COMBINATION THERAPY COMPOSITIONS AND METHODS FOR TREATING CANCERS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of, and priority to, Chinese Patent Application Serial No. 201410325480.9, filed July 9, 2014, the entire disclosure of which is hereby incorporated by reference in their entireties.

FIELD OF THE INVENTION

[0002] The present invention relates to therapeutic combinations and methods for treating cancers using combination therapy.

BACKGROUND OF THE INVENTION

[0003] Therapeutic antibodies have been used in clinical applications for over twenty years. Currently, there are fifteen anti-tumor antibody drugs in clinic, including Rituxan (1997), Herceptin (1998), Mylotarg (2000), Campath (2001), Zevalin (2002), Bexxer (2003), Avastin (2004), Erbitux (2004), Vectibix (2006), Arzerra (2009); Benlysta (2011); Yervoy (2011), Adcetris (2011), Perjeta (2012), and Kadcyla (2013). These antibodies target mainly four molecules: EGFR, Her2, CD20 and VEGF.

[0004] In general, therapeutic antibodies kill tumor cells via three mechanisms (Scott AM, Wolchok JD, Old LJ. Antibody therapy of cancer. Nat Rev Cancer. (2012), 12:278-87): (1) Direct antibody action, that is, blockade or agonist activity of ligand / receptor signaling, induction of apoptosis, and delivery of drugs or cytotoxic agents. Antibody receptor activation activity can produce direct tumor cell killing effect. For example, some antibodies can bind to receptors on the surface of tumor cells, activate the receptor, leading to apoptosis (e.g., in mitochondria). Antibodies can also mediate tumor cell killing by receptor-antagonistic activity. For example, certain antibodies can bind to cell surface receptors and block dimerization, kinase activation and downstream signaling, thereby inhibiting proliferation and promote apoptosis. Binding of antibodies to an enzyme can lead to neutralization, signal abrogation, and cell death. (2) Through immune-mediated cell killing mechanisms include complement-dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC), T cell function regulation, etc. Immune-mediated killing of tumor cells can be accomplished through the following ways: induction of phagocytosis, complement activation, antibody-dependent cell-mediated cytotoxicity, genetically modified T cells being targeted to the tumor by single-chain variable fragment (scFv), through antibody-

mediated antigenic cross presentation to dendritic cell to activate T cells, inhibition of T cell inhibitory receptors, such as cytotoxic T lymphocyte-associated antigen 4 (CTLA4). Of them, the Fc portion of the antibody feature is especially important for CDC and ADCC-mediated tumor cell killing effect. (3) Specific effect of antibody on tumor vasculature and matrix, through trapping of vascular receptor antagonist or ligand to induce vascular and stromal cells ablation, including: stromal cell inhibition, delivery of toxins to stromal cells, and delivery of toxins to the vasculature. (Scott AM, Wolchok JD, Old LJ. Antibody therapy of cancer. Nat Rev Cancer. 2012, 12 (4):278-87).

[0005] Therapeutic monoclonal antibody drugs have advanced anti-cancer drug research and development. However, some issues still need further study to be solved, such as antibody immunogenicity, tolerance of long-term use of tumor target, and long-term effects of simple single blockade of signal transduction pathway. In short, a simple majority of antibodies are difficult to achieve long-term efficient inhibition and killing of tumor cells.

[0006] Antibody - drug conjugates combines targeting function and small molecule drug with particular pharmacokinetics. The structure of antibody-drug conjugates is the attachment of a monoclonal antibody with targeting function to a compound with specific pharmacological properties. This technique requires the therapeutic antibody have binding specificity to a target, to be coupled to a molecule with therapeutic effect or other functions such as cyto-toxins. Many factors affect the effect of this type of antibodies, such as endocytosis of the coupled antibody, stability of the coupling, and release and killing activity of the toxins.

[0007] Antibodies - drug conjugates have direct and indirect anti-cancer effect. The antibody blocks or activates ligand / receptor signaling, induces apoptosis, and at the same time can present or deliver payload drug directly or indirectly (such as a drug, toxin, small interfering RNA or radioisotope) to the tumor cells. Therapeutic antibody drug conjugate utilizes dual characteristics of the antibody and the coupled drug, first is the binding function that it specifically binds to the target molecule, second is the tumor cell killing function of the antibody itself, and the third is the particular effect of the conjugated drug. Current antibody - drug conjugates drugs are limited in how to kill tumor cells directly. However, because of the tough requirement of technologies in antibody, linker molecule, toxin molecules, and conjugation, as well as the limitation of bringing toxins within the tumor microenvironment molecules, there are still some difficulties in actual clinical studies.

SUMMARY OF THE INVENTION

[0008] In general, the present invention directs to therapeutic combinations and methods for treatment of cancers.

[0009] In one aspect, the present invention provides a pharmaceutical composition, comprising: (i) a therapeutic effective amount of a targeted therapeutic against a cancer; (ii) a therapeutic effective amount of an immunotherapeutic that is capable of activating a human plasmacytoid dendritic cell, myeloid dendritic cell, NK cell, or tumor cell, or a combination thereof; and optionally (iii) one or more pharmaceutically acceptable carriers.

[0010] In some embodiments, the targeted therapeutic is capable of binding to a tumor cell specifically or preferably in comparison to a non-tumor cell. In some embodiments, the tumor cell is of a carcinoma, a sarcoma, a lymphoma, a myeloma, or a central nervous system cancer.

[0011] In some embodiments, the targeted therapeutic is capable of binding to a tumor antigen specifically or preferably in comparison to a non-tumor antigen.

[0012] In some embodiments, the tumor antigen is selected from the group consisting of: CD2, CD19, CD20, CD22, CD27, CD33, CD37, CD38, CD40, CD44, CD47, CD52, CD56, CD70, CD79, and CD137.

[0013] In some embodiments, the tumor antigen is selected from the group consisting of: 4-1BB, 5T4, AGS-5, AGS-16, Angiopoietin 2, B7.1, B7.2, B7DC, B7H1, B7H2, B7H3, BT-062, BTLA, CAIX, Carcinoembryonic antigen, CTLA4, Cripto, ED-B, ErbB1, ErbB2, ErbB3, ErbB4, EGFL7, EpCAM, EphA2, EphA3, EphB2, FAP, Fibronectin, Folate Receptor, Ganglioside GM3, GD2, glucocorticoid-induced tumor necrosis factor receptor (GITR), gp100, gpA33, GPNMB, ICOS, IGF1R, Integrin αv, Integrin $\alpha v\beta$, KIR, LAG-3, Lewis Y, Mesothelin, c-MET, MN Carbonic anhydrase IX, MUC1, MUC16, Nectin-4, NKGD2, NOTCH, OX40, OX40L, PD-1, PDL1, PSCA, PSMA, RANKL, ROR1, ROR2, SLC44A4, Syndecan-1, TACI, TAG-72, Tenascin, TIM3, TRAILR1, TRAILR2,VEGFR-1, VEGFR-2, VEGFR-3, and variants thereof.

[0014] In some embodiments, the targeted therapeutic comprises an immunoglobulin, a protein, a peptide, a small molecule, a nanoparticle, or a nucleic acid.

[0015] In some embodiments, the targeted therapeutic comprises an antibody, or a functional fragment thereof.

[0016] In some embodiments, the antibody is selected from the group consisting of: Rituxan (rituximab), Herceptin (trastuzumab), Erbitux (cetuximab), Vectibix (Panitumumab), Arzerra (Ofatumumab), Benlysta (belimumab), Yervoy (ipilimumab), Perjeta (Pertuzumab), Tremelimumab, Opdivo (Nivolumab), Dacetuzumab, Urelumab, MPDL3280A, Lambrolizumab, Blinatumomab, CT-011, MK-3475, BMS-936559, MED14736, MSB0010718C, and margetuximab (MGAH22).

[0017] In some embodiments, the targeted therapeutic comprises a Fab, Fab', F(ab')2, single domain antibody, T and Abs dimer, Fv, scFv, dsFv, ds-scFv, Fd, linear antibody, minibody, diabody, bispecific antibody fragment, bibody, tribody, sc-diabody, kappa (lamda) body, BiTE, DVD-Ig, SIP, SMIP, DART, or an antibody analogue comprising one or more CDRs.

[0018] In some embodiments, the targeted therapeutic comprises a ATWLPPR polypeptide of VEGFR, Thrombospondin-1 mimetics, CDCRGDCFCG (cyclic) polypeptide, SCH 221153 fragment, NCNGRC (cyclic) polypeptide, CTTHWGFTLC polypeptide, CGNKRTRGC polypeptide (LyP-1), Octreotide, Vapreotide, Lanreotide, C-3940 polypeptide, Decapeptyl, Lupron, Zoladex, or Cetrorelix.

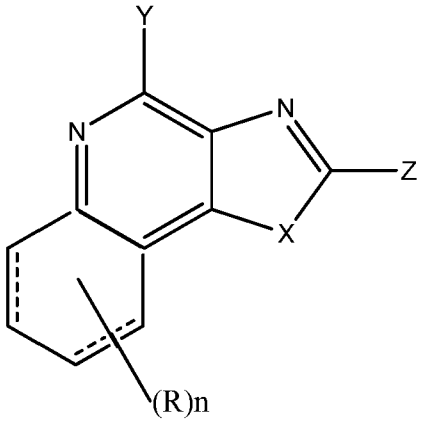
[0019] In some embodiments, the targeted therapeutic comprises extracellular domains (ECD) or soluble form of PD-1, PDL-1, CTLA4, BTLA, KIR, TIM3, 4-1BB, LAG3, full length of partial of a surface ligand amphiregulin, betacellulin, EGF, ephrin, epigen, epiregulin, IGF, neuregulin, TGF, TRAIL, or VEGF.

[0020] In some embodiments, the targeted therapeutic comprises a nanoparticle or an aptamer.

[0021] In some embodiments, the immunotherapeutic is capable of binding specifically to human TLR7 and/or TLR8.

[0022] In some embodiments, the immunotherapeutic comprises: (a) single-stranded RNA (ssRNA), preferably ORN02, ORN06, ssPoly(U), ssRNA40, ssRNA41, ssRNA-DR, or Poly(dT); or (b) a receptor ligand analogs, preferably CL075, CL097, CL264, CL307, Gardiquimod, Loxoribine, Imiquimod, or Resiquimod.

[0023] In some embodiments, the immunotherapeutic has a structure of Formula (I):



(I)

wherein dashed line represents bond or absence of bond;

X is S or $-NR_1$, R_1 is $-W_0—W_1—W_2—W_3—W_4$,

W_0 is a bond, alkyl, alkenyl, alkynyl, alkoxy, or –alkyl-S-alkyl--,

W_1 is a bond, --O--, or $–NR_2$--, wherein R_2 is hydrogen, alkyl or alkenyl,

W_2 is a bond, --O--, --C(O)--, --C(S)--, or $–S(O)_2$—,

W_3 is a bond, $--NR_3$--, wherein R_3 is hydrogen, alkyl or alkenyl,

W_4 is hydrogen, alkyl, alkenyl, alkynyl, alkoxy, cycloalkyl, aryl, aryloxy, heteroaryl, or heterocyclyl, each of which is optionally substituted by one or more substituents selected from the group consisting of hydroxyl, alkoxy, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, heterocyclyl, $--NH_2$, nitro, --alkyl-hydroxyl, --alkyl-aryl, --alkyl-heteroaryl, --alkyl-heterocyclyl, $--O-R_4$, $--O-alkyl-R_4$, $--alkyl-O-R_4$, $--C(O)-R_4$, $--alkyl-C(O)-R_4$, $--alkyl-C(O)-O-R_4$, $--C(O)-O-R_4$, $--S-R_4$, $--S(O)_2-R_4$, $--NH-S(O)_2-R_4$, --alkyl-S-R4, $--alkyl-S(O)_2-R_4$, $--NHR_4$, $--NR_4R_4$,$--NH-alkyl-R_4$, halogen, --CN, $--NO_2$, and –SH, wherein R_4 is independently hydrogen, alkyl, alkenyl, --alkyl-hydroxyl, aryl, heteroaryl, heterocyclyl, or haloalkyl;

Z is hydrogen, alkyl, alkenyl, alkynyl, alkoxy, aryl, haloalkyl, heteroaryl, heterocyclyl, each of which can be optionally substituted by one or more substituents selected from the group consisting of hydroxyl, alkoxy, alkyl, alkenyl, alkynyl, aryl, heteroaryl, heterocyclyl, halogen, cyano, nitro, $--N(R_5)_2$, --alkoxy-alkyl, --alkoxy-alkenyl, --C(O)-alkyl, --C(O)-O-alkyl, --O-C(O)-alkyl, $--C(O)-N(R_5)_2$, aryl, heteroaryl, --CO-aryl, and –CO-heteroaryl, wherein each R_5 is independently hydrogen, alkyl, haloalkyl, --alkyl-aryl, or –alkyl-heteroaryl;

R is hydrogen, alkyl, alkoxy, haloalkyl, halogen, aryl, heteroaryl, heterocyclyl, each of which is optionally substituted by one or more substituents selected from the group consisting of hydroxyl, alkoxy, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, heterocyclyl, $--NH_2$, nitro, --alkyl-hydroxyl, --alkyl-aryl, --alkyl-heteroaryl, --alkyl-heterocyclyl, $--O-R_4$, $--O-alkyl-R_4$, $--alkyl-O-R_4$, $--C(O)-R_4$, $--C(O)-NH-R_4$, $--C(O)-NR_4R_4$, $--alkyl-C(O)-R_4$, $--alkyl-C(O)-O-R_4$, $--C(O)-O-R_4$, $--O-C(O)-R_4$, $--S-R_4$, $--C(O)-S-R_4$, $--S-C(O)-R_4$, $--S(O)_2-R_4$, $--NH-S(O)_2-R_4$, $--alkyl-S-R_4$, $--alkyl-S(O)_2-R_4$, $--NHR_4$, $--NR_4R_4$,$--NH-alkyl-R_4$, halogen, --CN, and –SH, wherein R_4 is independently hydrogen, alkyl, alkenyl, alkoxy, --alkyl-hydroxyl, aryl, heteroaryl, heterocyclyl, or haloalkyl;

n is 0, 1, 2, 3, or 4;

Y is $–NR_6R_7$, $–CR_6R_7R_8$, or $–alkyl-NH_2$, each of which can be optionally substituted by one or more substituents selected from the group consisting of hydroxyl, alkoxy, alkyl, alkenyl, alkynyl, $--NH_2$, halogen, $--N(R_5)_2$, --alkoxy-alkyl, --alkoxy-alkenyl, --C(O)-alkyl, --C(O)-O-alkyl, $--C(O)-N(R_5)_2$, aryl, heteroaryl, --CO-aryl, and –CO-heteroaryl,

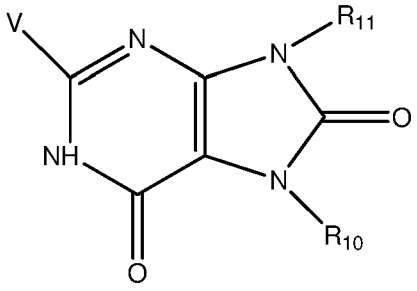
wherein R_6, R_7 and R_8 are independently hydrogen, alkyl, alkenyl, alkoxy, alkylamino, dialkylamino, alkylthio, arylthio, --alkyl-hydroxyl, --alkyl-C(O)-O-R_9, --alkyl-C(O)-R_9, or –alkyl-O-C(O)-R_9, wherein each R_5 is independently hydrogen, alkyl, haloalkyl, --alkyl-aryl, or –alkyl-heteroaryl, wherein R_9 is hydrogen, alkyl, alkenyl, halogen, or haloalkyl; and

X and Z taken together may optionally form a (5-9)-membered ring.

[0024] In some embodiments, the immunotherapeutic is a compound selected from the group consisting of: 2-propylthiazolo[4,5-c]quinolin-4-amine, 1-(2-methylpropyl)-1H-imidazo[4,5-c]quinolin-4-amine, 4-amino-2-(ethoxymethyl)-a,a-di-methyl-1H-imidazo[4,5-c]quinoline-1-ethanol, 1-(4-amino-2-ethylaminomethylimidazo-[4,5-c]quinolin-1-yl)-2-methylpropan-2-ol, N-[4-(4-amino-2-ethyl-1H-imidazo[4,5-c]quinolin-1-yl)butyl-]methanesulfonamide, 4-amino-2-ethoxymethyl-aa-dimethyl-6,7,8,9-tetrahydro-1h-imidazo[4,5-c]quinoline-1-ethanol, 4-amino-aa-dimethyl-2-methoxyethyl-1h-imidazo[4,5-c]quinoline-1-ethanol, 1-{2-[3-(benzyloxy)propoxy]ethyl}-2-(ethoxymethyl)-1H-imidazo[4,5-c]quinolin-4-amine, N-[4-(4-amino-2-butyl-1H-imidazo[4,5- c][1,5]naphthyridin-1-yl)butyl]-n'-butylurea, N1-[2-(4-amino-2-butyl-1H-imidazo[4,5-c][1,5] naphthyridin-1-yl)ethyl]-2-amino-4- methylpentanamide, N-(2-{2-[4-amino-2-(2-methoxyethyl)-1H- imidazo[4,5-c]quinolin-1-yl]ethoxy}ethyl)-n'- phenylurea, 1-(2-amino-2-methylpropyl)-2-(ethoxymethyl)-1H-imidazo[4,5-c]quinolin-4-amine, 1-{4-[(3,5-dichlorophenyl)sulfonyl]butyl}-2-ethyl- 1H-imidazo[4,5-c]quinolin-4-amine, N-(2-{2-[4-amino-2-(ethoxymethyl)-1H-imidazo[4,5- c]quinolin-1-yl]ethoxy}ethyl)-n'-cyclohexylurea, N-{3-[4-amino-2-(ethoxymethyl)-1H-imidazo[4,5- c]quinolin-1-yl]propyl}-n'-(3-cyanophenyl)thiourea, N-[3-(4-amino-2-butyl-1H-imidazo[4,5-c]quinolin-1- yl)-2,2-dimethylpropyl]benzamide, 2-butyl-1-[3-(methylsulfonyl)propyl]-1H- imidazo[4,5-c]quinolin-4-amine, N-{2-[4-amino-2-(ethoxymethyl)-1H-imidazo[4,5- c]quinolin-1-yl]-1,1-dimethylethyl}-2- ethoxyacetamide, 1-[4-amino-2-ethoxymethyl-7-(pyridin-4-yl)-1H- imidazo[4,5-c]quinolin-1-yl]-2-methylpropan-2-ol, 1-[4-amino-2-(ethoxymethyl)-7-(pyridin-3-yl)-1H- imidazo[4,5-c]quinolin-1-yl]-2-methylpropan-2-ol, N-{3-[4-amino-1-(2-hydroxy-2-methylpropyl)-2- (methoxyethyl)-1H-imidazo[4,5-c]quinolin-7-yl]phenyl}methanesulfonamide, 1-[4-amino-7-(5-hydroxymethylpyridin-3-yl)-2-(2- methoxyethyl)-1H-imidazo[4,5-c]quinolin-1-yl]-2-methylpropan-2-ol, 3-[4-amino-2-(ethoxymethyl)-7-(pyridin-3-yl)-1H- imidazo[4,5-c]quinolin-1-yl]propane-1,2-diol, 1-[2-(4-amino-2-ethoxymethyl-1H-imidazo[4,5- c]quinolin-1-yl)-1,1-dimethylethyl]-3-propylurea, 1-[2-(4-amino-2-ethoxymethyl-1H-imidazo[4,5- c]quinolin-1-yl)-1,1-dimethylethyl]-3-cyclopentylurea, 1-[(2,2-dimethyl-1,3-dioxolan-4-yl)methyl]-2- (ethoxymethyl)-7-(4-hydroxymethylphenyl)-1H- imidazo[4,5-c]quinolin-4-amine, 4-[4-amino-2-ethoxymethyl-1-(2-hydroxy-2-methylpropyl)-1H-imidazo[4,5-c]quinolin-7-yl]-N- methoxy-N-methylbenzamide, 2-ethoxymethyl-N1-isopropyl-6,7,8,9-tetrahydro-1H-imidazo[4,5-c]quinoline-1,4-diamine, 1-[4-amino-2-ethyl-7-(pyridin-4-

yl)-1H-imidazo[4,5- c]quinolin-1-yl]-2-methylpropan-2-ol, N-[4-(4-amino-2-ethyl-1H-imidazo[4,5-c]quinolin-1-yl)butyl]methanesulfonamide, and N-[4-(4-amino-2-butyl-1H-imidazo[4,5-c][1,5]naphthyridin-1-yl)butyl]-n'-cyclohexylurea.

[0025] In some embodiments, the immunotherapeutic has a structure of Formula (II):



(II)

wherein V is $-NR_6R_7$, wherein each of R_6 and R_7 is independently hydrogen, alkyl, alkenyl, alkoxy, alkylamino, dialkylamino, alkylthio, arylthio, --alkyl-hydroxyl, --alkyl-C(O)-O-R_9, --alkyl-C(O)-R_9, or –alkyl-O-C(O)-R_9, wherein R_9 is hydrogen, alkyl, alkenyl, halogen, or haloalkyl;

R_{10} and R_{11} are independently hydrogen, alkyl, alkenyl, aryl, haloalkyl, heteroaryl, heterocyclyl, or cycloalkyl, each of which is optionally substituted by one or more substituents selected from the group consisting of hydroxyl, alkoxy, alkyl, alkenyl, alkynyl, halogen, --$N(R_5)_2$, --alkoxy-alkyl, --alkoxy-alkenyl, --C(O)-alkyl, --C(O)-O-alkyl, --C(O)-$N(R_5)_2$, aryl, heteroaryl, --CO-aryl, and –CO-heteroaryl, wherein each R_5 is independently hydrogen, alkyl, haloalkyl, --alkyl-aryl, or –alkyl-heteroaryl.

[0026] In some embodiments, the therapeutic combination or pharmaceutical composition of the present invention further comprisse an effective amount of an additional therapeutic agent, such as an anticancer agent.

[0027] In some embodiments, the anticancer agent is an antimetabolite, an inhibitor of topoisomerase I and II, an alkylating agent, a microtubule inhibitor, an antiandrogen agent, a GNRh modulator or mixtures thereof.

[0028] In some embodiments, the additional therapeutic agent is a chemotherapeutic agent selected from the group consisting of tamoxifen, raloxifene, anastrozole, exemestane, letrozole, imatanib, paclitaxel, cyclophosphamide, lovastatin, minosine, gemcitabine, cytarabine, 5- fluorouracil, methotrexate, docetaxel, goserelin, vincristine, vinblastine,nocodazole, teniposide etoposide, gemcitabine, epothilone, vinorelbine, camptothecin, daunorubicin, actinomycin D, mitoxantrone, acridine, doxorubicin, epirubicin, or idarubicin.

[0029] In another aspect, the present invention provides a method for treating a disease condition in a subject that is in need of such treatment, comprising administering to the subject the therapeutic combination or pharmaceutical composition provided herein.

[0030] In some embodiments, the diseases condition is tumor. In some embodiments, the disease condition comprises abnormal cell proliferation.

[0031] In some embodiments, the abnormal cell proliferation comprises a pre-cancerous lesion. In some embodiments, the abnormal proliferation is of cancer cells.

[0032] In some embodiments, the cancer is selected from the group consisting of: breast cancer, colorectal cancer, diffuse large B-cell lymphoma, endometrial cancer, follicular lymphoma, gastric cancer, glioblastoma, head and neck cancer, hepatocellular cancer, lung cancer, melanoma, multiple myeloma, ovarian cancer, pancreatic cancer, prostate cancer, and renal cell carcinoma.

[0033] In some embodiments, the immunotherapeutic is of an amount that is capable of:

(1) inducing IFN-α in a enriched human blood DCs;

(2) inducing TNF-α in a enriched human blood DCs; and/or

(3) inducing IL-12-α in a enriched human blood DCs.

[0034] In some embodiments, the method comprisesvadministering to the subject an oral formulation comprising the immunotherapeutic (such as R848 and its analogues) in a dose of between about 0.0005 mg/kg, 0.0006 mg/kg, 0.0007 mg/kg, 0.0008 mg/kg, 0.0009 mg/kg, 0.001 mg/kg, 0.002 mg/kg, 0.003 mg/kg, 0.004 mg/kg, 0.005 mg/kg, 0.006 mg/kg, 0.007 mg/kg, 0.008 mg/kg, 0.009 mg/kg, or 0.01 mg/kg, to about 0.02 mg/kg, twice per week.

[0035] In some embodiments, the method comprises administering to the subject an oral formulation comprising the immunotherapeutic (such as R848 and its analogues) in a dose of less than or about 0.005 mg/kg, 0.006 mg/kg, 0.007 mg/kg , 0.008 mg/kg, 0.009 mg/kg, or 0.01 mg/kg, twice per week.

[0036] In some embodiments, the method comprises administering to said subject an intravenous formulation comprising said immunotherapeutic (such as R848 and its analogues) in a dose of between about 0.0005 mg/kg, 0.0006 mg/kg, 0.0007 mg/kg, 0.0008mg/kg, 0.0009mg/kg, 0.001 mg/kg, 0.002 mg/kg, 0.003 mg/kg, 0.004 mg/kg, 0.005 mg/kg, or 0.006 mg/kg to about 0.015 mg/kg, weekly. In some embodiments, the method comprises administering to said subject an intravenous formulation comprising said immunotherapeutic (such as R848 and its analogues) in a dose of between about 0.0008 mg/kg to about 0.0067 mg/kg, weekly.

[0037] In some embodiments, the method comprises administering to said subject an intravenous formulation comprising said immunotherapeutic in a dose of less than or about 0.003 mg/kg, 0.004 mg/kg, 0.005 mg/kg, or 0.006 mg/kg to about 0.01 mg/kg, weekly.

[0038] In some embodiments, the immunotherapeutic in the subject has a local concentration that is between about 0.005 μg/ml to about 12 μg/ml.

[0039] In some embodiments, the immunotherapeutic in the subject has a local concentration that is is between about 0.05 μg/ml, 0.1 μg/ml, 0.15 μg/ml, 0.2 μg/ml, 0.3 μg/ml, or 0.4 μg/ml, to about 0.5 μg/ml.

[0040] In a further aspec, the present invention provide a kit that contains the therapeteutic combination provided herein, and optionally with an instruction.

BRIEF DESCRIPTION OF THE DRAWINGS

[0041] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0042] **Figures 1A-1G** depict analysis of cytokine production by enriched human DCs from three healthy donors. Enriched human DCs were plated in a 96-well plate and cultured with allogeneic untreated (medium) or treated different concentration of TLRL directly for 20-22h in 37°C incubator. The supernatant were collected and human IFN-α, IL-12(p70) and TNF-α were analyzed by ELISA. Data are given as mean ± SD of triplicate cultures. Three independent experiments from three healthy donors were performed. **A**: TLRL induced IFN-α expression in enriched human blood DCs (CD3+/CD19+/CD14+/CD16+) from donor 1. **B**: TLRL induced IFN-α expression in enriched human blood DCs in Experiment #2 from donor 2. **C**: TLRL induced TNF-α expression in enriched human blood DCs in Experiment #2 from donor 2. **D**: TLRL induced IL-12 expression in enriched human blood DCs in Experiment #2 from donor 2. **E**: TLRL induced IFN-α expression in enriched human blood DCs Experiment #3 from donor 3. **F**: TLRL induced TNF-α expression in enriched human blood DCsin Experiment #3 from donor 3. **G**: TLRL induced IL-12 expression in enriched human blood DCs in Experiment #3 from donor 3.

[0043] **Figure 2** depicts that combination therapy of Resiquimod with Herceptin has better effect than compound alone in PDX tumor model (nude). It is Her2 expression PDX, Experiment #1.

[0044] **Figure 3** depicts that combination therapy of Resiquimod with Herceptin has better effect than compound alone in PDX tumor model (nude). This model is Herceptin-resistant gastric cancer.

[0045] **Figure 4** depicts that combination therapy of anti-neu and TLRL synergistically amplify the antitumor effect on TUBO tumor model. WT BALB/c mice (n=8/group) were injected s.c. with $5x10^5$ TUBO cells and treated with 75μg of anti-neu antibody (clone: 7.16.4) alone or combination with TLRL on days 13, 16, 20 and 24. Tumor vulome and weight were measured every three days for 28 days.

[0046] **Figure 5** depicts that TLRL enhances the efficacy of therapy with anti-neu on TUBO lung tumor in Babl/c mice. WT BALB/c mice (n=10/group) were injected i.v. at mouse tail with $4x10^5$ TUBO cells and were started to treat with 100 μg of anti-neu antibody (clone: 7.16.4) alone, TLRL alone or antibody combination with various dose of TLRL on days 12, q7d, for three times. Survival rate, wright and daily activity were observed for up to 100 days.

[0047] **Figure 6** depicts expression of IFN inducible genes in mouse PBMC after TLRL injection. RNA was isolated from PBMCs cryopreserved with TRIzol reagent at variable time points and Relative expression of IFN inducible genes were determined by quantitative RT-PCR. MX2 gene was detected over time course of 5 hours post TLRL injection (**6A**) and MX2 and ISG15 genes were measured with various dose of TLRL at 2 hours post injection (**6B**). Values indicate the mRNA expression of indicated IFN inducible genes relative to housekeeping gene Actin. Bar graphs represent data from 3 individual animals. $**P < 0.01$; $***P < 0.001$.

DETAILED DESCRIPTION OF THE INVENTION

[0048] Several aspects of the invention are described below with reference to example applications for illustration. It should be understood that numerous specific details, relationships, and methods are set forth to provide a full understanding of the invention. One having ordinary skill in the relevant art, however, will readily recognize that the invention can be practiced without one or more of the specific details or with other methods. The present invention is not limited by the illustrated ordering of acts or events, as some acts may occur in different orders and/or concurrently with other acts or events.

[0049] Furthermore, not all illustrated acts or events are required to implement a methodology in accordance with the present invention.

[0050] The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention. As used herein, the singular forms "a", "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise. Furthermore, to the extent that the terms "including", "includes", "having", "has", "with", or variants thereof are used in either the detailed description and/or the claims, such terms are intended to be inclusive in a manner similar to the term "comprising".

[0051] The term "about" or "approximately" means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, i.e., the limitations of the measurement system. For example, "about" can mean within 1 or more than 1 standard deviation, per the practice in the art. Alternatively, "about" can mean a range of up to 20%, preferably up to 10%, more preferably up to 5%, and more preferably still up to 1% of a given value. Alternatively, particularly with respect to biological systems or processes, the term can mean within an order of magnitude, preferably within 5-fold, and more preferably within 2-fold, of a value. Where particular values are described in the application and claims, unless otherwise stated the term "about" meaning within an acceptable error range for the particular value should be assumed.

I. Definitions and Abbreviations

[0052] Unless defined otherwise, all technical and scientific terms used herein generally have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Generally, the nomenclature used herein and the laboratory procedures in cell culture, molecular genetics, organic chemistry and nucleic acid chemistry and hybridization are those well-known and commonly employed in the art. Standard techniques are used for nucleic acid and peptide synthesis. The techniques and procedures are generally performed according to conventional methods in the art and various general references, which are provided throughout this document. The nomenclature used herein and the laboratory procedures in analytical chemistry, and organic synthetic described below are those well-known and commonly employed in the art. Standard techniques, or modifications thereof, are used for chemical syntheses and chemical analyses.

[0053] The term "alkyl," by itself or as part of another substituent, means, unless otherwise stated, a straight or branched chain, or cyclic hydrocarbon radical, or combination thereof, which may be fully saturated, mono- or polyunsaturated and can include di- and multivalent radicals, having the number of carbon atoms designated (*i.e.* C_1-C_{10} means one to ten carbons). Examples of saturated hydrocarbon radicals include, but are not limited to, groups such as methyl, ethyl, n-propyl, isopropyl, n-butyl, t-butyl, isobutyl, sec-butyl, cyclohexyl, (cyclohexyl)methyl, cyclopropylmethyl, homologs and isomers of, for example, n-pentyl, n-hexyl, n-heptyl, n-octyl, and the like. An unsaturated alkyl group is one having one or more double bonds or triple bonds. Examples of unsaturated alkyl groups include, but are not limited to, vinyl, 2-propenyl, crotyl, 2-isopentenyl, 2-(butadienyl), 2,4-pentadienyl, 3-(1,4-pentadienyl), ethynyl, 1- and 3-propynyl, 3-butynyl, and the higher homologs and isomers. The term "alkyl," unless otherwise noted, is also meant to include those derivatives of alkyl defined in more detail below, such as "heteroalkyl." Alkyl groups, which are limited to hydrocarbon groups, are termed "homoalkyl".

[0054] The term "alkylene" by itself or as part of another substituent means a divalent radical derived from an alkane, as exemplified, but not limited, by $-CH_2CH_2CH_2CH_2-$, and further includes those groups described below as "heteroalkylene." Typically, an alkyl (or alkylene) group will have from 1 to 24 carbon atoms, with those groups having 10 or fewer carbon atoms being preferred in the present invention. A "lower alkyl" or "lower alkylene" is a shorter chain alkyl or alkylene group, generally having eight or fewer carbon atoms.

[0055] The terms "alkoxy," "alkylamino" and "alkylthio" (or thioalkoxy) are used in their conventional sense, and refer to those alkyl groups attached to the remainder of the molecule via an oxygen atom, an amino group, or a sulfur atom, respectively.

[0056] The term "heteroalkyl," by itself or in combination with another term, means, unless otherwise stated, a stable straight or branched chain, or cyclic hydrocarbon radical, or combinations thereof, consisting of the stated number of carbon atoms and at least one heteroatom selected from the group consisting of O, N, Si and S, and wherein the nitrogen and sulfur atoms may optionally be oxidized and the nitrogen heteroatom may optionally be quaternized. The heteroatom(s) O, N and S and Si may be placed at any interior position of the heteroalkyl group or at the position at which the alkyl group is attached to the remainder of the molecule. Examples include, but are not limited to, $-CH_2-CH_2-O-CH_3$, $-CH_2-CH_2-NH-CH_3$, $-CH_2-CH_2-N(CH_3)-CH_3$, $-CH_2-S-CH_2-CH_3$, $-CH_2-CH_2,-S(O)-CH_3$, $-CH_2-CH_2-S(O)_2-CH_3$, $-CH=CH-O-CH_3$, $-Si(CH_3)_3$, $-CH_2-CH=N-OCH_3$, and $-CH=CH-N(CH_3)-CH_3$. Up to two heteroatoms may be consecutive, such as, for example, $-CH_2-NH-OCH_3$ and $-CH_2-O-Si(CH_3)_3$. Similarly, the term "heteroalkylene" by itself or as part of another substituent means a divalent radical derived from heteroalkyl, as exemplified, but not limited by, $-CH_2-CH_2-S-CH_2-CH_2-$ and $-CH_2-S-CH_2-CH_2-NH-CH_2-$. For heteroalkylene groups, heteroatoms can also occupy either or both of the chain termini (*e.g.*, alkyleneoxy, alkylenedioxy, alkyleneamino, alkylenediamino, and the like). Still further, for alkylene and heteroalkylene linking groups, no orientation of the linking group is implied by the direction in which the formula of the linking group is written. For example, the formula $-C(O)_2R'-$ represents both $-C(O)_2R'-$ and $-R'C(O)_2-$.

[0057] In general, an "acyl substituent" is also selected from the group set forth above. As used herein, the term "acyl substituent" refers to groups attached to, and fulfilling the valence of a carbonyl carbon that is either directly or indirectly attached to the polycyclic nucleus of the compounds of the present invention.

[0058] The terms "cycloalkyl" and "heterocycloalkyl", by themselves or in combination with other terms, represent, unless otherwise stated, cyclic versions of "alkyl" and "heteroalkyl", respectively. Additionally, for heterocycloalkyl, a heteroatom can occupy the position at which the heterocycle is attached to the

remainder of the molecule. Examples of cycloalkyl include, but are not limited to, cyclopentyl, cyclohexyl, 1-cyclohexenyl, 3-cyclohexenyl, cycloheptyl, and the like. Examples of heterocycloalkyl include, but are not limited to, 1 –(1,2,5,6-tetrahydropyridyl), 1-piperidinyl, 2-piperidinyl, 3-piperidinyl, 4-morpholinyl, 3-morpholinyl, tetrahydrofuran-2-yl, tetrahydrofuran-3-yl, tetrahydrothien-2-yl, tetrahydrothien-3-yl, 1 –piperazinyl, 2-piperazinyl, and the like.

[0059] The terms “halo” or “halogen,” by themselves or as part of another substituent, mean, unless otherwise stated, a fluorine, chlorine, bromine, or iodine atom. Additionally, terms such as “haloalkyl,” are meant to include monohaloalkyl and polyhaloalkyl. For example, the term “halo(C_1-C_4)alkyl” is mean to include, but not be limited to, trifluoromethyl, 2,2,2-trifluoroethyl, 4-chlorobutyl, 3-bromopropyl, and the like.

[0060] As used herein, the term “haloalkyl” refers to an alkyl as defined herein, that is substituted by one or more halo groups as defined herein. Preferably the haloalkyl can be monohaloalkyl, dihaloalkyl or polyhaloalkyl including perhaloalkyl. A monohaloalkyl can have one iodo, bromo, chloro or fluoro within the alkyl group. Dihaloalkyl and polyhaloalkyl groups can have two or more of the same halo atoms or a combination of different halo groups within the alkyl. Preferably, the polyhaloalkyl contains up to 12, 10, or 8, or 6, or 4, or 3, or 2 halo groups. Non-limiting examples of haloalkyl include fluoromethyl, difluoromethyl, trifluoromethyl, chloromethyl, dichioromethyl, trichioromethyl, pentafluoroethyl, heptafluoropropyl, difluorochioromethyl, dichiorofluoromethyl, difluoroethyl, difluoropropyl, dichloroethyl and dichioropropyl. A perhaloalkyl refers to an alkyl having all hydrogen atoms replaced with halo atoms.

[0061] As used herein, the term "heteroaryl" refers to a 5-14 membered monocyclic- or bicyclic- or fused polycyclic-ring system, having 1 to 8 heteroatoms selected from N, O, S or Se. Preferably, the heteroaryl is a 5-10 membered ring system. Typical heteroaryl groups include 2- or 3-thienyl, 2- or 3-furyl, 2- or 3-pyrrolyl, 2-, 4-, or 5-imidazolyl, 3-, 4-, or 5- pyrazolyl, 2-, 4-, or 5-thiazolyl, 3-, 4-, or 5-isothiazolyl, 2-, 4-, or 5-oxazolyl, 3-, 4-, or 5-isoxazolyl, 3- or 5-1,2,4-triazolyl, 4- or 5-1,2, 3-triazolyl, tetrazolyl, 2-, 3-, or 4-pyridyl, 3- or 4-pyridazinyl, 3-, 4-, or 5-pyrazinyl, 2-pyrazinyl, 2-, 4-, or 5-pyrimidinyl.

[0062] The term “heteroaryl” also refers to a group in which a heteroaromatic ring is fused to one or more aryl, cycloaliphatic, or heterocycloalkyl rings, where the radical or point of attachment is on the heteroaromatic ring. Nonlimiting examples include but are not limited to 1-, 2-, 3-, 5-, 6-, 7-, or 8-indolizinyl, 1-, 3-, 4-, 5-, 6-, or 7-isoindolyl, 2-, 3-, 4-, 5-, 6-, or 7-indolyl, 2-, 3-, 4-, 5-, 6-, or 7-indazolyl, 2-, 4-, 5-, 6-, 7-, or 8- purinyl, 1-, 2-, 3-, 4-, 6-, 7-, 8-, or 9-quinolizinyl, 2-, 3-, 4-, 5-, 6-, 7-, or 8-quinoliyl, 1-, 3-, 4-, 5-, 6-, 7-, or 8-isoquinoliyl, 1-, 4-, 5-, 6-, 7-, or 8-phthalazinyl, 2-, 3-, 4-, 5-, or 6-naphthyridinyl, 2-, 3-, 5-, 6-, 7-, or 8-quinazolinyl, 3-, 4-, 5-, 6-, 7-, or 8-cinnolinyl, 2-, 4-, 6-, or 7-pteridinyl, 1-, 2-, 3-, 4-,

5-, 6-, 7-, or 8-4aH carbazolyl, 1-, 2-, 3-, 4-, 5-, 6-, 7-, or 8-carbzaolyl, 1-, 3-, 4-, 5-, 6-, 7-, 8-, or 9-carbolinyl, 1-, 2-, 3-, 4-, 6-, 7-, 8-, 9-, or 10-phenanthridinyl, 1- , 2-, 3-, 4-, 5-, 6-, 7-, 8-, or 9-acridinyl, 1-, 2-, 4-, 5-, 6-, 7-, 8-, or 9-perimidinyl, 2-, 3-, 4-, 5-, 6-, 8-, 9-, or 10-phenathrolinyl, 1-, 2- , 3-, 4-, 6-, 7-, 8-, or 9-phenazinyl, 1-, 2-, 3-, 4-, 6-, 7-, 8-, 9-, or 10-phenothiazinyl, 1-, 2-, 3-, 4-, 6-, 7-, 8-, 9-, or 10-phenoxazinyl, 2-, 3-, 4-, 5-, 6-, or l-, 3-, 4-, 5-, 6-, 7-, 8-, 9-, or 10- benzisoqinolinyl, 2-, 3-, 4-, or 5-thieno[2,3-b]furanyl, 2-, 3-, 5-, 6-, 7-, 8-, 9-, 10 -, or 11-7H-pyrazino[2,3-c]carbazolyl,2-, 3-, 5-, 6-, or 7-2H- furo[3,2-b]-pyranyl, 2-, 3-, 4-, 5-, 7-, or 8-5H-pyrido[2,3-d]-o-oxazinyl, 1-, 3-, or 5-1H-pyrazolo[4,3-d]-oxazolyl, 2-, 4-, or 54H-imidazo[4,5-d] thiazolyl, 3-, 5-, or 8-pyrazino[2,3-d]pyridazinyl, 2-, 3-, 5-, or 6- imidazo[2,1-b] thiazolyl, 1-, 3-, 6-, 7-, 8-, or 9-furo[3,4-c]cinnolinyl, 1-, 2-, 3-, 4-, 5-, 6-, 8-, 9-, 10, or 11-4H-pyrido[2,3-c]carbazolyl, 2-, 3-, 6-, or 7-imidazo[1,2-b][1,2,4]triazinyl, 7-benzo[b]thienyl, 2-, 4-, 5- , 6-, or 7-benzoxazolyl, 2-, 4-, 5-, 6-, or 7-benzimidazolyl, 2-, 4-, 4-, 5-, 6-, or 7-benzothiazolyl, 1-, 2-, 4-, 5-, 6-, 7-, 8-, or 9- benzoxapinyl, 2-, 4-, 5-, 6-, 7-, or 8-benzoxazinyl, 1-, 2-, 3-, 5-, 6-, 7-, 8-, 9-, 10-, or 11-1H-pyrrolo[1,2-b][2]benzazapinyl. Typical fused heteroaryl groups include, but are not limited to 2-, 3-, 4-, 5-, 6-, 7-, or 8-quinolinyl, 1-, 3-, 4-, 5-, 6-, 7-, or 8-isoquinolinyl, 2-, 3-, 4-, 5-, 6-, or 7-indolyl, 2-, 3-, 4-, 5-, 6-, or 7-benzo[b]thienyl, 2-, 4-, 5- , 6-, or 7-benzoxazolyl, 2-, 4-, 5-, 6-, or 7-benzimidazolyl, 2-, 4-, 5-, 6-, or 7-benzothiazolyl.

[0063] As used herein, the term "heterocyclyl" or "heterocyclo" refers to an optionally substituted, fully saturated or unsaturated, aromatic or nonaromatic cyclic group, e.g., which is a 4- to 7-membered monocyclic, 7- to 12-membered bicyclic or 10- to 15-membered tricyclic ring system, which has at least one heteroatom in at least one carbon atom-containing ring. Each ring of the heterocyclic group containing a heteroatom may have 1, 2 or 3 heteroatoms selected from nitrogen atoms, oxygen atoms and sulfur atoms, where the nitrogen and sulfur heteroatoms may also optionally be oxidized. The heterocyclic group may be attached at a heteroatom or a carbon atom.

