

## METHODS OF TREATING CONDITIONS WITH MUTANT P53 REACTIVATING COMPOUNDS

### CROSS REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application Number 63/669,209, filed July 9, 2024, U.S. Provisional Patent Application Number 63/632,458, filed April 10, 2024, U.S. Provisional Patent Application Number 63/566,417, filed March 18, 2024, and U.S. Provisional Patent Application Number 63/589,229, filed October 10, 2023, each of which is incorporated by reference herein in its entirety.

### BACKGROUND

[0002] Cancer, an uncontrolled proliferation of cells, is a multifactorial disease characterized by tumor formation, growth, and in some instances, metastasis. Cells carrying an activated oncogene, damaged genome, or other cancer-promoting alterations can be prevented from replicating through an elaborate tumor suppression network. A central component of this tumor suppression network is p53, one of the most potent tumor suppressors in the cell. Both the wild-type and mutant conformations of p53 are implicated in the progression of cancer.

### INCORPORATION BY REFERENCE

[0003] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

### SUMMARY

[0004] In some embodiments, the present disclosure provides a method of treating a condition in a subject in need thereof, the method comprising administering to the subject a therapeutically-effective amount of a small molecule compound that binds a p53 mutant protein, wherein a mutational status of a KRAS gene in the subject has been previously determined.

[0005] In some embodiments, the present disclosure provides a method of treating a condition in a subject in need thereof, the method comprising: a) based on a result of an assay performed on a biological sample of the subject, determining a mutational status of a KRAS gene in the subject; and b) based at least in part on the mutational status of the KRAS gene in the subject, administering to the subject a therapeutically-effective amount of a compound that binds a p53 mutant protein.

### BRIEF DESCRIPTION OF THE DRAWINGS

[0006] **FIGURE 1** illustrates the dose escalation scheme for a study evaluating Compound 1 in the treatment of cancer.

[0007] **FIGURE 2** is a chart illustrating day 15 (steady state) exposures of Compound 1 in human subjects.

[0008] FIGURE 3 is a chart summarizing target lesion reduction across tumor types (in patients administered 1150 mg QD to 1500 mg BID Compound 1) in the *TP53* Y220C/*KRAS* wild-type efficacy evaluable population.

[0009] FIGURE 4 is a chart illustrating the duration of Compound 1 therapy in patients in the *TP53* Y220C/*KRAS* wild-type efficacy evaluable population.

[0010] FIGURE 5 is a chart summarizing the percentage change in *TP53* Y220C variant allele frequency at Week 3 from baseline of Compound 1 treatment course.

[0011] FIGURE 6 provides patient images of triple negative breast cancer at baseline and post-cycle 3 of Compound 1 treatment course.

[0012] FIGURE 7 is a chart summarizing best percent change in sum of tumor target lesions amongst the 15 patients in the ovarian subgroup with measurable disease.

[0013] FIGURE 8 is a chart summarizing the best percent change in CA-125 levels of the 15 patients in the ovarian subgroup with measurable CA-125 at baseline.

[0014] FIGURE 9 is a chart summarizing the time to response and duration of treatment for patients in the ovarian subgroup with measurable disease at baseline at  $\geq 1$  post baseline tumor assessment.

[0015] FIGURE 10 shows a linear plot of plasma concentration over time of 1000 mg Compound 1 in fed or fasted states.

[0016] FIGURE 11 shows a semi-logarithmic plot of plasma concentration over time of 1000 mg Compound 1 in fed or fasted states.

[0017] FIGURE 12 shows a linear plot of plasma concentration over time of 2000 mg Compound 1 according to ethnicity in fed or fasted states.

[0018] FIGURE 13 shows a semi-logarithmic plot of plasma concentration over time of 2000 mg Compound 1 according to ethnicity in fed or fasted states.

[0019] FIGURE 14 shows a ladder plot of 1000 mg Compound 1 plasma pharmacokinetic parameter  $AUC_{0-inf}$  in fed or fasted states.

[0020] FIGURE 15 shows a ladder plot of 1000 mg Compound 1 plasma pharmacokinetic parameter  $AUC_{0-last}$  in fed or fasted states.

[0021] FIGURE 16 shows a ladder plot of 1000 mg Compound 1 plasma pharmacokinetic parameter  $AUC_{0-last}$  in fed or fasted states.

[0022] FIGURE 17 shows a ladder plot of 2000 mg Compound 1 plasma pharmacokinetic parameter  $AUC_{0-inf}$  in fed or fasted states for Caucasian/non-Asian subjects.

[0023] FIGURE 18 shows a ladder plot of 2000 mg Compound 1 plasma pharmacokinetic parameter  $AUC_{0-last}$  in fed or fasted states for Caucasian/non-Asian subjects.

[0024] FIGURE 19 shows a ladder plot of 2000 mg Compound 1 plasma pharmacokinetic parameter  $C_{max}$  in fed or fasted states for Caucasian/non-Asian subjects.

[0025] FIGURE 20 shows a plot of arithmetic mean urinary cumulative excretion fraction (%) of 1000 mg Compound 1 over time in fed or fasted states.

[0026] **FIGURE 21** shows a plot of arithmetic mean urinary cumulative excretion fraction (%) of 1000 mg Compound 1 over time in fed or fasted states for Caucasian/non-Asian subjects.

[0027] **FIGURE 22** shows a plot of arithmetic mean urinary cumulative excretion fraction (%) of 1000 mg Compound 1 over time in fasted states for Japanese subjects.

#### DETAILED DESCRIPTION

[0028] The present disclosure provides, for example, methods for restoring wild-type function to mutant p53 using compounds disclosed herein. The compounds can bind to mutant p53 and restore the ability of the p53 mutant to bind DNA. The restoration of activity of the p53 mutant can allow for the activation of downstream effectors of p53 leading to inhibition of cancer progression. The disclosure further provides methods of treatment of a cancerous lesion or a tumor harboring a p53 mutation.

[0029] Cancer is a collection of related diseases characterized by uncontrolled proliferation of cells with the potential to metastasize throughout the body. Cancer can be classified into five broad categories including, for example: carcinomas, which can arise from cells that cover internal and external parts of the body such as the lung, breast, and colon; sarcomas, which can arise from cells that are located in bone, cartilage, fat, connective tissue, muscle, and other supportive tissues; lymphomas, which can arise in the lymph nodes and immune system tissues; leukemia, which can arise in the bone marrow and accumulate in the bloodstream; and adenomas, which can arise in the thyroid, the pituitary gland, the adrenal gland, and other glandular tissues.

[0030] Although different cancers can develop in virtually any of the body's tissues, and contain unique features, the basic processes that cause cancer can be similar in all forms of the disease. Cancer begins when a cell breaks free from the normal restraints on cell division and begins to grow and divide out of control. Genetic mutations in the cell can preclude the ability of the cell to repair damaged DNA or initiate apoptosis, and can result in uncontrolled growth and division of cells.

[0031] The ability of tumor cell populations to multiply is determined not only by the rate of cell proliferation but also by the rate of cell attrition. Programmed cell death, or apoptosis, represents a major mechanism of cellular attrition. Cancer cells can evade apoptosis through a variety of strategies, for example, through the suppression of p53 function, thereby suppressing expression of pro-apoptotic proteins.

[0032] Oncogenes and tumor suppressor genes can regulate the proliferation of cells. Genetic mutations can affect oncogenes and tumor suppressors, potentially activating or suppressing activity abnormally, further facilitating uncontrolled cell division. Whereas oncogenes assist in cellular growth, tumor suppressor genes slow cell division by repairing damaged DNA and activating apoptosis. Cellular oncogenes that can be mutated in cancer include, for example, Cdk1, Cdk2, Cdk3, Cdk4, Cdk6, EGFR, PDGFR, VEGF, HER2, Raf, KRAS, and myc. Tumor suppressor genes that can be mutated in cancer include, for example, BRCA1, BRCA2, cyclin-dependent kinase inhibitor 1C, Retinoblastoma protein (pRb), PTEN, p16, p27, p53, and p73.

**K-Ras.**

**[0033]** K-Ras is a Ras-family GTPase encoded by the KRAS oncogene. In mammals, KRAS can be expressed from alternative splicing in either of two isoforms, K-Ras4A and K-RasB. In wild type K-Ras, the binding of GTP activates intracellular signaling pathways operative in cell proliferation and angiogenesis. Oncogenic mutations in the KRAS gene can place K-Ras in a constitutively active state, thereby conferring ligand-independent activation leading to tumorigenesis. Mutations in KRAS associated with cancer include mutations listed in **TABLE 1**.

