days, with no mice surviving beyond about day 60; mice in Group 3 (CPI alone) had a median survival of 20.5 days, with no mice surviving beyond about day 60; and mice in Group 4 (CpG + CPI) had a median survival of 48.5 days with mice still surviving after more than 60 days.

SEQUENCE LISTING

[0326]

<110> Checkmate Pharmaceuticals, LLC

<120> COMBINATION TUMOR IMMUNOTHERAPY

<130> CPW-003.25

<150> US 62/098,568

<151> 2014-12-31

<150> US 62/106,526

<151> 2015-01-22

<150> US 62/118,165

<151> 2015-02-19

<160> 550

<170> PatentIn version 3.5

<210> 1

<211> 18

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (9)..(9)

<223> 5-iodo-2'-deoxyuridine

<400> 1

ggggacganc gtcggggg 18

<210> 2

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (9)..(9)

<223> 5-iodo-2'-deoxyuridine

<400> 2

ggggacgana tcgtcggggg 20

<210> 3

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (11)..(11)

<223> 5-iodo-2'-deoxyuridine

<400> 3

ggggacgagc ngctcggggg 20

<210> 4

<211> 19

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (5)..(5)

<223> 5-iodo-2'-deoxyuridine

<400> 4

ggggncaccg gtgaggggg 9

<210> 5

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (5)..(5)

<223> 5-iodo-2'-deoxyuridine

<400> 5

ggggncgacg tacgtcgagg ggg 23

<210> 6

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (5)..(5)

<223> 5-iodo-2'-deoxyuridine

<220>

<221> misc_feature

<222> (11)..(11)

<223> 5-iodo-2'-deoxyuridine

<400> 6

ggggncgacg nacgtcgagg ggg 23

<210> 7

<211> 23
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (5)..(5)
<223> 5-iodo-2'-deoxyuridine

<220>
<221> misc_feature
<222> (15)..(15)
<223> 5-iodo-2'-deoxyuridine

<400> 7
ggggncgacg tacgncgagg ggg 23

<210> 8
<211> 23
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (5)..(5)
<223> 5-iodo-2'-deoxyuridine

<220>
<221> misc_feature
<222> (11)..(11)
<223> 5-iodo-2'-deoxyuridine

<220>
<221> misc_feature
<222> (15)..(15)
<223> 5-iodo-2'-deoxyuridine

<400> 8
ggggncgacg nacgncgagg ggg 23

<210> 9
<211> 18

<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (8)..(8)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (9)..(9)
<223> 5-iodo-2'-deoxyuridine

<400> 9
ggggacgncg acgtgggg 18

<210> 10
<211> 26
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (5)..(5)
<223> 5-iodo-2'-deoxyuridine

<400> 10
ggggncgacg tcgacgtcga gggggg 26

<210> 11
<211> 26
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (5)..(5)
<223> 5-iodo-2'-deoxyuridine

<220>

<221> misc_feature

<222> (11)..(11)

<223> 5-iodo-2'-deoxyuridine

<400> 11

ggggncgacg ncgacgtcga gggggg 26

<210> 12

<211> 26

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (5)..(5)

<223> 5-iodo-2'-deoxyuridine

<220>

<221> misc_feature

<222> (17)..(17)

<223> 5-iodo-2'-deoxyuridine

<400> 12

ggggncgacg tcgacgncga gggggg 26

<210> 13

<211> 26

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (5)..(5)

<223> 5-iodo-2'-deoxyuridine

<220>

<221> misc_feature

<222> (11)..(11)

<223> 5-iodo-2'-deoxyuridine

<220>

<221> misc_feature

<222> (17)..(17)

<223> 5-iodo-2'-deoxyuridine

<400> 13

ggggncgacg ncgacgncga gggggg 26

<210> 14

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (11)..(11)

<223> 5-iodo-2'-deoxyuridine

<220>

<221> misc_feature

<222> (14)..(14)

<223> 5-iodo-2'-deoxyuridine

<400> 14

ggggacgacg ncgngggggg 20

<210> 15

<211> 19

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (5)..(5)

<223> 5-iodo-2'-deoxyuridine

<400> 15

ggggncgtcg acgagggg 19

<210> 16

<211> 19

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (8)..(8)

<223> 5-iodo-2'-deoxyuridine

<400> 16

ggggtcgncg acgaggggg 19

<210> 17

<211> 19

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (5)..(5)

<223> 5-iodo-2'-deoxyuridine

<220>

<221> misc_feature

<222> (8)..(8)

<223> 5-iodo-2'-deoxyuridine

<400> 17

ggggncgncg acgaggggg 19

<210> 18

<211> 21

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (11)..(11)

<223> 5-iodo-2'-deoxyuridine

<400> 18

ggggacgagc ncgtcggggg g 21

<210> 19
<211> 21
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (12)..(12)
<223> 5-iodo-2'-deoxyuridine

<400> 19
gggggacgag cncgtcgggg g 21

<210> 20
<211> 20
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (11)..(11)
<223> 5-iodo-2'-deoxyuridine

<400> 20
ggggacgagc ncgtcggggg 20

<210> 21
<211> 19
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (11)..(11)
<223> 5-iodo-2'-deoxyuridine

<400> 21
ggggacgagc ncgtcgggg 19

<210> 22
<211> 20
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (9)..(9)
<223> 5-iodo-2'-deoxyuridine

<400> 22
ggggacganc gtcggggggg 20

<210> 23
<211> 20
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (10)..(10)
<223> 5-iodo-2'-deoxyuridine

<400> 23
gggggacgan cgtcgggggg 20

<210> 24
<211> 19
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (9)..(9)
<223> 5-iodo-2'-deoxyuridine

<400> 24
ggggacganc gtcggggggg 19

<210> 25
<211> 20
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (9)..(9)
<223> 5-iodo-2'-deoxyuridine

<220>
<221> misc_feature
<222> (12)..(12)
<223> 5-iodo-2'-deoxyuridine

<400> 25
ggggacganc gncggggggg 20

<210> 26
<211> 20
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (10)..(10)
<223> 5-iodo-2'-deoxyuridine

<220>
<221> misc_feature
<222> (13)..(13)
<223> 5-iodo-2'-deoxyuridine

<400> 26
gggggacgan cgncgggggg 20

<210> 27
<211> 19
<212> DNA
<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (9)..(9)

<223> 5-iodo-2'-deoxyuridine

<220>

<221> misc_feature

<222> (12)..(12)

<223> 5-iodo-2'-deoxyuridine

<400> 27

ggggacganc gncggggggg 19

<210> 28

<211> 18

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (9)..(9)

<223> 5-iodo-2'-deoxyuridine

<220>

<221> misc_feature

<222> (12)..(12)

<223> 5-iodo-2'-deoxyuridine

<400> 28

ggggacganc gncggggggg 18

<210> 29

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (9)..(9)

<223> 5-iodo-2'-deoxyuridine

<400> 29
 ggggacganc gaacgtgggg gg 22

<210> 30
 <211> 21
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<220>
 <221> misc_feature
 <222> (9)..(9)
 <223> 5-iodo-2'-deoxyuridine

<400> 30
 ggggacganc gaacgtgggg g 21

<210> 31
 <211> 20
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<220>
 <221> misc_feature
 <222> (9)..(9)
 <223> 5-iodo-2'-deoxyuridine

<400> 31
 ggggacganc gaacgtgggg 20

<210> 32
 <211> 22
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<220>
 <221> misc_feature
 <222> (9)..(9)
 <223> 5-iodo-2'-deoxyuridine

<220>

<221> misc_feature

<222> (16).. (16)

<223> 5-iodo-2'-deoxyuridine

<400> 32

ggggacganc gaacgngggg gg 22

<210> 33

<211> 21

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (9)..(9)

<223> 5-iodo-2'-deoxyuridine

<220>

<221> misc_feature

<222> (16)..(16)

<223> 5-iodo-2'-deoxyuridine

<400> 33

ggggacganc gaacgngggg g 21

<210> 34

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (9)..(9)

<223> 5-iodo-2'-deoxyuridine

<220>

<221> misc_feature

<222> (16)..(16)

<223> 5-iodo-2'-deoxyuridine

<400> 34
 ggggacganc gaacgngggg 20

<210> 35
 <211> 27
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<220>
 <221> misc_feature
 <222> (5)..(5)
 <223> 5-iodo-2'-deoxyuridine

<400> 35
 ggggncgacg tcgacgtcga gggggggg 27

<210> 36
 <211> 25
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<220>
 <221> misc_feature
 <222> (5)..(5)
 <223> 5-iodo-2'-deoxyuridine

<400> 36
 ggggncgacg tcgacgtcga gggggg 25

<210> 37
 <211> 27
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<220>
 <221> misc_feature
 <222> (5)..(5)
 <223> 5-iodo-2'-deoxyuridine

<220>

<221> misc_feature

<222> (11)..(11)

<223> 5-iodo-2'-deoxyuridine

<400> 37

ggggnccgacg nccgacgtcga gggggggg 27

<210> 38

<211> 25

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (5)..(5)

<223> 5-iodo-2'-deoxyuridine

<220>

<221> misc_feature

<222> (11)..(11)

<223> 5-iodo-2'-deoxyuridine

<400> 38

ggggnccgacg nccgacgtcga gggggg 25

<210> 39

<211> 27

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (5)..(5)

<223> 5-iodo-2'-deoxyuridine

<220>

<221> misc_feature

<222> (17)..(17)

<223> 5-iodo-2'-deoxyuridine

<400> 39

ggggncgacg tcgacgncga gggggggg 27

<210> 40

<211> 25

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (5)..(5)

<223> 5-iodo-2'-deoxyuridine

<220>

<221> misc_feature

<222> (17)..(17)

<223> 5-iodo-2'-deoxyuridine

<400> 40

ggggncgacg tcgacgncga gggggg 25

<210> 41

<211> 27

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (5)..(5)

<223> 5-iodo-2'-deoxyuridine

<220>

<221> misc_feature

<222> (11)..(11)

<223> 5-iodo-2'-deoxyuridine

<220>

<221> misc_feature

<222> (17)..(17)

<223> 5-iodo-2'-deoxyuridine

<400> 41

ggggncgacg ncgacgncga gggggggg 27

<210> 42
<211> 25
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (5)..(5)
<223> 5-iodo-2'-deoxyuridine

<220>
<221> misc_feature
<222> (11)..(11)
<223> 5-iodo-2'-deoxyuridine

<220>
<221> misc_feature
<222> (17)..(17)
<223> 5-iodo-2'-deoxyuridine

<400> 42
ggggncgacg ncgacgncga ggggg 25

<210> 43
<211> 20
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<400> 43
ggggtcaacg ttgagggggg 20

<210> 44
<211> 24
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<400> 44
tcgtcgtttt gtcgttttgt cggt 24

<210> 45
 <211> 20
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<400> 45
 ggggtcgtcg tttgggggg 20

<210> 46
 <211> 24
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<400> 46
 tcgtcgttt gtcgttttg gggg 24

<210> 47
 <211> 20
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<400> 47
 ggggtcgacg tcgagggggg 20

<210> 48
 <211> 20
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<400> 48
 ggggtcatcg atgagggggg 20

<210> 49
 <211> 20
 <212> DNA
 <213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 49

gggggacgat cgtcgggggg 20

<210> 50

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 50

gggggtcgta cgacgggggg 20

<210> 51

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 51

gggggacgat atcgtcgggg gg 22

<210> 52

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 52

gggggacgac gtcgtcgggg gg 22

<210> 53

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 53

gggggacgag ctgctcgggg gg 22

<210> 54

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 54

gggggacgta cgtcgggggg 20

<210> 55

<211> 21

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 55

gggggacgat cgttgggggg g 21

<210> 56

<211> 19

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 56

ggggaacgat cgtcggggg 19

<210> 57

<211> 21

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 57

ggggggacga tcgtcggggg g 21

<210> 58

<211> 21

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 58

gggggacgat cgtcgggggg g 21

<210> 59

<211> 21

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 59

gggggtcatc gatgaggggg g 21

<210> 60

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 60

ggggtcgtcg acgagggggg 20

<210> 61

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 61

ggggtcgttc gaacgagggg gg 22

<210> 62

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 62

ggggacgttc gaacgtgggg gg 22

<210> 63

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 63

ggggaacgac gtcgttgggg gg 22

<210> 64

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 64

ggggaacgta cgtcgggggg 20

<210> 65

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 65

ggggaacgta cgtacgttgg gggg 24

<210> 66

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 66

ggggtcaccg gtgagggggg 20

<210> 67
 <211> 24
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Synthetic oligonucleotide

 <400> 67
 ggggtcgacg tacgtcgagg gggg 24

 <210> 68
 <211> 22
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Synthetic oligonucleotide

 <400> 68
 ggggaccggt accggtgggg gg 22

 <210> 69
 <211> 19
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Synthetic oligonucleotide

 <400> 69
 gggtcgacgt cgagggggg 19

 <210> 70
 <211> 18
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Synthetic oligonucleotide

 <400> 70
 ggggtcgacg tcgagggg 18

 <210> 71
 <211> 22
 <212> DNA
 <213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 71

ggggaacgtt aacgttgggg gg 22

<210> 72

<211> 19

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 72

ggggacgtcg acgtggggg 19

<210> 73

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 73

gggggtcgtt cggtggggg 20

<210> 74

<211> 19

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 74

gggacgatcg tcggggggg 19

<210> 75

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 75

gggtcgtcga cgagggggggg 20

<210> 76
 <211> 19
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<400> 76
 ggtcgtcgac gagggggggg 19

<210> 77
 <211> 20
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<400> 77
 ggggacgatc gtcggggggg 20

<210> 78
 <211> 27
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<400> 78
 ggggtcgacg tcgacgtcga gggggggg 27

<210> 79
 <211> 21
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<400> 79
 ggggacgacg tcgtggggggg g 21

<210> 80
 <211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 80

gggggacgag ctcgtcgggg gg 22

<210> 81

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 81

ggggacgatac gaacgtgggg gg 22

<210> 82

<211> 30

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 82

ggggggggggg gacgatacgtc gggggggggggg 30

<210> 83

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 83

tcgtcgttt cggcgcgcgc cg 22

<210> 84

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 84

tcgtcgtttt cggcggccgc cg 22

<210> 85

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 85

tcgtcgtttt cggcgcgccg cg 22

<210> 86

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 86

tcgtcgtttt cggcgcgggc cg 22

<210> 87

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 87

tcgtcgtttt cggcccgcgc gg 22

<210> 88

<211> 27

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 88

tcgtcgtttt cggcgcgcgc cgtttt 27

<210> 89
 <211> 22
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Synthetic oligonucleotide

 <400> 89
 tcctgacgtt cggcgcgcgc cg 22

<210> 90
 <211> 22
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<220>
 <221> misc_feature
 <222> (2) . . (2)
 <223> m5c

<220>
 <221> misc_feature
 <222> (5)..(5)
 <223> m5c

<220>
 <221> misc_feature
 <222> (11)..(11)
 <223> m5c

<220>
 <221> misc_feature
 <222> (14)..(14)
 <223> m5c

<220>
 <221> misc_feature
 <222> (16) . . (16)
 <223> m5c

<220>
 <221> misc_feature
 <222> (18)..(18)

<223> m5c

<220>

<221> misc_feature

<222> (20) . . (21)

<223> m5c

<400> 90

tngtngtttt nggnngngngn ng 22

<210> 91

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 91

tcctgacgtt cggcgcgcgc cc 22

<210> 92

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 92

tcggcgcgcg ccgtcgtcgt tt 22

<210> 93

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 93

tcgtcgtttt cggcggccga cg 22

<210> 94

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 94

tcgtcgtttt cgtcggccgc cg 22

<210> 95

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 95

tcgtcgtttt cgacggccgc cg 22

<210> 96

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 96

tcgtcgtttt cggcggccgt cg 22

<210> 97

<211> 21

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 97

tcgtcgtttc gacggccgtc g 21

<210> 98

<211> 21

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 98

tcgtcgtttc gacgatcgtc g 21

<210> 99

<211> 21

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 99

tcgtcgtttc gacgtacgtc g 21

<210> 100

<211> 18

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 100

tcgtcgcgac ggccgtcg 18

<210> 101

<211> 18

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 101

tcgtcgcgac gatcgtcg 18

<210> 102

<211> 18

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 102

tcgtcgcgac gtacgtcg 18

<210> 103

<211> 22

<212> DNA
 <213> Artificial sequence

 <220>
 <223> Synthetic oligonucleotide

 <400> 103
 tcgtttttt cgacggccgt cg 22

 <210> 104
 <211> 22
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Synthetic oligonucleotide

 <400> 104
 tcgtttttt cgacgatcgt cg 22

 <210> 105
 <211> 22
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Synthetic oligonucleotide

 <400> 105
 tcgtttttt cgacgtacgt cg 22

 <210> 106
 <211> 18
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Synthetic oligonucleotide

 <400> 106
 ttctgctgtt tcgtcgtt 18

 <210> 107
 <211> 18
 <212> DNA
 <213> Artificial sequence

 <220>

<223> Synthetic oligonucleotide

<400> 107

tcgtcggttcg gcgcgccg 18

<210> 108

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 108

tcgtcgtcgt tcggcgcgcg ccg 23

<210> 109

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 109

tcgtcgacga tcggcgcgcg ccg 23

<210> 110

<211> 17

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 110

ttcgtcgttt tgcgtt 17

<210> 111

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 111

tcgtcgtcgt tcggcgcgcg cc 22

<210> 112
 <211> 17
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<400> 112
 tcgtcgtcgt tcggcgc 17

<210> 113
 <211> 21
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<400> 113
 tcgtcgtcgt tcggcgcgcg c 21

<210> 114
 <211> 22
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<400> 114
 cgtcgtcgtt cggcgcgcgc cg 22

<210> 115
 <211> 21
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<400> 115
 gtcgtcgttc ggcgcgcgcc g 21

<210> 116
 <211> 20
 <212> DNA
 <213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<400> 116
tcgtcggttcg gcgcgcgccg 20

<210> 117
<211> 19
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<400> 117
cgtcggttcgg cgcgcgccg 19

<210> 118
<211> 18
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<400> 118
gtcgttcggc gcgcgccg 18

<210> 119
<211> 17
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<400> 119
tcgttcggcg cgcgccg 17

<210> 120
<211> 28
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<400> 120

tcgtcgacga ttttacgacg tcgttttt 28

<210> 121

<211> 27

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 121

tcgtcgacga ttttacgacg tcgttttt 27

<210> 122

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 122

tcgtcgacga acgacgtcgt 20

<210> 123

<211> 27

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 123

tcgtcgacga ttttcgtcg acgattt 27

<210> 124

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 124

tcgtcgacga tcgtcgacga 20

<210> 125

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 125

cgcgcgcgcg cgcgcgcgcg 20

<210> 126

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (20) . . (20)