[0064] Exemplary monocyclic heterocyclic groups include pyrrolidinyl, pyrrolyl, pyrazolyl, oxetanyl, pyrazolinyl, imidazolyl, imidazolinyl, imidazolidinyl, triazolyl, oxazolyl, oxazolidinyl, isoxazolinyl, isoxazolyl, thiazolyl, thiadiazolyl, thiazolidinyl, isothiazolyl, isothiazolidinyl, furyl, tetrahydrofuryl, thienyl, oxadiazolyl, piperidinyl, piperazinyl, 2-oxopiperazinyl, 2-oxopiperidinyl, 2-oxopyrrolodinyl, 2-oxoazepinyl, azepinyl, 4-piperidonyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, tetrahydropyranyl, morpholinyl, thiamorpholinyl, thiamorpholinyl sulfoxide, thiamorpholinyl sulfone, 1,3-dioxolane and tetrahydro-1,1-dioxothienyl, 1,1,4-trioxo-1,2,5-thiadiazolidin-2-yl and the like.

[0065] Exemplary bicyclic heterocyclic groups include indolyl, dihydroidolyl, benzothiazolyl, benzoxazinyl, benzoxazolyl, benzothienyl, benzothiazinyl, quinuclidinyl, quinolinyl, tetrahydroquinolinyl, decahydroquinolinyl, isoquinolinyl, tetrahydroisoquinolinyl, decahydroisoquinolinyl, benzimidazolyl,

benzopyranyl, indolizinyl, benzofuryl, chromonyl, coumarinyl, benzopyranyl, cinnolinyl, quinoxalinyl, indazolyl, pyrrolopyridyl, furopyridinyl (such as furo[2,3-c]pyridinyl, furo[3,2-b]-pyridinyl] or furo[2,3-b]pyridinyl), dihydroisoindolyl, 1,3-dioxo-1,3-dihydroisoindol-2-yl, dihydroquinazolinyl (such as 3,4-dihydro-4-oxo-quinazolinyl), phthalazinyl and the like.

[0066] Exemplary tricyclic heterocyclic groups include carbazolyl, dibenzoazepinyl, dithienoazepinyl, benzindolyl, phenanthrolinyl, acridinyl, phenanthridinyl, phenoxazinyl, phenothiazinyl, xanthenyl, carbolinyl and the like.

[0067] The term "heterocyclyl" further refers to heterocyclic groups as defined herein substituted with 1, 2 or 3 substituents selected from the groups consisting of the following:

(a) alkyl;

(b) hydroxy (or protected hydroxy);

(c) halo;

(d) oxo, i.e., =O;

(e) amino, alkylamino or dialkylamino;

(f) alkoxy;

(g) cycloalkyl;

(h) carboxy;

(i) heterocyclooxy, wherein heterocyclooxy denotes a heterocyclic group bonded through an oxygen bridge;

(j) alkyl-O-C(O)--;

(k) mercapto;

(l) nitro;

(m) cyano;

(n) sulfamoyl or sulfonamido;

(o) aryl;

(p) alkyl-C(O)-O--;

(q) aryl-C(O)-O--;

(r) aryl-S--;

(s) aryloxy;

(t) alkyl-S--;

(u) formyl, i.e., HC(O)--;

(v) carbamoyl;

(w) aryl-alkyl--; and

(x) aryl substituted with alkyl, cycloalkyl, alkoxy, hydroxy, amino, alkyl-C(O)-NH--, alkylamino, dialkylamino or halogen.

[0068] As used herein, the term "alkenyl" refers to a straight or branched hydrocarbon group having 2 to 20 carbon atoms and that contains at least one double bonds. The alkenyl groups preferably have about 2 to 8 carbon atoms.

[0069] The term "aryl" means, unless otherwise stated, a polyunsaturated, aromatic, hydrocarbon substituent, which can be a single ring or multiple rings (preferably from 1 to 3 rings), which are fused together or linked covalently. The term "heteroaryl" refers to aryl groups (or rings) that contain from one to four heteroatoms selected from N, O, and S, wherein the nitrogen and sulfur atoms are optionally oxidized, and the nitrogen atom(s) are optionally quaternized. A heteroaryl group can be attached to the remainder of the molecule through a heteroatom. Non-limiting examples of aryl and heteroaryl groups include phenyl, 1-naphthyl, 2-naphthyl, 4-biphenyl, 1-pyrrolyl, 2-pyrrolyl, 3-pyrrolyl, 3-pyrazolyl, 2-imidazolyl, 4-imidazolyl, pyrazinyl, 2-oxazolyl, 4-oxazolyl, 2-phenyl-4-oxazolyl, 5-oxazolyl, 3-isoxazolyl, 4-isoxazolyl, 5-isoxazolyl, 2-thiazolyl, 4-thiazolyl, 5-thiazolyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-pyrimidyl, 4-pyrimidyl, 5-benzothiazolyl, purinyl, 2-benzimidazolyl, 5-indolyl, 1-isoquinolyl, 5-isoquinolyl, 2-quinoxalinyl, 5-quinoxalinyl, 3-quinolyl, and 6-quinolyl. Substituents for each of the above noted aryl and heteroaryl ring systems are selected from the group of acceptable substituents described below.

[0070] For brevity, the term "aryl" when used in combination with other terms (*e.g.*, aryloxy, arylthioxy, arylalkyl) includes both aryl and heteroaryl rings as defined above. Thus, the term "arylalkyl" is meant to include those radicals in which an aryl group is attached to an alkyl group (*e.g.*, benzyl, phenethyl, pyridylmethyl and the like) including those alkyl groups in which a carbon atom (*e.g.*, a methylene group) has been replaced by, for example, an oxygen atom (*e.g.*, phenoxymethyl, 2-pyridyloxymethyl, 3-(1-naphthyloxy)propyl, and the like).

[0071] Each of the above terms (*e.g.*, "alkyl," "heteroalkyl," "aryl" and "heteroaryl") include both substituted and unsubstituted forms of the indicated radical. Preferred substituents for each type of radical are provided below.

[0072] Substituents for the alkyl, and heteroalkyl radicals (including those groups often referred to as alkylene, alkenyl, heteroalkylene, heteroalkenyl, alkynyl, cycloalkyl, heterocycloalkyl, cycloalkenyl, and heterocycloalkenyl) are generally referred to as "alkyl substituents" and "heteroakyl substituents," respectively, and they can be one or more of a variety of groups selected from, but not limited to: -OR', =O, =NR', =N-OR', -NR'R", -SR', -halogen, -SiR'R"R"', -OC(O)R', -C(O)R', -CO_2R', -CONR'R", -OC(O)NR'R", -NR"C(O)R', -NR'-C(O)NR"R"', -NR"$C(O)_2$R', -NR-C(NR'R"R"')=NR"", -NR-C(NR'R")=NR"', -S(O)R', -$S(O)_2$R', -$S(O)_2$NR'R", -$NRSO_2$R', -CN and –NO_2 in a number ranging from zero to (2m'+1), where m' is the total number of carbon atoms in such radical. R', R", R"' and R"" each preferably independently refer to hydrogen, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, e.g., aryl substituted with 1-3 halogens, substituted or unsubstituted alkyl, alkoxy or thioalkoxy groups, or arylalkyl groups. When a compound of the invention includes more than one R group, for example, each of the R groups is independently selected as are each R', R", R"' and R"" groups when more than one of these groups is present. When R' and R" are attached to the same nitrogen atom, they can be combined with the nitrogen atom to form a 5-, 6-, or 7-membered ring. For example, -NR'R" is meant to include, but not be limited to, 1-pyrrolidinyl and 4-morpholinyl. From the above discussion of substituents, one of skill in the art will understand that the term "alkyl" is meant to include groups including carbon atoms bound to groups other than hydrogen groups, such as haloalkyl (*e.g.*, -CF_3 and –CH_2CF_3) and acyl (*e.g.*, -C(O)CH_3, -C(O)CF_3, -C(O)CH_2OCH_3, and the like).

[0073] Similar to the substituents described for the alkyl radical, the aryl substituents and heteroaryl substituents are generally referred to as "aryl substituents" and "heteroaryl substituents," respectively and are varied and selected from, for example: halogen, -OR', =O, =NR', =N-OR', -NR'R", -SR', -halogen, -SiR'R"R"', -OC(O)R', -C(O)R', -CO_2R', -CONR'R", -OC(O)NR'R", -NR"C(O)R', -NR'-C(O)NR"R"', -NR"$C(O)_2$R', -NR-C(NR'R")=NR"', -S(O)R', -$S(O)_2$R', -$S(O)_2$NR'R", -$NRSO_2$R', -CN and –NO_2, -R', -N_3, -$CH(Ph)_2$, fluoro(C_1-C_4)alkoxy, and fluoro(C_1-C_4)alkyl, in a number ranging from zero to the total number of open valences on the aromatic ring system; and where R', R", R"' and R"" are preferably independently selected from hydrogen, (C_1-C_8)alkyl and heteroalkyl, unsubstituted aryl and heteroaryl, (unsubstituted aryl)-(C_1-C_4)alkyl, and (unsubstituted aryl)oxy-(C_1-C_4)alkyl. When a compound of the invention includes more than one R group, for example, each of the R groups is independently selected as are each R', R", R"' and R"" groups when more than one of these groups is present.

[0074] Two of the aryl substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula –T-C(O)-(CRR')$_q$-U-, wherein T and U are independently –NR-, -O-, -CRR'- or a single bond, and q is an integer of from 0 to 3. Alternatively, two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula –A-(CH$_2$)$_r$-B-, wherein A and B are independently –CRR'-, -O-, -NR-, -S-, -S(O)-, -S(O)$_2$-, -S(O)$_2$NR'- or a single bond, and r is an integer of from 1 to 4. One of the single bonds of the new ring so formed may optionally be replaced with a double bond. Alternatively, two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula –(CRR')$_s$-X-(CR"R"')$_d$-, where s and d are independently integers of from 0 to 3, and X is –O-, -NR'-, -S-, -S(O)-, -S(O)$_2$-, or –S(O)$_2$NR'-. The substituents R, R', R" and R"' are preferably independently selected from hydrogen or substituted or unsubstituted (C$_1$-C$_6$) akyl.

[0075] As used herein, the term "heteroatom" includes oxygen (O), nitrogen (N), sulfur (S), phosphorus (P) and silicon (Si).

[0076] As used herein, the term "aryloxy" refers to both an -O-aryl and an -O-heteroaryl group, wherein aryl and heteroaryl are defined herein.

[0077] As used herein, the term "pharmaceutically acceptable salts" refers to salts that retain the biological effectiveness and properties of the compounds of this invention and, which are not biologically or otherwise undesirable. In many cases, the compounds of the present invention are capable of forming acid and/or base salts by virtue of the presence of amino and/or carboxyl groups or groups similar thereto (e.g., phenol or hydroxyamic acid). Pharmaceutically acceptable acid addition salts can be formed with inorganic acids and organic acids. Inorganic acids from which salts can be derived include, for example, hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like. Organic acids from which salts can be derived include, for example, acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, *p*- toluenesulfonic acid, salicylic acid, and the like. Pharmaceutically acceptable base addition salts can be formed with inorganic and organic bases. Inorganic bases from which salts can be derived include, for example, sodium, potassium, lithium, ammonium, calcium, magnesium, iron, zinc, copper, manganese, aluminum, and the like; particularly preferred are the ammonium, potassium, sodium, calcium and magnesium salts. Organic bases from which salts can be derived include, for example, primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines, basic ion exchange resins, and the like, specifically such as isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, and ethanolamine. The pharmaceutically acceptable salts of the present

invention can be synthesized from a parent compound, a basic or acidic moiety, by conventional chemical methods. Generally, such salts can be prepared by reacting free acid forms of these compounds with a stoichiometric amount of the appropriate base (such as Na, Ca, Mg, or K hydroxide, carbonate, bicarbonate, or the like), or by reacting free base forms of these compounds with a stoichiometric amount of the appropriate acid. Such reactions are typically carried out in water or in an organic solvent, or in a mixture of the two. Generally, non-aqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are preferred, where practicable. Lists of additional suitable salts can be found, e.g., in *Remington's Pharmaceutical Sciences,* 20th ed., Mack Publishing Company, Easton, Pa., (1985), which is herein incorporated by reference.

[0078] As used herein, the term "pharmaceutically acceptable carrier/excipient" includes any and all solvents, dispersion media, coatings, surfactants, antioxidants, preservatives (e.g., antibacterial agents, antifungal agents), isotonic agents, absorption delaying agents, salts, drugs, drug stabilizers, binders, excipients, disintegration agents, lubricants, sweetening agents, flavoring agents, dyes, such like materials and combinations thereof, as would be known to one of ordinary skill in the art (see, for example, Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, pp. 1289- 1329, incorporated herein by reference). Except in so far as any conventional carrier is incompatible with the active ingredient, its use in the therapeutic or pharmaceutical compositions is contemplated.

[0079] As used herein, the term "subject" refers to an animal. Preferably, the animal is a mammal. A subject also refers to for example, primates (e.g., humans), cows, sheep, goats, horses, dogs, cats, rabbits, rats, mice, fish, birds and the like. In a preferred embodiment, the subject is a human.

[0080] As used herein, the term "therapeutic combination" or "combination" refers to a combination of one or more active drug substances, i.e., compounds having a therapeutic utility. Typically, each such compound in the therapeutic combinations of the present invention will be present in a pharmaceutical composition comprising that compound and a pharmaceutically acceptable carrier. The compounds in a therapeutic combination of the present invention may be administered simultaneously or separately, as part of a regimen.

II. Compositions

[0081] In general, the present invention provides therapeutic combinations, pharmaceutical compostions, and methods for treating cancers using combination therapy. More specifically, the combination of immunotherapy (such as using Toll-like Receptor Ligand "TLRL" to activate DCs in innate immunity and link to adaptive immunity) and targeted therapy (such as antibodies against tumor antigens, e.g., Her-2 or EGFR) are used to treate cancers such as gastric cancer and lung cancers.

[0082] In one aspect, the present invention provides therapeutic combinations, or pharmaceutical compositions, comprising: (i) an effective amount of a targeted therapeutic against a cancer; (ii) an effective amount of an immunotherapeutic that is capable of activating a human dendritic cell, NK cell, Monocyte, Macrophage or tumor cell, or a combination thereof; and optionally (iii) one or more pharmaceutically acceptable carriers.

[0083] Atherapeutic combination may be provided in a single pharmaceutical composition so that both the targeted therapeutics and the immunotherapeutic can be administered together. In alternative embodiments, a therapetuic combination may be provided using more than one pharmaceutical composition. In such embodiments, a targeted therapeutic may be provided in one pharmaceutical composition and an immunotherapeutic may be provided in a second pharmaceutical composition so that the two compounds can be administered separately such as, for example, at different times, by different routes of administration, and the like. Thus, it also may be possible to provide the targeted therapeutic and the immunotherapeutic in different dosing regimens.

[0084] Unless otherwise indicated, reference to a compound can include the compound in any pharmaceutically acceptable form, including any isomer (e.g., diastereomer or enantiomer), salt, solvate, polymorph, and the like. In particular, if a compound is optically active, reference to the compound can include each of the compound's enantiomers as well as racemic mixtures of the enantiomers.

[0085] In general, the targeted therapeutics and the immunotherapeutics are not linked to each other, such as by a covalent linker.

A. Targeted Therapeutics

[0086] In general, the compositons provided herein comprise a targeted therapeutic.

[0087] By "targeted therapeutic" herein is meant a therapeutic agent that binds specifically or selectively to a target molecule, cell, particle, tissue or aggregate, which generally is referred to as a "target" or a "marker," and these are discussed in further detail herein.

[0088] By "therapeutic agent" herein is meant an agent that has therapeutic effect, such as in ameliorating or treating cancers.

[0089] In some embodiments, the targeted therapeutic comprises an immunoglobulin, a protein, a peptide, a small molecule, a nanoparticle, or a nucleic acid.

[0090] In some embodiments, the targeted therapeutic comprises an antibody drug conjugate (ADC), as provided herein.

[0091] In some embodiments, the targeting therapeutic comprises an immunoglobulin, a protein, or a peptide, but does not contain a small molecule.

[0092] In some embodiments, the targeted therapeutic is not an ADC.

[0093] Exemplary targeted therapetutic such as antibodies (e.g., chimeric, humanized and human) are recognized in the art and are useful without limitation in practicing the present invention.

[0094] In some embodiments, a targeted therapeutic agent is an antibody, antibody fragment, bispecific antibody or other antibody-based molecule or compound.

[0095] In some embodiments, a targeted therapeutic agent is an aptamers, avimers, receptor-binding ligands, nucleic acids, biotin-avidin binding pairs, binding peptides or proteins, etc that both binds specifically or preferably to a target molecule and has therapeutic effect such as against cancers or tumors.

[0096] By "target" or "marker" herein is meant any entity that is capable of specifically binding to a particular targeted therapeutic, such as Her2/Neu. In some embodiments, targets are specifically associated with one or more particular cell or tissue types. In some embodiments, targets are specifically associated with one or more particular disease states. In some embodiments, targets are specifically associated with one or more particular developmental stages. For example, a cell type specific marker is typically expressed at levels at least 2 fold greater in that cell type than in a reference population of cells. In some embodiments, the cell type specific marker is present at levels at least 3 fold, at least 4 fold, at least 5 fold, at least 6 fold, at least 7 fold, at least 8 fold, at least 9 fold, at least 10 fold, at least 50 fold, at least 100 fold, or at least 1,000 fold greater than its average expression in a reference population. Detection or measurement of a cell type specific marker may make it possible to distinguish the cell type or types of interest from cells of many, most, or all other types. In some embodiments, a target can comprise a protein, a carbohydrate, a lipid, and/or a nucleic acid, as described herein.

[0097] By "specifically binds" or "preferably binds" herein is meant that the binding between two binding partners (e.g., between a targeting moiety and its binding partner) is selective for the two binding partners and can be discriminated from unwanted or non-specific interactions. For example, the ability of an antigen-binding moiety to bind to a specific antigenic determinant can be measured either through an enzyme- linked immunosorbent assay (ELISA) or other techniques familiar to one of skill in the art, e.g. surface plasmon resonance technique (analyzed on a BIAcore instrument) (Liljeblad et al., Glyco J 17, 323-329 (2000)), and traditional binding assays (Heeley, Endocr Res 28, 217-229 (2002)). The terms "anti- [antigen] antibody" and "an antibody that binds to [antigen]" refer to an antibody that is capable of binding the respective antigen with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting the antigen. In some embodiments, the extent of binding of an anti-

[antigen] antibody to an unrelated protein is less than about 10% of the binding of the antibody to the antigen as measured, e.g., by a radioimmunoassay (RIA). In some embodiments, an antibody that binds to[antigen has a dissociation constant (KD) of < IμM, < 100 nM, < 10 nM, < 1 nM, < 0.1 nM, < 0.01 nM, or < 0.001 nM (e.g. 10^{-8} M or less, e.g. from 10^{-8} M to 10^{-13} M, e.g., from 10^{-9} M to 10^{-13} M). It is understood that the above definition is also applicable to antigen-binding moieties that bind to an antigen.

[0098] In certain specific embodiments, a target is a tumor marker. In some embodiments, a tumor marker is an antigen that is present in a tumor that is not present in normal organs, tissues, and/or cells. In some embodiments, a tumor marker is an antigen that is more prevalent in a tumor than in normal organs, tissues, and/or cells. In some embodiments, a tumor marker is an antigen that is more prevalent in malignant cancer cells than in normal cells.

[0099] By "tumor antigen" herein is meant an antigenic substance produced in tumor cells, i.e., it triggers an immune response in the host. Normal proteins in the body are not antigenic because of self-tolerance, a process in which self-reacting cytotoxic T lymphocytes (CTLs) and autoantibody-producing B lymphocytes are culled "centrally" in primary lymphatic tissue (BM) and "peripherally" in secondary lymphatic tissue (mostly thymus for T-cells and spleen/lymph nodes for B cells). Thus any protein that is not exposed to the immune system triggers an immune response. This may include normal proteins that are well sequestered from the immune system, proteins that are normally produced in extremely small quantities, proteins that are normally produced only in certain stages of development, or proteins whose structure is modified due to mutation.

[0100] In some embodiments, a target is preferentially expressed in tumor tissues and/or cells versus normal tissues and/or cells.

[0101] In some embodiments of the invention a marker is a tumor marker. The marker may be a polypeptide that is expressed at higher levels on dividing than on non-dividing cells. For example, Her-2/neu (also known as ErbB-2) is a member of the EGF receptor family and is expressed on the cell surface of tumors associated with breast cancer. Another example is a peptide known as F3 that is a suitable targeting agent for directing a nanoparticle to nucleolin (Porkka et al., 2002, Proc. Natl. Acad. Sci., USA, 99:7444; and Christian et al., 2003, J. Cell Biol., 163:871). It has been shown that targeted particles comprising a nanoparticle and the A10 aptamer (which specifically binds to PSMA) were able to specifically and effectively deliver docetaxel to prostate cancer tumors.

[0102] Antibodies or other drug that specifically target these tumor targets specifically interfere with and regulate signaling pathways of the biological behavior of tumor cells regulate directly, or block signaling pathway to inhibit tumor cell growth or induce apoptosis. To date, there are dozens of target

drugs have been approved for solid tumors or hematological malignancies clinical research and treatment, and there are number of targeted drugs for hematological malignancies.

[0103] In some embodiments, the tumor antigen (or tumor target) is selected from the group consisting of: CD2, CD19, CD20, CD22, CD27, CD33, CD37, CD38, CD40, CD44, CD47, CD52, CD56, CD70, CD79, and CD137.

[0104] In some embodiments, the tumor antigen (or tumor target) is selected from the group consisting of: 4-1BB, 5T4, AGS-5, AGS-16, Angiopoietin 2, B7.1, B7.2, B7DC, B7H1, B7H2, B7H3, BT-062, *BTLA,* CAIX, Carcinoembryonic antigen, CTLA4, Cripto, ED-B, ErbB1, ErbB2, ErbB3, ErbB4, EGFL7, EpCAM, EphA2, EphA3, EphB2, FAP, Fibronectin, Folate Receptor, Ganglioside GM3, GD2, glucocorticoid-induced tumor necrosis factor receptor (GITR), gp100, gpA33, GPNMB, ICOS, IGF1R, Integrin αv, Integrin $\alpha v\beta$, KIR, LAG-3, Lewis Y antigen, Mesothelin, c-MET, MN Carbonic anhydrase IX, MUC1, MUC16, Nectin-4, NKGD2, NOTCH，OX40, OX40L, PD-1, PDL1, PSCA, PSMA, RANKL, ROR1, ROR2, SLC44A4, Syndecan-1, TACI, TAG-72, Tenascin, TIM3, TRAILR1, TRAILR2, VEGFR-1, VEGFR-2, VEGFR-3, and variants thereof. The variants of the tumor antigen encompass various mutants or polympormisms known in the art and/or naturally occurred.

[0105] In some embodiments, the targeted therapeutic comprises an antibody, or a functional fragment thereof.

[0106] By immunoglobulin" or "antibody" herein is meant a full-length (i.e., naturally occurring or formed by normal immunoglobulin gene fragment recombinatorial processes) immunoglobulin molecule (e.g., an IgG antibody) or an immunologically active (i.e., specifically binding) portion of an immunoglobulin molecule, like an antibody fragment. An antibody or antibody fragment may be conjugated or otherwise derivatized within the scope of the claimed subject matter. Such antibodies include IgGl, lgG2a, IgG3, IgG4 (and IgG4 subforms), as well as IgA isotypes.

[0107] The term "antibody" herein is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g. bispecific antibodies), and antibody fragments so long as they exhibit the desired antigen-binding activity and comprise an Fc region or a region equivalent to the Fc region of an immunoglobulin The terms "full-length antibody", "intact antibody", "and "whole antibody" are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure or having heavy chains that contain an Fc region as defined herein.

[0108] By "native antibodies" herein is meant naturally occurring immunoglobulin molecules with varying structures. For example, native IgG antibodies are heterotetrameric glycoproteins of about

150,000 daltons, composed of two identical light chains and two identical heavy chains that are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable region (VH), also called a variable heavy domain or a heavy chain variable domain, followed by three constant domains (CHI, CH2, and CH3), also called a heavy chain constant region. Similarly, from N- to C-terminus, each light chain has a variable region (VL), also called a variable light domain or a light chain variable domain, followed by a constant light (CL) domain, also called a light chain constant region. The light chain of an antibody may be assigned to one of two types, called kappa (κ) and lambda (λ), based on the amino acid sequence of its constant domain.

[0109] By "antibody fragment" herein is meant a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, F(ab')2, diabodies, linear antibodies, single-chain antibody molecules (e.g. scFv), single-domain antibodies, and multispecific antibodies formed from antibody fragments. For a review of certain antibody fragments, see Hudson et al., Nat Med 9, 129-134 (2003). For a review of scFv fragments, see e.g. Pliickthun, in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds., Springer- Verlag, New York, pp. 269-315 (1994); see also WO 93/16185; and U.S. Patent Nos. 5,571,894 and 5,587,458. For discussion of Fab and F(ab')2 fragments comprising salvage receptor binding epitope residues and having increased in vivo half-life, see U.S. Patent No. 5,869,046. Diabodies are antibody fragments with two antigen-binding sites that may be bivalent or bispecific. See, for example, EP 404,097; WO 1993/01161; Hudson et al., Nat Med 9, 129- 134 (2003); and Hollinger et al., Proc Natl Acad Sci USA 90, 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al., Nat Med 9, 129-134 (2003). Single-domain antibodies are antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, MA; see e.g. U.S. Patent No. 6,248,516 B l). Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells (e.g. E. coli or phage), as described herein.

[0110] By "antigen binding domain" herein is meant the part of an antibody that comprises the area which specifically binds to and is complementary to part or all of an antigen. An antigen binding domain may be provided by, for example, one or more antibody variable domains (also called antibody variable regions). Particularly, an antigen binding domain comprises an antibody light chain variable region (VL) and an antibody heavy chain variable region (VH).

[0111] By "variable region" or "variable domain" herein is meant the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (HVRs). See, e.g., Kindt et al., Kuby Immunology, 6th ed., W.H. Freeman and Co., page 91 (2007). A single VH or VL domain may be sufficient to confer antigen-binding specificity.

[0112] By "hypervariable region" or "HVR" herein is meant each of the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops ""hypervariable loops"). Generally, native four-chain antibodies comprise six HVRs; three in the VH (HI, H2, H3), and three in the VL (LI, L2, L3). HVRs generally comprise amino acid residues from the hypervariable loops and/or from the complementarity determining regions (CDRs), the latter being of highest sequence variability and/or involved in antigen recognition. With the exception of CDRl in VH, CDRs generally comprise the amino acid residues that form the hypervariable loops. Hypervariable regions (HVRs) are also referred to as "complementarity determining regions" (CDRs), and these terms are used herein interchangeably in reference to portions of the variable region that form the antigen binding regions. This particular region has been described by Kabat et al., U.S. Dept. of Health and Human Services, Sequences of Proteins of Immunological Interest (1983) and by Chothia et al., J Mol Biol 196:901-917 (1987), where the definitions include overlapping or subsets of amino acid residues when compared against each other. Nevertheless, application of either definition to refer to a CDR of an antibody or variants thereof is intended to be within the scope of the term as defined and used herein. The exact residue numbers which encompass a particular CDR will vary depending on the sequence and size of the CDR. Those skilled in the art can routinely determine which residues comprise a particular CDR given the variable region amino acid sequence of the antibody.

[0113] The antibody of the present invention can be chimeric antibodies, humanized antibodies, human antibodies, or antibody fusion proteins.

[0114] By "chimeric antibody" herein is meant a recombinant protein that contains the variable domains of both the heavy and light antibody chains, including the complementarity determining regions (CDRs) of an antibody derived from one species, preferably a rodent antibody, more preferably a murine antibody, while the constant domains of the antibody molecule are derived from those of a human antibody. For veterinary applications, the constant domains of the chimeric antibody may be derived from that of other species, such as a subhuman primate, cat or dog.

[0115] By "humanized antibody" herein is meant a recombinant protein in which the CDRs from an antibody from one species; e.g., a rodent antibody, are transferred from the heavy and light variable

chains of the rodent antibody into human heavy and light variable domains. The constant domains of the antibody molecule are derived from those of a human antibody. In some embodiments, specific residues of the framework region of the humanized antibody, particularly those that are touching or close to the CDR sequences, may be modified, for example replaced with the corresponding residues from the original rodent, subhuman primate, or other antibody.

[0116] By "human antibody" herein is meant an antibody obtained, for example, from transgenic mice that have been "engineered" to produce specific human antibodies in response to antigenic challenge. In this technique, elements of the human heavy and light chain locus are introduced into strains of mice derived from embryonic stem cell lines that contain targeted disruptions of the endogenous heavy chain and light chain loci. The transgenic mice can synthesize human antibodies specific for human antigens, and the mice can be used to produce human antibody-secreting hybridomas. Methods for obtaining human antibodies from transgenic mice are described by Green et al, Nature Genet. 7: 13 (1994), Lonberg et al, Nature 368:856 (1994), and Taylor et al, Int. Immun. 6:579 (1994). A fully human antibody also can be constructed by genetic or chromosomal transfection methods, as well as phage display technology, all of which are known in the art. See for example, McCafferty et al, Nature 348:552-553 (1990) for the production of human antibodies and fragments thereof in vitro, from immunoglobulin variable domain gene repertoires from unimmunized donors. In this technique, antibody variable domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. In this way, the phage mimics some of the properties of the B cell. Phage display can be performed in a variety of formats, for their review, see e.g. Johnson and Chiswell, Current Opinion in Structural Biology 3:5564-571 (1993). Human antibodies may also be generated by in vitro activated B cells. See U.S. Patent Nos. 5,567,610 and 5,229,275, which are incorporated herein by reference in their entirety.

[0117] By "antibody fusion protein" herein is meant a recombinantly-produced antigen- binding molecule in which two or more of the same or different natural antibody, single-chain antibody or antibody fragment segments with the same or different specificities are linked. A fusion protein comprises at least one specific binding site. Valency of the fusion protein indicates the total number of binding arms or sites the fusion protein has to antigen(s) or epitope(s); i.e., monovalent, bivalent, trivalent or mutlivalent. The multivalency of the antibody fusion protein means that it can take advantage of multiple interactions in binding to an antigen, thus increasing the avidity of binding to the antigen, or to different antigens. Specificity indicates how many different types of antigen or epitope an antibody

fusion protein is able to bind; i.e., monospecific, bispecific, trispecific, multispecific. Using these definitions, a natural antibody, e.g., an IgG, is bivalent because it has two binding arms but is monospecific because it binds to one type of antigen or epitope. A monospecific, multivalent fusion protein has more than one binding site for the same antigen or epitope. For example, a monospecific diabody is a fusion protein with two binding sites reactive with the same antigen. The fusion protein may comprise a multivalent or multispecific combination of different antibody components or multiple copies of the same antibody component. The fusion protein may additionally comprise a therapeutic agent.

[0118] In some embodiments, the targeting moiety comprises a probody, such as those disclosed in US Patent Nos,: 8,518,404;8,513,390; and US Pat. Appl. Pub. Nos.; 20120237977A1, 20120149061A1, 20130150558A1, the disclosures of which are incorporated by reference in their entireties.

[0119] Probodies are monoclonal antibodies that are selectively activated within the cancer microenvironment, focusing the activity of therapeutic antibodies to tumors and sparing healthy tissue.

[0120] In general, the porbody comprises at least an antibody or antibody fragment thereof (collectively referred to as "AB"), capable of specifically binding a target, wherein the AB is modified by a masking moiety (MM). When the AB is modified with a MM and is in the presence of the target, specific binding of the AB to its target is reduced or inhibited, as compared to the specific binding of the AB not modified with an MM or the specific binding of the parental AB to the target. The dissociation constant (Kd) of the MM towards the AB is generally greater than the Kd of the AB towards the target. When the AB is modified with a MM and is in the presence of the target, specific binding of the AB to its target can be reduced or inhibited, as compared to the specific binding of the AB not modified with an MM or the specific binding of the parental AB to the target. When an AB is coupled to or modified by a MM, the MM can 'mask' or reduce, or inhibit the specific binding of the AB to its target. When an AB is coupled to or modified by a MM, such coupling or modification can effect a structural change which reduces or inhibits the ability of the AB to specifically bind its target.

[0121] In some embodiments, the probody is an activatable antibodies (AAs) where the AB modified by an MM can further include one or more cleavable moieties (CM). Such AAs exhibit activatable/switchable binding, to the AB's target. AAs generally include an antibody or antibody fragment (AB), modified by or coupled to a masking moiety (MM) and a modifiable or cleavable moiety (CM). In some embodiments, the CM contains an amino acid sequence that serves as a substrate for a protease of interest. In other embodiments, the CM provides a cysteine-cysteine disulfide bond that is cleavable by reduction. In yet other embodiments the CM provides a photolytic substrate that is activatable by photolysis.

[0122] The CM and AB of the AA may be selected so that the AB represents a binding moiety for a target of interest, and the CM represents a substrate for a protease that is co-localized with the target at a treatment site in a subject. Alternatively or in addition, the CM is a cysteine-cysteine disulfide bond that is cleavable as a result of reduction of this disulfide bond. AAs contain at least one of a protease-cleavable CM or a cysteine-cysteine disulfide bond, and in some embodiments include both kinds of CMs. The AAs can alternatively or further include a photolabile substrate, activatable by a light source. The AAs disclosed herein find particular use where, for example, a protease capable of cleaving a site in the CM is present at relatively higher levels in target-containing tissue of a treatment site (for example diseased tissue; for example for therapeutic treatment or diagnostic treatment) than in tissue of non-treatment sites (for example in healthy tissue). The AAs disclosed herein also find particular use where, for example, a reducing agent capable of reducing a site in the CM is present at relatively higher levels in target-containing tissue of a treatment or diagnostic site than in tissue of non-treatment non-diagnostic sites. The AAs disclosed herein also find particular use where, for example, a light source, for example, by way of laser, capable of photolysing a site in the CM is introduced to a target-containing tissue of a treatment or diagnostic site.

[0123] In some embodiments, AAs can provide for reduced toxicity and/or adverse side effects that could otherwise result from binding of the AB at non-treatment sites if the AB were not masked or otherwise inhibited from binding its target. Where the AA contains a CM that is cleavable by a reducing agent that facilitates reduction of a disulfide bond, the ABs of such AAs may selected to exploit activation of an AB where a target of interest is present at a desired treatment site characterized by elevated levels of a reducing agent, such that the environment is of a higher reduction potential than, for example, an environment of a non-treatment site.

[0124] In general, an AA can be designed by selecting an AB of interest and constructing the remainder of the AA so that, when conformationally constrained, the MM provides for masking of the AB or reduction of binding of the AB to its target. Structural design criteria to be taken into account to provide for this functional feature.

[0125] In some embodiments, the targeted therapeutic is an antibody, or antibody fragment, that is selected based on its specificity for an antigen expressed on a target cell, or at a target site, of interest. A wide variety of tumor-specific or other disease-specific antigens have been identified and antibodies to those antigens have been used or proposed for use in the treatment of such tumors or other diseases. The antibodies that are known in the art can be used in the compositions of the invention, in particular for the treatment of the disease with which the target antigen is associated. Examples of target antigens (and their associated diseases) to which a target therapeutic of the invention can be targeted include: CD2,

CD19, CD20, CD22, CD27, CD33, CD37, CD38, CD40, CD44, CD47, CD52, CD56, CD70, CD79, CD137, 4-1BB, 5T4，AGS-5，AGS-16, Angiopoietin 2, B7.1, B7.2, B7DC, B7H1, B7H2, B7H3, BT-062, BTLA, CAIX, Carcinoembryonic antigen, CTLA4, Cripto, ED-B, ErbB1, ErbB2, ErbB3, ErbB4, EGFL7, EpCAM, EphA2, EphA3, EphB2, FAP, Fibronectin, Folate Receptor, Ganglioside GM3, GD2, glucocorticoid-induced tumor necrosis factor receptor (GITR), gp100, gpA33, GPNMB, ICOS, IGF1R, Integrin αv, Integrin $\alpha v\beta$, KIR, LAG-3, Lewis Y, Mesothelin, c-MET, MN Carbonic anhydrase IX, MUC1, MUC16, Nectin-4, NKGD2, NOTCH，OX40, OX40L, PD-1, PDL1, PSCA, PSMA, RANKL, ROR1, ROR2, SLC44A4, Syndecan-1, TACI, TAG-72, Tenascin, TIM3, TRAILR1, TRAILR2,VEGFR-1, VEGFR-2, VEGFR-3.

[0126] In some embodiments, the antibody is selected from the group consisting of: Rituxan (rituximab), Herceptin (trastuzumab), Erbitux (cetuximab), Vectibix (Panitumumab), Arzerra (Ofatumumab), Benlysta (belimumab), Yervoy (ipilimumab), Perjeta (Pertuzumab), Tremelimumab, Opdivo (Nivolumab, ONO-4538, BMS-936558, or MDX1106), Dacetuzumab, Urelumab, MPDL3280A, Lambrolizumab, and Blinatumomab, CT-011, Keytruda (pembrolizumab, MK-3475), BMS-936559, MPDL3280A, MED14736, or MSB0010718C.

[0127] Rituxan (Rituximab) is a chimeric antibody used for the treatment of B-cell non-Hodgkin's lymphoma. It acts on the surface of B cells expressing the CD20 antigen that is expressed on 90% of B-cell non-Hodgkin's lymphoma. Rituxan binds CD20 to induce B cell lysis through CDC and ADCC, as well as sensitize human lymphocytes that are drug resistance for some cytotoxic chemotherapeutics.

[0128] Herceptin (Trastuzumab) is a humanized monoclonal antibody that acts on human epidermal growth factor receptor extracellular domain of Her2, which is expressed in 25% -30% of breast cancer. It is believed that Trastuzumab has anti-tumor effect through (1) down-regulation Her2 receptor, inhibition of Her2 intracellular signaling transduction pathways and induction of apoptosis; (2) immune mechanisms related antibody dependent ADCC and CDC to kill tumor cells; (3) enhance the effects of chemotherapy.

[0129] Erbitux (Cetuximab) is a chimeric antibody that acts on epidermal growth factor receptor (EGFR). Erbitux binds EGFR to inhibit its signal transduction pathway, affecting cell proliferation, invasion and metastasis, and angiogenesis. Inhibition of EGFR signal transduction pathway can enhance chemotherapy drugs and radiation therapy efficacy.

[0130] Avastin (Bevacizumab) is a humanized monoclonal antibody that targets vascular endothelial growth factor (VEGF). Its binding of VEGFR inhibits VEGF and signal transduction, resulting in inhibition of tumor angiogenesis.

[0131] Other antibodies that currently under development can also be used as targeted therapeutic. For example, therapeutic monoclonal antibodies against the following targets are under development for treatment of tumors: CD2, CD19, CD20, CD22, CD27, CD33, CD37, CD38, CD40, CD44, CD47, CD52, CD56, CD70, CD79, and CD137 and the following targets for treatment of tumors: 4-1BB, 5T4，AGS-5，AGS-16, Angiopoietin 2, B7.1, B7.2, B7DC, B7H1, B7H2, B7H3, BT-062, BTLA, CAIX, Carcinoembryonic antigen, CTLA4, Cripto, ED-B, ErbB1, ErbB2, ErbB3, ErbB4, EGFL7, EpCAM, EphA2, EphA3, EphB2, FAP, Fibronectin, Folate Receptor, Ganglioside GM3, GD2, glucocorticoid-induced tumor necrosis factor receptor (GITR), gp100, gpA33, GPNMB, ICOS, IGF1R, Integrin αv, Integrin αvβ , KIR, LAG-3, Lewis, Mesothelin, c-MET, MN Carbonic anhydrase IX, MUC1, MUC16, Nectin-4, NKGD2, NOTCH，OX40, OX40L, PD-1, PDL1, PSCA, PSMA, RANKL, ROR1, ROR2, SLC44A4, Syndecan-1, TACI, TAG-72, Tenascin, TIM3, TRAILR1, TRAILR2, VEGFR-1, VEGFR-2, and VEGFR-3 and their variant. (Scott AM, Wolchok JD, Old LJ. Antibody Therapy of Cancer. Nat Rev Cancer. 2012 Mar 22;12(4):278-87).

[0132] In some embodiments, the targeted therapeutic comprises a Fab, Fab', F(ab')2, single domain antibody, T and Abs dimer, Fv, scFv, dsFv, ds-scFv, Fd, linear antibody, minibody, diabody, bispecific antibody fragment, bibody, tribody, sc-diabody, kappa (lamda) body, BiTE, DVD-Ig, SIP, SMIP, DART, or an antibody analogue comprising one or more CDRs.

[0133] The following table shows the various antibody structures and the targets being studied.

Table 1

Antibody Structure	Exemplary Target
scFv	CC49, ERBB2, Ley
Diabody	Ley and TAG-72
Affibody	ERBB2
Minibody	CEA, ERBB2
Protein–Fc	Angiopoietin 1, angiopoietin 2, VEGFR1, VEGFR2
Intact IgG	CD20, CD33, EGFR, ERBB2, VEGF
IgE and IgM	GM2
Drug conjugates	CD30, CD33 and ERBB2
Loaded nanoparticles	A33, EGFR and transferrin
Bispecifics	CD19–CD3, EPCAM–CD3, gp100–CD3

Targeted Therapeutics Comprising a Targeting Moiety

[0134] In some aspects, the targeted therapeutic of the present invention comprise a targeting moiety, such as an ADC.

[0135] By "targeting moiety (TM)" or "targeting agent" here in is meant a molecule, complex, or aggregate, that binds specifically or selectively to a target molecule, cell, particle, tissue or aggregate, which generally is referred to as a "target" or a "marker," and these are discussed in further detail herein.

[0136] In some embodiments, the targeting moiety comprises an immunoglobulin, a protein, a peptide, a small molecule, a nanoparticle, or a nucleic acid.

[0137] Exemplary targeting agents such as antibodies (e.g., chimeric, humanized and human), ligands for receptors, lecitins, and saccharides, and substrate for certain enzymes are recognized in the art and are useful without limitation in practicing the present invention. Other targeting agents include a class of compounds that do not include specific molecular recognition motifs include nanoparticles, macromolecules such as poly(ethylene glycol), polysaccharide, and polyamino acids which add molecular mass to the activating moiety. The additional molecular mass affects the pharmacokinetics of the activating moiety, e.g., serum half-life.

[0138] In some embodiments, a targeting moiety is an antibody, antibody fragment, bispecific antibody or other antibody-based molecule or compound. However, other examples of targeting moieties are known in the art and may be used, such as aptamers, avimers, receptor-binding ligands, nucleic acids, biotin-avidin binding pairs, binding peptides or proteins, etc. The terms "targeting moiety" and "binding moiety" are used synonymously herein.

[0139] By "target" or "marker" herein is meant any entity that is capable of specifically binding to a particular targeting moiety. In some embodiments, targets are specifically associated with one or more particular cell or tissue types. In some embodiments, targets are specifically associated with one or more particular disease states. In some embodiments, targets are specifically associated with one or more particular developmental stages. For example, a cell type specific marker is typically expressed at levels at least 2 fold greater in that cell type than in a reference population of cells. In some embodiments, the cell type specific marker is present at levels at least 3 fold, at least 4 fold, at least 5 fold, at least 6 fold, at least 7 fold, at least 8 fold, at least 9 fold, at least 10 fold, at least 50 fold, at least 100 fold, or at least 1,000 fold greater than its average expression in a reference population. Detection or measurement of a cell type specific marker may make it possible to distinguish the cell type or types of interest from cells of

many, most, or all other types. In some embodiments, a target can comprise a protein, a carbohydrate, a lipid, and/or a nucleic acid, as described herein.

[0140] A substance is considered to be "targeted" for the purposes described herein if it specifically binds to a nucleic acid targeting moiety. In some embodiments, a nucleic acid targeting moiety specifically binds to a target under stringent conditions. An inventive complex or compound comprising targeting moiety is considered to be "targeted" if the targeting moiety specifically binds to a target, thereby delivering the entire complex or compound composition to a specific organ, tissue, cell, extracellular matrix component, and/or intracellular compartment.

[0141] In certain embodiments, compound in accordance with the present invention comprise a targeting moiety which specifically binds to one or more targets (e.g. antigens) associated with an organ, tissue, cell, extracellular matrix component, and/or intracellular compartment. In some embodiments, compounds comprise a targeting moiety which specifically binds to targets associated with a particular organ or organ system. In some embodiments, compounds in accordance with the present invention comprise a nuclei targeting moiety which specifically binds to one or more intracellular targets (e.g. organelle, intracellular protein). In some embodiments, compounds comprise a targeting moiety which specifically binds to targets associated with diseased organs, tissues, cells, extracellular matrix components, and/or intracellular compartments. In some embodiments, compounds comprise a targeting moiety which specifically binds to targets associated with particular cell types (e.g. endothelial cells, cancer cells, malignant cells, prostate cancer cells, etc.).

