

USE OF CYCLOSPORINE ANALOGUES FOR TREATING CANCER

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority to U.S. Provisional Patent Application No. 62/981,383, filed February 25, 2020, the content of which is incorporated herein by reference in its entirety.

BACKGROUND

Field

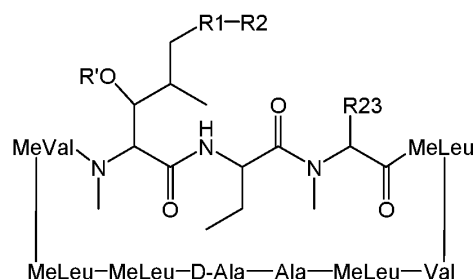
[0002] The present disclosure relates generally to the fields of molecular biology and medicine. One aspect relates to preventing and treating cancer with cyclophilin inhibitors.

Description of the Related Art

[0003] Cancer remains one of the leading causes of death globally. Although treatment options are available for various cancers, there is a need to find therapeutic agents that are effective in preventing and treating cancer.

SUMMARY

[0004] Disclosed herein include a method for treating a proliferative disease in a subject suffering from the proliferative disease. The method can, for example, comprise administering to the subject a composition comprising cyclosporine analogue of Formula L, or a pharmaceutically acceptable salt, solvate, stereoisomer thereof,



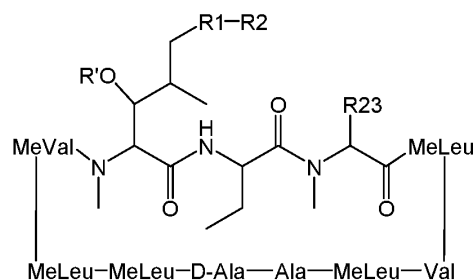
Formula L

wherein:

- a. R' is H or acetyl;
- b. R1 is a saturated or unsaturated straight or branched aliphatic carbon chain from 2 to 15 carbon atoms in length;
- c. R2 is selected from the group consisting of:
 - i. H;
 - ii. an unsubstituted, N-substituted, or N,N-disubstituted amide;

- iii. a N-substituted or unsubstituted acyl protected amine;
 - iv. a N-substituted or unsubstituted amine;
 - v. a carboxylic acid;
 - vi. a nitrile;
 - vii. an ester;
 - viii. a ketone;
 - ix. a hydroxy, dihydroxy, trihydroxy, or polyhydroxy alkyl; and
 - x. a substituted or unsubstituted aryl;
 - xi. a saturated or unsaturated, straight or branched aliphatic chain optionally containing a substituent selected from the group consisting of a hydrogen, a ketone, a hydroxyl, a nitrile, a carboxylic acid, an ester, a 1,3-dioxolane, a halogen, and an oxo;
 - xii. an aromatic group containing a substituent selected from the group consisting of a halogen, an ester, and a nitro; and
 - xiii. a combination of the saturated or unsaturated, straight or branched aliphatic chain of (xi) and the aromatic group of (xii); and
- d. R23 is a saturated or unsaturated straight chain or branched optionally substituted aliphatic carbon chain.

[0005] Also disclosed herein include a method for alleviating one or more symptoms of a proliferative disease, or preventing or delaying the onset of one or more symptoms of a proliferative disease, in a subject suffering from the proliferative disease, comprising administering to the subject a composition comprising cyclosporine analogue of Formula L, or a pharmaceutically acceptable salt, solvate, stereoisomer thereof,



Formula L

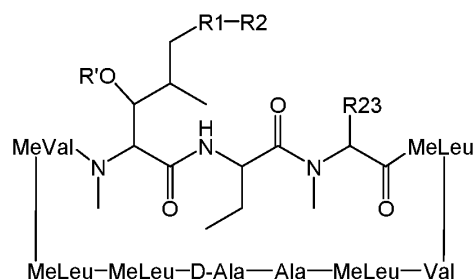
wherein:

- a. R' is H or acetyl;
- b. R1 is a saturated or unsaturated straight or branched aliphatic carbon chain from 2 to 15 carbon atoms in length;
- c. R2 is selected from the group consisting of:

- i. H;
 - ii. an unsubstituted, N-substituted, or N,N-disubstituted amide;
 - iii. a N-substituted or unsubstituted acyl protected amine;
 - iv. a N-substituted or unsubstituted amine;
 - v. a carboxylic acid;
 - vi. a nitrile;
 - vii. an ester;
 - viii. a ketone;
 - ix. a hydroxy, dihydroxy, trihydroxy, or polyhydroxy alkyl; and
 - x. a substituted or unsubstituted aryl;
 - xi. a saturated or unsaturated, straight or branched aliphatic chain optionally containing a substituent selected from the group consisting of a hydrogen, a ketone, a hydroxyl, a nitrile, a carboxylic acid, an ester, a 1,3-dioxolane, a halogen, and an oxo;
 - xii. an aromatic group containing a substituent selected from the group consisting of a halogen, an ester, and a nitro; and
 - xiii. a combination of the saturated or unsaturated, straight or branched aliphatic chain of (xi) and the aromatic group of (xii); and
- d. R23 is a saturated or unsaturated straight chain or branched optionally substituted aliphatic carbon chain.

[0006] The subject can be in a partial remission of the proliferative disease. In some embodiments, the method comprises identifying a subject suffering from the proliferative disease.

[0007] Disclosed herein includes a method for preventing a proliferative disease in a subject in need thereof, comprising administering to the subject a composition comprising cyclosporine analogue of Formula L, or a pharmaceutically acceptable salt, solvate, stereoisomer thereof,



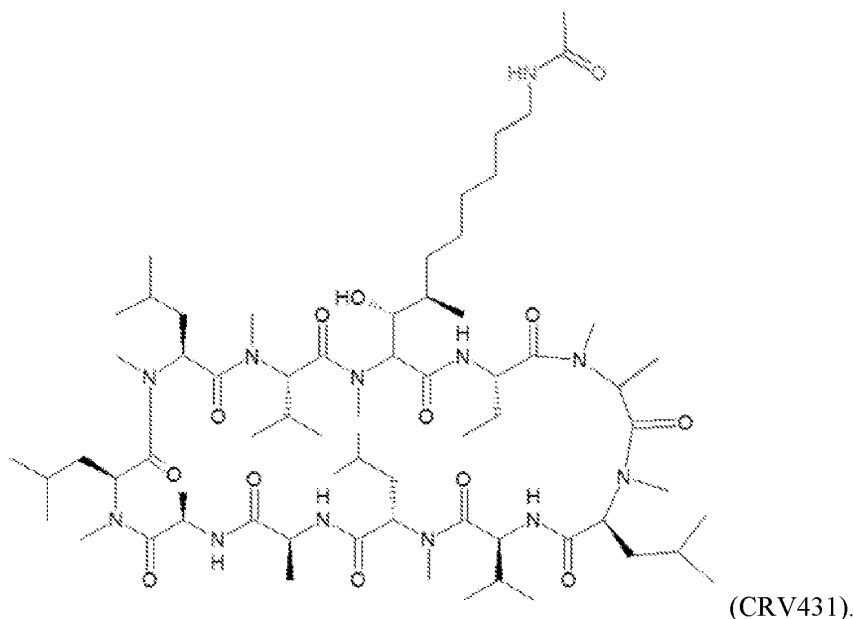
Formula L

wherein:

- a. R' is H or acetyl;
- b. R1 is a saturated or unsaturated straight or branched aliphatic carbon chain from 2 to 15 carbon atoms in length;
- c. R2 is selected from the group consisting of:
 - i. H;
 - ii. an unsubstituted, N-substituted, or N,N-disubstituted amide;
 - iii. a N-substituted or unsubstituted acyl protected amine;
 - iv. a N-substituted or unsubstituted amine;
 - v. a carboxylic acid;
 - vi. a nitrile;
 - vii. an ester;
 - viii. a ketone;
 - ix. a hydroxy, dihydroxy, trihydroxy, or polyhydroxy alkyl; and
 - x. a substituted or unsubstituted aryl;
 - xi. a saturated or unsaturated straight or branched aliphatic chain optionally containing a substituent selected from the group consisting of a hydrogen, a ketone, a hydroxyl, a nitrile, a carboxylic acid, an ester, a 1,3-dioxolane, a halogen, and an oxo;
 - xii. an aromatic group containing a substituent selected from the group consisting of a halogen, an ester, and a nitro; and
 - xiii. a combination of the saturated or unsaturated, straight or branched aliphatic chain of (xi) and the aromatic group of (xii); and
- d. R23 is a saturated or unsaturated straight chain or branched optionally substituted aliphatic carbon chain.

[0008] The subject in need thereof can be a subject at a risk of developing the proliferative disease, or a subject at a complete remission of the proliferative disease. The method can, for example, comprises identifying a subject at risk of development the proliferative disease.

[0009] In some embodiments, the cyclosporine analogue of Formula L is CRV431:



[0010] The proliferative disease can be, for example, cancer. Non-limiting examples of cancer include carcinoma, squamous carcinoma, adenocarcinoma, sarcomata, endometrial cancer, breast cancer, ovarian cancer, cervical cancer, fallopian tube cancer, primary peritoneal cancer, colon cancer, colorectal cancer, squamous cell carcinoma of the anogenital region, melanoma, renal cell carcinoma, lung cancer, non-small cell lung cancer, squamous cell carcinoma of the lung, stomach cancer, bladder cancer, gall bladder cancer, liver cancer, thyroid cancer, laryngeal cancer, salivary gland cancer, esophageal cancer, head and neck cancer, glioblastoma, glioma, squamous cell carcinoma of the head and neck, prostate cancer, pancreatic cancer, mesothelioma, sarcoma, hematological cancer, leukemia, lymphoma, neuroma, multiple myeloma, and any combination thereof. In some embodiments, the cancer is liver cancer, for example primary liver cancer or secondary liver cancer. In some embodiments, the liver cancer is hepatocellular carcinoma (HCC), bile duct cancer, Angiosarcoma, hemangiosarcoma, hepatoblastoma, hemangioma, hepatic adenoma, focal nodular hyperplasia, or a combination thereof.

[0011] The proliferative disease can be a solid tumor (including but not limited to neuroblastoma, Ewing sarcoma or Wilms tumor), or a liquid tumor.

[0012] In some embodiments, the composition comprises a therapeutically or prophylactically effective amount of cyclosporine analogue of Formula L, or a pharmaceutically acceptable salt, solvate, stereoisomer thereof. The subject can be a mammal, for example a human. The composition can comprise one or more pharmaceutically acceptable excipients.

[0013] The composition comprises one or more additional therapeutic agents. In some embodiments, the method further comprises administering to the subject one or more additional therapeutic agents, administering to the subject one or more cancer therapies, or both.

[0014] The one or more additional therapeutic agents can comprise, for example, a radiotherapeutic agent, an anti-immunosuppressive agent or immunostimulatory agent, a chemotherapeutic agent, or a combination thereof. In some embodiments, the one or more additional therapeutic agents comprise an anti-PD-1 agent, an anti-PD-L1 agent, an anti-CTLA4 agent, an anti-TIM-3 agent, an anti-LAG-3 agent, a GITR (glucocorticoid-induced TNFR-related protein) stimulating agent, an anti-IDO agent, an anti-ICOS agent, a proteasome inhibitor(s), an anti-OX40 agent, an anti-CSF1R agent, a chemokine signaling agent, a cytokine signal stimulating agent, or a combination thereof. In some embodiments, the one or more additional therapeutic agents comprise bevacizumab, pembrolizumab, nivolumab, PDR001, REGN2810 (SAR-439684), BGB-A317, BI 754091, IBI308, INCSHR-1210, JNJ-63723283, JS-001, MEDI0680 (AMP-514), MGA-012, PF-06801591, REGN-2810, TSR-042, atezolizumab, avelumab, CX-072, durvalumab, FAZ053, LY3300054, PD-L1 millamolecule, atezolizumab, durvalumab, avelumab, LY3300054, aminoglutethimide, amsacrine, anastrozole, asparaginase, bcg, beta-hydroxy beta-methylbutyrate, bicalutamide, bleomycin, bortezomib, buserelin, busulfan, camptothecin, capecitabine, carfilzomib, carboplatin, carmustine, chlorambucil, cisplatin, cladribine, clodronate, colchicine, cyclophosphamide, cyproterone, cytarabine, dacarbazine, dactinomycin, daunorubicin, delanzomib, dienestrol, diethylstilbestrol, disulfiram, docetaxel, doxorubicin, epigallocatechin-3-gallate, epirubicin, epoxomicin, estradiol, estramustine, etoposide, exemestane, filgrastim, fludarabine, fludrocortisone, fluorouracil, fluoxymesterone, flutamide, gemcitabine, genistein, goserelin, hydroxyurea, idarubicin, ifosfamide, imatinib, interferon, irinotecan, ironotecan, letrozole, leucovorin, leuprolide, levamisole, lomustine, ixazomib, marizomib, mechlorethamine, medroxyprogesterone, megestrol, melphalan, mercaptopurine, mesna, methotrexate, mitomycin, mitotane, mitoxantrone, nilutamide, nocodazole, octreotide, oprozomib, oxaliplatin, paclitaxel, pamidronate, pentostatin, plicamycin, porfimer, procarbazine, raltitrexed, rituximab, streptozocin, suramin, tamoxifen, temozolomide, teniposide, testosterone, thioguanine, thiotepa, titanocene dichloride, topotecan, trastuzumab, tretinoin, vinblastine, vincristine, vindesine, vinorelbine, or a combination thereof.

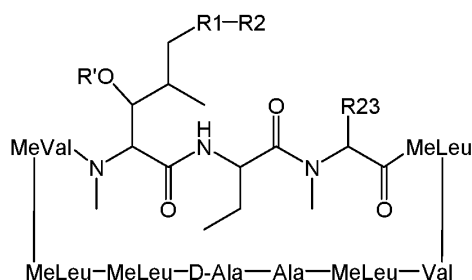
[0015] The one or more cancer therapies can, for example, comprise surgery, chemotherapy, radiotherapy, immunotherapy, or a combination thereof.

[0016] In some embodiments, the proliferative disease is multiple myeloma. In some embodiments, the method comprises administering to the subject a proteasome inhibitor, and the

cyclosporine analogue of Formula L, or a pharmaceutically acceptable salt, solvate, stereoisomer thereof. The cyclosporine analogue of Formula L can be CRV431. In some embodiments, the proteasome inhibitor is beta-hydroxy beta-methylbutyrate, bortezomib, carfilzomib, delanzomib, disulfiram, epigallocatechin-3-gallate, epoxomicin, ixazomib, marizomib, or oprozomib,

[0017] In some embodiments, at least one of the one or more additional therapeutic agents and/or the one or more cancer therapies is co-administered to the subject with the composition. In some embodiments, at least one of the one or more additional therapeutic agents and/or the one or more cancer therapies is administered to the subject before the administration of the composition, after the administration of the composition, or both. The composition can be, for example, administered to the subject by intravenous administration, oral administration, parenteral administration. The composition can be, for example, in the form of powder, pill, tablet, microtablet, pellet, micropellet, capsule, capsule containing microtablets, liquid, aerosols, or nanoparticles. In some embodiments, the composition is administered to the subject at an effective daily dose of the cyclosporine analogue or a pharmaceutically acceptable salt, solvate, stereoisomer thereof at from 10 mg to 250 mg.

[0018] Also disclosed herein include a pharmaceutical composition which comprises a cyclosporine analogue of Formula L, or a pharmaceutically acceptable salt, solvate, stereoisomer thereof, for use in preventing or treating a proliferative disease, or alleviating one or more symptoms of a proliferative disease, or preventing or delaying the onset of one or more symptoms of a proliferative disease, or preventing or delaying the onset of a proliferative disease,



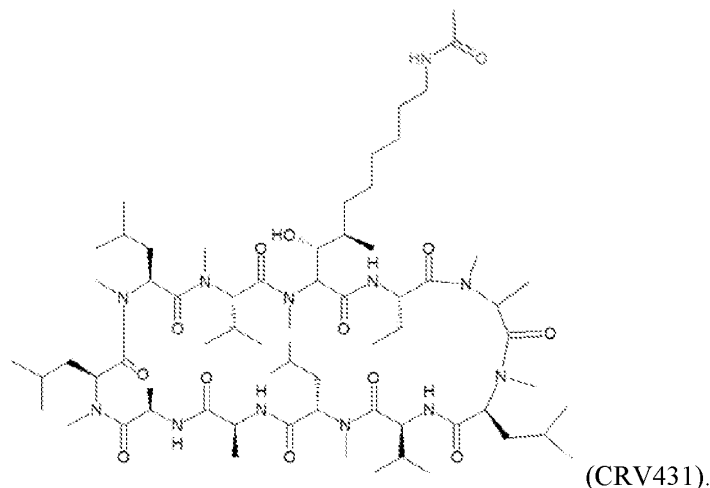
Formula L

wherein:

- a. R' is H or acetyl;
- b. R1 is a saturated or unsaturated straight or branched aliphatic carbon chain from 2 to 15 carbon atoms in length;
- c. R2 is selected from the group consisting of:
 - i. H;
 - ii. an unsubstituted, N-substituted, or N,N-disubstituted amide;

- iii. a N-substituted or unsubstituted acyl protected amine;
 - iv. a N-substituted or unsubstituted amine;
 - v. a carboxylic acid;
 - vi. a nitrile;
 - vii. an ester;
 - viii. a ketone;
 - ix. a hydroxy, dihydroxy, trihydroxy, or polyhydroxy alkyl; and
 - x. a substituted or unsubstituted aryl;
 - xi. a saturated or unsaturated, straight or branched aliphatic chain optionally containing a substituent selected from the group consisting of a hydrogen, a ketone, a hydroxyl, a nitrile, a carboxylic acid, an ester, a 1,3-dioxolane, a halogen, and an oxo;
 - xii. an aromatic group containing a substituent selected from the group consisting of a halogen, an ester, and a nitro; and
 - xiii. a combination of the saturated or unsaturated, straight or branched aliphatic chain of (xi) and the aromatic group of (xii); and
- d. R23 is a saturated or unsaturated straight chain or branched optionally substituted aliphatic carbon chain.

[0019] In some embodiments, the cyclosporine analogue is CRV431:

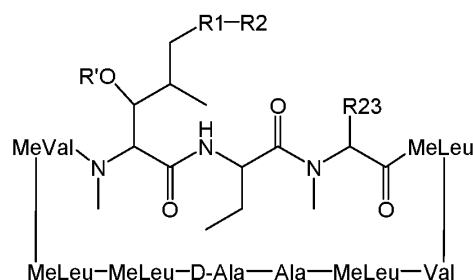


[0020] The pharmaceutical composition can be, for example, for intravenous administration, oral administration, or parenteral administration. The pharmaceutical composition can be, for example, in the form of powder, pill, tablet, microtablet, pellet, micropellet, capsule, capsule containing microtablets, liquid, aerosols, or nanoparticles. In some embodiments, the proliferative disease is cancer, including but are not limited to, squamous carcinoma, adenocarcinoma, sarcomata, endometrial cancer, breast cancer, ovarian cancer,

cervical cancer, fallopian tube cancer, primary peritoneal cancer, colon cancer, colorectal cancer, squamous cell carcinoma of the anogenital region, melanoma, renal cell carcinoma, lung cancer, non-small cell lung cancer, squamous cell carcinoma of the lung, stomach cancer, bladder cancer, gall bladder cancer, liver cancer, thyroid cancer, laryngeal cancer, salivary gland cancer, esophageal cancer, head and neck cancer, glioblastoma, glioma, squamous cell carcinoma of the head and neck, prostate cancer, pancreatic cancer, mesothelioma, sarcoma, hematological cancer, leukemia, lymphoma, neuroma, or a combination thereof. In some embodiments, the cancer is liver cancer.

[0021] Disclosed herein include a kit, comprising any one of the pharmaceutical composition disclosed herein; and a label, wherein the label indicating one or more of: (a) the kit is for preventing or treating a proliferative disease, (b) the kit is for alleviating one or more symptoms of a proliferative disease, or preventing or delaying the onset of one or more symptoms of a proliferative disease, and (c) the kit is for preventing or delaying the onset of a proliferative disease. In some embodiments, the kit further comprises instructions for identifying a subject at risk of development the proliferative disease, instructions for identifying a subject suffering from the proliferative disease, or both.

[0022] Disclosed herein include a method of sensitizing cancer cells to anti-cancer agents or therapies. The method can, for example, comprises contacting cancer cells with a composition comprising cyclosporine analogue of Formula L, or a pharmaceutically acceptable salt, solvate, stereoisomer thereof, thereby sensitizing the cancer cells to one or more anticancer agents, one or more cancer therapies, or both,



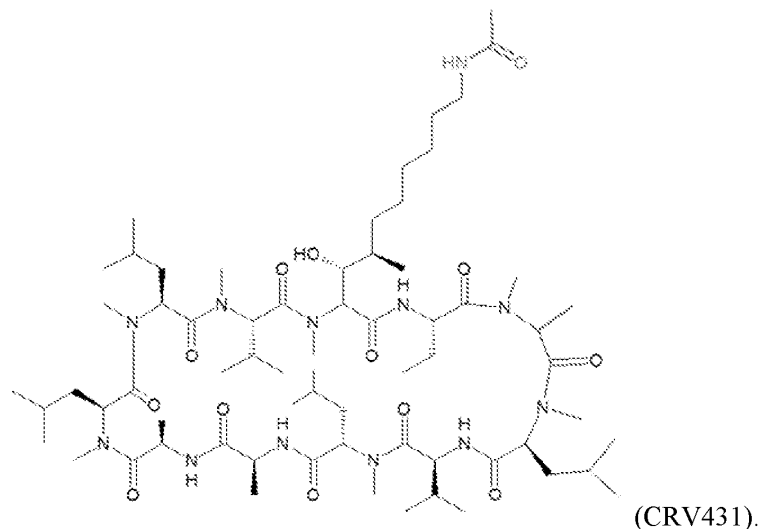
Formula L

wherein:

- a. R' is H or acetyl;
- b. R1 is a saturated or unsaturated straight or branched aliphatic carbon chain from 2 to 15 carbon atoms in length;
- c. R2 is selected from the group consisting of:
 - i. H;
 - ii. an unsubstituted, N-substituted, or N,N-disubstituted amide;

- iii. a N-substituted or unsubstituted acyl protected amine;
 - iv. a N-substituted or unsubstituted amine;
 - v. a carboxylic acid;
 - vi. a nitrile;
 - vii. an ester;
 - viii. a ketone;
 - ix. a hydroxy, dihydroxy, trihydroxy, or polyhydroxy alkyl; and
 - x. a substituted or unsubstituted aryl;
 - xi. a saturated or unsaturated, straight or branched aliphatic chain optionally containing a substituent selected from the group consisting of a hydrogen, a ketone, a hydroxyl, a nitrile, a carboxylic acid, an ester, a 1,3-dioxolane, a halogen, and an oxo;
 - xii. an aromatic group containing a substituent selected from the group consisting of a halogen, an ester, and a nitro; and
 - xiii. a combination of the saturated or unsaturated, straight or branched aliphatic chain of (xi) and the aromatic group of (xii); and
- d. R23 is a saturated or unsaturated straight chain or branched optionally substituted aliphatic carbon chain.

[0023] In some embodiments, the cyclosporine analogue is CRV431:



[0024] In some embodiments, contacting cancer cells with the composition occurs *in vitro*, *ex vivo*, and/or *in vivo*. In some embodiments, contacting cancer cells with the composition is in a subject. In some embodiments, the subject did not respond to, or is known to be resistant to, the one or more anticancer agents alone, the one or more cancer therapies alone, or both. In some embodiments, the subject had prior treatment with the one or more anticancer

agents, the one or more cancer therapies, or both. The subject can be a mammal, for example a human.

[0025] In some embodiments, the method comprises determining sensitization of the cancer cells to the one or more anticancer agents, the one or more cancer therapies, or both, after being contacted with the composition. In some embodiments, the method comprises contacting the cancer cells with the one or more anticancer agents, the one or more cancer therapies, or both. In some embodiments, contacting the cancer cells with the one or more anticancer agents, the one or more cancer therapies, or both, occurs in the subject. In some embodiments, the method comprises determining the response of the subject to the one or more anticancer agents, the one or more cancer therapies, or both. In some embodiments, contacting the cancer cells with the one or more anticancer agents, the one or more cancer therapies, or both, is concurrent with the contacting the cancer cells with the composition, or after the contacting the cancer cells with the composition.