<223> biotin appended

<400> 126

gagaacgctc gaccttcgat 20

<210> 127

<211> 19

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 127

agctccatgg tgctcactg 19

<210> 128

<211> 18

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 128

tctcccagcg tgcgcat 18

<210> 129

<211> 20
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<400> 129
 tccatgacgt tcctgaggtt 20

<210> 130
 <211> 20
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<400> 130
 tccaggactt ctctcaggtt 20

<210> 131
 <211> 20
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<400> 131
 tccacgacgt ttgcgacgtt 20

<210> 132
 <211> 24
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<400> 132
 tcgtcgtttt gacgttttga cggt 24

<210> 133
 <211> 27
 <212> DNA
 <213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 133

tcgctgctgt tttgtcgttt tgacgtt 27

<210> 134

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 134

tcgctgacgtt cggcgcgcgc cg 22

<210> 135

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(1)

<223> digoxigenin appended

<400> 135

ccggccggcc ggccggccgg 20

<210> 136

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(1)

<223> digoxigenin appended

<400> 136

cgcgcgcgcg cgcgcgcgcg 20

<210> 137

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 137

tccaggactt ctctcaggtt tttt 24

<210> 138

<211> 21

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 138

gtgctcgagg atgcgcttcg c 21

<210> 139

<211> 21

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 139

gccgaggtcc atgtcgtacg c 21

<210> 140

<211> 30

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 140

accgataccg gtgccggtga cggcaccacg 30

<210> 141

<211> 30

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 141

accgataacg ttgccggtga cggcaccacg 30

<210> 142

<211> 30

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 142

accgatgacg tcgccggtga cggcaccacg 30

<210> 143

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 143

cggcgcgcgc cgccggcgcgc gccg 24

<210> 144

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 144

tcgatcggtt ttcgtgcgtt ttt 23

<210> 145

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 145

tcgtccagga cttctctcag gtt 23

<210> 146

<211> 26

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 146

tcgtcgtcca ggacttctct caggtt 26

<210> 147

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 147

tcgtgacggg cggcgcgcgcc cc 22

<210> 148

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 148

acgacgtcgt tcggcggccg ccg 23

<210> 149

<211> 27

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 149

ggggacgacg tcgtgcggcg gccgccg 27

<210> 150

<211> 29

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 150

ccacgacgtc gtcgaagacg acgtcgtgg 29

<210> 151

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 151

ctgcagctgc agctgcagct gcag 24

<210> 152

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 152

cggccgctgc agcggccgct gcag 24

<210> 153

<211> 18

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 153

catgacgttt ttgatgtt 18

<210> 154

<211> 17

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 154

atgacgtttt tgatgtt 17

<210> 155

<211> 16

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 155

tgacgttttt gatgtt 16

<210> 156

<211> 19

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 156

atgacgtttt tgatgtgt 19

<210> 157

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 157

tccatgacgt tttgatgtt 20

<210> 158

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 158

tccatgcgtt tttgaatgtt 20

<210> 159

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 159

tccatgacgt ctttgatgtc 20

<210> 160

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (22) . . (22)

<223> cholesterol appended

<400> 160

acgacgtcgt tcacgacgtc gt 22

<210> 161

<211> 27

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (25) . . (27)

<223> 1'2'-dideoxyribose (D spacer)

<400> 161

acgacgtcgt ggccacgacg tcgtnnn 27

<210> 162

<211> 27

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (11)..(14)

<223> 1'2'-dideoxyribose (D spacer)

<220>

<221> misc_feature

<222> (25) . . (27)

<223> 1'2'-dideoxyribose (D spacer)

<400> 162

acgacgtcgt nnnnacgacg tcgtnnn 27

<210> 163

<211> 30

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(3)

<223> 1'2'-dideoxyribose (D spacer)

<220>

<221> misc_feature

<222> (14)..(17)

<223> 1'2'-dideoxyribose (D spacer)

<220>

<221> misc_feature

<222> (28)..(30)

<223> 1'2'-dideoxyribose (D spacer)

<400> 163

nnnacgacgt cgtnnnnacg acgtcgtnnn 30

<210> 164

<211> 27

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(3)

<223> 1'2'-dideoxyribose (D spacer)

<220>

<221> misc_feature

<222> (14)..(17)

<223> 1'2'-dideoxyribose (D spacer)

<220>

<221> misc_feature

<222> (27) . . (27)

<223> cholesterol appended

<400> 164

nnnacgacgt cgtnnnnacg acgtcgt 27

<210> 165

<211> 30

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 165

gggacgacgt cgtggccacg acgtcgtccc 30

<210> 166

<211> 16

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 166

cccacgacgt cgtggg 16

<210> 167

<211> 18

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 167

cccvacgacg tcgtgggg 18

<210> 168

<211> 17

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 168

atgacgtttt tgacgtt 17

<210> 169

<211> 17

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 169

acgacgtttt tgatgtt 17

<210> 170

<211> 17

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 170

atgatgtttt tgatgtt 17

<210> 171

<211> 16

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 171

atgacgtttt gatgtt 16

<210> 172
 <211> 17
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<400> 172
 atgacgtttg tgatgtt 17

<210> 173
 <211> 17
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<400> 173
 ttgacgtttt tgatgtt 17

<210> 174
 <211> 17
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<400> 174
 atgagctttt gtatgtt 17

<210> 175
 <211> 22
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<400> 175
 tcgacgtttt cggcggccgc cg 22

<210> 176
 <211> 24
 <212> DNA
 <213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 176

tcctgacgtt ttcggcggcc gccg 24

<210> 177

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 177

tcctgacgtt cggcggccgc cg 22

<210> 178

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 178

tccatgacgt tcggcgcgcg ccc 23

<210> 179

<211> 21

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 179

tcctgacgtt cggcgcgcg c 21

<210> 180

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 180

tcgacgtttt cggcgcgcgcg cg 22

<210> 181

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 181

tcgacgtcga cgtaggggtt aggg 24

<210> 182

<211> 21

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 182

acgacgtcgt tagggtagg g 21

<210> 183

<211> 12

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 183

gtcggcggttg ac 12

<210> 184

<211> 25

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (13)..(16)

<223> 1'2'-dideoxyribose (D spacer)

<400> 184
acgacgtcgt cgnnnncggc cgccg 25

<210> 185
<211> 18
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<400> 185
tcgtcgacga cgtcgtcg 18

<210> 186
<211> 22
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (19) . . (22)
<223> 1'2'-dideoxyribose (D spacer)

<400> 186
tcgtcgacga cgtcgtcgnn nn 22

<210> 187
<211> 25
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (25) . . (25)
<223> triethylene glycol

<400> 187
acgacgtcgt ttttacgacg tcgtn 25

<210> 188
<211> 27

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (25) . . (27)

<223> 1'2'-dideoxyribose (D spacer)

<400> 188

acgacgtcgt ttttacgacg tcgtnnn 27

<210> 189

<211> 27

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 189

acgacgtcgt ttttacgacg tcgtttt 27

<210> 190

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 190

acgacgtcgt ttttacgacg tcgt 24

<210> 191

<211> 25

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (11)..(14)

<223> 1'2'-dideoxyribose (D spacer)

<220>

<221> misc_feature

<222> (25) . . (25)

<223> hexaethylene glycol

<400> 191

acgacgtcgt nnnnacgacg tcgtn 25

<210> 192

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (11)..(11)

<223> hexaethylene glycol

<220>

<221> misc_feature

<222> (22) . . (22)

<223> hexaethylene glycol

<400> 192

acgacgtcgt nacgacgtcg tn 22

<210> 193

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (11)..(12)

<223> triethylene glyccol

<220>

<221> misc_feature

<222> (23) . . (23)

<223> triethylene glyccol

<400> 193
acgacgtcgt nnacgacgtc gtn 23

<210> 194
<211> 25
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (10)..(13)
<223> 1'2'-dideoxyribose (D spacer)

<220>
<221> misc_feature
<222> (23) . . (25)
<223> 1'2'-dideoxyribose (D spacer)

<400> 194
cgacgtcgt nnnacgacgt cgnnn 25

<210> 195
<211> 25
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (10)..(13)
<223> 1'2'-dideoxyribose (D spacer)

<220>
<221> misc_feature
<222> (23) . . (25)
<223> 1'2'-dideoxyribose (D spacer)

<400> 195
acgacgtcgn nnnacgacgtc gtann 25

<210> 196
<211> 23
<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (9)..(12)

<223> n is a, c, g, or t

<220>

<221> misc_feature

<222> (21) . . (23)

<223> n is a, c, g, or t

<400> 196

cgacgtcgnn nncgacgtcg nnn 23

<210> 197

<211> 27

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (11)..(14)

<223> 1'2'-dideoxyribose (D spacer)

<220>

<221> misc_feature

<222> (25) . . (27)

<223> 1'2'-dideoxyribose (D spacer)

<400> 197

tcgacgtcgt nnnnacgacg tcgannn 27

<210> 198

<211> 27

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature
<222> (11)..(14)
<223> 1'2'-dideoxyribose (D spacer)

<220>
<221> misc_feature
<222> (25) . . (27)
<223> 1'2'-dideoxyribose (D spacer)

<400> 198
acgtcgtcgt nnnnacgacg acgtnnn 27

<210> 199
<211> 27
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (11)..(14)
<223> 1'2'-dideoxyribose (D spacer)

<220>
<221> misc_feature
<222> (25) . . (27)
<223> 1'2'-dideoxyribose (D spacer)

<400> 199
tcgtcgacgt nnnnacgtcg acgannn 27

<210> 200
<211> 27
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (11)..(14)
<223> 1'2'-dideoxyribose (D spacer)

<220>
<221> misc_feature

<222> (25) . . (27)

<223> 1'2'-dideoxyribose (D spacer)

<400> 200

tcgacgtcgt nnnnacgacg tcgtnnn 27

<210> 201

<211> 27

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (11)..(14)

<223> 1'2'-dideoxyribose (D spacer)

<220>

<221> misc_feature

<222> (25) . . (27)

<223> 1'2'-dideoxyribose (D spacer)

<400> 201

acgacgtcgt nnnnacgtcg tcgtnnn 27

<210> 202

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (9)..(12)

<223> 1'2'-dideoxyribose (D spacer)

<220>

<221> misc_feature

<222> (21) . . (23)

<223> 1'2'-dideoxyribose (D spacer)

<400> 202

acgacgttnn nnaacgtcgt nnn 23

<210> 203
 <211> 21
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<220>
 <221> misc_feature
 <222> (8)..(11)
 <223> 1'2'-dideoxyribose (D spacer)

<220>
 <221> misc_feature
 <222> (19) . . (21)
 <223> 1'2'-dideoxyribose (D spacer)

<400> 203
 acgtcgtnnn nacgacgtnn n 21

<210> 204
 <211> 23
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<220>
 <221> misc_feature
 <222> (9)..(12)
 <223> 1'2'-dideoxyribose (D spacer)

<220>
 <221> misc_feature
 <222> (21) . . (23)
 <223> 1'2'-dideoxyribose (D spacer)

<400> 204
 ggcggccggnn nncggccgcc nnn 23

<210> 205
 <211> 23
 <212> DNA
 <213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (9)..(12)

<223> 1'2'-dideoxyribose (D spacer)

<220>

<221> misc_feature

<222> (21) . . (23)

<223> 1'2'-dideoxyribose (D spacer)

<400> 205

gcggccggnn nnccggccgc nnn 23

<210> 206

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (8)..(11)

<223> 1'2'-dideoxyribose (D spacer)

<220>

<221> misc_feature

<222> (22) . . (24)

<223> 1'2'-dideoxyribose (D spacer)

<400> 206

acgtcgtnnn nacgacgtcg tnnn 24

<210> 207

<211> 26

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(1)

<223> 1'2'-dideoxyribose (D spacer)

<220>

<221> misc_feature

<222> (12)..(15)

<223> 1'2'-dideoxyribose (D spacer)

<220>

<221> misc_feature

<222> (26) . . (26)

<223> 1'2'-dideoxyribose (D spacer)

<400> 207

nacgacgtcg tnnnnacgac gtcgtn 26

<210> 208

<211> 28

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (26).. (27)

<223> 1'2'-dideoxyribose (D spacer)

<400> 208

acgacgtcgt cgaagacgac gtcgtnt 28

<210> 209

<211> 28

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (26) . . (27)

<223> 1'2'-dideoxyribose (D spacer)

<400> 209

tcgacgtcgt cgaagacgtc gtcgtnt 28

<210> 210

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (5)..(5)

<223> 1'2'-dideoxyribose (D spacer)

<400> 210

tccangacgt ttttgatggt 20

<210> 211

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (12)..(12)

<223> 1'2'-dideoxyribose (D spacer)

<400> 211

tccatgacgt tnttgatggt 20

<210> 212

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (5)..(5)

<223> 1,3-propane diol

<400> 212

tccangacgt ttttgatggt 20

<210> 213

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (12)..(12)

<223> 1,3-propane diol

<400> 213

tccatgacgt tnttgatgtt 20

<210> 214

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (20) . . (20)

<223> bis-hydroxypropyl-3,3,3',3'-tetramethy-4,5-benzindocarbocyanine chloride appended

<400> 214

tccatgacgt ttttgatgtt 20

<210> 215

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(5)

<223> 1,3-propane diol

<400> 215

nnnnngacgt ttttgatgtt 20

<210> 216

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (5)..(5)

<223> 1,3-propane diol

<220>

<221> misc_feature

<222> (12)..(12)

<223> 1,3-propane diol

<400> 216

tccangacgt tnttgatgtt 20

<210> 217

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (5)..(5)

<223> 1'2'-dideoxyribose (D spacer)

<220>

<221> misc_feature

<222> (12)..(12)

<223> 1'2'-dideoxyribose (D spacer)

<400> 217

tccangacgt tnttgatgtt 20

<210> 218

<211> 28

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (11)..(14)
<223> 1'2'-dideoxyribose (D spacer)

<220>
<221> misc_feature
<222> (25) . . (27)
<223> 1'2'-dideoxyribose (D spacer)

<220>
<221> misc_feature
<222> (28) . . (28)
<223> ribouracil

<400> 218
acgacgtcgt nnnnacgacg tcgtnnnn 28

<210> 219
<211> 28
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (11)..(14)
<223> 1'2'-dideoxyribose (D spacer)

<220>
<221> misc_feature
<222> (25) . . (27)
<223> 1'2'-dideoxyribose (D spacer)

<220>
<221> misc_feature
<222> (28) . . (28)
<223> riboguanidine

<400> 219
acgacgtcgt nnnnacgacg tcgtnnng 28

<210> 220
<211> 28
<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (11)..(14)

<223> 1'2'-dideoxyribose (D spacer)

<220>

<221> misc_feature

<222> (25) . . (27)

<223> 1'2'-dideoxyribose (D spacer)

<220>

<221> misc_feature

<222> (28) . . (28)

<223> riboadenine

<400> 220

acgacgtcgt nnnnacgacg tcgtnna 28

<210> 221

<211> 31

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(3)

<223> 1'2'-dideoxyribose (D spacer)

<220>

<221> misc_feature

<222> (14)..(17)

<223> 1'2'-dideoxyribose (D spacer)

<220>

<221> misc_feature

<222> (28)..(30)

<223> 1'2'-dideoxyribose (D spacer)

<220>

<221> misc_feature

<222> (31)..(31)

<223> ribouracil

<400> 221

nnnacgacgt cgtnnnnacg acgtcgtnnn n 31

<210> 222

<211> 31

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (11)..(14)

<223> 1'2'-dideoxyribose (D spacer)

<220>

<221> misc_feature

<222> (25) . . (27)

<223> 1'2'-dideoxyribose (D spacer)

<220>

<221> misc_feature

<222> (28)..(31)

<223> riboadenine

<400> 222

acgacgtcgt nnnnacgacg tcgtnnnaaa a 31

<210> 223

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 223

tcgatgacgt tcctgacgtt 20

<210> 224

<211> 31

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (14)..(17)

<223> 1'2'-dideoxyribose (D spacer)

<220>

<221> misc_feature

<222> (28)..(30)

<223> 1'2'-dideoxyribose (D spacer)

<220>

<221> misc_feature

<222> (31)..(31)

<223> ribouracil

<400> 224

tttacgacgt cgtnnnnacg acgtcgtnnn n 31

<210> 225

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 225

tcgcgacgtt cgcgcgcgcg 20

<210> 226

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(1)

<223> 1'2'-dideoxyribose (D spacer)

<400> 226

nccatgacgt ttttgatgtt 20

<210> 227

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (2) . . (2)

<223> 1'2'-dideoxyribose (D spacer)

<400> 227

tncatgacgt ttttgatgtt 20

<210> 228

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (3)..(3)

<223> 1'2'-dideoxyribose (D spacer)

<400> 228

tcnatgacgt ttttgatgtt 20

<210> 229

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (4) . . (4)

<223> 1'2'-dideoxyribose (D spacer)

<400> 229

tccntgacgt ttttgatgtt 20

<210> 230
 <211> 20
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<220>
 <221> misc_feature
 <222> (14)..(14)
 <223> 1'2'-dideoxyribose (D spacer)

<400> 230
 tccatgacgt ttngatgtt 20

<210> 231
 <211> 20
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<220>
 <221> misc_feature
 <222> (13)..(13)
 <223> 1'2'-dideoxyribose (D spacer)

<400> 231
 tccatgacgt ttntgatgtt 20

<210> 232
 <211> 21
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<400> 232
 tcgaacgttc ggcgcgcgcc g 21

<210> 233
 <211> 24
 <212> DNA
 <213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 233

tcgtcgaacg ttcggcgcgc gccg 24

<210> 234

<211> 25

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 234

tcgtcgaacg ttcggcgcgtg cgccg 25

<210> 235

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 235

tcgcgacgtt cgttgcgcgcc gccg 24

<210> 236

<211> 21

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 236

tacgtcggtc ggcgcgcgcc g 21

<210> 237

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 237

ttcgcgacgt tcggcgcgcg ccg 23

<210> 238

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 238

tcggcgcgcg ccgtcgcgac gt 22

<210> 239

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 239

tagcgtgcgt ttgacgttt tttt 24

<210> 240

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 240

tagcgagcgt ttgacgttt tttt 24

<210> 241

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 241

ttgcgagcgt ttgacgttt tttt 24

<210> 242

<211> 24

<212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<400> 242
 atgcgtgcgt ttgacgttt tttt 24

<210> 243
 <211> 24
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<400> 243
 ttacgtgcgt ttgacgttt tttt 24

<210> 244
 <211> 24
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<400> 244
 ttgcatgcgt ttgacgttt tttt 24

<210> 245
 <211> 24
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<400> 245
 ttgcgtacgt ttgacgttt tttt 24