**TABLE 1**

| Codon        | Mutation in K-ras | Mutation in KRAS              |
|--------------|-------------------|-------------------------------|
| 12 (exon 2)  | G12C              | (c.34G>T)                     |
|              | G12R              | (c.34G>C)                     |
|              | C12S              | (c.34G>A)                     |
|              | G12A              | (c.35G>C)                     |
|              | G12D              | (c.35G>A)                     |
|              | G12V              | (c.35G>T)                     |
| 13 (exon 2)  | G13D              | (c.38G>A)                     |
| 59 (exon 3)  | A59E              | (c.176C>A)                    |
|              | A59G              | (c.176C>G)                    |
|              | A59T              | (c.175G>A)                    |
| 61 (exon 3)  | Q61K              | (c.181C>A; c.180_181delinsAA) |
|              | Q61L              | (c.182A>T)                    |
|              | Q61R              | (c.182A>G)                    |
|              | Q61H              | (c.183A>C; c.183A>T)          |
| 117 (exon 4) | K117N             | (c.351A>C; c.351A>T)          |
| 146 (exon 4) | A146P             | (c.436G>C)                    |
|              | A146T             | (c.436G>A)                    |
|              | A146V             | (c.437C>T)                    |

**[0034]** In some embodiments, a compound of the disclosure that binds p53 is more efficacious in the treatment of cancer in a subject that does not contain an oncogenic KRAS mutation relative to treatment of cancer in a subject that contains an oncogenic KRAS mutation. In some embodiments, a subject is selected for treatment with a p53-activating compound of the disclosure at least in part on the basis that the subject does not exhibit a mutation in codons 12, 13, and 61 of a KRAS gene in the subject. In some embodiments, the subject is selected for treatment on the basis that the subject does not exhibit a mutation in codons 12, 13, 59, 61, 117, and 146 of a KRAS gene in the subject. In some embodiments, the KRAS gene in the subject is a gene of a cancer cell in the subject.

**[0035]** In some embodiments, a cancer cell of the subject contains a KRAS gene that expresses a wildtype K-ras protein. A wild-type K-ras protein can be, for example, K-Ras4A:

MTEYKLVVVG AGGVGKSALT IQLIQNHFVD EYDPTIEDSY RKQVVIDGET CLLDILDTAG



QEEYSAMRDQ YMRGTGEGFLC VFAINNTKSF EDIHHYREQI KRVKDSSEDP MVLVGNKCDL  
 PSRTVDTKQA QDLARSYGIP FIETSAKTRQ RVEDAFYTLV REIRQYRLKK ISKEEKTPGC  
 VKIKKCIIM (SEQ ID NO 1),

or K-Ras4B:

MTEYKLVVVG AGGVGKSALT IQLIQNHFVD EYDPTIEDSY RKQVVIDGET CLLDILD<sup>1</sup>TAG  
 QEEYSAMRDQ YMRGTGEGFLC VFAINNTKSF EDIHHYREQI KRVKDSSEDP MVLVGNKCDL  
 PSRTVDTKQA QDLARSYGIP FIETSAKTRQ GVDDAFYTLV REIRKHKEKM SKDGK<sup>2</sup>KKKKK  
 SKTKCVIM (SEQ ID NO 2).

#### BRCA1/2.

[0036] BRCA1 and BRCA2 are human proteins encoded by the *BRCA1* and *BRCA2* tumor suppressor genes, respectively. Both proteins are involved in repairing double-stranded breaks in DNA. In some embodiments, a compound of the disclosure that binds p53 is more efficacious in the treatment of cancer in a subject that does not contain an oncogenic BRCA1 or BRCA2 mutation relative to treatment of cancer in a subject that contains an oncogenic BRCA1 or BRCA2 mutation. In some embodiments, a compound of the disclosure that binds p53 is more efficacious in the treatment of cancer in a subject that does not contain an oncogenic BRCA1 mutation relative to treatment of cancer in a subject that contains an oncogenic BRCA1 mutation. In some embodiments, a compound of the disclosure that binds p53 is more efficacious in the treatment of cancer in a subject that does not contain an oncogenic BRCA2 mutation relative to treatment of cancer in a subject that contains an oncogenic BRCA2 mutation.

#### PIK3CA.

[0037] The PIK3CA gene encodes the p110 alpha (p110 $\alpha$ ) protein, which forms the catalytic subunit of phosphatidylinositol 3-kinase (PI3K). Mutations in the p110 $\alpha$  subunit can be found in many forms of cancer, including ovarian, head and neck lung skin, brain, breast, prostate, colorectal, cervical, esophageal, skin, thyroid, gastric, liver, and pancreatic cancers, and often co-occur with HER-2 protein overexpression in breast cancer. In some embodiments, a compound of the disclosure that binds p53 is more efficacious in the treatment of cancer in a subject that does not contain an oncogenic PIK3CA mutation relative to treatment of cancer in a subject that contains an oncogenic PIK3CA mutation.

Mutations in PIK3CA associated with cancer include mutations listed in TABLE 2.

TABLE 2

| Mutation Name | Mutation in PIK3CA |
|---------------|--------------------|
| E542K         | c.1624G>A          |
| E545K         | c.1633G>A          |
| E545Q         | c.1633G>C          |
| H1047L        | c.3140A>T          |
| H1047R        | c.3140A>G          |

**Tumor suppressor p53.**

**[0038]** The tumor suppressor protein p53 is a 393 amino acid transcription factor that can regulate cell growth in response to cellular stresses including, for example, UV radiation, hypoxia, oncogene activation, and DNA damage. p53 has various mechanisms for inhibiting the progression of cancer including, for example, initiation of apoptosis, maintenance of genomic stability, cell cycle arrest, induction of senescence, and inhibition of angiogenesis. Due to the critical role of p53 in tumor suppression, p53 is inactivated in almost all cancers either by direct mutation or through perturbation of associated signaling pathways involved in tumor suppression. Homozygous loss of the p53 gene occurs in almost all types of cancer, including carcinomas of the breast, colon, and lung. The presence of certain p53 mutations in several types of human cancer can correlate with less favorable patient prognosis.

**[0039]** In the absence of stress signals, p53 levels are maintained at low levels via the interaction of p53 with Mdm2, an E3 ubiquitin ligase. In an unstressed cell, Mdm2 can target p53 for degradation by the proteasome. Under stress conditions, the interaction between Mdm2 and p53 is disrupted, and p53 accumulates. The critical event leading to the activation of p53 is phosphorylation of the N-terminal domain of p53 by protein kinases, thereby transducing upstream stress signals. The phosphorylation of p53 leads to a conformational change, which can promote DNA binding by p53 and allow transcription of downstream effectors. The activation of p53 can induce, for example, the intrinsic apoptotic pathway, the extrinsic apoptotic pathway, cell cycle arrest, senescence, and DNA repair. p53 can activate proteins involved in the above pathways including, for example, Fas/Apo1, KILLER/DR5, Bax, Puma, Noxa, Bid, caspase-3, caspase-6, caspase-7, caspase-8, caspase-9, and p21 (WAF1). Additionally, p53 can indirectly repress the transcription of a variety of genes including, for example, c-MYC, Cyclin B, VEGF, RAD51, and hTERT.

**[0040]** Each chain of the p53 tetramer is composed of several functional domains including the transactivation domain (amino acids 1-100), the DNA-binding domain (amino acids 101-306), and the tetramerization domain (amino acids 307-355), which are highly mobile and largely unstructured. Most p53 cancer mutations are located in the DNA-binding core domain of the protein, which contains a central  $\beta$ -sandwich of anti-parallel  $\beta$ -sheets that serves as a basic scaffold for the DNA-binding surface. The DNA-binding surface is composed of two  $\beta$ -turn loops, L2 and L3, which are stabilized by a zinc ion, for example, at Arg175 and Arg248, and a loop-sheet-helix motif. Altogether, these structural elements form an extended DNA-binding surface that is rich in positively-charged amino acids, and makes specific contact with various p53 response elements.

**[0041]** Due to the prevalence of p53 mutations in virtually every type of cancer, the reactivation of wild-type p53 function in a cancerous cell can be an effective therapy. Mutations in p53 located in the DNA-binding domain of the protein or periphery of the DNA-binding surface can result in aberrant protein folding required for DNA recognition and binding or reduction in DNA binding affinity. Mutations in p53 can occur, for example, at amino acids Val143, His168, Arg175, Tyr220, Gly245, Arg248, Arg249, Phe270, Arg273, and Arg282. p53 mutations that can abrogate the activity of p53 include, for example, R175H, Y220C, G245S, R248Q, R248W, R273C, R273H, and R282H. p53 mutations can distort the

structure of the DNA-binding site, thermodynamically destabilize the folded protein at body temperature, or weaken consensus DNA binding. Wild-type function of p53 mutants can be recovered by binding of the p53 mutant to a compound that can shift the folding-unfolding equilibrium towards the folded state, thereby reducing the rate of unfolding and destabilization; or by conjugating a small molecule to the DNA binding interface to restore consensus DNA binding.