[0026] The cancer cells can, for example, comprise cells of carcinoma, squamous carcinoma, adenocarcinoma, sarcomata, endometrial cancer, breast cancer, ovarian cancer, cervical cancer, fallopian tube cancer, primary peritoneal cancer, colon cancer, colorectal cancer, squamous cell carcinoma of the anogenital region, melanoma, renal cell carcinoma, lung cancer, non-small cell lung cancer, squamous cell carcinoma of the lung, stomach cancer, bladder cancer, gall bladder cancer, liver cancer, thyroid cancer, laryngeal cancer, salivary gland cancer, esophageal cancer, head and neck cancer, glioblastoma, glioma, squamous cell carcinoma of the head and neck, prostate cancer, pancreatic cancer, mesothelioma, sarcoma, hematological cancer, leukemia, lymphoma, neuroma, multiple myeloma, or a combination thereof. In some embodiments, the cancer cells comprise cells of liver cancer. In some embodiments, the one or more anticancer agents comprise a radiotherapeutic agent, an anti-immunosuppressive agent or immunostimulatory agent, a chemotherapeutic agent, or a combination thereof. In some embodiments, the one or more anticancer agents comprise an anti-PD-1 agent, an anti-PD-L1 agent, an anti-CTLA4 agent, an anti-TIM-3 agent, an anti-LAG-3 agent, a GITR (glucocorticoid-induced TNFR-related protein) stimulating agent, an anti-IDO agent, an anti-ICOS agent, a proteasome inhibitor, an anti-OX40 agent, an anti-CSF1R agent, a chemokine signaling agent, a cytokine signal stimulating agent, or a combination thereof. In some embodiments, the one or more anticancer agents comprise bevacizumab, pembrolizumab, nivolumab, PDR001, REGN2810 (SAR-439684), BGB-A317, BI 754091, IBI308, INCSR-1210, JNJ-63723283, JS-001, MEDI0680 (AMP-514), MGA-012, PF-06801591, REGN-2810, TSR-042, atezolizumab, avelumab, CX-072, durvalumab, FAZ053, LY3300054, PD-L1 millamolecule, atezolizumab, durvalumab, avelumab, LY3300054, aminoglutethimide,

amsacrine, anastrozole, asparaginase, bcg, beta-hydroxy beta-methylbutyrate, bicalutamide, bleomycin, bortezomib, buserelin, busulfan, camptothecin, capecitabine, carfilzomib, carboplatin, carmustine, chlorambucil, cisplatin, cladribine, clodronate, colchicine, cyclophosphamide, cyproterone, cytarabine, dacarbazine, dactinomycin, daunorubicin, delanzomib, dienestrol, diethylstilbestrol, disulfiram, docetaxel, doxorubicin, epigallocatechin-3-gallate, epirubicin, epoxomicin, estradiol, estramustine, etoposide, exemestane, filgrastim, fludarabine, fludrocortisone, fluorouracil, fluoxymesterone, flutamide, gemcitabine, genistein, goserelin, hydroxyurea, idarubicin, ifosfamide, imatinib, interferon, irinotecan, ironotecan, letrozole, leucovorin, leuprolide, levamisole, lomustine, ixazomib, marizomib, mechlorethamine, medroxyprogesterone, megestrol, melphalan, mercaptopurine, mesna, methotrexate, mitomycin, mitotane, mitoxantrone, nilutamide, nocodazole, octreotide, oprozomib, oxaliplatin, paclitaxel, pamidronate, pentostatin, plicamycin, porfimer, procarbazine, raltitrexed, rituximab, streptozocin, suramin, tamoxifen, temozolomide, teniposide, testosterone, thioguanine, thiotepa, titanocene dichloride, topotecan, trastuzumab, tretinoin, vinblastine, vincristine, vindesine, vinorelbine, or a combination thereof.

[0027] In some embodiments, the one or more cancer therapies comprise surgery, chemotherapy, radiotherapy, immunotherapy, or a combination thereof

BRIEF DESCRIPTION OF THE DRAWINGS

[0028] **FIGS. 1A-C** are plots showing antifibrotic activity of CRV431 in human precision cut liver slices (PCLS).

[0029] **FIGS. 2A-B** are PCA plots showing the distribution of the sample and donor for comparison of TGFb/PDGF + CRV431 vs TGFb/PDGF + Vehicle by group.

[0030] **FIG. 2C** is a MA plot, and **FIGS. 3A-B** are heatmap, generated for comparison of TGFb/PDGF + CRV431 vs TGFb/PDGF + Vehicle by group.

[0031] **FIG. 4** is a volcano plot showing significant differently expressed genes identified in the comparison of TGFb/PDGF + CRV431 vs TGFb/PDGF + Vehicle by group.

[0032] **FIGS. 5A-B** are venn diagrams showing significant gene overlapping between all three donors.

[0033] **FIGS. 6A-B** are PCA plots showing the distribution of the sample and donor for comparison of Nonstimulated + CRV431 vs Nonstimulated + Vehicle by group.

[0034] **FIG. 6C** is a MA plot, and **FIGS. 7A-B** are heatmap generated for comparison of Nonstimulated + CRV431 vs Nonstimulated + Vehicle by group.

[0035] **FIG. 8** is a volcano plot showing significant differently expressed genes identified in the comparison of Nonstimulated + CRV431 vs Nonstimulated + Vehicle by group.

[0036] **FIGS. 9A-B** are venn diagrams showing significant gene overlapping between all three donors for the comparison of Nonstimulated + CRV431 vs Nonstimulated + Vehicle by donor.

[0037] **FIGS. 10A-C** are plots showing CRV431 sensitization of HepG2 hepatocellular carcinoma cells to daunorubicin. **FIGS. 10D-F** are plots showing CRV431 sensitization of Huh7 hepatocellular carcinoma cells to daunorubicin.

[0038] **FIGS. 11A** and **12A** show tumor burden at the end of treatment was assessed by the number of tumors. **FIGS. 11B** and **12B** show a composite score based on the number and size of tumors (0–7 scale).

DETAILED DESCRIPTION

[0039] In the following detailed description, reference is made to the accompanying drawings, which form a part hereof. In the drawings, similar symbols typically identify similar components, unless context dictates otherwise. The illustrative embodiments described in the detailed description, drawings, and claims are not meant to be limiting. Other embodiments may be utilized, and other changes may be made, without departing from the spirit or scope of the subject matter presented herein. It will be readily understood that the aspects of the present disclosure, as generally described herein, and illustrated in the Figures, can be arranged, substituted, combined, separated, and designed in a wide variety of different configurations, all of which are explicitly contemplated herein and made part of the disclosure herein.

[0040] All patents, published patent applications, other publications, and sequences from GenBank, and other databases referred to herein are incorporated by reference in their entirety with respect to the related technology.

Definitions

[0041] Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the present disclosure belongs. *See, e.g.* Singleton et al., Dictionary of Microbiology and Molecular Biology 2nd ed., J. Wiley & Sons (New York, NY 1994); Sambrook et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press (Cold Spring Harbor, NY 1989). For purposes of the present disclosure, the following terms are defined below.

[0042] As used herein, a "subject" refers to an animal that is the object of treatment, observation or experiment. "Animals" include cold- and warm-blooded vertebrates and invertebrates such as fish, shellfish, reptiles and, in particular, mammals. "Mammal" includes,

without limitation, mice; rats; rabbits; guinea pigs; dogs; cats; sheep; goats; cows; horses; primates, such as monkeys, chimpanzees, and apes, and, in particular, humans.

[0043] As used herein, a “patient” refers to a subject that is being treated by a medical professional, such as a Medical Doctor (*i.e.*, Doctor of Allopathic medicine or Doctor of Osteopathic medicine) or a Doctor of Veterinary Medicine, to attempt to cure, or at least ameliorate the effects of, a particular disease or disorder or to prevent the disease or disorder from occurring in the first place.

[0044] As used herein, “administration” or “administering” refers to a method of giving a dosage of a pharmaceutically active ingredient to a vertebrate. The administration can be, for example, oral administration, administration as a suppository, topical contact, intravenous, intraperitoneal, intramuscular, intralesional, intranasal or subcutaneous administration, or the implantation of a slow-release device *e.g.*, a mini-osmotic pump, to a subject. Administration can be by any suitable route, including parenteral and transmucosal (*e.g.*, buccal, sublingual, palatal, gingival, nasal, vaginal, rectal, or transdermal). Parenteral administration includes, *e.g.*, intravenous, intramuscular, intra-arteriole, intradermal, subcutaneous, intraperitoneal, intraventricular, and intracranial. Other modes of delivery of pharmaceutical compositions and therapeutic substances disclosed herein include, but are not limited to, the use of liposomal formulations, intravenous infusion, transdermal patches, or a combination thereof.

[0045] As used herein, a “dosage” refers to the combined amount of the active ingredients (*e.g.*, cyclosporine analogues, including CRV431).

[0046] As used herein, a “unit dosage” refers to an amount of therapeutic agent administered to a patient in a single dose.

[0047] As used herein, a “daily dosage” refers to the total amount of therapeutic agent administered to a patient in a day,

[0048] As used herein, “therapeutically effective amount” or “pharmaceutically effective amount” is meant an amount of therapeutic agent, which has a therapeutic effect. The dosages of a pharmaceutically active ingredient which are useful in treatment when administered alone or in combination with one or more additional therapeutic agents are therapeutically effective amounts. Thus, as used herein, a therapeutically effective amount means an amount of therapeutic agent which produces the desired therapeutic effect as judged by clinical trial results and/or model animal studies.

[0049] As used herein, the term “treat,” “treatment,” or “treating,” refers to administering a therapeutic agent or pharmaceutical composition to a subject for prophylactic and/or therapeutic purposes. The term “prophylactic treatment” refers to treating a subject who

does not yet exhibit symptoms of a disease or condition, but who is susceptible to, or otherwise at risk of, a particular disease or condition, whereby the treatment reduces the likelihood that the patient will develop the disease or condition. The term "therapeutic treatment" refers to administering treatment to a subject already suffering from a disease or condition. As used herein, a "therapeutic effect" relieves, to some extent, one or more of the symptoms of a disease or disorder. For example, a therapeutic effect may be observed by a reduction of the subjective discomfort that is communicated by a subject (e.g., reduced discomfort noted in self-administered patient questionnaire).

[0050] As used herein, the term "prophylaxis" or "prevention" refers the preventive treatment of a subclinical disease-state in a subject, e.g., a mammal (including a human), for reducing the probability of the occurrence of a clinical disease-state. The subject is selected for preventative therapy based on factors that are known to increase risk of suffering a clinical disease state compared to the general population. "Prophylaxis" therapies can be divided into (a) primary prevention and (b) secondary prevention. Primary prevention is defined as treatment in a subject that has not yet presented with a clinical disease state, whereas secondary prevention is defined as preventing a second occurrence of the same or similar clinical disease state.

[0051] As used herein, the term "formulated" or "formulation" refers to the process in which different chemical substances, including one or more pharmaceutically active ingredients, are combined to produce a dosage form. In some embodiments, two or more pharmaceutically active ingredients can be co-formulated into a single dosage form or combined dosage unit, or formulated separately and subsequently combined into a combined dosage unit. A sustained release formulation is a formulation which is designed to slowly release a therapeutic agent in the body over an extended period of time, whereas an immediate release formulation is a formulation which is designed to quickly release a therapeutic agent in the body over a shortened period of time.

[0052] As used herein, the term "hydrate" refers to a complex formed by combination of water molecules with molecules or ions of the solute. As used herein, the term "solvate" refers to a complex formed by combination of solvent molecules with molecules or ions of the solute. The solvent can be an organic compound, an inorganic compound, or a mixture of both. Solvate is meant to include hydrate, hemi-hydrate, channel hydrate etc. Some examples of solvents include, but are not limited to, methanol, *N,N*-dimethylformamide, tetrahydrofuran, dimethylsulfoxide, and water.

Diseases

[0053] The methods, compositions and kits disclosed herein can be used to treat, to prevent, to delay the onset, and/or to slow down the progress of proliferative diseases such as cancer. Also provided are methods, compositions and kits for alleviating, preventing, and/or to delaying the onset of one or more symptoms of the proliferative diseases.

[0054] As described herein, a proliferative disease can, for example, be a hyperproliferative disease. In some embodiments, the proliferative disease is cancer. Cancer is an abnormal growth of cells which tend to proliferate in an uncontrolled way and, in some cases, to metastasize (spread). Cancer can involve any tissue of the body and have many different forms in each body area. A tumor can be cancerous or benign. A benign tumor means the tumor can grow but does not spread. A cancerous tumor is malignant, meaning it can grow and spread to other parts of the body. If a cancer spreads (metastasizes), the new tumor bears the same name as the original (primary) tumor. In some embodiments, the methods, compositions and kits disclosed herein are used to treat, to prevent, to delay the onset, to slow down the progress, and/or to alleviate one or more symptoms of primary cancer and/or secondary cancer.

[0055] The methods, compositions and kits disclosed herein can be used to various types of cancer, including but are not limited to, melanoma (e.g., metastatic malignant melanoma), renal cancer (e.g., clear cell carcinoma), prostate cancer (e.g., hormone refractory prostate adenocarcinoma), pancreatic adenocarcinoma, breast cancer, colon cancer, lung cancer (e.g., non-small cell lung cancer (NSCLC) and small-cell lung cancer (SCLC)), esophageal cancer, squamous cell carcinoma of the head and neck, liver cancer, ovarian cancer, cervical cancer, thyroid cancer, glioblastoma, glioma, leukemia, lymphoma, and other neoplastic malignancies. Additionally, the disease or condition provided herein includes refractory or recurrent malignancies whose growth may be inhibited using the methods and compositions disclosed herein. In some embodiments, the cancer is carcinoma, squamous carcinoma, adenocarcinoma, sarcomata, endometrial cancer, breast cancer, ovarian cancer, cervical cancer, fallopian tube cancer, primary peritoneal cancer, colon cancer, colorectal cancer, squamous cell carcinoma of the anogenital region, melanoma, renal cell carcinoma, lung cancer, non-small cell lung cancer, squamous cell carcinoma of the lung, stomach cancer, bladder cancer, gall bladder cancer, liver cancer, thyroid cancer, laryngeal cancer, salivary gland cancer, esophageal cancer, head and neck cancer, glioblastoma, glioma, squamous cell carcinoma of the head and neck, prostate cancer, pancreatic cancer, mesothelioma, sarcoma, hematological cancer, leukemia, lymphoma, neuroma, or a combination thereof. In some embodiments, the cancer is carcinoma, squamous carcinoma (e.g., cervical canal, eyelid, tunica conjunctiva, vagina, lung, oral cavity, skin, urinary bladder, tongue, larynx, and gullet), and adenocarcinoma (for example, prostate,

small intestine, endometrium, cervical canal, large intestine, lung, pancreas, gullet, rectum, uterus, stomach, mammary gland, and ovary). In some embodiments, the cancer is sarcomata (e.g., myogenic sarcoma), leukosis, neuroma, melanoma, and lymphoma. In some embodiments, the cancer is liver cancer, for example primary liver cancer and secondary liver cancer. Non-limiting examples of liver cancer include hepatocellular carcinoma (HCC), bile duct cancer, Angiosarcoma, hemangiosarcoma, hepatoblastoma, hemangioma, hepatic adenoma, focal nodular hyperplasia, and any combination thereof.

[0056] The cancer can be a solid tumor, a liquid tumor, or a combination thereof. In some embodiments, the cancer is a solid tumor, including but are not limited to, melanoma, renal cell carcinoma, lung cancer, bladder cancer, breast cancer, cervical cancer, colon cancer, gall bladder cancer, laryngeal cancer, liver cancer, thyroid cancer, stomach cancer, salivary gland cancer, prostate cancer, pancreatic cancer, Merkel cell carcinoma, brain and central nervous system cancers, and any combination thereof. In some embodiments, the cancer is a liquid tumor. In some embodiments, the cancer is a hematological cancer. Non-limiting examples of hematological cancer include Diffuse large B cell lymphoma (“DLBCL”), Hodgkin's lymphoma (“HL”), Non-Hodgkin's lymphoma (“NHL”), Follicular lymphoma (“FL”), acute myeloid leukemia (“AML”), and Multiple myeloma (“MM”).

[0057] Non-limiting examples of cancers that can be prevented and/or treated using the methods, compositions and kits disclosed herein include: renal cancer; kidney cancer; glioblastoma multiforme; metastatic breast cancer; breast carcinoma; breast sarcoma; neurofibroma; neurofibromatosis; pediatric tumors; neuroblastoma; malignant melanoma; carcinomas of the epidermis; leukemias such as but not limited to, acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemias such as myeloblastic, promyelocytic, myelomonocytic, monocytic, erythroleukemia leukemias and myelodysplastic syndrome, chronic leukemias such as but not limited to, chronic myelocytic (granulocytic) leukemia, chronic lymphocytic leukemia, hairy cell leukemia; polycythemia vera; lymphomas such as but not limited to Hodgkin's disease, non-Hodgkin's disease; multiple myelomas such as but not limited to smoldering multiple myeloma, nonsecretory myeloma, osteosclerotic myeloma, plasma cell leukemia, solitary plasmacytoma and extramedullary plasmacytoma; Waldenstrom's macroglobulinemia; monoclonal gammopathy of undetermined significance; benign monoclonal gammopathy; heavy chain disease; bone cancer and connective tissue sarcomas such as but not limited to bone sarcoma, myeloma bone disease, multiple myeloma, cholesteatoma-induced bone osteosarcoma, Paget's disease of bone, osteosarcoma, chondrosarcoma, Ewing's sarcoma, malignant giant cell tumor, fibrosarcoma of bone, chordoma, periosteal sarcoma, soft-tissue sarcomas, angiosarcoma (hemangiosarcoma), fibrosarcoma, Kaposi's sarcoma, leiomyosarcoma,

liposarcoma, lymphangio sarcoma, neurilemmoma, rhabdomyosarcoma, and synovial sarcoma; brain tumors such as but not limited to, glioma, astrocytoma, brain stem glioma, ependymoma, oligodendroglioma, nonglial tumor, acoustic neurinoma, craniopharyngioma, medulloblastoma, meningioma, pineocytoma, pineoblastoma, and primary brain lymphoma; breast cancer including but not limited to adenocarcinoma, lobular (small cell) carcinoma, intraductal carcinoma, medullary breast cancer, mucinous breast cancer, tubular breast cancer, papillary breast cancer, Paget's disease (including juvenile Paget's disease) and inflammatory breast cancer; adrenal cancer such as but not limited to pheochromocytom and adrenocortical carcinoma; thyroid cancer such as but not limited to papillary or follicular thyroid cancer, medullary thyroid cancer and anaplastic thyroid cancer; pancreatic cancer such as but not limited to, insulinoma, gastrinoma, glucagonoma, vipoma, somatostatin-secreting tumor, and carcinoid or islet cell tumor; pituitary cancers such as but limited to Cushing's disease, prolactin-secreting tumor, acromegaly, and diabetes insipidus; eye cancers such as but not limited to ocular melanoma such as iris melanoma, choroidal melanoma, and ciliary body melanoma, and retinoblastoma; vaginal cancers such as squamous cell carcinoma, adenocarcinoma, and melanoma; vulvar cancer such as squamous cell carcinoma, melanoma, adenocarcinoma, basal cell carcinoma, sarcoma, and Paget's disease; cervical cancers such as but not limited to, squamous cell carcinoma, and adenocarcinoma; uterine cancers such as but not limited to endometrial carcinoma and uterine sarcoma; ovarian cancers such as but not limited to, ovarian epithelial carcinoma, borderline tumor, germ cell tumor, and stromal tumor; cervical carcinoma; esophageal cancers such as but not limited to, squamous cancer, adenocarcinoma, adenoid cystic carcinoma, mucoepidermoid carcinoma, adenosquamous carcinoma, sarcoma, melanoma, plasmacytoma, verrucous carcinoma, and oat cell (small cell) carcinoma; stomach cancers such as but not limited to, adenocarcinoma, fungating (polypoid), ulcerating, superficial spreading, diffusely spreading, malignant lymphoma, liposarcoma, fibrosarcoma, and carcinosarcoma; colon cancers; colorectal cancer, KRAS mutated colorectal cancer; colon carcinoma; rectal cancers; liver cancers such as but not limited to hepatocellular carcinoma and hepatoblastoma, gallbladder cancers such as adenocarcinoma; cholangiocarcinomas such as but not limited to papillary, nodular, and diffuse; lung cancers such as KRAS-mutated non-small cell lung cancer, non-small cell lung cancer, squamous cell carcinoma (epidermoid carcinoma), adenocarcinoma, large-cell carcinoma and small-cell lung cancer; lung carcinoma; testicular cancers such as but not limited to germinal tumor, seminoma, anaplastic, classic (typical), spermatocytic, nonseminoma, embryonal carcinoma, teratoma carcinoma, choriocarcinoma (yolk-sac tumor), prostate cancers such as but not limited to, androgen-independent prostate cancer, androgen-dependent prostate cancer, adenocarcinoma, leiomyosarcoma, and rhabdomyosarcoma; penal

cancers; oral cancers such as but not limited to squamous cell carcinoma; basal cancers; salivary gland cancers such as but not limited to adenocarcinoma, mucoepidermoid carcinoma, and adenoid cystic carcinoma; pharynx cancers such as but not limited to squamous cell cancer, and verrucous; skin cancers such as but not limited to, basal cell carcinoma, squamous cell carcinoma and melanoma, superficial spreading melanoma, nodular melanoma, lentigo malignant melanoma, acral lentiginous melanoma; kidney cancers such as but not limited to renal cell cancer, adenocarcinoma, hypernephroma, fibrosarcoma, transitional cell cancer (renal pelvis and/or uterine); renal carcinoma; Wilms' tumor; and bladder cancers such as but not limited to transitional cell carcinoma, squamous cell cancer, adenocarcinoma, carcinosarcoma. In some embodiments, the cancer is myxosarcoma, osteogenic sarcoma, endotheliosarcoma, lymphangioendotheliosarcoma, mesothelioma, synovioma, hemangioblastoma, epithelial carcinoma, cystadenocarcinoma, bronchogenic carcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, or papillary adenocarcinomas.

[0058] The methods, compositions and kits disclosed herein can be used to alleviate, prevent, or delay the onset of, one or more symptoms of proliferative diseases such as cancer. The symptom can be, for example, fibrosis. Fibrosis is a pathological condition in which excess accumulation of fibrous connective tissue occurs. Cancer-associated fibrosis is an important regulator of cancer, for example in the tumor microenvironment (TME). In some embodiments, the methods, compositions and kits disclosed herein are used to alleviate or prevent chronic inflammation-related fibrosis (either from infectious or autoimmune etiologies) associated with cancers, including but not limited to, hepatocellular cancer, gastric cancer, esophageal cancer, head and neck cancer, colon cancer, pancreatic cancer, cervix cancer, breast cancer, prostate cancer, and vulvar cancer. In some embodiments, the methods, compositions and kits are used to alleviate or prevent fibrosis affecting, for example, the heart, liver, lung, muscle (e.g., skeletal muscle), kidney, eyes, blood vessel, skin, brain, bone marrow, gastrointestinal tract, peritoneum, and vasculature. In some embodiments, the methods, compositions and kits are used to alleviate or prevent fibrosis caused by inflammation associated cancer. In some embodiments, the methods, compositions and kits are used to alleviate or prevent fibrosis caused by medical procedures (e.g., surgeries, chemotherapies, immunotherapies) for treating cancer. Fibrosis includes, but is not limited to, pulmonary fibrosis, liver fibrosis, myelofibrosis, skin fibrosis (e.g., nephrogenic systemic fibrosis and keloid fibrosis), mediastinal fibrosis, cardiac fibrosis, kidney fibrosis, stromal fibrosis, epidural fibrosis, epithelial fibrosis, idiopathic fibrosis, cirrhosis, and any combination thereof.

Methods

[0059] Disclosed herein include methods, compositions and kits for treating, preventing, delaying the onset, and/or slowing down the progress of proliferative diseases such as cancer, in subjects in need thereof. Also disclosed are methods, compositions and kits that can be used to alleviate, to prevent, and/or to delay the onset of one or more symptoms of the proliferative diseases in subjects in need thereof. The cancer is, in some embodiments, liver cancer.

[0060] In some embodiments, the method comprises identifying a subject having a proliferative disease. In some embodiments, the method comprises identifying a subject at a risk of developing a proliferative disease. The kit can comprise instructions for identifying a subject having a proliferative disease, instructions for identifying a subject at a risk of developing a proliferative disease, or both.

[0061] The method can, for example, comprise: administering to a subject in need thereof a composition comprising a cyclosporine analogue (e.g., CRV431), or a pharmaceutically acceptable salt, solvate, stereoisomer thereof. In some embodiments, the subject in need thereof is a subject at a risk of developing a proliferative disease. In some embodiments, the subject in need thereof is a subject suffering from a proliferative disease. In some embodiments, the subject in need thereof is a subject having one or more symptoms of a proliferative disease, for example fibrosis. In some embodiments, the subject in need thereof is a subject in complete or partial remission of a proliferative disease. The proliferative disease (e.g., cancer) can be prevented from occurring, delayed for onset, or slowed down in disease progression. In some embodiments, the subject in need thereof is a subject in complete remission of liver cancer. In some embodiments, the subject in need thereof is a subject in incomplete remission of liver cancer.

[0062] The methods, compositions, and kits can, for example, prevent, slow down, or reduce the progression of cancer. For example, the weight and/or size of the tumor can be reduced. The method can comprise measuring weight, size and/or morphology of the tumor to determine the responsiveness and/or efficacy of the tumor to the treatment.