<210> 246
 <211> 24
 <212> DNA
 <213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 246

ttgcgtgcat ttgacgttt tttt 24

<210> 247

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 247

ttgcgtgcga ttgacgttt tttt 24

<210> 248

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 248

ttgcgcgcgt ttgacgttt tttt 24

<210> 249

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 249

ttgcgtgcgc ttgacgttt tttt 24

<210> 250

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 250

ttgcgtgcgt ttgacgttt tttt 24

<210> 251
 <211> 25
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Synthetic oligonucleotide

 <400> 251
 tcgtcggacg ttcggcgctg cgccg 25

 <210> 252
 <211> 24
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Synthetic oligonucleotide

 <400> 252
 tggcgacgtt cgttgcgcgc gccg 24

 <210> 253
 <211> 20
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Synthetic oligonucleotide

 <400> 253
 tcgcgacgtt ttgcgcgcgc 20

 <210> 254
 <211> 23
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Synthetic oligonucleotide

 <400> 254
 tcgcgacgtc gttgcgcgcg ccg 23

 <210> 255
 <211> 24
 <212> DNA
 <213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 255

tcgcgacggt cgaagcgcg gccg 24

<210> 256

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 256

tcgcgacgaa cggtgcgcg gccg 24

<210> 257

<211> 27

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (11)..(14)

<223> 1'2'-dideoxyribose (D spacer)

<220>

<221> misc_feature

<222> (25) . . (27)

<223> 1'2'-dideoxyribose (D spacer)

<400> 257

tcgacgtcgt nnnntcgacg tcgtnnn 27

<210> 258

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 258

tcgtcgtag ctcgtagct cggt 24

<210> 259
<211> 24
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<400> 259
tcgtcggtac gtaattacgt cggt 24

<210> 260
<211> 24
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<400> 260
tcgtcggtac gtcgttacgt aatt 24

<210> 261
<211> 24
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<400> 261
tcgtcggtac gtaattacgt aatt 24

<210> 262
<211> 19
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<400> 262
tcgacgtcga cgtgacggg 19

<210> 263
<211> 23
<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 263

tcgcgacgtt cggcgcgctg ccg 23

<210> 264

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 264

tcgcgacgtt cggcgcgctcg ccg 23

<210> 265

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 265

tcgcgacgtt cggcggctcg ccg 23

<210> 266

<211> 10

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (10)..(10)

<223> vitamin E appended

<400> 266

tcgacgtcgt 10

<210> 267

<211> 19

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 267

tcgacgtcga cgtgacgtc 19

<210> 268

<211> 17

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 268

tcgacgtcga cgtgacg 17

<210> 269

<211> 10

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (10)..(10)

<223> vitamin E appended

<400> 269

tcgacgtcga 10

<210> 270

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 270

tcgtcggttac gtaactacgt cggt 24

<210> 271

<211> 24
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<400> 271
 tcgtcggttac gtaacgacgt cggt 24

<210> 272
 <211> 24
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<400> 272
 tcgtcggttag ctaattagct cggt 24

<210> 273
 <211> 24
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<400> 273
 tcgtcggttac gtaattagct cggt 24

<210> 274
 <211> 20
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<400> 274
 cccatgacgt tcctgacgt 20

<210> 275
 <211> 20
 <212> DNA
 <213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 275

gccatgacgt tcctgacgtt 20

<210> 276

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 276

accatgacgt tcctgacgtt 20

<210> 277

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 277

tgcatgacgt tcctgacgtt 20

<210> 278

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 278

tttatgacgt tcctgacgtt 20

<210> 279

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 279

taaatgacgt tcctgacgtt 20

<210> 280

<211> 19

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 280

ccatgacgtt cctgacgtt 19

<210> 281

<211> 18

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 281

catgacgttc ctgacgtt 18

<210> 282

<211> 17

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 282

atgacgttcc tgacgtt 17

<210> 283

<211> 16

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 283

tgacgttcct gacgtt 16

<210> 284

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (11)..(14)

<223> 1'2'-dideoxyribose (D spacer)

<220>

<221> misc_feature

<222> (24) . . (24)

<223> cholesterol appended

<400> 284

tcgacgtcga nnnntcgacg tcga 24

<210> 285

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(1)

<223> triethylene glycol

<220>

<221> misc_feature

<222> (12)..(15)

<223> n is a, c, g, or t

<220>

<221> misc_feature

<222> (22) . . (22)

<223> cholesterol appended

<400> 285

nagctgcagc tnnntcgac ga 22

<210> 286

<211> 21

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 286

tcgcgacgtt cgggcgcgcc g 21

<210> 287

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 287

tcgtcgacgt tcggcgcgcg ccg 23

<210> 288

<211> 21

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 288

tcggacgttc ggcgcgcgcc g 21

<210> 289

<211> 19

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 289

tcggacgttc ggcgcgccg 19

<210> 290

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 290
tcgcgacgtt cggcgcgccg 20

<210> 291
<211> 20
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<400> 291
tcgacgttcg gcgcgcgccg 20

<210> 292
<211> 18
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<400> 292
tcgacgttcg gcgcgccg 18

<210> 293
<211> 18
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<400> 293
tcgcgacgtt cggcgccg 18

<210> 294
<211> 16
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<400> 294
tcgcgacgtt cggccg 16

<210> 295

<211> 16
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<400> 295
tcgacgttcg gcgccg 16

<210> 296
<211> 21
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<400> 296
tcgtcgacgt tcggcgggcc g 21

<210> 297
<211> 20
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<400> 297
tcgtcgacgt tcgggcgccg 20

<210> 298
<211> 23
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<400> 298
tcgacgacgt tcggcgcgcg ccg 23

<210> 299
<211> 23
<212> DNA
<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 299

tcgacgtcgt tcggcgcgcg ccg 23

<210> 300

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 300

tcgtcgacgt tcgccgcgcg gcg 23

<210> 301

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 301

tcgtcgacgt tcggcgccgt gccg 24

<210> 302

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 302

tcgtcgacgt tcgactcgag tcg 23

<210> 303

<211> 19

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 303

tcgacgtcga cgtgacgtt 19

<210> 304

<211> 21

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 304

tcgtcgacgt tcggcgcgcc g 21

<210> 305

<211> 28

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (11)..(14)

<223> 1'2'-dideoxyribose (D spacer)

<220>

<221> misc_feature

<222> (25) .. (27)

<223> 1'2'-dideoxyribose (D spacer)

<220>

<221> misc_feature

<222> (28)..(28)

<223> inverse orientation ribouracil

<400> 305

acgacgtcgt nnnnacgacg tcgtnnnn 28

<210> 306

<211> 25

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 306

tcgtcgacga tcgggcgccg tgccg 25

<210> 307

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 307

tcgtcgacga tcgggcgccgt gccg 24

<210> 308

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 308

tcgtcgacga cggcgccgtg ccg 23

<210> 309

<211> 19

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 309

tcgtcgacga cgtgtcgat 19

<210> 310

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 310

tcgtcgacga cggcgccgtg ccgt 24

<210> 311

<211> 25

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 311

tcgtcgacga tcggcggcgt gccgt 25

<210> 312

<211> 25

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 312

tcgtcgacgt tcggcgccgt gccgt 25

<210> 313

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 313

tcgtcgacgt cggcgccgtg ccgt 24

<210> 314

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 314

tcgtcgacgc ggcgccgtgc cgt 23

<210> 315

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 315

tcgtcgacga agtcgacgat 20

<210> 316

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 316

tcgtcgacga gaatcgtcga cgat 24

<210> 317

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 317

tcgtcgtagc gcgccgtgcc gt 22

<210> 318

<211> 25

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 318

tcgtcgacga tcggcgccgt gccgt 25

<210> 319

<211> 19

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 319

tcgtcgacga cgtgtcgat 19

<210> 320
 <211> 20
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<400> 320
 tcgacgtcga agacgtcgat 20

<210> 321
 <211> 24
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<400> 321
 tcgacgtcga gaatcgacgt cgat 24

<210> 322
 <211> 21
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<400> 322
 tcgtcgacga cggcgaagcc g 21

<210> 323
 <211> 22
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<400> 323
 tcgtcgacga cggcgaagcc gt 22

<210> 324
 <211> 21
 <212> DNA
 <213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 324

tcgacgtcga cgtgacgttg t 21

<210> 325

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (23) .. (23)

<223> butyrate appended

<400> 325

tcgtcgacga tcggcgcgcg ccg 23

<210> 326

<211> 17

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 326

acgacgtttt cgacgtt 17

<210> 327

<211> 25

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 327

tcgtcgacga tcggcgcgcg cccgt 25

<210> 328

<211> 26

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 328

ttcgtcgacg atcggcgcgcg gcccggt 26

<210> 329

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 329

tcgtcgacga tcgacgcgcg tcg 23

<210> 330

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 330

tcgtcgacga tcaacgcgcg ttg 23

<210> 331

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 331

tcgtcgacga tcggcacgtg ccg 23

<210> 332

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 332

tcgtcgacga tcggcatatg ccg 23

<210> 333

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 333

tcgtcgacga tgccgcgcgc ggc 23

<210> 334

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 334

tcgtcgacga tgccgcgctg cggc 24

<210> 335

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 335

tcgtcgtagc atgccgcgcg cggc 24

<210> 336

<211> 25

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 336

tcgtcgtagc atgccgcgct gcggc 25

<210> 337

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 337

tcgtcgacga tcggcgcgcg ccgt 24

<210> 338

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 338

tcgtgcacga tcggcgcgcg ccg 23

<210> 339

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (2) .. (2)

<223> m5c

<400> 339

tngtcgacga tcggcgcgcg ccg 23

<210> 340

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (5)..(5)

<223> m5c

<400> 340
tcgtngacga tcggcgcgcg ccg 23

<210> 341
<211> 23
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (8)..(8)
<223> m5c

<400> 341
tcgtcganga tcggcgcgcg ccg 23

<210> 342
<211> 23
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (12)..(12)
<223> m5c

<400> 342
tcgtcgacga tngcgcgcg ccg 23

<210> 343
<211> 18
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<400> 343
tcgacgtcga cgtcgacg 18

<210> 344
<211> 25

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 344

tcgtcgacgt tcggcgccgt gccgt 25

<210> 345

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 345

gccgcgcgcg gctagcagct gct 23

<210> 346

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 346

cggcgcgcgc cgtagcagct gct 23

<210> 347

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 347

cggcgccgtg ccgttcagc tgct 24

<210> 348

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 348

gccgtgccgc ggcttcagc tgct 24

<210> 349

<211> 25

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 349

tcggcgcgcg ccgatagcag ctgct 25

<210> 350

<211> 25

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 350

tcggcgcgcg gccgttcag ctgct 25

<210> 351

<211> 13

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (7)..(7)

<223> n is a, c, g, or t

<400> 351

cggcgcngcg ccg 13

<210> 352

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 352

tcgtcgacga cggcgcgcg cg 22

<210> 353

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (11)..(11)

<223> 1,3-propane diol

<400> 353

tcgtcgacga ncggcgcgcg ccg 23

<210> 354

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (11)..(11)

<223> hexaethylene glycol

<220>

<221> misc_feature

<222> (11)..(11)

<223> 1'2'-dideoxyribose (D spacer)

<400> 354

tcgtcgacga ncggcgcgcg ccg 23

<210> 355

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (11)..(11)

<223> n is a, c, g, or t

<400> 355

tcgtcgacga ncggcgcgcg ccg 23

<210> 356

<211> 15

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 356

tcgacgtcgt ggggg 15

<210> 357

<211> 16

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 357

tccaggactt ctctca 16

<210> 358

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (6) .. (6)

<223> 2'-O-methyl guanidine

<220>

<221> misc_feature

<222> (7) .. (7)

<223> 2'-O-methyl adenine

<220>

<221> misc_feature

<222> (9)..(9)

<223> 2'-O-methyl guanidine

<220>

<221> misc_feature

<222> (10)..(10)

<223> 2'-O-methyl adenosine

<220>

<221> misc_feature

<222> (13)..(14)

<223> 2'-O-methyl guanidine

<220>

<221> misc_feature

<222> (16) .. (16)

<223> 2'-O-methyl guanidine

<220>

<221> misc_feature

<222> (18)..(18)

<223> 2'-O-methyl guanidine

<220>

<221> misc_feature

<222> (20).. (20)

<223> 2'-O-methyl guanidine

<220>

<221> misc_feature

<222> (23) .. (23)

<223> 2'-O-methyl guanidine

<400> 358

tcgtcnnccn tcnnccnccn ccn 23

<210> 359

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (3)..(3)

<223> 2'-O-methyl guanidine

<220>

<221> misc_feature

<222> (6) .. (6)

<223> 2'-O-methyl guanidine

<220>

<221> misc_feature

<222> (7) .. (7)

<223> 2'-O-methyl adenine

<220>

<221> misc_feature

<222> (9)..(9)

<223> 2'-O-methyl guanidine

<220>

<221> misc_feature

<222> (10)..(10)

<223> 2'-O-methyl adenosine

<220>

<221> misc_feature

<222> (13)..(14)

<223> 2'-O-methyl guanidine

<220>

<221> misc_feature

<222> (16) .. (16)

<223> 2'-O-methyl guanidine

<220>

<221> misc_feature

<222> (18)..(18)

<223> 2'-O-methyl guanidine

<220>

<221> misc_feature

<222> (20).. (20)

<223> 2'-O-methyl guanidine

<220>

<221> misc_feature

<222> (23) .. (23)

<223> 2'-O-methyl guanidine

<400> 359

tcntcnnccn tcnnccnccn ccn 23

<210> 360

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (3)..(3)

<223> 2,4-difluorotoluene

<400> 360

tgncgttttt ttttttttt 20

<210> 361

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (6) .. (6)

<223> 2,4-difluorotoluene

<400> 361

tgtcgntttt ttttttttt 20

<210> 362

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (3)..(3)

<223> 2,4-difluorotoluene

<220>

<221> misc_feature

<222> (6) .. (6)

<223> 2,4-difluorotoluene

<400> 362

tgncgntttt tttttttt 20

<210> 363

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (4) .. (4)

<223> 2,4-difluorotoluene

<400> 363

tgtngtttt tttttttt 20

<210> 364

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (5)..(5)

<223> 2,4-difluorotoluene

<400> 364

tgtcntttt tttttttt 20

<210> 365

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (2) .. (2)

<223> 2,4-difluorotoluene

<400> 365

tncgtttttt ttttttttt 20

<210> 366

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (7) .. (7)

<223> 2,4-difluorotoluene

<400> 366

tgtcgtnttt ttttttttt 20

<210> 367

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (3)..(3)

<223> 5-bromo-2'-deoxyuridine

<400> 367

tgncgttttt ttttttttt 20

<210> 368

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (6) .. (6)

<223> 5-bromo-2'-deoxyuridine

<400> 368

tgtcgntttt ttttttttt 20

<210> 369

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (3)..(3)

<223> 5-bromo-2'-deoxyuridine

<220>

<221> misc_feature

<222> (6) .. (6)

<223> 5-bromo-2'-deoxyuridine

<400> 369

tgncgntttt ttttttttt 20

<210> 370

<211> 19

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (3)..(3)

<223> 5-iodo-2'-deoxyuridine

<400> 370

tgncgttttt tttttttt 19

<210> 371

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (6) .. (6)

<223> 5-iodo-2'-deoxyuridine

<400> 371

tgtcgntttt tttttttt 20

<210> 372

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (3)..(3)

<223> 5-iodo-2'-deoxyuridine

<220>

<221> misc_feature

<222> (6) .. (6)

<223> 5-iodo-2'-deoxyuridine

<400> 372

tgncgntttt tttttttt 20

<210> 373

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (3)..(3)

<223> uridine

<400> 373

tgncgttttt ttttttttt 20

<210> 374

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (6) .. (6)

<223> uridine

<400> 374

tgtcgntttt ttttttttt 20

<210> 375

<211> 19

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (3)..(3)

<223> uridine

<220>

<221> misc_feature

<222> (6) .. (6)

<223> uridine

<400> 375

tgncgntttt ttttttttt 19

<210> 376

<211> 21

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(1)

<223> 5-iodo-2'-deoxyuridine

<400> 376

ncgtcgtttt tcggtcgttt t 21

<210> 377

<211> 21

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (3)..(3)

<223> 5-iodo-2'-deoxyuridine

<220>

<221> misc_feature

<222> (4)..(4)

<223> n is a, c, g, or t

<400> 377

tcgncgtttt tcggtcgttt t 21

<210> 378

<211> 21

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (15)..(15)

<223> 5-iodo-2'-deoxyuridine

<400> 378

tcgtcgtttt tcggncgttt t 21

<210> 379
<211> 21
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (1)..(1)
<223> 5-iodo-2'-deoxyuridine

<220>
<221> misc_feature
<222> (4)..(4)
<223> 5-iodo-2'-deoxyuridine

<400> 379
ncgncgtttt tcggtcgttt t 21

<210> 380
<211> 21
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (4) .. (4)
<223> 5-iodo-2'-deoxyuridine

<220>
<221> misc_feature
<222> (7)..(7)
<223> 5-iodo-2'-deoxyuridine

<400> 380
tcgncgnttt tcggtcgttt t 21

<210> 381
<211> 21
<212> DNA
<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (15)..(15)

<223> 5-iodo-2'-deoxyuridine

<220>

<221> misc_feature

<222> (18)..(18)

<223> 5-iodo-2'-deoxyuridine

<400> 381

tcgtcgtttt tcggncgntt t 21

<210> 382

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(1)

<223> 5-iodo-2'-deoxyuridine

<400> 382

ncgtcgtttt acggcgccgt gccg 24

<210> 383

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (4) .. (4)

<223> 5-iodo-2'-deoxyuridine

<400> 383

tcgncgtttt acggcgccgt gccg 24

<210> 384
 <211> 20
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Synthetic oligonucleotide

 <220>
 <221> misc_feature
 <222> (6) .. (6)
 <223> 5-ethyl-2'-deoxyuridine

 <400> 384
 tgtcgntttt tttttttt 20

 <210> 385
 <211> 20
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Synthetic oligonucleotide

 <220>
 <221> misc_feature
 <222> (3)..(3)
 <223> 5-ethyl-2'-deoxyuridine

 <220>
 <221> misc_feature
 <222> (6) .. (6)
 <223> 5-ethyl-2'-deoxyuridine

 <400> 385
 tgnccgntttt tttttttt 20

 <210> 386
 <211> 23
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Synthetic oligonucleotide

 <220>
 <221> misc_feature
 <222> (1)..(1)

<223> 5-iodo-2'-deoxyuridine

<400> 386

ncgtcgacga tcggcgcgcg ccg 23

<210> 387

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (4) .. (4)