**[0042]** Non-limiting examples of amino acids include: alanine (A, Ala); arginine (R, Arg); asparagine (N, Asn); aspartic acid (D, Asp); cysteine (C, Cys); glutamic acid (E, Glu); glutamine (Q, Gln); glycine (G, Gly); histidine (H, His); isoleucine (I, Ile); leucine (L, Leu); lysine (K, Lys); methionine (M, Met); phenylalanine (F, Phe); proline (P, Pro); serine (S, Ser); threonine (T, Thr); tryptophan (W, Trp); tyrosine (Y, Tyr); and valine (V, Val).

#### **CA-125.**

**[0043]** CA-125 is a cancer antigen. A CA-125 test can be used to measure the amount of the CA-125 protein in the blood of a patient. A CA-125 test can be used to monitor certain cancers during and after treatment. In some embodiments, the disclosure provides a method of treating a cancer, the method comprising: a) administering to a subject in need thereof a therapeutically-effective amount of a compound herein (e.g., Compound 1); and b) subsequent to the administering, monitoring a level of CA-125 protein in the subject. In some embodiments, the disclosure provides a method comprising monitoring a level of CA-125 in a subject who is undergoing therapy for a cancer with a compound herein (e.g., Compound 1).

#### **HRD.**

**[0044]** Homologous recombination is a process wherein nucleotide sequences between distinct stands of DNA are exchanged. Homologous recombination is involved in a number of different biological processes, for example, homologous recombination occurs as part of the DNA repair process (e.g., doubled-strand break repair pathway and synthesis-dependent strand annealing pathway) and during process of meiosis/gametogenesis of eukaryotic organisms. Homologous recombination deficiency, also known as homologous recombination repair deficiency, homologous repair deficiency, or HRD is a reduction or impairment of the homologous recombination process. A sample that suffers from HRD can accumulate genomic errors or chromosomal aberrations that can be used as a biomarker for HRD. Chromosomal aberration (CA) is a detectable variation in a sample's chromosomal DNA. In some embodiments, CA falls into at least one of three overlapping categories: loss of heterozygosity (LOH), allelic imbalance (e.g., telomeric allelic imbalance (TAI)), or large scale transition (LST).

**[0045]** In some embodiments, HRD status is determined by the detection of CA in a sample (e.g., a tumor sample) obtained from a patient. In some embodiments, a sample having a positive HRD status meets or exceeds a threshold number or level of CAs at a specified number of chromosomal indicator regions. In some embodiments, HRD status is determined using a commercially available diagnostic to detect chromosomal aberrations in a sample (e.g. a tumor sample) and/or to assess whether a sample is able to repair double-strand DNA breaks. Non-limiting examples of diagnostics suitable to assess HRD status include the myChoice HRD™ diagnostic kit (Myriad Genetics, South San Francisco).

[0046] In some embodiments, a compound of the disclosure that binds p53 is more efficacious in the treatment of cancer in a subject that is HRD negative relative to treatment of cancer in a subject that is HRD positive.

**Mechanism of compounds of the disclosure.**

[0047] The compounds of the present disclosure can selectively bind to a p53 mutant and can recover wild-type activity of the p53 mutant including, for example, DNA binding function and activation of downstream targets involved in tumor suppression. In some embodiments, a compound of the disclosure selectively binds to the p53 Y220C mutant. The Y220C mutant is a temperature sensitive mutant, which binds to DNA at lower temperature and is denatured at body temperature. A compound of the disclosure can stabilize the Y220C mutant to reduce the likelihood of denaturation of the protein at body temperature.

[0048] In some embodiments, the compounds of the disclosure stabilize a mutant p53 and allows the mutant p53 to bind to DNA, thereby shifting the equilibrium of wild type and mutant p53 proteins to wild type p53. In some embodiments, the compounds of the disclosure reactivate the mutant p53 protein to provide wild type p53 activity. In some embodiments, the compounds of the disclosure reactivate the mutant p53 protein to provide pro-apoptotic p53 activity. In some embodiments, the compounds of the disclosure reactivate the mutant p53 protein to block angiogenesis. In some embodiments, the compounds of the disclosure reactivate the mutant p53 protein to induce cellular senescence. In some embodiments, the compounds of the disclosure reactivate the mutant p53 protein to induce cell cycle arrest.

[0049] Located in the periphery of the p53  $\beta$ -sandwich connecting  $\beta$ -strands S7 and S8, the aromatic ring of Y220 is an integral part of the hydrophobic core of the  $\beta$ -sandwich. The Y220C mutation can be highly destabilizing, due to the formation of an internal surface cavity. A compound of the disclosure can bind to and occupy this surface crevice to stabilize the  $\beta$ -sandwich, thereby restoring wild-type p53 DNA-binding activity.

[0050] To determine the ability of a compound of the disclosure to bind and stabilize mutant p53, assays can be employed to detect, for example, a conformational change in the p53 mutant or activation of wild-type p53 targets. Conformational changes in p53 can be measured by, for example, differential scanning fluorimetry (DSF), isothermal titration calorimetry (ITC), nuclear magnetic resonance spectrometry (NMR), or X-ray crystallography. Additionally, antibodies specific for the wild-type of mutant conformation of p53 can be used to detect a conformational change via, for example, immunoprecipitation (IP), immunofluorescence (IF), or immunoblotting.

[0051] Methods used to detect the ability of the p53 mutant to bind DNA can include, for example, DNA affinity immunoblotting, modified enzyme-linked immunosorbent assay (ELISA), electrophoretic mobility shift assay (EMSA), fluorescence resonance energy transfer (FRET), homogeneous time-resolved fluorescence (HTRF), and a chromatin immunoprecipitation (ChIP) assay.

[0052] To determine whether a compound described herein is able to reactivate the transcriptional activity of p53, the activation of downstream targets in the p53 signaling cascade can be measured. Activation of p53 effector proteins can be detected by, for example, immunohistochemistry (IHC-P),

reverse transcription polymerase chain reaction (RT-PCR), and western blotting. The activation of p53 can also be measured by the induction of apoptosis via the caspase cascade and using methods including, for example, Annexin V staining, TUNEL assays, pro-caspase and caspase levels, and cytochrome c levels. Another consequence of p53 activation is senescence, which can be measured using methods such as  $\beta$ -galactosidase staining.

**[0053]** A p53 mutant that can be used to determine the effectiveness of a compound of the disclosure to increase the DNA binding ability of a p53 mutant is a p53 truncation mutant, which contains only amino acids 94-312, encompassing the DNA-binding domain of p53. For example, the sequence of the p53 Y220C mutant used for testing compound efficacy can be:

SSSVPSQ KTYQGSYGFR LGFLHSGTAK SVTCTYSPAL NKMFCQLAKT  
CPVQLWVDST PPPGTRVRAM AIYKQSQHMT EVVRRCPHHE  
RCSDSGLAP PQHLIRVEGN LRVEYLDDRN TFRHSVVVPC EPPEVGSDCT  
TIHYNYMCNS SCMGGMNRPP ILTIITLED SGNLLGRNSF EVHVCACPGR  
DRRTEENLR KKGEPHHELP PGSTKRALSNT (SEQ ID NO. 3)

**[0054]** A compound of the disclosure can increase the ability of a p53 mutant to bind DNA by at least or up to about 0.1%, at least or up to about 0.2%, at least or up to about 0.3%, at least or up to about 0.4%, at least or up to about 0.5%, at least or up to about 0.6%, at least or up to about 0.7%, at least or up to about 0.8%, at least or up to about 0.9%, at least or up to about 1%, at least or up to about 2%, at least or up to about 3%, at least or up to about 4%, at least or up to about 5%, at least or up to about 6%, at least or up to about 7%, at least or up to about 8%, at least or up to about 9%, at least or up to about 10%, at least or up to about 11%, at least or up to about 12%, at least or up to about 13%, at least or up to about 14%, at least or up to about 15%, at least or up to about 16%, at least or up to about 17%, at least or up to about 18%, at least or up to about 19%, at least or up to about 20%, at least or up to about 21%, at least or up to about 22%, at least or up to about 23%, at least or up to about 24%, at least or up to about 25%, at least or up to about 26%, at least or up to about 27%, at least or up to about 28%, at least or up to about 29%, at least or up to about 30%, at least or up to about 31%, at least or up to about 32%, at least or up to about 33%, at least or up to about 34%, at least or up to about 35%, at least or up to about 36%, at least or up to about 37%, at least or up to about 38%, at least or up to about 39%, at least or up to about 40%, at least or up to about 41%, at least or up to about 42%, at least or up to about 43%, at least or up to about 44%, at least or up to about 45%, at least or up to about 46%, at least or up to about 47%, at least or up to about 48%, at least or up to about 49%, at least or up to about 50%, at least or up to about 51%, at least or up to about 52%, at least or up to about 53%, at least or up to about 54%, at least or up to about 55%, at least or up to about 56%, at least or up to about 57%, at least or up to about 58%, at least or up to about 59%, at least or up to about 60%, at least or up to about 61%, at least or up to about 62%, at least or up to about 63%, at least or up to about 64%, at least or up to about 65%, at least or up to about 66%, at least or up to about 67%, at least or up to about 68%, at least or up to about 69%, at least or up to about 70%, at

least or up to about 71%, at least or up to about 72%, at least or up to about 73%, at least or up to about 74%, at least or up to about 75%, at least or up to about 76%, at least or up to about 77%, at least or up to about 78%, at least or up to about 79%, at least or up to about 80%, at least or up to about 81%, at least or up to about 82%, at least or up to about 83%, at least or up to about 84%, at least or up to about 85%, at least or up to about 86%, at least or up to about 87%, at least or up to about 88%, at least or up to about 89%, at least or up to about 90%, at least or up to about 91%, at least or up to about 92%, at least or up to about 93%, at least or up to about 94%, at least or up to about 95%, at least or up to about 96%, at least or up to about 97%, at least or up to about 98%, at least or up to about 99%, at least or up to about 100%, at least or up to about 125%, at least or up to about 150%, at least or up to about 175%, at least or up to about 200%, at least or up to about 225%, or at least or up to about 250% as compared to the ability of the p53 mutant to bind DNA in the absence of a compound of the disclosure.