[0063] The methods, compositions and kits disclosed herein can be used to alleviate, prevent, or delay the onset of, one or more symptoms of the proliferative disease, for example fibrosis. In some embodiments, the fibrosis is prevented from occurring. In some embodiments, fibrosis formation is prohibited in the subject. In some embodiments, the onset of fibrosis is delayed. The delay can be, for example, one or more seconds, minutes, hours, days, weeks, months, or years. In some embodiments, the delay in the treated subject is relative to the same subject had he/she received no treatment. In some embodiments, the delay in the treated subject

is relative to untreated subjects. In some embodiments, the onset of fibrosis is delayed by, or by about, 5, 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, 150, 200, 250, 300, 350, or a range between any of these values, days. In some embodiments, the onset of fibrosis is delayed by, or by about, one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, or a range between any of these values, months or years. In some embodiments, the onset of fibrosis is delayed by at least, or at least about, one, two, three, four, five, six, seven, eight, nine, ten, months or years. In some embodiments, the onset of fibrosis is delayed by at least, or at least about, one, two, three, four, five, six, seven, eight, nine, ten, or more hours. The fibrosis can be, for example, fibrosis associated with a liver cancer.

[0064] In some embodiments, fibrosis (e.g., tissue fibrosis) is reversed in the subject. The reverse can be, or be about, 1%, 2%, 5%, 8%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or a range between any two of these values, of the existing fibrosis in the subject. In some embodiments, the reverse can be at least, or be at least about, 1%, 2%, 5%, 8%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more of the existing fibrosis in the subject.

[0065] In some embodiments, the amount of fibrosis (e.g., tissue fibrosis) is reduced in the subject. The reduction in the amount of fibrosis can be, or be about, 1%, 2%, 5%, 8%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or a range between any two of these values, in the subject. In some embodiments, the reduction in the amount of fibrosis can be at least, or be at least about, 1%, 2%, 5%, 8%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more in the subject.

[0066] In some embodiments, formation of fibrosis (e.g., tissue fibrosis) is reduced in the subject. The reduction in fibrosis formation can be, or be about, 1%, 2%, 5%, 8%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or a range between any two of these values, in the subject. In some embodiments, the reduction in fibrosis formation can be at least, or be at least about, 1%, 2%, 5%, 8%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more in the subject. The fibrosis is, in some embodiments, non-liver fibrosis. The reduction in fibrosis formation the treated subject can be relative to the same subject had he/she received no treatment. In some embodiments, the reduction in fibrosis formation in the treated subject is relative to untreated subjects.

[0067] Therapeutic effectiveness of one or more of the cyclosporine analog (e.g., CRV431) or a pharmaceutically acceptable salt, solvate, stereoisomer thereof, disclosed herein, in alleviating, preventing or delaying the onset of, fibrosis (a symptom of the proliferative disease) can be determined using methods known for measuring the amount of fibrosis (e.g., fibrosis in affected organ(s), tissue(s) or area(s)) in a subject. The subject can be, for example, a

patient suffering from fibrosis or a patient recently suffered from fibrosis. The amount of fibrosis in the subject can be determined by methods known by one of skill in the art for determining the amount of fibrosis. For example, and without limitation, the amount of fibrosis can be determined by taking a muscle biopsy from the subject, sectioning the muscle onto slides and assessing the amount of fibrosis as revealed by staining techniques known in the art (e.g., Hematoxylin and Eosin (H&E) staining and/or Masson's trichrome staining). As another example, the amount of fibrosis can be determined *in vivo* by using magnetic resonance imaging (MRI).

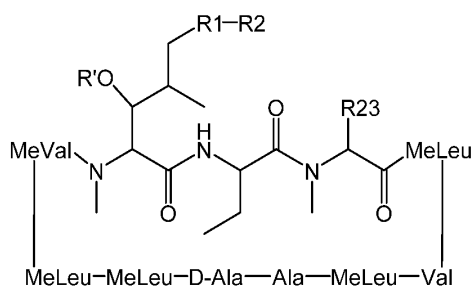
[0068] An exemplary therapeutic endpoint that can be achieved by the compositions, methods or kits disclosed herein can be a reduction in the amount of fibrosis in a subject being administered with one or more the cyclosporine analogs (e.g., CRV431) disclosed herein or a pharmaceutically acceptable salt, solvate, stereoisomer thereof, and, optionally one or more additional therapeutic agents (e.g., antifibrotic agents). Relative amounts of fibrosis in the subject can be quantitated, for example, by tissue biopsy and subsequent histology, including but not limited by, by quantifying Evans blue dye uptake as a measure of myofiber or cellular damage (described for example in Heydemann et al., *Neuromuscular Disorders* 15(9-10): 601-9 (2005), quantitation of hydroxyproline content as described in Swaggart et al., *Physiol Genomics* 43: 24-31 (2011), or both. In some embodiments, the amount of fibrosis in the subject being administered with one or more the cyclosporine analogs (e.g., CRV431) disclosed herein, or a pharmaceutically acceptable salt, solvate, stereoisomer thereof and, optionally one or more additional therapeutic agents (e.g., anticancer agents), is reduced by, or reduced by about, 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 100%, or any value within 1% to 100%, or a range between any two of these values, as compared to a patient not so treated. In some embodiments, the amount of fibrosis in the subject being administered with one or more the cyclosporine analogs (e.g., CRV431) disclosed herein, or a pharmaceutically acceptable salt, solvate, stereoisomer thereof and, optionally one or more additional therapeutic agents (e.g., anticancer agents), is reduced by at least, or reduced by at least about, 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%, as compared to a patient not so treated.

Therapeutic Agents

[0069] Anticancer agents, including cyclosporine analogues (e.g., CRV431) disclosed herein, or a pharmaceutically acceptable salt, solvate, stereoisomer thereof, can be used to treat proliferative diseases (e.g., cancer), or for primary prophylaxis or secondary

prophylaxis of proliferative diseases (e.g., cancer) in a subject. The cyclosporine analogues, or a pharmaceutically acceptable salt, solvate, stereoisomer thereof, can, for example, delay the onset of proliferative diseases (e.g., cancer), in a subject (e.g., a subject at a risk of developing the proliferative disease). The cyclosporine analogues, or a pharmaceutically acceptable salt, solvate, stereoisomer thereof, can, for example, treat or prevent cancer, in a subject. The cancer is, in some embodiments, liver cancer.

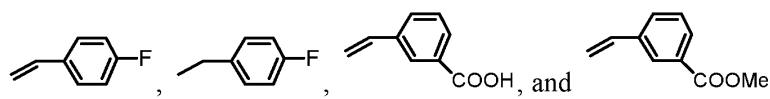
[0070] In some embodiments, a cyclosporine analogue is a compound of Formula L:



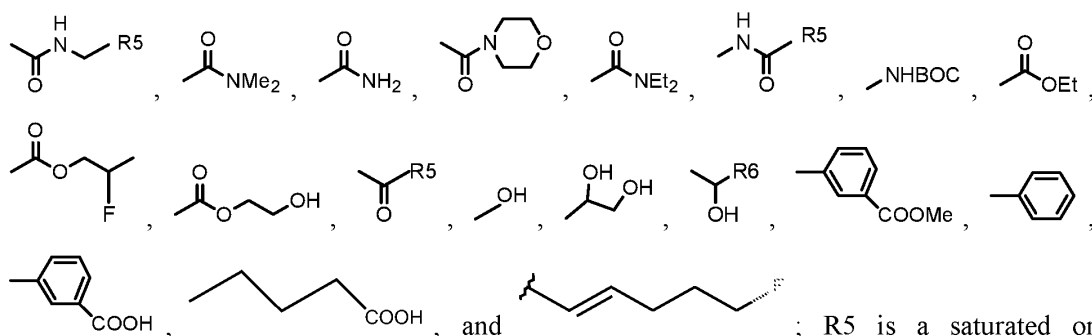
Formula L

wherein:

- a. R' is H or acetyl;
- b. R1 is a saturated or unsaturated straight or branched aliphatic carbon chain from 2 to 15 carbon atoms in length;
- c. R2 is selected from the group consisting of:
 - i. H;
 - ii. an unsubstituted, N-substituted, or N,N-disubstituted amide;
 - iii. a N-substituted or unsubstituted acyl protected amine;
 - iv. a N-substituted or unsubstituted amine;
 - v. a carboxylic acid;
 - vi. a nitrile;
 - vii. an ester;
 - viii. a ketone;
 - ix. a hydroxy, dihydroxy, trihydroxy, or polyhydroxy alkyl; and
 - x. a substituted or unsubstituted aryl;
 - xi. a saturated or unsaturated straight or branched aliphatic chain optionally containing a substituent selected from the group consisting of a hydrogen, a ketone, a hydroxyl, a nitrile, a carboxylic acid, an ester, a 1,3-dioxolane, a halogen, and an oxo;
 - xii. an aromatic group containing a substituent selected from the group consisting of a halogen, an ester, and a nitro; and

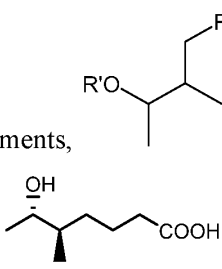
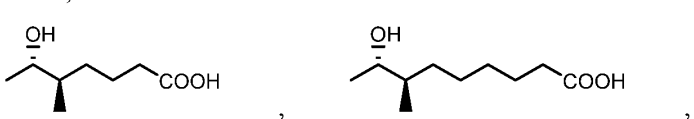
. In some embodiments, R1-R2 comprises a saturated or unsaturated, straight or branched aliphatic chain of between 2 and 5 carbons optionally substituted with a substituent selected from the group consisting of a hydrogen, a ketone, a hydroxyl, a nitrile, a halogen, an oxo, a carboxylic acid, an ester, and an 1,3-dioxolane.

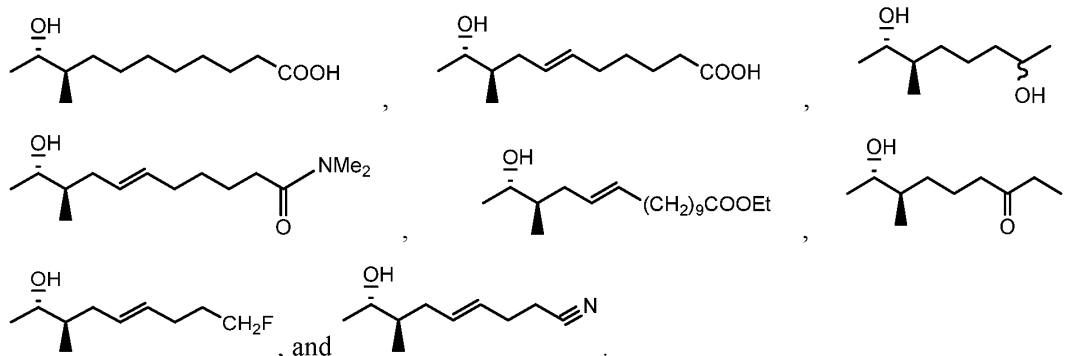
[0072] In some embodiments, R2 is selected from the group consisting of



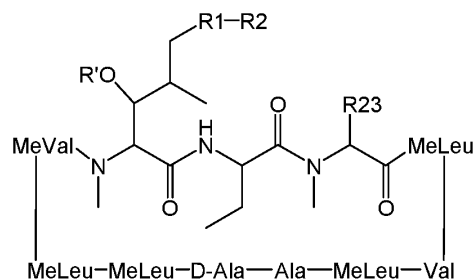
; R5 is a saturated or unsaturated straight or branched aliphatic carbon chain between 1 and 10 carbons in length; and R6 is a monohydroxylated, dihydroxylated, trihydroxylated or polyhydroxylated saturated or unsaturated straight chain or branched aliphatic carbon chain between 1 and 10 carbons in length.

[0073] In some embodiments, R23 is selected from the group consisting of: $-\text{CH}_3$, $-\text{CH}_2\text{CH}_3$, $-\text{CH}_2\text{CHCH}_2$, $-\text{CH}_2\text{CH}_2\text{CH}_2\text{I}$, $-(\text{CH}_2)_3\text{CH}_2\text{I}$, $-(\text{CH}_2)_3\text{N}^+(\text{CH}_3)_3$, $-\text{CH}_2\text{CCH}$, $-\text{CH}_2\text{CO}_2(\text{t-Bu})$, $-\text{CH}_2\text{Ph}$, $-\text{CH}_2\text{OH}$, $-\text{CH}(\text{OH})\text{CH}_3$, $-\text{CH}(\text{OH})(\text{t-Bu})$, $-\text{CH}(\text{OH})\text{Ph}$, $-\text{COOH}$, $-\text{SCH}_3$, and $-\text{S}(\text{p-Tol})$. In some embodiments, R23 comprises an optionally substituted alkyl, including optionally substituted C1-C3 alkyl. The alkyl can be substituted with amino and may comprise a C1-C3-Ala, wherein the compound comprises the D-epimer of amino acid 3 which is the amino acid to which R23 is attached. In some embodiments, R23 can be MeAla. In some embodiments, R23 is a straight or branched aliphatic carbon chain of 1 to 6, 1 to 5, 1 to 4, 1 to 3 or 2 carbons in length.

[0074] In some embodiments,  in Formula L is selected from the group consisting of: 



[0075] In some embodiments, a cyclosporine analogue is a compound of Formula L:

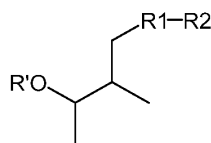


Formula L

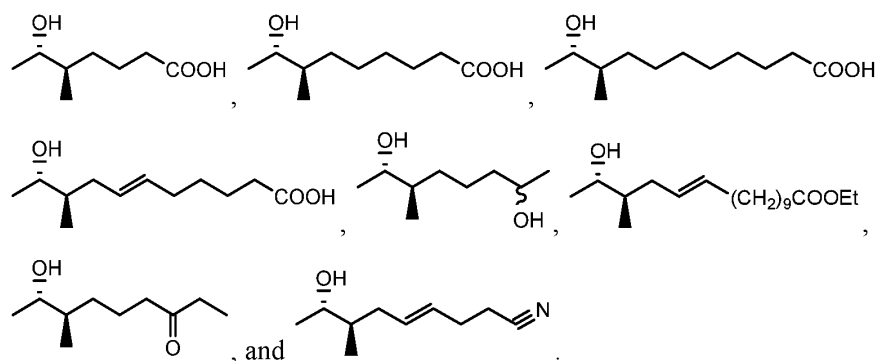
wherein:

- a. R' is H or acetyl;
- b. R1 is a saturated or unsaturated straight or branched aliphatic carbon chain from 2 to 15 carbon atoms in length;
- c. R2 is selected from the group consisting of:
 - i. an unsubstituted, N-substituted, or N,N-disubstituted amide;
 - ii. a carboxylic acid;
 - iii. a nitrile;
 - iv. an ester;
 - v. a ketone;
 - vi. a hydroxy, dihydroxy, trihydroxy, or polyhydroxy alkyl;
 - vii. a substituted or unsubstituted aryl;
 - viii. a saturated or unsaturated straight or branched aliphatic carbon chain substituted with a substituent selected from the group consisting of a ketone, a hydroxy, a nitrile, a carboxylic acid, an ester, a 1,3-dioxolane, and an oxo;
 - ix. an aromatic group substituted with a substituent selected from the group consisting of a halogen, an ester, and a nitro; and
 - x. a combination of the saturated or unsaturated straight or branched aliphatic carbon chain of viii) and the aromatic group of ix); and

substituted with a substituent selected from the group consisting of a ketone, a hydroxy, a nitrile, an oxo, a carboxylic acid, an ester, and a 1,3-dioxolane. In some embodiments, R1-R2 is at least 6 carbon atoms in length.



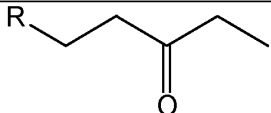
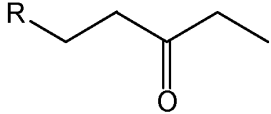
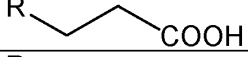
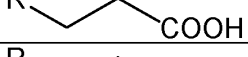
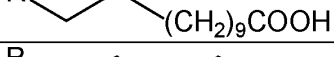
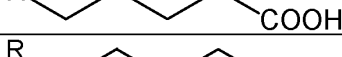
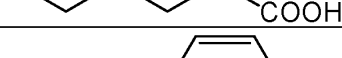
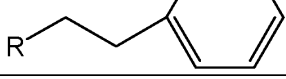
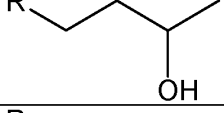
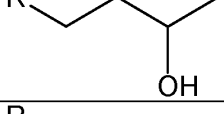
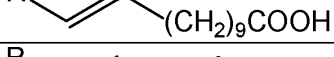
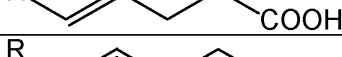
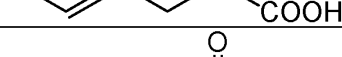
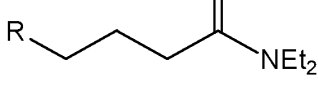
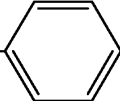
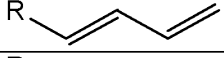
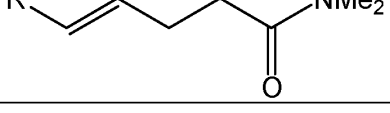
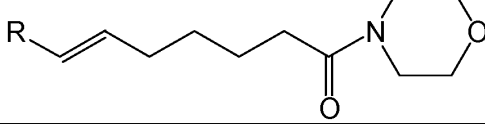
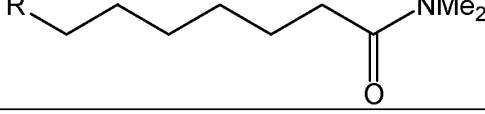
[0080] In some embodiments, in Formula L is selected from the group consisting of:



[0081] In some embodiments, R23 is selected from the group consisting of: $-\text{CH}_3$ and $-\text{CH}_2\text{CH}_3$. In some embodiments, R23 is methyl. In some embodiments, the compound comprises the D-epimer of amino acid 3 which is the amino acid to which R23 is attached.

[0082] In some embodiments, a cyclosporine analogue is a compound selected from the group consisting of:

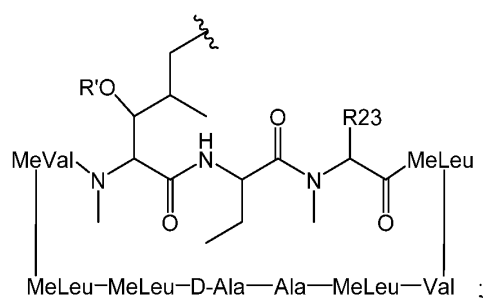
		R23	Isomer
a)		$-\text{CH}_3$	L
b)		$-\text{CH}_3$	D
c)		$-\text{CH}_3$	L
d)		$-\text{CH}_2\text{CH}_3$	D
e)		$-\text{CH}_3$	D
f)		$-\text{CH}_3$	L
g)		$-\text{CH}_2\text{CH}_3$	D
h)		$-\text{CH}_3$	D
i)		$-\text{CH}_2\text{CH}_3$	L
j)		$-\text{CH}_2\text{CH}_3$	D

k)		-CH ₃	L
l)		-CH ₃	D
m)		-CH ₃	D
n)		-CH ₂ CH ₃	D
o)		-CH ₃	D
r)		-CH ₃	D
s)		-CH ₃	L
t)		-CH ₃	D
u)		-CH ₃	D
v)		-CH ₃	L
w)		-CH ₂ CH ₃	L
x)		-SCH ₃	D/L
z)		-(CH ₂) ₃ N ⁺ (CH ₃) ₃	D/L
aa)		-CH ₂ - 	D
bb)		-CH ₃	D
cc)		-CH ₃	L
dd)		-CH ₃	L
ee)		-CH ₃	D

ff)		-CH ₂ CH ₃	D
gg)		-CH ₃	D
hh)		-CH ₃	D
ii)		-CH ₂ CH ₃	D
jj)		-CH ₃	D

wherein:

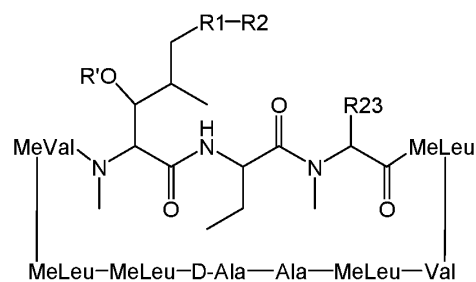
R is



R' is H or acetyl; and

the isomer is the isomeric form of amino acid 3 which is the amino acid to which R23 is attached.

[0083] In some embodiments, a cyclosporine analogue is a compound of Formula L:



Formula L

wherein:

- a. R' is H or acetyl;
- b. R1 is a saturated or unsaturated straight or branched aliphatic carbon chain from 2 to 15 carbon atoms in length;
- c. R2 is selected from the group consisting of:
 - i. an unsubstituted, N-substituted, or N,N-disubstituted amide;
 - ii. a carboxylic acid;

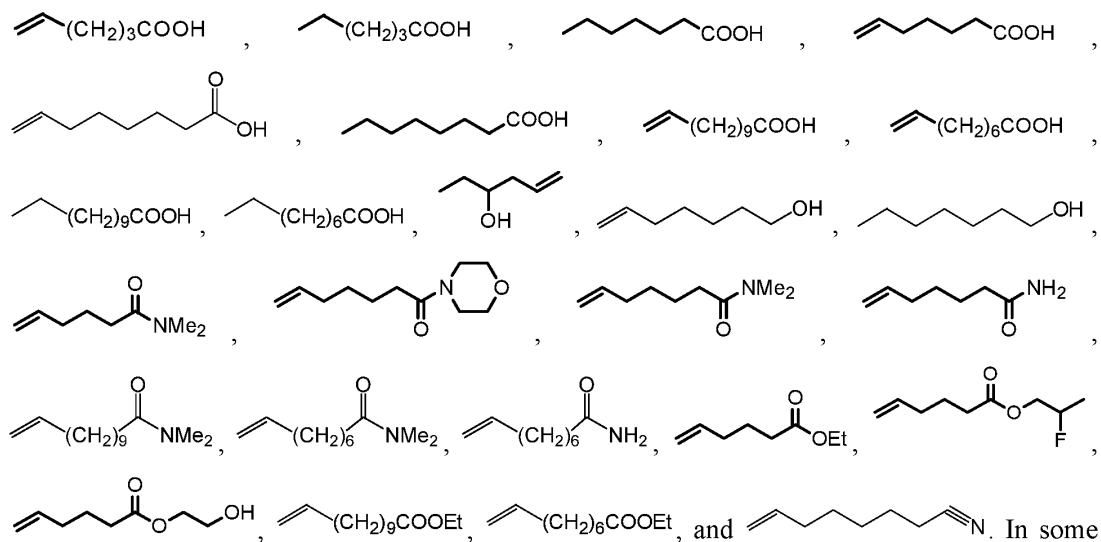
- iii. a nitrile;
 - iv. an ester;
 - v. a ketone;
 - vi. a hydroxy, dihydroxy, trihydroxy, or polyhydroxy alkyl;
 - vii. a substituted or unsubstituted aryl;
 - viii. a saturated or unsaturated straight or branched aliphatic carbon chain substituted with a substituent selected from the group consisting of a ketone, a hydroxy, a nitrile, a carboxylic acid, an ester, a 1,3-dioxolane, and an oxo;
 - ix. an aromatic group substituted with a substituent selected from the group consisting of a halogen, an ester, and a nitro; and
 - x. a combination of the saturated or unsaturated straight or branched aliphatic carbon chain of (viii) and the aromatic group of (ix); and
- d. R23 is a saturated or unsaturated straight or branched optionally substituted aliphatic carbon chain,

wherein R1-R2 is at least 6 carbon atoms in length.

[0084] In some embodiments, R' is H.

[0085] In some embodiments, R1 is a saturated or unsaturated straight or branched aliphatic carbon chain from 5 to 8 carbon atoms in length.

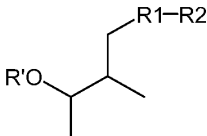
[0086] In some embodiments, R1-R2 is selected from the group consisting of:

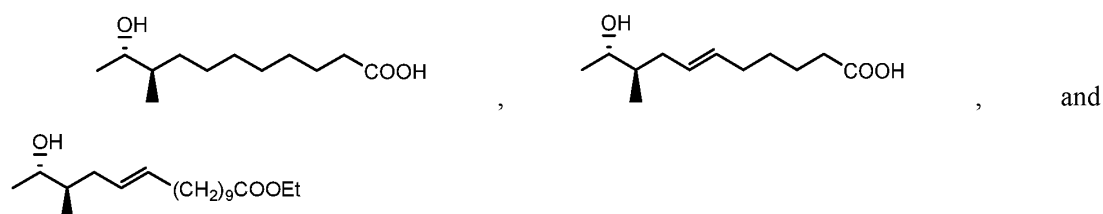


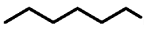
In some embodiments, R1-R2 is substituted with a substituent selected from the group consisting of a ketone, a hydroxy, a nitrile, an oxo, a carboxylic acid, an ester, and a 1,3-dioxolane.