<223> 5-iodo-2'-deoxyuridine

<400> 387

tcgncgacga tcggcgcgcg ccg 23

<210> 388

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(1)

<223> 5-iodo-2'-deoxyuridine

<220>

<221> misc_feature

<222> (4) .. (4)

<223> 5-iodo-2'-deoxyuridine

<400> 388

ncgncgacga tcggcgcgcg ccg 23

<210> 389

<211> 15

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(1)

<223> 5-iodo-2'-deoxyuridine

<400> 389

ncgacgtcgt ggggg 15

<210> 390

<211> 15

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (7) .. (7)

<223> 5-iodo-2'-deoxyuridine

<400> 390

tcgacgncgt ggggg 15

<210> 391

<211> 15

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (7)..(7)

<223> 5-iodo-2'-deoxyuridine

<220>

<221> misc_feature

<222> (10)..(10)

<223> 5-iodo-2'-deoxyuridine

<400> 391

tcgacgncgn ggggg 15

<210> 392

<211> 20
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Synthetic oligonucleotide

 <220>
 <221> misc_feature
 <222> (3)..(3)
 <223> 5-chloro-2'-deoxyuridine

 <400> 392
 tgncgttttt ttttttttt 20

 <210> 393
 <211> 20
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Synthetic oligonucleotide

 <220>
 <221> misc_feature
 <222> (3)..(3)
 <223> 5-ethyl-2'-deoxyuridine

 <400> 393
 tgncgttttt ttttttttt 20

 <210> 394
 <211> 24
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Synthetic oligonucleotide

 <220>
 <221> misc_feature
 <222> (1)..(1)
 <223> 5-iodo-2'-deoxyuridine

 <220>
 <221> misc_feature
 <222> (4) .. (4)
 <223> 5-iodo-2'-deoxyuridine

<400> 394
ncgncgtttt acggcgccgt gccg 24

<210> 395
<211> 24
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (4) .. (4)
<223> 5-iodo-2'-deoxyuridine

<220>
<221> misc_feature
<222> (7) .. (7)
<223> 5-iodo-2'-deoxyuridine

<400> 395
tcgncgnttt acggcgccgt gccg 24

<210> 396
<211> 22
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (10)..(10)
<223> 5-iodo-2'-deoxyuridine

<400> 396
tcttttttgn cgttttttt tt 22

<210> 397
<211> 22
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (10)..(10)

<223> 5-iodo-2'-deoxyuridine

<220>

<221> misc_feature

<222> (13)..(13)

<223> 5-iodo-2'-deoxyuridine

<400> 397

tcttttttgn cgntttttt tt 22

<210> 398

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(1)

<223> 5-iodo-2'-deoxyuridine

<400> 398

ncttttttgt cgttttttt tt 22

<210> 399

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (9) .. (9)

<223> uridine

<220>

<221> misc_feature

<222> (11)..(11)

<223> n is a, c, g, or t

<400> 399
tcttttttgc ngttttttt tt 22

<210> 400
<211> 23
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (1)..(1)
<223> 5-iodo-2'-deoxyuridine

<400> 400
ncgacgtcga tcggcgcgcg ccg 23

<210> 401
<211> 24
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (1)..(1)
<223> 5-iodo-2'-deoxyuridine

<400> 401
ncgacgtcga tcggcgcgcg ccgt 24

<210> 402
<211> 23
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (1)..(1)
<223> 5-ethyl-2'-deoxyuridine

<400> 402
ncgacgtcga tcggcgcgcg ccg 23

<210> 403
<211> 24
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (1)..(1)
<223> 5-iodo-2'-deoxyuridine

<400> 403
ncgtcgacga tcggcggccg ccgt 24

<210> 404
<211> 23
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (1)..(1)
<223> 5-ethyl-2'-deoxyuridine

<400> 404
ncgtcgacga tcggcggccg ccg 23

<210> 405
<211> 23
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (1)..(1)
<223> 5-iodo-2'-deoxyuridine

<400> 405
ncgtcgacga tcggcggccg ccg 23

<210> 406
<211> 20
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (6) .. (6)
<223> 5-fluorodeoxyuridine

<400> 406
tgtcgntttt tttttttt 20

<210> 407
<211> 20
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (3)..(3)
<223> 5-fluorodeoxyuridine

<220>
<221> misc_feature
<222> (6) .. (6)
<223> 5-fluorodeoxyuridine

<400> 407
tgncgntttt tttttttt 20

<210> 408
<211> 20
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (3)..(3)

<223> uridine

<220>

<221> misc_feature

<222> (6) .. (6)

<223> uridine

<400> 408

tgncgntttt ttttttttt 20

<210> 409

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (5)..(5)

<223> 6-nitro-benzimidizol

<400> 409

tgtcnttttt ttttttttt 20

<210> 410

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (4)..(4)

<223> 6-nitro-benzimidazol

<400> 410

tgtngttttt ttttttttt 20

<210> 411

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(1)

<223> 5-iodo-2'-deoxyuridine

<400> 411

ngtcgttttt ttttttttt 20

<210> 412

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(1)

<223> 5-iodo-2'-deoxyuridine

<220>

<221> misc_feature

<222> (3)..(3)

<223> 5-iodo-2'-deoxyuridine

<400> 412

ngncgttttt ttttttttt 20

<210> 413

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (7) .. (7)

<223> 5-iodo-2'-deoxyuridine

<400> 413

tgtcgntttt tttttttttt 20

<210> 414

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (3)..(3)

<223> a,a,a-trifluoro-dT

<400> 414

tgncgttttt tttttttttt 20

<210> 415

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (6) .. (6)

<223> a,a,a-trifluoro-dT

<400> 415

tgtcgntttt tttttttttt 20

<210> 416

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (3)..(6)

<223> a,a,a-trifluoro-dT

<220>

<221> misc_feature

<222> (6) .. (6)

<223> a,a,a-trifluoro-dT

<400> 416

tgncgntttt ttttttttt 20

<210> 417

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (6) .. (6)

<223> 5-chloro-2'-deoxyuridine

<400> 417

tgtcgntttt ttttttttt 20

<210> 418

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (3)..(3)

<223> 5-chloro-2'-deoxyuridine

<220>

<221> misc_feature

<222> (6) .. (6)

<223> 5-chloro-2'-deoxyuridine

<400> 418

tgncgntttt ttttttttt 20

<210> 419

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (2) .. (2)

<223> 5-iodo-2'-deoxyuridine

<400> 419

tncgtttttt ttttttttt 20

<210> 420

<211> 15

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (3)..(3)

<223> 5-iodo-2'-deoxyuridine

<400> 420

tgncgttttg tcgtt 15

<210> 421

<211> 18

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (3)..(3)

<223> 5-iodo-2'-deoxyuridine

<400> 421

ttncgtcgtt tcgtcgtt 18

<210> 422

<211> 15

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(1)

<223> 5-bromo-2'-deoxyuridine

<400> 422

ncgacgtcgt ggggg 15

<210> 423

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (3)..(3)

<223> 5-iodo-2'-deoxyuridine

<400> 423

tgngcttttt tttttttt 20

<210> 424

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(1)

<223> 5-iodo-2'-deoxyuridine

<400> 424

ncgtcgtttt cggcgcgcgc cg 22

<210> 425

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (4) .. (4)

<223> 5-iodo-2'-deoxyuridine

<400> 425

tcgncgtttt cggcgcgcgc cg 22

<210> 426

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (10)..(10)

<223> 5-iodo-2'-deoxyuridine

<400> 426

tcgtcgtttt cggcgcgcgc cg 22

<210> 427

<211> 21

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(1)

<223> 5-iodo-2'-deoxyuridine

<220>

<221> misc_feature

<222> (15)..(15)

<223> 5-iodo-2'-deoxyuridine

<400> 427

ncgtcgtttt tcgncgttt t 21

<210> 428
 <211> 21
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Synthetic oligonucleotide

 <220>
 <221> misc_feature
 <222> (4)..(4)
 <223> 5-iodo-2'-deoxyuridine

 <220>
 <221> misc_feature
 <222> (15)..(15)
 <223> 5-iodo-2'-deoxyuridine

 <400> 428
 tcgncgtttt tcggncgttt t 21

 <210> 429
 <211> 20
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Synthetic oligonucleotide

 <220>
 <221> misc_feature
 <222> (3)..(3)
 <223> 5-iodo-2'-deoxyuridine

 <220>
 <221> misc_feature
 <222> (16) .. (16)
 <223> 5-iodo-2'-deoxyuridine

 <400> 429
 tgncgttttt ttttgncgtt 20

 <210> 430
 <211> 20
 <212> DNA
 <213> Artificial sequence

 <220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (3)..(3)

<223> 5-iodo-2'-deoxyuridine

<400> 430

tgncgttttt tttttttt 20

<210> 431

<211> 15

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(1)

<223> 5-iodo-2'-deoxyuridine

<220>

<221> misc_feature

<222> (13)..(13)

<223> 7-deaza-dG

<400> 431

ncgacgtcgt ggngg 15

<210> 432

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (2) .. (2)

<223> 5-methyl guanidine

<220>

<221> misc_feature

<222> (3)..(3)

<223> 5-iodo-2'-deoxyuridine

<400> 432

tnncgttttt ttttttttt 20

<210> 433

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (3)..(3)

<223> 5-iodo-2'-deoxyuridine

<220>

<221> misc_feature

<222> (5)..(5)

<223> 5-methyl guanidine

<400> 433

tgncttttt ttttttttt 20

<210> 434

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (2) .. (2)

<223> 5-methyl guanidine

<220>

<221> misc_feature

<222> (3)..(3)

<223> 5-iodo-2'-deoxyuridine

<220>

<221> misc_feature

<222> (5)..(5)

<223> 5-methyl guanidine

<400> 434

tnncnttttt ttttttttt 20

<210> 435

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (3)..(3)

<223> 5-proynyl-dU

<400> 435

tgncgttttt ttttttttt 20

<210> 436

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (6) .. (6)

<223> 5-proynyl-dU

<400> 436

tgtcgntttt ttttttttt 20

<210> 437

<211> 21

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (3)..(3)

<223> 5-iodo-2'-deoxyuridine

<400> 437
tgncgttttc ggcgcgcgcc g 21

<210> 438
<211> 21
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (2) .. (2)
<223> 5-iodo-2'-deoxyuridine

<400> 438
tncgttttcg gcgcgcgccg t 21

<210> 439
<211> 20
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (2) .. (2)
<223> 5-ethyl-2'-deoxyuridine

<400> 439
tncgtttttt tttttttt 20

<210> 440
<211> 20
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (3)..(3)
<223> 5-ethyl-2'-deoxyuridine

<400> 440

tgngcttttt ttttttttt 20

<210> 441

<211> 21

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(1)

<223> 5-ethyl-2'-deoxyuridine

<400> 441

ncgtcgtttt tcggtcgttt t 21

<210> 442

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(1)

<223> 5-ethyl-2'-deoxyuridine

<400> 442

ncgtcgtttt acggcgccgt gccg 24

<210> 443

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (4) .. (4)

<223> 5-ethyl-2'-deoxyuridine

<400> 443
tcgncgtttt acggcgccgt gccg 24

<210> 444
<211> 23
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (1)..(1)
<223> 5-ethyl-2'-deoxyuridine

<400> 444
ncgtcgacga tcggcgcgcg ccg 23

<210> 445
<211> 17
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (1)..(1)
<223> 5-iodo-2'-deoxyuridine

<400> 445
nctttttttt tttttt 17

<210> 446
<211> 22
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (1)..(1)
<223> 5-iodo-2'-deoxyuridine

<400> 446
nctttttttt cgtttttttt tt 22

<210> 447
<211> 22
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (10)..(10)
<223> 5-iodo-2'-deoxyuridine

<400> 447
tctttttttt cgtttttttt tt 22

<210> 448
<211> 22
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (1)..(1)
<223> 5-iodo-2'-deoxyuridine

<220>
<221> misc_feature
<222> (10)..(10)
<223> 5-iodo-2'-deoxyuridine

<400> 448
nctttttttt cgtttttttt tt 22

<210> 449
<211> 24
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(1)

<223> 5-iodo-2'-deoxyuridine

<400> 449

ncgtcgtttc gtcgttttgt cggt 24

<210> 450

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (20) .. (20)

<223> 5-iodo-2'-deoxyuridine

<400> 450

tcgtcgtttc gtcgttttgn cggt 24

<210> 451

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(1)

<223> 5-iodo-2'-deoxyuridine

<220>

<221> misc_feature

<222> (20) .. (20)

<223> 5-iodo-2'-deoxyuridine

<400> 451

ncgtcgtttc gtcgttttgn cggt 24

<210> 452

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (3)..(3)

<223> 5-iodo-2'-deoxyuridine

<220>

<221> misc_feature

<222> (5)..(5)

<223> 7-deaza-dG

<400> 452

tgncnttttt tttttttt 20

<210> 453

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (3)..(3)

<223> 5-iodo-2'-deoxyuridine

<220>

<221> misc_feature

<222> (5)..(5)

<223> inosine

<400> 453

tgncnttttt tttttttt 20

<210> 454

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature
<222> (3)..(3)
<223> 5-iodo-2'-deoxyuridine

<220>
<221> misc_feature
<222> (4)..(4)
<223> m5c

<400> 454
tgnngttttt ttttttttt 20

<210> 455
<211> 20
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (8)..(8)
<223> 5-iodo-2'-deoxyuridine

<400> 455
tgtcgttntt ttttttttt 20

<210> 456
<211> 20
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (9) .. (9)
<223> 5-iodo-2'-deoxyuridine

<400> 456
tgtcgttntt ttttttttt 20

<210> 457
<211> 23
<212> DNA
<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(1)

<223> 5-iodo-2'-deoxyuridine

<400> 457

ncgtcgtttt cggcgcgcgc cgt 23

<210> 458

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(1)

<223> 5-ethyl-2'-deoxyuridine

<400> 458

ncgtcgtttt cggcgcgcgc cgt 23

<210> 459

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (4) .. (4)

<223> 5-ethyl-2'-deoxyuridine

<400> 459

tcgncgtttt cggcgcgcgc cgt 23

<210> 460

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (10)..(10)

<223> 5-iodo-2'-deoxyuridine

<400> 460

tcgtcgtttn cggcgcgcgc cgt 23

<210> 461

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (10)..(10)

<223> 5-ethyl-2'-deoxyuridine

<400> 461

tcgtcgtttn cggcgcgcgc cgt 23

<210> 462

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(1)

<223> 5-ethyl-2'-deoxyuridine

<220>

<221> misc_feature

<222> (10)..(10)

<223> 5-ethyl-2'-deoxyuridine

<400> 462

ncgtcgtttn cggcgcgcgc cgt 23

<210> 463
<211> 23
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (1)..(1)
<223> 5-ethyl-2'-deoxyuridine

<220>
<221> misc_feature
<222> (4) .. (4)
<223> 5-ethyl-2'-deoxyuridine

<400> 463
ncgncgtttt cggcgcgcgc cgt 23

<210> 464
<211> 23
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (1)..(1)
<223> 5-iodo-2'-deoxyuridine

<220>
<221> misc_feature
<222> (4) .. (4)
<223> 5-ethyl-2'-deoxyuridine

<400> 464
ncgncgtttt cggcgcgcgc cgt 23

<210> 465
<211> 24
<212> DNA
<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(1)

<223> 5-iodo-2'-deoxyuridine

<400> 465

ncgtcgtttt gtcgttttgt cgtt 24

<210> 466

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(1)

<223> 5-ethyl-2'-deoxyuridine

<400> 466

ncgtcgtttt gtcgttttcg tt 22

<210> 467

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (3)..(3)

<223> 5-(d-bromo-vinyl)-uridine

<400> 467

tgncgttttt ttttttttt 20

<210> 468

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (6) .. (6)

<223> 5-(d-bromo-vinyl)-uridine

<400> 468

tgtcgntttt tttttttt 20

<210> 469

<211> 13

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(1)

<223> 5-iodo-2'-deoxyuridine

<400> 469

ncggcggccg ccg 13

<210> 470

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(1)

<223> 5-iodo-2'-deoxyuridine

<220>

<221> misc_feature

<222> (24)..(24)

<223> 3'-O-methyl riboguanidine

<400> 470

ncgtcgttt acggcgccgt gccn 24

<210> 471

<211> 24
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Synthetic oligonucleotide

 <220>
 <221> misc_feature
 <222> (1)..(1)
 <223> 5-ethyl-2'-deoxyuridine

 <220>
 <221> misc_feature
 <222> (24)..(24)
 <223> 3'-O-methyl riboguanidine

 <400> 471
 ncgtcgtttt acggcgccgt gccn 24

 <210> 472
 <211> 24
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Synthetic oligonucleotide

 <220>
 <221> misc_feature
 <222> (1)..(1)
 <223> 5-ethyl-2'-deoxyuridine

 <220>
 <221> misc_feature
 <222> (4)..(4)
 <223> 5-ethyl-2'-deoxyuridine

 <220>
 <221> misc_feature
 <222> (24)..(24)
 <223> 3'-O-methyl riboguanidine

 <400> 472
 ncgncgtttt acggcgccgt gccn 24

 <210> 473
 <211> 25

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(1)

<223> 5-ethyl-2'-deoxyuridine

<400> 473

ncgtcgtttt acggcgccgt gccgt 25

<210> 474

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(1)

<223> 5-iodo-2'-deoxyuridine

<220>

<221> misc_feature

<222> (22)..(22)

<223> 3'-O-methyl riboguanidine

<400> 474

ncgtcgtttt cggcgcgcgcn 22

<210> 475

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(1)

<223> 5-ethyl-2'-deoxyuridine

<220>

<221> misc_feature

<222> (22)..(22)

<223> 3'-O-methyl riboguanidine

<400> 475

ncgtcgtttt cggcgcgcgc cn 22

<210> 476

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(1)

<223> 5-ethyl-2'-deoxyuridine

<220>

<221> misc_feature

<222> (4)..(4)

<223> 5-ethyl-2'-deoxyuridine

<220>

<221> misc_feature

<222> (22)..(22)

<223> 3'-O-methyl riboguanidine

<400> 476

ncgncgtttt cggcgcgcgc cn 22

<210> 477

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(1)

<223> 5-ethyl-2'-deoxyuridine

<220>

<221> misc_feature
<222> (10)..(10)
<223> 5-ethyl-2'-deoxyuridine

<220>
<221> misc_feature
<222> (22)..(22)
<223> 3'-O-methyl riboguanidine

<400> 477
ncgtcgtttn cggcgcgcgcg cn 22

<210> 478
<211> 22
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (1)..(1)
<223> 5-iodo-2'-deoxyuridine