**[0055]** A compound described herein can increase the activity of the p53 mutant that is, for example, at least or up to about 2-fold, at least or up to about 3-fold, at least or up to about 4-fold, at least or up to about 5-fold, at least or up to about 6-fold, at least or up to about 7-fold, at least or up to about 8-fold, at least or up to about 9-fold, at least or up to about 10-fold, at least or up to about 11-fold, at least or up to about 12-fold, at least or up to about 13-fold, at least or up to about 14-fold, at least or up to about 15-fold, at least or up to about 16-fold, at least or up to about 17-fold, at least or up to about 18-fold, at least or up to about 19-fold, at least or up to about 20-fold, at least or up to about 25-fold, at least or up to about 30-fold, at least or up to about 35-fold, at least or up to about 40-fold, at least or up to about 45-fold, at least or up to about 50-fold, at least or up to about 55-fold, at least or up to about 60-fold, at least or up to about 65-fold, at least or up to about 70-fold, at least or up to about 75-fold, at least or up to about 80-fold, at least or up to about 85-fold, at least or up to about 90-fold, at least or up to about 95-fold, at least or up to about 100-fold, at least or up to about 110-fold, at least or up to about 120-fold, at least or up to about 130-fold, at least or up to about 140-fold, at least or up to about 150-fold, at least or up to about 160-fold, at least or up to about 170-fold, at least or up to about 180-fold, at least or up to about 190-fold, at least or up to about 200-fold, at least or up to about 250-fold, at least or up to about 300-fold, at least or up to about 350-fold, at least or up to about 400-fold, at least or up to about 450-fold, at least or up to about 500-fold, at least or up to about 550-fold, at least or up to about 600-fold, at least or up to about 650-fold, at least or up to about 700-fold, at least or up to about 750-fold, at least or up to about 800-fold, at least or up to about 850-fold, at least or up to about 900-fold, at least or up to about 950-fold, at least or up to about 1,000-fold, at least or up to about 1,500-fold, at least or up to about 2,000-fold, at least or up to about 3,000-fold, at least or up to about 4,000-fold, at least or up to about 5,000-fold, at least or up to about 6,000-fold, at least or up to about 7,000-fold, at least or up to about 8,000-fold, at least or up to about 9,000-fold, or at least or up to about 10,000-fold greater than the activity of the p53 mutant in the absence of the compound.

**[0056]** A compound of the disclosure can be used, for example, to induce apoptosis, cell cycle arrest, or senescence in a cell. In some embodiments, the cell is a cancer cell. In some embodiments, the cell carries a mutation in p53. A patient can be treated with a compound of the disclosure on the basis of

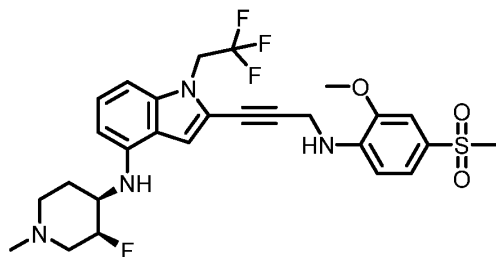
whether the patient suffers from a cancer that is platinum-sensitive, platinum resistant, or platinum refractory. Platinum resistant cancer includes, for example, cancer that progresses within 6 months of treatment with platinum-based chemotherapy, such as treatment with cisplatin or carboplatin. Platinum refractory cancer includes, for example, cancer that either progresses during treatment with platinum-based chemotherapy or within four weeks of the last dose of platinum-based chemotherapy. In some embodiments, the disclosure provides a method of treating a cancer in a subject in need thereof, the method comprising administering to the subject a therapeutically-effective amount of a compound herein (e.g., Compound 1), wherein the cancer is platinum-sensitive, platinum resistant, or platinum refractory. In some embodiments, the disclosure provides a method of treating a cancer in a subject in need thereof, the method comprising administering to the subject a therapeutically-effective amount of a compound herein (e.g., Compound 1), wherein the subject had previously received a platinum-based therapy for the cancer and the platinum-based therapy was ineffective at treating the cancer. In some embodiments, the disclosure provides a method of treating a cancer in a subject in need thereof, the method comprising administering to the subject a therapeutically-effective amount of a compound herein (e.g., Compound 1) at least partially on a basis that the subject had previously received a platinum-based therapy for the cancer and the platinum-based therapy was ineffective at treating the cancer.

**Compounds of the disclosure.**

[0057] In some embodiments, a compound of the disclosure comprises a substituted heterocyclyl group, wherein the compound binds a mutant p53 protein and increases wild-type p53 activity of the mutant protein. In some embodiments, a compound of the disclosure comprises a heterocyclyl group comprising a halo substituent, wherein the compound binds a mutant p53 protein and increases wild-type p53 activity of the mutant protein. In some embodiments, the compound further comprises an indole group. In some embodiments, the indole group has a 2,2,2,-trifluoroethyl substituent at a 1-position of the indole group.

[0058] In some embodiments, the indole group has a propargyl substituent at a 2-position of the indole group. In some embodiments, the propargyl substituent is attached to the indole group via an sp carbon atom of the propargyl substituent. In some embodiments, the propargyl substituent is attached to a nitrogen atom of an aniline group via a methylene group of the propargyl substituent. In some embodiments, the indole group comprises an amino substituent at a 4-position of the indole group. In some embodiments, the amino substituent is attached to the heterocyclyl group. In some embodiments, the heterocyclyl group is a piperidine group. In some embodiments, the halo substituent is a fluoro group. In some embodiments, the halo substituent is a chloro group. In some embodiments, the compound has oral bioavailability that is at least about 50% greater than that of an analogous compound that lacks the halo substituent on the heterocyclyl group.

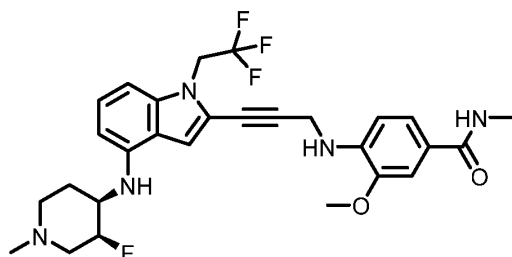
[0059] In some embodiments, the compound is



, or a pharmaceutically-acceptable salt thereof.

[0060] In some embodiments, the compound is N-((3S,4R)-3-fluoro-1-methylpiperidin-4-yl)-2-((2-methoxy-4-(methylsulfonyl)phenyl)amino)prop-1-yn-1-yl)-1-(2,2,2-trifluoroethyl)-1H-indol-4-amine, or a pharmaceutically-acceptable salt thereof.

[0061] In some embodiments, the compound is



(Compound 1), or a pharmaceutically-acceptable salt

thereof.

or a pharmaceutically-acceptable salt thereof.

[0062] In some embodiments, the compound is 4-((3-((3S,4R)-3-fluoro-1-methylpiperidin-4-yl)amino)-1-(2,2,2-trifluoroethyl)-1H-indol-2-yl)prop-2-yn-1-yl)amino)-3-methoxy-N-methylbenzamide, or a pharmaceutically-acceptable salt thereof.

[0063] Suitable p53-activating compounds of the present disclosure also can include, for example, p53-activating compounds provided in US10138219B2, US11814373B2, US11807644B2, US11963953B2, US20230024905A1, US20230049952A1, PCT/US2024/046050, and PCT/US2024/046043, each of which is incorporated by reference in its entirety.

[0064] Compounds herein can include all stereoisomers, enantiomers, diastereomers, mixtures, racemates, atropisomers, and tautomers thereof.