[0087] In some embodiments, R23 is selected from the group consisting of: $-\text{CH}_3$, $-\text{CH}_2\text{CH}_3$, $-\text{CH}_2\text{CHCH}_3$, $-\text{CH}_2\text{CH}_2\text{CH}_2\text{I}$, $-(\text{CH}_2)_3\text{CH}_2\text{I}$, $-(\text{CH}_2)_3\text{N}^+(\text{CH}_3)_3$,

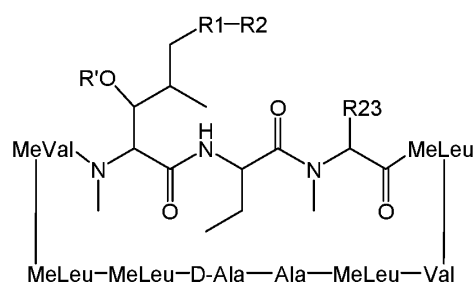
$-\text{CH}_2\text{CCH}$, $-\text{CH}_2\text{CO}_2(\text{t-Bu})$, $-\text{CH}_2\text{Ph}$, $-\text{CH}_2\text{OH}$, $-\text{CH}(\text{OH})\text{CH}_3$, $-\text{CH}(\text{OH})(\text{t-Bu})$, $-\text{CH}(\text{OH})\text{Ph}$, $-\text{COOH}$, $-\text{SCH}_3$, and $-\text{S}(\text{p-Tol})$. In some embodiments, R23 comprises an optionally substituted $\text{C}_1\text{-C}_3$ alkyl. In some embodiments, R23 is substituted with amino. In some embodiments, R23 is $\text{C}_1\text{-C}_3$ alkyl and the compound comprises the D-epimer of amino acid 3 which is the amino acid to which R23 is attached. In some embodiments, R23 is methyl. In some embodiments, R23 is a straight or branched aliphatic carbon chain of 1 to 6 carbons in length.

[0088] In some embodiments,  in Formula L is selected from the group consisting of:



[0089] In some embodiments, R1-R2 is , R23 is methyl, and the compound is a D-epimer of amino acid 3 which is the amino acid to which R23 is attached.

[0090] In some embodiments, a cyclosporine analogue is a compound of Formula L:



Formula L

wherein:

R' is H or acetyl;

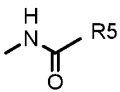
R1 is a saturated or unsaturated straight chain or branched aliphatic carbon chain from 2 to 15 carbon atoms in length;

R2 is a N-substituted or unsubstituted acyl protected amine; and

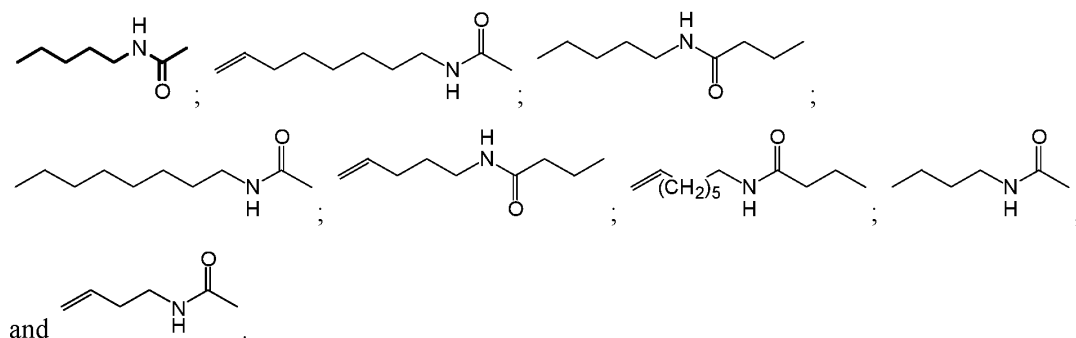
R23 is methyl or ethyl.

[0091] In some embodiments, R' is H.

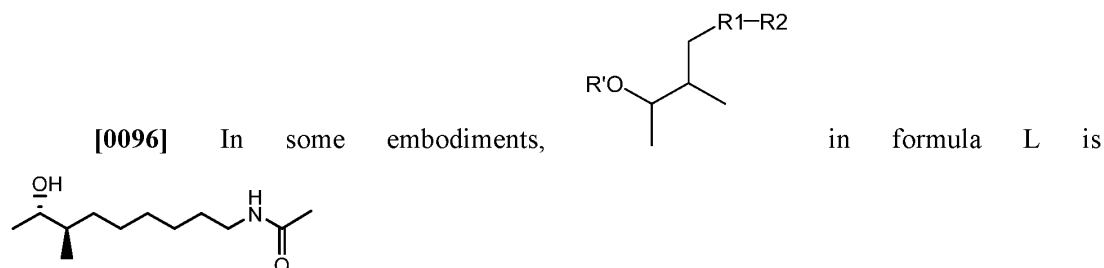
[0092] In some embodiments, R1 is a saturated or unsaturated straight or branched aliphatic carbon chain from 5 to 8 carbon atoms in length.

[0093] In some embodiments, R2 is ; wherein R5 is a saturated or unsaturated straight or branched aliphatic carbon chain between 1 and 10 carbons in length.

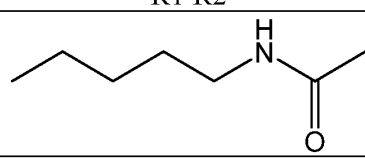
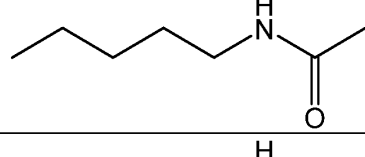
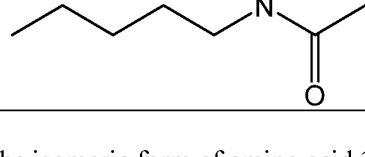
[0094] In some embodiments, R1-R2 is selected from the group consisting of:



[0095] In some embodiments, R23 is methyl.

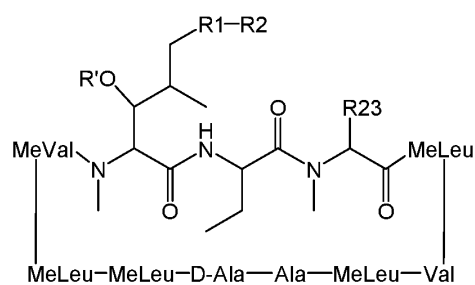


[0097] In some embodiments, R', R1-R2, and R23 and the isomer of said compound are selected from the following:

R'	R1-R2	R23	Isomer
H		-CH ₃	D
H		-CH ₂ CH ₃	L
H		-CH ₂ CH ₃	D

wherein the isomer is the isomeric form of amino acid 3 which is the amino acid to which R23 is attached.

[0098] In some embodiments, a cyclosporine analogue is a compound of Formula L:

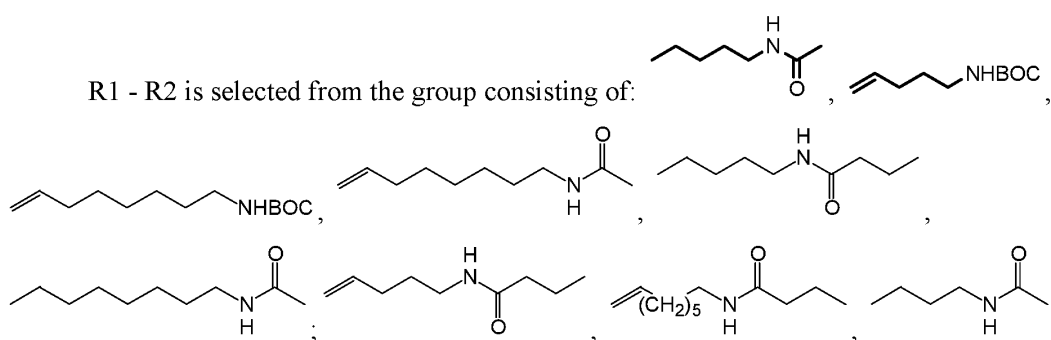


Formula L

wherein:

R' is H or acetyl;

R1 - R2 is selected from the group consisting of:



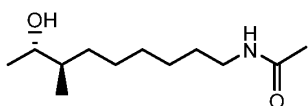
and and

R23 is a saturated or unsaturated straight chain or branched optionally substituted aliphatic carbon chain.

[0099] In some embodiments, R' is H.

[0100] In some embodiments, R1-R2 is .

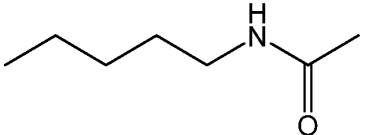
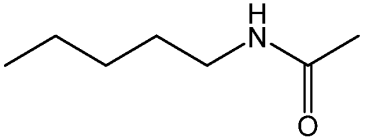
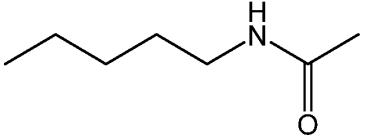
[0101] In some embodiments, in Formula L is



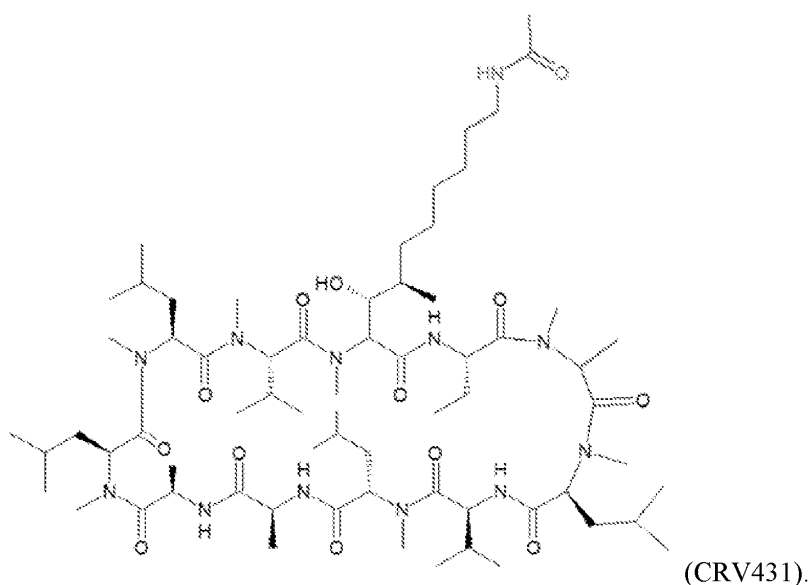
[0102] In some embodiments, R23 is selected from the group consisting of: $-\text{CH}_3$, $-\text{CH}_2\text{CH}_3$, $-\text{CH}_2\text{CHCH}_2$, $-\text{CH}_2\text{CH}_2\text{CH}_2\text{I}$, $-(\text{CH}_2)_3\text{CH}_2\text{I}$, $-(\text{CH}_2)_3\text{N}^+(\text{CH}_3)_3$, $-\text{CH}_2\text{CCH}$, $-\text{CH}_2\text{CO}_2(\text{t-Bu})$, $-\text{CH}_2\text{Ph}$, $-\text{CH}_2\text{OH}$, $-\text{CH}(\text{OH})\text{CH}_3$, $-\text{CH}(\text{OH})(\text{t-Bu})$, $-\text{CH}(\text{OH})\text{Ph}$, $-\text{COOH}$, $-\text{SCH}_3$, and $-\text{S}(\text{p-Tol})$. In some embodiments, R23 is $-\text{CH}_3$ or

$-\text{CH}_2\text{CH}_3$. In some embodiments, R23 is $-\text{CH}_3$. In some embodiments, R23 (a) comprises an optionally substituted C1-C3 alkyl; (b) is substituted with an amino; (c) is a C1-C3-Ala and said compound comprises the D-epimer; (d) is MeAla; and/or (e) is a straight or branched aliphatic carbon chain of 1 to 6, 1 to 5, 1 to 4, 1 to 3 or 2 carbons in length.

[0103] In some embodiments, R', R1-R2 and R23 and the isomer of said compound are selected from the following:

R'	R1-R2	R23	Isomer
H		$-\text{CH}_3$	D
H		$-\text{CH}_2\text{CH}_3$	L
H		$-\text{CH}_2\text{CH}_3$	D

[0104] The cyclosporine analogue can be a small molecule cyclophilin inhibitor CRV431 (shown below) which is a derivative of cyclosporine A (CsA), a neutral cyclic peptide consisting of eleven amino acids, wherein amino acids 1 and 3 have been chemically modified.



[0105] CRV431 is a small molecule cyclophilin inhibitor under clinical development for the treatment of liver diseases including liver fibrosis and hepatocellular carcinoma. In preclinical studies, CRV431 shows anti-viral activity against a number of viruses including

hepatitis B, hepatitis C, and HIV and anti-fibrotic activity in the liver in a number of *in vivo* models. CRV431 can reduce liver fibrosis arising from non-alcoholic steatohepatitis (“NASH”) and hepatocellular carcinoma tumor burden in experimental models of NASH.

[0106] For example, as described herein, CRV431 can regulate oncogenes, and have anti-oncogenic activities. CRV431 can decrease production of the extracellular matrix (ECM) molecules, collagen and fibronectin, from fibroblastic cells derived from five cell types, including lung fibroblasts from a patient with idiopathic pulmonary fibrosis (“IPF”), cardiac fibroblasts, dermal (skin) fibroblasts, renal mesangial cells, and the LX2 hepatic stellate cell line. It is known that IPF is an aggressive fibrotic disease in tremendous need of new treatments. As described herein, CRV431 dose-dependently decreased procollagen and fibronectin secretion from all cell types with similar magnitude, as measured by enzyme-linked immunosorbent assay (ELISA). The extent of inhibition was similar whether or not the cells were stimulated with the profibrotic agent, transforming growth factor-beta (TGF β), consistent with direct effects on ECM synthesis. CRV431 dose-dependently decreased ECM production by up to 55% at clinically relevant concentrations, without causing any reduction in cell viability. As disclosed herein, CRV431 can be used to reduce ECM production by inhibiting cyclophilin B, and consistent with this observation, downregulation of cyclophilin B with small interfering RNA (siRNA) similarly decreased procollagen and fibronectin secretion.

[0107] Fibrotic scarring is a major pathological feature and driver of organ dysfunction in many diseases, including cancer. But very few treatments are available to attenuate the scarring. Most treatments attempt to reduce fibrosis by targeting the stimulation of fibroblastic cells, but these signaling events may vary by patient, type of fibrotic disease, or disease stage. Without being limited to any particular theory, it can be advantageous, in some embodiments, to use CRV431 for treating fibrosis associated with cancer (in liver as well as in organs other than liver) since the effects of CRV431 can be independent of the type of stimulatory signal.

[0108] The methods, compositions and kits can also be used to sensitize cancer cells to one or more anticancer agents, one or more cancer therapies, or both. The method can comprise contacting cancer cells with a composition comprising a cyclosporine analogue disclosed herein, or a pharmaceutically acceptable salt, solvate, stereoisomer thereof, thereby sensitizing the cancer cells to the one or more anticancer agents, the one or more cancer therapies, or both. Contacting cancer cells with the composition can occur *in vitro*, *ex vivo*, *in vivo*, or in any combination. In some embodiments, contacting cancer cells with the composition is in a subject’s body. In some embodiments, cancer cells are contacted with the composition in a cell culture. The subject can be a mammal, for example a human. The sensitization of the

cancer cells can increase the responsiveness of the cancer cells to the one or more anticancer agents, one or more cancer therapies, or both, by, or by about, 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or a range between any two of these values. The sensitization of the cancer cells can increase the responsiveness of the cancer cells to the one or more anticancer agents, one or more cancer therapies, or both, by at least, or by at least about, 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or a range between any two of these values. The increase of the responsiveness of the cancer cells is, in some embodiments, relative to the untreated cancer cells. The sensitization of the cancer cells can increase the responsiveness of the subject having the cancer cells to the one or more anticancer agents, one or more cancer therapies, or both, by, or by about, 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or a range between any two of these values. The sensitization of the cancer cells can increase the responsiveness of the subject having the cancer cells to the one or more anticancer agents, one or more cancer therapies, or both, by at least, or by at least about, 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or a range between any two of these values. The increase of the responsiveness of the subject having the cancer cells is, in some embodiments, relative to the subjects untreated with the composition.

[0109] The method can comprise determining sensitization of the cancer cells to the one or more anticancer agents, the one or more cancer therapies, or both, after being contacted with the composition. The method can comprise contacting the cancer cells with the one or more anticancer agents, the one or more cancer therapies, or both, concurrently and/or after being contacted with the composition. In some embodiments, contacting the cancer cells with the one or more anticancer agents, the one or more cancer therapies, or both, occurs in the body of a subject. The subject can be a mammal, for example human. The subject can be, for example, a subject that did not respond to, or is known to be resistant to, the one or more anticancer agents alone, the one or more cancer therapies alone, or both. The subject can be, for example, a subject that had prior treatment with the one or more anticancer agents, the one or more cancer therapies, or both. In some embodiments, the method comprises determining the response of the subject to the one or more anticancer agents, the one or more cancer therapies, or both.

[0110] The cancer cells can comprise, for example, cells of carcinoma, squamous carcinoma, adenocarcinoma, sarcomata, endometrial cancer, breast cancer, ovarian cancer, cervical cancer, fallopian tube cancer, primary peritoneal cancer, colon cancer, colorectal cancer, squamous cell carcinoma of the anogenital region, melanoma, renal cell carcinoma, lung cancer, non-small cell lung cancer, squamous cell carcinoma of the lung, stomach cancer,

bladder cancer, gall bladder cancer, liver cancer, thyroid cancer, laryngeal cancer, salivary gland cancer, esophageal cancer, head and neck cancer, glioblastoma, glioma, squamous cell carcinoma of the head and neck, prostate cancer, pancreatic cancer, mesothelioma, sarcoma, hematological cancer, leukemia, lymphoma, neuroma, multiple myeloma, or a combination thereof. In some embodiments, the cancer cells comprise cells of liver cancer. In some embodiments, the cancer cells comprise cells isolated from a patient suffering from liver cancer. In some embodiments, the cancer cells comprise cells of multiple myeloma.

[0111] Anticancer agents can be, for example, radiotherapeutic agents, anti-immunosuppressive agents, immunostimulatory agents, chemotherapeutic agents, or any combination thereof. For example, the anticancer agent can be an anti-PD-1 agent, an anti-PD-L1 agent, an anti-CTLA4 agent, an anti-TIM-3 agent, an anti-LAG-3 agent, a GITR (glucocorticoid-induced TNFR-related protein) stimulating agent, an anti-IDO agent, an anti-ICOS agent, an anti-OX40 agent, an anti-CSF1R agent, a chemokine signaling agent, a cytokine signal stimulating agent, or a combination thereof. Non-limiting examples of anticancer agent include bevacizumab, pembrolizumab, nivolumab, PDR001, REGN2810 (SAR-439684), BGB-A317, BI 754091, IBI308, INCSHR-1210, JNJ-63723283, JS-001, MEDI0680 (AMP-514), MGA-012, PF-06801591, REGN-2810, TSR-042, atezolizumab, avelumab, CX-072, durvalumab, FAZ053, LY3300054, PD-L1 millamolecule, atezolizumab, durvalumab, avelumab, LY3300054, aminoglutethimide, amsacrine, anastrozole, asparaginase, bcr, beta-hydroxy beta-methylbutyrate, bicalutamide, bleomycin, bortezomib, buserelin, busulfan, camptothecin, capecitabine, carfilzomib, carboplatin, carmustine, chlorambucil, cisplatin, cladribine, clodronate, colchicine, cyclophosphamide, cyproterone, cytarabine, dacarbazine, dactinomycin, daunorubicin, delanzomib, dienestrol, diethylstilbestrol, disulfiram, docetaxel, doxorubicin, epigallocatechin-3-gallate, epirubicin, epoxomicin, estradiol, estramustine, etoposide, exemestane, filgrastim, fludarabine, fludrocortisone, fluorouracil, fluoxymesterone, flutamide, gemcitabine, genistein, goserelin, hydroxyurea, idarubicin, ifosfamide, imatinib, interferon, irinotecan, ironotecan, letrozole, leucovorin, leuprolide, levamisole, lomustine, ixazomib, marizomib, mechlorethamine, medroxyprogesterone, megestrol, melphalan, mercaptopurine, mesna, methotrexate, mitomycin, mitotane, mitoxantrone, nilutamide, nocodazole, octreotide, oxaliplatin, paclitaxel, pamidronate, pentostatin, plicamycin, porfimer, procarbazine, raltitrexed, rituximab, streptozocin, suramin, tamoxifen, temozolomide, teniposide, testosterone, thioguanine, thiotepa, titanocene dichloride, topotecan, trastuzumab, tretinoin, vinblastine, vincristine, vindesine, vinorelbine, and a combination thereof. Non-limiting examples of cancer therapies include surgery, chemotherapy, radiotherapy, immunotherapy, and a combination thereof.

Combination Therapy

[0112] The methods, compositions and kits disclosed herein can be used in combination in any treatment methods, agents, or therapies to treat, prevent, inhibit or delay the onset, to slow down the progress of proliferative diseases, and/or to alleviate, prevent or delay the onset of one or more symptoms of proliferative diseases. The proliferative disease is cancer, in some embodiments. For example, the methods disclosed herein can comprise administering to the subject one or more cancer therapies or one or more additional therapeutic agents. Examples of the cancer therapies include, but are not limited to, surgery, chemotherapy, radiation therapy, hormonal therapy, immunotherapy, complementary or alternative therapy, and any combination thereof. In some embodiments, the cancer therapies or the additional therapeutic agents are part of the current standard of care for the respective cancer.

[0113] The additional therapeutic agents can comprises one or more chemotherapeutics, including but are not limited to, mitotic inhibitors, alkylating agents, anti-metabolites, intercalating antibiotics, growth factor inhibitors, cell cycle inhibitors, enzymes, topoisomerase inhibitors, biological response modifiers, anti-hormones, angiogenesis inhibitors, proteasome inhibitors, and anti-androgens. Non-limiting examples of the additional therapeutic agents include chemotherapeutic agents, cytotoxic agents, and non-peptide small molecules such as Gleevec® (Imatinib Mesylate), Kyprolis® (carfilzomib), Velcade® (bortezomib), Casodex (bicalutamide), Iressa® (gefitinib), venetoclax, and Adriamycin. Non-limiting examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclophosphamide (CYTOXANTM); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphoramide and trimethylolomelamine; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carabycin, carminomycin, carzinophilin, CasodexTM, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as

denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, androgens such as calusterone, dromostanolone propionate, epitio stanol, mepitio stanane, testolactone; anti-adrenals such as aminogluthethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diazi quone; elfomithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK; razoxane; sizofiran; spirogermanium; tenuazonic acid; triazi quone; 2,2',2"-trichlorotriethylamine; urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxanes, e.g. paclitaxel and docetaxel; retinoic acid; esperamicins; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

[0114] In some embodiments, the one or more additional agents comprise anti-hormonal agents capable of regulating or inhibiting hormone action on tumors such as anti-estrogens including for example tamoxifen, (NolvadexTM), raloxifene, aromatase inhibiting 4(5)-imidazoles, 4-hydroxytamoxifen, trioxifene, keoxifene, LY 117018, onapristone, and toremifene (Fareston); and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; xeloda; ibandronate; camptothecin-11 (CPT-11); topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO).

[0115] In some embodiment, the one or more additional therapeutic agents that can be administered to the subject receiving, has received, or will receive, the administration of the compositions disclosed herein comprise currently prescribed anti-cancer drugs such as Herceptin®, Avastin®, Erbitux®, Rituxan®, Taxol®, Arimidex®, Taxotere®, ABVD, AVICINE, Abagovomab, Acridine carboxamide, Adecatumumab, 17-N-Allylamino-17-demethoxygeldanamycin, Alfaradin, Alvocidib, 3-Aminopyridine-2-carboxaldehyde thiosemicarbazone, Amonafide, Anthracenedione, Anti-CD22 immunotoxins, Antineoplastic, Antitumorigenic herbs, Apazi quone, Atiprimod, Azathioprine, Belotecan, Bendamustine, BIBW 2992, Biricodar, Brostallicin, Bryostatin, Buthionine sulfoximine, CBV (chemotherapy), Calyculin, cell-cycle nonspecific antineoplastic agents, Dichloroacetic acid, Discodermolide, Elsamitrucin, Enocitabine, Epopthilone, Eribulin, Everolimus, Exatecan, Exisulind, Ferruginol,

Forodesine, Fosfestrol, ICE chemotherapy regimen, IT-101, Imexon, Imiquimod, Indolocarbazole, Irofulven, Laniquidar, Larotaxel, Lenalidomide, Lucanthone, Lurtotecan, Mafosfamide, Mitozolomide, Nafoxidine, Nedaplatin, Olaparib, Ortataxel, PAC-1, Pawpaw, Pixantrone, Proteasome inhibitor, Rebeccamycin, Resiquimod, Rubitecan, SN-38, Salinosporamide A, Sapacitabine, Stanford V, Swainsonine, Talaporfin, Tariquidar, Tegafur-uracil, Temodar, Tesetaxel, Triplatin tetranitrate, Tris(2-chloroethyl)amine, Troxacitabine, Uramustine, Vadimezan, Vinflunine, ZD6126, Zosuquidar, immunomodulatory agents, histone deacetylase (HDAC) inhibitors, antibodies, or a combination thereof.

[0116] The methods, compositions and kits disclosed herein can be, in some embodiments, used in combination with radiation therapy for inhibiting abnormal cell growth or treating the proliferative disease such as a hyperproliferative disorder. Non-limiting examples of radiation therapy include, but are not limited to, external-beam therapy, internal radiation therapy, implant radiation, stereotactic radiosurgery, systemic radiation therapy, radiotherapy and permanent or temporary interstitial brachytherapy.