<220>
<221> misc_feature
<222> (10)..(10)
<223> 5-iodo-2'-deoxyuridine

<220>
<221> misc_feature
<222> (22)..(22)
<223> 3-methyl guanidine

<220>
<221> misc_feature
<222> (22)..(22)
<223> 3'-O-methyl riboguanidine

<400> 478
ncgtcgtttn cggcgcgcgcg cn 22

<210> 479
<211> 23
<212> DNA
<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> N_region

<222> (1)..(1)

<223> 5-ethyl-2'-deoxyuridine

<220>

<221> misc_feature

<222> (1)..(1)

<223> n is a, c, g, or t

<400> 479

ncgtcgtttt cggcgcgcgc cgt 23

<210> 480

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(1)

<223> 5-iodo-2'-deoxyuridine

<400> 480

ncgtcgtttt cggcgcgcgc cgt 23

<210> 481

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(1)

<223> 5-ethyl-2'-deoxyuridine

<220>

<221> misc_feature

<222> (24)..(24)

<223> 3'-O-methyl riboguanidine

<400> 481

ncgtcgacgt tcggcgccgt gccn 24

<210> 482

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(1)

<223> 5-iodo-2'-deoxyuridine

<220>

<221> misc_feature

<222> (24)..(24)

<223> 3'-O-methyl riboguanidine

<400> 482

ncgtcgacgt tcggcgccgt gccn 24

<210> 483

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(1)

<223> 5-iodo-2'-deoxyuridine

<220>

<221> misc_feature

<222> (23)..(23)

<223> 3'-O-methyl riboguanidine

<400> 483

ncgtcgacga tcggcgcgcg ccn 23

<210> 484

<211> 23
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (1)..(1)
<223> 5-ethyl-2'-deoxyuridine

<220>
<221> misc_feature
<222> (23)..(23)
<223> 3'-O-methyl riboguanidine

<400> 484
ncgtcgacga tcggcgcgcg ccn 23

<210> 485
<211> 25
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (1)..(1)
<223> 5-ethyl-2'-deoxyuridine

<400> 485
ncgtcgacgt tcggcgccgt gccgt 25

<210> 486
<211> 24
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (1)..(1)
<223> 5-ethyl-2'-deoxyuridine

<400> 486
ncgtcgacga tcggcgcgcg ccgt 24

<210> 487
<211> 20
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (3)..(3)
<223> nitroindole

<400> 487
tgncgttttt ttttttttt 20

<210> 488
<211> 20
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (3)..(3)
<223> nitropyrrole

<400> 488
tgncgttttt ttttttttt 20

<210> 489
<211> 20
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (3)..(3)
<223> 6-nitro-benzimidazole

<400> 489

tgncgttttt ttttttttt 20

<210> 490

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(1)

<223> 5-iodo-2'-deoxyuridine

<400> 490

ncgtcgacga tggcggcgcc gcc 23

<210> 491

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(1)

<223> 5-ethyl-2'-deoxyuridine

<400> 491

ncgtcgacga tggcggcgcc gcc 23

<210> 492

<211> 21

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 492

ttcgttttcg gcgcgcgccg t 21

<210> 493

<211> 21

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (2)..(2)

<223> 5-ethyl-2'-deoxyuridine

<400> 493

tncgttttcg gcgcgcgccg t 21

<210> 494

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(1)

<223> 5-iodo-2'-deoxyuridine

<400> 494

ncgttttcgg cgcgccggt 20

<210> 495

<211> 21

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(2)

<223> 5-iodo-2'-deoxyuridine

<400> 495

nncgttttcg gcgcgcgccg t 21

<210> 496

<211> 25

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(1)

<223> 5-ethyl-2'-deoxyuridine

<400> 496

ncgtcgtttt acggcgccgt gccgt 25

<210> 497

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (2)..(2)

<223> 5-ethyl-2'-deoxyuridine

<400> 497

tncgttttac ggcgccgtgc cgt 23

<210> 498

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (2)..(2)

<223> 5-iodo-2'-deoxyuridine

<400> 498

tncgttttac ggcgccgtgc cgt 23

<210> 499

<211> 17

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(1)

<223> 5-iodo-2'-deoxyuridine

<220>

<221> misc_feature

<222> (12)..(13)

<223> uridine

<220>

<221> misc_feature

<222> (15)..(15)

<223> uridine

<220>

<221> misc_feature

<222> (17)..(17)

<223> uridine

<400> 499

ncgtcgtttt gnnngnn 17

<210> 500

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(1)

<223> 5-ethyl-2'-deoxyuridine

<400> 500

ncgtcgacga tcggcggccg ccgt 24

<210> 501

<211> 116

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 501

aggtaggtaac ccctaggggt taccaccttc attggaaaac gttcttcggg gcgttcttag 60

gtggtaaccc ctaggggtta ccaccttcac tggaaaacgt tcttcggggc gttctt 116

<210> 502

<211> 18

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 502

ggggacgatc gtcggggg 18

<210> 503

<211> 23

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 503

ggggtcgacg tacgtcagg ggg 23

<210> 504

<211> 19

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 504

ggggtcgtcg acgagggg 19

<210> 505

<211> 21

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 505

ggggacgagc tcgtcggggg g 21

<210> 506

<211> 21

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 506

gggggacgag ctcgtcgggg g 21

<210> 507

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 507

ggggacgagc tcgtcggggg 20

<210> 508

<211> 19

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 508

ggggacgagc tcgtcggggg 19

<210> 509

<211> 19

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 509

ggggacgatc gtcggggggg 19

<210> 510
 <211> 21
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<400> 510
 ggggacgatc gaacgtgggg g 21

<210> 511
 <211> 20
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<400> 511
 ggggacgatc gaacgtgggg 20

<210> 512
 <211> 26
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<400> 512
 ggggtcgacg tcgacgtcga gggggg 26

<210> 513
 <211> 25
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Synthetic oligonucleotide

<400> 513
 ggggtcgacg tcgacgtcga ggggg 25

<210> 514
 <211> 20
 <212> DNA
 <213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 514

ggggacgacg tcgtgggggg 20

<210> 515

<211> 19

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 515

ggggacgacg tcgtggggg 19

<210> 516

<211> 18

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 516

gggtcgtcga cgaggggg 18

<210> 517

<211> 21

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 517

gggggacgag ctcgtcgggg g 21

<210> 518

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (11)..(11)

<223> 5-iodo-2'-deoxyuridine

<220>

<221> misc_feature

<222> (14)..(14)

<223> 5-iodo-2'-deoxyuridine

<400> 518

ggggacgacg ncgngggggg 20

<210> 519

<211> 30

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 519

tcgaacgttc gaacgttcga acgttcgaat 30

<210> 520

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (1)..(4)

<223> n is a, c, g, or t

<220>

<221> misc_feature

<222> (16)..(20)

<223> n is a, c, g, or t

<400> 520

nnnnacgacg tcgtgnnnnn 20

<210> 521

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (20)..(20)

<223> n is a, c, g, or t

<400> 521

ggggacgacg tcgtgggggn 20

<210> 522

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 522

ggggacgacg tcgtgggggg tt 22

<210> 523

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (21)..(22)

<223> n is a, c, g, or t

<400> 523

ggggacgacg tcgtgggggg nn 22

<210> 524

<211> 10

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 524

tcgtcgacga 10

<210> 525

<211> 10

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 525

gacgatcgtc 10

<210> 526

<211> 10

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 526

acgacgtcgt 10

<210> 527

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 527

gggggagcat gcctgggggg 20

<210> 528

<211> 27

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 528

gggggggggac gatcgtcggg gggggggg 27

<210> 529

<211> 27

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 529

ggggggggggg gacgatcgtc gggggggg 27

<210> 530

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 530

gggggggggac gatcgtcggg gggg 24

<210> 531

<211> 30

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 531

ggggggggggg tcgtcgacga gggggggggggg 30

<210> 532

<211> 31

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 532

ggggggggggg acgagctcgt cggggggggggg g 31

<210> 533

<211> 29

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 533

ggggggggggg acgatcgctcg gggggggggg 29

<210> 534

<211> 36

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 534

ggggggggggg tcgacgtcga cgctcgagggg gggggg 36

<210> 535

<211> 30

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 535

ggggggggggg acgacgtcgt gggggggggg 30

<210> 536

<211> 32

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 536

ggggggggggg aacgacgtcg ttggggggggg gg 32

<210> 537

<211> 40

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 537

ggggggggggg acgacgacga tcgtcgctcgt gggggggggg 40

<210> 538

<211> 31

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 538

ggggggggggg cacgacgtcg tgggggggggg g 31

<210> 539

<211> 34

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 539

ggggggggggg aacgttcgaa cggtggggggg gggg 34

<210> 540

<211> 50

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 540

ggggggggggg aacgttcgaa cggtcgaacg ttcgaacgtt ggggggggggg 50

<210> 541

<211> 34

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 541

ggggggggggg ttcgaacgtt cgaagggggg gggg 34

<210> 542

<211> 31

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 542

ggggggggggg acgtcgacgt cgggggggggg g 31

<210> 543

<211> 31

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 543

ggggggggggg tcgacgtcga cgggggggggg g 31

<210> 544

<211> 40

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 544

ggggggggggg acgtcgacgt acgtcgacgt ggggggggggg 40

<210> 545

<211> 32

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 545

ggggggggggg tacgatatcg taggggggggg gg 32

<210> 546

<211> 32

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 546

ggggggggggg tacgtatacg taggggggggg gg 32

<210> 547

<211> 30

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 547

ggggggggggg cagcatgctg ggggggggggg 30

<210> 548

<211> 10

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 548

acgacgacga 10

<210> 549

<211> 10

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 549

acgtcgacgt 10

<210> 550

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 550

tcgtcggttac gtaacgacgt cggt 24

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- [US5922845A \[0046\]](#)
- [US5837243A \[0046\]](#)
- [US8017591B \[0086\]](#)
- [US7635468B \[0086\]](#)
- [US7074772B \[0086\]](#)
- [US6849725B \[0086\]](#)
- [US6451593B \[0086\]](#)
- [US6451563B \[0086\]](#)
- [US20030125279A \[0086\]](#)
- [WO9602555A \[0094\]](#)
- [WO9818810PCT \[0094\]](#)
- [US6194388B1 \[0094\]](#)
- [US6239116B1 \[0094\]](#)
- [US6949520B \[0095\]](#)
- [US7776344B \[0095\]](#)
- [WO2003024481A \[0097\]](#)
- [US20030099668A \[0097\]](#)
- [US20120301499A \[0097\]](#)
- [WO2004084940A \[0097\]](#)
- [US7517520B \[0097\]](#)
- [US20100098722A \[0097\]](#)
- [WO2007068747A \[0097\]](#)
- [US20070184068A \[0097\]](#)
- [US8574564B \[0097\]](#)
- [WO2007144150A \[0097\]](#)
- [US8541559B \[0097\]](#)
- [WO2008073960A \[0097\]](#)
- [US8586728B \[0097\]](#)
- [US4469863A \[0101\]](#)
- [US5023243A \[0101\]](#)
- [EP092574A \[0101\]](#)

- [US7566703B \[0101\]](#)
- [US7795235B \[0101\] \[0291\]](#)
- [US8283328B \[0101\]](#)
- [US8304396B \[0101\]](#)
- [US5177198A \[0112\]](#)
- [US5859231A \[0112\]](#)
- [US6160109A \[0112\]](#)
- [US6207819B \[0112\]](#)
- [WO0006588A \[0112\]](#)
- [WO9501363A \[0118\]](#)
- [US5658738A \[0126\]](#)
- [US5668265A \[0126\]](#)
- [US6808710B \[0136\]](#)
- [US7488802B \[0136\]](#)
- [US8728474B \[0136\]](#)
- [US6682736B \[0137\] \[0138\]](#)
- [US20020088014 \[0137\]](#)
- [US5770429A \[0139\]](#)
- [US20040120948 \[0140\]](#)
- [US4816567A \[0143\]](#)
- [EP0216846A \[0146\]](#)
- [EP0256055A \[0146\]](#)
- [EP0323997A \[0146\]](#)
- [EP0338841A \[0146\]](#)
- [US20030207336 \[0148\]](#)
- [US20030157108A \[0148\]](#)
- [WO0181405A \[0148\]](#)
- [WO0024893A \[0148\]](#)
- [WO03031464A \[0149\]](#)
- [WO9858964A \[0149\]](#)
- [WO9922764A \[0149\]](#)
- [US20040063911 \[0149\]](#)
- [US20040132640A \[0149\] \[0150\]](#)
- [US20040142856A \[0149\]](#)
- [US20040072290A \[0149\]](#)
- [US6602684B \[0149\]](#)
- [US20030207346 \[0150\]](#)
- [US4640835A \[0150\]](#)
- [US4496689A \[0150\]](#)
- [US4301144A \[0150\]](#)
- [US4670417A \[0150\]](#)
- [US4791192A \[0150\]](#)
- [US4179337A \[0150\]](#)
- [US4816397A \[0152\]](#)

- [US5916771A \[0152\]](#)
- [US20040198680A \[0261\]](#)
- [US8580268B \[0287\] \[0291\]](#)
- [US20140163213A \[0287\]](#)
- [US62098568B \[0326\]](#)
- [US62106526B \[0326\]](#)
- [US62118165B \[0326\]](#)

Non-patent literature cited in the description

- CHENMELLMAN Immunity, 2013, vol. 39, 11-10 [\[0001\]](#)
- VICARITRINCHIERI Immunol. Rev., 2004, [\[0002\]](#)
- LU Frontiers Immunol, 2014, [\[0002\]](#)
- MANGBO et al. Journal of Immunotherapy, 2010, [\[0002\]](#)
- KIMCANTOR Cancer Immunol Res, 2014, vol. 2, 929-936 [\[0003\]](#)
- POWLES et al. Nature, 2014, vol. 515, 558- [\[0004\]](#)
- HERBST et al. Nature, 2014, vol. 515, 563- [\[0004\]](#)
- TUMEH et al. Nature, 2014, vol. 515, 568- [\[0004\]](#)
- CHENMELLMAN Immunity, 2013, [\[0023\] \[0023\]](#)
- KIMCANTOR Cancer Immunol. Res., 2014, vol. 2, 926-936 [\[0023\]](#)
- KRIEG Nucleic Acid Ther., 2012, vol. 22, 277-89 [\[0025\]](#)
- KRUIT et al. J Clin Oncol, 2013, [\[0026\]](#)
- TARHINI et al. J Immunother, 2013, [\[0026\]](#)
- LOVGREN et al. Cancer Immunol Immunother, 2012, [\[0026\]](#)
- KARBACH et al. Clin Cancer Res, 2011, [\[0026\]](#)
- KARBACH et al. Int J Cancer, 2010, [\[0026\]](#)
- SPEISER et al. JCI, 2005, [\[0026\]](#)
- SPEISER et al. J. Immunother, 2010, [\[0026\]](#)
- RAJASAGI et al. Blood, 2014, vol. 124, 3453-462 [\[0026\]](#)
- PRASANNA et al. J Thoracic Dis., 2014, vol. 6, 4287-302 [\[0028\]](#)
- POSTOW et al. N. Engl. J. Med., 2012, vol. 366, 10925-31 [\[0029\]](#)
- GRIMALDI et al. Oncoimmunol, 2014, vol. 3, e28780- [\[0030\]](#)
- SAMBROOK RUSSELL Molecular Cloning, A Laboratory Approach Cold Spring Harbor Press 2001 0000 [\[0032\] \[0146\]](#)
- AUSUBEL et al. Current Protocols in Molecular Biology John Wiley & Sons 2002 0000 [\[0032\] \[0146\]](#)
- HARLOW LANE Antibodies: A Laboratory Manual Cold Spring Harbor Laboratory Press 1990 0000 [\[0032\]](#)
- Immunology--A Synthesis Sinauer Associates 1991 0000 [\[0035\]](#)
- PEARSON Methods Mol. Biol., 1994, vol. 243, 307-31 [\[0037\]](#)

- GONNET et al.Science, 1992, vol. 256, 1443-45 [0039]
- Proteins, Structures and Molecular PrinciplesW. H. Freeman and Company19840000 [0040]
- Introduction to Protein StructureGarland Publishing19910000 [0040]
- THORNTON et al.Nature, 1991, vol. 354, 105- [0040]
- PEARSONMethods Enzymol., 1990, vol. 183, 63-98 [0041]
- PEARSONMethods Mol. Biol., 2000, vol. 132, 185-219 [0041]
- ALTSCHUL et al.J. Mol. Biol., 1990, vol. 215, 403-410 [0041]
- ALTSCHUL et al.Nucleic Acids Res., 1997, vol. 25, 3389-402 [0041]
- Fundamental ImmunologyRaven Press19890000 [0042]
- KABATSequences of Proteins of Immunological InterestNational Institutes of Health19870000 [0042]
- CHOTHIALESKJ. Mol. Biol., 1987, vol. 196, 901-917 [0042]
- CHOTHIA et al.Nature, 1989, vol. 342, 878-883 [0042]
- WARD et al.Nature, 1989, vol. 341, 544-546 [0045]
- BIRD et al.Science, 1988, vol. 242, 423-426 [0045]
- HUSTON et al.Proc. Natl. Acad. Sci. USA, 1988, vol. 85, 5879-5883 [0045]
- ZEILDERJ. Immunol, 1999, vol. 163, 1246-1252 [0046]
- SOMASUNDARAMHum. Antibodies, 1999, vol. 9, 47-54 [0046]
- KELERCancer Res., 1997, vol. 57, 4008-4014 [0046]
- HOLLIGER et al.Proc. Natl. Acad. Sci. USA, 1993, vol. 90, 6444-6448 [0048]
- POLLAK et al.Structure, 1994, vol. 2, 1121-1123 [0048]
- KRIEG et al.J. Immunother., 2004, vol. 27, 460-471 [0061]
- TUMEHNature, 2014, vol. 515, 568-571 [0062]
- HERBST et al.Nature, 2014, vol. 515, 563-567 [0062]
- TAUBE et al.Clin Cancer Res., 2014, [0062]
- SIEGAL et al.Science, 1999, [0063]
- MANGSBO et al.J. Immunother, 2010, vol. 33, 225- [0066]
- HARTMANN et al.Eur. J. Immunol., 2003, vol. 33, 1633-1641 [0085]
- MARSHALL et al.J. Leukocyte Biol., 2003, vol. 73, 781-792 [0085]
- VOLLMER et al.Eur. J. Immunol., 2004, vol. 34, 251-262 [0085]
- MOSEMAN et al.J. Immunol., 2004, vol. 173, 74433-4442 [0085]
- CHEN et al.J. Immunol., 2008, vol. 181, 85396-5404 [0085]
- MOLENKAMP BG et al.Clin Cancer Res., 2007, vol. 13, 102961-2969 [0088]
- MUMMOFTBioessays, 2013, vol. 35, 7623-631 [0090]
- SATO et al.Immunol Res., 2011, vol. 51, 2-3170-182 [0090]
- UHLMANN E et al.Chem Rev, 1990, vol. 90, 544- [0101]
- GOODCHILD JBioconjugate Chem, 1990, vol. 1, 165- [0101]
- F. E. ECKSTEINOligonucleotides and Analogues--A Practical ApproachIRL Press19910000 [0102]
- M. D. MATTEUCCIM. H. CARUTHERSTetrahedron Lett., 1980, vol. 21, 719- [0102]
- R. P. IYERW. EGANJ. B. REGANS. L. BEAUCAGEJ. Am. Chem. Soc., 1990, vol. 112, 1253- [0102]
- SERGUEEVJAm Chem Soc, 1998, vol. 120, 9417-27 [0112]