[0065] As used herein, the term “hydrogen”, the atomic symbol “H”, implicit hydrogen atoms in chemical structures, and hydrogen atoms encompassed by specific and generic chemical radical terms shall be understood to include all atoms with atomic number 1, including  $^1\text{H}$  (i.e. “protium”),  $^2\text{H}$  (i.e. “deuterium” or “D”), and  $^3\text{H}$  (i.e. “tritium”). For example, the terms “alkoxy” and “methoxy” shall be understood to be inclusive of the radical  $-\text{OCD}_3$ , and the term “ $\text{C}_1$ - $\text{C}_6$ alkyl” shall be inclusive of the term “ $\text{C}_1$ - $\text{C}_6$ deuteroalkyl”. Likewise, all references to a given atomic element shall be understood to include all natural and unnatural isotopes of that element.

[0066] In certain embodiments, the compounds disclosed herein have some or all of the  $^1\text{H}$  atoms replaced with  $^2\text{H}$  atoms. The methods of synthesis for deuterium-containing compounds are known in the art and include, by way of non-limiting example only, the following synthetic methods. Such deuterated forms can be made by the procedure described in U.S. Patent Nos. 5,846,514 and 6,334,997. As



described in U.S. Patent Nos. 5,846,514 and 6,334,997, deuteration can improve the metabolic stability and or efficacy, thus increasing the duration of action of drugs. Deuterium substituted compounds are synthesized using various methods such as described in: Dean, Dennis C.; Editor. Recent Advances in the Synthesis and Applications of Radiolabeled Compounds for Drug Discovery and Development. [In: Curr., Pharm. Des., 2000; 6(10)] **2000**, 110 pp; George W.; Varna, Rajender S. The Synthesis of Radiolabeled Compounds via Organometallic Intermediates, Tetrahedron, **1989**, 45(21), 6601-21; and Evans, E. Anthony. Synthesis of radiolabeled compounds, J. Radioanal. Chem., **1981**, 64(1-2), 9-32. Deuterated starting materials are readily available and are subjected to the synthetic methods described herein to provide for the synthesis of deuterium-containing compounds. Deuterium-containing reagents and building blocks are available commercially from chemical vendors, such as Aldrich Chemical Co.

**[0067]** Compositions comprising the compounds disclosed herein, in some embodiments, can be prepared whereby a proportion of atoms at a given position are of a specific isotope, and said proportion differs from the natural abundance of said isotope. In some embodiments, the present disclosure provides a composition comprising a population of molecules, wherein each molecule has a structure according to a compound provided herein (e.g., Compound 1), wherein the population of molecules has a mass of at least 1  $\mu$ g, wherein at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 50%, at least 75%, at least 90%, at least 95%, at least 97%, at least 99%, at least 99%, at least 99.9%, or at least 99.99%, of the molecules in the population of molecules each independently comprise a deuterium atom.

**[0068]** In some embodiments, the present disclosure provides a composition comprising a population of molecules having a mass of at least 1  $\mu$ g, wherein each molecule has a structure according to a compound provided herein (e.g., Compound 1), wherein if, in a mass spectrometry experiment, a mass spectrum of the population is obtained, the mass spectrometry experiment determines that at least 10% of the molecules in the population of molecules each independently comprise a deuterium atom.

**[0069]** Any compound herein can be purified. A compound herein can be least 1% pure, at least 2% pure, at least 3% pure, at least 4% pure, at least 5% pure, at least 6% pure, at least 7% pure, at least 8% pure, at least 9% pure, at least 10% pure, at least 11% pure, at least 12% pure, at least 13% pure, at least 14% pure, at least 15% pure, at least 16% pure, at least 17% pure, at least 18% pure, at least 19% pure, at least 20% pure, at least 21% pure, at least 22% pure, at least 23% pure, at least 24% pure, at least 25% pure, at least 26% pure, at least 27% pure, at least 28% pure, at least 29% pure, at least 30% pure, at least 31% pure, at least 32% pure, at least 33% pure, at least 34% pure, at least 35% pure, at least 36% pure, at least 37% pure, at least 38% pure, at least 39% pure, at least 40% pure, at least 41% pure, at least 42% pure, at least 43% pure, at least 44% pure, at least 45% pure, at least 46% pure, at least 47% pure, at least 48% pure, at least 49% pure, at least 50% pure, at least 51% pure, at least 52% pure, at least 53% pure, at least 54% pure, at least 55% pure, at least 56% pure, at least 57% pure, at least 58% pure, at least 59% pure, at least 60% pure, at least 61% pure, at least 62% pure, at least 63% pure, at least 64% pure, at least 65% pure, at least 66% pure, at least 67% pure, at least 68% pure, at least 69% pure, at least 70% pure, at least 71% pure, at least 72% pure, at least 73% pure, at least 74% pure, at least 75% pure, at least 76% pure, at least 77% pure, at least 78% pure, at least 79% pure, at least 80% pure, at least 81% pure, at least

82% pure, at least 83% pure, at least 84% pure, at least 85% pure, at least 86% pure, at least 87% pure, at least 88% pure, at least 89% pure, at least 90% pure, at least 91% pure, at least 92% pure, at least 93% pure, at least 94% pure, at least 95% pure, at least 96% pure, at least 97% pure, at least 98% pure, at least 99% pure, at least 99.1% pure, at least 99.2% pure, at least 99.3% pure, at least 99.4% pure, at least 99.5% pure, at least 99.6% pure, at least 99.7% pure, at least 99.8% pure, or at least 99.9% pure.

#### **Therapeutic Methods.**

**[0070]** In some embodiments, compounds of the disclosure can be used to treat cancer in a subject. A compound of the disclosure can, for example, slow the proliferation of cancer cell lines, or kill cancer cells. Non-limiting examples of cancer that can be treated by a compound of the disclosure include: acute lymphoblastic leukemia, acute myeloid leukemia, adrenocortical carcinoma, AIDS-related cancers, AIDS-related lymphoma, anal cancer, appendix cancer, astrocytomas, basal cell carcinoma, bile duct cancer, bladder cancer, bone cancers, brain tumors, such as cerebellar astrocytoma, cerebral astrocytoma/malignant glioma, ependymoma, medulloblastoma, supratentorial primitive neuroectodermal tumors, visual pathway and hypothalamic glioma, breast cancer, bronchial adenomas, Burkitt lymphoma, carcinoma of unknown primary origin, central nervous system lymphoma, cerebellar astrocytoma, cervical cancer, childhood cancers, chronic lymphocytic leukemia, chronic myelogenous leukemia, chronic myeloproliferative disorders, colon cancer, cutaneous T-cell lymphoma, desmoplastic small round cell tumor, endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma, germ cell tumors, gallbladder cancer, gastric cancer, gastrointestinal carcinoid tumor, gastrointestinal stromal tumor, gliomas, hairy cell leukemia, head and neck cancer, heart cancer, hepatocellular (liver) cancer, Hodgkin lymphoma, Hypopharyngeal cancer, intraocular melanoma, islet cell carcinoma, Kaposi sarcoma, kidney cancer, laryngeal cancer, lip and oral cavity cancer, liposarcoma, liver cancer, lung cancers, such as non-small cell and small cell lung cancer, lymphomas, leukemias, macroglobulinemia, malignant fibrous histiocytoma of bone, medulloblastoma, melanomas, mesothelioma, metastatic squamous neck cancer with occult primary, mouth cancer, multiple endocrine neoplasia syndrome, myelodysplastic syndromes, myeloid leukemia, nasal cavity and paranasal sinus cancer, nasopharyngeal carcinoma, neuroblastoma, non-Hodgkin lymphoma, non-small cell lung cancer, oral cancer, oropharyngeal cancer, osteosarcoma, ovarian cancer, high-grade serous ovarian cancer, ovarian cancer with homologous recombination deficiency, ovarian epithelial cancer, ovarian germ cell tumor, pancreatic cancer, pancreatic cancer islet cell, paranasal sinus and nasal cavity cancer, parathyroid cancer, penile cancer, pharyngeal cancer, pheochromocytoma, pineal astrocytoma, pineal germinoma, pituitary adenoma, pleuropulmonary blastoma, plasma cell neoplasia, primary central nervous system lymphoma, prostate cancer, rectal cancer, renal cell carcinoma, renal pelvis and ureter transitional cell cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, sarcomas, skin cancers, skin carcinoma merkel cell, small intestine cancer, soft tissue sarcoma, squamous cell carcinoma, stomach cancer, T-cell lymphoma, throat cancer, thymoma, thymic carcinoma, thyroid cancer, trophoblastic tumor (gestational), cancers of unknown primary site, urethral cancer, uterine sarcoma, vaginal cancer, vulvar cancer, Waldenström macroglobulinemia, and Wilms tumor.