[0142] In some embodiments, compounds in accordance with the present invention comprise a targeting moiety which binds to a target that is specific for one or more particular tissue types (e.g. liver tissue vs. prostate tissue). In some embodiments, compounds in accordance with the present invention comprise a targeting moiety which binds to a target that is specific for one or more particular cell types (e.g. T cells vs. B cells). In some embodiments, compounds in accordance with the present invention comprise a targeting moiety which binds to a target that is specific for one or more particular disease states (e.g. tumor cells vs. healthy cells). In some embodiments, compounds in accordance with the present invention comprise a targeting moiety which binds to a target that is specific for one or more particular developmental stages (e.g. stem cells vs. differentiated cells).

[0143] In some embodiments, a target may be a marker that is exclusively or primarily associated with one or a few cell types, with one or a few diseases, and/or with one or a few developmental stages. A cell type specific marker is typically expressed at levels at least 2 fold greater in that cell type than in a reference population of cells which may consist, for example, of a mixture containing cells from a plurality (e.g., 5-10 or more) of different tissues or organs in approximately equal amounts. In some

embodiments, the cell type specific marker is present at levels at least 3 fold, at least 4 fold, at least 5 fold, at least 6 fold, at least 7 fold, at least 8 fold, at least 9 fold, at least 10 fold, at least 50 fold, at least 100 fold, or at least 1000 fold greater than its average expression in a reference population. Detection or measurement of a cell type specific marker may make it possible to distinguish the cell type or types of interest from cells of many, most, or all other types.

[0144] In some embodiments, a target comprises a protein, a carbohydrate, a lipid, and/or a nucleic acid. In some embodiments, a target comprises a protein and/or characteristic portion thereof, such as a tumor-marker, integrin, cell surface receptor, transmembrane protein, intercellular protein, ion channel, membrane transporter protein, enzyme, antibody, chimeric protein, glycoprotein, etc. In some embodiments, a target comprises a carbohydrate and/or characteristic portion thereof, such as a glycoprotein, sugar (e.g., monosaccharide, disaccharide, polysaccharide), glycocalyx (i.e., the carbohydrate-rich peripheral zone on the outside surface of most eukaryotic cells) etc. In some embodiments, a target comprises a lipid and/or characteristic portion thereof, such as an oil, fatty acid, glyceride, hormone, steroid (e.g., cholesterol, bile acid), vitamin (e.g. vitamin E), phospholipid, sphingolipid, lipoprotein, etc. In some embodiments, a target comprises a nucleic acid and/or characteristic portion thereof, such as a DNA nucleic acid; RNA nucleic acid; modified DNA nucleic acid; modified RNA nucleic acid; nucleic acid that includes any combination of DNA, RNA, modified DNA, and modified RNA.

[0145] Numerous markers are known in the art. Typical markers include cell surface proteins, e.g., receptors. Exemplary receptors include, but are not limited to, the transferrin receptor; LDL receptor; growth factor receptors such as epidermal growth factor receptor family members (e.g., EGFR, Her2, Her3, Her4) or vascular endothelial growth factor receptors, cytokine receptors, cell adhesion molecules, integrins, selectins, and CD molecules. The marker can be a molecule that is present exclusively or in higher amounts on a malignant cell, e.g., a tumor antigen.

[0146] In some embodiments, the targeting moiety binds to a tumor cell specifically or preferably in comparison to a non-tumor cell.

[0147] The binding of target moiety to tumor cell can be measured using assays known in the art.

[0148] In some embodiments, the tumor cell is of a carcinoma, a sarcoma, a lymphoma, a myeloma, or a central nervous system cancer.

[0149] In some embodiments, the targeting moiety is capable of binding to a tumor antigen specifically or preferably in comparison to a non-tumor antigen.

[0150] In certain specific embodiments, a target is a tumor marker. In some embodiments, a tumor marker is an antigen that is present in a tumor that is not present in normal organs, tissues, and/or cells. In some embodiments, a tumor marker is an antigen that is more prevalent in a tumor than in normal organs, tissues, and/or cells. In some embodiments, a tumor marker is an antigen that is more prevalent in malignant cancer cells than in normal cells.

[0151] In some embodiments, the targeting moiety comprises folic acid or a derivative thereof.

[0152] In recent years, research on folic acid had made great progress. Folic acid is a small molecule vitamin that is necessary for cell division. Tumor cells divide abnormally and there is a high expression of folate receptor (FR) on tumor cell surface to capture enough folic acid to support cell division.

[0153] Data indicate FR expression in tumor cells is 20-200 times higher than normal cells. The expression rate of FR in various malignant tumors are: 82% in ovarian cancer, 66% in non-small cell lung cancer, 64% in kidney cancer, 34% in colon cancer, and 29% in breast cancer (Xia W, Low PS. Late-targeted therapies for cancer. J Med Chem. 2010; 14; 53 (19):6811-24). The expression rate of FA and the degree of malignancy of epithelial tumor invasion and metastasis is positively correlated. FA enters cell through FR mediated endocytosis, and FA through its carboxyl group forms FA complexes with drugs which enter the cells. Under acidic conditions (pH value of 5), FR separates from the FA, and FA releases drugs into the cytoplasm.

[0154] Clinically, the system can be used to deliver drugs selectively attack the tumor cells. Folic acid has small molecular weight, has non-immunogenicity and high stability, and is inexpensive to synthesis. More importantly, chemical coupling between the drug and the carrier is simple, and as such using FA as targeting molecule to construct drug delivery system has become a research hotspot for cancer treatment. Currently EC145 (FA chemotherapy drug conjugate compound) that is in clinical trials can effectively attack cancer cells (Pribble P and Edelman MJ. EC145: a novel targeted agent for adenocarcinoma of the lung. Expert Opin. Investig. Drugs (2012) 21:755-761).

[0155] In some embodiments, the targeting moiety comprises extracellular domains (ECD) or soluble form of PD-1, PDL-1, CTLA4, CD47, BTLA, KIR, TIM3, 4-1BB, and LAG3, full length of partial of a surface ligand Amphiregulin, Betacellulin, EGF, Ephrin, Epigen, Epiregulin, IGF, Neuregulin, TGF, TRAIL, or VEGF.

[0156] In some embodiments, the targeting moiety comprises a Fab, Fab', F(ab')2, single domain antibody, T and Abs dimer, Fv, scFv, dsFv, ds-scFv, Fd, linear antibody, minibody, diabody, bispecific antibody fragment, bibody, tribody, sc-diabody, kappa (lamda) body, BiTE, DVD-Ig, SIP, SMIP, DART, or an antibody analogue comprising one or more CDRs.

[0157] In some embodiments, the targeting moiety is an antibody, or antibody fragment, that is selected based on its specificity for an antigen expressed on a target cell, or at a target site, of interest. A wide variety of tumor-specific or other disease-specific antigens have been identified and antibodies to those antigens have been used or proposed for use in the treatment of such tumors or other diseases. The antibodies that are known in the art can be used in the compounds of the invention, in particular for the treatment of the disease with which the target antigen is associated. Examples of target antigens (and their associated diseases) to which an antibody-linker-drug conjugate of the invention can be targeted include: CD2, CD19, CD20, CD22, CD27, CD33, CD37, CD38, CD40, CD44, CD47, CD52, CD56, CD70, CD79, CD137, 4-1BB, 5T4，AGS-5，AGS-16, Angiopoietin 2, B7.1, B7.2, B7DC, B7H1, B7H2, B7H3, BT-062, *BTLA,* CAIX, Carcinoembryonic antigen, CTLA4, Cripto, ED-B, ErbB1, ErbB2, ErbB3, ErbB4, EGFL7, EpCAM, EphA2, EphA3, EphB2, FAP, Fibronectin, Folate Receptor, Ganglioside GM3, GD2, glucocorticoid-induced tumor necrosis factor receptor (GITR), gp100, gpA33, GPNMB, ICOS, IGF1R, Integrin αv, Integrin $\alpha v\beta$, KIR, LAG-3, Lewis Y, Mesothelin, c-MET, MN Carbonic anhydrase IX, MUC1, MUC16, Nectin-4, NKGD2, NOTCH，OX40, OX40L, PD-1, PDL1, PSCA, PSMA, RANKL, ROR1, ROR2, SLC44A4, Syndecan-1, TACI, TAG-72, Tenascin, TIM3, TRAILR1, TRAILR2,VEGFR-1, VEGFR-2, VEGFR-3.

[0158] In some embodiments, the targeting moiety comprises a particle (target particle), preferably a nanoparticle, optionally a targeted nanoparticle that attached to a targeting molecule that can binds specifically or preferably to a target. In some embodiments, the targeting particle by itself guides the compound of the present invention (such as by enrichment in tumor cells or tissue) and there is no additional targeting molecules attached therein.

[0159] By "nanoparticle" herein is meant any particle having a diameter of less than 1000 nm. In some embodiments, a therapeutic agent and/or targeting molecule can be associated with the polymeric matrix. In some embodiments, the targeting molecule can be covalently associated with the surface of a polymeric matrix. In some embodiments, covalent association is mediated by a linker. In some embodiments, the therapeutic agent can be associated with the surface of, encapsulated within, surrounded by, and/or dispersed throughout the polymeric matrix. US Pat. No. 8,246,968, which is incorporated by reference in its entirety.

[0160] In general, nanoparticles of the present invention comprise any type of particle. Any particle can be used in accordance with the present invention. In some embodiments, particles are biodegradable and biocompatible. In general, a biocompatible substance is not toxic to cells. In some embodiments, a substance is considered to be biocompatible if its addition to cells results in less than a certain threshold of cell death. In some embodiments, a substance is considered to be biocompatible if its addition to cells

does not induce adverse effects. In general, a biodegradable substance is one that undergoes breakdown under physiological conditions over the course of a therapeutically relevant time period (e.g., weeks, months, or years). In some embodiments, a biodegradable substance is a substance that can be broken down by cellular machinery. In some embodiments, a biodegradable substance is a substance that can be broken down by chemical processes. In some embodiments, a particle is a substance that is both biocompatible and biodegradable. In some embodiments, a particle is a substance that is biocompatible, but not biodegradable. In some embodiments, a particle is a substance that is biodegradable, but not biocompatible.

[0161] In some embodiments, particles are greater in size than the renal excretion limit (e.g. particles having diameters of greater than 6 nm). In some embodiments, particles are small enough to avoid clearance of particles from the bloodstream by the liver (e.g. particles having diameters of less than 1000 nm). In general, physiochemical features of particles should allow a targeted particle to circulate longer in plasma by decreasing renal excretion and liver clearance.

[0162] It is often desirable to use a population of particles that is relatively uniform in terms of size, shape, and/or composition so that each particle has similar properties. For example, at least 80%, at least 90%, or at least 95% of the particles may have a diameter or greatest dimension that falls within 5%, 10%, or 20% of the average diameter or greatest dimension. In some embodiments, a population of particles may be heterogeneous with respect to size, shape, and/or composition.

[0163] Zeta potential is a measurement of surface potential of a particle. In some embodiments, particles have a zeta potential ranging between −50 mV and +50 mV. In some embodiments, particles have a zeta potential ranging between −25 mV and +25 mV. In some embodiments, particles have a zeta potential ranging between −10 mV and +10 mV. In some embodiments, particles have a zeta potential ranging between −5 mV and +5 mV. In some embodiments, particles have a zeta potential ranging between 0 mV and +50 mV. In some embodiments, particles have a zeta potential ranging between 0 mV and +25 mV. In some embodiments, particles have a zeta potential ranging between 0 mV and +10 mV. In some embodiments, particles have a zeta potential ranging between 0 mV and +5 mV. In some embodiments, particles have a zeta potential ranging between −50 mV and 0 mV. In some embodiments, particles have a zeta potential ranging between −25 mV and 0 mV. In some embodiments, particles have a zeta potential ranging between −10 mV and 0 mV. In some embodiments, particles have a zeta potential ranging between −5 mV and 0 mV. In some embodiments, particles have a substantially neutral zeta potential (i.e. approximately 0 mV).

[0164] A variety of different particles can be used in accordance with the present invention. In some embodiments, particles are spheres or spheroids. In some embodiments, particles are spheres or spheroids.

In some embodiments, particles are flat or plate-shaped. In some embodiments, particles are cubes or cuboids. In some embodiments, particles are ovals or ellipses. In some embodiments, particles are cylinders, cones, or pyramids.

[0165] In some embodiments, particles are microparticles (e.g. microspheres). In general, a "microparticle" refers to any particle having a diameter of less than 1000 μm. In some embodiments, particles are picoparticles (e.g. picospheres). In general, a "picoparticle" refers to any particle having a diameter of less than 1 nm. In some embodiments, particles are liposomes. In some embodiments, particles are micelles.

[0166] Particles can be solid or hollow and can comprise one or more layers (e.g., nanoshells, nanorings). In some embodiments, each layer has a unique composition and unique properties relative to the other layer(s). For example, particles may have a core/shell structure, wherein the core is one layer and the shell is a second layer. Particles may comprise a plurality of different layers. In some embodiments, one layer may be substantially cross-linked, a second layer is not substantially cross-linked, and so forth. In some embodiments, one, a few, or all of the different layers may comprise one or more therapeutic or diagnostic agents to be delivered. In some embodiments, one layer comprises an agent to be delivered, a second layer does not comprise an agent to be delivered, and so forth. In some embodiments, each individual layer comprises a different agent or set of agents to be delivered.

[0167] In some embodiments, a particle is porous, by which is meant that the particle contains holes or channels, which are typically small compared with the size of a particle. For example a particle may be a porous silica particle, e.g., a mesoporous silica nanoparticle or may have a coating of mesoporous silica (Lin et al., 2005, J. Am. Chem. Soc., 17:4570). Particles may have pores ranging from about 1 nm to about 50 nm in diameter, e.g., between about 1 and 20 nm in diameter. Between about 10% and 95% of the volume of a particle may consist of voids within the pores or channels.

[0168] Particles may have a coating layer. Use of a biocompatible coating layer can be advantageous, e.g., if the particles contain materials that are toxic to cells. Suitable coating materials include, but are not limited to, natural proteins such as bovine serum albumin (BSA), biocompatible hydrophilic polymers such as polyethylene glycol (PEG) or a PEG derivative, phospholipid-(PEG), silica, lipids, polymers, carbohydrates such as dextran, other nanoparticles that can be associated with inventive nanoparticles etc. Coatings may be applied or assembled in a variety of ways such as by dipping, using a layer-by-layer technique, by self-assembly, conjugation, etc. Self-assembly refers to a process of spontaneous assembly of a higher order structure that relies on the natural attraction of the components of the higher order structure (e.g., molecules) for each other. It typically occurs through random movements of the molecules and formation of bonds based on size, shape, composition, or chemical properties.

[0169] Examples of polymers include polyalkylenes (e.g. polyethylenes), polycarbonates (e.g. poly(1,3-dioxan-2one)), polyanhydrides (e.g. poly(sebacic anhydride)), polyhydroxyacids (e.g. poly(β-hydroxyalkanoate)), polyfumarates, polycaprolactones, polyamides (e.g. polycaprolactam), polyacetals, polyethers, polyesters (e.g. polylactide, polyglycolide), poly(orthoesters), polyvinyl alcohols, polyurethanes, polyphosphazenes, polyacrylates, polymethacrylates, polycyanoacrylates, polyureas, polystyrenes, and polyamines. In some embodiments, polymers in accordance with the present invention include polymers which have been approved for use in humans by the U.S. Food and Drug Administration (FDA) under 21 C.F.R. §177.2600, including but not limited to polyesters (e.g. polylactic acid, polyglycolic acid, poly(lactic-co-glycolic acid), polycaprolactone, polyvalerolactone, poly(1,3-dioxan-2one)); polyanhydrides (e.g. poly(sebacic anhydride)); polyethers (e.g., polyethylene glycol); polyurethanes; polymethacrylates; polyacrylates; and polycyanoacrylates.

[0170] In some embodiments, particles can be non-polymeric particles (e.g. metal particles, quantum dots, ceramic particles, polymers comprising inorganic materials, bone-derived materials, bone substitutes, viral particles, etc.). In some embodiments, a therapeutic or diagnostic agent to be delivered can be associated with the surface of such a non-polymeric particle. In some embodiments, a non-polymeric particle is an aggregate of non-polymeric components, such as an aggregate of metal atoms (e.g. gold atoms). In some embodiments, a therapeutic or diagnostic agent to be delivered can be associated with the surface of and/or encapsulated within, surrounded by, and/or dispersed throughout an aggregate of non-polymeric components.

[0171] Particles (e.g. nanoparticles, microparticles) may be prepared using any method known in the art. For example, particulate formulations can be formed by methods as nanoprecipitation, flow focusing fluidic channels, spray drying, single and double emulsion solvent evaporation, solvent extraction, phase separation, milling, microemulsion procedures, microfabrication, nanofabrication, sacrificial layers, simple and complex coacervation, and other methods well known to those of ordinary skill in the art. Alternatively or additionally, aqueous and organic solvent syntheses for monodisperse semiconductor, conductive, magnetic, organic, and other nanoparticles have been described (Pellegrino et al., 2005, Small, 1:48; Murray et al., 2000, Ann. Rev. Mat. Sci., 30:545; and Trindade et al., 2001, Chem. Mat., 13:3843).

[0172] Methods for making microparticles for delivery of encapsulated agents are described in the literature (see, e.g., Doubrow, Ed., "Microcapsules and Nanoparticles in Medicine and Pharmacy," CRC Press, Boca Raton, 1992; Mathiowitz et al., 1987, J. Control. Release, 5:13; Mathiowitz et al., 1987, Reactive Polymers, δ: 275; and Mathiowitz et al., 1988, J. Appl. Polymer Sci., 35:755).

[0173] In some embodiments, the targeting moiety comprises an nucleic acid targeting moiety.

[0174] In general, a nucleic acid targeting moiety is any polynucleotide that binds to a component associated with an organ, tissue, cell, extracellular matrix component, and/or intracellular compartment (the target).

[0175] In some embodiments, the nucleic acid targeting moieties are aptamers.

[0176] An aptamer is typically a polynucleotide that binds to a specific target structure that is associated with a particular organ, tissue, cell, extracellular matrix component, and/or intracellular compartment. In general, the targeting function of the aptamer is based on the three-dimensional structure of the aptamer. In some embodiments, binding of an aptamer to a target is typically mediated by the interaction between the two- and/or three-dimensional structures of both the aptamer and the target. In some embodiments, binding of an aptamer to a target is not solely based on the primary sequence of the aptamer, but depends on the three-dimensional structure(s) of the aptamer and/or target. In some embodiments, aptamers bind to their targets via complementary Watson-Crick base pairing which is interrupted by structures (e.g. hairpin loops) that disrupt base pairing.

[0177] In some embodiments, the nucleic acid targeting moieties are spiegelmers (PCT Publications WO 98/08856, WO 02/100442, and WO 06/117217). In general, spiegelmers are synthetic, mirror-image nucleic acids that can specifically bind to a target (i.e. mirror image aptamers). Spiegelmers are characterized by structural features which make them not susceptible to exo- and endo-nucleases.

[0178] One of ordinary skill in the art will recognize that any nucleic acid targeting moiety (e.g. aptamer or spiegelmer) that is capable of specifically binding to a target can be used in accordance with the present invention. In some embodiments, nucleic acid targeting moieties to be used in accordance with the present invention may target a marker associated with a disease, disorder, and/or condition. In some embodiments, nucleic acid targeting moieties to be used in accordance with the present invention may target cancer-associated targets. In some embodiments, nucleic acid targeting moieties to be used in accordance with the present invention may target tumor markers. Any type of cancer and/or any tumor marker may be targeted using nucleic acid targeting moieties in accordance with the present invention. To give but a few examples, nucleic acid targeting moieties may target markers associated with prostate cancer, lung cancer, breast cancer, colorectal cancer, bladder cancer, pancreatic cancer, endometrial cancer, ovarian cancer, bone cancer, esophageal cancer, liver cancer, stomach cancer, brain tumors, cutaneous melanoma, and/or leukemia.

[0179] Nucleic acids of the present invention (including nucleic acid nucleic acid targeting moieties and/or functional RNAs to be delivered, e.g., RNAi-inducing entities, ribozymes, tRNAs, etc., described in further detail below) may be prepared according to any available technique including, but not limited to

chemical synthesis, enzymatic synthesis, enzymatic or chemical cleavage of a longer precursor, etc. Methods of synthesizing RNAs are known in the art (see, e.g., Gait, M. J. (ed.) Oligonucleotide synthesis: a practical approach, Oxford [Oxfordshire], Washington, D.C.: IRL Press, 1984; and Herdewijn, P. (ed.) Oligonucleotide synthesis: methods and applications, Methods in molecular biology, v. 288 (Clifton, N.J.) Totowa, N.J.: Humana Press, 2005).

[0180] The nucleic acid that forms the nucleic acid nucleic acid targeting moiety may comprise naturally occurring nucleosides, modified nucleosides, naturally occurring nucleosides with hydrocarbon linkers (e.g., an alkylene) or a polyether linker (e.g., a PEG linker) inserted between one or more nucleosides, modified nucleosides with hydrocarbon or PEG linkers inserted between one or more nucleosides, or a combination of thereof. In some embodiments, nucleotides or modified nucleotides of the nucleic acid nucleic acid targeting moiety can be replaced with a hydrocarbon linker or a polyether linker provided that the binding affinity and selectivity of the nucleic acid nucleic acid targeting moiety is not substantially reduced by the substitution (e.g., the dissociation constant of the nucleic acid nucleic acid targeting moiety for the target should not be greater than about 1×10^{-3} M).

[0181] It will be appreciated by those of ordinary skill in the art that nucleic acids in accordance with the present invention may comprise nucleotides entirely of the types found in naturally occurring nucleic acids, or may instead include one or more nucleotide analogs or have a structure that otherwise differs from that of a naturally occurring nucleic acid. U.S. Pat. Nos. 6,403,779; 6,399,754; 6,225,460; 6,127,533; 6,031,086; 6,005,087; 5,977,089; and references therein disclose a wide variety of specific nucleotide analogs and modifications that may be used. See Crooke, S. (ed.) Antisense Drug Technology: Principles, Strategies, and Applications (1st ed), Marcel Dekker; ISBN: 0824705661; 1st edition (2001) and references therein. For example, 2′-modifications include halo, alkoxy and allyloxy groups. In some embodiments, the 2′-OH group is replaced by a group selected from H, OR, R, halo, SH, SR, NH2, NHR, NR2 or CN, wherein R is C1-C6 alkyl, alkenyl, or alkynyl, and halo is F, Cl, Br, or I. Examples of modified linkages include phosphorothioate and 5′-N-phosphoramidite linkages.

[0182] Nucleic acids comprising a variety of different nucleotide analogs, modified backbones, or non-naturally occurring internucleoside linkages can be utilized in accordance with the present invention. Nucleic acids of the present invention may include natural nucleosides (i.e., adenosine, thymidine, guanosine, cytidine, uridine, deoxyadenosine, deoxythymidine, deoxyguanosine, and deoxycytidine) or modified nucleosides. Examples of modified nucleotides include base modified nucleoside (e.g., aracytidine, inosine, isoguanosine, nebularine, pseudouridine, 2,6-diaminopurine, 2-aminopurine, 2-thiothymidine, 3-deaza-5-azacytidine, 2′-deoxyuridine, 3-nitorpyrrole, 4-methylindole, 4-thiouridine, 4-thiothymidine, 2-aminoadenosine, 2-thiothymidine, 2-thiouridine, 5-bromocytidine, 5-iodouridine,

inosine, 6-azauridine, 6-chloropurine, 7-deazaadenosine, 7-deazaguanosine, 8-azaadenosine, 8-azidoadenosine, benzimidazole, M1-methyladenosine, pyrrolo-pyrimidine, 2-amino-6-chloropurine, 3-methyl adenosine, 5-propynylcytidine, 5-propynyluridine, 5-bromouridine, 5-fluorouridine, 5-methylcytidine, 7-deazaadenosine, 7-deazaguanosine, 8-oxoadenosine, 8-oxoguanosine, O(6)-methylguanine, and 2-thiocytidine), chemically or biologically modified bases (e.g., methylated bases), modified sugars (e.g., 2′-fluororibose, 2′-aminoribose, 2′-azidoribose, 2′-O-methylribose, L-enantiomeric nucleosides arabinose, and hexose), modified phosphate groups (e.g., phosphorothioates and 5′-N-phosphoramidite linkages), and combinations thereof. Natural and modified nucleotide monomers for the chemical synthesis of nucleic acids are readily available. In some cases, nucleic acids comprising such modifications display improved properties relative to nucleic acids consisting only of naturally occurring nucleotides. In some embodiments, nucleic acid modifications described herein are utilized to reduce and/or prevent digestion by nucleases (e.g. exonucleases, endonucleases, etc.). For example, the structure of a nucleic acid may be stabilized by including nucleotide analogs at the 3′ end of one or both strands order to reduce digestion.

[0183] Modified nucleic acids need not be uniformly modified along the entire length of the molecule. Different nucleotide modifications and/or backbone structures may exist at various positions in the nucleic acid. One of ordinary skill in the art will appreciate that the nucleotide analogs or other modification(s) may be located at any position(s) of a nucleic acid such that the function of the nucleic acid is not substantially affected. To give but one example, modifications may be located at any position of a nucleic acid targeting moiety such that the ability of the nucleic acid targeting moiety to specifically bind to the target is not substantially affected. The modified region may be at the 5′-end and/or the 3′-end of one or both strands. For example, modified nucleic acid targeting moieties in which approximately 1-5 residues at the 5′ and/or 3′ end of either of both strands are nucleotide analogs and/or have a backbone modification have been employed. The modification may be a 5′ or 3′ terminal modification. One or both nucleic acid strands may comprise at least 50% unmodified nucleotides, at least 80% unmodified nucleotides, at least 90% unmodified nucleotides, or 100% unmodified nucleotides.

[0184] Nucleic acids in accordance with the present invention may, for example, comprise a modification to a sugar, nucleoside, or internucleoside linkage such as those described in U.S. Patent Application Publications 2003/0175950, 2004/0192626, 2004/0092470, 2005/0020525, and 2005/0032733. The present invention encompasses the use of any nucleic acid having any one or more of the modification described therein. For example, a number of terminal conjugates, e.g., lipids such as cholesterol, lithocholic acid, aluric acid, or long alkyl branched chains have been reported to improve cellular uptake. Analogs and modifications may be tested using, e.g., using any appropriate assay known

in the art, for example, to select those that result in improved delivery of a therapeutic or diagnostic agent, improved specific binding of an nucleic acid targeting moiety to a target, etc. In some embodiments, nucleic acids in accordance with the present invention may comprise one or more non-natural nucleoside linkages. In some embodiments, one or more internal nucleotides at the 3′-end, 5′-end, or both 3′- and 5′-ends of the nucleic acid targeting moiety are inverted to yield a linkage such as a 3′-3′ linkage or a 5′-5′ linkage.

[0185] In some embodiments, nucleic acids in accordance with the present invention are not synthetic, but are naturally-occurring entities that have been isolated from their natural environments.

[0186] Any method can be used to design novel nucleic acid targeting moieties (see, e.g., U.S. Pat. Nos. 6,716,583; 6,465,189; 6,482,594; 6,458,543; 6,458,539; 6,376,190; 6,344,318; 6,242,246; 6,184,364; 6,001,577; 5,958,691; 5,874,218; 5,853,984; 5,843,732; 5,843,653; 5,817,785; 5,789,163; 5,763,177; 5,696,249; 5,660,985; 5,595,877; 5,567,588; and 5,270,163; and U.S. Patent Application Publications 2005/0069910, 2004/0072234, 2004/0043923, 2003/0087301, 2003/0054360, and 2002/0064780). The present invention provides methods for designing novel nucleic acid targeting moieties. The present invention further provides methods for isolating or identifying novel nucleic acid targeting moieties from a mixture of candidate nucleic acid targeting moieties.

[0187] Nucleic acid targeting moieties that bind to a protein, a carbohydrate, a lipid, and/or a nucleic acid can be designed and/or identified. In some embodiments, nucleic acid targeting moieties can be designed and/or identified for use in the complexes of the invention that bind to proteins and/or characteristic portions thereof, such as tumor-markers, integrins, cell surface receptors, transmembrane proteins, intercellular proteins, ion channels, membrane transporter proteins, enzymes, antibodies, chimeric proteins etc. In some embodiments, nucleic acid targeting moieties can be designed and/or identified for use in the complexes of the invention that bind to carbohydrates and/or characteristic portions thereof, such as glycoproteins, sugars (e.g., monosaccharides, disaccharides and polysaccharides), glycocalyx (i.e., the carbohydrate-rich peripheral zone on the outside surface of most eukaryotic cells) etc. In some embodiments, nucleic acid targeting moieties can be designed and/or identified for use in the complexes of the invention that bind to lipids and/or characteristic portions thereof, such as oils, saturated fatty acids, unsaturated fatty acids, glycerides, hormones, steroids (e.g., cholesterol, bile acids), vitamins (e.g. vitamin E), phospholipids, sphingolipids, lipoproteins etc. In some embodiments, nucleic acid targeting moieties can be designed and/or identified for use in the complexes of the invention that bind to nucleic acids and/or characteristic portions thereof, such as DNA nucleic acids; RNA nucleic acids; modified DNA nucleic acids; modified RNA nucleic acids; and nucleic acids that include any combination of DNA, RNA, modified DNA, and modified RNA; etc.

[0188] Nucleic acid targeting moieties (e.g. aptamers or spiegelmers) may be designed and/or identified using any available method. In some embodiments, nucleic acid targeting moieties are designed and/or identified by identifying nucleic acid targeting moieties from a candidate mixture of nucleic acids. Systemic Evolution of Ligands by Exponential Enrichment (SELEX), or a variation thereof, is a commonly used method of identifying nucleic acid targeting moieties that bind to a target from a candidate mixture of nucleic acids.

[0189] Nucleic acid targeting moieties that bind selectively to any target can be isolated by the SELEX process, or a variation thereof, provided that the target can be used as a target in the SELEX process.

B. Immunotherapeutics

[0190] In genenal, the combination or compostion of the present invention comprises an immunotherapeutic.

[0191] By "immunotherapeutics" herein is meant a compound, a molecule, or an agent that is capable of stimulating or enhancing the body's immune system or tumor cells. Immunotherapetuics are used for the treatment of disease by inducing, enhancing, or suppressing an immune response. Immunotherapeutics of the present invention generally are designed to elicit or amplify an immune response, rather than suppresss an immune response.

[0192] In general, the immunoethreapeutics of the present invention act, directly or indirectly, on toll like receptors, nucleotide-oligomerization domain-like receptors, RIG-I-Like receptors, c-type lectin receptors, or cytosolic DNA Sensors, or a combination thereof. Particually, the immunotherapeutics of the present invention are capable of activating a human plasmacytoid dendritic cell, myeloid dendritic cell, NK cell, or tumor cell, or a combination thereof.

[0193] In some embodiments, the immunotherepetuics of the present invention activate human immune cells, including but not limited to dendritic cells, macrophages, monocytes, myeloid-derived suppressor cells, NK cells, B cells, T cells, or tumor cells, or a combination thereof.

[0194] Dendritic cells are the most powerful antigen-presenting cells. Dendritic cells play an essential role for the initiation of both innate and adaptive immune responses. Dendritic cells also play a key role in the induction and maintenance of immune tolerance.

[0195] By "dendritic cells" (DC) herein is meant a heterogeneous cell population including two main subtypes: namely, myeloid DC (mDC) and plasmacytoid DC (pDC) (Steinman et al., 1979, J. Exp. Med., 149, 1-16). These two blood DC subsets were originally differentiated by their expression of CD11c

(integrin complement receptor) and CD123 (IL-3Rα). Each of the pDC and mDC populations constitutes between about 0.2 to about 0.6% of the PBMC population in humans.

[0196] By "pDC" herein is meant plasmacytoid dendritic cells and they represent a subtype of dendritic cells found in the blood and peripheral lymphoid organs. These cells express the surface markers CD123, BDCA-2(CD303) and BDCA-4(CD304) and HLA-DR, but do not express CD11c, CD14, CD3, CD20 or CD56, which distinguishes them from conventional dendritic cells, monocytes, T-cells, B cells and NK cells. As components of the innate immune system, these cells express intracellular Toll-like receptors 7 and 9, which enable the detection of viral and bacterial nucleic acids, such as ssRNA or CpG DNA motifs. Upon stimulation and subsequent activation, these cells produce large amounts of Type I interferon (mainly IFN-α and IFN-β) and Type III interferon (e.g., IFN-λ), which are critical pleiotropic anti-viral compounds mediating a wide range of effects. By generating a large number of type I interferon, cytokines and chemokines, plasmacytoid dendritic cells are widely involved in the body's innate and adaptive immune responses. They can regulate NK cells, T cells, B cells and other cells involved in immune response intensity, duration, and response mode, thus play a very important function in tumor, infection and autoimmune disease. (Liu YJ. IPC: professional type 1 interferon-producing cells and plasmacytoid dendritic cell precursors. Annu Rev Immunol. 2005; 23:275-306. Gilliet M, Cao W, Liu YJ. Plasmacytoid dendritic cells: sensing nucleic acids in viral infection and autoimmune diseases. Nat Rev Immunol. 2008 Aug; 8 (8) :594-606).

[0197] By "mDC" herein is meant myeloid dendritic cells and they represent a subtype of circulating dendritic cellsfound in blood and peripheral lymphoid organs. These cells express the surface markers CD11c, CD1a, HLA-DR and either BDCA-1 (CD1c) or BDCA-3 (CD141). They do not express BDCA-2 or CD123, which distinguishes them from pDC. mDC also do not express CD3, CD20 or CD56. As components of the innate immune system, mDC express Toll-like receptors (TLR), including TLR2, 3, 4, 5, 6 and 8, which enable the detection of bacterial and viral components. Upon stimulation and subsequent activation, these cells are the most potent antigen presenting cells to activate antigen-specific CD4 as well as CD8 T cells. In addition, mDCs has the ability to produce large amounts of IL-12 and IL23, which is critical for the induction of Th1-mediated or Th17 cell-mediated immunity.

[0198] Study found that many solid tumors such as breast cancer and head and neck cancer, ovarian cancer has pDC's invasion (Treilleux I, Blay JY, Bendriss-Vermare N et al. Dendritic cell infiltration and prognosis of early stage breast cancer. Clin Cancer Res 2004; 10:7466-7474. Hartmann E, Wollenberg B, Rothenfusser S et al. Identification and functional analysis of tumor-infiltrating plasmacytoid dendritic cells in head and neck cancer. Cancer Res 2003; 63:6478-6487. Zou WP, Machelon V, Coulomb-L'Hermin A, et al. Stromal-derived factor-1 in human tumors recruits and alters the function of

plasmacytoid precursor dendritic cells. Nat Med 2001; 7:1339-1346) and factors secreted by tumor cells inhibit DC maturation. (Gabrilovich DI, Corak J, Ciernik IF et al. Decreased antigen presentation by dendritic cells in patients with breast cancer. Clin Cancer Res 1997; 3:483-490. Bell D, Chomarat P, Broyles D et al. In breast carcinoma tissue, immature dendritic cells reside within the tumor, whereas mature dendritic cells are located in peritumoral areas. J Exp Med 1999; 190:1417-1425. Menetrier-Caux C, Montmain G, Dieu MC et al. Inhibition of the differentiation of dendritic cells from CD34 (+) progenitors by tumor cells: role of interleukin-6 and macrophage colony-stimulating factor. Blood 1998; 92:4778-4791). These immature DC cells did not play a role in promoting anti-tumor immunity. By contrast, DCs within the tumor microenvironment promote tumor growth by inhibiting antitumor immunity and by promoting angiogenesis. There is evidence that Toll-like receptor 7 agonist Imiquimod, and Toll-like receptor 9 agonist CpG drugs can stimulate pDC within the tumor microenvironment to inhibit tumor development. (Dummer R, Urosevic M, Kempf W et al. Imiquimod in basal cell carcinoma: how does it work? Br J Dermatol 2003; 149:57-58. Miller RL, Gerster JF, Owens ML et al Imiquimod applied topically: a novel immune response modifier and new class of drug. Int J Immunopharmacol 1999; 21:1-14. Hofmann MA, Kors C, Audring H et al Phase 1 evaluation of intralesionally injected TLR9-agonist PF-3512676 in patients with basal cell carcinoma or metastatic melanoma. J Immunother 2008; 31:520-527).

[0199] Natural killer (NK) cells are a type of cytotoxic lymphocyte that constitutes a major component of the immune system. NK cells are a subset of peripheral blood lymphocytes defined by the expression of CD56 or CD 16 and the absence of the T cell receptor (CD3). They recognize and kill transformed cell lines without priming in an MHC-unrestricted fashion. NK cells play a major role in the rejection of tumors and cells infected by viruses. The process by which an NK cell recognizes a target cell and delivers a sufficient signal to trigger target lysis is determined by an array of inhibitory and activating receptors on the cell surface. NK discrimination of self from altered self involves inhibitory receptor recognition of MHC-I molecules and non-MHC ligands like CD48 and Clr-1b. NK recognition of infected or damaged cells (altered self) is coordinated through stress induced ligands (e.g., MICA, MICB, Rae1, H60, Mult1) or virally encoded ligands (e.g., m157, hemagluttinin) recognized by various activating receptors, including NKG2D, Ly49H and NKp46/Ncr1.

[0200] NK cells represent the predominant lymphoid cell in the peripheral blood for many months after allogeneic or autologous stem cell transplant and they have a primary role in immunity to pathogens during this period (Reittie et al (1989) Blood 73: 1351-1358; Lowdell et al (1998) Bone Marrow Transplant 21: 679-686). The role of NK cells in engraftment, graft- versus-host disease, anti-leukemia activity and post-transplant infection is reviewed in Lowdell (2003) Transfusion Medicine 13:399-404.

[0201] Human NK cells mediate the lysis of tumor cells and virus-infected cells via natural cytotoxicity and antibody-dependent cellular cytotoxicity (ADCC).

[0202] Human NK cells are controlled by positive and negative cytolytic signals. Negative (inhibitory) signals are transduced by C-lectin domain containing receptors CD94/NKG2A and by some Killer Immunoglobulin- like Receptors (KIRs). The regulation of NK lysis by inhibitory signals is known as the "missing self" hypothesis in which specific HLA-class I alleles expressed on the target cell surface ligate inhibitory receptors on NK cells. The down- regulation of HLA molecules on tumor cells and some virally infected cells (e.g. CMV) lowers this inhibition below a target threshold and the target cells may become susceptible to NK cell- mediated lysis if the target cells also carry NK-priming and activating molecules. TLR7, TLR8 or TLR9 agonists can activate both mDC and pDCs to produce type I IFNs and express costimulatory molecules such as GITR-ligand, which subsequently activate NK cells to produce IFN-g and potently promote NK cell killing function.

[0203] Inhibitory receptors fall into two groups, those of the Ig-superfamily called Killer Immunoglobulin- like Receptors (KIRs) and those of the lectin family, the NKG2, which form dimers with CD94 at the cell surface. KIRs have a 2- or 3-domain extracellular structure and bind to HLA-A, -B or -C. The NKG2/CD94 complexes ligate HLA-E.

[0204] Inhibitory KIRs have up to 4 intracellular domains which contain ITIMs and the best characterized are KIR2DL1, KIR2DL2 and KIR2DL3 which are known to bind HLA-C molecules. KIR2DL2 and KIR2DL3 bind the group 1 HLA-C alleles while KIR2DL1 binds to group 2 alleles. Certain leukemia/lymphoma cells express both group 1 and 2 HLA-C alleles and are known to be resistant to NK-mediated cell lysis.

[0205] With regards to positive activating signals, ADCC is thought to be mediated via CD 16, and a number of triggering receptors responsible for natural cytotoxicity have been identified, including CD2, CD38, CD69, NKRP-I, CD40, B7-2, NK-TR, NKp46, NKp30 and NKp44. In addition, several KIR molecules with short intracytoplasmic tails are also stimulatory. These KIRs (KIR2DS1, KIR2DS2 and KIR2DS4) are known to bind to HLA-C; their extracellular domains being identical to their related inhibitory KIRs. The activatory KIRs lack the ITIMs and instead associate with DAP 12 leading to NK cell activation. The mechanism of control of expression of inhibitory versus activatory KIRs remains unknown.

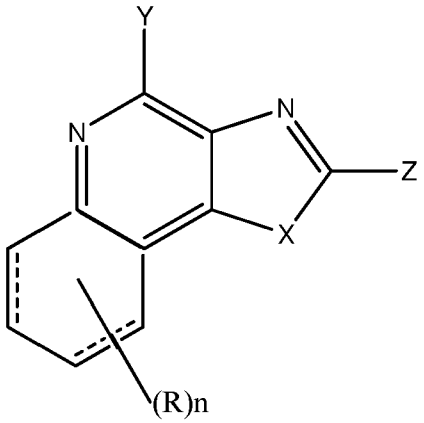
[0206] Several reports have described the expression of TLRs in mouse or human cancer or cancer cell lines. For example, TLR1 to TLR6 are expressed by colon, lung, prostate, and melanoma mouse tumor cell lines (Huang B, et al. Toll-like receptors on tumor cells facilitate evasion of immune surveillance.

Cancer Res. 2005;65(12):5009–5014.), TLR3 is expressed in human breast cancer cells (Salaun B, Coste I, Rissoan MC, Lebecque SJ, Renno T. TLR3 can directly trigger apoptosis in human cancer cells. J Immunol. 2006;176(8):4894–4901.), hepatocarcinoma and gastric carcinoma cells express TLR2 and TLR4 (Huang B, et al. Listeria monocytogenes promotes tumor growth via tumor cell toll-like receptor 2 signaling. Cancer Res. 2007;67(9):4346–4352), and TLR9 (Droemann D, et al. Human lung cancer cells express functionally active Toll-like receptor 9. Respir Res. 2005;6:1.) and TLR4 (He W, Liu Q, Wang L, Chen W, Li N, Cao X. TLR4 signaling promotes immune escape of human lung cancer cells by inducing immunosuppressive cytokines and apoptosis resistance. Mol Immunol. 2007;44(11):2850–2859.) are expressed by human lung cancer cells. TLR7 and TLR8 are found in tumor cells of human lung cancer (Cherfils-Vicini J, Platonova S, Gillard M, Laurans L, Validire P, Caliandro R, Magdeleinat P, Mami-Chouaib F, Dieu-Nosjean MC, Fridman WH, Damotte D, Sautès-Fridman C, Cremer I. J. Clin Invest. 2010;120(4):1285–1297).

[0207] TLR are a family of proteins that sense a microbial product and/or initiates an adaptive immune response. TLRs activate a dendritic cell (DC). TLRs are conserved membrane spanning molecules containing an ectodomain of leucine-rich repeats, a transmembrane domain and an intracellular TIR (Toll/interleukin receptor) domain. TLRs recognize distinct structures in microbes, often referred to as "PAMPs" (pathogen associated molecular patterns). Ligand binding to TLRs invokes a cascade of intra-cellular signaling pathways that induce the production of factors involved in inflammation and immunity.

[0208] In some embodiments, the immunotherapeutic is a TLR7 and/or TLR8 agonist. TLR7 and TLR8 are phylogenetically and structurally related. TLR7 is selectively expressed by human pDCs and B cells. TLR8 is predominantly expressed mDCs, monocytes, macrophages and myeloid suppressor cells. TLR7-specific agonists activate plasmacytoid DCs (pDCs) to produce large amounts of type 1 IFNs and expressing high levels of costimulatory molecules that promote activation of T cells, NK cells, B cells and mDCs. TLR8-specific agonists activate myeloid DCs, monocytes, macrophages or myeloid-derived suppressor cells to produce large amounts of type 1 IFN, IL-12 and IL-23, and express high levels of MHC class I, MHC class II and costimulatory molecules that promote the activation of antigen specific CD4 and CD8+ T cells.