- **KRIEG et al.**Oligonucleotides, 2003, vol. 13, 6491-499 [\[0113\]](#)
- **UHLMANN E et al.**Chem Rev, 1990, vol. 90, 543- [\[0116\]](#)
- **Protocols for Oligonucleotides and Analogs**Synthesis and Properties & Synthesis and Analytical TechniquesHumana Press19930000 [\[0116\]](#)
- **CROOKE S T et al.**Annu Rev Pharmacol Toxicol, 1996, vol. 36, 107-129 [\[0116\]](#)
- **HUNZIKER J et al.**Mod Synth Methods, 1995, vol. 7, 331-417 [\[0116\]](#)
- **Protocols for Oligonucleotides and Analogs**UHLMANN EPEYMAN AMethods in Molecular BiologyHumana Press19930000vol. 20, 355- [\[0119\]](#)
- **STIRCHAK E P et al.**Oligonucleotides Res, 1989, vol. 17, 6129-41 [\[0120\]](#)
- **NIELSEN P E et al.**Bioconj Chem, 1994, vol. 5, 3-7 [\[0120\]](#)
- **FROEHLER JJ** Am Chem Soc, 1992, vol. 114, 8320- [\[0121\]](#)
- **VANDENDRIESSCHE et al.**Tetrahedron, 1993, vol. 49, 7223- [\[0121\]](#)
- **TARKOV M et al.**Helv Chim Acta, 1993, vol. 76, 481- [\[0121\]](#)
- **SELIGER, H. et al.**Oligonucleotide analogs with terminal 3'-3'- and 5'-5'-internucleotidic linkages as antisense inhibitors of viral gene expressionNucleosides & Nucleotides, 1991, vol. 10, 1-3469-77 [\[0125\]](#)
- **JIANG et al.**Pseudo-cyclic oligonucleotides: in vitro and in vivo propertiesBioorganic & Medicinal Chemistry, 1999, vol. 7, 122727-2735 [\[0125\]](#)
- **DURAND, M. et al.**Triple-helix formation by an oligonucleotide containing one (dA)₁₂ and two (dT)₁₂ sequences bridged by two hexaethylene glycol chainsBiochemistry, 1992, vol. 31, 389197-204 [\[0126\]](#)
- **FONTANELMARIE LAURENCE et al.**Sterical recognition by T4 polynucleotide kinase of non-nucleosidic moieties 5'-attached to oligonucleotidesOligonucleotides Research, 1994, vol. 22, 112022-7 [\[0126\]](#)
- **SCHMIDT M et al.**Allergy, 2006, vol. 61, 56-63 [\[0127\]](#)
- **KAPP, K et al.**Mol Ther Nucleic Acids, 2014, vol. 3, e170- [\[0127\]](#)
- **RAMALHO ORTIGAO et al.**Antisense Research and Development, 1992, vol. 2, 129-46 [\[0129\]](#)
- **LONBERG et al.**Nature, 1994, vol. 368, 856-859 [\[0139\]](#)
- **TOMIZUKA et al.**Proc Natl Acad Sci USA, 2000, vol. 97, 722- [\[0140\]](#)
- **KUROIWA et al.**Nature Biotechnol, 2000, vol. 18, 1086- [\[0140\]](#)
- **WINTERHARRIS**Immunol. Today, 1993, vol. 14, 43-46 [\[0143\]](#)
- **WRIGHT et al.**Crit. Reviews in Immunol., 1992, vol. 12, 125-168 [\[0143\]](#)
- **MAGELAMOYI**Monoclonal Antibody Production Techniques and ApplicationsMarcel Dekker, Inc.1987000079-97 [\[0143\]](#)
- **BARNES et al.**Biotech & Bioengineering, 2001, vol. 73, 261-270 [\[0146\]](#)
- **THOTAKURA et al.**Meth. Enzymol., 1987, vol. 138, 350- [\[0147\]](#)
- **HAKIMUDDIN et al.**Arch. Biochem. Biophys., 1987, vol. 259, 52- [\[0147\]](#)
- **MILLWARD M et al.**Br. J. Cancer, 2013, vol. 108, 101998-2004 [\[0154\]](#)
- **ZITVOGEL L. et al.**Immunity, 2013, vol. 39, 174-88 [\[0225\]](#)
- **BRODY et al.**J. Clin. Oncol., 2010, vol. 28, 284324-4332 [\[0230\]](#)
- **KIM et al.**Blood, 2012, vol. 119, 2355-363 [\[0230\]](#)
- **BURNETTE et al.**Cancer Res., 2011, vol. 71, 2488-2496 [\[0231\]](#)
- **GOMES DOS SANTOS AL et al.**Curr Pharm Biotechnol., 2005, vol. 6, 17-15 [\[0240\]](#)

- **JOSHI VB et al.**AAPS J., 2013, vol. 15, 185-94 [\[0240\]](#)
- **ARIMA H et al.**Curr Top Med Chem., 2014, vol. 14, 4465-77 [\[0240\]](#)
- **MILLWARD M. et al.**Br. J. Cancer, 2013, vol. 108, 101998-2004 [\[0257\]](#)
- Sustained and Controlled Release Drug Delivery Systems Marcel Dekker, Inc. 19780000 [\[0278\]](#)
- **KRIEG AM et al.**Oligonucleotide, 2003, vol. 13, 6491-9 [\[0286\]](#)
- **BEEH et al.**J Allergy Clin Immunol, 2013, vol. 131, 866-74 [\[0304\]](#)

Patentkrav

1. TLR9-agonist, der er et A-klasse CpG DNA og et antistof eller antigenbindingsfragment deraf, der specifikt binder til PD-1, til anvendelse i en fremgangsmåde til behandling af en
5 cancertumor hos et individ,
 hvor A-klasse CpG DNA'et indgives i eller stødende op til tumoren; og
 antistoffet eller antigenbindingsfragmentet deraf, der specifikt binder til PD-1, indgives systemisk.
- 10 2. A-klasse CpG DNA og et antistof eller antigenbindingsfragment deraf, der specifikt binder til PD-1, til anvendelse ifølge krav 1, hvor fremgangsmåden endvidere omfatter anvendelse af radioterapi til individet, hvilken radioterapi initieres før indgivelse af A-klasse CpG DNA'et.
- 15 3. A-klasse CpG DNA og et antistof eller antigenbindingsfragment deraf, der specifikt binder til PD-1, til anvendelse ifølge krav 1 eller krav 2, hvor der endvidere anvendes et andet antistof eller antigenbindingsfragment deraf, der specifikt binder til PD-L1.
- 20 4. A-klasse CpG DNA og et antistof eller antigenbindingsfragment deraf, der specifikt binder til PD-1, til anvendelse ifølge et hvilket som helst af de foregående krav, hvor A-klasse CpG DNA'et indgives før indgivelse af antistoffet eller antigenbindingsfragmentet deraf, der specifikt binder til PD-1, eller i alt væsentligt samtidigt.
- 25 5. A-klasse CpG DNA og et antistof eller antigenbindingsfragment deraf, der specifikt binder til PD-1, til anvendelse ifølge et hvilket som helst af de foregående krav,, hvor A-klasse CpG DNA'et og en yderligere tjekpunkthæmmer (CPI) indgives i eller stødende op til tumoren.
- 30 6. A-klasse CpG DNA og et antistof eller antigenbindingsfragment deraf, der specifikt binder til PD-1, til anvendelse ifølge krav 5, hvor den yderligere CPI omfatter et andet antistof eller antigenbindingsfragment deraf, der specifikt binder til PD-L1.
7. A-klasse CpG DNA og et antistof eller antigenbindingsfragment deraf, der specifikt binder til PD-1, til anvendelse ifølge krav 5 eller krav 6, hvor A-klasse CpG DNA'et indgives før indgivelse af antistoffet eller antigenbindingsfragmentet deraf, der specifikt binder til PD-1,

A-klasse CpG DNA'et og antistoffet eller antigenbindingsfragmentet deraf, der specifikt binder til PD-1, indgives i alt væsentligt samtidigt, eller A-klasse CpG DNA'et indgives efter indgivelse af antistoffet eller antigenbindingsfragmentet deraf, der specifikt binder til PD-1.

5 **8.** A-klasse CpG DNA og et antistof eller antigenbindingsfragment deraf, der specifikt binder til PD-1, til anvendelse ifølge et hvilket som helst af kravene 1 til 7, hvor A-klasse CpG DNA'et har sekvensen GGGGGGGGGGGGACGATCGTCGGGGGGGGGGG (SEQ ID NO:82).

10 **9.** A-klasse CpG DNA og et antistof eller antigenbindingsfragment deraf, der specifikt binder til PD-1, til anvendelse, ifølge krav 8, hvor A-klasse CpG DNA'et er formuleret som en viruslignende partikel.

15 **10.** A-klasse CpG DNA og et antistof eller antigenbindingsfragment deraf, der specifikt binder til PD-1, til anvendelse ifølge et hvilket som helst af kravene 1 til 9, hvor cancertumoren er et lymfom eller en cancertumor i et væv eller organ udvalgt fra gruppen bestående af hud, hoved og hals, øsophagus, mave, lever, colon, rectum, pancreas, lunge, bryst, cervix, ovarie, nyre, blære, prostata, skjoldbruskkirtel, hjerne, muskel og knogle, fortrinsvis et melanom eller et lymfom.

20 **11.** A-klasse CpG DNA og et antistof eller antigenbindingsfragment deraf, der specifikt binder til PD-1, til anvendelse ifølge et hvilket som helst af kravene 1 til 10, hvor cancertumoren er resistent over for en behandlingsplan, der omfatter indgivelse af antistoffet eller antigenbindingsfragmentet deraf, der specifikt binder til PD-1, uden indgivelse af A-klasse CpG DNA'et.

25 **12.** A-klasse CpG DNA og et antistof eller antigenbindingsfragment deraf, der specifikt binder til PD-1, til anvendelse ifølge et hvilket som helst af kravene 1 til 11, hvor individet er et menneske.

DRAWINGS

Figure 1

(PRIOR ART)

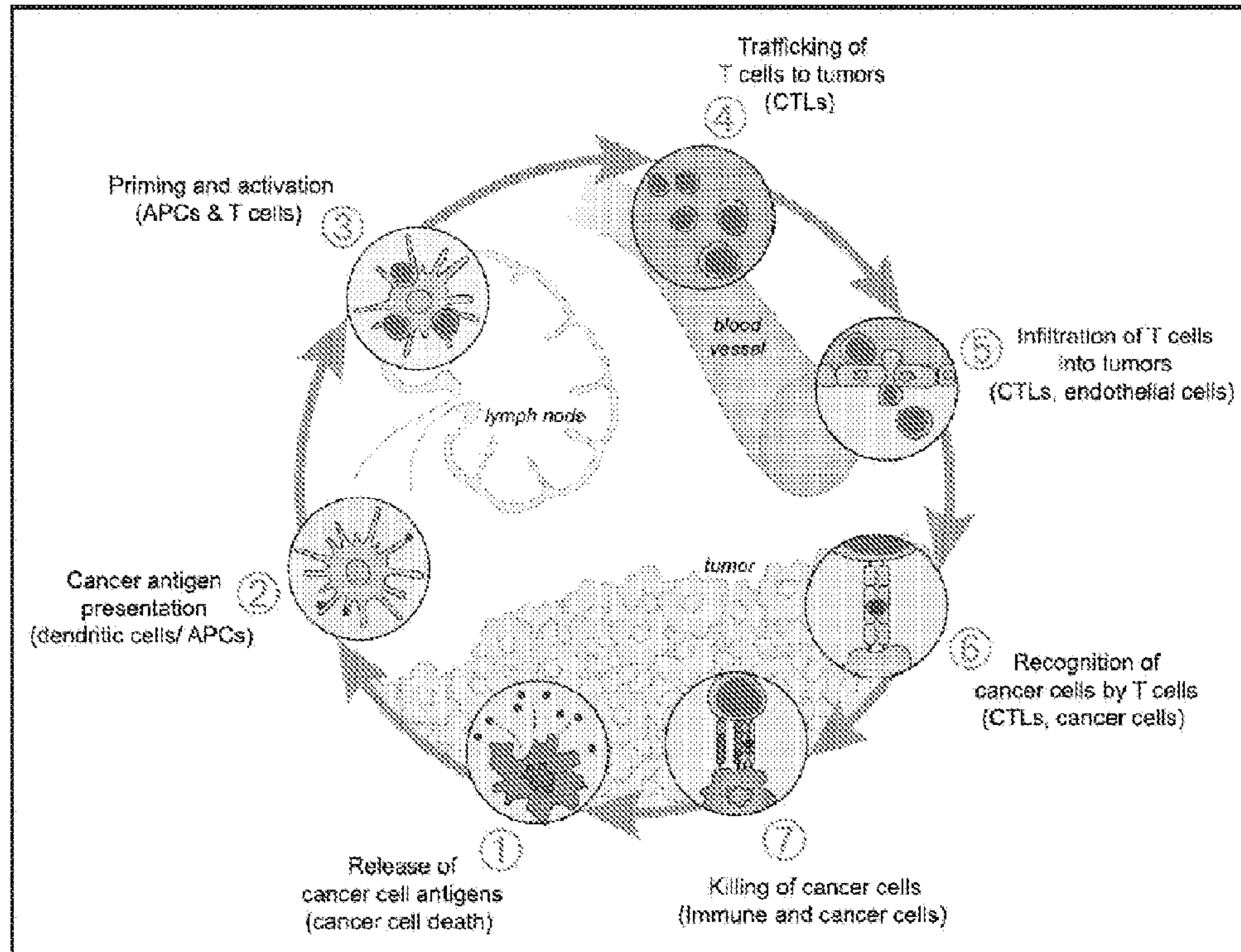


Figure 2

(PRIOR ART)