[0117] In some embodiments, the methods, compositions or kits disclosed herein is used in combination with one or more of anti-angiogenesis agents, chemotherapeutic agents, anti-neoplastic agents, steroids, signal transduction inhibitors, antiproliferative agents, glycolysis inhibitors, and autophagy inhibitors. The anti-angiogenesis agents can be MMP-2 (matrix-metalloproteinase 2) inhibitors, MMP-9 (matrix-metalloproteinase 9) inhibitors, and COX-11 (cyclooxygenase 11) inhibitors. Anti-angiogenesis agents include, for example, rapamycin, temsirolimus (CCI-779), everolimus (RAD001), sorafenib, sunitinib, and bevacizumab. Non-limiting examples of COX-II inhibitors include alecoxib, valdecoxib, and rofecoxib. Non-limiting examples of anti-neoplastic agents include acemannan, aclarubicin, aldesleukin, alemtuzumab, alitretinoin, altretamine, amifostine, aminolevulinic acid, amrubicin, amsacrine, anagrelide, anastrozole, ANCER, aneastim, ARGLABIN, arsenic trioxide, BAM 002 (Novelos), bexarotene, bicalutamide, broxuridine, capecitabine, celmoleukin, cetorelix, cladribine, clotrimazole, cytarabine ocfosphate, DA 3030 (Dong-A), daclizumab, denileukin diftitox, deslorelin, dexrazoxane, dilazep, docetaxel, docosanol, doxercalciferol, doxifluridine, doxorubicin, bromocriptine, carmustine, cytarabine, fluorouracil, HIT diclofenac, interferon alfa, daunorubicin, doxorubicin, tretinoin, edelfosine, edrecolomab, eflornithine, emitefur, epirubicin, epoetin beta, etoposide phosphate, exemestane, exisulind, fadrozole, filgrastim, finasteride, fludarabine phosphate, formestane, fotemustine, gallium nitrate, gemcitabine, gemtuzumab, zogamicin, gimeracil/oteracil/tegafur combination, glycopine, goserelin, heptaplatin, human chorionic gonadotropin, human fetal alpha fetoprotein, ibandronic acid, idarubicin, (imiquimod, interferon alfa, interferon alfa, natural, interferon alfa-2, interferon alfa-2a, interferon alfa-2b,

interferon alfa-N1, interferon alfa-n3, interferon alfacon-1, interferon alpha, natural, interferon beta, interferon beta-1a, interferon beta-1b, interferon gamma, natural interferon gamma-1a, interferon gamma-1b, interleukin-1 beta, iobenguane, irinotecan, irsogladine, lanreotide, LC 9018 (Yakult), leflunomide, lenograstim, lentinan sulfate, letrozole, leukocyte alpha interferon, leuporelin, levamisole + fluorouracil, liarozole, lobaplatin, lonidamine, lovastatin, masoprocol, melarsoprol, metoclopramide, mifepristone, miltefosine, mirimostim, mismatched double stranded RNA, mitoguazone, mitolactol, mitoxantrone, molgramostim, nafarelin, naloxone + pentazocine, nartogastim, nedaplatin, nilutamide, noscapine, novel erythropoiesis stimulating protein, NSC 631570 octreotide, oprelvekin, osaterone, oxaliplatin, paclitaxel, pamidronic acid, pegaspargase, peginterferon alfa-2b, pentosan polysulfate sodium, pentostatin, picibanil, pirarubicin, rabbit antithymocyte polyclonal antibody, polyethylene glycol interferon alfa-2a, porfimer sodium, raloxifene, raltitrexed, rasburiembodiment, rhenium Re 186 etidronate, RII retinamide, rituximab, romurtide, samarium (153 Sm) lexidronam, sargramostim, sizofiran, sobuzoxane, sonermin, strontium-89 chloride, suramin, tasonermin, tazarotene, tegafur, temoporfin, temozolomide, teniposide, tetrachlorodecaoxide, thalidomide, thymalfasin, thyrotropin alfa, topotecan, toremifene, tositumomab-iodine 131, trastuzumab, treosulfan, tretinoin, trilostane, trimetrexate, triptorelin, tumor necrosis factor alpha, natural, ubenimex, bladder cancer vaccine, Maruyama vaccine, melanoma lysate vaccine, valrubicin, verteporfin, vinorelbine, VIRULIZIN, zinostatin stimalamer, or zoledronic acid; abarelix; AE 941 (Aeterna), ambamustine, antisense oligonucleotide, bcl-2 (Genta), APC 8015 (Dendreon), cetuximab, decitabine, dexaminoglutethimide, diaziquone, EL 532 (Elan), EM 800 (Endorecherche), eniluracil, etanidazole, fenretinide, filgrastim SD01 (Amgen), fulvestrant, galocitabine, gastrin 17 immunogen, HLA-B7 gene therapy (Vical), granulocyte macrophage colony stimulating factor, histamine dihydrochloride, ibritumomab tiuxetan, ilomastat, IM 862 (Cytran), interleukin-2, iproxifene, LDI 200 (Milkhaus), leridistim, lintuzumab, CA 125 MAb (Biomira), cancer MAb (Japan Pharmaceutical Development), HER-2 and Fc MAb (Medarex), idiotypic 105AD7 MAb (CRC Technology), idiotypic CEA MAb (Trilex), LYM-1-iodine 131 MAb (Techniclone), polymorphic epithelial mucin-yttrium 90 MAb (Antisoma), marimastat, menogaril, mitumomab, motexafin gadolinium, MX 6 (Galderma), nelarabine, nolatrexed, P 30 protein, pegvisomant, pemetrexed, porfiromycin, prinomastat, RL 0903 (Shire), rubitecan, satraplatin, sodium phenylacetate, sparfosic acid, SRL 172 (SR Pharma), SU 5416 (SUGEN), TA 077 (Tanabe), tetrathiomolybdate, thaliblastine, thrombopoietin, tin ethyl etiopurpurin, tirapazamine, cancer vaccine (Biomira), or valspodar.

[0118] Examples of anti-angiogenic agent include, but are not limited to, ERBITUX™ (IMC-C225), KDR (kinase domain receptor) inhibitory agents (e.g., antibodies and

antigen binding regions that specifically bind to the kinase domain receptor), anti-VEGF agents (e.g., antibodies or antigen binding regions that specifically bind VEGF, or soluble VEGF receptors or a ligand binding region thereof) such as AVASTIN™ or VEGF-TRAP™, and anti-VEGF receptor agents (e.g., antibodies or antigen binding regions that specifically bind thereto), EGFR inhibitory agents (e.g., antibodies or antigen binding regions that specifically bind thereto) such as Vectibix (panitumumab), IRESSA™ (gefitinib), TARCEVA™ (erlotinib), anti-Ang1 and anti-Ang2 agents (e.g., antibodies or antigen binding regions specifically binding thereto or to their receptors, e.g., Tie2/Tek), anti-Tie2 kinase inhibitory agents (e.g., antibodies or antigen binding regions that specifically bind thereto), Campath, IL-8, B-FGF, Tek antagonists, anti-TWEAK agents (e.g., specifically binding antibodies or antigen binding regions, or soluble TWEAK receptor antagonists), ADAM disintegrin domain to antagonize the binding of integrin to its ligands, specifically binding anti-eph receptor and/or anti-ephrin antibodies or antigen binding regions, anti-PDGF-BB antagonists (e.g., specifically binding antibodies or antigen binding regions) as well as antibodies or antigen binding regions specifically binding to PDGF-BB ligands, and PDGFR kinase inhibitory agents (e.g., antibodies or antigen binding regions that specifically bind thereto). Autophagy inhibitors include, but are not limited to, chloroquine, 3-methyladenine, hydroxychloroquine (Plaquenil™), bafilomycin A1, 5-amino-4-imidazole carboxamide riboside (AICAR), okadaic acid, autophagy-suppressive algal toxins which inhibit protein phosphatases of type 2A or type 1, analogues of cAMP, and drugs which elevate cAMP levels such as adenosine, LY204002, N6-mercaptopurine riboside, and vinblastine.

[0119] Non-limiting chemotherapeutic agents include, natural products such as vinca alkaloids (e.g., vinblastine, vincristine, and vinorelbine), paclitaxel, epidipodophyllotoxins (e.g., etoposide and teniposide), antibiotics (e.g., dactinomycin (actinomycin D), daunorubicin, doxorubicin, and idarubicin), anthracyclines, mitoxantrone, bleomycins, plicamycin (mithramycin), mitomycin, enzymes (e.g., L-asparaginase which systemically metabolizes L-asparagine and deprives cells which do not have the capacity to synthesize their own asparagine), antiplatelet agents, antiproliferative/antimitotic alkylating agents such as nitrogen mustards (e.g., mechlorethamine, cyclophosphamide and analogs, melphalan, and chlorambucil), ethylenimines and methylmelamines (e.g., hexamethylmelamine and thiotepa), CDK inhibitors (e.g., seliciclib, UCN-01, P1446A-05, PD-0332991, dinaciclib, P27-00, AT-7519, RGB286638, and SCH727965), alkyl sulfonates (e.g., busulfan), nitrosoureas (e.g., carmustine (BCNU) and analogs, and streptozocin), trazenes-dacarbazine (DTIC), antiproliferative/antimitotic antimetabolites such as folic acid analogs (e.g., methotrexate), pyrimidine analogs (e.g., fluorouracil, floxuridine, and cytarabine), purine analogs and related

inhibitors (e.g., mercaptopurine, thioguanine, pentostatin and 2-chlorodeoxyadenosine), aromatase inhibitors (e.g., anastrozole, exemestane, and letrozole), and platinum coordination complexes (e.g., cisplatin and carboplatin), procarbazine, hydroxyurea, mitotane, aminoglutethimide, histone deacetylase (HDAC) inhibitors (e.g., trichostatin, sodium butyrate, apicidan, suberoyl anilide hydroamic acid, vorinostat, LBH 589, romidepsin, ACY-1215, and panobinostat), mTor inhibitors (e.g., temsirolimus, everolimus, ridaforolimus, and sirolimus), KSP(Eg5) inhibitors (e.g., Array 520), DNA binding agents (e.g., Zalypsis), PI3K delta inhibitor (e.g., GS-1101 and TGR-1202), PI3K delta and gamma inhibitor (e.g., CAL-130), multi-kinase inhibitor (e.g., TG02 and sorafenib), hormones (e.g., estrogen) and hormone agonists such as leutinizing hormone releasing hormone (LHRH) agonists (e.g., goserelin, leuprolide and triptorelin), BAFF-neutralizing antibody (e.g., LY2127399), IKK inhibitors, p38MAPK inhibitors, anti-IL-6 (e.g., CNTO328), telomerase inhibitors (e.g., GRN 163L), aurora kinase inhibitors (e.g., MLN8237), cell surface monoclonal antibodies (e.g., anti-CD38 (HUMAX-CD38), anti-CS1 (e.g., elotuzumab), HSP90 inhibitors (e.g., 17 AAG and KOS 953), P13K / Akt inhibitors (e.g., perifosine), Akt inhibitor (e.g., GSK-2141795), PKC inhibitors (e.g., enzastaurin), FTIs (e.g., Zarnestra™), anti-CD138 (e.g., BT062), Torc1/2 specific kinase inhibitor (e.g., INK128), kinase inhibitor (e.g., GS-1101), ER/UPR targeting agent (e.g., MKC-3946), cFMS inhibitor (e.g., ARRY-382), JAK1/2 inhibitor (e.g., CYT387), PARP inhibitor (e.g., olaparib and veliparib (ABT-888)), BCL-2 antagonist. Other chemotherapeutic agents may include mechlorethamine, camptothecin, ifosfamide, tamoxifen, raloxifene, gemcitabine, navelbine, sorafenib, or any analog or derivative variant of the foregoing.

[0120] The methods, compositions and kits as disclosed herein, can be used in combination with radiation therapy, hormone therapy, surgery and immunotherapy, which therapies are well known to those of skill in the art.

[0121] Non-limiting examples of steroids include 21-acetoxypregnenolone, alclometasone, algestone, amcinonide, beclomethasone, betamethasone, budesonide, chloroprednisone, clobetasol, clocortolone, cloprednol, corticosterone, cortisone, cortivazol, deflazacort, desonide, desoximetasone, dexamethasone, diflorasone, difluocortolone, difuprednate, enoxolone, fluazacort, flucloronide, flumethasone, flunisolide, fluocinolone acetonide, fluocinonide, fluocortin butyl, fluocortolone, fluorometholone, fluperolone acetate, fluprednidene acetate, fluprednisolone, flurandrenolide, fluticasone propionate, formocortal, halcinonide, halobetasol propionate, halometasone, hydrocortisone, loteprednol etabonate, maziopredone, medrysone, meprednisone, methylprednisolone, mometasone furoate, paramethasone, prednicarbate, prednisolone, prednisolone 25-diethylaminoacetate, prednisolone sodium phosphate, prednisone, prednival, prednylidene, rimexolone, tixocortol, triamcinolone,

triamcinolone acetonide, triamcinolone benetonide, triamcinolone hexacetonide, and salts and/or derivatives thereof. In a particular embodiment, the compounds of the present invention can also be used in combination with additional pharmaceutically active agents that treat nausea. Examples of agents that can be used to treat nausea include: dronabinol; granisetron; metoclopramide; ondansetron; and prochlorperazine; or a pharmaceutically acceptable salt thereof.

[0122] In some embodiments, the one or more additional therapeutic agents that are administered to the subject comprises one or more PD-1 antagonists, PD-L1 antagonists, EGFR inhibitors, MEK inhibitors, PI3K inhibitors, AKT inhibitors, TOR inhibitors, Mcl-1 inhibitors, BCL-2 inhibitors, SHP2 inhibitors, proteasome inhibitors, and immune therapies, including monoclonal antibodies, immunomodulatory imides (IMiDs), anti-PD-1, anti-PDL-1, anti-CTLA4, anti-LAG1, and anti-OX40 agents, GTR agonists, CAR-T cells, and BiTEs. Proteasome inhibitors include, but are not limited to, Kyprolis®(carfilzomib), Velcade®(bortezomib), and oprozomib. Monoclonal antibodies include, but are not limited to, Darzalex® (daratumumab), Herceptin® (trastuzumab), Avastin® (bevacizumab), Rituxan® (rituximab), Lucentis® (ranibizumab), and Eylea® (aflibercept). In some embodiments, the one or more additional therapeutic agents comprise bevacizumab, pembrolizumab, nivolumab, PDR001, REGN2810 (SAR-439684), BGB-A317, BI 754091, IBI308, INCSHR-1210, JNJ-63723283, JS-001, MEDI0680 (AMP-514), MGA-012, PF-06801591, REGN-2810, TSR-042, atezolizumab, avelumab, CX-072, durvalumab, FAZ053, LY3300054, PD-L1 millamolecule, atezolizumab, durvalumab, avelumab, LY3300054, aminoglutethimide, amsacrine, anastrozole, asparaginase, bcr, beta-hydroxy beta-methylbutyrate, bicalutamide, bleomycin, bortezomib, buserelin, busulfan, camptothecin, capecitabine, carfilzomib, carboplatin, carmustine, chlorambucil, cisplatin, cladribine, clodronate, colchicine, cyclophosphamide, cyproterone, cytarabine, dacarbazine, dactinomycin, daunorubicin, delanzomib, dienestrol, diethylstilbestrol, disulfiram, docetaxel, doxorubicin, epigallocatechin-3-gallate, epirubicin, epoxomicin, estradiol, estramustine, etoposide, exemestane, filgrastim, fludarabine, fludrocortisone, fluorouracil, fluoxymesterone, flutamide, gemcitabine, genistein, goserelin, hydroxyurea, idarubicin, ifosfamide, imatinib, interferon, irinotecan, ironotecan, letrozole, leucovorin, leuprolide, levamisole, lomustine, lxazomib, marizomib, mechlorethamine, medroxyprogesterone, megestrol, melphalan, mercaptopurine, mesna, methotrexate, mitomycin, mitotane, mitoxantrone, nilutamide, nocodazole, octreotide, oprozomib, oxaliplatin, paclitaxel, pamidronate, pentostatin, plicamycin, porfimer, procarbazine, raltitrexed, rituximab, streptozocin, suramin, tamoxifen, temozolomide, teniposide, testosterone, thioguanine, thiotepa,

titanocene dichloride, topotecan, trastuzumab, tretinoin, vinblastine, vincristine, vindesine, vinorelbine, or a combination thereof.

[0123] In some embodiments, the method comprising administering a standard of care treatment and a cyclosporine analogue (e.g., CRV431) disclosed herein (or a pharmaceutically acceptable salt, solvate, stereoisomer thereof) for treating multiple myeloma in a subject in need. The standard of care treatment can comprise, for example, one or more proteasome inhibitors (e.g., Velcade (bortezomib), Kyprolis (carfilzomib), and Ninlaro (ixazomib), beta-hydroxy beta-methylbutyrate, delanzomib, disulfiram, epigallocatechin-3-gallate, epoxomicin, marizomib, and oprozomib).

Kits, Compositions and Methods of Administration

[0124] Provided in some embodiments include kits comprising: a cyclosporine analogue (e.g., CRV431) or a pharmaceutically acceptable salt, solvate, stereoisomer thereof, and a label indicating the use of the kit. In some embodiments, the label indicates that the kit is for treating proliferative diseases, for example cancer, in a subject. In some embodiments, the label indicates that the kit is for preventing proliferative diseases, for example cancer, in a subject. In some embodiments, the label indicates that the kit is for alleviating one or more symptoms of proliferative diseases, or preventing or delaying the onset of one or more symptoms of proliferative diseases. In some embodiments, the label indicates the kit is for preventing or delaying onset of proliferative diseases. In some embodiments, the methods, compositions and kits can prevent fibrosis (e.g., a liver cancer-associated fibrosis), treating fibrosis (e.g., a liver cancer-associated fibrosis), reducing the amount of fibrosis, delaying the onset of fibrosis, reducing or inhibiting fibrosis formation, reversing fibrosis, or any combination thereof.

[0125] Also provided herein, in some embodiments, are compositions comprising: one or more of the cyclosporine analogues disclosed herein (e.g., CRV431), or a pharmaceutically acceptable salt, solvate, stereoisomer thereof, for use in treating proliferative diseases, preventing proliferative diseases, for alleviating one or more symptoms of proliferative diseases, or preventing or delaying the onset of one or more symptoms of proliferative diseases.

[0126] The proliferative disease can, for example, be a hyperproliferative disease. In some embodiments, the proliferative disease is cancer. The cancer can be primary cancer and/or secondary cancer. The cancer can be a solid tumor or a liquid tumor. In some embodiments, the cancer is a solid tumor, including but are not limited to, melanoma, renal cell carcinoma, lung cancer, bladder cancer, breast cancer, cervical cancer, colon cancer, gall bladder cancer, laryngeal cancer, liver cancer, thyroid cancer, stomach cancer, salivary gland cancer, prostate

cancer, pancreatic cancer, Merkel cell carcinoma, brain and central nervous system cancers, and any combination thereof. In some embodiments, the cancer is a liquid tumor. In some embodiments, the cancer is a hematological cancer, including but not limited to, Diffuse large B cell lymphoma (“DLBCL”), Hodgkin's lymphoma (“HL”), Non-Hodgkin's lymphoma (“NHL”), Follicular lymphoma (“FL”), acute myeloid leukemia (“AML”), and Multiple myeloma (“MM”).

[0127] Fibrosis can be fibrosis affecting the heart, liver, lung, skeletal muscle, kidney, eyes, blood vessel, skin, brain, bone marrow, gastrointestinal tract, peritoneum, vasculature, or any combination thereof. In some embodiments, the fibrosis is non-liver fibrosis. The fibrotic disorder can be any of the fibrotic disorders disclosed herein, including but not limited to retinal fibrosis, corneal fibrosis, conjunctival fibrosis, fibrosis of the trabecular meshwork, renal fibrosis, pulmonary fibrosis, idiopathic pulmonary fibrosis (IPF), usual interstitial pneumonitis (UIP), interstitial lung disease (ILD), cryptogenic fibrosing alveolitis (CFA), bronchiolitis obliterans, bronchiectasis, cirrhosis, hepatic fibrosis, fibrotic vascular disease, cystic fibrosis, pulmonary fibrosis, idiopathic pulmonary fibrosis, musculoskeletal fibrosis, renal fibrotic disease, lymph node fibrosis associated with HIV, inflammatory pulmonary fibrosis, pancreatic fibrosis, cardiac fibrosis, vascular fibrosis, myocardial fibrosis, or a combination thereof.

[0128] In some embodiments, the composition is a stable self-microemulsifying drug delivery systems (“SMEDDS”) formulation comprising a derivative or an analog, of cyclosporine A (e.g., CRV431), or a pharmaceutically acceptable salt, solvate, stereoisomer thereof. The composition can, for example, enables good solubility of a derivative of cyclosporine A (e.g., CRV431), or a pharmaceutically acceptable salt, solvate, stereoisomer thereof, and enables significant blood exposure in humans. In some embodiments, the composition further comprises polyoxyl castor oil (also known as polyoxyl 40 hydrogenated castor oil, macrogolglycerol hydroxystearate, and PEG-40 hydrogenated castor oil, such as Cremophor® RH40 and Kolliphor® RH40). In some embodiments, the composition comprises ethanol. In some embodiments, the composition comprises diethylene glycol monoethyl ether (also known as 2-(2-Ethoxyethoxy)ethanol, such as Transcutol®). In some embodiments, the composition comprises propylene glycol (PG). In some embodiments, the composition comprises glyceryl monolinoleate, such as Maisine® CC. In some embodiments, the composition comprises Vitamin E. Various pharmaceutical compositions/drug delivery system (e.g., SMEDDS formulations) comprising cyclosporine analogues (e.g., CRV431) or pharmaceutically acceptable salts thereof, have been described in PCT patent application

published as WO 2020/112562, the content of which is incorporated herein by reference in its entirety.

[0129] In some embodiments, the system comprises Vitamin E, Maisine® CC, propylene glycol, Transcutol®, ethanol, and Cremophor® RH40. The weight ratios of non-cyclosporine A analog components in the system can be different in different embodiments. In some embodiments, the weight ratio of a non-cyclosporine A analog component (e.g., Vitamin E, Maisine® CC, propylene glycol, Transcutol®, ethanol, or Cremophor® RH40) relative to another non-cyclosporine A analogy components (e.g., Vitamin E, Maisine® CC, propylene glycol, Transcutol®, ethanol, or Cremophor® RH40) in the system can be between about 0.1 and about 10. In some embodiments, the weight ratio of a non-cyclosporine A analog component relative to all other non-cyclosporine A components in the system can be between about 0.1 and about 10. In some embodiments, the weight ratio of a non-cyclosporine A analog component relative to another non-cyclosporine A analogy components (or relative to all other non-cyclosporine A components) in the system can be, be about, be at least, be at least about, be at most, or be at most about, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, 0.55, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, 0.9, 0.95, 1, 1.05, 1.1, 1.15, 1.2, 1.25, 1.3, 1.35, 1.4, 1.45, 1.5, 1.55, 1.6, 1.65, 1.7, 1.75, 1.8, 1.85, 1.9, 1.95, 2, 2.05, 2.1, 2.15, 2.2, 2.25, 2.3, 2.35, 2.4, 2.45, 2.5, 2.55, 2.6, 2.65, 2.7, 2.75, 2.8, 2.85, 2.9, 2.95, 3, 3.05, 3.1, 3.15, 3.2, 3.25, 3.3, 3.35, 3.4, 3.45, 3.5, 3.55, 3.6, 3.65, 3.7, 3.75, 3.8, 3.85, 3.9, 3.95, 4, 4.05, 4.1, 4.15, 4.2, 4.25, 4.3, 4.35, 4.4, 4.45, 4.5, 4.55, 4.6, 4.65, 4.7, 4.75, 4.8, 4.85, 4.9, 4.95, 5, 5.05, 5.1, 5.15, 5.2, 5.25, 5.3, 5.35, 5.4, 5.45, 5.5, 5.55, 5.6, 5.65, 5.7, 5.75, 5.8, 5.85, 5.9, 5.95, 6, 6.05, 6.1, 6.15, 6.2, 6.25, 6.3, 6.35, 6.4, 6.45, 6.5, 6.55, 6.6, 6.65, 6.7, 6.75, 6.8, 6.85, 6.9, 6.95, 7, 7.05, 7.1, 7.15, 7.2, 7.25, 7.3, 7.35, 7.4, 7.45, 7.5, 7.55, 7.6, 7.65, 7.7, 7.75, 7.8, 7.85, 7.9, 7.95, 8, 8.05, 8.1, 8.15, 8.2, 8.25, 8.3, 8.35, 8.4, 8.45, 8.5, 8.55, 8.6, 8.65, 8.7, 8.75, 8.8, 8.85, 8.9, 8.95, 9, 9.05, 9.1, 9.15, 9.2, 9.25, 9.3, 9.35, 9.4, 9.45, 9.5, 9.55, 9.6, 9.65, 9.7, 9.75, 9.8, 9.85, 9.9, 9.95, 10, or number or a range between any two of these values. For example, the system comprises Vitamin E, Maisine® CC, propylene glycol, Transcutol®, ethanol, and Cremophor® RH40 at a weight ratio of 1/1/5/5/2.4/4 to 1/1.5/2.5/5/2.4/5.