[0071] In some embodiments, compounds of the disclosure can be used to treat breast cancer in a subject. In some embodiments, the breast cancer is human epidermal growth receptor 2-positive (HER2) breast cancer. In some embodiments, the breast cancer is hormone receptor-negative (HR1) breast cancer. In some embodiments, the breast cancer is human epidermal growth receptor 2-negative (HER2) breast cancer. In some embodiments, the breast cancer is hormone receptor-positive (HR1) and human epidermal growth receptor 2-positive (HER2) breast cancer. In some embodiments, the breast cancer is hormone receptor-negative (HR1) and human epidermal growth receptor 2-negative (HER2) breast cancer. In some embodiments, the breast cancer is hormone receptor-positive (HR1) and human epidermal growth receptor 2-negative (HER2) breast cancer. In some embodiments, the breast cancer is hormone receptor-negative (HR1) and human epidermal growth receptor 2-positive (HER2) breast cancer.

[0072] In some embodiments, the present disclosure provides a method of treating triple-negative breast cancer (TNBC), comprising administering to a subject in need thereof a therapeutically-effective amount of a compound of the disclosure. Triple-negative breast cancer (TNBC) refers to any breast cancer that does not express the genes for estrogen receptor (ER), progesterone receptor (PR), or Her2/neu.

[0073] In some embodiments, the present disclosure provides a method of treating cancer-associated edema, the method comprising administering to a subject in need thereof a therapeutically-effective amount of a compound of the disclosure. In some embodiments, the cancer-associated edema is associated with breast cancer. In some embodiments, the cancer-associated edema further comprises limited limb or digit mobility. In some embodiments, the cancer-associated edema further comprises limited arm mobility. In some embodiments, the cancer-associated edema is lymphedema.

[0074] In some embodiments, the disclosure provides a method of improving cancer-associated edema in a cancer subject in need thereof, the method comprising administering to the cancer subject a therapeutically-effective amount of a compound of the disclosure, wherein the cancer subject suffers from cancer-associated edema prior to the administering and experiences improvement in the cancer-associated edema after the administering, for example, after a course of therapy of the compound of the disclosure. In some embodiments, the cancer-associated edema is lymphedema. In some embodiments, the cancer subject has breast cancer in a breast. In some embodiments, the cancer-associated edema is in the breast that has breast cancer. In some embodiments, the cancer-associated edema is in a limb. In some embodiments, the limb is an arm. In some embodiments, the limb is an arm, wherein the breast is ipsilateral with the arm. In some embodiments, the cancer-associated edema is in a digit. In some embodiments, the digit is a finger. In some embodiments, the digit is a finger, wherein the breast is ipsilateral with the finger.

[0075] In some embodiments, the disclosure provides a method of improving limb mobility in a cancer subject in need thereof, the method comprising administering to the cancer subject a therapeutically-effective amount of a compound of the disclosure, wherein the cancer subject suffers from limited mobility of the limb prior to the administering and experiences improvement in mobility of the limb after the administering, for example, after a course of therapy of the compound of the disclosure. In some

embodiments, the limb is an arm. In some embodiments, the cancer subject has breast cancer. In some embodiments, the limb is an arm, wherein the breast cancer is in a breast that is ipsilateral with the arm.

[0076] In some embodiments, the disclosure provides a method comprising: a) administering to a subject in need of cancer therapy a compound of the disclosure; b) observing that the subject exhibits an improvement in limb mobility after the administering; and c) based at least in part on the observing that the subject exhibits an improvement in limb mobility after the administering, performing further administration of the compound of the disclosure. In some embodiments, the subject has breast cancer and the cancer therapy is breast cancer therapy. In some embodiments, the method further comprises, prior to the administering, observing that the subject exhibits inhibited mobility of the limb. In some embodiments, the limb is an arm. In some embodiments, the cancer subject has breast cancer. In some embodiments, the limb is an arm, wherein the breast cancer is in a breast that is ipsilateral with the arm.

[0077] In some embodiments, the disclosure provides a method of improving digit mobility in a cancer subject in need thereof, the method comprising administering to the cancer subject a therapeutically-effective amount of a compound of the disclosure, wherein the cancer subject suffers from limited mobility of the digit prior to the administering and experiences improvement in mobility of the digit after the administering, for example, after a course of therapy of the compound of the disclosure. In some embodiments, the digit is a finger. In some embodiments, the cancer subject has breast cancer. In some embodiments, the digit is a finger, wherein the breast cancer is in a breast that is ipsilateral with the finger.

[0078] In some embodiments, the disclosure provides a method comprising: a) administering to a subject in need of cancer therapy a compound of the disclosure; b) observing that the subject exhibits an improvement in digit mobility after the administering; and c) based at least in part on the observing that the subject exhibits an improvement in digit mobility after the administering, performing further administrations of the compound of the disclosure. In some embodiments, the subject has breast cancer and the cancer therapy is breast cancer therapy. In some embodiments, the method further comprises, prior to the administering, observing that the subject exhibits inhibited mobility of the digit. In some embodiments, the digit is a finger. In some embodiments, the cancer subject has breast cancer. In some embodiments, the digit is a finger, wherein the breast cancer is in a breast that is ipsilateral with the finger.

[0079] In some embodiments, the compounds of the disclosure show non-lethal toxicity.

#### **Pharmacokinetic and pharmacodynamic properties**

[0080] Pharmacokinetic and pharmacodynamic data can be obtained by various experimental techniques. Appropriate pharmacokinetic and pharmacodynamic profile components describing a particular composition can vary due to variations in drug metabolism in human subjects. Pharmacokinetic and pharmacodynamic profiles can be based on the determination of the mean parameters of a group of subjects. The group of subjects includes any reasonable number of subjects suitable for determining a representative mean, for example, 5 subjects, 10 subjects, 15 subjects, 20 subjects, 25 subjects, 30 subjects, 35 subjects, or more. The mean is determined, for example, by calculating the average of all

subject's measurements for each parameter measured. A dose can be modulated to achieve a desired pharmacokinetic or pharmacodynamics profile, such as a desired or effective blood profile, as described herein.

[0081] The pharmacodynamic parameters can be any parameters suitable for describing compositions of the invention. For example, the pharmacodynamic profile can be obtained at a time after dosing of, for example, about zero minutes, about 1 minute, about 2 minutes, about 3 minutes, about 4 minutes, about 5 minutes, about 6 minutes, about 7 minutes, about 8 minutes, about 9 minutes, about 10 minutes, about 11 minutes, about 12 minutes, about 13 minutes, about 14 minutes, about 15 minutes, about 16 minutes, about 17 minutes, about 18 minutes, about 19 minutes, about 20 minutes, about 21 minutes, about 22 minutes, about 23 minutes, about 24 minutes, about 25 minutes, about 26 minutes, about 27 minutes, about 28 minutes, about 29 minutes, about 30 minutes, about 31 minutes, about 32 minutes, about 33 minutes, about 34 minutes, about 35 minutes, about 36 minutes, about 37 minutes, about 38 minutes, about 39 minutes, about 40 minutes, about 41 minutes, about 42 minutes, about 43 minutes, about 44 minutes, about 45 minutes, about 46 minutes, about 47 minutes, about 48 minutes, about 49 minutes, about 50 minutes, about 51 minutes, about 52 minutes, about 53 minutes, about 54 minutes, about 55 minutes, about 56 minutes, about 57 minutes, about 58 minutes, about 59 minutes, about 60 minutes, about zero hours, about 0.5 hours, about 1 hour, about 1.5 hours, about 2 hours, about 2.5 hours, about 3 hours, about 3.5 hours, about 4 hours, about 4.5 hours, about 5 hours, about 5.5 hours, about 6 hours, about 6.5 hours, about 7 hours, about 7.5 hours, about 8 hours, about 8.5 hours, about 9 hours, about 9.5 hours, about 10 hours, about 10.5 hours, about 11 hours, about 11.5 hours, about 12 hours, about 12.5 hours, about 13 hours, about 13.5 hours, about 14 hours, about 14.5 hours, about 15 hours, about 15.5 hours, about 16 hours, about 16.5 hours, about 17 hours, about 17.5 hours, about 18 hours, about 18.5 hours, about 19 hours, about 19.5 hours, about 20 hours, about 20.5 hours, about 21 hours, about 21.5 hours, about 22 hours, about 22.5 hours, about 23 hours, about 23.5 hours, or about 24 hours.