[0209] In some embodiments, the immunotherapeutic is a TLR7 and/or TLR8 agonist that is represented by the structure of Formula (I):



(I)

wherein dashed line represents bond or absence of bond;

X is S or $-NR_1$, R_1 is $-W_0—W_1—W_2—W_3—W_4$,

W_0 is a bond, alkyl alkenyl, alkynyl, alkoxy, or –alkyl-S-alkyl--,

W_1 is a bond, --O--, or $-NR_2$--, wherein R_2 is hydrogen, alkyl or alkenyl,

W_2 is a bond, --O--, --C(O)--, --C(S)--, or $-S(O)_2—$,

W_3 is a bond, $--NR_3$--, wherein R_3 is hydrogen, alkyl or alkenyl,

W_4 is hydrogen, alkyl, alkenyl, alkynyl, alkoxy, cycloalkyl, aryl, aryloxy, heteroaryl, or heterocyclyl, each of which is optionally substituted by one or more substituents selected from the group consisting of hydroxyl, alkoxy, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, heterocyclyl, $--NH_2$, nitro, --alkyl-hydroxyl, --alkyl-aryl, --alkyl-heteroaryl, --alkyl-heterocyclyl, $--O-R_4$, $--O-alkyl-R_4$, $--alkyl-O-R_4$, $--C(O)-R_4$, $--alkyl-C(O)-R_4$, $--alkyl-C(O)-O-R_4$, $--C(O)-O-R_4$, $--S-R_4$, $--S(O)_2-R_4$, $--NH-S(O)_2-R_4$, --alkyl-S-R4, $--alkyl-S(O)_2-R_4$, $--NHR_4$, $--NR_4R_4$,--NH-alkyl-R_4, halogen, --CN, $--NO_2$, and –SH, wherein R_4 is independently hydrogen, alkyl, alkenyl, --alkyl-hydroxyl, aryl, heteroaryl, heterocyclyl, or haloalkyl;

Z is hydrogen, alkyl, alkenyl, alkynyl, alkoxy, aryl, haloalkyl, heteroaryl, heterocyclyl, each of which can be optionally substituted by one or more substituents selected from the group consisting of hydroxyl, alkoxy, alkyl, alkenyl, alkynyl, aryl, heteroaryl, heterocyclyl, halogen, cyano, nitro, $--N(R_5)_2$, --alkoxy-alkyl, --alkoxy-alkenyl, --C(O)-alkyl, --C(O)-O-alkyl, --O-C(O)-alkyl, $--C(O)-N(R_5)_2$, aryl, heteroaryl, --CO-aryl, and –CO-heteroaryl, wherein each R_5 is independently hydrogen, alkyl, haloalkyl, --alkyl-aryl, or –alkyl-heteroaryl;

R is hydrogen, alkyl, alkoxy, haloalkyl, halogen, aryl, heteroaryl, heterocyclyl, each of which is optionally substituted by one or more substituents selected from the group consisting of hydroxyl, alkoxy, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, heterocyclyl, $--NH_2$, nitro, --alkyl-hydroxyl, --alkyl-

aryl, --alkyl-heteroaryl, --alkyl-heterocyclyl, --O-R_4, --O-alkyl-R_4, --alkyl-O-R_4, --C(O)-R_4, --C(O)-NH-R_4, --C(O)-NR_4R_4, --alkyl-C(O)-R_4, --alkyl-C(O)-O-R_4, --C(O)-O-R_4, --O-C(O)-R_4, --S-R_4, --C(O)-S-R_4, --S-C(O)-R_4, --$S(O)_2$-R_4, --NH-$S(O)_2$-R_4, --alkyl-S-R_4, --alkyl-$S(O)_2$-R_4, --NHR_4, --NR_4R_4,--NH-alkyl-R_4, halogen, --CN, and –SH, wherein R_4 is independently hydrogen, alkyl, alkenyl, alkoxy, --alkyl-hydroxyl, aryl, heteroaryl, heterocyclyl, or haloalkyl;

n is 0, 1, 2, 3, or 4;

Y is –NR_6R_7, –$CR_6R_7R_8$, or –alkyl-NH_2, each of which can be optionally substituted by one or more substituents selected from the group consisting of hydroxyl, alkoxy, alkyl, alkenyl, alkynyl, --NH_2, halogen, --$N(R_5)_2$, --alkoxy-alkyl, --alkoxy-alkenyl, --C(O)-alkyl, --C(O)-O-alkyl, --C(O)-$N(R_5)_2$, aryl, heteroaryl, --CO-aryl, and –CO-heteroaryl,

wherein R_6, R_7 and R_8 are independently hydrogen, alkyl, alkenyl, alkoxy, alkylamino, dialkylamino, alkylthio, arylthio, --alkyl-hydroxyl, --alkyl-C(O)-O-R_9, --alkyl-C(O)-R_9, or –alkyl-O-C(O)-R_9, wherein each R_5 is independently hydrogen, alkyl, haloalkyl, --alkyl-aryl, or –alkyl-heteroaryl, wherein R_9 is hydrogen, alkyl, alkenyl, halogen, or haloalkyl;

X and Z taken together may optionally form a (5-9)-membered ring;

or a pharmaceutically acceptable salt or solvate thereof.

[0210] In some embodiments, X of Formula (I) is S.

[0211] In some embodiments, X of Formula (I) is –NR_1, R_1 is alkyl, --alkyl-W_4, --alkyl-O-W_4, --alkyl-NH-C(O)-W_4, --alkoxy-NH-C(O)-W_4, --alkyl-NH-C(O)-NH-W_4, --alkoxy-NH-C(O)-NH-W_4, --alkyl-$S(O)_2$-W_4, or --alkyl-NH-C(S)-W_4, wherein W_4 is defined above.

[0212] In some embodiments, Z of Formula (I) is hydrogen, alkyl, alkoxy, aryl, heteroaryl, haloalkyl, each of which is optionally substituted by one to three substituents selected from the group consisting of hydroxyl, alkyl, aryl, heteroaryl, heterocyclyl, cyano, --alkoxy-alkyl, nitro, and –$N(R_5)_2$, wherein each R_5 is independently hydrogen, alkyl, haloalkyl, --alkyl-aryl, or –alkyl-heteroaryl.

[0213] In some embodiments, Y of Formula (I) is –NH_2, --alkyl-NH_2, each of which is optionally substituted by one to three substituents selected from the group consisting of alkyl, alkoxy, alkenyl, and alkynyl.

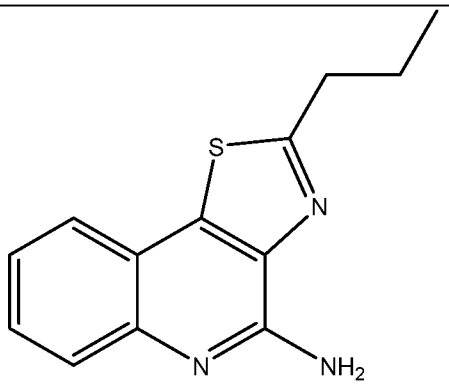
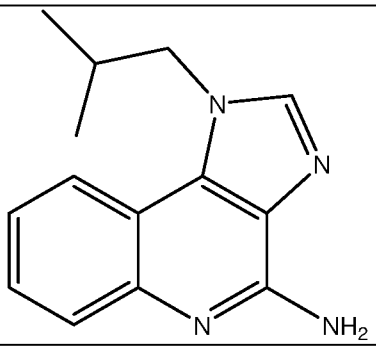
[0214] In some embodiments, n of Formula (I) is 1 or 2.

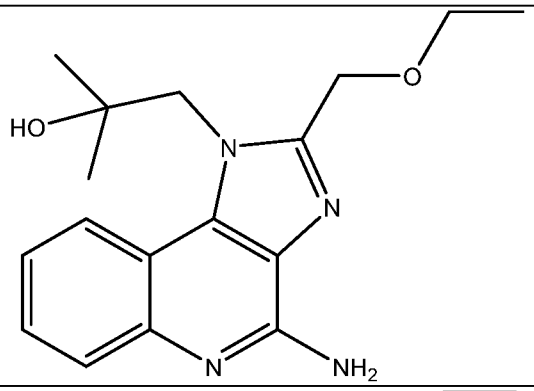
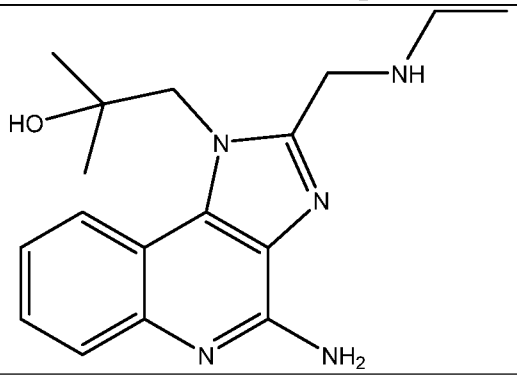
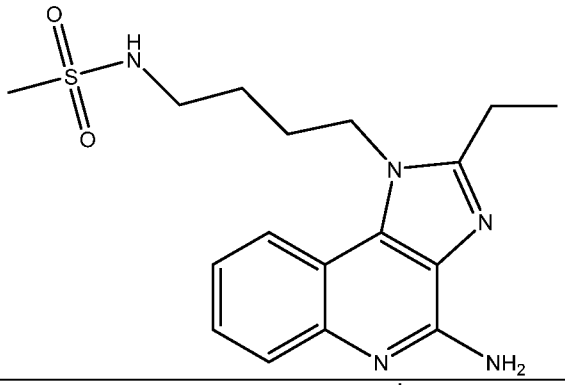
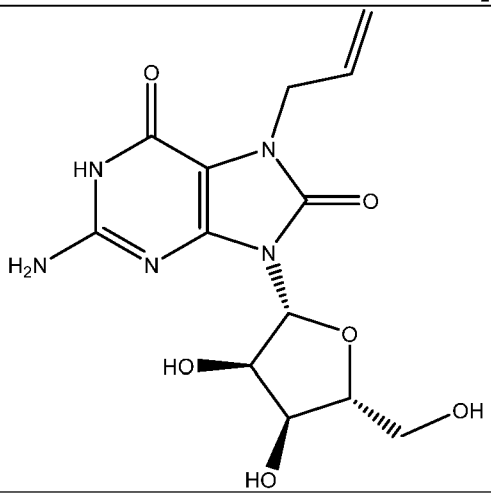
[0215] In some embodiments, R of Formula (I) is aryl or heteroaryl each of which is optionally substituted by one to three substituents selected from the group consisting of hydroxyl, alkoxy, --alkyl-hydroxyl, --O-R_4, --O-alkyl-R_4, --alkyl-O-R_4, --C(O)-R_4, --C(O)-NH-R_4, --C(O)-NR_4R_4, --alkyl-C(O)-R_4,

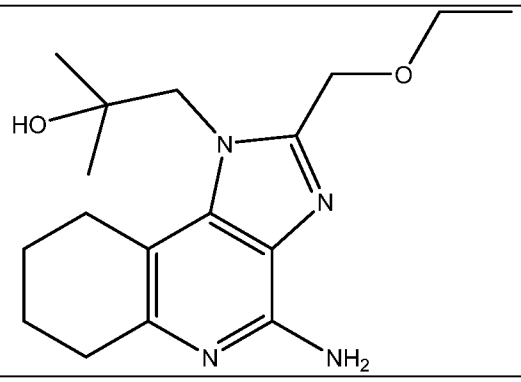
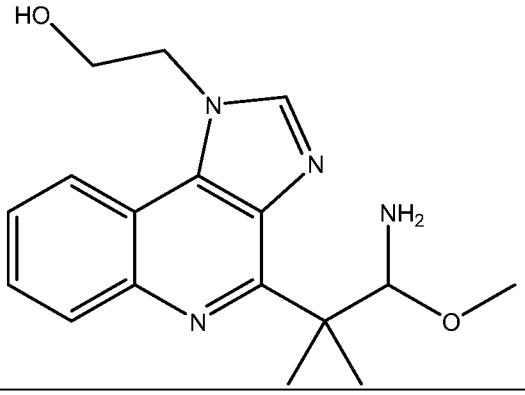
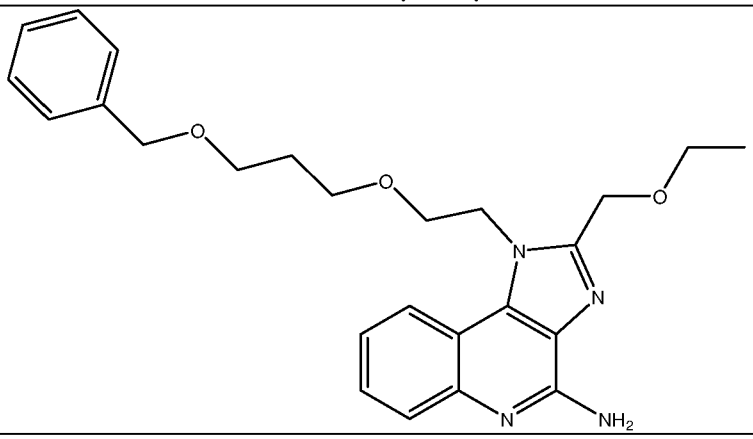
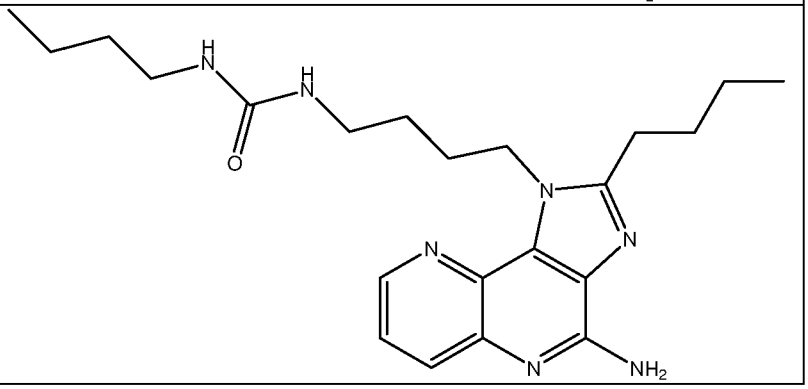
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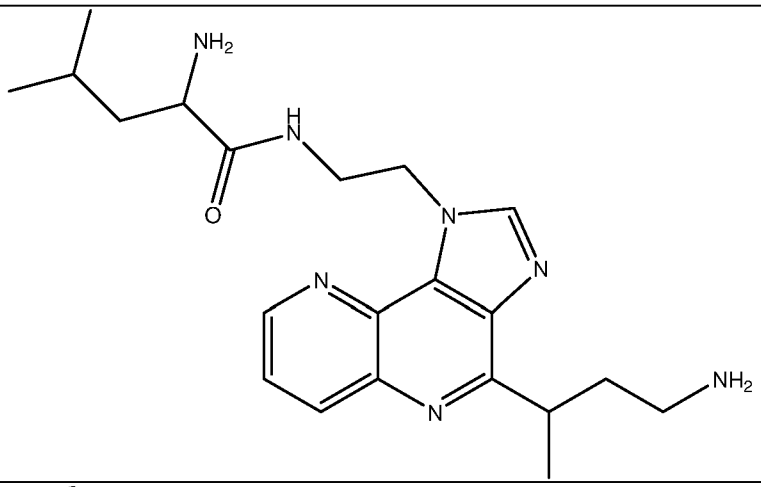
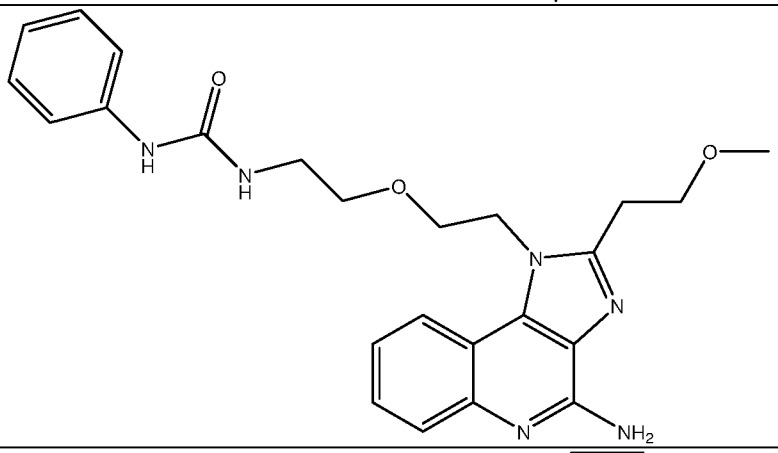
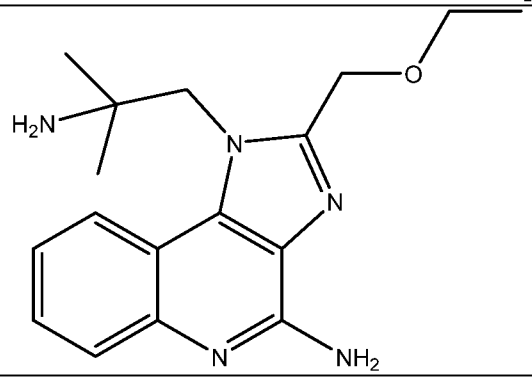
[0216] In some embodiments, the immunotherapeutic is a TLR7 and/or TLR8 agonist that is selected from Table 2. The compounds in Table 2 are described and characterized in more details in US4,689,338, US5,389,640, US5,226,575, US6,110,929, US6,194,425, US5,352,784, US6,331,539, US5,482,936, US6,451810, WO2002/46192, WO2002/46193, WO2002/46194, US2004/0014779 and US2004/0162309.

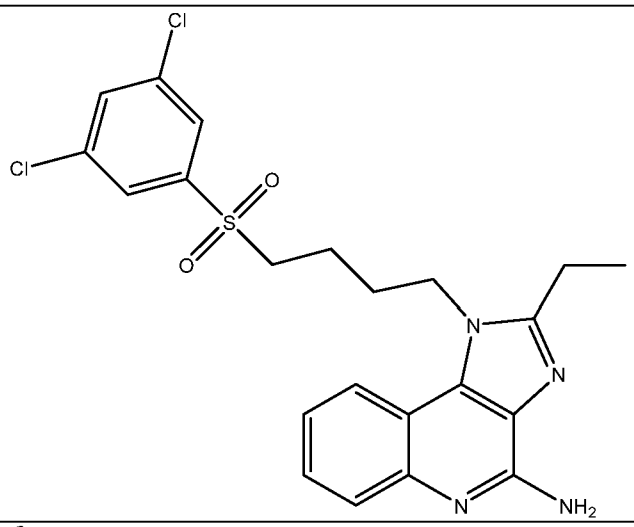
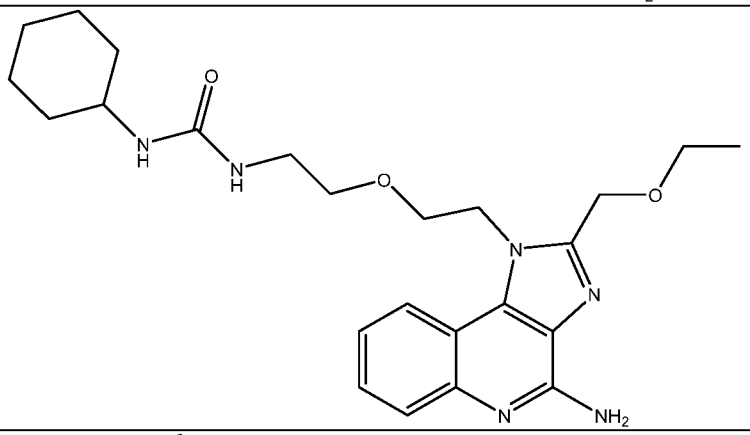
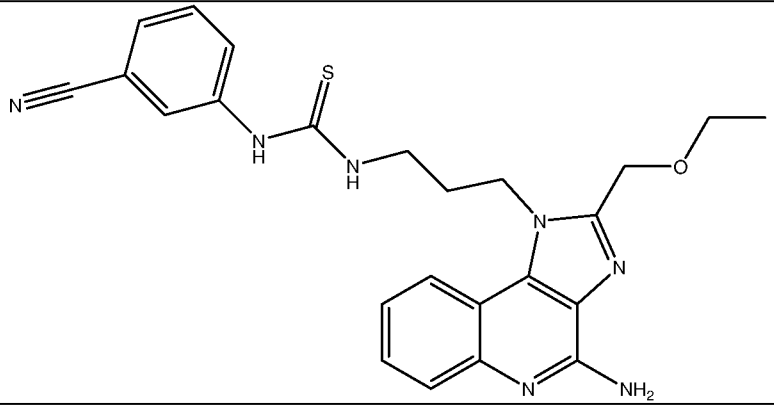
Table 2 Representative TLR7 and/or TLR8 Agonists

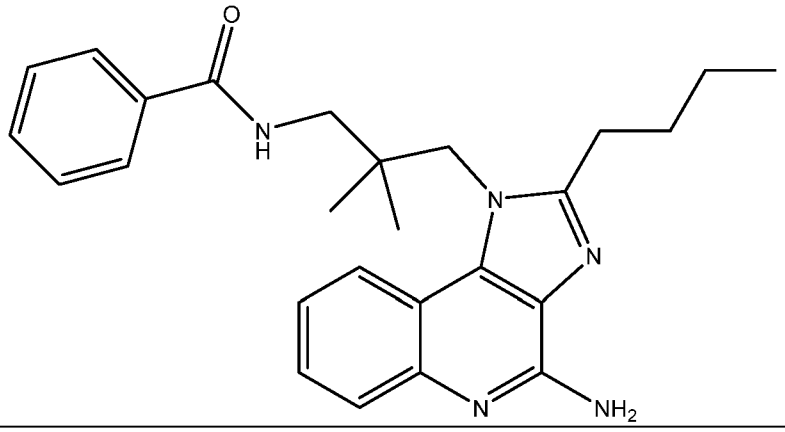
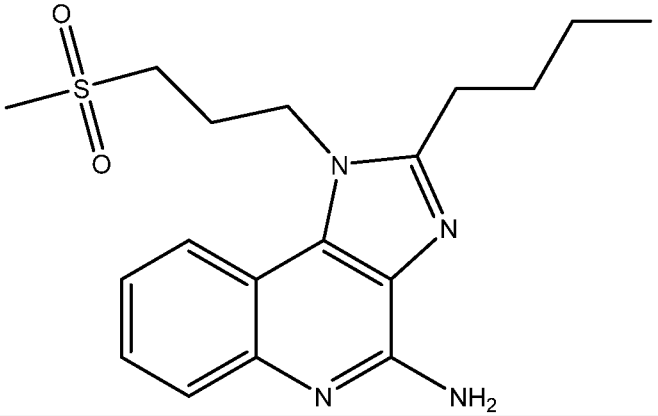
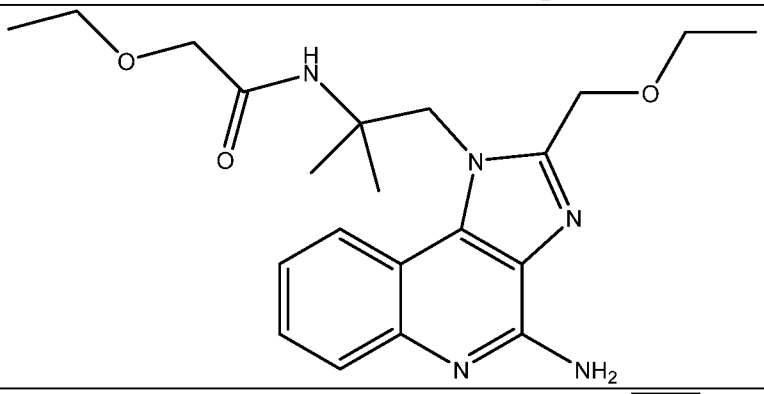
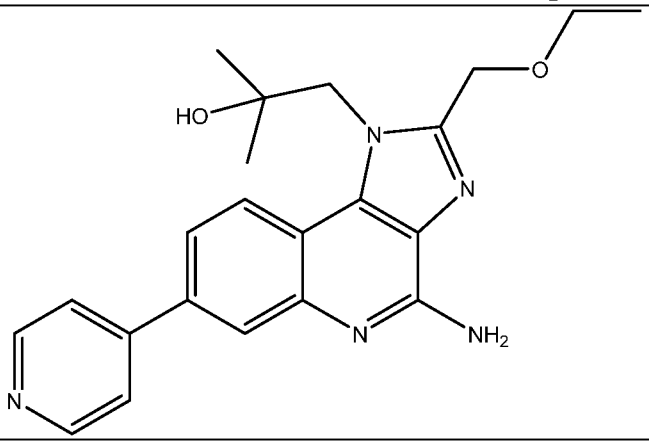
Name	Structure
2-propylthiazolo[4,5-c]quinolin-4-amine (CL075)	
1-(2-methylpropyl)-1H-imidazo[4,5-c]quinolin-4-amine (Imiquimod)	

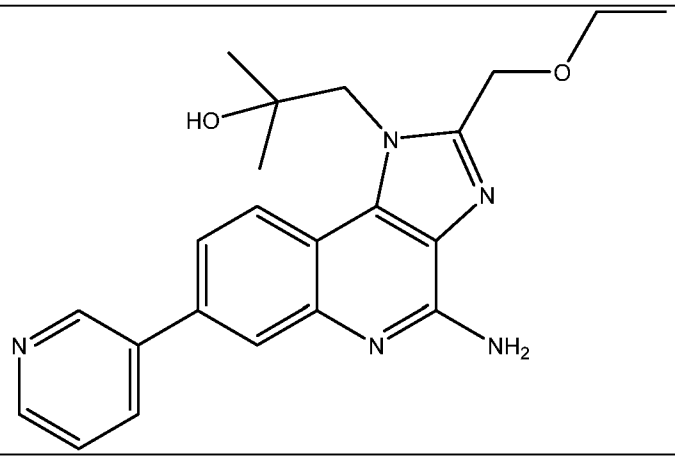
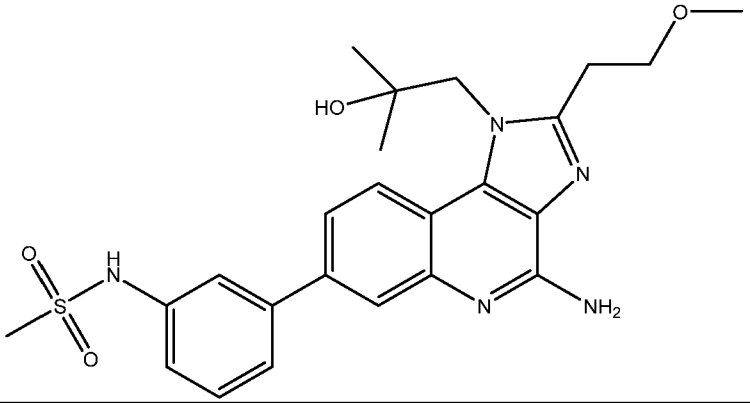
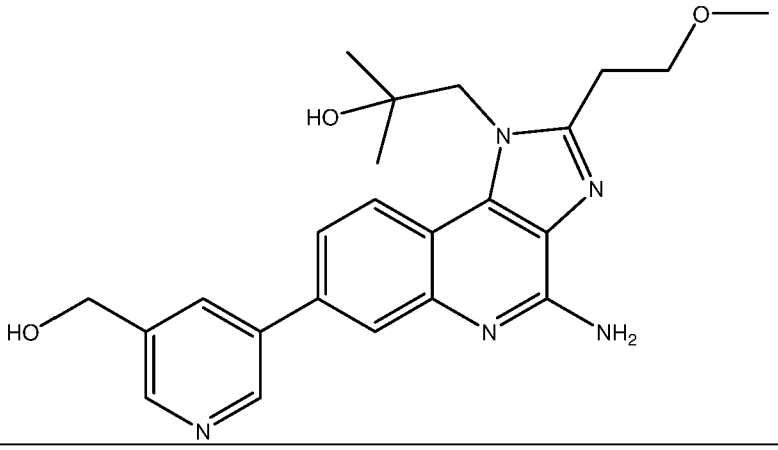
4-amino-2-(ethoxymethyl)-a,a-dimethyl-1H-imidazo[4,5-c]quinoline-1-ethanol (Resiquimod)	
1-(4-amino-2-ethylaminomethylimidazo-[4,5-c]quinolin-1-yl)-2-methylpropan-2-ol (Gardiquimod)	
N-[4-(4-amino-2-ethyl-1H-imidazo[4,5-c]quinolin-1-yl)butyl-]methanesulfonamide (CM001)	
7-allyl-7,8-dihydro-8-oxo-guanosine (Loxoribine)	

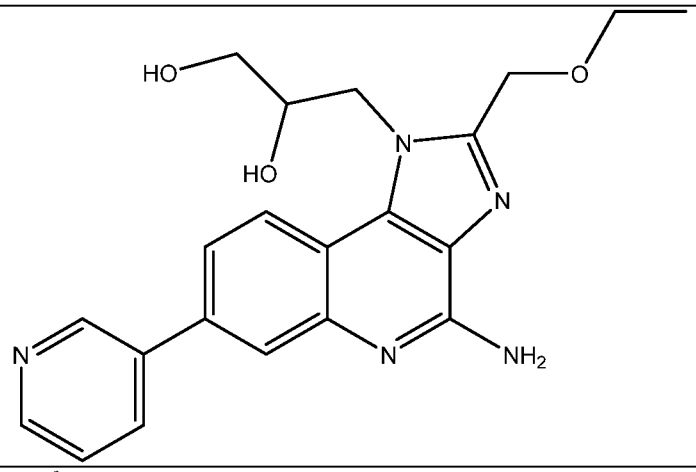
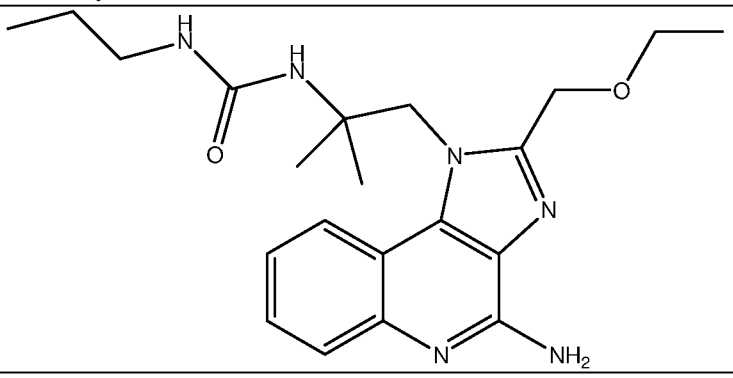
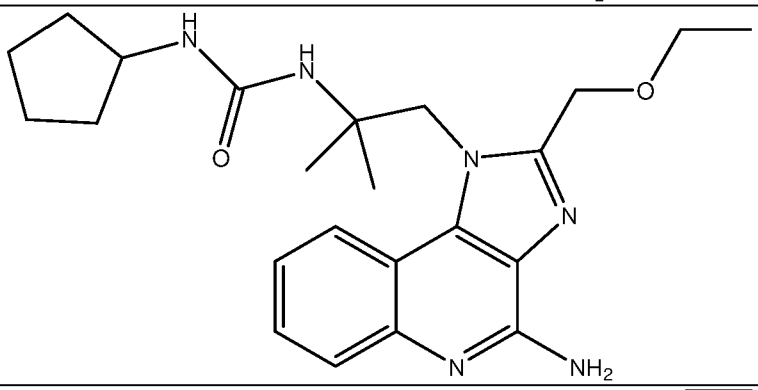
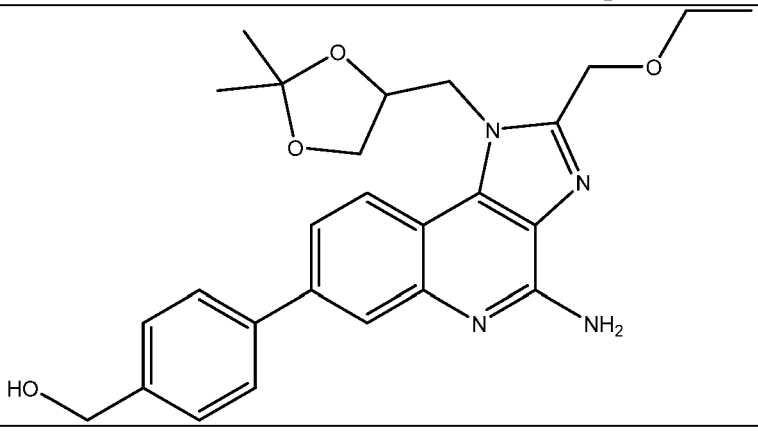
4-amino-2-ethoxymethyl-aa-dimethyl-6,7,8,9-tetrahydro-1h-imidazo[4,5-c]quinoline-1-ethanol ol	
4-amino-aa-dimethyl-2-methoxyethyl-1h-imidazo[4,5-c]quinoline-1-ethanol	
1-(2-(3-(benzyloxy)propoxy)ethyl)-2-(ethoxymethyl)-1H-imidazo[4,5-c]quinolin-4-amine	
N-[4-(4-amino-2-butyl-1H-imidazo[4,5-c][1,5]naphthyridin-1-yl)butyl]-n'-butylurea	

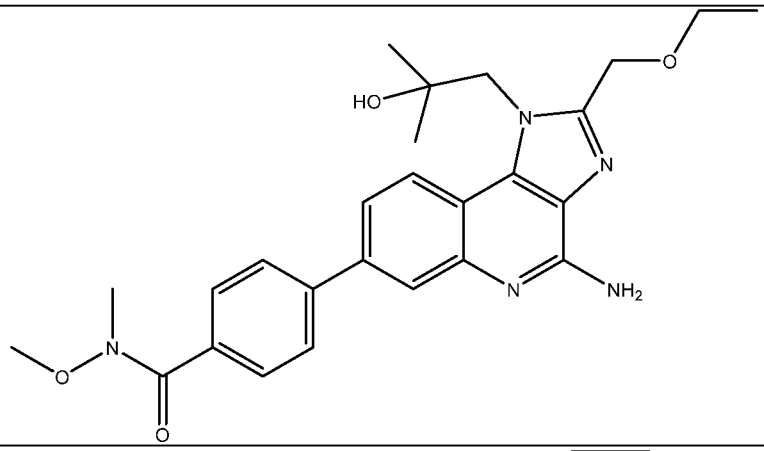
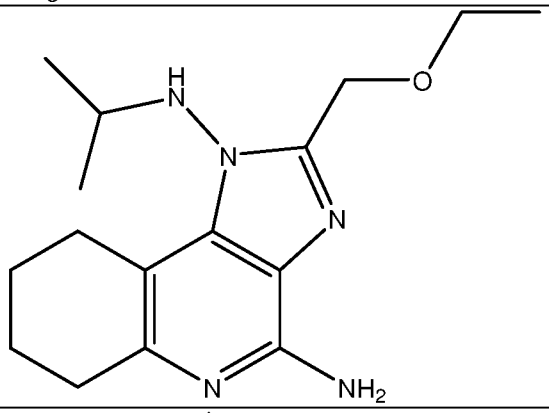
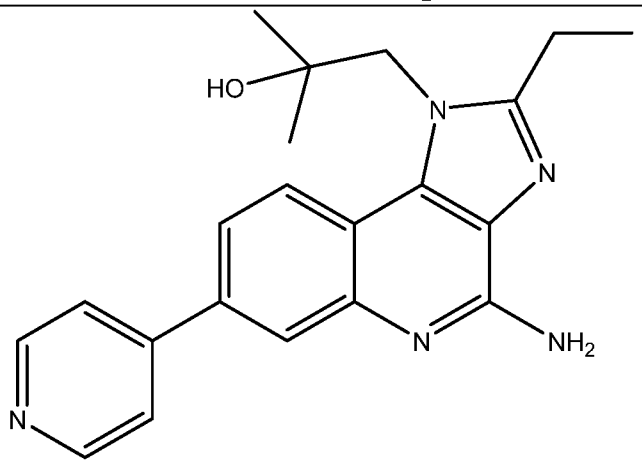
N1-[2-(4-amino-2-butyl-1H-imidazo[4,5-c][1,5] naphthyridin-1-yl)ethyl]-2-amino-4-methylpentanamide	
N-(2-{2-[4-amino-2-(2-methoxyethyl)-1H-imidazo[4,5-c]quinolin-1-yl]ethoxy}ethyl)-n'-phenylurea	
1-(2-amino-2-methylpropyl)-2-(ethoxymethyl)-1H-imidazo[4,5-c]quinolin-4-amine	

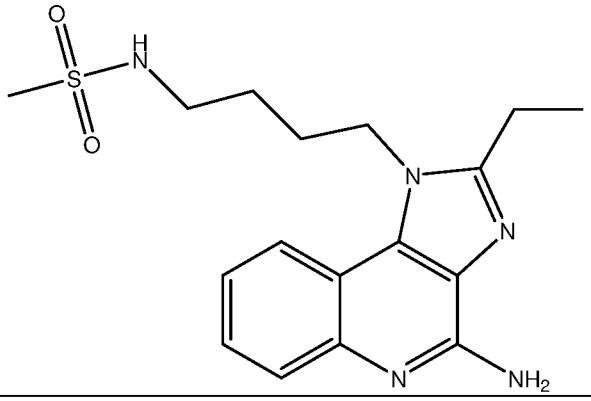
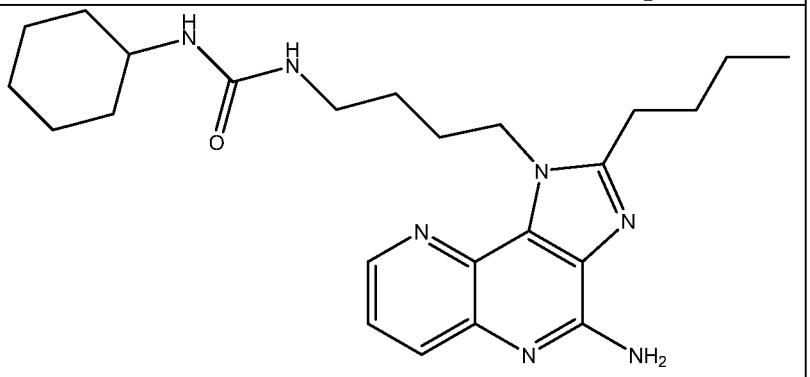
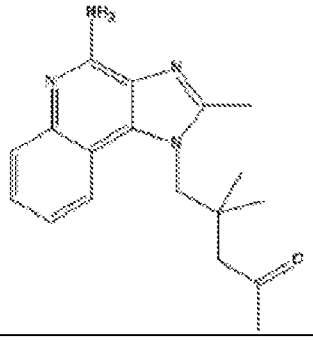
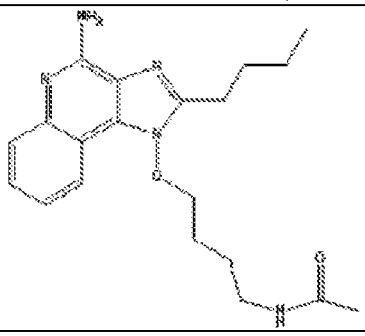
1-{4-[(3,5-dichlorophenyl)sulfonyl]butyl}-2-ethyl-1H-imidazo[4,5-c]quinolin-4-amine	
N-(2-{2-[4-amino-2-(ethoxymethyl)-1H-imidazo[4,5-c]quinolin-1-yl]ethoxy}ethyl)-n'-cyclohexylurea	
N-{3-[4-amino-2-(ethoxymethyl)-1H-imidazo[4,5-c]quinolin-1-yl]propyl}-n'-(3-cyanophenyl)thiourea	

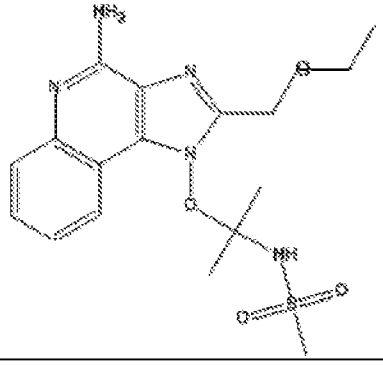
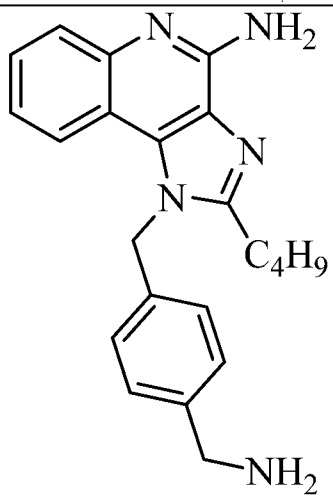
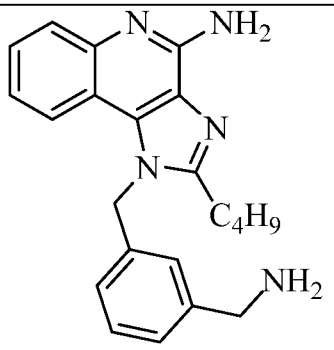
N-[3-(4-amino-2-butyl-1H-imidazo[4,5-c]quinolin-1-yl)-2,2-dimethylpropyl]benzamide	
2-butyl-1-[3-(methylsulfonyl)propyl]-1H-imidazo[4,5-c]quinolin-4-amine	
N-{2-[4-amino-2-(ethoxymethyl)-1H-imidazo[4,5-c]quinolin-1-yl]-1,1-dimethylethyl}-2-ethoxyacetamide	
1-[4-amino-2-ethoxymethyl-7-(pyridin-4-yl)-1H-imidazo[4,5-c]quinolin-1-yl]-2-methylpropan-2-ol	

1-[4-amino-2-(ethoxymethyl)-7-(pyridin-3-yl)-1H-imidazo[4,5-c]quinolin-1-yl]-2-methylpropan-2-ol	
N-{3-[4-amino-1-(2-hydroxy-2-methylpropyl)-2-(methoxyethyl)-1H-imidazo[4,5-c]quinolin-7-yl]phenyl}methanesulfonamide	
1-[4-amino-7-(5-hydroxymethylpyridin-3-yl)-2-(2-methoxyethyl)-1H-imidazo[4,5-c]quinolin-1-yl]-2-methylpropan-2-ol	

3-[4-amino-2-(ethoxymethyl)-7-(pyridin-3-yl)-1H-imidazo[4,5-c]quinolin-1-yl]propane-1,2-diol	
1-[2-(4-amino-2-ethoxymethyl-1H-imidazo[4,5-c]quinolin-1-yl)-1,1-dimethylethyl]-3-propylurea	
1-[2-(4-amino-2-ethoxymethyl-1H-imidazo[4,5-c]quinolin-1-yl)-1,1-dimethylethyl]-3-cyclopentylurea	
1-[(2,2-dimethyl-1,3-dioxolan-4-yl)methyl]-2-(ethoxymethyl)-7-(4-hydroxymethylphenyl)-1H-imidazo[4,5-c]quinolin-4-amine	

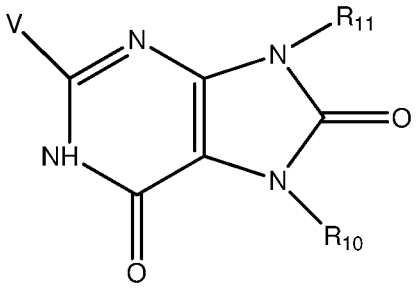
4-[4-amino-2-ethoxymethyl-1-(2-hydroxy-2-methylpropyl)-1H-imidazo[4,5-c]quinolin-7-yl]-N-methoxy-N-methylbenzamide	
2-ethoxymethyl-N1-isopropyl-6,7,8,9-tetrahydro-1H-imidazo[4,5-c]quinoline-1,4-diamine	
1-[4-amino-2-ethyl-7-(pyridin-4-yl)-1H-imidazo[4,5-c]quinolin-1-yl]-2-methylpropan-2-ol	

N-[4-(4-amino-2-ethyl-1H-imidazo[4,5-c]quinolin-1-yl)butyl]methanesulfonamide	
N-[4-(4-amino-2-butyl-1H-imidazo[4,5-c][1,5]naphthyridin-1-yl)butyl]-n'-cyclohexylurea	
3M-34240	
3M-052	

3M-854A	
	 **p-IMDQ**
	 **m-IMDQ**

[0217] Preferably in some embodiments, the immunotherapeutic is Resiquimod or Imiquimod.

[0218] In some embodiments, the immunotherapeutic is a TLR modulator (e.g., TLR7 and/or TLR8 agonist) that is represented by structure of Formula (II):



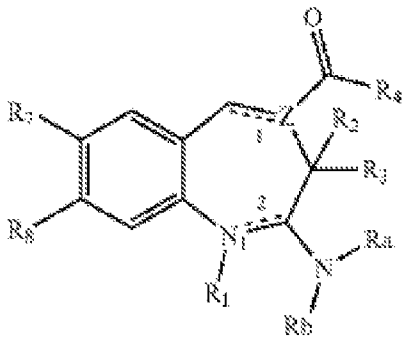
(II)

wherein V is $-NR_6R_7$, wherein each of R_6 and R_7 is independently hydrogen, alkyl, alkenyl, alkoxy, alkylamino, dialkylamino, alkylthio, arylthio, --alkyl-hydroxyl, --alkyl-C(O)-O-R_9, --alkyl-C(O)-R_9, or –alkyl-O-C(O)-R_9, wherein R_9 is hydrogen, alkyl, alkenyl, hydrogen, or haloalkyl;

R_{10} and R_{11} are independently hydrogen, alkyl, alkenyl, aryl, haloalkyl, heteroaryl, heterocyclyl, or cycloalkyl, each of which is optionally substituted by one or more substituents selected from the group consisting of hydroxyl, alkoxy, alkyl, alkenyl, alkynyl, halogen, --$N(R_5)_2$, --alkoxy-alkyl, --alkoxy-alkenyl, --C(O)-alkyl, --C(O)-O-alkyl, --C(O)-$N(R_5)_2$, aryl, heteroaryl, --CO-aryl, and –CO-heteroaryl, wherein each R_5 is independently hydrogen, alkyl, haloalkyl, --alkyl-aryl, or –alkyl-heteroaryl,

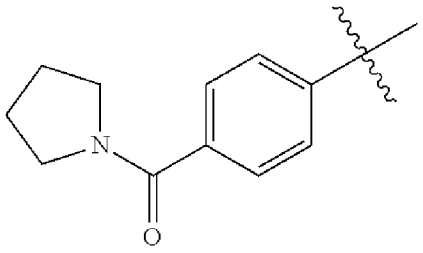
or a pharmaceutically acceptable salt or solvate thereof.