[0130] The system can, for example, comprise the cyclosporine analogue (e.g., CRV431) at a concentration of from about 10 mg/mL to about 90 mg/mL, including 10 mg/mL, 20 mg/mL, 30 mg/mL, 40 mg/mL, 50 mg/mL, 60 mg/mL, 70 mg/mL, 80 mg/mL, 90 mg/mL, a range between any two of these values, or any value within 10 mg/mL to 90 mg/mL. In some embodiments, the system comprises the cyclosporine analogue (e.g., CRV431) at a concentration of about 90 mg/mL. In some embodiments, the system comprises the cyclosporine analogue (e.g., CRV431) at a concentration of, or of about, 70 mg/mL.

[0131] The composition can be, for example, a pharmaceutical composition comprising a cyclosporine analogue (e.g., CRV431), or a pharmaceutically acceptable salt, solvate, stereoisomer thereof, and one or more pharmaceutically acceptable excipients. In some embodiments, the composition is administered to the subject by intravenous administration, nasal administration, pulmonary administration, oral administration, or parenteral administration. In some embodiments, the composition is in the form of powder, pill, tablet, microtablet, pellet, micropellet, capsule, capsule containing microtablets, liquid, aerosols, suspension, or nanoparticles. In some embodiments, the composition is administered to the subject once, twice, or three times a day. In some embodiments, the composition is administered to the subject once or twice in an emergency situation (e.g., in an ongoing surgery). In some embodiments, the composition is administered to the subject over the course of at least a day, at least two days, at least three days, at least a week, or more. In some embodiments, the composition is administered to the subject at an effective daily dose of the cyclosporine analogue (e.g., CRV431) or a pharmaceutically acceptable salt, solvate, stereoisomer thereof at from 10 mg to 250 mg.

[0132] The therapeutically effective amount and the frequency of administration of, and the length of treatment with, the cyclosporine analogue (e.g., CRV431) may depend on various factors, including the nature and the severity of the proliferative disease, the potency of the cyclosporine analogue (e.g., CRV431), the mode of administration, the age, the body weight, the general health, the gender and the diet of the subject, and the response of the subject to the treatment, and can be determined by the treating physician. In some embodiments, a therapeutically effective amount of the cyclosporine analogue (e.g., CRV431) for treating or preventing a proliferative disease, or for reducing or inhibiting progression of the proliferative disease, as described herein, is about 0.1-200 mg, 0.1-150 mg, 0.1-100 mg, 0.1-50 mg, 0.1-30 mg, 0.5-20 mg, 0.5-10 mg or 1-10 mg (e.g., per day or per dose), or as deemed appropriate by the treating physician, which can be administered in a single dose or in divided doses. In some embodiments, the therapeutically effective dose (e.g., per day or per dose) of the cyclosporine analogue (e.g., CRV431) for treating or preventing a proliferative disease, or for reducing or inhibiting progression of the proliferative disease, as described herein, is about 0.1-1 mg (e.g., about 0.1 mg, 0.5 mg or 1 mg), about 1-5 mg (e.g., about 1 mg, 2 mg, 3 mg, 4 mg or 5 mg), about 5-10 mg (e.g., about 5 mg, 6 mg, 7 mg, 8 mg, 9 mg or 10 mg), about 10-20 mg (e.g., about 10 mg, 15 mg or 20 mg), about 20-30 mg (e.g., about 20 mg, 25 mg or 30 mg), about 30-40 mg (e.g., about 30 mg, 35 mg or 40 mg), about 40-50 mg (e.g., about 40 mg, 45 mg or 50 mg), about 50-100 mg (e.g., about 50 mg, 60 mg, 70 mg, 80 mg, 90 mg or 100 mg), about 100-150 mg (e.g., about 100 mg, 125 mg or 150 mg), or about 150-200 mg (e.g., about 150 mg, 175 mg or 200

mg). In some embodiments, the therapeutically effective dose of the cyclosporine analogue (e.g., CRV431) is administered one or more (e.g., two, three or more) times a day, or once every two or three days, or once, twice or thrice a week, or as deemed appropriate by the treating physician. In some embodiments, the composition comprises a therapeutically or prophylactically effective amount of the cyclosporine analogue (e.g., CRV431) or a pharmaceutically acceptable salt, solvate, stereoisomer thereof.

[0133] The cyclosporine analogue (e.g., CRV431) can also be dosed in an irregular manner. For example, the cyclosporine analogue (e.g., CRV431) can be administered once, twice or thrice in a period of 30 minutes, one hour, two hours or more, in an irregular manner. Furthermore, the cyclosporine analogue (e.g., CRV431) can be taken pro re rata (as needed). For instance, the cyclosporine analogue (e.g., CRV431) can be administered 1, 2, 3, 4, 5 or more times, whether in a regular or irregular manner, until the disease condition improves. Once relief from the disorder/condition is achieved, dosing of the cyclosporine analogue (e.g., CRV431) can optionally be discontinued. If the disorder/condition returns, administration of the cyclosporine analogue (e.g., CRV431), whether in a regular or irregular manner, can be resumed. The appropriate dosage of, frequency of dosing of and length of treatment with the cyclosporine analogue (e.g., CRV431) can be determined by the treating physician.

[0134] The cyclosporine analogue (e.g., CRV431) can also be used prophylactically to treat or prevent a proliferative disease, or to prevent or reduce the onset of the proliferative disease, or to reduce or inhibit progression of the proliferative disease. The prophylactically effective amount of a cyclosporine analogue (e.g., CRV431) can be any therapeutically effective amount of the cyclosporine analogue (e.g., CRV431) described herein.

[0135] The cyclosporine analogue (e.g., CRV431) can be administered via any suitable route. Potential routes of administration of the cyclosporine analogue (e.g., CRV431) include without limitation oral, parenteral (including intramuscular, subcutaneous, intradermal, intravascular, intravenous, intraarterial, intramedullary and intrathecal), intracavitary, intraperitoneal, and topical (including dermal/epicutaneous, transdermal, mucosal, transmucosal, intranasal [e.g., by nasal spray or drop], intraocular [e.g., by eye drop], pulmonary [e.g., by oral or nasal inhalation], buccal, sublingual, rectal and vaginal). In some embodiments, the cyclosporine analogue (e.g., CRV431) is administered orally (e.g., as a capsule or tablet, optionally with an enteric coating). In other embodiments, the cyclosporine analogue (e.g., CRV431) is administered parenterally (e.g., intravenously, subcutaneously or intradermally). In further embodiments, the cyclosporine analogue (e.g., CRV431) is administered topically (e.g., dermally, epicutaneously, transdermally, mucosally, transmucosally, buccally or sublingually).

[0136] In some embodiments, the cyclosporine analogue (e.g., CRV431) is administered without food. In some embodiments, the cyclosporine analogue (e.g., CRV431) is administered at least about 1 or 2 hours before or after a meal. In some embodiments, the cyclosporine analogue (e.g., CRV431) is administered at least about 2 hours after an evening meal. The cyclosporine analogue (e.g., CRV431) can also be taken substantially concurrently with food (e.g., within about 0.5, 1 or 2 hours before or after a meal, or with a meal).

[0137] In some embodiments where a more rapid establishment of a therapeutic level of the cyclosporine analogue (e.g., CRV431) is desired, the cyclosporine analogue (e.g., CRV431) is administered under a dosing schedule in which a loading dose is administered, followed by (i) one or more additional loading doses and then one or more therapeutically effective maintenance doses, or (ii) one or more therapeutically effective maintenance doses without an additional loading dose, as deemed appropriate by the treating physician. A loading dose of a drug is typically larger (e.g., about 1.5, 2, 3, 4 or 5 times larger) than a subsequent maintenance dose and is designed to establish a therapeutic level of the drug more quickly. The one or more therapeutically effective maintenance doses can be any therapeutically effective dose described herein. In some embodiments, the loading dose is about three times greater than the maintenance dose. In some embodiments, a loading dose of the cyclosporine analogue (e.g., CRV431) is administered, followed by administration of a maintenance dose of the cyclosporine analogue (e.g., CRV431) after an appropriate time (e.g., after about 12 or 24 hours) and thereafter for the duration of therapy--e.g., a loading dose of the cyclosporine analogue (e.g., CRV431) is administered on day 1 and a maintenance dose is administered on day 2 and thereafter for the duration of therapy. In some embodiments, the cyclosporine analogue (e.g., CRV431) is administered in a loading dose of about 1.5, 3, 15 or 30 mg (e.g., 3×about 0.5, 1, 5 or 10 mg) orally (e.g., as a tablet) on day 1, followed by a maintenance dose of about 0.5, 1, 5 or 10 mg orally (e.g., as a tablet) once daily, optionally at bedtime, for at least about 2 weeks, 1 month (4 weeks), 6 weeks, 2 months, 10 weeks, 3 months, 4 months, 5 months, 6 months, 1 year, 1.5 years, 2 years, 3 years or longer (e.g., at least about 6 weeks, 2 months, 3 months or 6 months). In some embodiments, the cyclosporine analogue (e.g., CRV431) is administered in a loading dose of about 15 mg (e.g., 3×about 5 mg) orally (e.g., as a tablet) on day 1, followed by a maintenance dose of about 5 mg orally (e.g., as a tablet) once daily, optionally at bedtime, for at least about 2 weeks, 1 month, 6 weeks, 2 months, 3 months, 6 months, 1 year, 1.5 years, 2 years, 3 years or longer (e.g., at least about 6 weeks, 2 months, 3 months or 6 months).

[0138] In some embodiments, a first loading dose of the cyclosporine analogue (e.g., CRV431) is administered on day 1, a second loading dose is administered on day 2, and a maintenance dose is administered on day 3 and thereafter for the duration of therapy. In some

embodiments, the first loading dose is about three times greater than the maintenance dose, and the second loading dose is about two times greater than the maintenance dose.

[0139] As disclosed herein, the therapeutic agent (e.g., the cyclosporine analogue (e.g., CRV431)) can be formulated for administration in a pharmaceutical composition comprising a physiologically acceptable surface active agents, carriers, diluents, excipients, smoothing agents, suspension agents, film forming substances, coating assistants, or a combination thereof. In some embodiments, the therapeutic agent (e.g., the cyclosporine analogue (e.g., CRV431)) are formulated for administration with a pharmaceutically acceptable carrier or diluent. The therapeutic agent (e.g., the cyclosporine analogue (e.g., CRV431)) can be formulated as a medicament with a standard pharmaceutically acceptable carrier(s) and/or excipient(s) as is routine in the pharmaceutical art. The exact nature of the formulation will depend upon several factors including the desired route of administration. In some embodiments, the cyclosporine analogue (e.g., CRV431) is formulated for oral, intravenous, intragastric, intravascular or intraperitoneal administration. Standard pharmaceutical formulation techniques may be used, such as those disclosed in Remington's The Science and Practice of Pharmacy, 21st Ed., Lippincott Williams & Wilkins (2005), incorporated herein by reference in its entirety.

[0140] The term "pharmaceutically acceptable carrier" or "pharmaceutically acceptable excipient" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. In addition, various adjuvants such as are commonly used in the art may be included. Considerations for the inclusion of various components in pharmaceutical compositions are described, e.g., in Gilman et al. (Eds.) (1990); Goodman and Gilman's: The Pharmacological Basis of Therapeutics, 8th Ed., Pergamon Press, which is incorporated herein by reference in its entirety.

[0141] Some examples of substances, which can serve as pharmaceutically-acceptable carriers or components thereof, are sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose and its derivatives, such as sodium carboxymethyl cellulose, powdered tragacanth; malt; gelatin; talc; solid lubricants, such as stearic acid and magnesium stearate; calcium sulfate; vegetable oils, such as peanut oil, cottonseed oil, sesame oil, olive oil, corn oil and theobroma oil; polyols such as propylene glycol, glycerin, sorbitol, mannitol, and polyethylene glycol; alginic acid; emulsifiers, such as the TWEENS; wetting agents, such sodium lauryl sulfate; coloring agents; flavoring agents;

tableting agents, stabilizers; antioxidants; preservatives; pyrogen-free water; isotonic saline; and phosphate buffer solutions.

[0142] The choice of a pharmaceutically-acceptable carrier to be used in conjunction with the subject therapeutic agent is basically determined by the way the composition is to be administered.

[0143] The compositions described herein are preferably provided in unit dosage form. As used herein, a "unit dosage form" is a composition containing an amount of a therapeutic agent (e.g., a cyclosporine analogue (e.g., CRV431)) that is suitable for administration to an animal, preferably mammal subject, in a single dose, according to good medical practice. The preparation of a single or unit dosage form however, does not imply that the dosage form is administered once per day or once per course of therapy. Such dosage forms are contemplated to be administered once, twice, thrice or more per day and may be administered as infusion over a period of time (e.g., from about 30 minutes to about 2-6 hours), or administered as a continuous infusion, and can be given more than once during a course of therapy, though a single administration is not specifically excluded. The skilled artisan will recognize that the formulation does not specifically contemplate the entire course of therapy and such decisions are left for those skilled in the art of treatment rather than formulation.

[0144] The compositions useful as described above may be in any of a variety of suitable forms for a variety of routes for administration, for example, for oral, nasal, rectal, topical (including transdermal), ocular, intracerebral, intracranial, intrathecal, intra-arterial, intravenous, intramuscular, or other parental routes of administration. The skilled artisan will appreciate that oral and nasal compositions include compositions that are administered by inhalation, and made using available methodologies. Depending upon the particular route of administration desired, a variety of pharmaceutically-acceptable carriers well-known in the art may be used. Pharmaceutically-acceptable carriers include, for example, solid or liquid fillers, diluents, hydrotropes, surface-active agents, and encapsulating substances. Optional pharmaceutically-active materials may be included, which do not substantially interfere with the inhibitory activity of the therapeutic agent (e.g., the cyclosporine analogue (e.g., CRV431)). The amount of carrier employed in conjunction with the therapeutic agent (e.g., the cyclosporine analogue (e.g., CRV431)) is sufficient to provide a practical quantity of material for administration per unit dose of the therapeutic agent (e.g., the cyclosporine analogue (e.g., CRV431)). Techniques and compositions for making dosage forms useful in the methods described herein are described in the following references, all incorporated by reference herein: Modern Pharmaceutics, 4th Ed., Chapters 9 and 10 (Banker & Rhodes, editors, 2002);

Lieberman et al., *Pharmaceutical Dosage Forms: Tablets* (1989), and Ansel, *Introduction to Pharmaceutical Dosage Forms* 8th Edition (2004).

[0145] Various oral dosage forms can be used, including such solid forms as tablets, capsules, and granules. Tablets can be compressed, tablet triturates, enteric-coated, sugar-coated, film-coated, or multiple-compressed, containing suitable binders, lubricants, diluents, disintegrating agents, coloring agents, flavoring agents, flow-inducing agents, and melting agents. Liquid oral dosage forms include aqueous solutions, emulsions, suspensions, solutions and/or suspensions reconstituted from non-effervescent granules, and effervescent preparations reconstituted from effervescent granules, containing suitable solvents, preservatives, emulsifying agents, suspending agents, diluents, sweeteners, melting agents, coloring agents and flavoring agents.

[0146] The pharmaceutically-acceptable carriers suitable for the preparation of unit dosage forms for peroral administration is well-known in the art. Tablets typically comprise conventional pharmaceutically-compatible adjuvants as inert diluents, such as calcium carbonate, sodium carbonate, mannitol, lactose and cellulose; binders such as starch, gelatin and sucrose; disintegrants such as starch, alginic acid and croscarmellose; lubricants such as magnesium stearate, stearic acid and talc. Glidants such as silicon dioxide can be used to improve flow characteristics of the powder mixture. Coloring agents, such as the FD&C dyes, can be added for appearance. Sweeteners and flavoring agents, such as aspartame, saccharin, menthol, peppermint, and fruit flavors, are useful adjuvants for chewable tablets. Capsules typically comprise one or more solid diluents disclosed above. The selection of carrier components depends on secondary considerations like taste, cost, and shelf stability, which are not critical, and can be readily made by a person skilled in the art.

[0147] Peroral compositions also include liquid solutions, emulsions, suspensions, and the like. The pharmaceutically-acceptable carriers suitable for preparation of such compositions are well known in the art. Typical components of carriers for syrups, elixirs, emulsions and suspensions include ethanol, glycerol, propylene glycol, polyethylene glycol, liquid sucrose, sorbitol and water. For a suspension, typical suspending agents include sodium carboxymethyl cellulose, AVICEL RC-591, tragacanth and sodium alginate; typical wetting agents include lecithin and polysorbate 80; and typical preservatives include methyl paraben and sodium benzoate. Peroral liquid compositions may also contain one or more components such as sweeteners, flavoring agents and colorants disclosed above.

[0148] Other compositions useful for attaining systemic delivery of the subject therapeutic agents include sublingual, buccal and nasal dosage forms. Such compositions typically comprise one or more of soluble filler substances such as sucrose, sorbitol and

mannitol; and binders such as acacia, microcrystalline cellulose, carboxymethyl cellulose and hydroxypropyl methyl cellulose. Glidants, lubricants, sweeteners, colorants, antioxidants and flavoring agents disclosed above may also be included.

[0149] For topical use, creams, ointments, gels, solutions or suspensions, etc., containing the therapeutic agent (e.g., the cyclosporine analogue (e.g., CRV431)) disclosed herein are employed. Topical formulations may generally be comprised of a pharmaceutical carrier, co-solvent, emulsifier, penetration enhancer, preservative system, and emollient.

[0150] For intravenous administration, the therapeutic agent (e.g., the cyclosporine analogue (e.g., CRV431)) and compositions described herein may be dissolved or dispersed in a pharmaceutically acceptable diluent, such as a saline or dextrose solution. Suitable excipients may be included to achieve the desired pH, including but not limited to NaOH, sodium carbonate, sodium acetate, HCl, and citric acid. In various embodiments, the pH of the final composition ranges from 2 to 8, or preferably from 4 to 7. Antioxidant excipients may include sodium bisulfite, acetone sodium bisulfite, sodium formaldehyde, sulfoxylate, thiourea, and EDTA. Other non-limiting examples of suitable excipients found in the final intravenous composition may include sodium or potassium phosphates, citric acid, tartaric acid, gelatin, and carbohydrates such as dextrose, mannitol, and dextran. Further acceptable excipients are described in Powell, et al., *Compendium of Excipients for Parenteral Formulations*, PDA J Pharm Sci and Tech 1998, 52 238-311 and Nema et al., *Excipients and Their Role in Approved Injectable Products: Current Usage and Future Directions*, PDA J Pharm Sci and Tech 2011, 65 287-332, both of which are incorporated herein by reference in their entirety. Antimicrobial agents may also be included to achieve a bacteriostatic or fungistatic solution, including but not limited to phenyl mercuric nitrate, thimerosal, benzethonium chloride, benzalkonium chloride, phenol, cresol, and chlorobutanol.

[0151] The compositions for intravenous administration may be provided to caregivers in the form of one more solids that are reconstituted with a suitable diluent such as sterile water, saline or dextrose in water shortly prior to administration. In other embodiments, the compositions are provided in solution ready to administer parenterally. In still other embodiments, the compositions are provided in a solution that is further diluted prior to administration. In embodiments that include administering a combination of a therapeutic agent (e.g., the cyclosporine analogue (e.g., CRV431)) described herein and another agent, the combination may be provided to caregivers as a mixture, or the caregivers may mix the two agents prior to administration, or the two agents may be administered separately.

[0152] In non-human animal studies, applications of potential products are commenced at higher dosage levels, with dosage being decreased until the desired effect is no

longer achieved or adverse side effects disappear. The dosage may range broadly, depending upon the desired effects and the therapeutic indication. Typically, dosages may be between about 0.1 mg/kg and 4000 mg/kg body weight, preferably between about 80 mg/kg and 1600 mg/kg body weight. Alternatively dosages may be based and calculated upon the surface area of the patient, as understood by those of skill in the art.

[0153] Depending on the severity and responsiveness of the condition to be treated, dosing can also be a single administration of a slow release composition, with course of treatment lasting from several days to several weeks or until cure is effected or diminution of the disease state is achieved. The amount of a composition to be administered will, of course, be dependent on many factors including the subject being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician. The therapeutic agent (e.g., cyclosporine analogue (e.g., CRV431)) or combination of therapeutic agents disclosed herein may be administered orally or via injection at a dose from 0, 1 mg/kg to 4000 mg/kg of the patient's body weight per day. The dose range for adult humans is generally from 1 g to 100 g/day. Tablets or other forms of presentation provided in discrete units may conveniently contain an amount of the therapeutic agent (e.g., cyclosporine analogue (e.g., CRV431)) or combination of therapeutic agents disclosed herein which is effective at such dosage or as a multiple of the same, for instance, units containing 1 g to 60 g (for example, from about 5 g to 20 g, from about 10 g to 50 g, from about 20 g to 40 g, or from about 25 g to 35 g). The precise amount of therapeutic agent administered to a patient will be the responsibility of the attendant physician. However, the dose employed will depend on a number of factors, including the age and sex of the patient, the precise disorder being treated, and its severity. Additionally, the route of administration may vary depending on the condition and its severity. A typical dose of the therapeutic agent (e.g., cyclosporine analogue (e.g., CRV431)) can be from 0.02 g to 1.25 g per kg of body weight, for example from 0.1 g to 0.5 g per kg of body weight, depending on such parameters. In some embodiments, a dosage of the therapeutic agent (e.g., cyclosporine analogue (e.g., CRV431)) can be from 1 g to 100 g, for example, from 10 g to 80 g, from 15 g to 60 g, from 20 g to 40 g, or from 25 g to 35 g. In A physician will be able to determine the required dosage of the therapeutic agent (e.g., cyclosporine analogue (e.g., CRV431)) for any particular subject.

[0154] The exact formulation, route of administration and dosage for the pharmaceutical compositions of the therapeutic agent (e.g., cyclosporine analogue (e.g., CRV431)) or combination of therapeutic agents disclosed herein can be chosen by the individual physician in view of the patient's condition. Typically, the dose range of the composition administered to the patient can be from about 0.1 to about 4000 mg/kg of the patient's body

weight. The dosage may be a single one or a series of two or more given in the course of one or more days, as is needed by the patient. In instances where human dosages for therapeutic agents have been established for at least some condition, the present disclosure will use those same dosages, or dosages that are between about 0.1% and about 5000%, more preferably between about 25% and about 1000% of the established human dosage. Where no human dosage is established, as will be the case for newly-discovered pharmaceutical compounds, a suitable human dosage can be inferred from ED₅₀ or ID₅₀ values, or other appropriate values derived from in vitro or in vivo studies, as qualified by toxicity studies and efficacy studies in animals.

[0155] It should be noted that the attending physician would know how to and when to terminate, interrupt, or adjust administration due to toxicity or organ dysfunctions. Conversely, the attending physician would also know to adjust treatment to higher levels if the clinical response were not adequate (precluding toxicity). The magnitude of an administered dose in the management of the disorder of interest will vary with the severity of the condition to be treated and to the route of administration. The severity of the condition may, for example, be evaluated, in part, by standard prognostic evaluation methods. Further, the dose and perhaps dose frequency, will also vary according to the age, body weight, and response of the individual patient. A program comparable to that discussed above may be used in veterinary medicine.

[0156] Although the exact dosage will be determined on a drug-by-drug basis, in most cases, some generalizations regarding the dosage can be made. In cases of administration of a pharmaceutically acceptable salt, dosages may be calculated as the free base. In some embodiments, the composition is administered 1 to 4 times per day. Alternatively the compositions disclosed herein may be administered by continuous intravenous infusion, e.g., at a dose of each active ingredient up to 100 g per day. As will be understood by those of skill in the art, in certain situations it may be necessary to administer the compositions disclosed herein in amounts that exceed, or even far exceed, the above-stated, preferred dosage range in order to effectively and aggressively treat particularly aggressive diseases or infections. In some embodiments, the therapeutic agent (e.g., cyclosporine analogue (e.g., CRV431)) or combination of therapeutic agents disclosed herein will be administered for a period of continuous therapy, for example for a week or more, or for months or years.

[0157] In some embodiments, the dosing regimen of the therapeutic agent (e.g., cyclosporine analogue (e.g., CRV431)) or combination of therapeutic agents disclosed herein is administered for a period of time, which time period can be, for example, from at least about 1 week to at least about 4 weeks, from at least about 4 weeks to at least about 8 weeks, from at least about 4 weeks to at least about 12 weeks, from at least about 4 weeks to at least about 16 weeks, or longer. The dosing regimen of the therapeutic agent (e.g., cyclosporine analogue (e.g.,

CRV431)) or combination of therapeutic agents disclosed herein can be administered three times a day, twice a day, daily, every other day, three times a week, every other week, three times per month, once monthly, substantially continuously or continuously.

[0158] The cyclosporine analogue (e.g., CRV431) can be administered alone or in the form of a composition (e.g., a pharmaceutical composition). In some embodiments, a pharmaceutical composition comprises a cyclosporine analogue (e.g., CRV431) or a pharmaceutically acceptable salt, solvate, hydrate, clathrate, polymorph, prodrug or metabolite thereof, and one or more pharmaceutically acceptable carriers or excipients. The composition can optionally contain one or more additional therapeutic agents as described herein. A pharmaceutical composition contains a therapeutically effective amount of a therapeutic agent (e.g., a cyclosporine analogue (e.g., CRV431)) and one or more pharmaceutically acceptable carriers or excipients, and is formulated for administration to a subject for therapeutic use. For purposes of the content of a pharmaceutical composition, the terms "therapeutic agent", "active ingredient", "active agent" and "drug" encompass prodrugs.