[0082] The pharmacokinetic parameters can be any parameters suitable for describing a compound. Non-limiting examples of pharmacodynamic and pharmacokinetic parameters that can be calculated for a compound that is administered with the methods of the invention include:

- a) the amount of drug administered, which can be represented as a dose  $D$ ;
- b) the dosing interval, which can be represented as  $\tau$ ;
- c) the apparent volume in which a drug is distributed, which can be represented as a volume of distribution  $V_d$ , where  $V_d = D/C_0$ ;
- d) the amount of drug in a given volume of plasma, which can be represented as concentration  $C_0$  or  $C_{ss}$ , where  $C_0$  or  $C_{ss} = D/V_d$  and can be represented as a mean plasma concentration over a plurality of samples;
- e) the half-life of a drug  $t_{1/2}$ , where  $t_{1/2} = \ln(2)/k_e$ ;
- f) the rate at which a drug is removed from the body  $k_e$ , where  $k_e = \ln(2)/t_{1/2} = CL/V_d$ ; g) the rate of infusion required to balance the equation  $K_{in}$ , where  $K_{in} = C_{ss} \cdot CL$ ;

- h) the integral of the concentration-time curve after administration of a single dose, which can be represented as  $AUC_{0-\infty}$ , wherein  $\int_0^{\infty} C dt$ , or in steady-state, which can be represented as  $AUC\tau_{ss}$ , wherein  $\int_t^{t+\tau} C dt$ ; i) the volume of plasma cleared of the drug per unit time, which can be represented as CL (clearance), wherein  $CL = V_d \cdot k_e = D/AUC$ ;
- j) the systemically available fraction of a drug, which can be represented as  $f$ , where  $f = \frac{AUC_{po, Div}}{AUC_{iv, Dpo}}$ ;
- k) the peak plasma concentration of a drug after administration,  $C_{max}$ ;
- l) the time taken by a drug to reach  $C_{max}$ ,  $t_{max}$ ;
- m) the lowest concentration that a drug reaches before the next dose is administered  $C_{min}$ ; and
- n) the peak trough fluctuation within one dosing interval at steady state, which can be represented as  $\%PTF = 100 \cdot \frac{(C_{max,ss} - C_{min,ss})}{C_{av,ss}}$  where  $C_{av,ss} = \frac{AUC\tau_{ss}}{\tau}$ .

**[0083]** Non-limiting examples of pharmacokinetic parameters that can be used to determine the effect of a treatment of a subject with a composition of the disclosure include: terminal elimination rate constant ( $\lambda_z$ ); percentage of  $AUC_{0-\infty}$  that is due to extrapolation beyond  $t_{last}$  ( $AUC\%_{extrap}$ ); area under the concentration-time curve from pre-dose (time 0) to 24 hours ( $AUC_{0-24}$ ); area under the concentration-time curve from pre-dose (time 0) to 96 hours ( $AUC_{0-96}$ ); area under the concentration-time curve from pre-dose (time 0) extrapolated to infinity ( $AUC_{0-\infty}$ ); area under the concentration-time curve from pre-dose (time 0) to  $t_{last}$  ( $AUC_{0-last}$ ); apparent total body clearance (CL/F); maximum observed plasma concentration ( $C_{max}$ ); apparent terminal elimination half-life ( $t_{1/2}$ ); time prior to the first measurable (non-zero) concentration ( $t_{lag}$ ); time of last quantifiable concentration ( $t_{last}$ ); time corresponding to the occurrence of  $C_{max}$  ( $T_{max}$ ); and apparent volume of distribution (Vz/F). The  $C_{max}$  can be, for example, not less than about 1 ng/mL; not less than about 5 ng/mL; not less than about 10 ng/mL; not less than about 15 ng/mL; not less than about 20 ng/mL; not less than about 25 ng/mL; not less than about 50 ng/mL; not less than about 75 ng/mL; not less than about 100 ng/mL; not less than about 200 ng/mL; not less than about 300 ng/mL; not less than about 400 ng/mL; not less than about 500 ng/mL; not less than about 600 ng/mL; not less than about 700 ng/mL; not less than about 800 ng/mL; not less than about 900 ng/mL; not less than about 1000 ng/mL; not less than about 1250 ng/mL; not less than about 1500 ng/mL; not less than about 1750 ng/mL; not less than about 2000 ng/mL; or any other  $C_{max}$  appropriate for describing a pharmacokinetic profile of a compound described herein. The  $C_{max}$  can be, for example, about 1 ng/mL to about 5,000 ng/mL; about 1 ng/mL to about 4,500 ng/mL; about 1 ng/mL to about 4,000 ng/mL; about 1 ng/mL to about 3,500 ng/mL; about 1 ng/mL to about 3,000 ng/mL; about 1 ng/mL to about 2,500 ng/mL; about 1 ng/mL to about 2,000 ng/mL; about 1 ng/mL to about 1,500 ng/mL; about 1 ng/mL to about 1,000 ng/mL; about 1 ng/mL to about 900 ng/mL; about 1 ng/mL to about 800 ng/mL; about 1 ng/mL to about 700 ng/mL; about 1 ng/mL to about 600 ng/mL; about 1 ng/mL to about 500 ng/mL; about 1 ng/mL to about 450 ng/mL; about 1 ng/mL to about 400 ng/mL; about 1 ng/mL to about 350 ng/mL; about 1 ng/mL to about 300 ng/mL; about 1 ng/mL to about 250 ng/mL; about 1 ng/mL to about 200 ng/mL; about 1 ng/mL to about 150 ng/mL; about 1 ng/mL to about 125 ng/mL; about 1 ng/mL to about 100 ng/mL; about 1 ng/mL to about 90 ng/mL; about 1 ng/mL to about 80 ng/mL; about 1 ng/mL

to about 70 ng/mL; about 1 ng/mL to about 60 ng/mL; about 1 ng/mL to about 50 ng/mL; about 1 ng/mL to about 40 ng/mL; about 1 ng/mL to about 30 ng/mL; about 1 ng/mL to about 20 ng/mL; about 1 ng/mL to about 10 ng/mL; about 1 ng/mL to about 5 ng/mL; about 10 ng/mL to about 4,000 ng/mL; about 10 ng/mL to about 3,000 ng/mL; about 10 ng/mL to about 2,000 ng/mL; about 10 ng/mL to about 1,500 ng/mL; about 10 ng/mL to about 1,000 ng/mL; about 10 ng/mL to about 900 ng/mL; about 10 ng/mL to about 800 ng/mL; about 10 ng/mL to about 700 ng/mL; about 10 ng/mL to about 600 ng/mL; about 10 ng/mL to about 500 ng/mL; about 10 ng/mL to about 400 ng/mL; about 10 ng/mL to about 300 ng/mL; about 10 ng/mL to about 200 ng/mL; about 10 ng/mL to about 100 ng/mL; about 10 ng/mL to about 50 ng/mL; about 25 ng/mL to about 500 ng/mL; about 25 ng/mL to about 100 ng/mL; about 50 ng/mL to about 500 ng/mL; about 50 ng/mL to about 100 ng/mL; about 100 ng/mL to about 500 ng/mL; about 100 ng/mL to about 400 ng/mL; about 100 ng/mL to about 300 ng/mL; or about 100 ng/mL to about 200 ng/mL.

[0084] In some embodiments, the  $C_{\max}$  is about 8 mg/mL to about 15 mg/mL. In some embodiments, the  $C_{\max}$  is about 8 mg/mL to about 12 mg/mL. In some embodiments, the  $C_{\max}$  is about 7 mg/mL to about 11 mg/mL. In some embodiments, the  $C_{\max}$  is about 12 mg/mL to about 28 mg/mL. In some embodiments, the  $C_{\max}$  is about 3 mg/mL to about 20 mg/mL. In some embodiments, the  $C_{\max}$  is about 7 mg/mL to about 20 mg/mL.

[0085] In some embodiments, in a method disclosed herein wherein a subject has consumed food within an amount of time prior to administering a compound of the disclosure, the  $C_{\max}$  is increased compared to the  $C_{\max}$  of the compound administered wherein a subject has not consumed food within an amount of time prior to administering a compound of the disclosure. In some embodiments, the  $C_{\max}$  of a compound disclosed herein is increased by about 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85% or more.

[0086] In some embodiments, the  $C_{\max}$  of a compound disclosed herein is increased by about 28% for a fed subject compared to that of a fasted subject. In some embodiments, the  $C_{\max}$  of a compound disclosed herein is increased by about 35% for a fed subject compared to that of a fasted subject. In some embodiments, the  $C_{\max}$  of a compound disclosed herein is increased by about 84% for a fed subject compared to that of a fasted subject.