[0219] In some embodiments, the immunotherapeutic is a TLR modulator (e.g., TLR7 and/or TLR8 agonist) that is represented by structure of Formula (III):

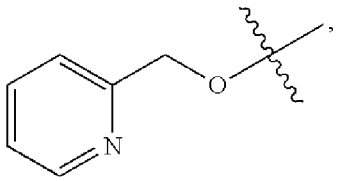


(III)

wherein ------ is a double bond or a single bond; R_2 and R_3 are independently selected from H and lower alkyl, or R_2 and R_3 are connected to form a saturated carbocycle having from 3 to 7 ring members; one of R_7 and R_8 is

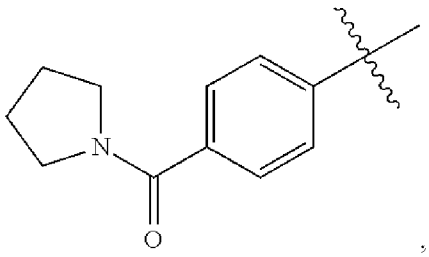


or

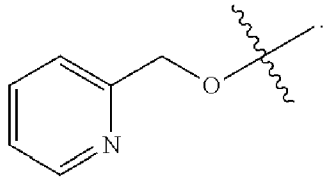


and the other is hydrogen; R_4 is —NR_cR_d or —OR_{10}; R_c and R_d are lower alkyl, where the alkyl is optionally substituted with one or more —OH; R_{10} is alkyl, where the alkyl is optionally substituted with one or more —OH; Z is C and $\overset{1}{\text{------}}$ is a double bond, or Z is N and $\overset{1}{\text{------}}$ is a single bond; R_a and R_b are independently selected from H, alkyl, alkenyl, alkynyl, and R_e, wherein the alkyl is optionally substituted with one or more —OR_{10}, or R_e, R_e is selected from —NH_2, —NH(alkyl), and —$N(alkyl)_2$; R_1 is absent when $\overset{2}{\text{------}}$ is a double bond, or when $\overset{2}{\text{------}}$ is a single bond, N_1—R_1 and one of R_a or R_b are connected to form a saturated, partially unsaturated, or unsaturated heterocycle having 5-7 ring members and the other of R_a or R_b may be hydrogen or absent as necessary to accommodate ring unsaturation; and at least one of the following A-D applies: A) R_7 is not hydrogen B) R_8 is not hydrogen and at least one of R_a and R_b is not hydrogen; C) Z is N; or D) N_1—R_1 and one of R_a or R_b are connected to form a saturated, partially unsaturated, or unsaturated heterocycle having 5-7 ring members. US 20140088085A1, the disclosure of which is incorporated by references in its entirety.

[0220] In some embodiments, R_7 of the compound of Formula (III) is



, or

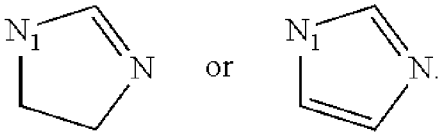


.

Additionally, at least one of R_a and R_b is not hydrogen in the compound of Formula (III), or, for example, one of R_a and R_b is alkyl and the other of R_a and R_b is hydrogen. Further, the alkyl of Formula (III) is

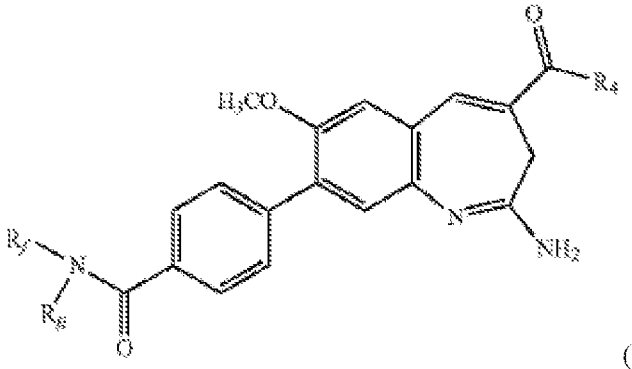
substituted with R_e. In a different embodiment, both R_a and R_b are alkyl or, one of R_a and R_b is R_e and the other $R_a$and R_b is hydrogen. For example, R_8 of formula (III) is not hydrogen.

[0221] In some alternative embodiments, N_1 and one of R_a or R_b of Formula (III) are connected to form a saturated, partially unsaturated, or unsaturated heterocycle having 5-7 ring members and the other of R_a or R_b is hydrogen, or absent as necessary to accommodate ring unsaturation, where the ring is a 5 membered ring, or, for example, the ring is:



[0222] In some embodiments, at least one of R_2 and R_3 in the compound of Formula (III) is not hydrogen, or, for example, R_2 and R_3 are connected to form a saturated carbocycle, where the saturated carbocycle is cyclopropyl. Alternatively, Z is N in the compound of Formula (III).

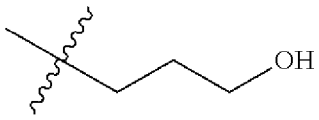
[0223] In some embodiments, the TLR agonist or modulator has the structure of Formula (IV):



(IV)

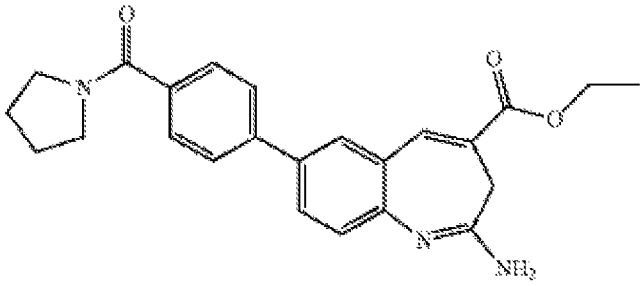
wherein R_4 is selected from ----NR_cR_d and ----OR_{10}; R_c and R_d are lower alkyl, where the alkyl is optionally substituted with one or more ----OH; $R_{10}$is alkyl, where the alkyl is optionally substituted with one or more ----OH; R_f and R_g are lower alkyl or R_f and R_g together with the nitrogen atom to which they are attached form a saturated heterocyclic ring having 4-6 ring members. For example, R_f and R_g in the compound of Formula (IV), together with the nitrogen atom to which they are attached form a saturated heterocyclic ring, where the heterocyclic ring is pyrrolidine.

[0224] In some alternative embodiments, R_4 of either Formula (III) or Formula (IV) is ----OR_{10}, where R_{10} is alkyl or is ethyl. In another embodiment, R_4 of either Fformula (III) or Fformula (IV) is —NR_cR_d, where both are alkyl or both are propyl. Moreover, in certain embodiments, at least one of R_c or R_d is alkyl substituted with one ----OH and at least one of R_c and R_d is

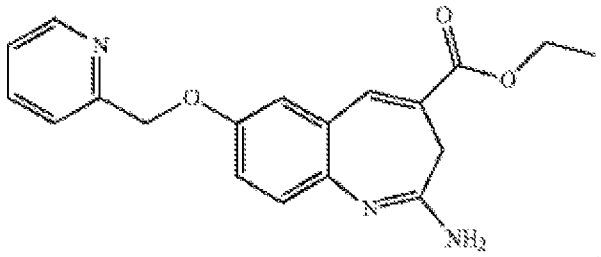


and the remaining R_c or R_d is propyl.

[0225] In some alternative embodiments, the TLR is a a compound selected from

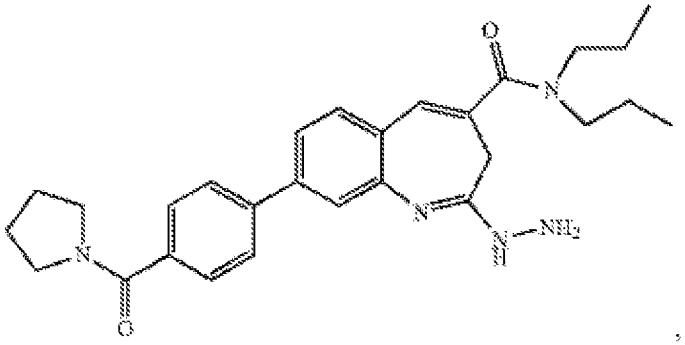


and

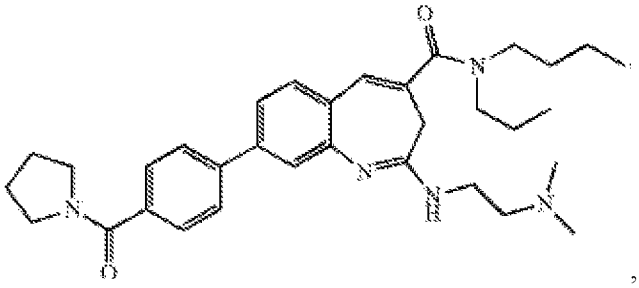


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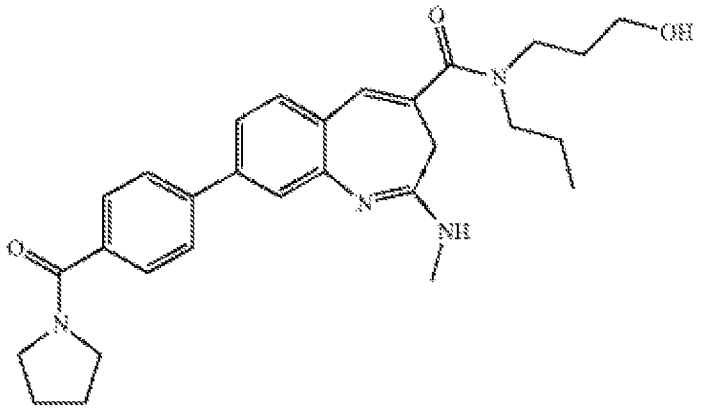
Alternatively, the compound is selected from



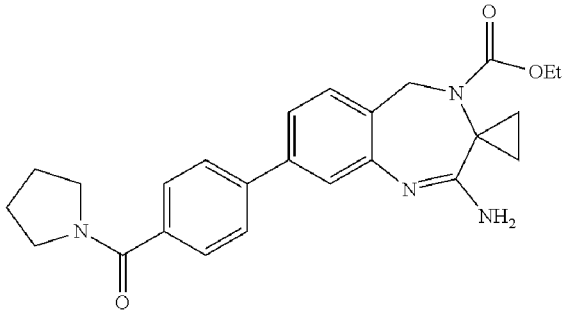
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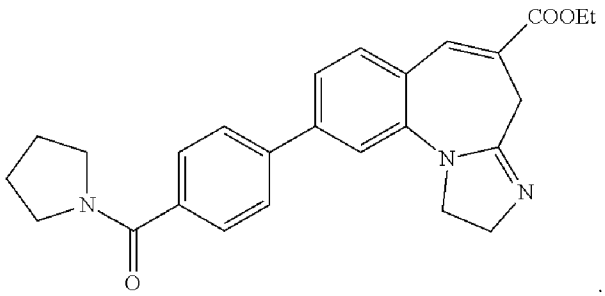
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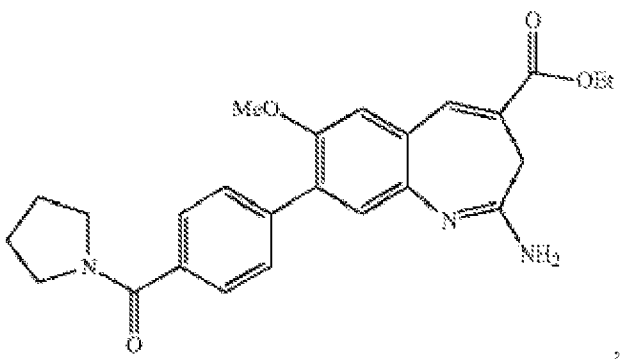
[0226] In some alternative embodiments, the TLR agonist ound is either

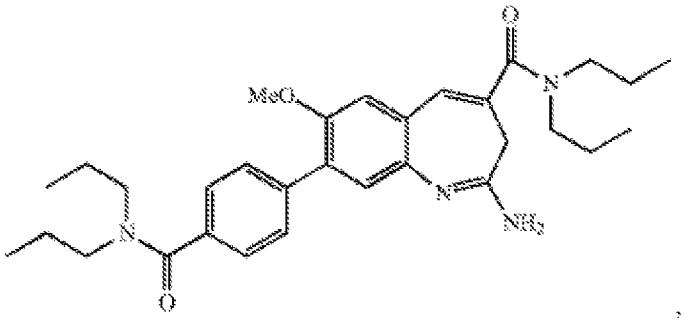


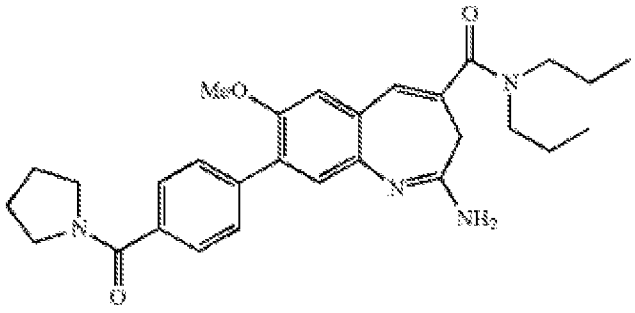
or



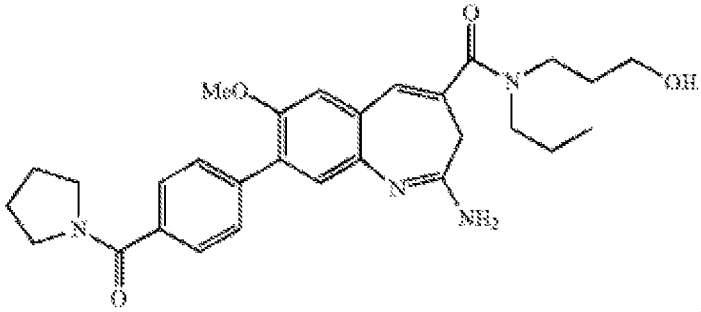
[0227] In some alternative embodiments, the TLR agonist is a compound selected from



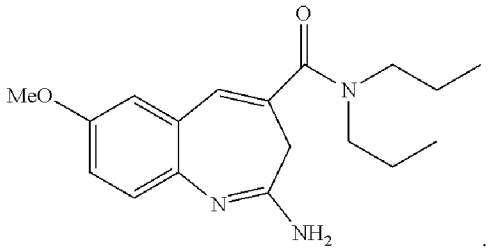




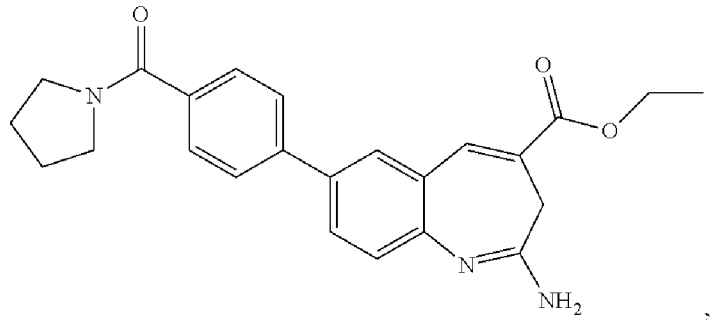
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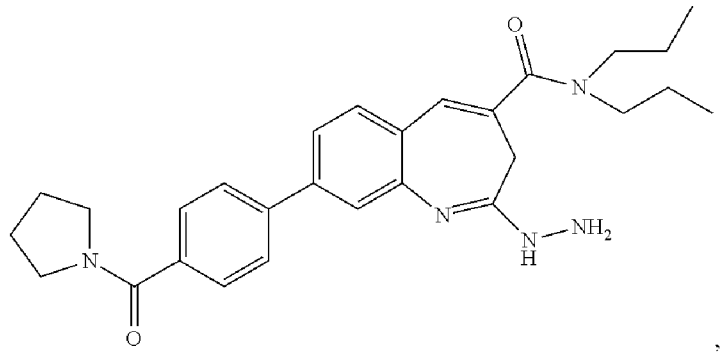


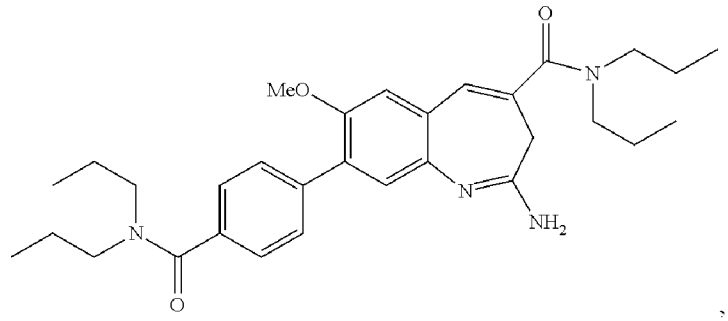
[0228] In some alternative embodiments, the TLR agonist is

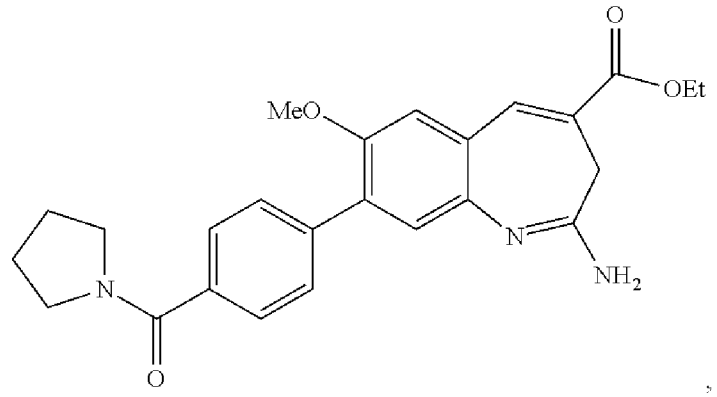


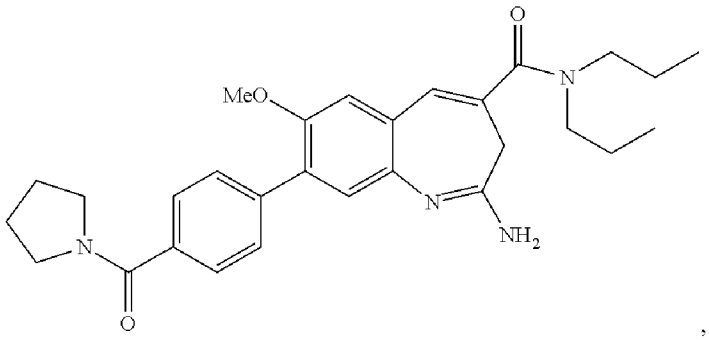
[0229] In some alternative embodiments, the TLR agonist is a compound selected from:



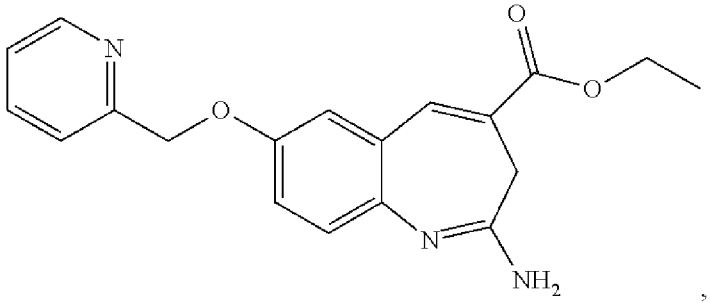




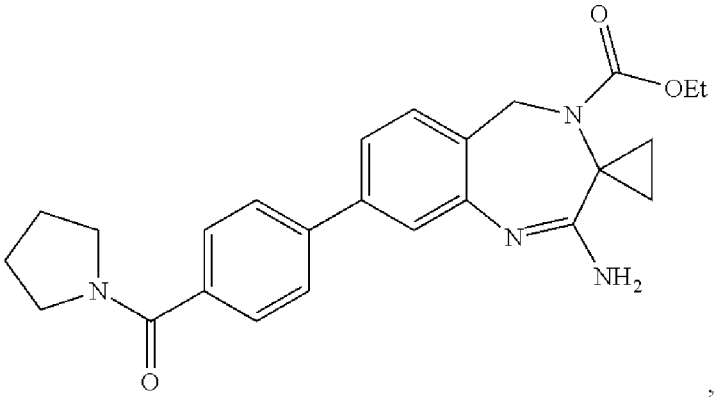




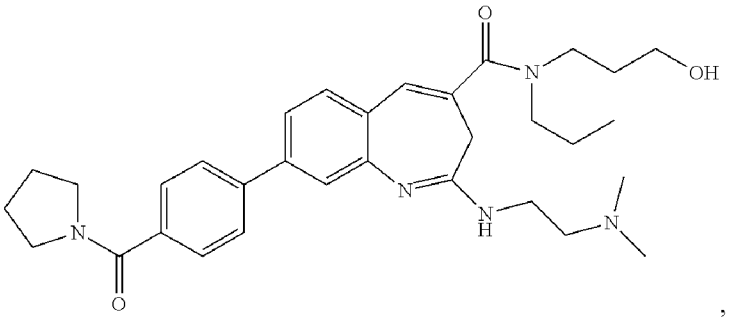
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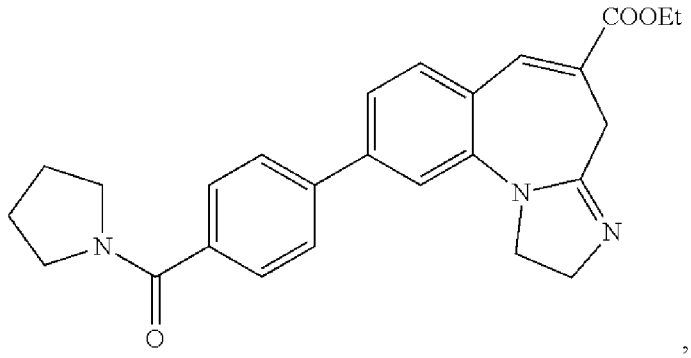
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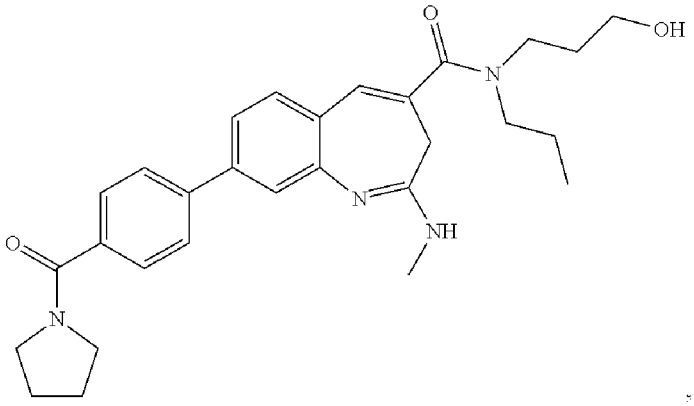
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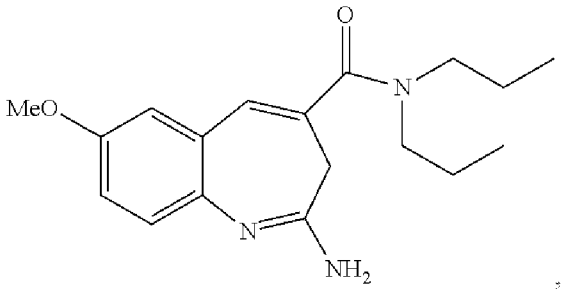
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COOEt
N
N
N
O

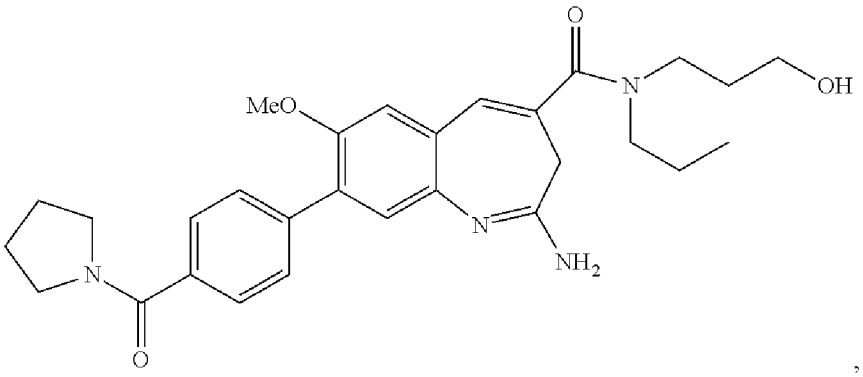
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O
N
OH
N
NH
O
N

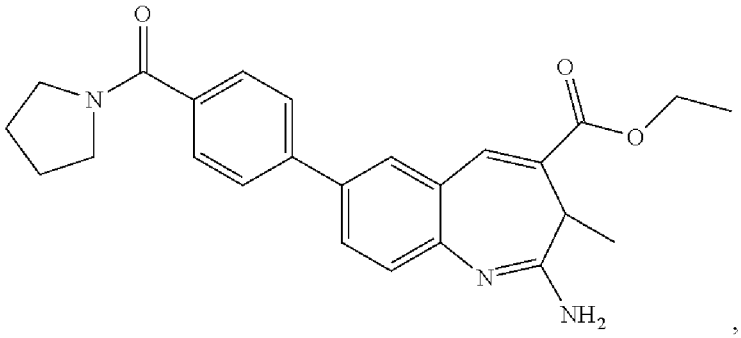
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O
N
MeO
N
NH2

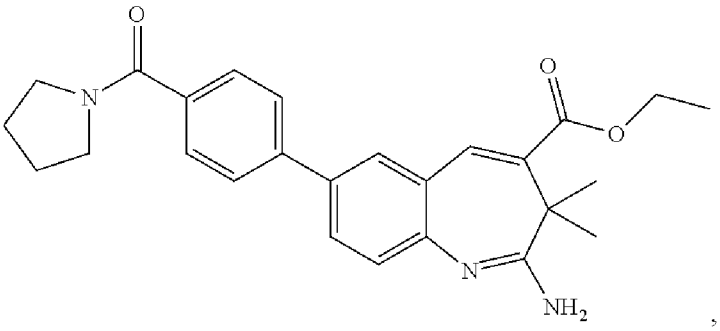
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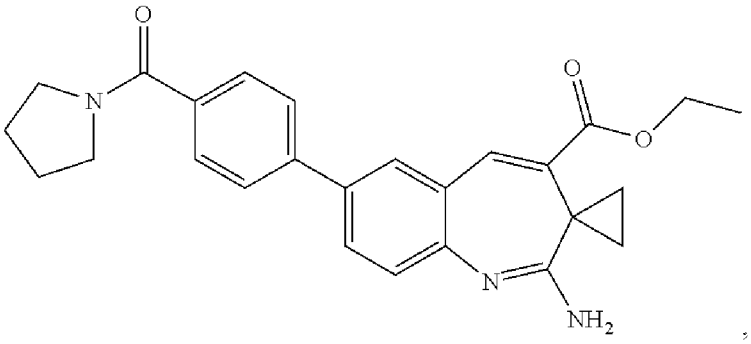
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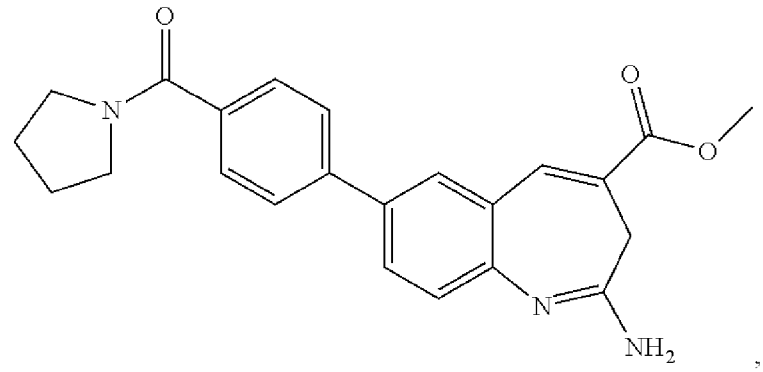
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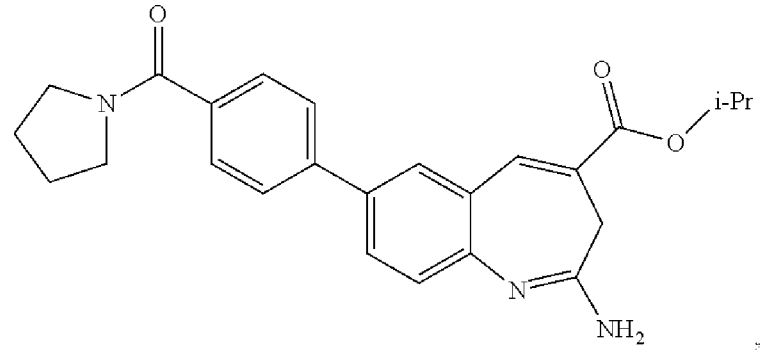


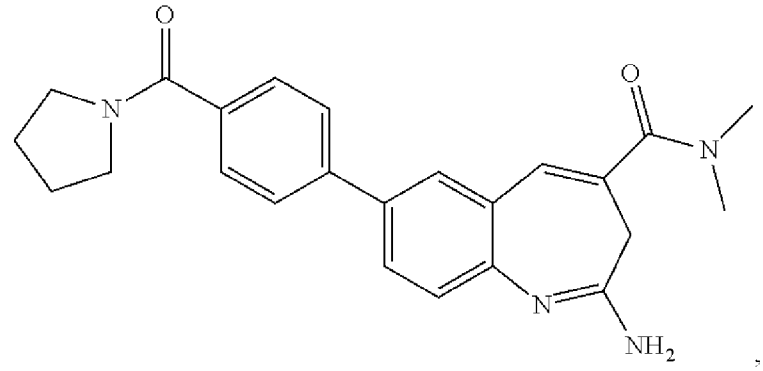
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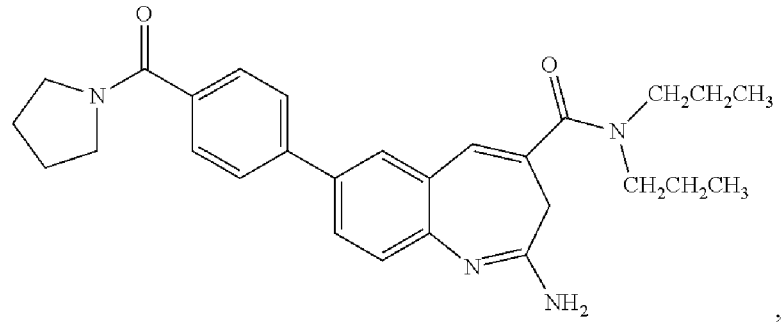


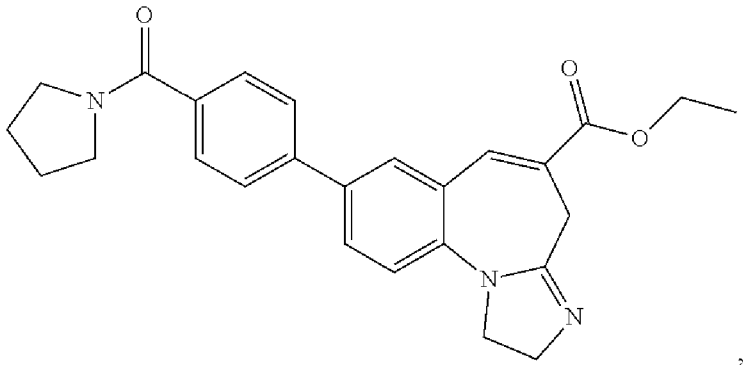
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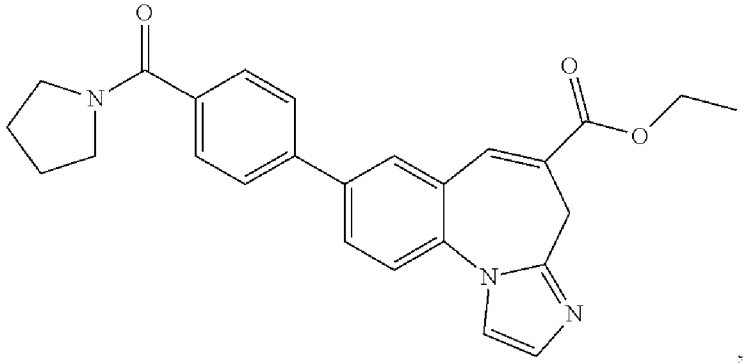




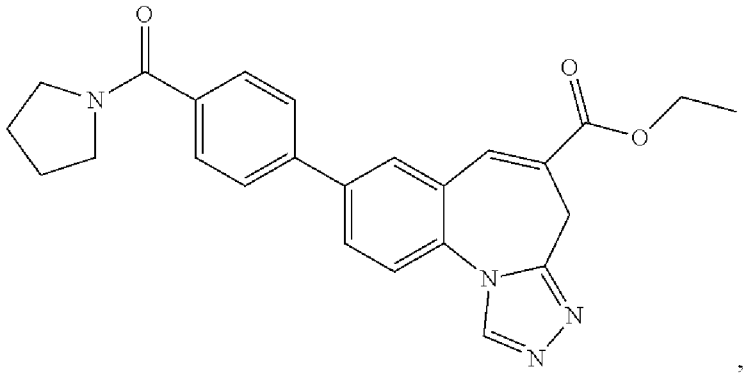




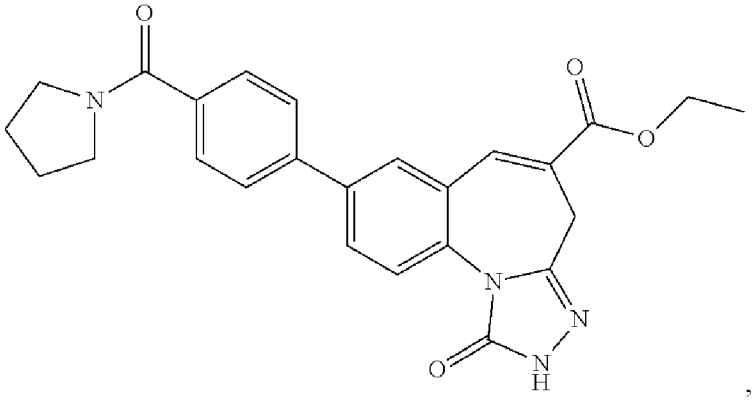
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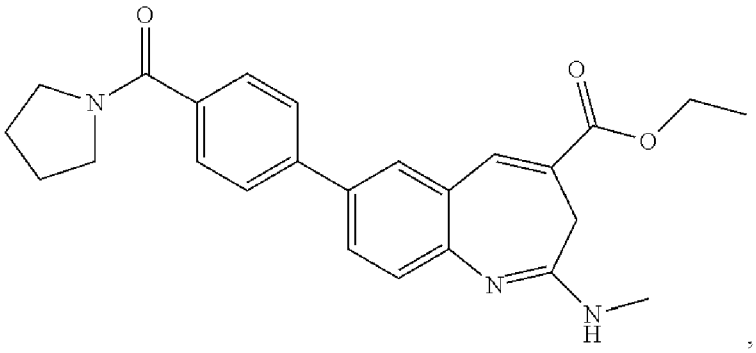
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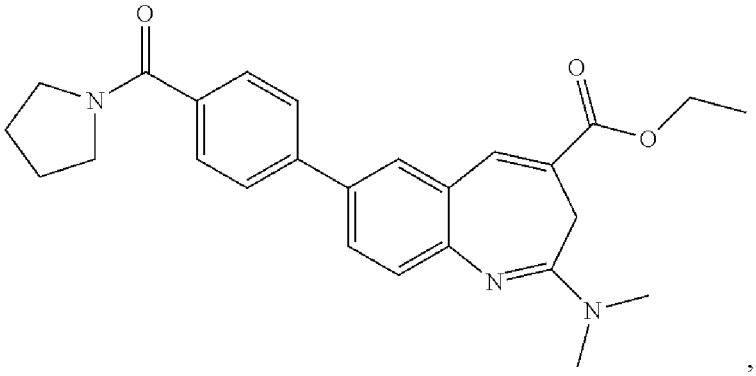
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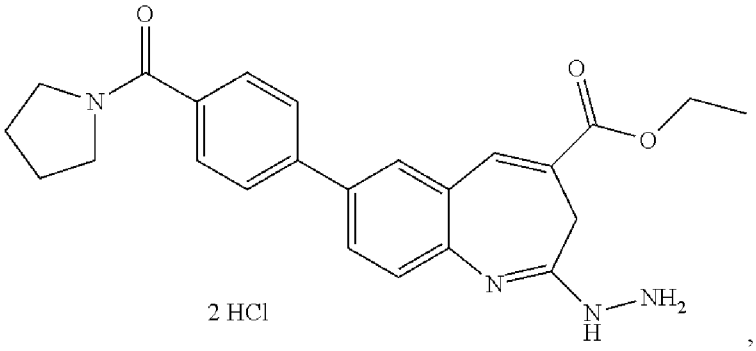
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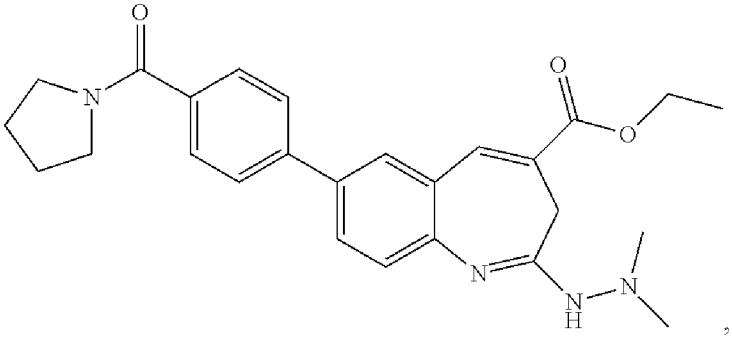


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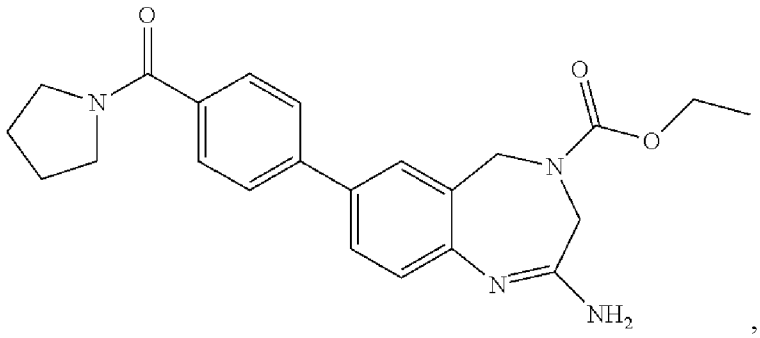


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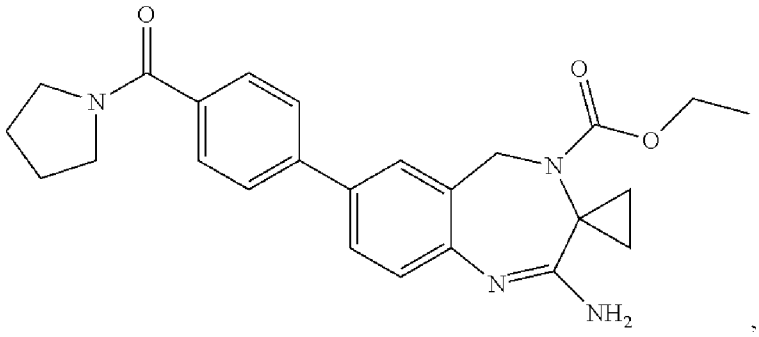
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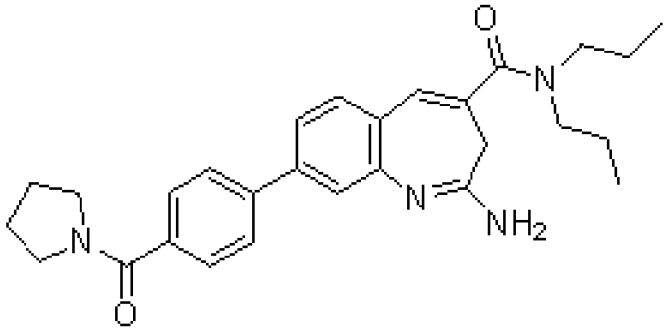
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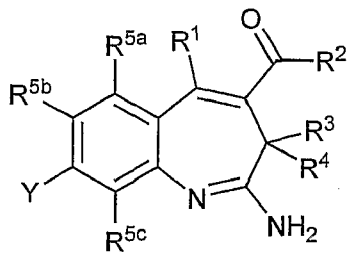


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(VTX-2337).

[0230] In some embodiments, the immunotherapeutic is a TLR modulator (e.g., TLR7 and/or TLR8 agonist) that is represented by structure of Formula (V):



(V)

and metabolites, solvates, tautomers, and prodrugs thereof, wherein:

Y is CF_2CF_3, $CF_2CF_2R^6$, or an aryl or heteroaryl ring, wherein said aryl and heteroaryl rings are substituted with one or more groups independently selected from alkenyl, alkynyl, Br, CN, OH, NR^6R^7, $C(=O)R^8$, $NR^6SO_2R^7$, (C_1-C_6 alkyl)amino, $R^6OC(=O)CH=CH_2$-, SR^6 and SO_2R^6, and wherein the aryl and

heteroaryl rings are optionally further substituted with one or more groups independently selected from F, Cl, CF_3, CF_3O-, HCF_2O-, alkyl, heteroalkyl and ArO-;

R^1, R^3 and R^4 are independently selected from H, alkyl, alkenyl, alkynyl, heteroalkyl, cycloalkyl, cycloalkenyl, heterocycloalkyl, aryl and heteroaryl, wherein the alkyl, alkenyl, alkynyl, heteroalkyl, cycloalkyl, cycloalkenyl, heterocycloalkyl, aryl and heteroaryl are optionally substituted with one or more groups independently selected from alkyl, alkenyl, alkynyl, F, Cl_5 Br, I, CN, OR^6, NR^6R^7, $C(=O)R^6$, $C(=O)OR^6$, $OC(=O)R^6$, $C(=O)NR^6R^7$, (C_1- C_6 alkyl)amino, CH_3OCH_2O-, $R^6OC(^O)CH=CH_2$-, $NR^6SO_2R^7$, SR^6 and SO_2R^6,

or R^3 and R^4 together with the atom to which they are attached form a saturated or partially unsaturated carbocyclic ring, wherein the carbocyclic ring is optionally substituted with one or more groups independently selected from alkyl, alkenyl, alkynyl, F, Cl, Br, I, CN, OR^6, NR^6R^7, $C(=O)R^6$, $C(=O)OR^6$, $0C(=0)R^6$, $C(=O)NR^6R^7$, (C_1-C_6 alkyl)amino, CH_3OCH_2O-, $R^6OC(=O)CH=CH_2$-, $NR^6SO_2R^7$, SR^6 and SO_2R^6;

R^2 and R^8 are independently selected from H, OR^6, NR^6R^7, alkyl, alkenyl, alkynyl, heteroalkyl, cycloalkyl, cycloalkenyl, heterocycloalkyl, aryl and heteroaryl, wherein the alkyl, alkenyl, alkynyl, heteroalkyl, cycloalkyl, cycloalkenyl, heterocycloalkyl, aryl and heteroaryl are optionally substituted with one or more groups independently selected from alkyl, alkenyl, alkynyl, F, Cl, Br_5 I, CN, OR^6, NR^6R^7, $C(=O)R^6$, $C(=O)OR^6$, $OC(=O)R^6$, $C(^O)NR^6R^7$, (C_1-C_6 alkyl)amino, CH_3OCH_2O-, $R^6OC(=O)CH=CH_2$-, $NR^6SO_2R^7$, SR^6 and SO_2R^6;

R^{5a}, R^{5b}, and R^{5c} are independently H, F, Cl, Br, I_5 OMe_5 CH_3, CH_2F_5 CHF_2 or CF_3; and

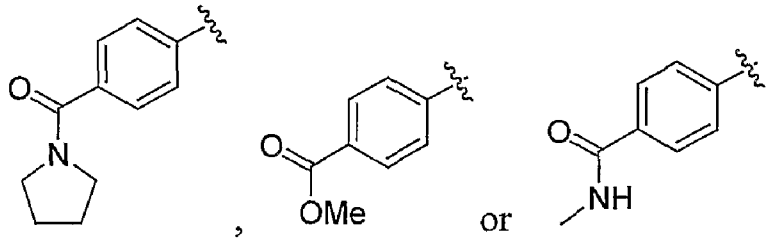
R^6 and R^7 are independently selected from H_5 alkyl, alkenyl, alkynyl, heteroalkyl, cycloalkyl, cycloalkenyl, heterocycloalkyl, aryl and lieteroaryl, wherein said alkyl, alkenyl, alkynyl, heteroalkyl, cycloalkyl, cycloalkenyl, heterocycloalkyl, aryl and heteroaryl are optionally substituted with one or more groups independently selected from alkyl, alkenyl, alkynyl, F, Cl, Br, I, CN, OR^6, NR^6R^7, $C(=O)R^6$, $C(=O)OR^6$, $OC(=O)R^6$, $C(=O)NR^6R^7$, (C_1- C_6 alkyl)amino, CH_3OCH_2O-, $R^6OC(^O)CH=CH_2$-, $NR^6SO_2R^7$, SR^6 and SO_2R^6,

or R^6 and R^7 together with the atom to which they are attached form a saturated or partially unsaturated heterocyclic ring, wherein said heterocyclic ring is optionally substituted with one or more groups independently selected from alkyl, alkenyl, alkynyl, F, Cl, Br, I, CN, OR^6, NR^6R^7, $C(=O)R^6$, $C(=O)OR^6$, $OC(=O)R^6$, $C(=O)NR^6R^7$, (C_1-C_6alkyl)amino, CH_3OCH_2O-, $R^6OC(=O)CH=CH_2$-, $NR^6SO_2R^7$, SR^6 and SO_2R^6. In certain embodiments, R^1, R^3 and R^4 are each hydrogen. In certain embodiments, R^{5a},

R^{5b} and R^{5c} are each hydrogen. WO 2007024612 A2, the disclosure of which is incorporated by reference in its entirety.