[0159] A pharmaceutical composition contains a therapeutic agent (e.g., a cyclosporine analogue (e.g., CRV431)) in substantially pure form. In some embodiments, the purity of the therapeutic agent is at least about 95%, 96%, 97%, 98% or 99%. In some embodiments, the purity of the therapeutic agent is at least about 98% or 99%. In addition, a pharmaceutical composition is substantially free of contaminants or impurities. In some embodiments, the level of contaminants or impurities other than residual solvent in a pharmaceutical composition is no more than about 5%, 4%, 3%, 2% or 1% relative to the combined weight of the intended active and inactive ingredients. In some embodiments, the level of contaminants or impurities other than residual solvent in a pharmaceutical composition is no more than about 2% or 1% relative to the combined weight of the intended active and inactive ingredients. Pharmaceutical compositions generally are prepared according to current good manufacturing practice (GMP), as recommended or required by, e.g., the Federal Food, Drug, and Cosmetic Act §501(a)(2)(B) and the International Conference on Harmonisation Q7 Guideline.

[0160] Pharmaceutically acceptable carriers and excipients include pharmaceutically acceptable materials, vehicles and substances. Non-limiting examples of excipients include liquid and solid fillers, diluents, binders, lubricants, glidants, solubilizers, surfactants, dispersing agents, disintegration agents, emulsifying agents, wetting agents, suspending agents, thickeners, solvents, isotonic agents, buffers, pH adjusters, stabilizers, preservatives, antioxidants, antimicrobial agents, antibacterial agents, antifungal agents, absorption- delaying agents, sweetening agents, flavoring agents, coloring agents, adjuvants, encapsulating materials and

coating materials. The use of such excipients in pharmaceutical formulations is known in the art. For example, conventional vehicles and carriers include without limitation oils (e.g., vegetable oils, such as sesame oil), aqueous solvents (e.g., saline, phosphate-buffered saline [PBS] and isotonic solutions [e.g., Ringer's solution]), and solvents (e.g., dimethyl sulfoxide [DMSO] and alcohols [e.g., ethanol, glycerol and propylene glycol]). Except insofar as any conventional carrier or excipient is incompatible with the active ingredient, the disclosure encompasses the use of conventional carriers and excipients in formulations containing a therapeutic agent (e.g., a cyclosporine analogue (e.g., CRV431)). See, e.g., Remington: The Science and Practice of Pharmacy, 21st Ed., Lippincott Williams & Wilkins (Philadelphia, Pennsylvania [2005]); Handbook of Pharmaceutical Excipients, 5th Ed., Rowe et al., Eds., The Pharmaceutical Press and the American Pharmaceutical Association (2005); Handbook of Pharmaceutical Additives, 3rd Ed., Ash and Ash, Eds., Gower Publishing Co. (2007); and Pharmaceutical Preformulation and Formulation, Gibson, Ed., CRC Press (Boca Raton, Florida, 2004).

[0161] Proper formulation can depend on various factors, such as the mode of administration chosen. Potential modes of administration of pharmaceutical compositions comprising a cyclosporine analogue (e.g., CRV431) include without limitation oral, parenteral (including intramuscular, subcutaneous, intradermal, intravascular, intravenous, intraarterial, intraperitoneal, intramedullary, intrathecal and topical), intracavitary, and topical (including dermal/epicutaneous, transdermal, mucosal, transmucosal, intranasal [e.g., by nasal spray or drop], pulmonary [e.g., by oral or nasal inhalation], buccal, sublingual, rectal [e.g., by suppository], and vaginal [e.g., by suppository]).

[0162] As an example, formulations of a cyclosporine analogue (e.g., CRV431) suitable for oral administration can be presented as, e.g., boluses; tablets, capsules, pills, cachets or lozenges; as powders or granules; as semisolids, electuaries, pastes or gels; as solutions or suspensions in an aqueous liquid or/and a non-aqueous liquid; or as oil-in-water liquid emulsions or water- in-oil liquid emulsions.

[0163] Tablets can contain a cyclosporine analogue (e.g., CRV431) in admixture with, e.g., a filler or inert diluent (e.g., calcium carbonate, calcium phosphate, lactose, mannitol or microcrystalline cellulose), a binding agent (e.g., a starch, gelatin, acacia, alginate or a salt thereof, or microcrystalline cellulose), a lubricating agent (e.g., stearic acid, magnesium stearate, talc or silicon dioxide), and a disintegrating agent (e.g., croscopolidone, croscarmellose sodium or colloidal silica), and optionally a surfactant (e.g., sodium lauryl sulfate). The tablets can be uncoated or can be coated with, e.g., an enteric coating that protects the active ingredient from the acidic environment of the stomach, or with a material that delays disintegration and absorption of the active ingredient in the gastrointestinal tract and thereby provides a sustained

action over a longer time period. In some embodiments, a tablet comprises a cyclosporine analogue (e.g., CRV431), mannitol, microcrystalline cellulose, magnesium stearate, silicon dioxide, croscarmellose sodium and sodium lauryl sulfate, and optionally lactose monohydrate, and the tablet is optionally film-coated (e.g., with Opadry®).

[0164] Push-fit capsules or two-piece hard gelatin capsules can contain a cyclosporine analogue (e.g., CRV431) in admixture with, e.g., a filler or inert solid diluent (e.g., calcium carbonate, calcium phosphate, kaolin or lactose), a binder (e.g., a starch), a glidant or lubricant (e.g., talc or magnesium stearate), and a disintegrant (e.g., crospovidone), and optionally a stabilizer or/and a preservative. For soft capsules or single-piece gelatin capsules, a cyclosporine analogue (e.g., CRV431) can be dissolved or suspended in a suitable liquid (e.g., liquid polyethylene glycol or an oil medium, such as a fatty oil, peanut oil, olive oil or liquid paraffin), and the liquid-filled capsules can contain one or more other liquid excipients or/and semi-solid excipients, such as a stabilizer or/and an amphiphilic agent (e.g., a fatty acid ester of glycerol, propylene glycol or sorbitol).

[0165] Compositions for oral administration can also be formulated as solutions or suspensions in an aqueous liquid or/and a non-aqueous liquid, or as oil-in-water liquid emulsions or water-in-oil liquid emulsions. Dispersible powder or granules of a cyclosporine analogue (e.g., CRV431) can be mixed with any suitable combination of an aqueous liquid, an organic solvent or/and an oil and any suitable excipients (e.g., any combination of a dispersing agent, a wetting agent, a suspending agent, an emulsifying agent or/and a preservative) to form a solution, suspension or emulsion.

[0166] A cyclosporine analogue (e.g., CRV431) can also be formulated for parenteral administration by injection or infusion to circumvent gastrointestinal absorption and first-pass metabolism. A representative parenteral route is intravenous.

[0167] Additional advantages of intravenous administration include direct administration of a therapeutic agent into systemic circulation to achieve a rapid systemic effect, and the ability to administer the agent continuously or/and in a large volume if desired. Formulations for injection or infusion can be in the form of, e.g., solutions, suspensions or emulsions in oily or aqueous vehicles, and can contain excipients such as suspending agents, dispersing agents or/and stabilizing agents. For example, aqueous or non-aqueous (e.g., oily) sterile injection solutions can contain a cyclosporine analogue (e.g., CRV431) along with excipients such as an antioxidant, a buffer, a bacteriostat and solutes that render the formulation isotonic with the blood of the subject. Aqueous or non-aqueous sterile suspensions can contain a cyclosporine analogue (e.g., CRV431) along with excipients such as a suspending agent and a thickening agent, and optionally a stabilizer and an agent that increases the solubility of the

cyclosporine analogue (e.g., CRV431) to allow for the preparation of a more concentrated solution or suspension. As another example, a sterile aqueous solution for injection or infusion (e.g., subcutaneously or intravenously) can contain a cyclosporine analogue (e.g., CRV431), NaCl, a buffering agent (e.g., sodium citrate), a preservative (e.g., meta-cresol), and optionally a base (e.g., NaOH) or/and an acid (e.g., HCl) to adjust pH.

[0168] For topical administration, a cyclosporine analogue (e.g., CRV431) can be formulated as, e.g., a buccal or sublingual tablet or pill. Buccal or sublingual tablets or pills may avoid first-pass metabolism and circumvention of gastrointestinal absorption. A buccal or sublingual tablet or pill can be designed to provide faster release of the cyclosporine analogue (e.g., CRV431) for more rapid uptake of it into systemic circulation. In addition to a therapeutically effective amount of the cyclosporine analogue (e.g., CRV431), the buccal or sublingual tablet or pill can contain suitable excipients, including without limitation any combination of fillers and diluents (e.g., mannitol and sorbitol), binding agents (e.g., sodium carbonate), wetting agents (e.g., sodium carbonate), disintegrants (e.g., crospovidone and croscarmellose sodium), lubricants (e.g., silicon dioxide [including colloidal silicon dioxide] and sodium stearyl fumarate), stabilizers (e.g., sodium bicarbonate), flavoring agents (e.g., spearmint flavor), sweetening agents (e.g., sucralose), and coloring agents (e.g., yellow iron oxide).

[0169] For topical administration, a cyclosporine analogue (e.g., CRV431) can also be formulated for intranasal administration. The nasal mucosa provides a big surface area, a porous endothelium, a highly vascular subepithelial layer and a high absorption rate, and hence allows for high bioavailability. Moreover, intranasal administration avoids first-pass metabolism and can introduce a significant concentration of the cyclosporine analogue (e.g., CRV431) to the central nervous system, allowing the cyclosporine analogue (e.g., CRV431) to block the central cough reflex via the nucleus tractus solitarius in the cough center in the medulla oblongata, where vagal afferent nerves terminate. An intranasal solution or suspension formulation can comprise a cyclosporine analogue (e.g., CRV431) along with excipients such as a solubility enhancer (e.g., propylene glycol), a humectant (e.g., mannitol or sorbitol), a buffer and water, and optionally a preservative (e.g., benzalkonium chloride), a mucoadhesive agent (e.g., hydroxyethyl cellulose) or/and a penetration enhancer. In some embodiments, a nasal spray formulation comprises a cyclosporine analogue (e.g., CRV431), microcrystalline cellulose, sodium carboxymethylcellulose, dextrose and water, and optionally an acid (e.g., HCl) to adjust pH. An intranasal solution or suspension formulation can be administered to the nasal cavity by any suitable means, including but not limited to a dropper, a pipette, or spray using, e.g., a metering atomizing spray pump. An additional mode of topical administration is pulmonary, including by oral inhalation and nasal inhalation.

[0170] In some embodiments, a cyclosporine analogue (e.g., CRV431) is delivered from a sustained-release composition. As used herein, the term "sustained-release composition" encompasses sustained-release, prolonged-release, extended-release, slow-release and controlled-release compositions, systems and devices. Use of a sustained-release composition can have benefits, such as an improved profile of the amount of the drug or an active metabolite thereof delivered to the target site(s) over a time period, including delivery of a therapeutically effective amount of the drug or an active metabolite thereof over a prolonged time period. In some embodiments, the sustained-release composition delivers the cyclosporine analogue (e.g., CRV431) over a period of at least about 1 day, 2 days, 3 days, 1 week, 2 weeks, 3 weeks, 1 month, 2 months, 3 months or longer. In some embodiments, the sustained-release composition is a drug-encapsulation system, such as nanoparticles, microparticles or a capsule made of, e.g., a biodegradable polymer or/and a hydrogel. In some embodiments, the sustained-release composition comprises a hydrogel. Non-limiting examples of polymers of which a hydrogel can be composed include polyvinyl alcohol, acrylate polymers (e.g., sodium poly acrylate), and other homopolymers and copolymers having a relatively large number of hydrophilic groups (e.g., hydroxyl or/and carboxylate groups). In some embodiments, the sustained-release drug-encapsulation system comprises a membrane-enclosed reservoir, wherein the reservoir contains a drug and the membrane is permeable to the drug. Such a drug-delivery system can be in the form of, e.g., a transdermal patch.

[0171] In some embodiments, the sustained-release composition is an oral dosage form, such as a tablet or capsule. For example, a drug can be embedded in an insoluble porous matrix such that the dissolving drug must make its way out of the matrix before it can be absorbed through the gastrointestinal tract. Alternatively, a drug can be embedded in a matrix that swells to form a gel through which the drug exits. Sustained release can also be achieved by way of a single-layer or multi-layer osmotic controlled-release oral delivery system (OROS). An OROS is a tablet with a semi-permeable outer membrane and one or more small laser-drilled holes in it. As the tablet passes through the body, water is absorbed through the semipermeable membrane via osmosis, and the resulting osmotic pressure pushes the drug out through the hole(s) in the tablet and into the gastrointestinal tract where it can be absorbed.

[0172] The sustained-release composition can be formulated as polymeric nanoparticles or microparticles, wherein the polymeric particles can be delivered, e.g., by inhalation or injection or from an implant. In some embodiments, the polymeric implant or polymeric nanoparticles or microparticles are composed of a biodegradable polymer. In some embodiments, the biodegradable polymer comprises lactic acid or/and glycolic acid [e.g., an L-lactic acid-based copolymer, such as poly(L-lactide-co-glycolide) or poly(L-lactic acid-co-D,L-

2-hydroxyoctanoic acid)). For example, biodegradable polymeric microspheres composed of polylactic acid or/and polyglycolic acid can serve as sustained-release pulmonary drug-delivery systems. The biodegradable polymer of the polymeric implant or polymeric nanoparticles or microparticles can be selected so that the polymer substantially completely degrades around the time the period of treatment is expected to end, and so that the byproducts of the polymer's degradation, like the polymer, are biocompatible.

[0173] For a delayed or sustained release of a cyclosporine analogue (e.g., CRV431), a composition can also be formulated as a depot that can be implanted in or injected into a subject, e.g., intramuscularly or subcutaneously. A depot formulation can be designed to deliver the cyclosporine analogue (e.g., CRV431) over a longer period of time, e.g., over a period of at least about 1 week, 2 weeks, 3 weeks, 1 month, 6 weeks, 2 months, 3 months or longer. For example, the cyclosporine analogue (e.g., CRV431) can be formulated with a polymeric material (e.g., polyethylene glycol (PEG), polylactic acid (PLA) or polyglycolic acid (PGA), or a copolymer thereof (e.g., PLGA)), a hydrophobic material (e.g., as an emulsion in an oil) or/and an ion- exchange resin, or as a sparingly soluble derivative (e.g., a sparingly soluble salt). As an illustrative example, a cyclosporine analogue (e.g., CRV431) can be incorporated or embedded in sustained-release microparticles composed of PLGA and formulated as a monthly depot.

[0174] A cyclosporine analogue (e.g., CRV431) can also be contained or dispersed in a matrix material. The matrix material can comprise a polymer (e.g., ethylene-vinyl acetate) and controls the release of the compound by controlling dissolution or/and diffusion of the compound from, e.g., a reservoir, and can enhance the stability of the compound while contained in the reservoir. Such a release system can be designed as a sustained-release system, can be configured as, e.g., a transdermal or transmucosal patch, and can contain an excipient that can accelerate the compound's release, such as a water- swellable material (e.g., a hydrogel) that aids in expelling the compound out of the reservoir.

[0175] The release system can provide a temporally modulated release profile (e.g., pulsatile release) when time variation in plasma levels is desired, or a more continuous or consistent release profile when a constant plasma level is desired. Pulsatile release can be achieved from an individual reservoir or from a plurality of reservoirs. For example, where each reservoir provides a single pulse, multiple pulses ("pulsatile" release) are achieved by temporally staggering the single pulse release from each of multiple reservoirs.

[0176] In addition, pharmaceutical compositions comprising a cyclosporine analogue (e.g., CRV431) can be formulated as, e.g., liposomes, micelles (e.g., those composed of biodegradable natural or/and synthetic polymers, such as lactosomes), microspheres, microparticles or nanoparticles, whether or not designed for sustained release.

[0177] The pharmaceutical compositions can be manufactured in any suitable manner known in the art, e.g., by means of conventional mixing, dissolving, suspending, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or compressing processes.

[0178] A pharmaceutical composition can be presented in unit dosage form as a single dose wherein all active and inactive ingredients are combined in a suitable system, and components do not need to be mixed to form the composition to be administered. The unit dosage form can contain an effective dose, or an appropriate fraction thereof, of a therapeutic agent (e.g., a cyclosporine analogue (e.g., CRV431). Representative examples of a unit dosage form include a tablet, capsule or pill for oral administration, and powder in a vial or ampoule for oral or nasal inhalation.

[0179] A pharmaceutical composition disclosed herein can be presented as a kit, wherein the active ingredient, excipients and carriers (e.g., solvents) are provided in two or more separate containers (e.g., ampoules, vials, tubes, bottles or syringes) and need to be combined to form the composition to be administered. The kit can contain instructions for storing, preparing and administering the composition (e.g., a solution to be injected intravenously).

[0180] A kit can contain all active and inactive ingredients in unit dosage form or the active ingredient and inactive ingredients in two or more separate containers, and can contain instructions for using the pharmaceutical composition. In some embodiments, a kit comprises a cyclosporine analogue (e.g., CRV431) or a pharmaceutically acceptable salt, solvate, hydrate, clathrate, polymorph, prodrug or metabolite thereof, and instructions for administering the compound.

EXAMPLES

[0181] Some aspects of the embodiments discussed above are disclosed in further detail in the following examples, which are not in any way intended to limit the scope of the present disclosure.

Example 1

CRV431 Treatment of Human Precision Cut Liver Slices (PCLS)

[0182] This example describes a study conducted with human PCLS to determine antifibrotic activity of CRV431, in which it was observed that PCLS from all 5 human donors had some pre-existing fibrosis which was increased by TGF β +PDGF-BB stimulation to 7-11% by fractional area. CRV431 was most effective of five NASH drug candidates in preventing TGF β +PDGF-BB-induced tissue fibrosis. Most CRV431-treated slices also showed less fibrosis than vehicle-treated slices after 6 days of culture in the absence of exogenous TGF β +PDGF-BB.

Decreased tissue fibrosis was accompanied by reduced secretion of collagen1 α 1, fibronectin, hyaluronic acid, IL-6, and MCP-1, and by reductions in RNA levels of collagen1 α 1, α SMA, TIMP1, IL-6, and MCP-1 as demonstrated by qRT-PCR. RNA-Seq analysis similarly showed that CRV431 decreased expression of many fibrosis-related genes, including more than 10 collagen genes, collagen hydroxylases and oxidases, ACTA2, VEGF, and TIMPs. Gene expression varied considerably among donors, such that the expression of fewer than 200 genes were universally changed by CRV431 in all donors. Many of these pan-donor transcriptional changes were consistent with anti-NASH, anti-fibrotic, and anti-oncogenic activities described for the genes in the literature. Notable genes universally influenced by CRV431 in the absence of TGF β +PDGF-BB were ESM1 (2.2-fold decrease in RNA; -2.2), NCOA3 (-2.7), IFI44L (-4.8), miR-194-2 (-7.9), and DKK1 (3.8-fold increase in RNA; +3.8). In the presence of exogenous TGF β +PDGF-BB, notable genes universally influenced by CRV431 were LOXL2 (-1.9) UBD/FAT10 (-2.0), ESM1 (-2.6), STRA6 (-3.1), RCCD1 (-3.6), and DUOX2 (-4.5). Without being bound by any particular theory, it is believed that the results described herein indicate CRV431 is capable of preventing fibrosis formation and reversing fibrosis.

[0183] PCLS were obtained from 5 human donors who underwent resection of liver cancer. Replicate slices were collected from healthy margins of the resections and cultured for 4 or 6 days depending on the experimental protocol. In the Nonstimulation Protocol slices were cultured for 6 days and treated for the entire period with DMSO vehicle or 5 μ M CRV431. In the Stimulation Protocol slices were rested for 1 day and then administered the cytokines, TGF β and PDGF-BB, for 3 days to stimulate inflammation and fibrosis. DMSO vehicle, CRV431 (1 and 5 μ M), Alk5i (10 μ M), obeticholic acid (5 μ M), elafibranor (5 μ M), resmetirom (5 μ M), and Aramchol (5 μ M) were administered individually as drug treatments in the Stimulation Protocol concurrent with TGF β + PDGF-BB. Culture medium treatments were replaced daily.

[0184] Four types of evaluations were conducted on the PCLS: (a) secretion of inflammation and fibrosis biomarkers into the culture medium, (b) histological staining and quantitation of Sirius red as a measure of tissue fibrosis, (c) gene expression of inflammation and fibrosis biomarkers by RT-PCR, and (d) RNA sequencing (RNA-Seq) of the complete transcriptome. Each of the four types of evaluation is described below:

[0185] (a) Biomarker secretion. Spent culture medium was collected daily from duplicate slices for each treatment. ELISAs were used to quantify secretion of monocyte chemoattractant protein (MCP-1), interleukin-6 (IL-6), tissue inhibitor of metalloproteinase-1 (TIMP1), hyaluronic acid, fibronectin, and collagen 1 α 1. For each donor the mean level of biomarker secretion in each treatment group was expressed as a percentage change relative to DMSO vehicle, the percentages averaged for all donors, and finally the mean daily percentage

change calculated from all days of evaluation. Results are shown in Figure 1A (secreted markers - % daily average change).

[0186] (b) Sirius red histological staining. PCLS were processed histologically at the end of the experiments and stained with Sirius red to demonstrate tissue fibrosis. Sirius red was quantified morphometrically in the histological sections as the percentage area with Sirius red staining in 10 sections from each treatment. In the Nonstimulation Protocol the quantity of Sirius red staining was expressed relative to the vehicle group. In the Stimulation Protocol the quantity of Sirius red staining was expressed relative to nonstimulated PCLS (0%) and TGF β + PDGF-BB-stimulated + vehicle PCLS (100%). Results are shown in Figure 1B (Sirius red staining for tissue fibrosis).

[0187] (c) Gene expression by RT-PCR. RNA was isolated from duplicate liver slices per treatment at the end of the experiments and evaluated by RT-PCR for 5 markers of inflammation and fibrosis: MCP-1, IL-6, TIMP1, α SMA, and collagen 1 α 1. β actin was used as a reference gene to calculate relative levels of each target gene RNA. CRV431 applied at 5 μ M concentration decreased the mean daily secretion and gene expression of all markers in similarity to Alk5i (inhibitor of TGF β receptor kinase), pirfenidone (approved treatment for IPF), and nintedanib (approved treatment for IPF). Results are shown in Figure 1C (gene expression by RT-PCR).

[0188] (d) Gene expression by RNA-Seq. RNA sequencing of the complete transcriptome (30 million reads per sample) was conducted on vehicle and 5 μ M CRV431 treatment groups from 3 donors both from the Nonstimulation and Stimulation Protocols. Data were analyzed by bioinformatics software programs to identify genes that were differentially expressed between vehicle and CRV431 treatments.

[0189] For the evaluation done for (d), 12 samples corresponding to 3 different donor slides which underwent 4 different treatments were studied. the comparisons carried out in this analysis are: (1) TGFb/PDGF + CRV vs TGFb/PDGF + Vehicle (V), and (2) Non-stimulated + CRV vs Non-stimulated + Vehicle. Table 1 shows sample ID and the corresponding treatment.

Table 1. Samples for evaluation (d)

Sample ID	Treatment
CVR_2.1_3	TGFb/PDGF+V
CVR_2.1_9	TGFb/PDGF+CRV
CVR_2.1_19	Control+V
CVR_2.1_21	Control+CRV
CVR_2.2_3	TGFb/PDGF+V
CVR_2.2_9	TGFb/PDGF+CRV
CVR_2.2_19	Control+V
CVR_2.2_21	Control+CRV
CVR_2.4_3	TGFb/PDGF+V

CVR 2.4 9	TGFb/PDGF+CRV
CVR 2.4 19	Control+V
CVR 2.4 21	Control+CRV

[0190] Quality Control check was performed in all samples. All samples had more than 30 million reads and a quality score average >35 for almost all the bases in the sequences. The duplication levels are expected as it is RNA sequencing, and different copies of the same RNA are expected to be present in the samples. All samples had good amount of reads and quality, therefore all samples passed the QC checked and were included in the analysis.

[0191] TGFb/PDGF + CRV431 vs TGFb/PDGF + Vehicle were compared by group. A Principal Components Analysis (PCA) plot was generated to analyze the distribution of the sample (Figure 2A), which shows a strong effect of the donor, as they cluster together based on donor; however, there are also differences between the treatment, as they separate in the PCA plot as shown in Figure 2B.

[0192] In the MA plot of Figure 2C, mean counts were plotted against the log fold change. Showing in red the significant genes differently expressed in the comparison TGFb/PDGF + CRV and TGFb/PDGF + Vehicle (p-adjust<0.05).

[0193] Heatmaps were also generated to plot significant differently expressed genes for the comparison TGFb/PDGF + CRV and TGFb/PDGF + Vehicle (p-adjust<0.05, log fold change > 0.5). The heatmap with the samples grouped by group is shown in Figure 3A to observe the different expression pattern present within the group (blue=vehicle; red=CRV). The same significant genes were also plotted into another heatmap (Figure 3B) but the samples are now plotted grouped by donor in order to observe the differences between donors (blue=vehicle; red=CRV).