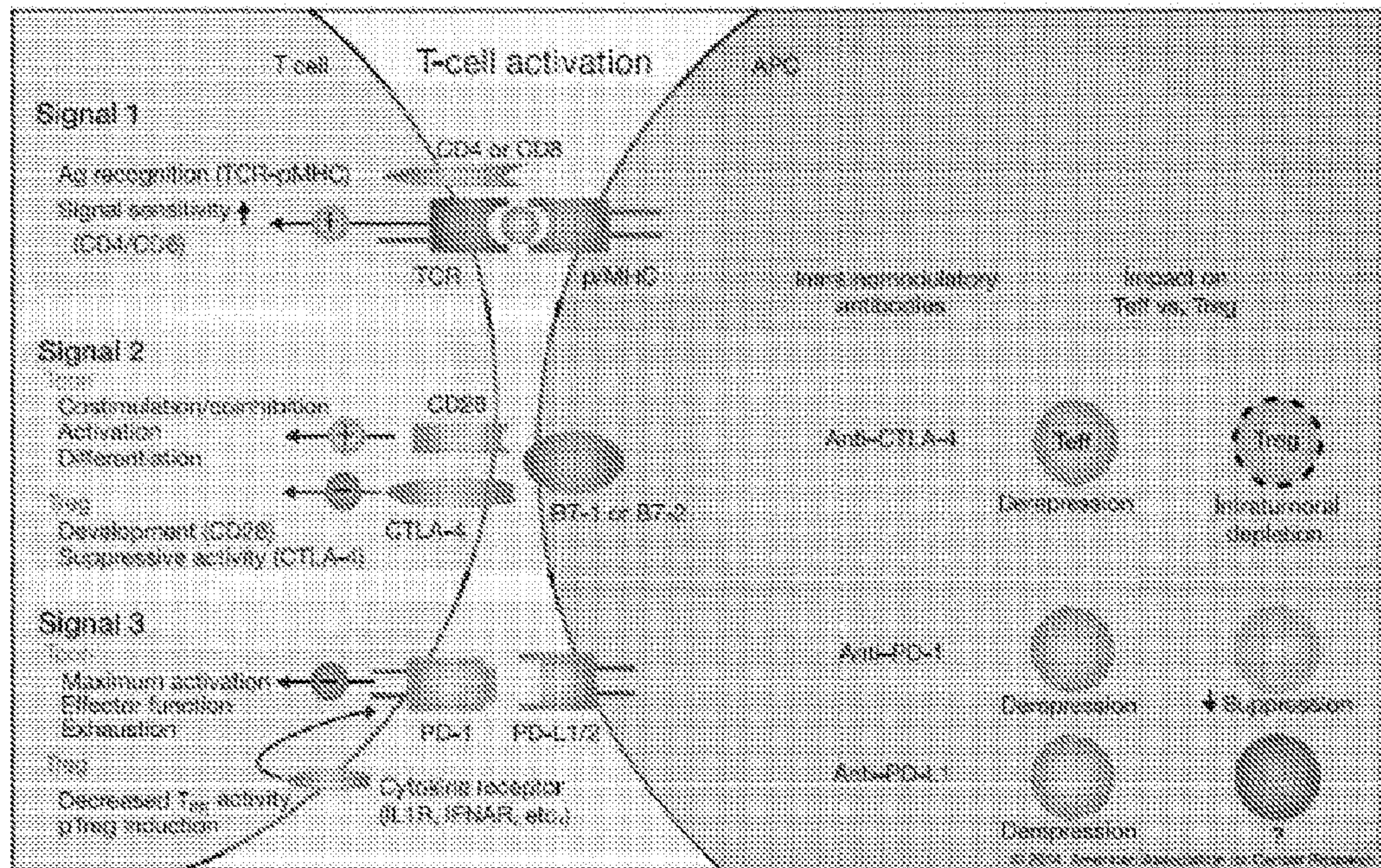


Figure 3

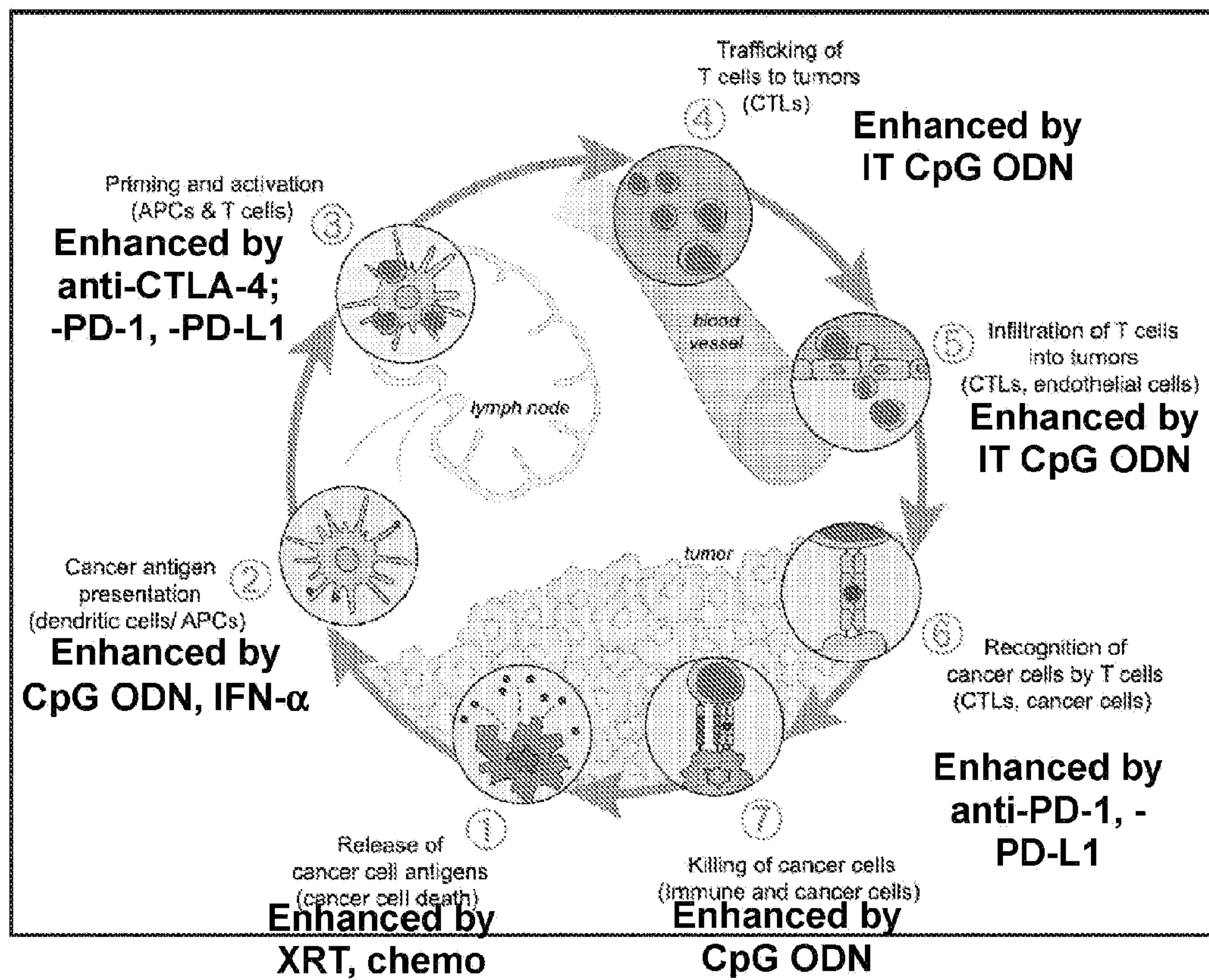


Figure 4

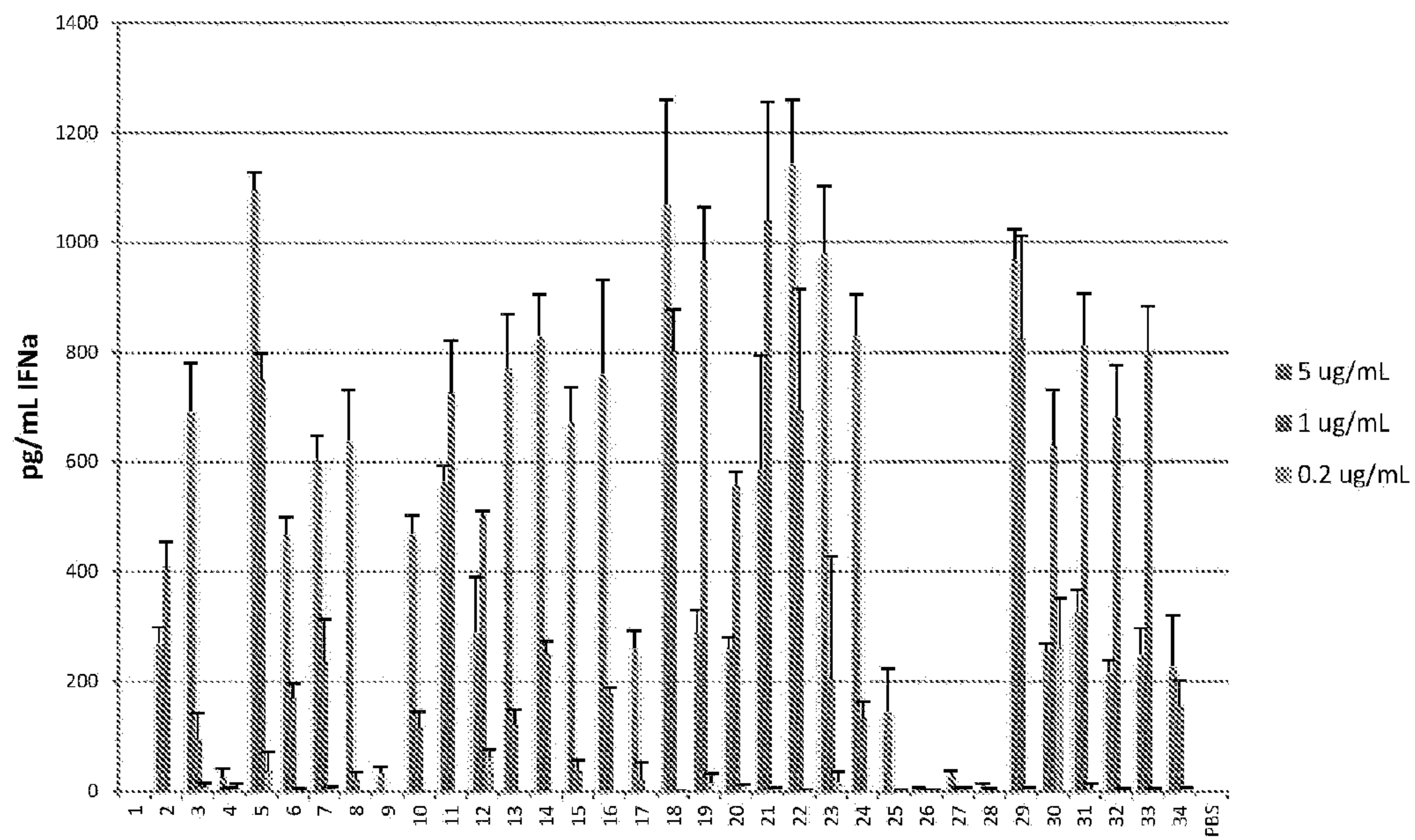


Figure 5

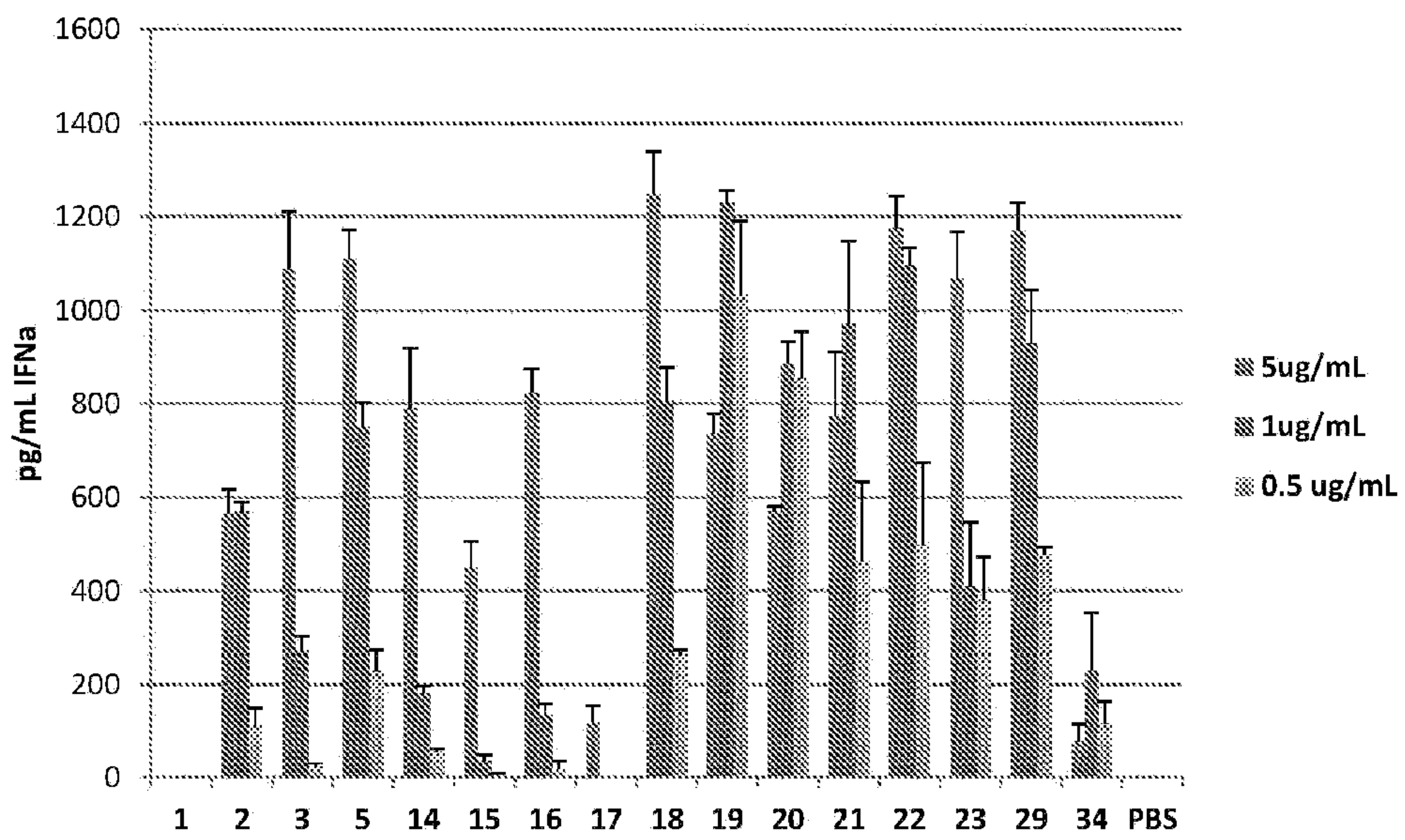


Figure 6

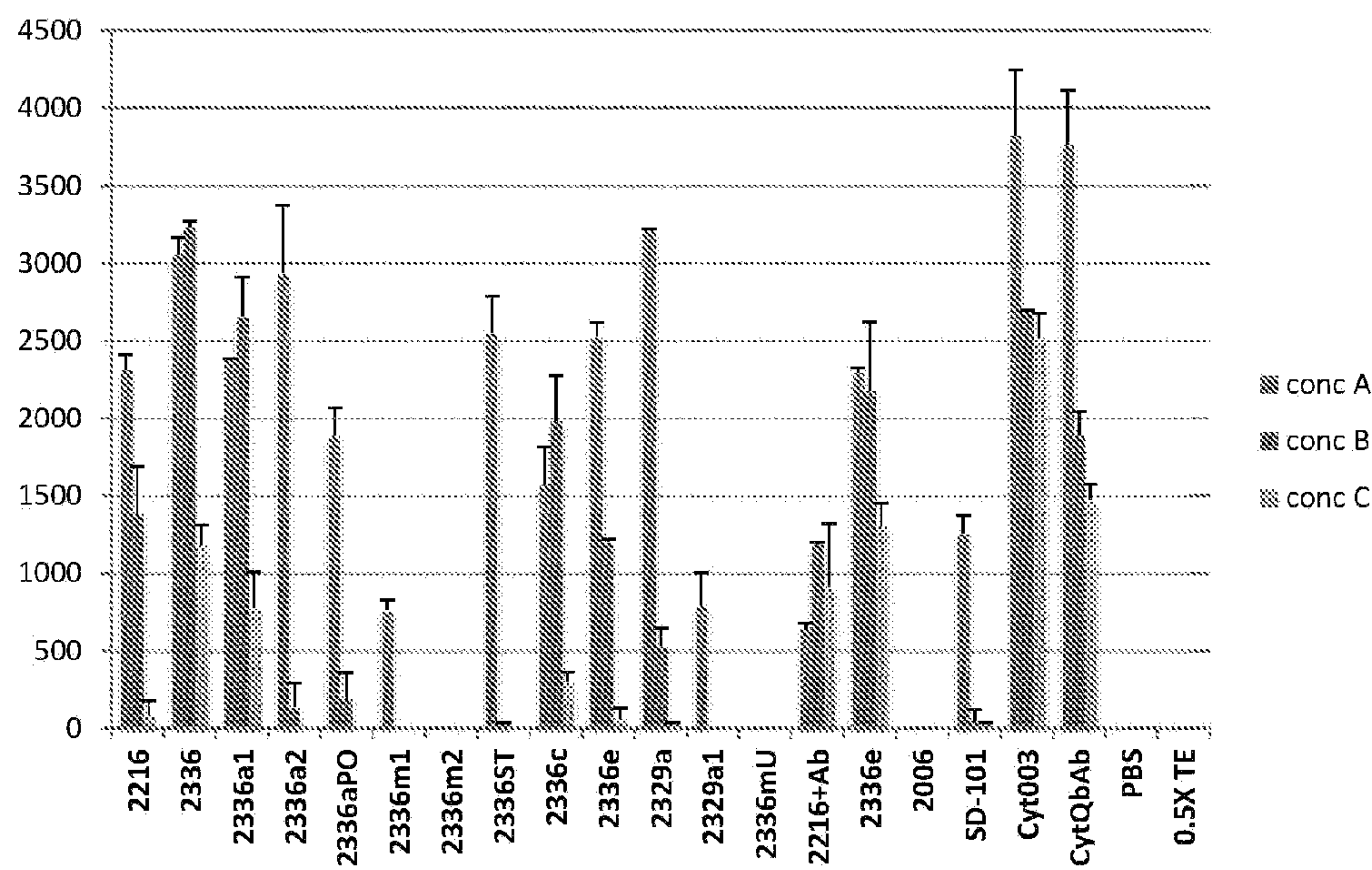


Figure 7

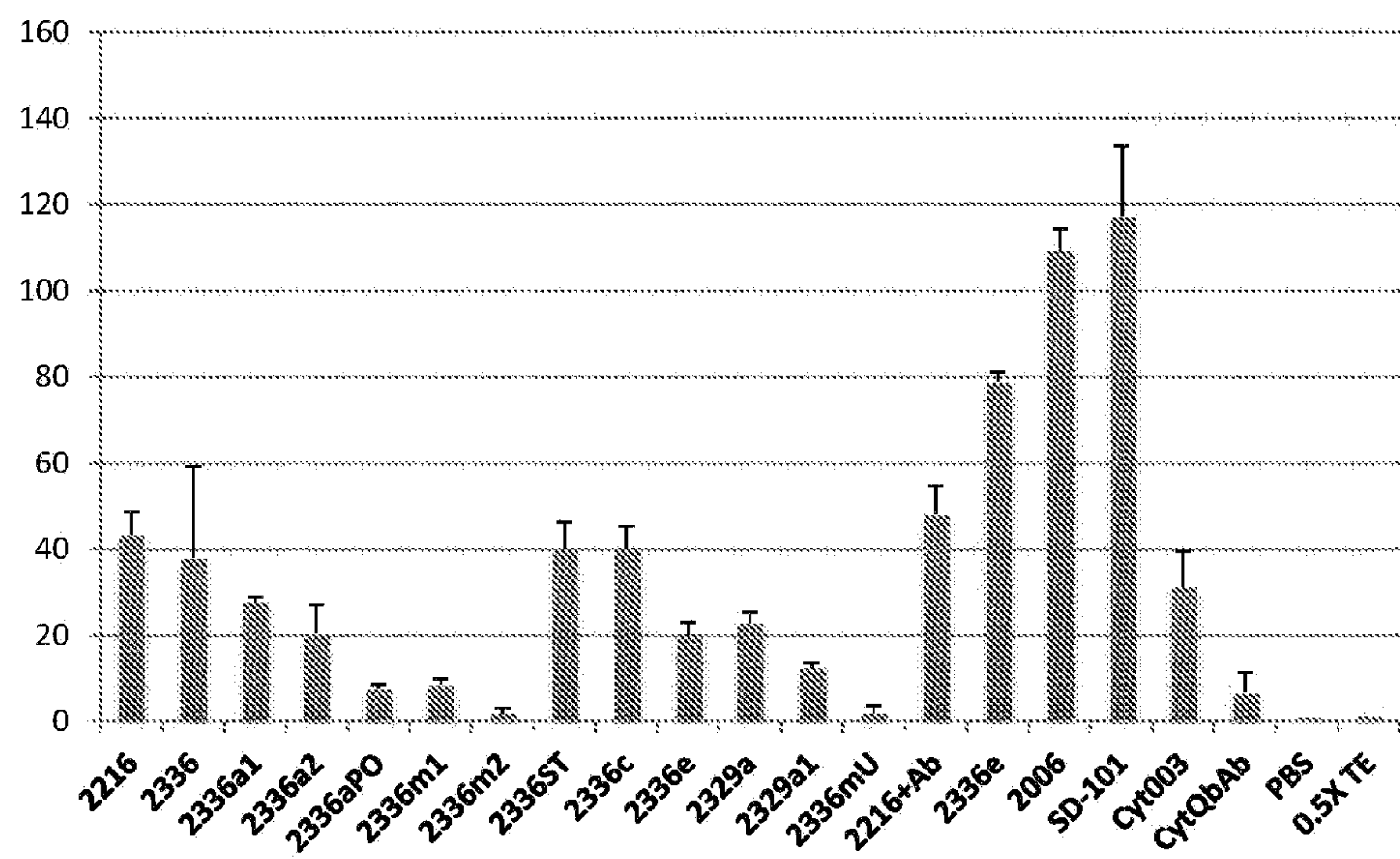