[0231] In some embodiments of the compound of Formula (V), R^2 is OR^6. In some embodiments, R^6 is alkyl, such as (1-4C)alkyl. In particular embodiments, R^6 is ethyl.

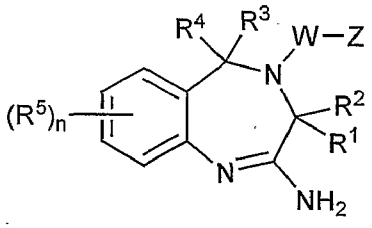
[0232] In some embodiments of the compound of Formula (V), R^2 is NR^6R^7. In some embodiments, R^6 and R^7 are independently H, alkyl, such as (1-6C)alkyl, or heteroalkyl, such as (1-4C)alkoxy(2-4C)alkyl. In particular embodiments, R^6 and R^7 are independently H, ethyl, propyl, or $CH_2CH_2OCH_3$. In some embodiments of the compound of Formula V, Y is aryl, such as phenyl. In some embodiments, the aryl is substituted with $C(=O)R^8$, such as in para-$R^8C(=O)$phenyl. In some embodiments, R^8 is OR^6, NR^6R^7 or heterocycloalkyl. In some embodiments, R^6 and R^7 are independently H or alkyl, such as (1-6C)alkyl. In some other embodiments, R^6 and R^7 together with the nitrogen atom to which they are attached form a 4-6 membered azacycloalkyl ring, such as pyrrolidinyl. In some embodiments, Y is



or

[0233] In some embodiments of the compound of Formula (V), Y is CF_2CF_3.

[0234] In some embodiments, the immunotherapeutic is a TLR modulator (e.g.,TLR8 agonist) that is represented by structure of formula (VI):



(VI)

and metabolites, solvates, tautomers, and pharmaceutically acceptable prodrugs and salts thereof, wherein:

Z is H, alkyl, alkenyl, alkynyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, OR^6 or NR^6R^7, wherein said alkyl, alkenyl, alkynyl; heteroalkyl, cycloalkyl, heterocycloalkyl, aryl and heteroaryl are optionally substituted with one or more groups independently selected from alkyl, alkenyl, alkynyl, F, Cl_3 Br, I, CN, OR^6, NR^6R^7, $C(=O)R^6$, $C(=O)OR^6$, $OC(=O)R^6$, $C(=O)NR^6R^7$, CCi-C_6alkyl)amino, CH_3OCH_2O-, $R^6OCC=O)CH=CH_2$-, $NR^6SO_2R^7$, SR^6 and SO_2R^6;

R^1, R^2, R^3 and R^4 are independently selected from H, alkyl, alkenyl, alkynyl, heteroalkyl, cycloalkyl, cycloalkenyl, heterocycloalkyl, aryl and heteroaryl, wherein said alkyl, alkenyl, alkynyl, heteroalkyl, cycloalkyl, cycloalkenyl, heterocycloalkyl, aryl, and heteroaryl are optionally substituted with one or more groups independently selected from alkyl, alkenyl, alkynyl, F, Cl, Br, I, CN, OR^6, NR^6R^7, CC=O)R^6, C(=O)OR^6, OC(=O)R^6, CC=O)NR^6R^7, (C_1-C_6 alkyl)amino, CH_3OCH_2O-, $R^6OCC=O)CH=CH_2$-, $NR^6SO_2R^7$, SR^6 and SO_2R^6,

or R^1 and R^2 together with the atom to which they are attached form a saturated or partially unsaturated carbocyclic ring, wherein said carbocyclic ring is optionally substituted with one or more groups independently selected from alkyl, alkenyl, alkynyl, F, Cl, Br, I, CN, OR^6, NR^6R^7, C(=O)R^6, CC=O)OR^6, OC(=O)R^6, CC=O)NR^6R^7, CCi-C_6 alkyl)amino, CH_3OCH_2O-, $R^6OCC=O)CH=CH_2$-, $NR^6SO_2R^7$, SR^6 and SO_2R^6;

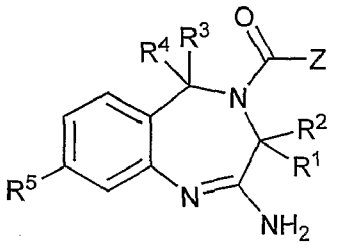
or R^3 and R^4 together are oxo;

each R^5 is independently selected from H, F, Cl, Br, I, OMe, CH_3, CH_2F, CHF_2, CF_3 and CF_2CF_3;

R^6 and R^7 are independently selected from H, alkyl, alkenyl, alkynyl, heteroalkyl, cycloalkyl, cycloalkenyl, heterocycloalkyl, aryl, and heteroaryl, wherein said alkyl, alkenyl, alkynyl, heteroalkyl, cycloalkyl, cycloalkenyl, heterocycloalkyl, aryl, and heteroaryl are optionally substituted with one or more groups independently selected from alkyl, alkenyl, alkynyl, F, Cl, Br, I, CN, OR^6, NR^6R^7, CC=O)R^6, C(=0)0R^6, 0C(=0)R^6, CC=O)NR^6R^7, (C_1- C_6 alkyl)amino, CH_3OCH_2O-, $R^6OC(=O)CH=CH_2$-, $NR^6SO_2R^7$, SR^6 and SO_2R^6;

or R^6 and R^7 together with the atom to which they are attached form a saturated or partially unsaturated heterocyclic ring, wherein the heterocyclic ring is optionally substituted with one or more groups independently selected from alkyl, alkenyl, alkynyl, F, Cl, Br, I, CN, OR^6, NR^6R^7, CC=O)R^6, C(=0)0R^6, 0C(=0)R^6, C(=O)NR^6R^7, (C_1-C_6alkyl)amino, CH_3OCH_2O-, $R^6OC(=O)CH=CH_2$-, $NR^6SO_2R^7$, SR^6 and SO_2R^6; and n is O, 1, 2, 3 or 4. WO2007040840A2, the disclosure of which is incorporated by reference in its entirety.

[0235] In some embodiments, the immunotherapeutic is a TLR modulator (e.g.,TLR8 agonist) that is represented by structure of Formula (VI):



(VII)

and metabolites, solvates, tautomers, and pharmaceutically acceptable salts and prodrugs thereof, wherein:

Z is H, alkyl, alkenyl, alkynyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, OR^6 or NR^6R^7, wherein the alkyl, alkenyl, alkynyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl and heteroaryl are optionally substituted with one or more groups independently selected from alkyl, alkenyl, alkynyl, F, Cl, Br, I, CN, OR^6, NR^6R^7, $C(=O)R^6$, $C(=O)OR^6$, $OC(=O)R^6$, $C(=O)NR^6R^7$, (C_1-C_6 alkyl)amino, CH_3OCH_2O-, $R^6OCC=O)CH=CH_2$-, $NR^6SO_2R^7$, SR^6 and SO_2R^6;

R^1, R^2, R^3 and R^4 are independently selected from H, alkyl, alkenyl, alkynyl, heteroalkyl, . cycloalkyl, cycloalkenyl, heterocycloalkyl, aryl and heteroaryl, wherein said alkyl, alkenyl, alkynyl, heteroalkyl, cycloalkyl, cycloalkenyl, heterocycloalkyl, aryl, and heteroaryl are optionally substituted with one or more groups independently selected from alkyl, alkenyl, alkynyl, F, Cl, Br, I_9 CN, OR^6, NR^6R^7, $C(=0)R^6$, $C(=O)OR^6$, $OC(=O)R^6$, $C(=O)NR^6R^7$, (C_1-C_6 alkyl)amino, CH_3OCH_2O-, $R^6OCC=O)CH=CH_2$-, $NR^6SO_2R^7$, SR^6 and SO_2R^6,

or R^1 and R^2 together with the atom to which they are attached form a saturated or partially unsaturated carbocyclic ring, wherein said carbocyclic ring is optionally substituted with one or more groups independently selected from alkyl, alkenyl, alkynyl, F, Cl, Br, I, CN, OR^6, NR^6R^7, $C(=0)R^6$, $C(=O)OR^6$, $OC(=O)R^6$, $C(=O)NR^6R^7$, (C_1-C_6 alkyl)amino, CH_3OCH_2O-, $R^6OC(=O)CH=CH_2$-, $NR^6SO_2R^7$, SR^6 and SO_2R^6,

or R^3 and R^4 together are oxo;

R^5 is H, F, Cl, Br, I, OMe, CH_3, CH_2F, CHF_2, CF_3 or CF_2CF_3;

R^6 and R^7 are independently selected from H, alkyl, alkenyl, alkynyl, heteroalkyl, cycloalkyl, cycloalkenyl, heterocycloalkyl, aryl, and heteroaryl, wherein said alkyl, alkenyl, alkynyl, heteroalkyl, cycloalkyl, cycloalkenyl, heterocycloalkyl, aryl, and heteroaryl are optionally substituted with one or more groups independently selected from alkyl, alkenyl, alkynyl, F, Cl, Br, I, CN, OR^6, NR^6R^7, $C(=O)R^6$, $C(=0)0R^6$, $0C(=0)R^6$, $C(=O)NR^6R^7$, (C_1- C_6 alkyl)amino$_5$ CH_3OCH_2O-, $R^6OC(=O)CH=CH_2$-, $NR^6SO_2R^7$, SR^6 and SO_2R^6;

or R^6 and R^7 together with the atom to which they are attached form a saturated or partially unsaturated heterocyclic ring, wherein said heterocyclic ring is optionally substituted with one or more groups independently selected from alkyl, alkenyl, alkynyl, F, Cl, Br, I, CN, OR^6, NR^6R^7, $C(=O)R^6$, $C(=O)OR^6$, $OC(=O)R^6$, $C(=O)NR^6R^7$, (C_1-C_6 alkyl)amino, CH_3OCH_2O-, $R^6OC(=O)CH=CH_2$-, $NR^6SO_2R^7$, SR^6 and SO_2R^6; and

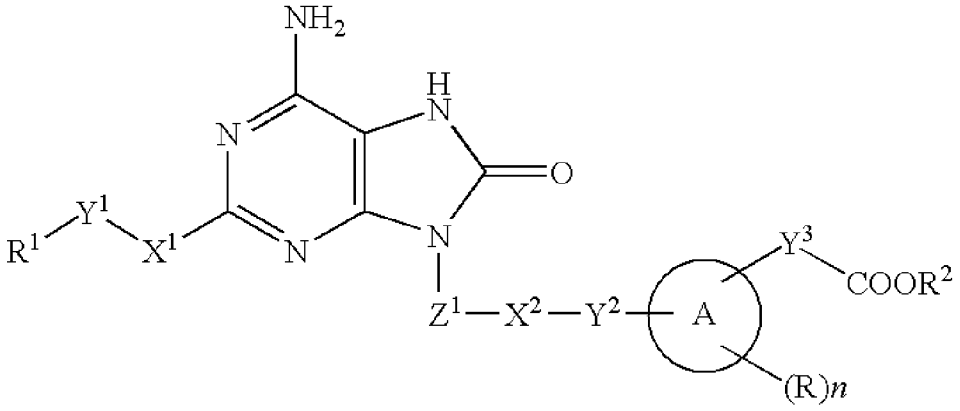
n is 0, 1, 2, 3 or 4.

[0236] In some embodiments, Z is OR^6. In some embodiments, R^6 is alkyl, such as (1- 6C)alkyl. In particular embodiments, R^6 is ethyl, propyl, isopropyl or isobutyl.

[0237] In some embodiments, Z is NR^6R^7. In some embodiments, R^6 and R^7 are independently H or alkyl, such as (1-6C)alkyl. In some embodiments, R^6 and R^7 are ethyl. In some embodiments, n is 0 or 1.

[0238] In some embodiments, R^5 is C F_2CF_3. In certain embodiments, R^3 is H or alkyl, such as (1-4C)alkyl, and R^4 is H. In certain embodiments, R is alkyl, such as (1-4C)alkyl. In some embodiments, R is methyl. In other particular embodiments, R^3 is H. In some embodiments, R is H or alkyl, such as (1-4C)alkyl and R is H. In some embodiments, R^1 is alkyl. In some embodiments, R^1 is methyl. In some particular embodiments, R^1 is H.

[0239] In some embodiments, the activating moiety is a TLR7 and/or TLR8 agonist that is represented by structure of Formula (XV):



(XV)

wherein ring A represents a 6-10 membered aromatic carbocyclic ring or a 5-10 membered heteroaromatic ring;

R represents a halogen atom, an alkyl group, a hydroxyalkyl group, a haloalkyl group, an alkoxy group, a hydroxyalkoxy group, a haloalkoxy group, amino group, an alkylamino group, a dialkylamino group, or a

4-7 membered cyclic group containing in the ring 1-2 hetero atoms selected from 1-2 nitrogen atoms and optionally 0-1 oxygen atom or 0-1 sulfur atom;

n represents an integer of 0-2, and when n is 2, the Rs may be the same or different;

Z^1 represents a substituted or unsubstituted alkylene group or a substituted or unsubstituted cycloalkylene group;

X^2 represents oxygen atom, sulfur atom, SO_2, NR^5, CO, $CONR^5$, NR^5CO, SO_2NR^5, NR^5SO_2, NR^5CONR^6 or NR^5CSNR^6 (in which R^5 and R^6 are each independently hydrogen atom, a substituted or unsubstituted alkyl group, or a substituted or unsubstituted cycloalkyl group);

Y^1, Y^2 and Y^3 represent each independently a single bond or an alkylene group;

X^1 represents oxygen atom, sulfur atom, SO_2, NR^4 (wherein R^4 is hydrogen atom or an alkyl group) or a single bond;

R^2 represents hydrogen atom, a substituted or unsubstituted alkyl group, a substituted or unsubstituted alkenyl group, a substituted or unsubstituted alkynyl group or a substituted or unsubstituted cycloalkyl group; and

R^1 represents hydrogen atom, hydroxy group, an alkoxy group, an alkoxycarbonyl group, a haloalkyl group, a haloalkoxy group, a substituted or unsubstituted aryl group, a substituted or unsubstituted heteroaryl group or a substituted or unsubstituted cycloalkyl group. The linker is linked to one of the possible linking site of the angonist, such as to $-NH_2$.

[0240] In some embodiments, R^1 represents hydrogen, hydroxyl, or a C_1-C_6 alkoxy, C_2-C_5alkoxycarbonyl, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, C_6-C_{10} aryl, C_5-C_{10}heteroaryl or C_3-C_8 cycloalkyl group, each group being optionally substituted by one or more substituents independently selected from halogen, hydroxyl, a C_1-C_6 alkyl, C_1-C_6 haloalkyl, C_1-C_6 alkoxy, C_1-C_6haloalkoxy, C_2-C_5 alkoxycarbonyl, amino (NH_2), (mono)-C_1-C_6alkylamino and (di)-C_1-C_6 alkylamino group;

Y^1 represents a single bond or C_1-C_6 alkylene;

X^1 represents a single bond, an oxygen, sulphur atom, sulphonyl (SO_2) or NR^3;

Z^1 represents a C_2-C_6 alkylene or C_3-C_8 cycloalkylene group, each group being optionally substituted by at least one hydroxyl;

X^2 represents NR^4;

Y^2 represents a single bond or C_1-C_6 alkylene;

Y^3 represents a single bond or C_1-C_6 alkylene;

n is an integer 0, 1 or 2;

R represents halogen or a C_1-C_6 alkyl, C_1-C_6 hydroxyalkyl, C_1-C_6haloalkyl, C_1-C_6 alkoxy, C_1-C_6 hydroxyalkoxy, C_1-C_6 haloalkoxy, amino (NH_2), (mono)-C_1-C_6 alkylamino, (di)-C_1-C_6 alkylamino group or a C_3-C_8saturated heterocyclic ring containing a ring nitrogen atom and optionally one or more further heteroatoms independently selected from nitrogen, oxygen and sulphur, the heterocyclic ring being optionally substituted by one or more substituents independently selected from halogen, hydroxyl, oxo, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_2-C_5 alkylcarbonyl and C_2-C_5alkoxycarbonyl;

R^2 represents hydrogen or a C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl or C_3-C_8 cycloalkyl group, each group being optionally substituted by one or more substituents independently selected from halogen, hydroxyl or a C_1-C_6 alkoxy, a C_2-C_{10} acyloxy, group selected from a C_2-$_5$alkylcarbonyloxy group, a C_2-C_5 alkenylcarbonyloxy group, a C_2-C_5alkynylcarbonyloxy group, a C_6-C_9 arylcarbonyloxy group and a C_5-C_9heteroarylcarbonyloxy group, each of which acyloxy groups may be optionally substituted by one or more substituents independently selected from halogen, hydroxyl, C_1-C_3 alkoxy and phenyl providing that the total number of carbon atoms in the acyloxy group does not exceed 10, amino (NH_2), (mono)-C_1-C_6 alkylamino, (di)-C_1-C_6 alkylamino group and a C_3-C_8 saturated heterocyclic ring containing a ring nitrogen atom and optionally one or more further heteroatoms independently selected from nitrogen, oxygen and sulphur, the heterocyclic ring in turn being optionally substituted by one or more substituents independently selected from halogen, hydroxyl, oxo, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_2-C_5alkylcarbonyl and C_2-C_5 alkoxycarbonyl group;

R^3 represents hydrogen or C_1-C_6 alkyl;

R^4 represents CO_2R^5, SO_2R^5, COR^5, $SO_2NR^6R^7$ and $CONR^6R^7$;

R^5 independently represents

(i) 3- to 8-membered heterocyclic ring containing 1 or 2 heteroatoms selected from a ring group NR^8, $S(O)_m$ or oxygen, the 3- to 8-membered heterocyclic ring being optionally substituted by one or more substituents independently selected from halogen, hydroxyl or a C_1-C_6 alkyl and C_1-C_6 alkoxy group, or

(ii) a C_6-C_{10} aryl or C_5-C_{10} heteroaryl group, each of which may be optionally substituted by one or more substituents independently selected from halogen, cyano, C_1-C_6 alkyl, C_1-C_3 haloalkyl, carboxyl, $S(O)_mR^9$, OR^{10}, CO_2R^{10}, $SO_2NR^{10}R^{11}$, $CONR^{10}R^{11}$, $NR^{10}R^{11}$, $NR^{10}SO_2R^9$, $NR^{10}CO_2R^9$, $NR^{10}COR^9$, or

(iii) a C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl or C_3-C_8 cycloalkyl group, each of which may be optionally substituted by one or more substituents independently selected from halogen, CN, C_3-C_8cycloalkyl, $S(O)_pR^{12}$, OR^{13}, COR^{13}, CO_2R^{13}, $SO_2NR^{13}R^{14}$, $CONR^{13}R^{14}$, $NR^{13}R^{14}$, $NR^{13}SO_2R^{12}$,

$NR^{13}CO_2R^{12}$, $NR^{13}COR^{12}$, $NR^{13}SO_2R^{12}$ or a C_6-C_{10} aryl or C_5-C_{10} heteroaryl group or a heterocyclic ring, the latter three groups may be optionally substituted by one or more substituents independently selected from C_1-C_6 alkyl (optionally substituted by hydroxy, C_1-C_6 alkoxy, C_1-C_6 alkoxycarbonyl, amino, C_1-C_6 alkylamino, di-C_1-C_6alkylamino, $NH_2C(O)$—, C_1-C_6 alkylNHC(O), di-C_1-C_6 alkyl NC(O), —OCH_2CH_2OH, pyrrolidinyl, pyrrolidinylcarbonyl, furanyl, piperidyl, methylpiperidyl or phenyl), C_2-C_6 alkenyl (optionally substituted by phenyl), halogen, hydroxy, cyano, carboxy, amino, C_1-C_6alkylamino, di-C_1-C_6 alkylamino, $NH_2C(O)$—, C_1-C_6 alkyl NHC(O)—,di-C_1-C_6 alkyl NC(O), C_1-C_6 alkoxycarbonyl, C_1-C_6alkylsulphonyl, C_1-C_6 alkylcarbonylamino, C_1-C_6alkylcarbonylmethylamino, phenyl (optionally substituted by hydroxy, fluoro or methyl), pyrrolidinyl, pyridyl, piperidinyl, benzothiazolyl or pyrimidinyl;

R^6 represents hydrogen or a C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_8 cycloalkyl group or heterocyclic ring, each of which may be optionally substituted by one or more substituents independently selected from halogen, hydroxyl, oxo, cyano, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_8 cycloalkyl, OR^{15}, $S(O)_qR^{15}$, CO_2R^{16}, COR^{16}, $NR^{16}R^{17}$, $CONR^{16}R^{17}$, $NR^{16}COR^{17}$, $NR^{16}CO_2R^{15}$, $SO_2NR^{16}R^{17}$, $NR^{16}SO_2R^{15}$, or a C_6-C_{10} aryl or C_5-C_{10} heteroaryl group or heterocyclic ring, the latter three groups being optionally substituted by one or more substituents independently selected from, C_1-C_6 alkyl, C_3-C_8 cycloalkyl, halogen, $S(O)_qR^{15}$, CO_2R^{16}, COR^{16}, hydroxy or cyano; and

R^7 represents hydrogen, a C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, or C_3-C_8 cycloalkyl group, each group may be optionally substituted by one or more substituents independently selected from halogen, C_3-C_8cycloalkyl, a C_6-C_{10} aryl or C_5-C_{10} heteroaryl group, carboxy, cyano, OR^{15}, hydroxy or $NR^{18}R^{19}$, or

R^6 and R^7 together with the nitrogen atom to which they are attached fowl a 3- to 8-membered saturated or partially saturated heterocyclic ring, optionally containing further heteroatoms or heterogroups selected from nitrogen, $S(O)_m$ or oxygen, the heterocyclic ring, may be optionally substituted by one or more substituents independently selected from halogen, hydroxyl, carboxyl, cyano, OR^{20}, $NR^{21}R^{22}$, $S(O)_qR^{23}$, COR^{24}, CO_2R^{24}, $NR^{24}R^{25}$, $CONR^{24}R^{25}$, $NR^{24}COR^{25}$, $NR^{24}CO_2R^{23}$, $SO_2NR^{24}R^{25}$, $NR^{24}SO_2R^{23}$, C_6-C_{10} aryl , C_5-C_{10} heteroaryl group, heterocyclic ring, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl or C_3-C_8cycloalkyl group, the latter seven groups being optionally substituted by one or more substituents independently selected from halogen, hydroxyl, oxo, cyano, OR^{20}, $S(O)_qR^{23}$, COR^{24}, CO_2R^{24}, $NR^{24}R^{25}$, $CONR^{24}R^{25}$, $NR^{24}CO_2R^{23}$, $NR^{24}COR^{25}$, $SO_2NR^{24}R^{25}$, $NR^{24}SO_2R^{23}$, a heterocyclic ring or a C_6-C_{10} aryl or C_5-C_{10} heteroaryl group, the latter three groups being optionally substituted by one or more substituents independently selected from C_1-C_6 alkyl, halogen, hydroxy or cyano;

R^8 represents hydrogen, CO_2R^{26}, COR^{26}, SO_2R^{26}, C_1-C_6 alkyl or C_3-C_6cycloalkyl group, each group may be optionally substituted by one or more substituents independently selected from halogen, hydroxyl, and $NR^{27}R^{28}$;

R^{10}, R^{11}, R^{16}, R^{17}, R^{18}, R^{19}, R^{21}, R^{22}, R^{26}, R^{27} or R^{28} each independently represents hydrogen, and a C_1-C_6 alkyl or C_3-C_6cycloalkyl group;

$R^{24}$and R^{25} each independently represents hydrogen, and a C_1-C_6 alkyl or C_3-C_6 cycloalkyl group; or

R^{24} and R^{25} together with the nitrogen atom to which they are attached form a 3- to 8-membered saturated or partially saturated heterocyclic ring, optionally containing further heteroatoms or heterogroups selected from nitrogen, $S(O)_m$ or oxygen;

R^9, R^{12}, R^{15} and R^{23} represent C_1-C_6 alkyl or C_3-C_6 cycloalkyl;

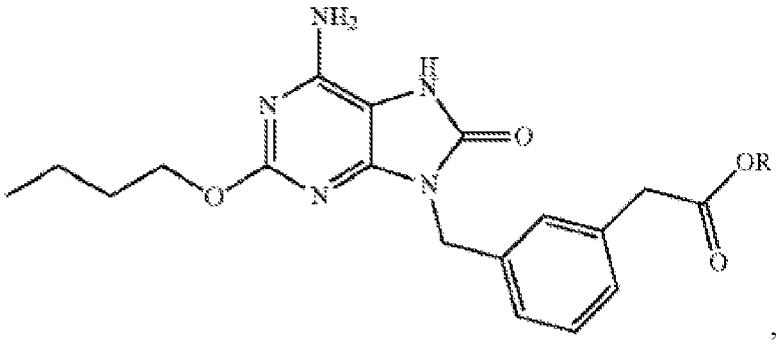
R^{13} and R^{14} are defined as for R^6 and R^7 respectively;

R^{20} represents a C_1-C_6 alkyl optionally substituted by one or more substituents independently selected from halogen, hydroxyl or OR^{23};

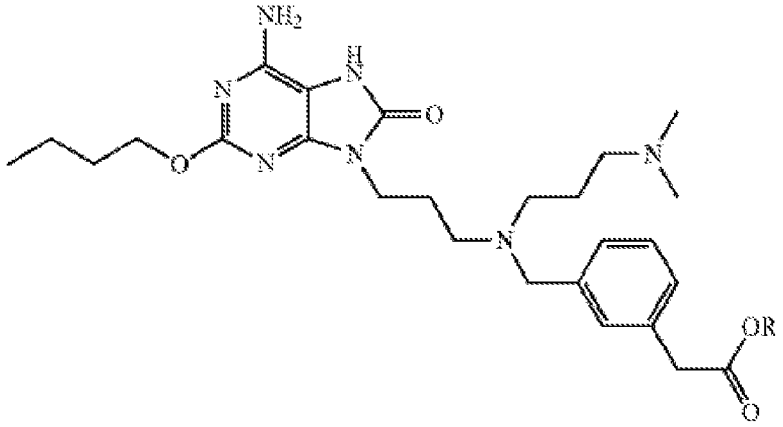
m, p, q and r each independently represent an integer 0, 1 or 2; and

A represents a C_6-C_{10} aryl or C_5-C_{12} heteroaryl group. See WO2008004948A1, US 8,138,172, and US 8,575,180, the disclosures of which are incorporpated by reference in their entirties.

[0241] In some embodiments, the activating moiety is a TLR7 and/or TLR8 agonist having the structure of:

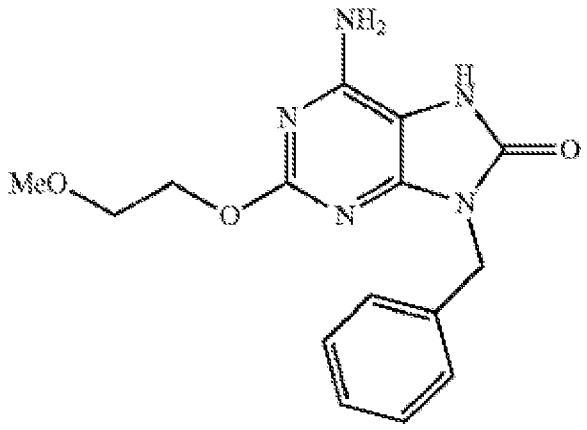


, or

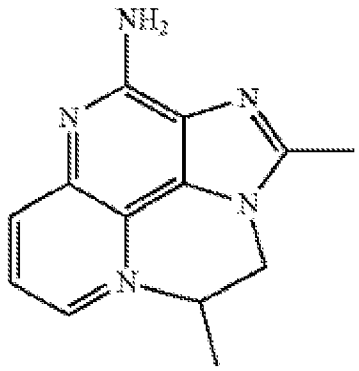


wherein R is Me or H.

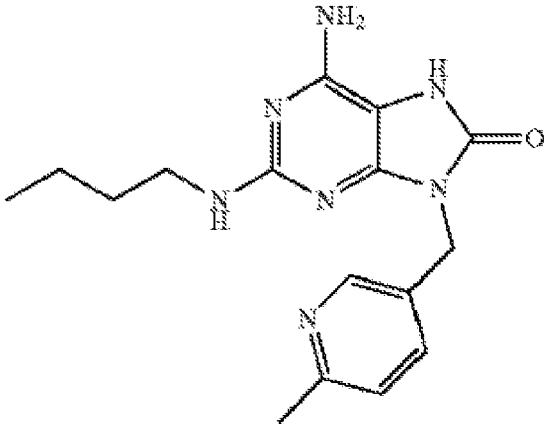
[0242] In some embodiments, the activating moiety is a TLR7 and/or TLR8 agonist having the structure of:



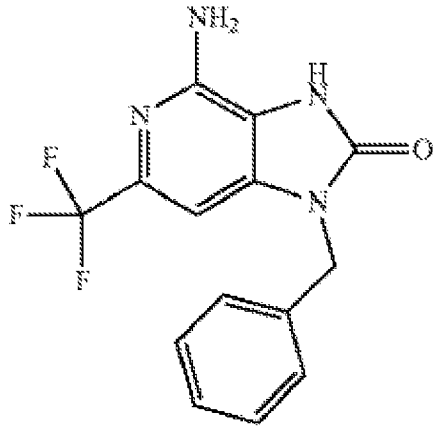
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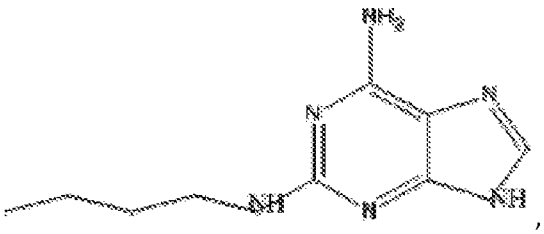
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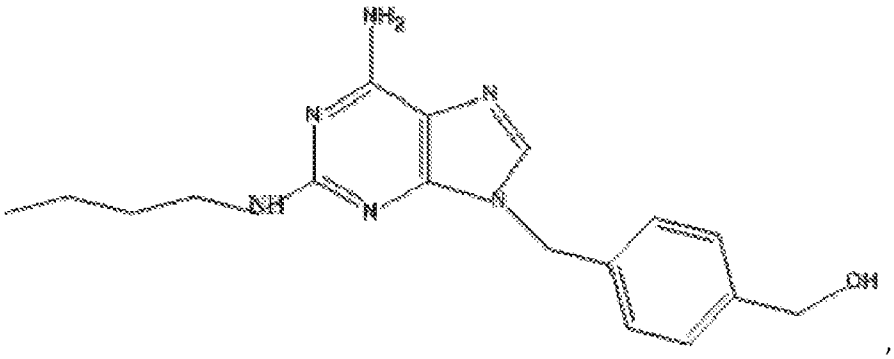
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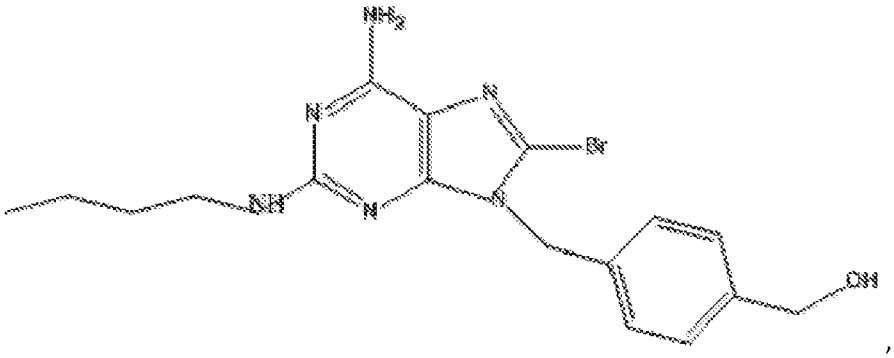
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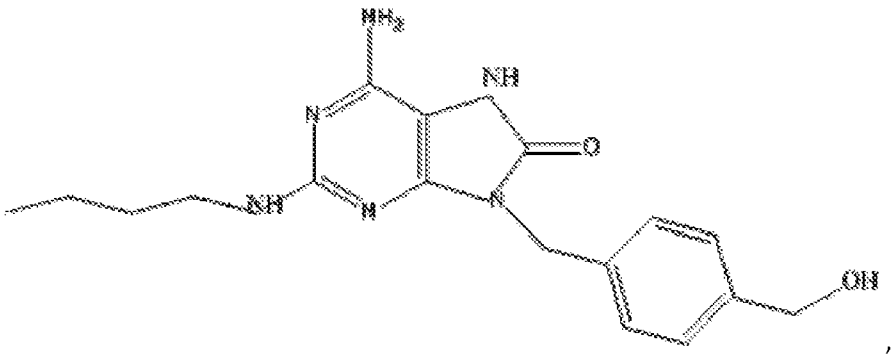
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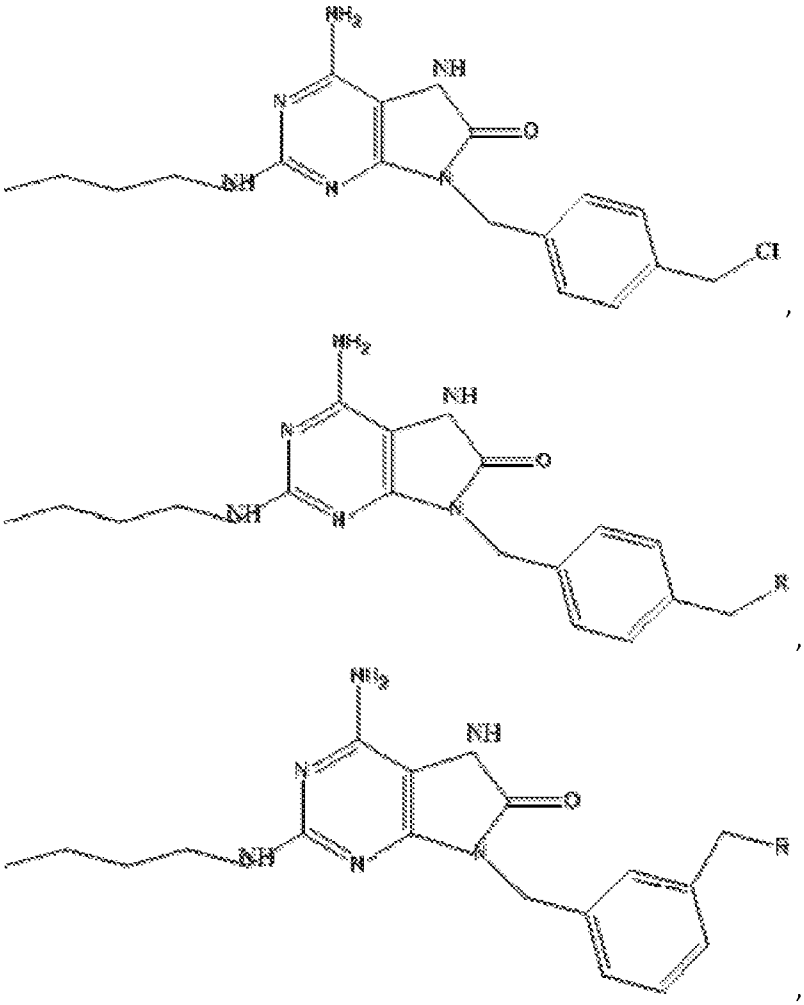
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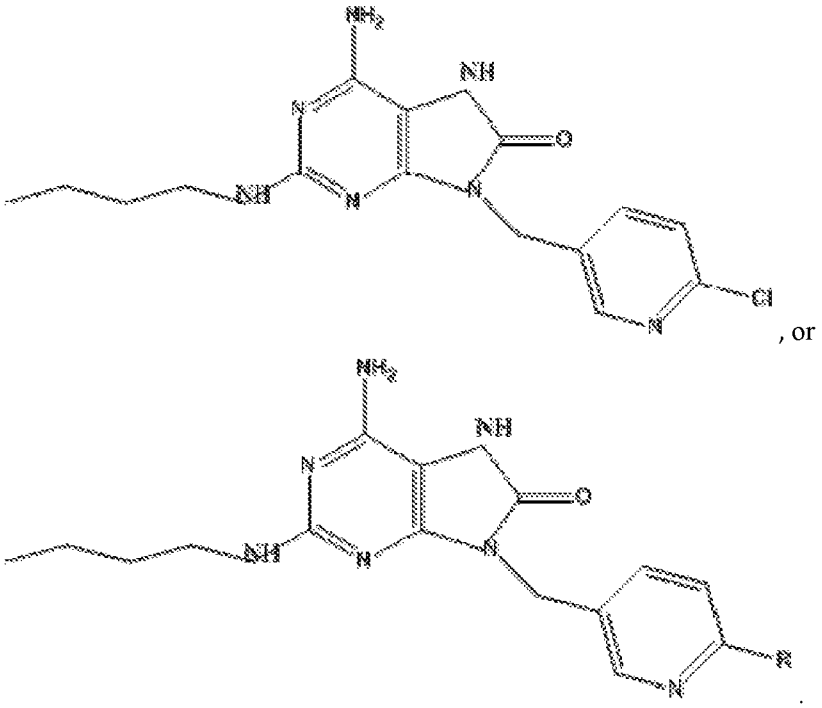


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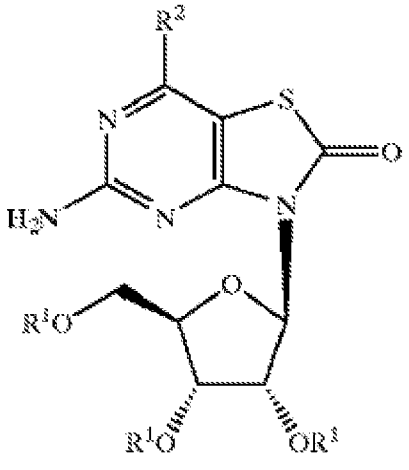




, or

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[0243] In some embodiments, the activating moiety is a TLR7 and/or TLR8 agonist having the structure of Formula (XVI):



(XVI),

wherein: R^1 is independently H, $-C(O)R^3$, or a racemic, L-, or D- amino acid group $-C(O)CHNH_2R^4$, wherein R^3 is a substituted or unsubstituted alkyl, and R^4 is H, or a substituted or unsubstituted alkyl;

R^2 is H, O, OR^5, or $N(R^6)_2$, wherein R^5 is independently H or alkyl, and wherein R^6 is independently H, substituted or unsubstituted alkyl, cycloalkyl, or together with nitrogen forms a substituted or unsubstituted heterocycloalkyl ring; and wherein if R is -OH, at least one of the R groups is a racemic, L-,

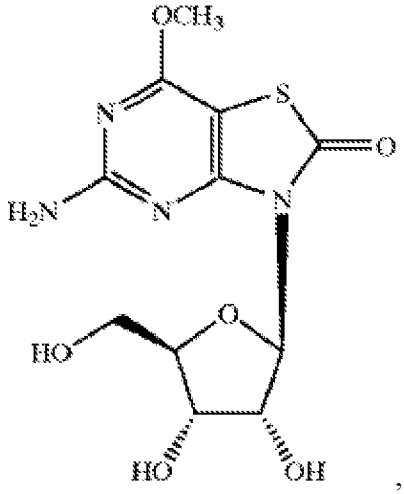
or D- amino acid group -C(O)CHNH$_2$R^4. See US 6,924,271, the disclosure of which is incorporated by reference in its entirety.

[0244] In some embodiments, at least one of the R^1 groups is a racemic, L-, or D- amino acid group -C(O)CHNH$_2$R^4, wherein R^4 is a substituted or unsubstituted alkyl, and wherein the remaining R^1 groups are H; R^2 is OR^5 or N(R^6)$_2$, wherein R^{5}is independently selected from H or alkyl, and wherein R is independently H, substituted or unsubstituted alkyl, cycloalkyl, or together with nitrogen forms a substituted or unsubstituted heterocycloalkyl ring.

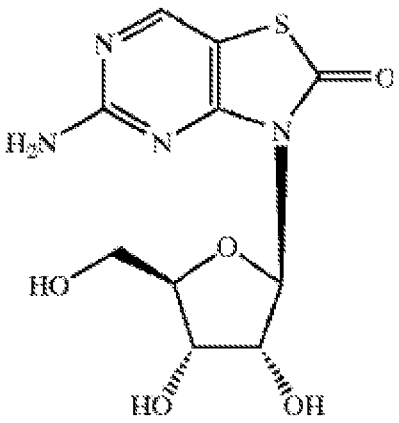
[0245] In some embodiments, at least one of the R^1 groups is a L- amino acid group -C(O)CHNH$_2$R^4, wherein R^4 is a substituted or unsubstituted alkyl, and wherein the remaining R^1 groups are H; R^2 is OR^5 or N(R^6)$_2$, wherein R^4 is a substituted alkyl, and wherein R^6 is independently H or substituted or unsubstituted alkyl.

[0246] In some embodiments, at least one of the R^1 groups is a L- amino acid group -C(O)CHNH$_2$R , wherein R^4 is -CH(CH$_3$)$_2$, and wherein the remaining R^1 groups are H; and R^2 is OH.