[0194] In addition, significant hits are plotted in a volcano plot (Figure 4), in which the significant genes were plotted in red and labelled with their correspondent symbol ID. Down and up-regulated are relative to CRV treatment.

[0195] Significant differently expressed genes that were identified in the comparison TGFb/PDGF + CRV and TGFb/PDGF + Vehicle (p-adjust>0.05, log fold change> 0.5) are shown in Table 2. The fold change is relative to the treatment (CRV).

Table 2. Significant differently expressed genes identified in the comparison TGFb/PDGF + CRV and TGFb/PDGF + Vehicle

ensembl_geneid	symbol	log2FoldChange	padj
ENSG00000164283	ESM1	-1.401	3.29283493401111e-10
ENSG00000172955	ADH6	1.903	2.94236428678419e-07
ENSG00000134013	LOXL2	-0.948	1.19798115569287e-06
ENSG00000124222	STX16	1.025	1.67355828789889e-06

ENSG00000180776	ZDHHC20	1.207	1.73084943004934e-06
ENSG00000078699	CBFA2T2	1.299	4.29111173342668e-06
ENSG00000198804	MT-CO1	-0.85	5.14890213770242e-06
ENSG00000213516	RBMXL1	-8.265	5.51686338456531e-06
ENSG00000196616	ADH1B	2.156	1.22290666093167e-05
ENSG00000080573	COL5A3	-0.842	5.37672079837298e-05
ENSG00000076706	MCAM	-0.841	6.63581694668706e-05
ENSG00000140279	DUOX2	-2.182	6.63581694668706e-05
ENSG00000166840	GLYATL1	1.438	6.63581694668706e-05
ENSG00000166840	GLYATL1	1.438	6.63581694668706e-05
ENSG00000211970	IGHV4-61	7.957	6.63581694668706e-05
ENSG00000095596	CYP26A1	-1.283	0.000663869405904214
ENSG00000135473	PAN2	1.019	0.000663869405904214
ENSG00000187048	CYP4A11	1.611	0.00131126021103255
ENSG00000050438	SLC4A8	-2.641	0.00135464161540089
ENSG00000198887	SMC5	0.799	0.00135464161540089
ENSG00000272196	HIST2H2AA4	-7.504	0.00135464161540089
ENSG00000282827	AC134772.2	-4.561	0.00135464161540089
ENSG00000283623	NA	-5.759	0.00135464161540089
ENSG00000198099	ADH4	2.123	0.00191949000960653
ENSG00000151790	TDO2	0.916	0.00312047886997339
ENSG00000175264	CHST1	-1.087	0.0033627319829465
ENSG00000087303	NID2	-0.755	0.00436564894103764
ENSG00000154639	CXADR	1.61	0.00465248444892784
ENSG00000101445	PPP1R16B	-1.016	0.00504512080532397
ENSG00000076356	PLXNA2	1.326	0.00524948770298015
ENSG00000166183	ASPG	0.91	0.00524948770298015
ENSG00000081189	MEF2C	-1.013	0.00569870441559758
ENSG00000166965	RCCD1	-1.838	0.00813696698391237
ENSG00000102763	VWA8	1.514	0.00820494655996272
ENSG00000248144	ADH1C	1.869	0.00820494655996272
ENSG00000128917	DLL4	-0.69	0.00990020877847807
ENSG00000256977	LIMS3	-1.705	0.00990020877847807
ENSG00000148671	ADIRF	1.402	0.0105361838033656
ENSG00000112936	C7	0.739	0.0107467217612096
ENSG00000177464	GPR4	-0.9	0.0116584644837567
ENSG00000137868	STRA6	-1.639	0.0126077678419959
ENSG00000091831	ESR1	3.688	0.0146645434418194
ENSG00000154016	GRAP	-0.772	0.0146645434418194
ENSG00000166341	DCHS1	-0.686	0.0157049728052682
ENSG00000140519	RHCG	-0.927	0.0157568332080644
ENSG00000175606	TMEM70	1.062	0.0157568332080644
ENSG00000113555	PCDH12	-0.741	0.0183319430393364
ENSG00000125810	CD93	-0.613	0.0183319430393364
ENSG00000184271	POU6F1	-1.716	0.0183319430393364
ENSG00000213886	UBD	-1.035	0.0187122936785154
ENSG00000147050	KDM6A	0.785	0.019070909371344
ENSG00000167178	ISLR2	-1.27	0.019070909371344
ENSG00000116191	RALGPS2	0.836	0.0205154157563514
ENSG00000112769	LAMA4	-0.939	0.0213416290882877

ENSG00000127249	ATP13A4	-3.082	0.0274603874188308
ENSG00000142549	IGLON5	-0.851	0.030298756625196
ENSG00000172943	PHF8	0.709	0.0303737817896317
ENSG00000197808	ZNF461	1.391	0.0303737817896317
ENSG00000263266	RPS7P1	2.668	0.0303737817896317
ENSG00000011028	MRC2	-0.773	0.0347062844025796
ENSG00000135482	ZC3H10	1.022	0.0347062844025796
ENSG00000141540	TTYH2	-1.114	0.0347062844025796
ENSG00000182379	NXPH4	-0.808	0.0347062844025796
ENSG00000214548	MEG3	-0.853	0.0347062844025796
ENSG00000260565	ERVK13-1	-1.125	0.0347062844025796
ENSG00000143627	PKLR	2.127	0.0389991819266929
ENSG00000003137	CYP26B1	-0.656	0.0406743042317417
ENSG00000063180	CA11	-1.573	0.0417678950796777
ENSG00000214331	AC009053.1	1.194	0.0417678950796777
ENSG00000225697	SLC26A6	-0.692	0.0417678950796777
ENSG00000107404	DVL1	0.635	0.0419108509612772
ENSG00000127533	F2RL3	-0.711	0.0462741524861874
ENSG00000134817	APLNR	0.844	0.0462741524861874
ENSG00000139540	SLC39A5	1.92	0.0484295024414694
ENSG00000149564	ESAM	-0.573	0.0484295024414694
ENSG00000185038	MROH2A	3.641	0.0484295024414694
ENSG00000120137	PANK3	0.687	0.0484878857306103

[0196] As shown in the PCA analysis, there is a strong effect of the donor. Therefore, each donor was analyzed individually. Counts comparison was performed for TGFb/PDGF + CRV341 vs TGFb/PDGF + Vehicle by donor. First, genes that had a difference in the number of counts (TGFb/PDGF + Vehicle - TGFb/PDGF + CRV) <-300 were selected. Therefore the vehicle group had at least 300 copies less than CRV treatment. In other words, the treatment had at least 300 copies more than the Vehicle. After doing this selection individually for each donor, a venn diagram was plotted showing the overlapping between all three donors. As shown in Figure 5A, 117 genes had at least 300 copies more in the CRV treated samples than in the vehicle.

[0197] Then, genes that had a difference in the number of counts (TGFb/PDGF + Vehicle - TGFb/PDGF + CRV) >300 were selected, therefore those genes that had at least 300 more copies in the vehicle sample, compared to the CRV treated, were selected. So, they had at least 300 copies less in the CRV treatment compared to the vehicle. After doing the selection individually for each donor, a venn diagram was plotted showing the overlapping between all three donors. As shown in Figure 5B, 279 genes had at least 300 copies less in the CRV treated than in the vehicle.

[0198] Nonstimulated + CRV431 vs Nonstimulated + Vehicle were then compared by group. A Principal Components Analysis (PCA) plot was generated to analyze the

distribution of the sample (Figure 6A). As expected, a strong effect of the donor was observed, as they cluster together base on donor and not on treatment. But there were differences between the treatment as they separate in the PCA plot (Figure 6B).

[0199] For MA plot shown in Figure 6C, mean counts were plotted against the log fold change. Showing in red the significant genes differently expressed in the comparison Non-stimulated + CRV and Non-stimulated + Vehicle (p-adjust<0.05).

[0200] Heatmaps were generated by plotting significant differently expressed genes for the comparison Non-stimulated + CRV and Non-stimulated + Vehicle (p-adjust<0.05, log fold change > 0.5). First, the heatmap with the samples grouped by group (Figure 7A) is shown in order to observe the different expression pattern present within the group (blue= vehicle; red= CRV). Then, the same significant genes were plotted into a heatmap (Figure 7B), but the samples are now plotted grouped by donor in order to observe the differences between donors (blue= vehicle; red= CRV).

[0201] Significant hits were plotted in a volcano plot (Figure 8). The significant genes are plotted in red and labelled with its correspondent symbol ID. Down and up-regulated genes are relative to the treatment CRV.

[0202] Significant differently expressed genes that were identified in the comparison Non-stimulated + CRV and Non-stimulated + Vehicle (p-adjust>0.05, log fold change> 0.5) are provided in **Table 3**. The fold changes shown are relative to the treatment CRV.

Table 3. Significant differently expressed genes identified in the comparison Non-stimulated + CRV and Non-stimulated + Vehicle

ensembl_geneid	symbol	log2FoldChange	padj
ENSG00000242265	PEG10	1.463	7.64497256360981e-13
ENSG00000198804	MT-CO1	-1.083	3.14975868134979e-08
ENSG00000146122	DAAM2	1.498	8.53610989000754e-08
ENSG00000137959	IFI44L	-2.254	5.03046934688257e-07
ENSG00000078403	MLLT10	-1.19	7.17576575470264e-07
ENSG00000076356	PLXNA2	1.719	7.55834893080804e-07
ENSG00000106538	RARRES2	-0.766	1.40363819097048e-05
ENSG00000070081	NUCB2	0.851	3.45044971944497e-05
ENSG00000144591	GMPPA	0.768	3.45044971944497e-05
ENSG00000013375	PGM3	0.751	4.29563263720564e-05
ENSG00000124151	NCOA3	-1.438	9.05565703215956e-05
ENSG00000128590	DNAJB9	0.721	0.000105934561817305
ENSG00000197632	SERPINB2	1.255	0.000223315832408411
ENSG00000028277	POU2F2	2.856	0.000298692252063288
ENSG00000109321	AREG	1.034	0.000298692252063288
ENSG00000135480	KRT7	0.563	0.000298692252063288
ENSG00000166562	SEC11C	0.844	0.000298692252063288
ENSG00000214548	MEG3	-0.682	0.000298692252063288

ENSG00000229719	MIR194-2HG	-2.973	0.000298692252063288
ENSG00000132530	XAF1	-0.725	0.000442143354650337
ENSG00000140525	FANCI	-1.319	0.000486460299226267
ENSG00000077044	DGKD	-0.85	0.000490134073876104
ENSG00000185513	L3MBTL1	-2.03	0.000606634757391397
ENSG00000205426	KRT81	1.137	0.000694123487010014
ENSG00000173540	GMPPB	0.715	0.000764920012703645
ENSG00000111452	ADGRD1	-1.126	0.00186864914063056
ENSG00000147854	UHRF2	-0.778	0.00186864914063056
ENSG00000164949	GEM	-0.975	0.00200478523553848
ENSG00000135709	KIAA0513	-0.98	0.00288083461992706
ENSG00000170525	PFKFB3	-0.545	0.00288083461992706
ENSG00000107984	DKK1	1.939	0.0038442433397899
ENSG00000197283	SYNGAP1	-1.718	0.0038442433397899
ENSG00000150961	SEC24D	0.58	0.00406643464218239
ENSG00000157654	PALM2-AKAP2	-1.035	0.00417168314039161
ENSG00000204642	HLA-F	-0.727	0.00417168314039161
ENSG00000186818	LILRB4	0.909	0.00417691201899714
ENSG00000117394	SLC2A1	-0.635	0.00426895599329709
ENSG00000132005	RFX1	-1.495	0.00426895599329709
ENSG00000163430	FSTL1	-0.595	0.00426895599329709
ENSG00000124222	STX16	0.577	0.00440249554429453
ENSG00000134709	HOOK1	-0.998	0.00506965939803037
ENSG00000133401	PDZD2	-1.468	0.00513572755975289
ENSG00000189233	NUGGC	1.136	0.00557406393671569
ENSG00000275066	SYNRG	-0.74	0.00557406393671569
ENSG00000073754	CD5L	1.675	0.00696061006715629
ENSG00000126709	IFI6	-1.273	0.00812520880633794
ENSG00000151893	CACUL1	-0.94	0.00812520880633794
ENSG00000237973	MTCO1P12	-1.183	0.00812520880633794
ENSG00000129128	SPCS3	0.715	0.00830997247774313
ENSG00000167861	HID1	0.716	0.00836179166380686
ENSG00000060237	WNK1	-0.698	0.00871291063051336
ENSG00000105609	LILRB5	1.075	0.00871291063051336
ENSG00000108679	LGALS3BP	-0.556	0.00931883426718094
ENSG00000164283	ESM1	-1.157	0.00982663156220979
ENSG00000157933	SKI	-0.931	0.0101551930217387
ENSG00000108599	AKAP10	-1.249	0.0104187139875557
ENSG00000141994	DUS3L	0.745	0.0107670886056319
ENSG00000163754	GYG1	0.94	0.0124948431676863
ENSG00000188404	SELL	2.035	0.0124948431676863
ENSG00000170006	TMEM154	-1.351	0.013539360764021
ENSG00000224389	C4B	-0.506	0.013539360764021
ENSG00000129009	ISLR	-0.815	0.0136013822876729
ENSG00000145050	MANF	0.682	0.0136013822876729
ENSG00000074181	NOTCH3	-0.564	0.013877649399757
ENSG00000196739	COL27A1	-0.545	0.013877649399757
ENSG00000272578	AP000347.1	1.809	0.013877649399757
ENSG00000135473	PAN2	0.742	0.0142537182784554
ENSG00000059145	UNKL	-0.916	0.0148533925176707

ENSG00000105738	SIPA1L3	-0.652	0.014997918499619
ENSG00000106803	SEC61B	0.602	0.015634934319665
ENSG00000180879	SSR4	0.564	0.015634934319665
ENSG00000160868	CYP3A4	-2.313	0.0171844499432494
ENSG00000112759	SLC29A1	-0.53	0.0171960065344814
ENSG00000135744	AGT	-0.627	0.0173515994784034
ENSG00000099889	ARVCF	-0.609	0.0181970100656262
ENSG00000149474	KAT14	0.874	0.0182111511073747
ENSG00000101294	HM13	0.528	0.0183391036656796
ENSG00000136383	ALPK3	-0.621	0.0183391036656796
ENSG00000179912	R3HDM2	-0.678	0.0183391036656796
ENSG00000125735	TNFSF14	-0.681	0.0186023821876669
ENSG00000135537	AFG1L	1.327	0.0186023821876669
ENSG00000226950	DANCR	0.693	0.020979092384978
ENSG00000180900	SCRIB	0.661	0.0249785512795709
ENSG00000163735	CXCL5	0.663	0.0251528991580969
ENSG00000214021	TTLL3	-0.579	0.0265583936173043
ENSG00000100979	PLTP	-0.584	0.0266061673079832
ENSG00000259970	AC099668.1	1.056	0.0273196728101709
ENSG00000105650	PDE4C	-0.669	0.028386879814035
ENSG00000105650	PDE4C	-0.669	0.028386879814035
ENSG00000090061	CCNK	0.545	0.0285388969844227
ENSG00000134061	CD180	1.051	0.0285388969844227
ENSG00000175265	GOLGA8A	-0.669	0.0305240081123133
ENSG00000185379	RAD51D	1.387	0.0308390955534405
ENSG00000119922	IFIT2	-0.812	0.0311836408752711
ENSG00000107282	APBA1	-1.158	0.0320978587782238
ENSG00000165125	TRPV6	0.83	0.0329864085040579
ENSG00000107562	CXCL12	0.623	0.0347973357286517
ENSG00000134986	NREP	-0.638	0.0347973357286517
ENSG00000158555	GDPD5	-0.606	0.0347973357286517
ENSG00000181982	CCDC149	0.76	0.0347973357286517
ENSG00000197629	MPEG1	0.584	0.0347973357286517
ENSG00000242125	SNHG3	0.665	0.0347973357286517
ENSG00000123130	ACOT9	0.543	0.0375735367132606
ENSG00000261578	AP003119.3	-0.67	0.0376685663501125
ENSG00000137497	NUMA1	-0.537	0.0397028864413373
ENSG00000137628	DDX60	-0.739	0.0409633137500865
ENSG0000019186	CYP24A1	-1.499	0.0424990125003709
ENSG00000196369	SRGAP2B	-0.729	0.0424990125003709
ENSG00000198142	SOWAHC	-0.601	0.0424990125003709
ENSG00000140937	CDH11	-0.915	0.0426576776979742
ENSG00000110079	MS4A4A	0.735	0.0492158532765886

[0203] Nonstimulated + CRV431 vs Nonstimulated + Vehicle were then compared by donor. As shown in the PCA, there was a strong effect of the donor. Therefore, the analysis was repeated as in the previous comparison. Firstly, genes that had a difference in the number of counts (Non-stimulated + Vehicle - Non-stimulated + CRV) < -300 were selected, therefore the

vehicle group had at least 300 copies less than CRV treatment. In other words, there were at least 300 copies more in the treatment CRV than in the vehicle. After doing this selection individually for each donor, a venn diagram was plotted showing the overlapping between all three donors. As shown in Figure 9A, 210 genes had at least 300 copies more in the CRV treatment than in the Vehicle. Then, genes that had a difference in the number of counts (Non-stimulated + Vehicle - Non-stimulated + CRV) >300 were selected, therefore those genes that had at least 300 more copies in the vehicle sample, compared to the CRV treated, were selected. So, there are at least 300 copies less in the treated group compared to the vehicle. After doing the selection individually for each donor, a venn diagram was plotted showing the overlapping between all three donors. As shown in Figure 9B, 255 genes had 300 or less copies in the CRV treated than in the vehicle.

Example 2

CRV431 Sensitization of Hepatocellular Carcinoma Cells to Daunorubicin

[0204] HepG2 hepatocellular carcinoma cell lines and Huh7 hepatocellular carcinoma cell lines were plated in 96 well plates at subconfluent densities. The chemotherapy compound, daunorubicin, and CRV431 were applied alone or in combination at the indicated concentrations in duplicate wells for each treatment. After 4 days incubation, cell viability was assessed with Cell Titer Glo 2.0.

[0205] As shown in Figures 10A-C, for HepG2 cells, CRV431 increased the cytotoxicity of daunorubicin. The strongest, dose-dependent, sensitizing effect of CRV431 was observed with 0.37 μ M daunorubicin and CRV431 at concentrations ranging from 0.25 – 4 μ M.

[0206] As shown in Figures 10D-F, for Huh7 cells, CRV431 increased the cytotoxicity of daunorubicin. The strongest, dose-dependent, sensitizing effect of CRV431 was observed with 0.37 μ M daunorubicin and CRV431 at concentrations ranging from 1 – 4 μ M.

Example 3

CRV431 Treatment

[0207] NASH was initiated in 2-day-old C57BL/6J male mice by intraperitoneal injection with 200 mg streptozotocin (S0130; Sigma-Aldrich) to disrupt pancreatic β cells, induce diabetes, and promote adiposity in the liver. Mice were weaned at 3 weeks of age and begun on a high-fat diet with 60%kcal fat (D12492N; Research Diets) for the duration of the studies. Additionally, mice with a regular diet (D12450KN; Research Diets) and without streptozotocin were included as negative controls. CRV431 was dissolved in a self-microemulsifying drug vehicle and then diluted with PBS and administered daily by oral gavage at 50 mg/kg per day. The start time and duration of vehicle and CRV431 treatments varied depending on the study.

[0208] Mouse livers examined at week 14 had no tumors, whereas vehicle-treated mice at week 30 had an extensive tumor load, indicating that liver tumors developed sometime during weeks 14–30. Histopathological assessment indicated that the tumors were cellular in nature and therefore likely represented hepatocellular carcinoma. Moreover, in the vehicle control group all mice had liver tumors, and 3 of 10 livers had nodules that were 1 cm or larger in diameter. In contrast, CRV431 treatment from weeks 20 to 30 resulted in half the number of tumors, with smaller tumors on average, with 2 of 10 livers of CRV431-treated mice lacking tumors. A scoring system reflecting a combination of tumor number and size revealed that CRV431 decreased the tumor burden by 52%. Further studies are required to determine whether the reduced tumor load is mechanistically linked to the lower fibrosis levels or reflects a more direct effect on the cells.

[0209] In two separate experiments, tumor burden at the end of treatment was assessed by the number of tumors (Figures 11A and 12A) and a composite score based on the number and size of tumors (0–7 scale; Figures 11B and 12B). **Table 4** shows the means of tumor number of tumor score in the two groups treated by the vehicle and by CRV431, respectively.

Table 4. Tumor number and score

	Mean in Vehicle	Mean in CRV431 50 mg/kg/day group	T-test
Tumor number	9.78	4.80	0.0240
Tumor score	4.22	2.40	0.0218

[0210] In at least some of the previously described embodiments, one or more elements used in an embodiment can interchangeably be used in another embodiment unless such a replacement is not technically feasible. It will be appreciated by those skilled in the art that various other omissions, additions and modifications may be made to the methods and structures described above without departing from the scope of the claimed subject matter. All such modifications and changes are intended to fall within the scope of the subject matter, as defined by the appended claims.

[0211] With respect to the use of substantially any plural and/or singular terms herein, those having skill in the art can translate from the plural to the singular and/or from the singular to the plural as is appropriate to the context and/or application. The various singular/plural permutations may be expressly set forth herein for sake of clarity. As used in this specification and the appended claims, the singular forms “a,” “an,” and “the” include plural references unless the context clearly dictates otherwise. Any reference to “or” herein is intended to encompass “and/or” unless otherwise stated.

[0212] It will be understood by those within the art that, in general, terms used herein, and especially in the appended claims (*e.g.*, bodies of the appended claims) are generally intended as “open” terms (*e.g.*, the term “including” should be interpreted as “including but not limited to,” the term “having” should be interpreted as “having at least,” the term “includes” should be interpreted as “includes but is not limited to,” etc.). It will be further understood by those within the art that if a specific number of an introduced claim recitation is intended, such an intent will be explicitly recited in the claim, and in the absence of such recitation no such intent is present. For example, as an aid to understanding, the following appended claims may contain usage of the introductory phrases “at least one” and “one or more” to introduce claim recitations. However, the use of such phrases should not be construed to imply that the introduction of a claim recitation by the indefinite articles “a” or “an” limits any particular claim containing such introduced claim recitation to embodiments containing only one such recitation, even when the same claim includes the introductory phrases “one or more” or “at least one” and indefinite articles such as “a” or “an” (*e.g.*, “a” and/or “an” should be interpreted to mean “at least one” or “one or more”); the same holds true for the use of definite articles used to introduce claim recitations. In addition, even if a specific number of an introduced claim recitation is explicitly recited, those skilled in the art will recognize that such recitation should be interpreted to mean at least the recited number (*e.g.*, the bare recitation of “two recitations,” without other modifiers, means at least two recitations, or two or more recitations). Furthermore, in those instances where a convention analogous to “at least one of A, B, and C, etc.” is used, in general such a construction is intended in the sense one having skill in the art would understand the convention (*e.g.*, “a system having at least one of A, B, and C” would include but not be limited to systems that have A alone, B alone, C alone, A and B together, A and C together, B and C together, and/or A, B, and C together, etc.). In those instances where a convention analogous to “at least one of A, B, or C, etc.” is used, in general such a construction is intended in the sense one having skill in the art would understand the convention (*e.g.*, “a system having at least one of A, B, or C” would include but not be limited to systems that have A alone, B alone, C alone, A and B together, A and C together, B and C together, and/or A, B, and C together, etc.). It will be further understood by those within the art that virtually any disjunctive word and/or phrase presenting two or more alternative terms, whether in the description, claims, or drawings, should be understood to contemplate the possibilities of including one of the terms, either of the terms, or both terms.

[0213] In addition, where features or aspects of the disclosure are described in terms of Markush groups, those skilled in the art will recognize that the disclosure is also thereby described in terms of any individual member or subgroup of members of the Markush group.

[0214] As will be understood by one skilled in the art, for any and all purposes, such as in terms of providing a written description, all ranges disclosed herein also encompass any and all possible sub-ranges and combinations of sub-ranges thereof. Any listed range can be easily recognized as sufficiently describing and enabling the same range being broken down into at least equal halves, thirds, quarters, fifths, tenths, etc. As a non-limiting example, each range discussed herein can be readily broken down into a lower third, middle third and upper third, etc. As will also be understood by one skilled in the art all language such as “up to,” “at least,” “greater than,” “less than,” and the like include the number recited and refer to ranges which can be subsequently broken down into sub-ranges as discussed above. Finally, as will be understood by one skilled in the art, a range includes each individual member. Thus, for example, a group having 1-3 articles refers to groups having 1, 2, or 3 articles. Similarly, a group having 1-5 articles refers to groups having 1, 2, 3, 4, or 5 articles, and so forth.

[0215] While various aspects and embodiments have been disclosed herein, other aspects and embodiments will be apparent to those skilled in the art. The various aspects and embodiments disclosed herein are for purposes of illustration and are not intended to be limiting, with the true scope and spirit being indicated by the following claims.