Figure 8

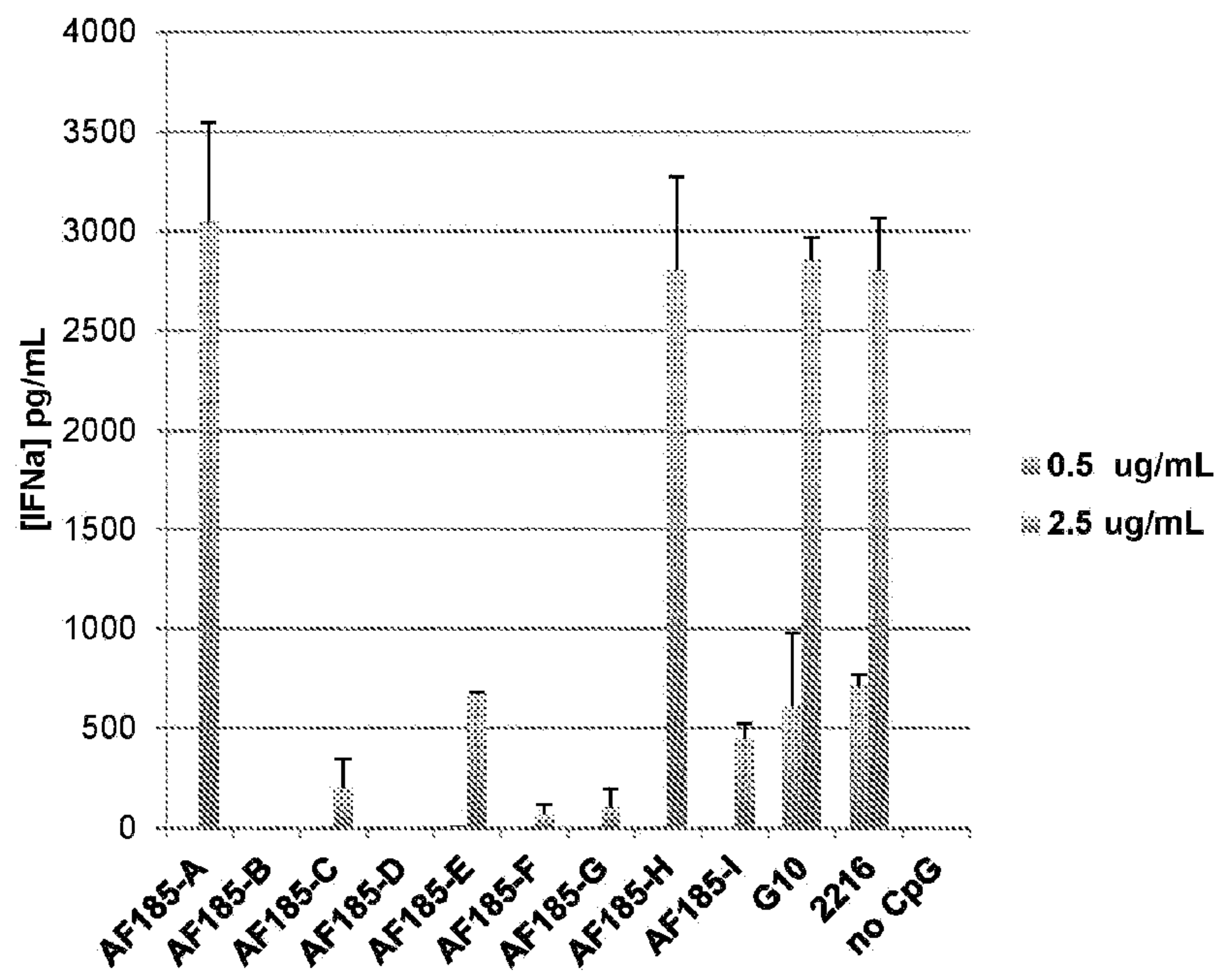


Figure 9

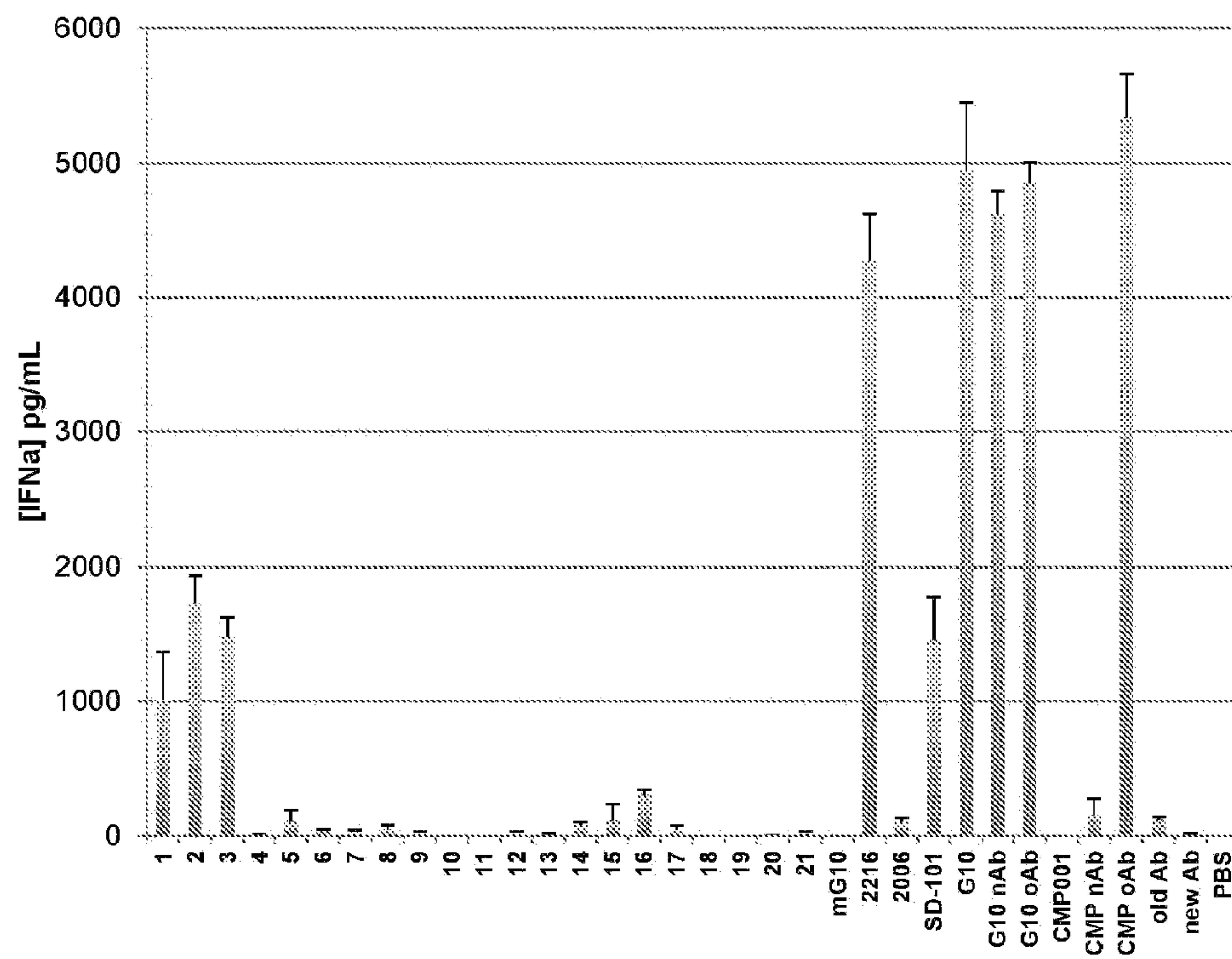


Figure 10

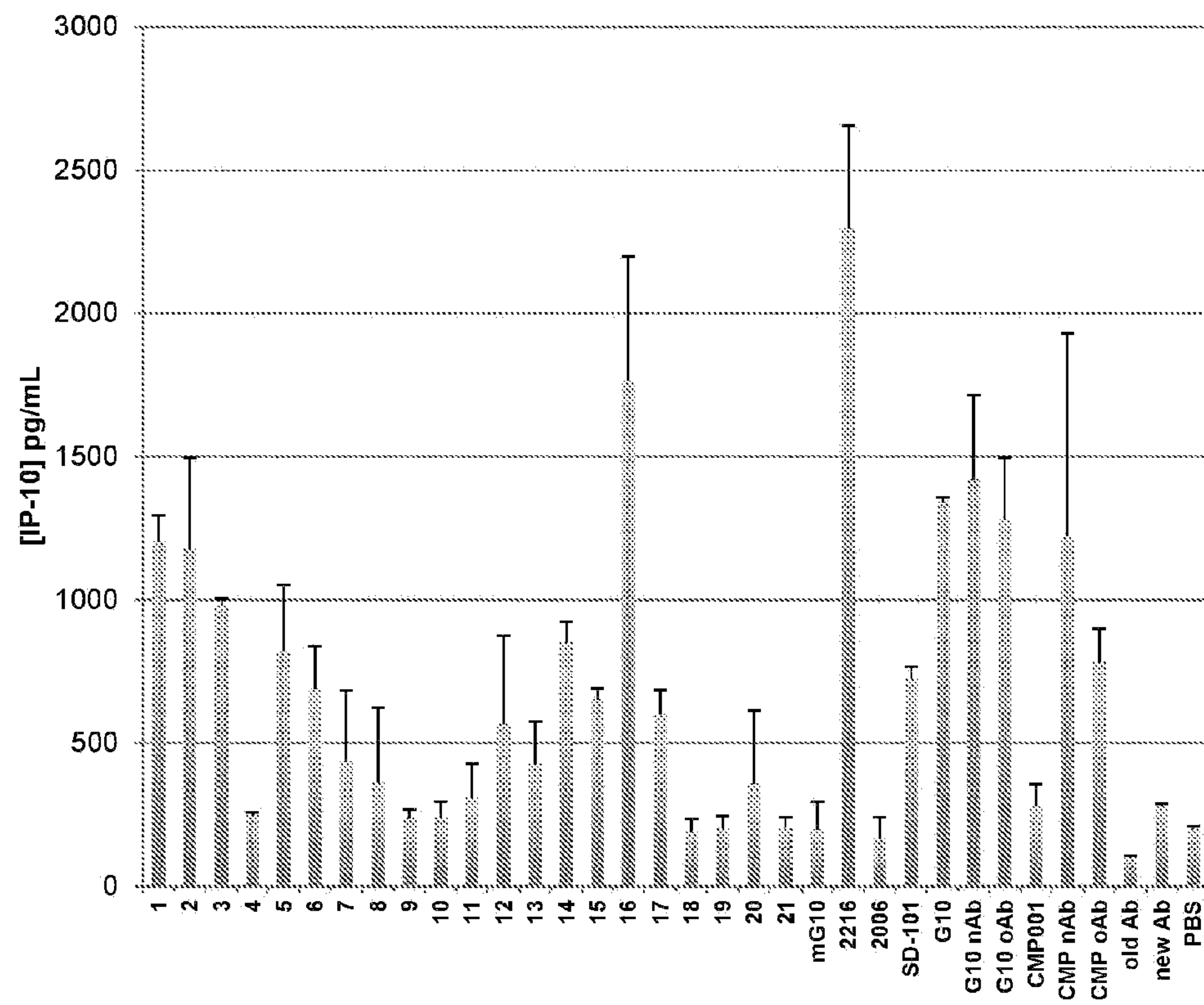


Figure 11

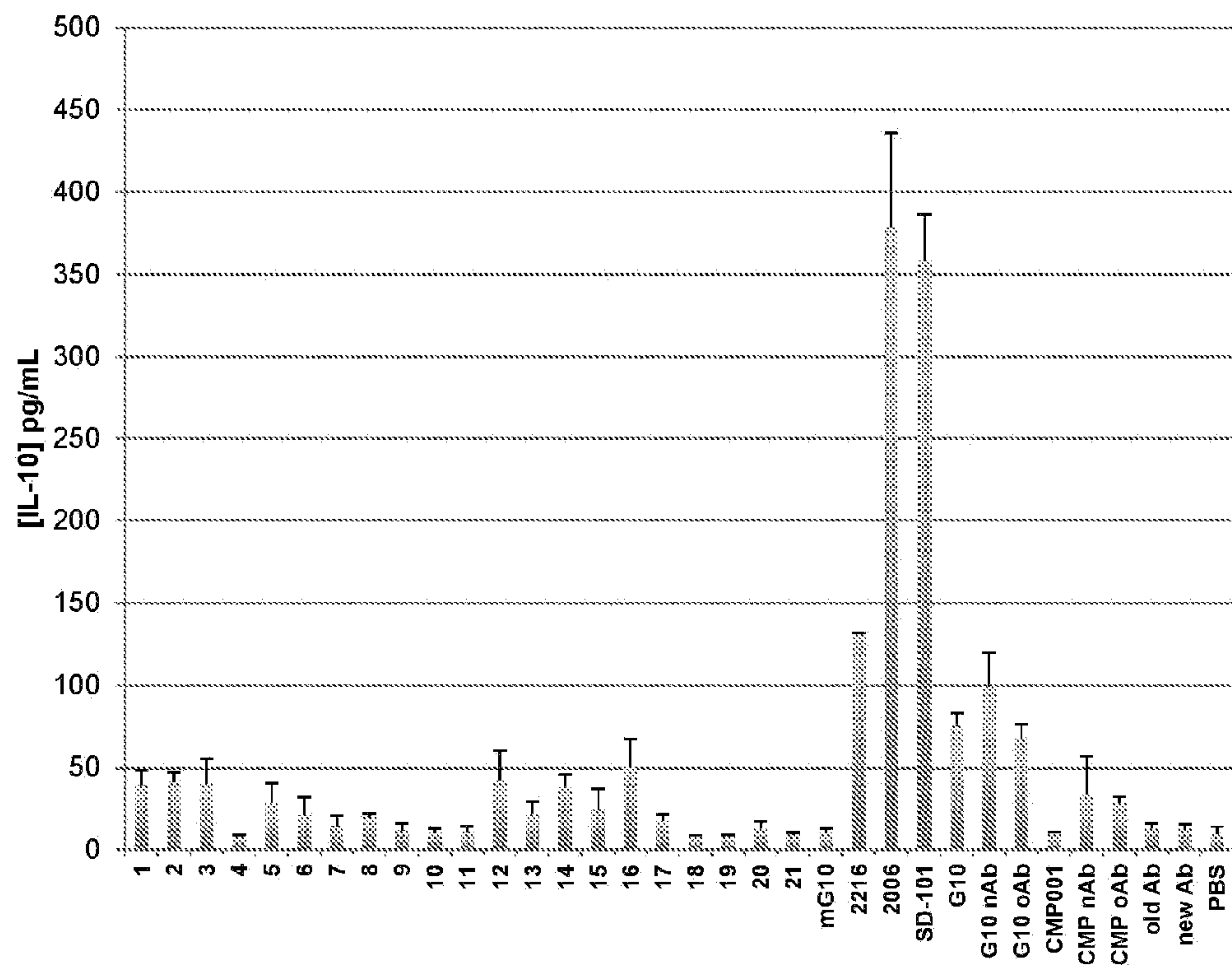


Figure 12

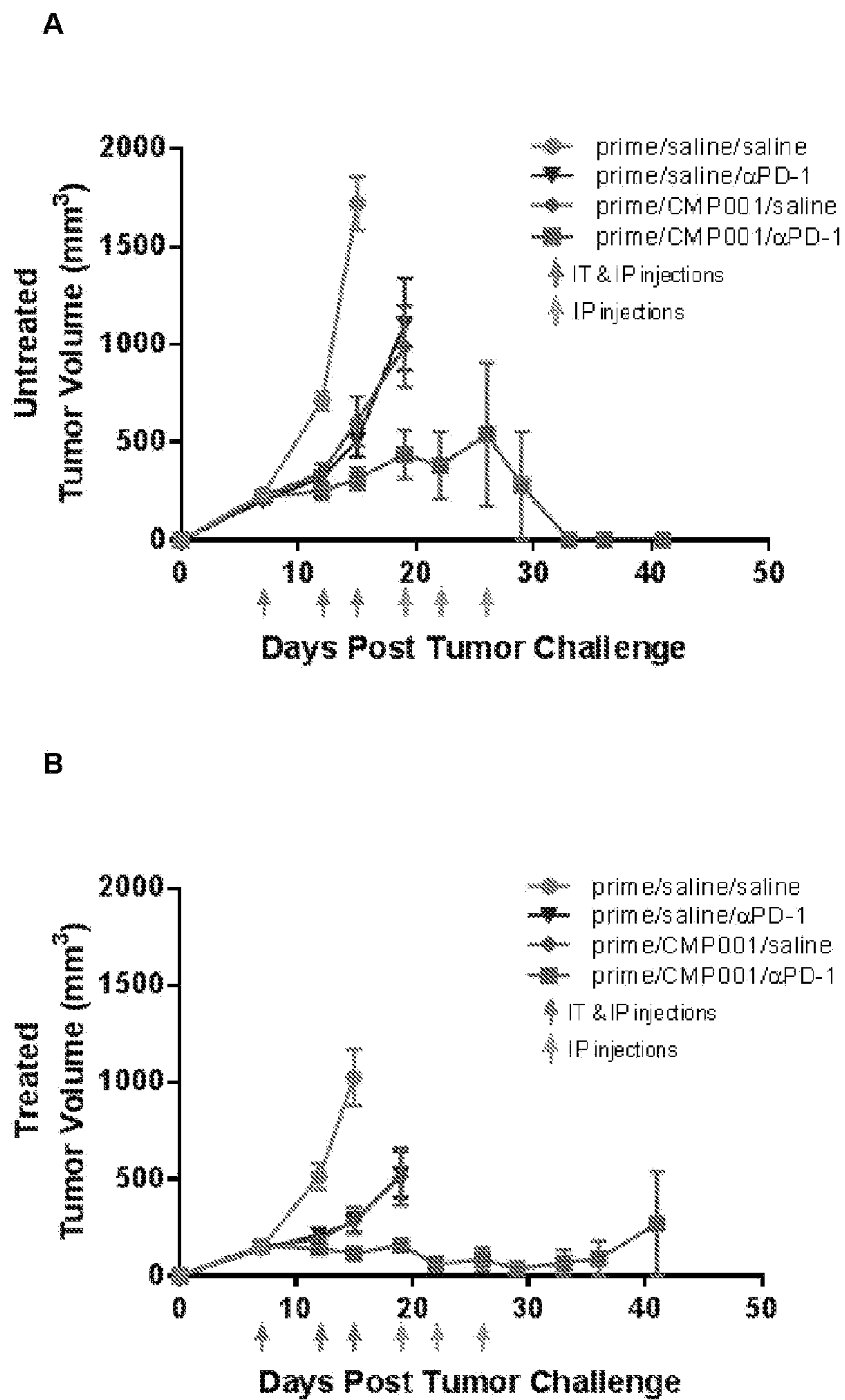


Figure 13