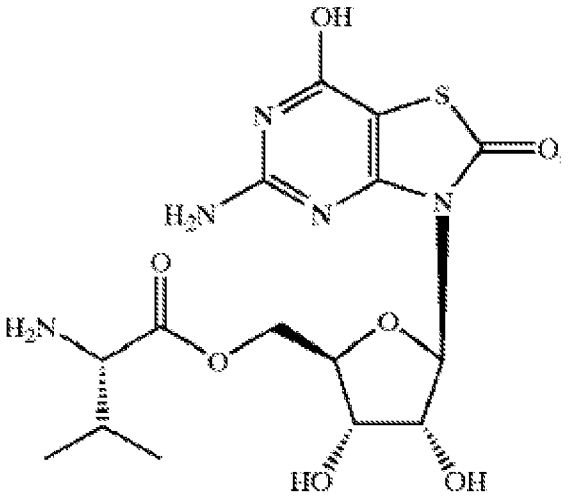
[0247] In some embodiments, the TLR7 and/or agonist is selected from the group consisting of:



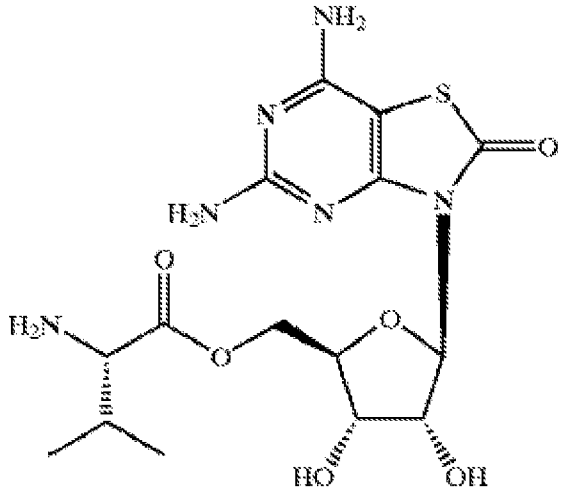
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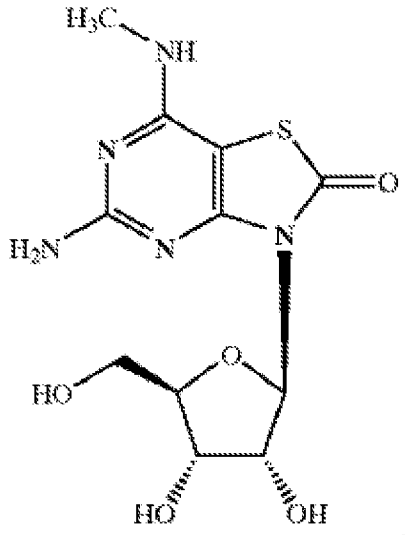
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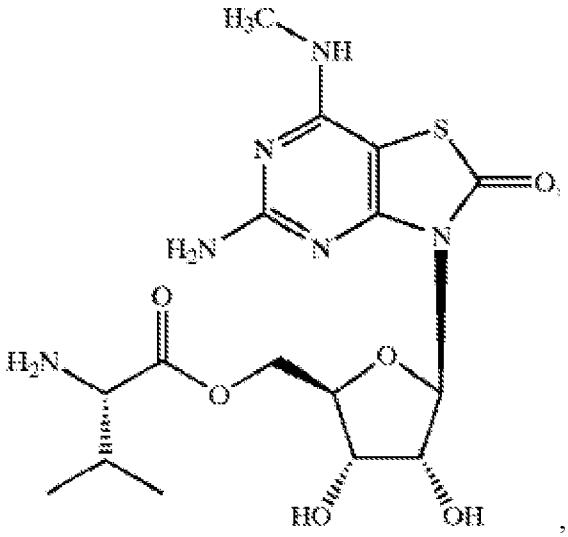
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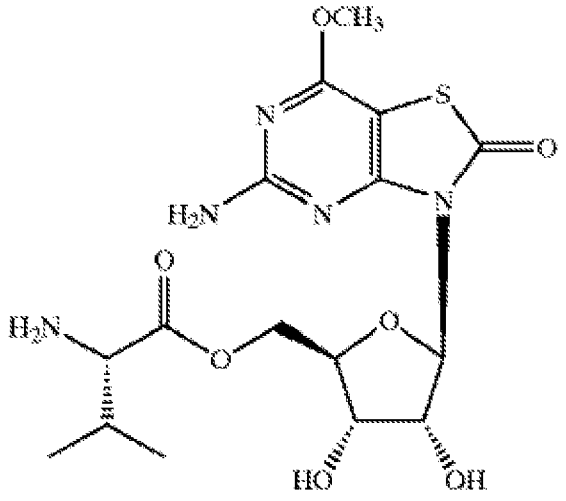
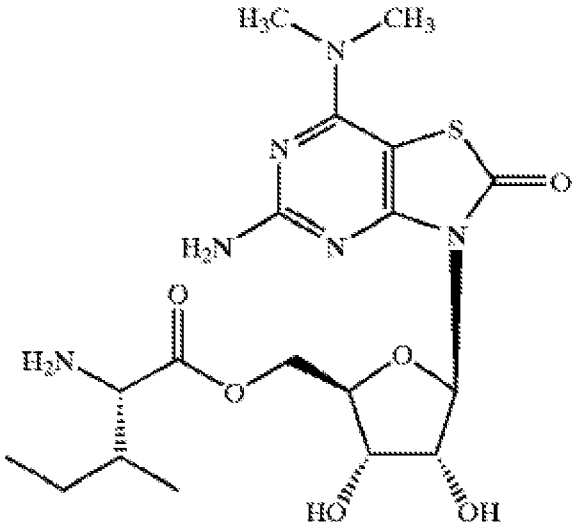
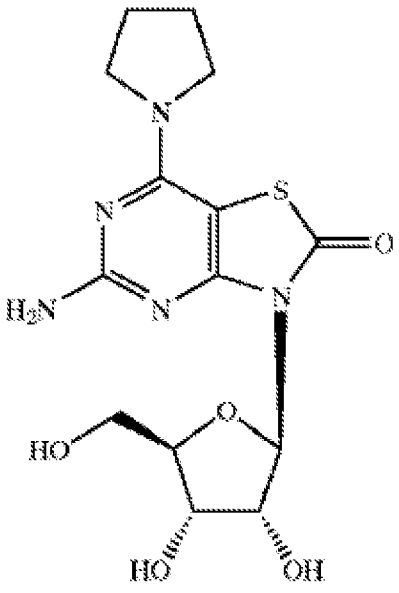
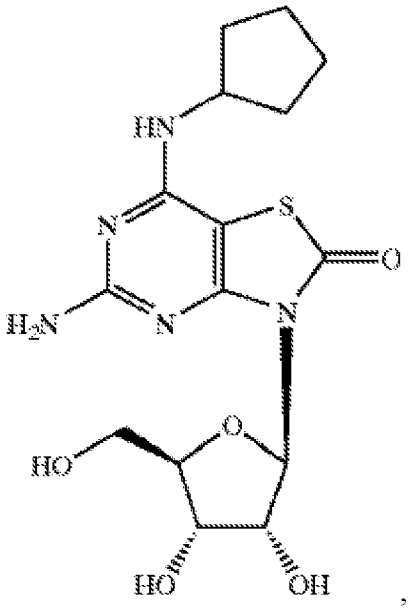
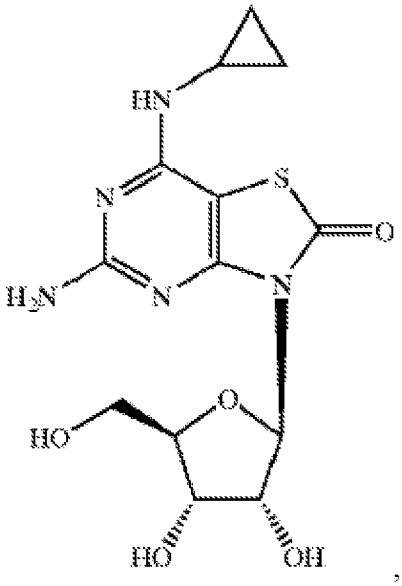
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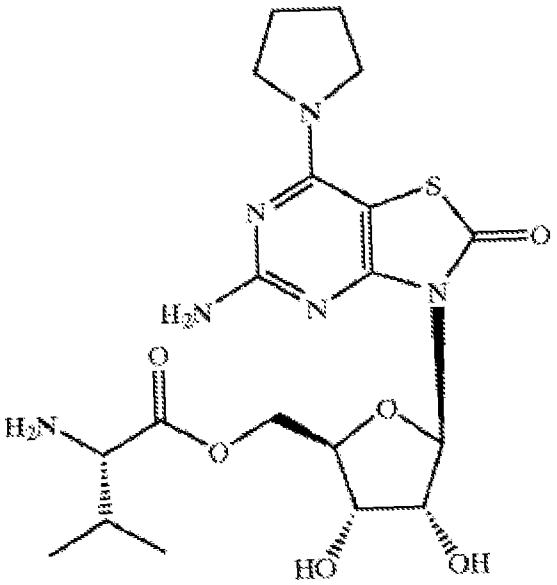


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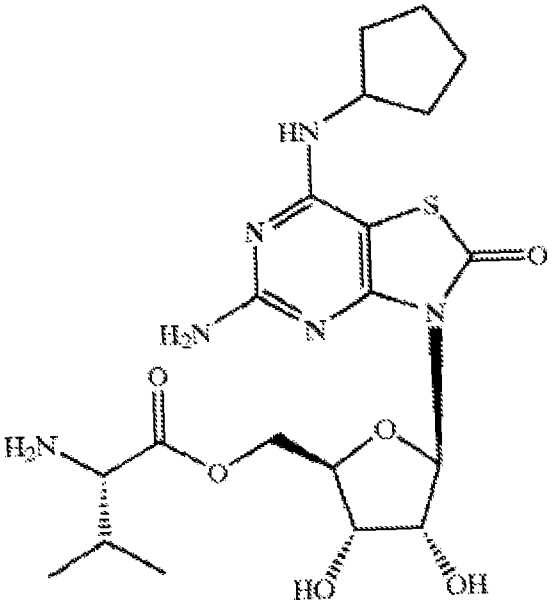


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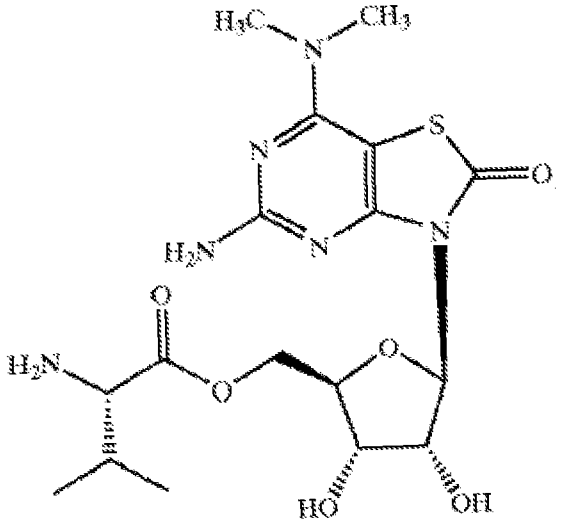

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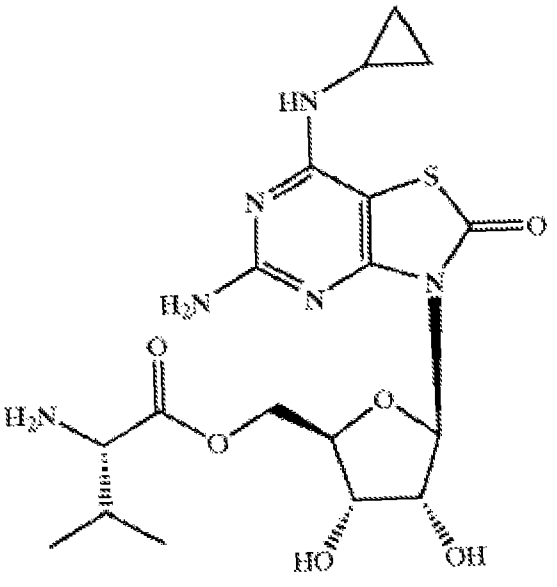
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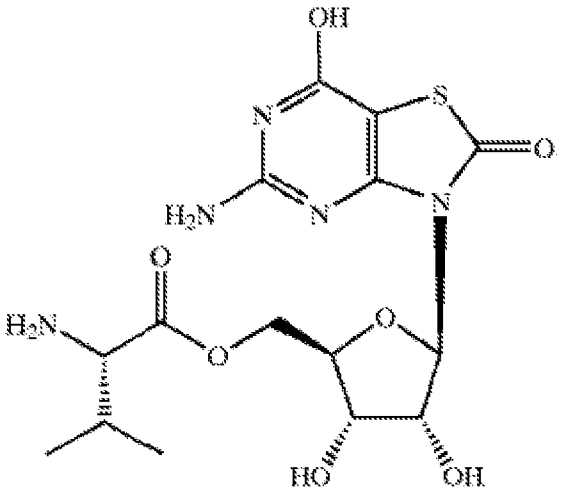
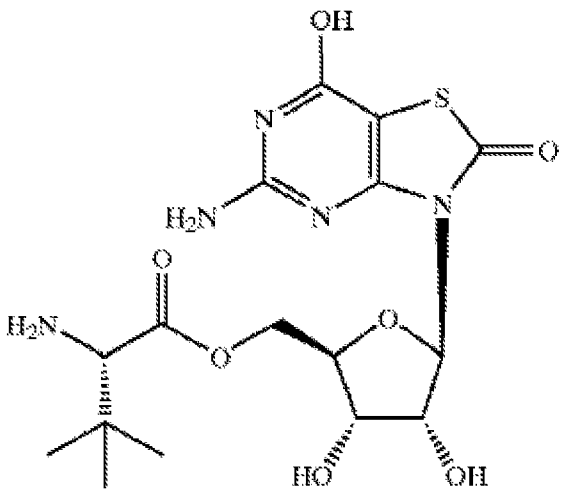
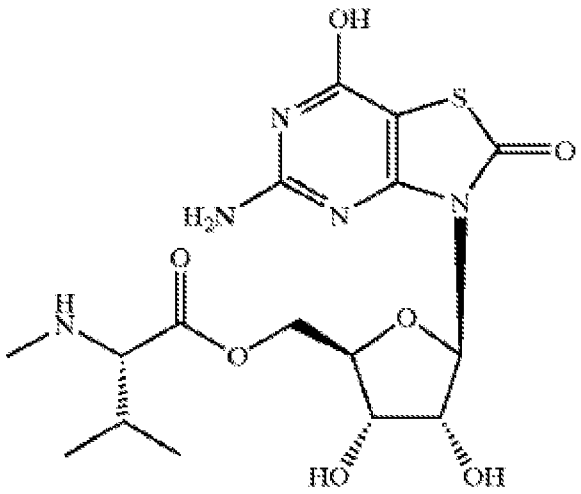
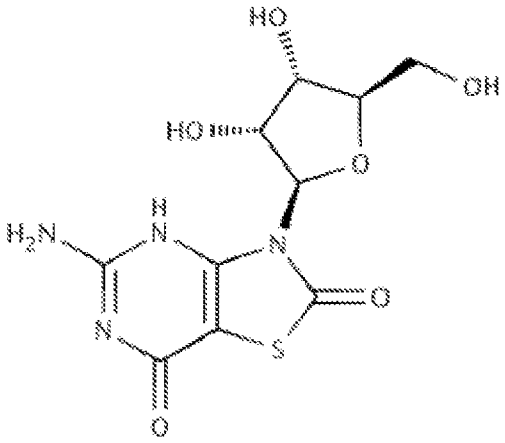
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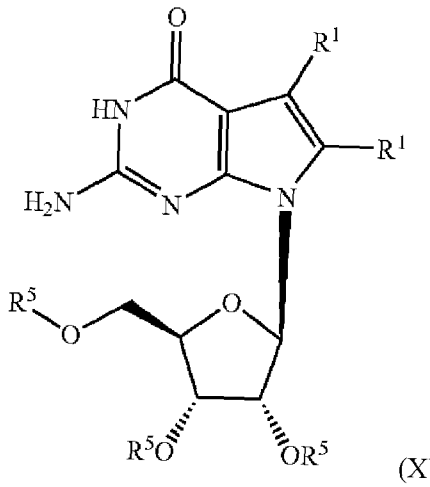
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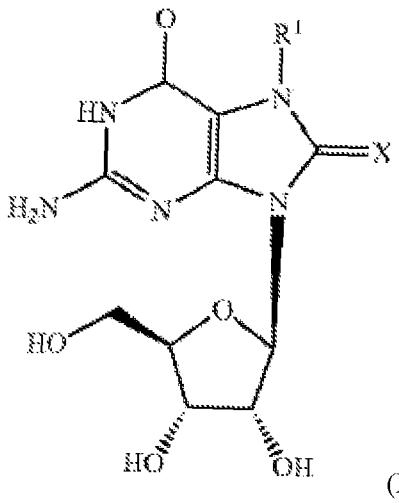
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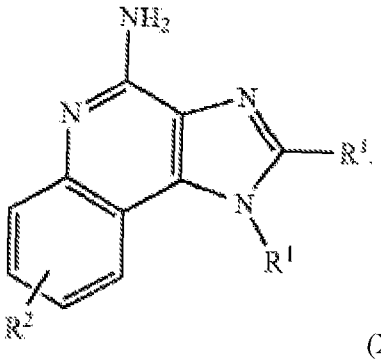
[0248] In some embodiments, the activating moiety is a TLR7 and/or TLR8 agonist having the structure of:



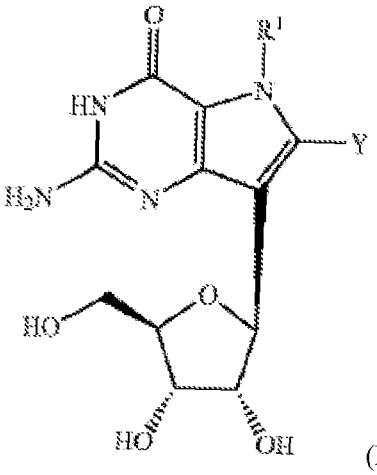
(XVIIa),



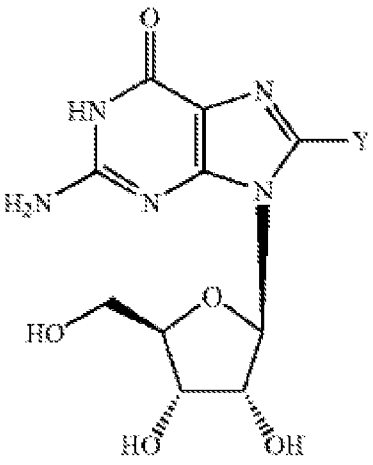
(XVIIb),



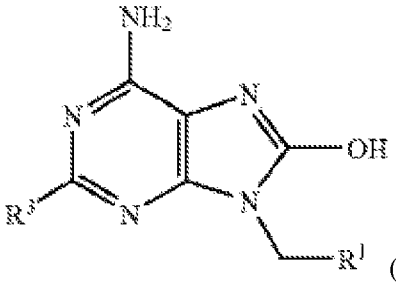
(XVIIc),



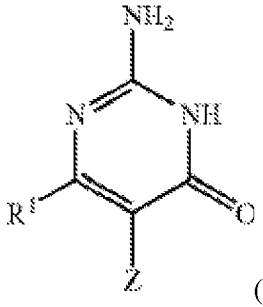
(XVIId),



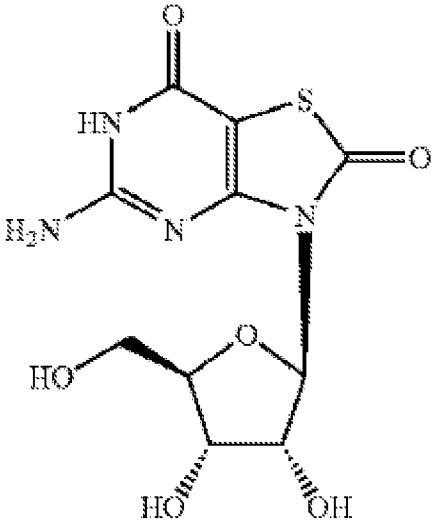
(XVIIe),



(XVIIf),



(XVIIg), and



(XVIIh),

wherein:

each R^1 is H, or a substituted or unsubstituted alkyl, alkenyl, or alkynyl, which may be interrupted by one or more O, S, or N heteroatoms, or a substituted or unsubstituted aryl or heteroaryl;

R^2 is H, OH, SH, halo, or a substituted or unsubstituted alkyl, alkenyl, or alkynyl, which may be interrupted by one or more O, S, or N heteroatoms, or a substituted or unsubstituted —O-(alkyl), —O-(aryl), —O-(heteroaryl), —S-(alkyl), —S-(aryl), —S-(heteroaryl), aryl, or heteroaryl;

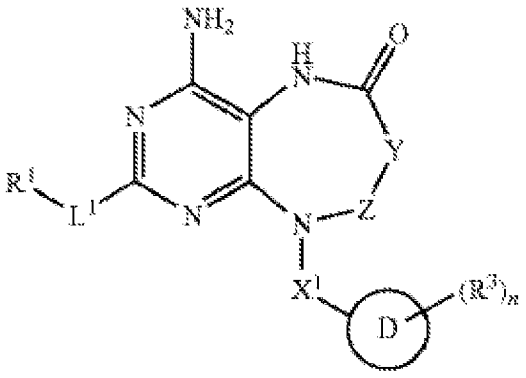
R^3 is H, OH, or SH, or a substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, heteroaryl, —O-(alkyl), —O-(aryl), —O-(heteroaryl), —S-(alkyl), —S-(aryl), —S-(heteroaryl), —NH(alkyl), —NH(aryl), —NH(heteroaryl), —NH(R^4)(alkyl), —NH(R^4)(aryl), or —NH(R^4)(heteroaryl), wherein R^4 is a substituted or unsubstituted alkyl;

X is O or S;

Y is H, halo, OH, OR^4, SH, SR^4, or a substituted or unsubstituted alkyl or aryl; and

Z is H, halo, OH, OR^4, SH, or SR^4. See US 7,576,068, the disclosure of which is incorporated by reference in its entirety.

[0249] In some embodiments, the activating moiety is a TLR7 and/or TLR8 agonist having the structure of Formular (XVIII):



(XVIII),

wherein:

Y—Z is —CR^4R^5—, —CR^4R^5—CR^4R^5—, —$C(O)CR^4R^5$—, —$CR^4R^5C(O)$—, —$NR^8C(O)$—, —$C(O)NR^8$—, —$CR^4R^5S(O)_2$—, or —CR^5=CR^5—;

L^1 is —NR^8—, —O—, —S—, —$N(R^8)C(O)$—, —$S(O)_2$—, —S(O) —$C(O)N(R^8)$—, —$N(R^8)S(O)_2$—, —$S(O)_2N(R^8)$— or a covalent bond;

R^1 is alkyl, substituted alkyl, haloalkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, heteroalkyl, substituted heteroalkyl, carbocyclyl, substituted carbocyclyl, carbocyclylalkyl, substituted carbocyclylalkyl, heterocyclyl, substituted heterocyclyl, heterocyclylalkyl, or substituted heterocyclylalkyl, arylalkyl, substituted arylalkyl, heteroarylalkyl, substituted heteroarylalkyl, carbocyclylheteroalkyl, substituted carbocyclylheteroalkyl, heterocyclylheteroalkyl, substituted heterocyclylheteroalkyl, arylheteroalkyl, substituted arylheteroalkyl, heteroarylheteroalkyl, or substituted heteroarylheteroalkyl;

X^1 is alkylene, substituted alkylene, heteroalkylene, substituted heteroalkylene, alkenylene, substituted alkenylene, alkynylene, substituted alkynylene, carbocyclylene, substituted carbocyclylene, heterocyclylene, substituted heterocyclylene, —NR^8—, —O—, —C(O)—, —S(O)—, $S(O)_2$—, or a bond;

D is carbocyclyl, substituted carbocyclyl, heterocyclyl or substituted heterocyclyl wherein said carbocyclyl, substituted carbocyclyl, heterocyclyl or substituted heterocyclyl is substituted with one or two -L^2-NR^6R^7; or

D is a heterocyclyl, substituted heterocyclyl, heteroaryl or substituted heteroaryl wherein said heterocyclyl, substituted heterocyclyl, heteroaryl or substituted heteroaryl comprises one to four nitrogen atoms;

each L^2 is independently alkylene, substituted alkylene, heteroalkylene, substituted heteroalkylene, or a covalent bond;

each R^3 is independently halogen, cyano, azido, nitro, alkyl, substituted alkyl, hydroxyl, amino, heteroalkyl, substituted heteroalkyl, alkoxy, haloalkyl, haloalkoxy, —CHO, —C(O)OR^8, —S(O)R^8, —$S(O)_2R^8$; —C(O)NR^9R^{10}, —N(R^9)C(O)R^8, carbocyclyl, substituted carbocyclyl, carbocyclylalkyl, substituted carbocyclylalkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, —$S(O)_2NR^9R^{10}$, —N(R^9)$S(O)_2R^8$, —N(R^9)$S(O)_2OR^{10}$, —$OS(O)_2NR^9R^{10}$;

n is 0, 1, 2, 3, 4 or 5;

R^4 and R^5 are each independently H, alkyl, substituted alkyl, haloalkyl, heteroalkyl, substituted heteroalkyl, carbocyclyl, substituted carbocyclyl, carbocyclylalkyl, substituted carbocyclylalkyl, heterocyclyl, substituted heterocyclyl, heterocyclylalkyl, substituted heterocyclylalkyl, arylalkyl, substituted arylalkyl, heteroarylalkyl, substituted heteroarylalkyl, carbocyclylheteroalkyl, substituted carbocyclylheteroalkyl, heterocyclylheteroalkyl, substituted heterocyclylheteroalkyl, arylheteroalkyl, substituted arylheteroalkyl, heteroarylheteroalkyl, or substituted heteroarylheteroalkyl, cyano, azido, OR^8, —C(O)H, —C(O)R^8, —S(O)R^8, —$S(O)_2R^8$, —C(O)OR^8, or —C(O)NR^9R^{10}; or

R^4 and R^5, taken together with the carbon to which they are both attached, form a carbocycle, substituted carbocycle, heterocycle or substituted heterocycle; or

R^4 and R^5, when on the same carbon atom, taken together with the carbon to which they are attached are —C(O)— or —C(NR^8)—; or

two R^4 or two R^5 on adjacent carbon atoms when taken together with the carbons to which they are attached form a 3 to 6 membered carbocycle, substituted carbocycle, heterocycle or substituted heterocycle;

R^6 and R7 are each independently H, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, haloalkyl, heteroalkyl, substituted heteroalkyl, carbocyclyl, substituted carbocyclyl, carbocyclylalkyl, substituted carbocyclylalkyl, heterocyclyl, substituted heterocyclyl, heterocyclylalkyl, substituted heterocyclylalkyl, arylalkyl, substituted arylalkyl, heteroarylalkyl, substituted heteroarylalkyl, carbocyclylheteroalkyl, substituted carbocyclylheteroalkyl, heterocyclylheteroalkyl, substituted heterocyclylheteroalkyl, arylheteroalkyl, substituted arylheteroalkyl, heteroarylheteroalkyl, or substituted

heteroarylheteroalkyl, —C(O)H, —C(O)R^8, —S(O)R^8, —$S(O)_2R^8$, —C(O)OR^8, or —C(O)NR^9R^{10}, $S(O)_2NR^9R^{10}$; or

R^6 and R^7, taken together with the nitrogen to which they are both attached, form a substituted or unsubstituted heterocycle, which may contain one or more additional heteroatoms selected from N, O, P, or S; or

R^7 taken together with L^2, and the N to which they are both attached, forms a substituted or unsubstituted 3 to 8 membered heterocycle which may contain one or more additional heteroatoms selected from N, O, S, or P;

R^8 is H, alkyl, substituted alkyl, haloalkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, heteroalkyl, substituted heteroalkyl, carbocyclyl, substituted carbocyclyl, carbocyclylalkyl, substituted carbocyclylalkyl, heterocyclyl, substituted heterocyclyl, heterocyclylalkyl, substituted heterocyclylalkyl, arylalkyl, substituted arylalkyl, heteroarylalkyl, substituted heteroarylalkyl, carbocyclylheteroalkyl, substituted carbocyclylheteroalkyl, heterocyclylheteroalkyl, substituted heterocyclylheteroalkyl, arylheteroalkyl, substituted arylheteroalkyl, heteroarylheteroalkyl, or substituted heteroarylheteroalkyl; and

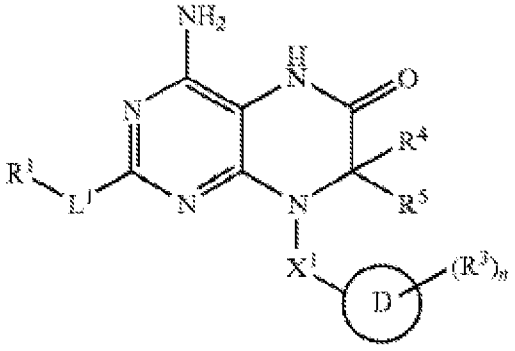
R^9 and R^{10} are each independently H, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, haloalkyl, heteroalkyl, substituted heteroalkyl, carbocyclyl, substituted carbocyclyl, carbocyclylalkyl, substituted carbocyclylalkyl, heterocyclyl, substituted heterocyclyl, heterocyclylalkyl, substituted heterocyclylalkyl, arylalkyl, substituted arylalkyl, heteroarylalkyl, substituted heteroarylalkyl, carbocyclylheteroalkyl, substituted carbocyclylheteroalkyl, heterocyclylheteroalkyl, substituted heterocyclylheteroalkyl, arylheteroalkyl, substituted arylheteroalkyl, heteroarylheteroalkyl, or substituted heteroarylheteroalkyl; or

R^9 and R^{10}, taken together with the nitrogen to which they are both bonded, form a substituted or unsubstituted heterocycle;

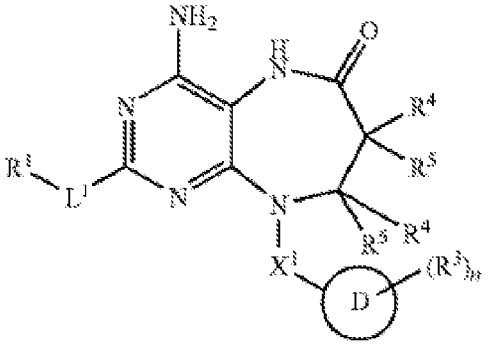
wherein each substituted alkyl, substituted alkenyl, substituted alkynyl, substituted heteroalkyl, substituted carbocyclyl, substituted carbocyclylalkyl, substituted heterocyclyl, substituted heterocyclylalkyl, substituted arylalkyl, substituted heteroarylalkyl, substituted carbocyclylheteroalkyl, substituted heterocyclylheteroalkyl, substituted arylheteroalkyl, substituted heteroarylheteroalkyl, substituted alkylene, substituted heteroalkylene, substituted alkenylene, substituted alkynylene, substituted carbocyclylene, or substituted heterocyclylene is independently substituted with one to four substituents selected from the group consisting of -halogen, —R, —O^-, =O, —OR, —SR, —S^-, —NR_2, —$N(+)R_3$, =NR, —$C(halogen)_3$, —$CR(halogen)_2$, —$CR_2(halogen)$, —CN, —OCN, —SCN, —N=C=O,

—NCS, —NO, —NO_2, =N_2, —N_3, —NRC(=O)R, —NRC(=O)OR, —NRC(=O)NRR, —C(=O)NRR, —C(=O)OR, —OC(=O)NRR, —OC(=O)OR, —C(=O)R, —$S(=O)_2OR$, —$S(=O)_2R$, —$OS(=O)_2OR$, —$S(=O)_2NR$, —S(=O)R, —$NRS(=O)_2R$, —$NRS(=O)_2NRR$, —$NRS(=O)_2OR$, —$OP(=O)(OR)_2$, —$P(=O)(OR)_2$, —P(O)(OR)(O)R, —C(=O)R, —C(=S)R, —C(=O)OR, —C(=S)OR, —C(=O)SR, —C(=S)SR, —C(=O)NRR, —C(=S)NRR, —C(=NR)NRR, and —NRC(=NR)NRR; wherein each R is independently H, alkyl, cycloalkyl, aryl, arylalkyl, or heterocyclyl. See US 20100143301 A1, the disclosure of which is incorporated by reference in its entirety.

[0250] In some embodiments, the activating moiety is a TLR7 and/or TLR8 agonist having the structure of:



(XIXa),



(XIXb),

wherein:

L^1 is —NH— or —O—;

R^1 is alkyl, substituted alkyl, heteroalkyl, substituted heteroalkyl, heterocyclylalkyl, substituted heterocyclylalkyl, carbocyclylalkyl or substituted carbocyclylalkyl;

each of R^4 and R^5 independently is H or C_1-C_6 alkyl or R^4 and R^5 taken together with the carbon to which they are attached is —C(O)—;

X^1 is C_1-C_6 alkylene, C_1-C_6 heteroalkylene or C_1-C_6 substituted heteroalkylene;

D is phenyl, biphenyl or pyridinyl, wherein said phenyl, biphenyl or pyridinyl is substituted with -L^2-NR^6R^7; or

D is pyridinyl, piperidinyl, piperazinyl or 1,2,3,4-tetrahydroisoquinolinyl;

n is 0 or 1;

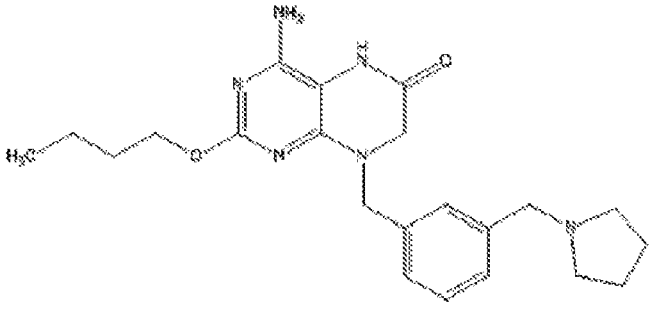
R^3 is halogen, cyano, alkyl, carbocyclyl, carbocyclylalkyl, haloalkyl, —$C(O)OR^6$, —$C(O)NR^9R^{10}$ or —CHO;

L^2 is C_1-C_6 alkylene or a covalent bond;

each of R^6 and R^7 independently is H, alkyl, or heteroaryl; or

R^6 and R^7 takentogether with the nitrogen to which they are attached form a substituted or unsubstituted 4-6 membered heterocycle comprising 0 to 2 heteroatoms selected from N, O or S.

[0251] In some embodiments, the activating moiety is a TLR7 and/or TLR8 agonist having the structure of:



GS-9620.

C. Amount of Immunotherapeutics in the Therapeutic Combinations

[0252] In another aspect, the present invention provides a therapeutic combination comprising a target therapeutic and an immunotherapeutic in an amount that is suitbale for the combination therapy treatment of diseases such as tumors and cancers.

[0253] In some embodiments, the immunotherapeutic is of an amount that is capable of: (1) inducing IFN-α in a enriched human blood DCs; (2) inducing TNF-α in a enriched human blood DCs; and/or (3) inducing IL-12-α in a enriched human blood DCs.

[0254] Methods for measuring the activity of the immunotherapeutics are: 1) an assay to measure cytokines release from human dendritic cell stimulated by immunotherapy; 2) an assay to detect antibody dependent cell mediated cytotoxicity enhanced by immunotherapy; and 3) an efficacy study in a tumor model treated by immunotherapy.

[0255] In some embodiments, the immunotherapeutic (e.g. resiquimod or its analogues) is adminstered, either orally or intravenously using oral formulation or intravenous formulation, of an amount so that the local concentration of the immunotherapetuics (e.g. near or at the tumor site of a solid tumor) is between about 0.005 μg/ml to about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 μg/ml (all inclusive).

[0256] The local concentration of the immunotherapetuics (e.g. near or at the tumor site of a solid tumor) can measured using methods known in the art, such as measuring the tissue or serum concentration. Local effective concentration of therapeutic agent is depended on its absorption from various routes, tissue distribution, and metabolism process, and plasma phamocokinetics of agent and tissue concentration could be measured routinely using methods known in the art.

[0257] In some embodiments, the immunotherapeutic is adminstered of an amount so that the local concentration of the immunotherapetuics (e.g. near or at the tumor site of a solid tumor) is between about 0.05 μg/ml, 0.1 μg/ml, 0.15 μg/ml, 0.2 μg/ml, 0.3 μg/ml, or 0.4 μg/ml, to about 0.5 μg/ml (all inclusive).

[0258] In some embodiments, the subject is administed an oral formulation comprising the immunotherapeutic (e.g. resiquimod or its analogues) in a dose of between about 0.0005 mg/kg, 0.0006 mg/kg, 0.0007 mg/kg, 0.0008 mg/kg, 0.0009 mg/kg, 0.001 mg/kg, 0.002 mg/kg, 0.003 mg/kg, 0.004 mg/kg, 0.005 mg/kg, 0.006 mg/kg, 0.007 mg/kg, 0.008 mg/kg, 0.009 mg/kg, 0.01 mg/kg, or 0.015 mg/kg, to about 0.02 mg/kg (all inclusive), two times per week. In some embodiments, the subject is administed an oral formulation comprising the immunotherapeutic (e.g. resiquimod or its analogues) in a dose of between about 0.0005 mg/kg, to about 0.0006 mg/kg, 0.0007 mg/kg, 0.0008 mg/kg, 0.0009 mg/kg, 0.001 mg/kg, 0.002 mg/kg, 0.003 mg/kg, 0.004 mg/kg, 0.005 mg/kg, 0.006 mg/kg, 0.007 mg/kg, 0.008 mg/kg, 0.009 mg/kg, 0.01 mg/kg, 0.015 mg/kg, or 0.02 mg/kg (all inclusive), two times per week.

[0259] In some embodiments, the subject is administed an oral formulation comprising the immunotherapeutic (e.g. resiquimod or its analogues) in a dose of less than or about 0.0005 mg/kg, 0.0006 mg/kg, 0.0007 mg/kg, 0.0008mg/kg, 0.0009 mg/kg, 0.001 mg/kg, 0.002 mg/kg, 0.003 mg/kg, 0.004 mg/kg,0.005 mg/kg, 0.006 mg/kg, 0.007 mg/kg , 0.008 mg/kg, 0.009 mg/kg, 0.01 mg/kg, two times per week.

[0260] In some embodiments, the subject is administed an intravenous formulation comprising the immunotherapeutic (e.g. resiquimod or its analogues) in a dose of between about 0.0005mg/kg, 0.0006 mg/kg, 0.0007mg/kg, 0.0008mg/kg, 0.0009mg/kg, 0.001 mg/kg, 0.002 mg/kg, 0.003 mg/kg, 0.004 mg/kg, 0.005 mg/kg, 0.006 mg/kg, 0.007 mg/kg, 0.008 mg/kg, 0.009 mg/kg, 0.01 mg/kg, or about 0.015 mg/kg, to about 0.02 mg/kg (inclusive), weekly. In some embodiments, the subject is administed an intravenous formulation comprising the immunotherapeutic (e.g. resiquimod or its analogues) in a dose of between about 0.0005mg/kg, to about 0.0006 mg/kg, 0.0007mg/kg, 0.0008mg/kg, 0.0009 mg/kg, 0.001 mg/kg, 0.002 mg/kg, 0.003 mg/kg, 0.004 mg/kg, 0.005 mg/kg, 0.006 mg/kg, 0.007 mg/kg, 0.008 mg/kg, 0.009 mg/kg, 0.01 mg/kg, 0.015 mg/kg, or 0.02 mg/kg (inclusive), weekly.

[0261] In some embodiments, the method comprises administering to said subject an intravenous formulation comprising said immunotherapeutic (e.g. resiquimod or its analogues) in a dose of between about from 0.0008 mg/kg to about 0.0133 mg/kg, weekly.

[0262] In some embodiments, the subject is administed an intravenous formulation comprising the immunotherapeutic (e.g. resiquimod or its analogues) in a dose of less than or about 0.003 mg/kg, 0.004 mg/kg, 0.005 mg/kg, or 0.006 mg/kg to about 0.01 mg/kg , weekly. For references regarding safe dosage

of immunotherapeutics, see Jurk et al., Nature Immunology, Vol. 4, No. 6”499 (2002), and Pockros et al., J. Hepatology, 47:174-182 (2007), the disclosure of which is incorporated by reference in their entirety.

III. Pharmaceutical Formulations and Administration

[0263] The present invention further relates to a pharmaceutical formulation comprising a compound of the invention or a pharmaceutically acceptable salt thereof, and one or more pharmaceutically acceptable carriers.

[0264] The compounds described herein including pharmaceutically acceptable carriers such as addition salts or hydrates thereof, can be delivered to a patient using a wide variety of routes or modes of administration. Suitable routes of administration include, but inhalation, transdermal, oral, rectal, transmucosal, intestinal and parenteral administration, including intramuscular, subcutaneous and intravenous injections. Preferably, the compouds of the invention comprising an antibody or antibody fragment as the targeting moiety are administered parenterally, more preferably intravenously.

[0265] As used herein, the terms “administering” or “administration” are intended to encompass all means for directly and indirectly delivering a compound to its intended site of action.

[0266] The compounds described herein, or pharmaceutically acceptable salts and/or hydrates thereof, may be administered singly, in combination with other compounds of the invention, and/or in cocktails combined with other therapeutic agents. Of course, the choice of therapeutic agents that can be co-administered with the compounds of the invention will depend, in part, on the condition being treated.

[0267] For example, when administered to patients suffering from a disease state caused by an organism that relies on an autoinducer, the compounds of the invention can be administered in cocktails containing agents used to treat the pain, infection and other symptoms and side effects commonly associated with the disease. Such agents include, e.g., analgesics, antibiotics, etc.

[0268] When administered to a patient undergoing cancer treatment, the compounds may be administered in cocktails containing anti-cancer agents and/or supplementary potentiating agents. The compounds may also be administered in cocktails containing agents that treat the side-effects of radiation therapy, such as anti-emetics, radiation protectants, etc.

[0269] Supplementary potentiating agents that can be co-administered with the compounds of the invention include,e.g., tricyclic anti-depressant drugs (e.g., imipramine, desipramine, amitriptyline, clomipramine, trimipramine, doxepin, nortriptyline, protriptyline, amoxapine and maprotiline); non-tricyclic and anti-depressant drugs (e.g., sertraline, trazodone and citalopram); Ca+2 antagonists (e.g.,

verapamil, nifedipine, nitrendipine and caroverine); amphotericin; triparanol analogues (e.g., tamoxifen); antiarrhythmic drugs (e.g., quinidine); antihypertensive drugs (e.g., reserpine); thiol depleters (e.g., buthionine and sulfoximine); and calcium leucovorin.

[0270] The active compound(s) of the invention are administered per se or in the form of a pharmaceutical composition wherein the active compound(s) is in admixture with one or more pharmaceutically acceptable carriers, excipients or diluents. Pharmaceutical compositions for use in accordance with the present invention are typically formulated in a conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active compounds into preparations which, can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

[0271] For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

[0272] For oral administration, the compounds can be formulated readily by combining the active compound(s) with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, and suspensions for oral ingestion by a patient to be treated. Pharmaceutical preparations for oral use can be obtained solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxyniethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

[0273] Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

[0274] Pharmaceutical preparations, which can be used orally, include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules,

the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for such administration.

[0275] For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

[0276] For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of e.g., gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

[0277] The compounds may be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Injection is a preferred method of administration for the compositions of the current invention. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

[0278] Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances, which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents, which increase the solubility of the compounds to allow for the preparation of highly, concentrated solutions. For injection, the agents of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer.

[0279] Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

[0280] The compounds may also be formulated in rectal compositions such as suppositories or retention enemas,e.g., containing conventional suppository bases such as cocoa butter or other glycerides.

[0281] In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation or transcutaneous delivery (e.g., subcutaneously or intramuscularly), intramuscular injection or a transdermal patch. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (e.g., as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

[0282] The pharmaceutical compositions also may comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include calcium carbonate, calcium phosate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols.

[0283] A preferred pharmaceutical composition is a composition formulated for injection such as intravenous injection and includes about 0.01 % to about 100% by weight of the compound of the present invention, based upon 100% weight of total pharmaceutical composition. The drug-ligand conjugate may be an antibody-cytotoxin conjugatewhere the antibody has been selected to target a particular cancer.

[0284] In some embodiments, the pharmaceutical composition of the present invention further comprises an additional therapeutic agent.

[0285] In some embodiments, the additional therapeutic agent is an anticancer agent.

[0286] In some embodiments, the additional anticancer agent is selected from an antimetabolite, an inhibitor of topoisomerase I and II, an alkylating agent, a microtubule inhibitor, an antiandrogen agent, a GNRh modulator or mixtures thereof.

[0287] In some embodiments, the additional therapeutic agent is a chemotherapeutic agent.

[0288] By “chemotherapeutic agent” herein is meant a chemical compound useful in the treatment of cancer. Examples are but not limited to: Gemcitabine, Irinotecan, Doxorubicin, 5-Fluorouracil, Cytosine arabinoside ("Ara-C"), Cyclophosphamide, Thiotepa, Busulfan, Cytoxin, TAXOL, Methotrexate, Cisplatin, Melphalan, Vinblastine and Carboplatin.

[0289] In some embodiments, the second chemotherapeutic agent is selected from the group consisting of tamoxifen, raloxifene, anastrozole, exemestane, letrozole, imatanib, paclitaxel, cyclophosphamide, lovastatin, minosine, gemcitabine, cytarabine, 5- fluorouracil, methotrexate, docetaxel, goserelin, vincristine, vinblastine,nocodazole, teniposide etoposide, gemcitabine, epothilone, vinorelbine,

camptothecin, daunorubicin, actinomycin D, mitoxantrone, acridine, doxorubicin, epirubicin, or idarubicin.

IV. Kits

[0290] In another aspect, the present invention provides kits containing the therapeutic combinations provided herein and directions for using the therapeutic combinations. The kit may also include a container and optionally one or more vial, test tube, flask, bottle, or syringe. Other formats for kits will be apparent to those of skill in the art and are within the scope of the present invention.

V. Medical Use

[0291] In another aspect, the present invention provides a method for treating a disease condition in a subject that is in need of such treatment, comprising: administering to the subject a therapeteutic combination or pharmaceutical composition comprising a therapeutically effective amount of the compound of the present invention or a pharmaceutically acceptable salt thereof, and a pharmaceutical acceptable carrier.

[0292] In addition to the compositions and constructs described above, the present invention also provides a number of uses of the combinations of the invention. Uses of the combinations of the current invention include: killing or inhibiting the growth, proliferation or replication of a tumor cell or cancer cell, treating cancer, treating a pre-cancerous condition, preventing the multiplication of a tumor cell or cancer cell, preventing cancer, preventing the multiplication of a cell that expresses an auto-immune antibody. These uses comprise administering to an animal such as a mammal or a human in need thereof an effective amount of a compound of the present invention.

[0293] The combination of the current invention is useful for treating diseases such as cancer in a subject, such as a human being. Combinations and uses for treating tumors by providing a subject the composition in a pharmaceutically acceptable manner, with a pharmaceutically effective amount of a composition of the present invention are provided.

[0294] By "cancer" herein is meant the pathological condition in humans that is characterized by unregulated cell proliferation. Examples include but are not limited to: carcinoma, lymphoma, blastoma, and leukemia. More particular examples of cancers include but are not limited to: lung (small cell and non-small cell), breast, prostate, carcinoid, bladder, gastric, pancreatic, liver (hepatocellular), hepatoblastoma, colorectal, head and neck squamous cell carcinoma, esophageal, ovarian, cervical, endometrial, mesothelioma, melanoma, sarcoma, osteosarcoma, liposarcoma, thyroid, desmoids, chronic myelocytic leukemia (AML), and chronic myelocytic leukemia (CML).

[0295] By "inhibiting" or "treating" or "treatment" herein is meant to reduction, therapeutic treatment and prophylactic or preventative treatment, wherein the objective is to reduce or prevent the aimed pathologic disorder or condition. In one example, following administering of a compound of the present invention, a cancer patient may experience a reduction in tumor size. "Treatment" or "treating" includes (1) inhibiting a disease in a subject experiencing or displaying the pathology or symptoms of the disease, (2) ameliorating a disease in a subject that is experiencing or displaying the pathology or symptoms of the disease, and/or (3) affecting any measurable decrease in a disease in a subject or patient that is experiencing or displaying the pathology or symptoms of the disease. To the extent a compound of the present invention may prevent growth and/or kill cancer cells, it may be cytostatic and/or cytotoxic.

[0296] By "therapeutically effective amount" herein is meant an amount of a compound provided herein effective to "treat" a disorder in a subject or mammal. In the case of cancer, the therapeutically effective amount of the drug may either reduce the number of cancer cells, reduce the tumor size, inhibit cancer cell infiltration into peripheral organs, inhibit tumor metastasis, inhibit tumor growth to certain extent, and/or relieve one or more of the symptoms associated with the cancer to some extent.

[0297] Administration "in combination with" one or more further therapeutic agents includes simultaneous (concurrent) and consecutive administration in any order. As used herein, the term "pharmaceutical combination" refers to a product obtained from mixing or combining active ingredients, and includes both fixed and non-fixed combinations of the active ingredients. The term "fixed combination" means that the active ingredients, e.g. a compound of Formula (1) and a co-agent, are both administered to a patient simultaneously in the form of a single entity or dosage. The term "non-fixed combination" means that the active ingredients, e.g. a compound of Formula (1) and a co-agent, are both administered to a patient as separate entities either simultaneously, concurrently or sequentially with no specific time limits, wherein such administration provides therapeutically effective levels of the active ingredients in the body of the patient. The latter also applies to cocktail therapy, e.g. the administration of three or more active ingredients.

[0298] In some embodiments, the diseases condition is tumor or cancer. In some embodiments, the cancer or tumor is selected from stomach, colon, rectal, liver, pancreatic, lung, breast, cervix uteri, corpus uteri, ovary, testis, bladder, renal, brain/CNS, head and neck, throat, Hodgkin's disease, non-Hodgkin's lymphoma, multiple myeloma, leukemia, melanoma, non-melanoma skin cancer, acute lymphocytic leukemia, acute myelogenous leukemia, Ewing's sarcoma, small cell lung cancer, choriocarcinoma, rhabdomyosarcoma, Wilms' tumor, neuroblastoma, hairy cell leukemia, mouth/pharynx, oesophagus, larynx, kidney cancer or lymphoma.

[0299] In some embodiments, the disease condition comprises abnormal cell proliferation, such as a pre-cancerous lesion.

[0300] The current invention is particularly useful for the treatment of cancer and for the inhibition of the multiplication of a tumor cell or cancer cell in an animal. Cancer, or a precancerous condition, includes a tumor, metastasis, or any disease or disorder characterized by uncontrolled cell growth, can be treated or prevented by administration the drug-ligand complex of the current invention. The compound delivers the activating moiety to a tumor cell or cancer cell. In some embodiments, the targeting moiety specifically binds to or associates with a cancer-cell or a tumor-cell-associated antigen. Because of its close proximity to the ligand, after being internalized, the activating moiety can be taken up inside a tumor cell or cancer cell through, for example, receptor-mediated endocytosis. The antigen can be attached to a tumor cell or cancer cell or can be an extracellular matrix protein associated with the tumor cell or cancer cell. Once inside the cell, the linker is hydrolytically or enzymatically cleaved by a tumor-cell or cancer-cell-associated proteases, thereby releasing the activating moiety. The released activating moiety is then free to diffuse and induce or enhance immune activity of immune cells or tumor cells. In an alternative embodiment, the activating moiety is cleaved from the compound tumor microenvironment, and the drug subsequently penetrates the cell.

[0301] Representative examples of precancerous conditions that may be targeted by the compounds of the present invention, include: metaplasia, hyperplysia, dysplasia, colorectal polyps, actinic ketatosis, actinic cheilitis, human papillomaviruses, leukoplakia, lychen planus and Bowen's disease.

[0302] Representative examples of cancers or tumors that may be targeted by compounds of the present invention include: lung cancer, colon cancer, prostate cancer, lymphoma, melanoma, breast cancer, ovarian cancer, testicular cancer, CNS cancer, renal cancer, kidney cancer, pancreatic cancer, stomach cancer, oral cancer, nasal cancer, cervical cancer and leukemia. It will be readily apparent to the ordinarily skilled artisan that the particular targeting moiety used in the compound can be chosen such that it targets the activating moiety to the tumor tissue to be treated with the drug (i.e., a targeting agent specific for a tumor-specific antigen is chosen). Examples of such targeting moiety are well known in the art, examples of which include anti-Her2 for treatment of breast cancer, anti-CD20 for treatment of lymphoma, anti-PSMA for treatment of prostate cancer and anti-CD30 for treatment of lymphomas, including non-Hodgkin's lymphoma.

[0303] In some embodiments, the abnormal proliferation is of cancer cells.

[0304] In some embodiments, the cancer is selected from the group consisting of: breast cancer, colorectal cancer, diffuse large B-cell lymphoma, endometrial cancer, follicular lymphoma, gastric cancer,

glioblastoma, head and neck cancer, hepatocellular cancer, lung cancer, melanoma, multiple myeloma, ovarian cancer, pancreatic cancer, prostate cancer, and renal cell carcinoma.

[0305] In some embodiments, the present invention provides a compound for use in killing a cell. The compound is administered to the cell in an amount sufficient to kill said cell. In an exemplary embodiment, the compound is administered to a subject bearing the cell. In a further exemplary embodiment, the administration serves to retard or stop the growth of a tumor that includes the cell (e.g., the cell can be a tumor cell). For the administration to retard the growth, the rate of growth of the cell should be at least 10% less than the rate of growth before administration. Preferably, the rate of growth will be retarded at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or completely stopped.

[0306] Additionally, the present invention provides a compound or a pharmaceutical composition of the present invention for use as a medicament. The present invention also provides a compound or a pharmaceutical composition for killing, inhibiting or delaying proliferation of a tumor or cancer cell, or for treating a disease wherein TLR7 and/or TLR8 are implicated.

Effective Dosages

[0307] Pharmaceutical compositions suitable for use with the present invention include compositions wherein the active ingredient is contained in a therapeutically effective amount, i.e., in an amount effective to achieve its intended purpose. The actual amount effective for a particular application will depend, inter alia, on the condition being treated. Determination of an effective amount is well within the capabilities of those skilled in the art, especially in light of the detailed disclosure herein.

[0308] For any compound described herein, the therapeutically effective amount can be initially determined from cell culture assays. Target plasma concentrations will be those concentrations of active compound(s) that are capable of inhibition cell growth or division. In preferred embodiments, the cellular activity is at least 25% inhibited. Target plasma concentrations of active compound(s) that are capable of inducing at least about 30%, 50%, 75%, or even 90% or higher inhibition of cellular activity are presently preferred. The percentage of inhibition of cellular activity in the patient can be monitored to assess the appropriateness of the plasma drug concentration achieved, and the dosage can be adjusted upwards or downwards to achieve the desired percentage of inhibition.

[0309] As is well known in the art, therapeutically effective amounts for use in humans can also be determined from animal models. For example, a dose for humans can be formulated to achieve a circulating concentration that has been found to be effective in animals. The dosage in humans can be adjusted by monitoring cellular inhibition and adjusting the dosage upwards or downwards, as described above.

[0310] A therapeutically effective dose can also be determined from human data for 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 as compared with the known compound.

[0311] Adjusting the dose to achieve maximal efficacy in humans 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.

[0312] In the case of local administration, the systemic circulating concentration of administered compound will not be of particular importance. In such instances, the compound is administered so as to achieve a concentration at the local area effective to achieve the intended result.

[0313] Therapeutic amounts of specific antibodies disclosed herein can also be administered, as a component of the combination, with the immunotherperutics, either in a single mixure form, or separately. In some embodiments, therapeutic amounts are amounts which eliminate or reduce the patient's tumor burden, or which prevent or reduce the proliferation of metastatic cells. The dosage will depend on many parameters, including the nature of the tumor, patient history, patient condition, the possible co-use of other oncolytic agents, and methods of administration. Methods of administration include injection (e.g., parenteral, subcutaneous, intravenous, intraperitoneal, etc.) for which the antibodies are provided in a nontoxic pharmaceutically acceptable carrier such as water, saline, Ringer's solution, dextrose solution, 5% human serum albumin, fixed oils, ethyl oleate, or liposomes. Typical dosages may range from about 0.01 to about 20 mg/kg, such as from about 0.1 to about 10 mg/kg. Other effective methods of administration and dosages may be determined by routine experimentation and are within the scope of this invention.

[0314] The therapeutically effective amount of the agents (disclosed herein) administered, when it is used for combination therapy, can vary depending upon the desired effects and the subject to be treated. For example the subject can receive at least 1mg/kg (such as 1 mg/kg to 20 mg/kg, 2.5 mg/kg to 10 mg/kg, or 3.75 mg/kg to 5 mg/kg) intravenously of each antibody agent. The dosage can be administered in divided doses (such as 2, 3, or 4 divided doses per day), or in a single dosage.

[0315] In the method for combined administration, the agent may be simultaneously administered with the antibody used in the present invention, or the agent may be administered before or after the administration of the antibody used in the present invention.

[0316] For other modes of administration, dosage amount and interval can be adjusted individually to provide plasma levels of the administered compound effective for the particular clinical indication being

treated. For example, in one embodiment, a compound according to the invention can be administered in relatively high concentrations multiple times per day. Alternatively, it may be more desirable to administer a compound of the invention at minimal effective concentrations and to use a less frequent administration regimen. This will provide a therapeutic regimen that is commensurate with the severity of the individual's disease.

[0317] Utilizing the teachings provided herein, an effective therapeutic treatment regimen can be planned which does not cause substantial toxicity and yet is entirely effective to treat the clinical symptoms demonstrated by the particular patient. This planning should involve the careful choice of active compound by considering factors such as compound potency, relative bioavailability, patient body weight, presence and severity of adverse side effects, preferred mode of administration and the toxicity profile of the selected agent.

[0318] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

EXAMPLES

[0319] The present invention is further exemplified, but not limited, by the following and Examples that illustrate the preparation of the compounds of the invention.

Example 1

[0320] Enrichment of human dendritic cells (DCs) from PBMC

[0321] Human PBMC was prepared from Buffy coats obtained from healthy volunteer donors by Ficoll centrifugation. Dendritic cells were enriched by using negative depletion with magnetic beads (Miltenyi Biotec Inc. San Diego, CA) with mixture of anti-CD3, CD19, CD20, CD14, and CD16 antibodies from human PBMC. The enrichment of DCs was stained with goat anti-mouse FITC (lineages), HLA-DR-APCCy7, CD123-BV421 and CD11C-APC. The stained cells were analyzed on BD LSR Fortessa (BD Biosciences). The anti-CD3, CD4, CD11C, CD19, CD14, CD16, CD123 monoclonal antibody were purchased from BD Biosciences, CA or Biolegend, San Diego, CA.

[0322] Stimulation of enriched human DCs and cytokines expression

[0323] 1-2 x 10^5 enriched DCs were plated in a 96-well plate in 100 μl media, 100 μl diluted stimulators (including TLRL were add to the plate and cultured for 20-22h in 37°C incubator. The supernatant were collected and human IFN-α, IL-12(p70) and TNF-α were analyzed by ELISA (Mabtech AB, Sweden).

[0324] **Figures 1A-1G** depict analysis of cytokine production by enriched human DCs from three healthy donors. Enriched human DCs were plated in a 96-well plate and cultured with allogeneic untreated (medium) or treated different concentration of TLRL directly for 20-22h in 37°C incubator. The supernatant were collected and human IFN-α, IL-12(p70) and TNF-α were analyzed by ELISA. Data are given as mean ± SD of triplicate cultures. Three independent experiments from three healthy donors were performed (Donor 1: Figure 1A, Donor 2: Figure 1B-D; Donor 3: Figure 1E-G).

Example 2

[0325] In vivo tumor cell killing assay using Trastuzumab combination therapy with resiquimod

[0326] For development of patient-derived gastric carcinoma xenograft (PDX) mouse models, female Balb/c nude mice (from SLAC, Shanghai, China) of 6-8 weeks old were used for tumor fragment implantation. Animals were fed with normal nude mice diet and housed in SPF animal facility in accordance with the Guide for Care and Use of Laboratory Animals and regulations of the Institutional Animal Care and Use Committee. Patient STO#69 (her2 overexpression) or STO#179 (drug resistant) gastric tumor fragments of about 15-30 mm^3 in size were implanted (s.c.) into right flanks of Balb/c nude mice.

[0327] Drugs were administered by i.v. route with 10 mg/kg of antibody combined with 0.085 or 0.170 mg/kg of resiquimod, QWKx4. Tumors were measured once a week by caliper to determine its subcutaneous growth. Tumors were measured twice a week in two dimensions with calipers. Tumor volume was calculated using following formula: tumor volume = (length x width2) x 0.5. Average tumor volumes or body weights were plotted using graph program Prism 5 (GraphPad). An endpoint for efficacy study was set at 30-45 days post-first treatment or when a tumor size reached above 2000 mm3, whichever came first. If a mouse lose more than 20% of body weight or is very sick and cannot get to adequate food or water, it will be removed from the study and euthanized. Tumors were collected from mice at the end point, half frozen in liquid N_2 and half fixed in formalin for preparing FFPE tissues.

[0328] **Figure 2** depicts therapeutic efficacy of resiquimod in PDX her2 overexpressed gastric tumor model. BALB/c nu/nu nude mice 6-8 week-old female mice bearing subcutaneous patient derived gastric tumor were treated intravenously with 10 mg/kg of antibody combined with 0.085 or 0.170 mg/kg of resiquimod, QWKx4 (10 mice per group). Treatment was performed weekly for a period of four weeks.

Data represents mean tumor volumes (mean ± SD). Tumor growth curves were stopped when tumors reached a size of 2000 mm^3.

[0329] **Figure 3** depict therapeutic efficacy of resiquimod in PDX drug resistant, her2 expressed gastric tumor model. BALB/c nu/nu nude mice 6-8 week-old female mice bearing subcutaneous patient derived gastric tumor were treated intravenously with 10 mg/kg of antibody combined with 0.085 or 0.170 mg/kg of resiquimod, QWKx4 (10 mice per group). Treatment was performed weekly for a period of four weeks. Data represents mean tumor volumes (mean ± SD). Tumor growth curves were stopped when tumors reached a size of 2000 mm^3.

Example 3

[0330] In vivo tumor cell killing using anti-neu with TLRL combination therapy in immune competent mice

[0331] All BALB/c mice were maintained under specific pathogen free conditions and used between 6-16 weeks of age in accordance to the animal experimental guidelines set by the Institutional Animal Care and Use Committee. All experiments have been approved by the Institutional Animal Care and Use Committee and conform to the relevant regulatory standards. TUBO which was cell line derived from a spontaneous mammary tumor in a BALB-neuT Tg mouse expressing transforming rat neu was cultured in 5% CO2, and maintained in vitro in DMEM supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Sigma), 10% NCTC 109 medium, 2 mmol/L L-glutamine, 0.1 mmol/L MEM nonessential amino acids, 100 units/mL penicillin, and 100 μg/mL streptomycin. The anti-neu mAb 7.16.4 (BioXcell, NH) recognizes the juxtamembrane region of rat neu and competes with 4D5, the precursor of trastuzumab, for binding and inhibition of tumor growth. Adherent TUBO cells were removed from culture flasks by incubating for 3-5 minutes in 1xTrypsin EDTA. Cells were washed 2-3 times in 1xPBS and counted by trypan exclusion. TUBO cells (3-5 x 10^5) were injected s.c. in the back of 6 to 8-week-old anesthetized mice. Tumor volumes were measured along three orthogonal axes (x, y, and z) and calculated as tumor volume = (xyz)/2. Mice were treated with i.v. injections of 100ug of anti-neu antibody (clone 7.16.4) diluted in 500 uL of 1x PBS or TLRL combination, twice a week. Injection time points are indicated by different triangles along the x-axis of tumor growth curves graphs.

[0332] **Figure 4** depicts that anti-neu and TLRL synergistically amplify the antitumor effect on TUBO tumor model. WT BALB/c mice (n=8/group) were injected s.c. with 5x10^5 TUBO cells and treated with 75μg of anti-neu antibody (clone: 7.16.4) alone or combination with TLRL on days 13, 16, 20 and 24. Tumor vulome and weight were measured every three days for 28 days.

[0333] In a separated experiment, 4x10^4 TUBO cells were washed 2-3 times in 1xPBS and counted by

trypan exclusion. Then cells were injected i.v. through mice tail vein. The metasis of tumor celss into the lung were established. Mice were treated with i.v. injections of 100ug of anti-neu antibody (clone 7.16.4) diluted in 500 uL of 1x PBS or various dose of TLRL combination at 12 days post injuction weekly for three times.

[0334] **Figures 5A-5E** depict that TLRL enhances the efficacy of therapy with anti-neu on TUBO lung tumor in Babl/c mice. WT BALB/c mice (n=10/group) were injected i.v. at mouse tail with $4x10^5$ TUBO cells and were started to treat with 100 μg of anti-neu antibody (clone: 7.16.4) alone, TLRL alone or antibody combination with various dose of TLRL on days 12, q7d, for three times. Survival rate, wright and daily activity were observed for up to 100 days.

[0335] The results showed that effective dose could be from 0.0008 mg/kg to 0.0067 mg/kg weekly in human, based on the dosage converted from the mouse experiments with both in vivo and in vitro data where 0.1, 0.2, 0.4, 0.8 and 1.6 μg/dose were tesed. Table 3 is the converting table.

Table 3

Amount/dose in experiment	mouse (20g)	monkey	human
μ/dose (i.v.)	mg/kg	mg/kg	mg/kg
0.1	0.0050	0.0013	0.0004
0.2	0.0100	0.0025	0.0008
0.4	0.0200	0.0050	0.0017
0.8	0.0400	0.0100	0.0033
1.6	0.0800	0.0200	0.0067
3.2	0.1600	0.0400	0.0133
6.4	0.3200	0.0800	0.0267

[0336] The above covension is based on the assumption that the mice weighs 20g (which is typically between 20 to 25g). For the the assumptions and constants for convension, see Freireich EJ et al, Quantitative Comparision of Toxicity of Anticancer Agents in Mouse, Rat, Dog, Monkey and Man. Cancer Chemother Rep 1966;50(4):219-244, which is incorporated by reference in its entirety.

Example 4

[0337] Detection of systemic immune activation with IFN inducible genes expression in mouse PBMC by TLRL

[0338] Balb/c mice, 6-8 weeks of age, female, purchased from Vital River were injected intravenously with TLRL, at indicated time point, mice were bled and IFN inducible genes were examed by qPCR.

Once pick time of expression IFN inducible genes was determined, a sperated experiment was performance with various dose of TLRL. At indicated time point, mice were bled and IFN inducible genes were examed. The Quantitative Real-Time PCR was performed and gene expression data were normalized relative to geometric mean of two housekeeping genes (Actin):

Mouse Actin: F:CATTGCTGACAGGATGCAGAAGG (SEQ ID NO.:1),

Mouse Actin R:TGCTGGAAGGTGGACAGTGAGG (SEQ ID NO.:2);

Mouse Inf-b: F: CTCCAGCACTGGGTGGAATG (SEQ ID NO.:3),

Mouse Inf-b R: AGTGGAGAGCAGTTGAGGAC (SEQ ID NO.:4);

Mouse Mx2: F; GTGGCAGAGGGAGAATGTCG (SEQ ID NO.:5),

Mouse Mx2 R:TAAAACAGCATAACCTTTTGCGA (SEQ ID NO.:6);

Mouse Ifn-a: F: CCTGAGAGAGAAGAAACACAGCC (SEQ ID NO.:7),

Mouse Ifn-a R: GGCTCTCCAGACTTCTGCTCTG (SEQ ID NO.:8);

Mouse ISG15: F: CAGCAATGGCCTGGGACCTAA (SEQ ID NO.:9),

Mouse ISG15R: GGAAAGCCGGCACACCAATC (SEQ ID NO.:10).

[0339] **Figures 6A-6C** depicts expression of IFN inducible genes in mouse PBMC after TLRL injection. RNA was isolated from PBMCs cryopreserved with TRIzol reagent at variable time points and Relative expression of IFN inducible genes were determined by quantitative RT-PCR. MX2 gene was detected over time course of 5 hours post TLRL injection (Figure 6A) and MX2 and ISG15 genes were measured with various dose of TLRL at 2 hours post injection (Figure 6B and Figure 6C). Values indicate the mRNA expression of indicated IFN inducible genes relative to housekeeping gene Actin. Bar graphs represent data from 3 individual animals. $^{**}P < 0.01$; $^{***}P < 0.001$.

[0340] Statistical Analysis

[0341] The significance of all comparisons was calculated using a Student's two-tailed t test assuming unequal variance between mock and sample groups, and results considered significant when $p<0.05$. Correlations between parameters were assessed using Spearman's rank correlation test, P values <0.05 were consider to be statistically significant.

Claims

1. A combination, comprising:

(i) an effective amount of a targeted therapeutic against a cancer; and

(ii) an effective amount of an immunotherapeutic that is capable of activating a human plasmacytoid dendritic cell, myeloid dendritic cell, or NK cell, or a combination thereof.

2. The combination of claim **1**, wherein said targeted therapeutic is capable of binding to a tumor cell specifically or preferably in comparison to a non-tumor cell.

3. The combination of claim **2**, wherein said tumor cell is of a carcinoma, a sarcoma, a lymphoma, a myeloma, or a central nervous system cancer.

4. The combination of any one of claims **1** to **3**, wherein said targeted therapeutic is capable of binding to a tumor antigen specifically or preferably in comparison to a non-tumor antigen.

5. The combination of claim **4**, wherein said tumor antigen is selected from the group consisting of: CD2, CD19, CD20, CD22, CD27, CD33, CD37, CD38, CD40, CD44, CD47, CD52, CD56, CD70, CD79, and CD137.

6. The combination of claim **4**, wherein said tumor antigen is selected from the group consisting of: 4-1BB, 5T4，AGS-5，AGS-16, Angiopoietin 2, B7.1, B7.2, B7DC, B7H1, B7H2, B7H3, BT-062, BTLA, CAIX, Carcinoembryonic antigen, CTLA4, Cripto, ED-B, ErbB1, ErbB2, ErbB3, ErbB4, EGFL7, EpCAM, EphA2, EphA3, EphB2, FAP, Fibronectin, Folate Receptor, Ganglioside GM3, GD2, glucocorticoid-induced tumor necrosis factor receptor (GITR), gp100, gpA33, GPNMB, ICOS, IGF1R, Integrin αv, Integrin $\alpha v\beta$, KIR, LAG-3, Lewis Y, Mesothelin, c-MET, MN Carbonic anhydrase IX, MUC1, MUC16, Nectin-4, NKGD2, NOTCH，OX40, OX40L, PD-1, PDL1, PSCA, PSMA, RANKL, ROR1, ROR2, SLC44A4, Syndecan-1, TACI, TAG-72, Tenascin, TIM3, TRAILR1, TRAILR2,VEGFR-1, VEGFR-2, VEGFR-3, and variants thereof.

7. The combination of any one of claims **1** to **6**, wherein said targeted therapeutic comprises an immunoglobulin, a protein, a peptide, a small molecule, a nanoparticle, or a nucleic acid.

8. The combination of any one of claims **1** to **6**, wherein said targeted therapeutic comprises an antibody, or a functional fragment thereof.

9. The combination of claim **8**, wherein said antibody is selected from the group consisting of: Rituxan (rituximab), Herceptin (trastuzumab), Erbitux (cetuximab), Vectibix (Panitumumab), Arzerra (Ofatumumab), Benlysta (belimumab), Yervoy (ipilimumab), Perjeta (Pertuzumab), Tremelimumab,

Nivolumab, Dacetuzumab, Urelumab, MPDL3280A, Lambrolizumab, Blinatumomab, CT-011, MK-3475, BMS-936559, MED14736, MSB0010718C, and margetuximab (MGAH22).

10. The combination of claim **8**, wherein said targeted therapeutic comprises a Fab, Fab', F(ab')2, single domain antibody, T and Abs dimer, Fv, scFv, dsFv, ds-scFv, Fd, linear antibody, minibody, diabody, bispecific antibody fragment, bibody, tribody, sc-diabody, kappa (lamda) body, BiTE, DVD-Ig, SIP, SMIP, DART, or an antibody analogue comprising one or more CDRs.

11. The combination of claim **1**, wherein said targeted therapeutic comprises a ATWLPPR polypeptide of VEGFR, Thrombospondin-1 mimetics, CDCRGDCFCG (cyclic) polypeptide, SCH 221153 fragment, NCNGRC (cyclic) polypeptide, CTTHWGFTLC polypeptide, CGNKRTRGC polypeptide (LyP-1), Octreotide, Vapreotide, Lanreotide, C-3940 polypeptide, Decapeptyl, Lupron, Zoladex, or Cetrorelix.

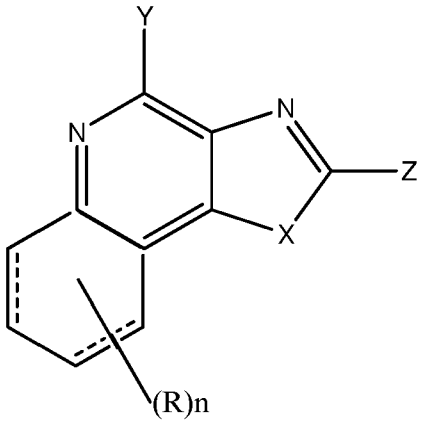
12. The combination of claim **1**, wherein said targeted therapeutic comprises extracellular domains (ECD) or soluble form of PD-1, PDL-1, CTLA4, BTLA, KIR, TIM3, 4-1BB, LAG3, full length of partial of a surface ligand amphiregulin, betacellulin, EGF, ephrin, epigen, epiregulin, IGF, neuregulin, TGF, TRAIL, or VEGF.

13. The combination of claim **1**, wherein said targeted therapeutic comprises a nanoparticle or an aptamer.

14. The combination of any one of claims **1** to **13**, wherein said immunotherapeutic is capable of binding specifically to human TLR7 and/or TLR8.

15. The combination of any one of claims **1** to **13**, wherein said immunotherapeutic comprises: (a) single-stranded RNA (ssRNA), preferably ORN02, ORN06, ssPoly(U), ssRNA40, ssRNA41, ssRNA-DR, or Poly(dT); or (b) a receptor ligand analogs, preferably CL075, CL097, CL264, CL307, Gardiquimod, Loxoribine, Imiquimod, or Resiquimod.

16. The combination of any one of claims **1** to **13**, wherein said immunotherapeutic has a structure of Formula (I):



(I)

wherein dashed line represents bond or absence of bond;

X is S or $-NR_1$, R_1 is $-W_0—W_1—W_2—W_3—W_4$,

W_0 is a bond, alkyl, alkenyl, alkynyl, alkoxy, or –alkyl-S-alkyl--,

W_1 is a bond, --O--, or $-NR_2$--, wherein R_2 is hydrogen, alkyl or alkenyl,

W_2 is a bond, --O--, --C(O)--, --C(S)--, or $-S(O)_2—$,

W_3 is a bond, $--NR_3$--, wherein R_3 is hydrogen, alkyl or alkenyl,

W_4 is hydrogen, alkyl, alkenyl, alkynyl, alkoxy, cycloalkyl, aryl, aryloxy, heteroaryl, or heterocyclyl, each of which is optionally substituted by one or more substituents selected from the group consisting of hydroxyl, alkoxy, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, heterocyclyl, $--NH_2$, nitro, --alkyl-hydroxyl, --alkyl-aryl, --alkyl-heteroaryl, --alkyl-heterocyclyl, $--O-R_4$, $--O-alkyl-R_4$, $--alkyl-O-R_4$, $--C(O)-R_4$, $--alkyl-C(O)-R_4$, $--alkyl-C(O)-O-R_4$, $--C(O)-O-R_4$, $--S-R_4$, $--S(O)_2-R_4$, $--NH-S(O)_2-R_4$, --alkyl-S-R4, $--alkyl-S(O)_2-R_4$, $--NHR_4$, $--NR_4R_4$,--NH-alkyl-R_4, halogen, --CN, $--NO_2$, and –SH, wherein R_4 is independently hydrogen, alkyl, alkenyl, --alkyl-hydroxyl, aryl, heteroaryl, heterocyclyl, or haloalkyl;

Z is hydrogen, alkyl, alkenyl, alkynyl, alkoxy, aryl, haloalkyl, heteroaryl, heterocyclyl, each of which can be optionally substituted by one or more substituents selected from the group consisting of hydroxyl, alkoxy, alkyl, alkenyl, alkynyl, aryl, heteroaryl, heterocyclyl, halogen, cyano, nitro, $--N(R_5)_2$, --alkoxy-alkyl, --alkoxy-alkenyl, --C(O)-alkyl, --C(O)-O-alkyl, --O-C(O)-alkyl, $--C(O)-N(R_5)_2$, aryl, heteroaryl, --CO-aryl, and –CO-heteroaryl, wherein each R_5 is independently hydrogen, alkyl, haloalkyl, --alkyl-aryl, or –alkyl-heteroaryl;

R is hydrogen, alkyl, alkoxy, haloalkyl, halogen, aryl, heteroaryl, heterocyclyl, each of which is optionally substituted by one or more substituents selected from the group consisting of hydroxyl, alkoxy,

alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, heterocyclyl, --NH_2, nitro, --alkyl-hydroxyl, --alkyl-aryl, --alkyl-heteroaryl, --alkyl-heterocyclyl, --O-R_4, --O-alkyl-R_4, --alkyl-O-R_4, --C(O)-R_4, --C(O)-NH-R_4, --C(O)-NR_4R_4, --alkyl-C(O)-R_4, --alkyl-C(O)-O-R_4, --C(O)-O-R_4, --O-C(O)-R_4, --S-R_4, --C(O)-S-R_4, --S-C(O)-R_4, --$S(O)_2$-R_4, --NH-$S(O)_2$-R_4, --alkyl-S-R_4, --alkyl-$S(O)_2$-R_4, --NHR_4, --NR_4R_4,--NH-alkyl-R_4, halogen, --CN, and –SH, wherein R_4 is independently hydrogen, alkyl, alkenyl, alkoxy, --alkyl-hydroxyl, aryl, heteroaryl, heterocyclyl, or haloalkyl;

n is 0, 1, 2, 3, or 4;

Y is –NR_6R_7, –$CR_6R_7R_8$, or –alkyl-NH_2, each of which can be optionally substituted by one or more substituents selected from the group consisting of hydroxyl, alkoxy, alkyl, alkenyl, alkynyl, --NH_2, halogen, --$N(R_5)_2$, --alkoxy-alkyl, --alkoxy-alkenyl, --C(O)-alkyl, --C(O)-O-alkyl, --C(O)-$N(R_5)_2$, aryl, heteroaryl, --CO-aryl, and –CO-heteroaryl,

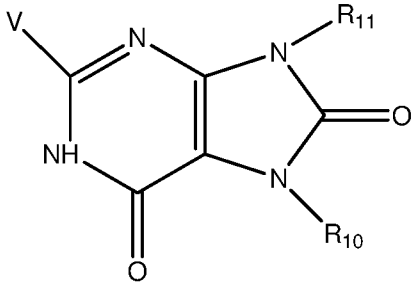
wherein R_6, R_7 and R_8 are independently hydrogen, alkyl, alkenyl, alkoxy, alkylamino, dialkylamino, alkylthio, arylthio, --alkyl-hydroxyl, --alkyl-C(O)-O-R_9, --alkyl-C(O)-R_9, or –alkyl-O-C(O)-R_9, wherein each R_5 is independently hydrogen, alkyl, haloalkyl, --alkyl-aryl, or –alkyl-heteroaryl, wherein R_9 is hydrogen, alkyl, alkenyl, halogen, or haloalkyl;

X and Z taken together may optionally form a (5-9)-membered ring.

17. The combination of claim **16**, wherein said immunotherapeutic is a compound selected from the group consisting of: 2-propylthiazolo[4,5-c]quinolin-4-amine, 1-(2-methylpropyl)-1H-imidazo[4,5-c]quinolin-4-amine, 4-amino-2-(ethoxymethyl)-a,a-di-methyl-1H-imidazo[4,5-c]quinoline-1-ethanol, 1-(4-amino-2-ethylaminomethylimidazo-[4,5-c]quinolin-1-yl)-2-methylpropan-2-ol, N-[4-(4-amino-2-ethyl-1H-imidazo[4,5-c]quinolin-1-yl)butyl-]methanesulfonamide, 4-amino-2-ethoxymethyl-aa-dimethyl-6,7,8,9-tetrahydro-1h-imidazo[4,5-c]quinoline-1-ethanol, 4-amino-aa-dimethyl-2-methoxyethyl-1h-imidazo[4,5-c]quinoline-1-ethanol, 1-{2-[3-(benzyloxy)propoxy]ethyl}-2-(ethoxymethyl)-1H-imidazo[4,5-c]quinolin-4-amine, N-[4-(4-amino-2-butyl-1H-imidazo[4,5- c][1,5]naphthyridin-1-yl)butyl]-n'-butylurea, N1-[2-(4-amino-2-butyl-1H-imidazo[4,5-c][1,5] naphthyridin-1-yl)ethyl]-2-amino-4-methylpentanamide, N-(2-{2-[4-amino-2-(2-methoxyethyl)-1H- imidazo[4,5-c]quinolin-1-yl]ethoxy}ethyl)-n'- phenylurea, 1-(2-amino-2-methylpropyl)-2-(ethoxymethyl)-1H-imidazo[4,5-c]quinolin-4-amine, 1-{4-[(3,5-dichlorophenyl)sulfonyl]butyl}-2-ethyl- 1H-imidazo[4,5-c]quinolin-4-amine, N-(2-{2-[4-amino-2-(ethoxymethyl)-1H-imidazo[4,5- c]quinolin-1-yl]ethoxy}ethyl)-n'-cyclohexylurea, N-{3-[4-amino-2-(ethoxymethyl)-1H-imidazo[4,5- c]quinolin-1-yl]propyl}-n'-(3-cyanophenyl)thiourea, N-[3-(4-amino-2-butyl-1H-imidazo[4,5-c]quinolin-1- yl)-2,2-dimethylpropyl]benzamide, 2-butyl-1-[3-(methylsulfonyl)propyl]-1H- imidazo[4,5-c]quinolin-4-amine,

N-{2-[4-amino-2-(ethoxymethyl)-1H-imidazo[4,5- c]quinolin-1-yl]-1,1-dimethylethyl}-2-ethoxyacetamide, 1-[4-amino-2-ethoxymethyl-7-(pyridin-4-yl)-1H- imidazo[4,5-c]quinolin-1-yl]-2-methylpropan-2-ol, 1-[4-amino-2-(ethoxymethyl)-7-(pyridin-3-yl)-1H- imidazo[4,5-c]quinolin-1-yl]-2-methylpropan-2-ol, N-{3-[4-amino-1-(2-hydroxy-2-methylpropyl)-2- (methoxyethyl)-1H-imidazo[4,5-c]quinolin-7-yl]phenyl}methanesulfonamide, 1-[4-amino-7-(5-hydroxymethylpyridin-3-yl)-2-(2-methoxyethyl)-1H-imidazo[4,5-c]quinolin-1-yl]-2-methylpropan-2-ol, 3-[4-amino-2-(ethoxymethyl)-7-(pyridin-3-yl)-1H- imidazo[4,5-c]quinolin-1-yl]propane-1,2-diol, 1-[2-(4-amino-2-ethoxymethyl-1H-imidazo[4,5- c]quinolin-1-yl)-1,1-dimethylethyl]-3-propylurea, 1-[2-(4-amino-2-ethoxymethyl-1H-imidazo[4,5- c]quinolin-1-yl)-1,1-dimethylethyl]-3-cyclopentylurea, 1-[(2,2-dimethyl-1,3-dioxolan-4-yl)methyl]-2- (ethoxymethyl)-7-(4-hydroxymethylphenyl)-1H- imidazo[4,5-c]quinolin-4-amine, 4-[4-amino-2-ethoxymethyl-1-(2-hydroxy-2-methylpropyl)-1H-imidazo[4,5-c]quinolin-7-yl]-N- methoxy-N-methylbenzamide, 2-ethoxymethyl-N1-isopropyl-6,7,8,9-tetrahydro-1H-imidazo[4,5-c]quinoline-1,4-diamine, 1-[4-amino-2-ethyl-7-(pyridin-4-yl)-1H-imidazo[4,5- c]quinolin-1-yl]-2-methylpropan-2-ol, N-[4-(4-amino-2-ethyl-1H-imidazo[4,5-c]quinolin-1-yl)butyl]methanesulfonamide, and N-[4-(4-amino-2-butyl-1H-imidazo[4,5- c][1,5]naphthyridin-1-yl)butyl]-n'-cyclohexylurea.

18. The combination of any one of claims **1** to **13**, wherein said immunotherapeutic has a structure of Formula (II):



(II)

wherein V is $-NR_6R_7$, wherein each of R_6 and R_7 is independently hydrogen, alkyl, alkenyl, alkoxy, alkylamino, dialkylamino, alkylthio, arylthio, --alkyl-hydroxyl, --alkyl-C(O)-O-R_9, --alkyl-C(O)-R_9, or –alkyl-O-C(O)-R_9, wherein R_9 is hydrogen, alkyl, alkenyl, halogen, or haloalkyl;

R_{10} and R_{11} are independently hydrogen, alkyl, alkenyl, aryl, haloalkyl, heteroaryl, heterocyclyl, or cycloalkyl, each of which is optionally substituted by one or more substituents selected from the group consisting of hydroxyl, alkoxy, alkyl, alkenyl, alkynyl, halogen, --$N(R_5)_2$, --alkoxy-alkyl, --alkoxy-alkenyl, --C(O)-alkyl, --C(O)-O-alkyl, --C(O)-$N(R_5)_2$, aryl, heteroaryl, --CO-aryl, and –CO-heteroaryl, wherein each R_5 is independently hydrogen, alkyl, haloalkyl, --alkyl-aryl, or –alkyl-heteroaryl.

19. The combination of any one of claims **1** to **18**, further comprising an effective amount of an additional therapeutic agent.

20. The combination of claim **19**, wherein said additional therapeutic agent is an anticancer agent.

21. The combination of claim **20**, wherein said anticancer agent is an antimetabolite, an inhibitor of topoisomerase I and II, an alkylating agent, a microtubule inhibitor, an antiandrogen agent, a GNRh modulator or mixtures thereof.

22. The combination of claim **21**, wherein said additional therapeutic agent is a chemotherapeutic agent selected from the group consisting of tamoxifen, raloxifene, anastrozole, exemestane, letrozole, imatanib, paclitaxel, cyclophosphamide, lovastatin, minosine, gemcitabine, cytarabine, 5- fluorouracil, methotrexate, docetaxel, goserelin, vincristine, vinblastine,nocodazole, teniposide etoposide, gemcitabine, epothilone, vinorelbine, camptothecin, daunorubicin, actinomycin D, mitoxantrone, acridine, doxorubicin, epirubicin, or idarubicin.

23. The combination of any one of claims **1** to **22**, wherein said immunotherapeutic is of an amount that is capable of:

(1) inducing IFN-α in a enriched human blood DCs;

(2) inducing TNF-α in a enriched human blood DCs; and/or

(3) inducing IL-12-α in a enriched human blood DCs.

24. A method for treating a disease condition in a subject that is in need of such treatment, comprising administering to said subject the combination of any one of claims **1-23**.

25. The method of claim **24**, wherein said diseases condition is tumor.

26. The method of claim **24**, wherein said disease condition comprises abnormal cell proliferation.

27. The method of claim **26**, wherein said abnormal cell proliferation comprises a pre-cancerous lesion.

28. The method of claim **26**, wherein said abnormal proliferation is of cancer cells.

29. The method of claim **28**, wherein said cancer is selected from the group consisting of: breast cancer, colorectal cancer, diffuse large B-cell lymphoma, endometrial cancer, follicular lymphoma, gastric cancer, glioblastoma, head and neck cancer, hepatocellular cancer, lung cancer, melanoma, multiple myeloma, ovarian cancer, pancreatic cancer, prostate cancer, and renal cell carcinoma.

30. The method of any one of claims **24** to **29**, comprising administering to said subject an oral formulation comprising said immunotherapeutic in a dose of between about 0.0005 mg/kg, 0.0006

mg/mg/kg, 0.0007 mg/kg, 0.0008 mg/kg, 0.0009 mg/kg, 0.001 mg/kg, 0.002 mg/kg, 0.003 mg/kg, 0.004 mg/kg, 0.005 mg/kg, 0.006 mg/kg, 0.007 mg/kg, 0.008 mg/kg, 0.009 mg/kg, or 0.01 mg/kg, to about 0.02 mg/kg, all inclusive, twice per week.

31. The method of any one of claims **24** to **29**, comprising administering to said subject an oral formulation comprising said immunotherapeutic in a dose of less than or about 0.0005mg/kg, 0.0006 mg/kg, 0.0007 mg/kg, 0.0008 mg/kg, 0.0009 mg/kg, 0.001 mg/kg, 0.002 mg/kg, 0.003 mg/kg, 0.004 mg/kg, 0.005 mg/kg, 0.006 mg/kg, 0.007 mg/kg, 0.008 mg/kg, 0.009 mg/kg, or 0.01 mg/kg, twice per week.

32. The method of any one of claims **24** to **29**, comprising administering to said subject an intravenous formulation comprising said immunotherapeutic in a dose of between about 0.0005 mg/kg, 0.0006 mg/kg, 0.0007 mg/kg, 0.0008 mg/kg, 0.0009 mg/kg, 0.001 mg/kg, 0.002 mg/kg, 0.003 mg/kg, 0.004 mg/kg, 0.005 mg/kg, or 0.006 mg/kg to about 0.015 mg/kg, all inclusive, weekly.

33. The method of any one of claims **24** to **29**, comprising administering to said subject an intravenous formulation comprising said immunotherapeutic in a dose of less than or about 0.003 mg/kg, 0.004 mg/kg, 0.005 mg/kg, or 0.006 mg/kg, to about 0.01 mg/kg, weekly.

34. The method of any one of claims **24** to **33**, wherein said immunotherapeutic in said subject has a local concentration that is between about 0.005 μg/ml to about 12 μg/ml.

35. The The method of any one of claims **24** to **33**, wherein said immunotherapeutic in said subject has a local concentration that is is between about 0.05 μg/ml, 0.1 μg/ml, 0.15 μg/ml, 0.2 μg/ml, 0.3 μg/ml, or 0.4 μg/ml, to about 0.5 μg/ml.

36. A kit, comprising the combination of any one of claims **1** to **23**.