[0087] The  $T_{\max}$  of a compound described herein can be, for example, not greater than about 0.5 hours, not greater than about 1 hours, not greater than about 1.5 hours, not greater than about 2 hours, not greater than about 2.5 hours, not greater than about 3 hours, not greater than about 3.5 hours, not greater than about 4 hours, not greater than about 4.5 hours, not greater than about 5 hours, or any other  $T_{\max}$  appropriate for describing a pharmacokinetic profile of a compound described herein. The  $T_{\max}$  can be, for example, about 0.1 hours to about 24 hours; about 0.1 hours to about 0.5 hours; about 0.5 hours to about 1 hour; about 1 hour to about 1.5 hours; about 1.5 hours to about 2 hour; about 2 hours to about 2.5 hours; about 2.5 hours to about 3 hours; about 3 hours to about 3.5 hours; about 3.5 hours to about 4 hours; about 4 hours to about 4.5 hours; about 4.5 hours to about 5 hours; about 5 hours to about 5.5 hours; about 5.5 hours to about 6 hours; about 6 hours to about 6.5 hours; about 6.5 hours to about 7

hours; about 7 hours to about 7.5 hours; about 7.5 hours to about 8 hours; about 8 hours to about 8.5 hours; about 8.5 hours to about 9 hours; about 9 hours to about 9.5 hours; about 9.5 hours to about 10 hours; about 10 hours to about 10.5 hours; about 10.5 hours to about 11 hours; about 11 hours to about 11.5 hours; about 11.5 hours to about 12 hours; about 12 hours to about 12.5 hours; about 12.5 hours to about 13 hours; about 13 hours to about 13.5 hours; about 13.5 hours to about 14 hours; about 14 hours to about 14.5 hours; about 14.5 hours to about 15 hours; about 15 hours to about 15.5 hours; about 15.5 hours to about 16 hours; about 16 hours to about 16.5 hours; about 16.5 hours to about 17 hours; about 17 hours to about 17.5 hours; about 17.5 hours to about 18 hours; about 18 hours to about 18.5 hours; about 18.5 hours to about 19 hours; about 19 hours to about 19.5 hours; about 19.5 hours to about 20 hours; about 20 hours to about 20.5 hours; about 20.5 hours to about 21 hours; about 21 hours to about 21.5 hours; about 21.5 hours to about 22 hours; about 22 hours to about 22.5 hours; about 22.5 hours to about 23 hours; about 23 hours to about 23.5 hours; or about 23.5 hours to about 24 hours. In some embodiments, the  $T_{\max}$  of a compound of the disclosure is about 2 hours. In some embodiments, the  $T_{\max}$  of a compound of the disclosure is about 4 hours. In some embodiments, the  $T_{\max}$  of a compound of the disclosure is about 6 hours. In some embodiments, the  $T_{\max}$  of a compound of the disclosure is about 8 hours.

**[0088]** In some embodiments, the  $T_{\max}$  is about 1.5 hours to about 5 hours. In some embodiments, the  $T_{\max}$  is about 1.5 hours to about 2.5 hours. In some embodiments, the  $T_{\max}$  is about 2 hours to about 8 hours. In some embodiments, the  $T_{\max}$  is about 1 hour to about 6 hours. In some embodiments, the  $T_{\max}$  is about 2 hours to about 5 hours. In some embodiments, the  $T_{\max}$  is about 2 hours. In some embodiments, the  $T_{\max}$  is about 3 hours. In some embodiments, the  $T_{\max}$  is about 4 hours.

**[0089]** In some embodiments, in a method disclosed herein wherein a subject has consumed food within an amount of time prior to administering a compound of the disclosure, the  $T_{\max}$  is increased compared to the  $T_{\max}$  of the compound administered wherein a subject has not consumed food within an amount of time prior to administering a compound of the disclosure. In some embodiments, the  $T_{\max}$  of a compound disclosed herein is increased by about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, or more.

**[0090]** The  $AUC_{(0-\infty)}$  or  $AUC_{(last)}$  of a compound described herein can be, for example, not less than about 1 ng•hr/mL, not less than about 5 ng•hr/mL, not less than about 10 ng•hr/mL, not less than about 20 ng•hr/mL, not less than about 30 ng•hr/mL, not less than about 40 ng•hr/mL, not less than about 50 ng•hr/mL, not less than about 100 ng•hr/mL, not less than about 150 ng•hr/mL, not less than about 200 ng•hr/mL, not less than about 250 ng•hr/mL, not less than about 300 ng•hr/mL, not less than about 350 ng•hr/mL, not less than about 400 ng•hr/mL, not less than about 450 ng•hr/mL, not less than about 500 ng•hr/mL, not less than about 600 ng•hr/mL, not less than about 700 ng•hr/mL, not less than about 800 ng•hr/mL, not less than about 900 ng•hr/mL, not less than about 1000 ng•hr/mL, not less than about 1250 ng•hr/mL, not less than about 1500 ng•hr/mL, not less than about 1750 ng•hr/mL, not less than about 2000 ng•hr/mL, not less than about 2500 ng•hr/mL, not less than about 3000 ng•hr/mL, not less than about 3500 ng•hr/mL, not less than about 4000 ng•hr/mL, not less than about 5000 ng•hr/mL, not less



than about 6000 ng•hr/mL, not less than about 7000 ng•hr/mL, not less than about 8000 ng•hr/mL, not less than about 9000 ng•hr/mL, not less than about 10,000 ng•hr/mL, or any other  $AUC_{(0-\text{inf})}$  or  $AUC_{(\text{last})}$  appropriate for describing a pharmacokinetic profile of a compound described herein. In some embodiments, the  $AUC_{(0-\text{inf})}$  or  $AUC_{(\text{last})}$  of a compound described herein can be, for example, not less than about 10,000 ng•hr/mL, not less than about 11,000 ng•hr/mL, not less than about 12,000 ng•hr/mL, not less than about 13,000 ng•hr/mL, not less than about 14,000 ng•hr/mL, not less than about 15,000 ng•hr/mL, not less than about 16,000 ng•hr/mL, not less than about 17,000 ng•hr/mL, not less than about 18,000 ng•hr/mL, not less than about 19,000 ng•hr/mL, not less than about 20,000 ng•hr/mL, not less than about 21,000 ng•hr/mL, not less than about 22,000 ng•hr/mL, not less than about 23,000 ng•hr/mL, not less than about 24,000 ng•hr/mL, or not less than about 25,000 ng•hr/mL.

[0091] The  $AUC_{(0-\text{inf})}$  or  $AUC_{(\text{last})}$  of a compound can be, for example, about 1 ng•hr/mL to about 10,000 ng•hr/mL; about 1 ng•hr/mL to about 10 ng•hr/mL; about 10 ng•hr/mL to about 25 ng•hr/mL; about 25 ng•hr/mL to about 50 ng•hr/mL; about 50 ng•hr/mL to about 100 ng•hr/mL; about 100 ng•hr/mL to about 200 ng•hr/mL; about 200 ng•hr/mL to about 300 ng•hr/mL; about 300 ng•hr/mL to about 400 ng•hr/mL; about 400 ng•hr/mL to about 500 ng•hr/mL; about 500 ng•hr/mL to about 600 ng•hr/mL; about 600 ng•hr/mL to about 700 ng•hr/mL; about 700 ng•hr/mL to about 800 ng•hr/mL; about 800 ng•hr/mL to about 900 ng•hr/mL; about 900 ng•hr/mL to about 1,000 ng•hr/mL; about 1,000 ng•hr/mL to about 1,250 ng•hr/mL; about 1,250 ng•hr/mL to about 1,500 ng•hr/mL; about 1,500 ng•hr/mL to about 1,750 ng•hr/mL; about 1,750 ng•hr/mL to about 2,000 ng•hr/mL; about 2,000 ng•hr/mL to about 2,500 ng•hr/mL; about 2,500 ng•hr/mL to about 3,000 ng•hr/mL; about 3,000 ng•hr/mL to about 3,500 ng•hr/mL; about 3,500 ng•hr/mL to about 4,000 ng•hr/mL; about 4,000 ng•hr/mL to about 4,500 ng•hr/mL; about 4,500 ng•hr/mL to about 5,000 ng•hr/mL; about 5,000 ng•hr/mL to about 5,500 ng•hr/mL; about 5,500 ng•hr/mL to about 6,000 ng•hr/mL; about 6,000 ng•hr/mL to about 6,500 ng•hr/mL; about 6,500 ng•hr/mL to about 7,000 ng•hr/mL; about 7,000 ng•hr/mL to about 7,500 ng•hr/mL; about 7,500 ng•hr/mL to about 8,000 ng•hr/mL; about 8,000 ng•hr/mL to about 8,500 ng•hr/mL; about 8,500 ng•hr/mL to about 9,000 ng•hr/mL; about 9,000 ng•hr/mL to about 9,500 ng•hr/mL; or about 9,500 ng•hr/mL to about 10,000 ng•hr/mL. In some embodiments, the  $AUC_{(0-\text{inf})}$  or  $AUC_{(\text{last})}$  of a compound described herein can be, for example, about 10,000 ng•hr/mL, about 11,000 ng•hr/mL, about 12,000 ng•hr/mL, about 13,000 ng•hr/mL, about 14,000 ng•hr/mL, about 15,000 ng•hr/mL, about 16,000 ng•hr/mL, about 17,000 ng•hr/mL, about 18,000 ng•hr/mL, about 19,000 ng•hr/mL, about 20,000 ng•hr/mL, about 21,000 ng•hr/mL, about 22,000 ng•hr/mL, about 23,000 ng•hr/mL, about 24,000 ng•hr/mL, or about 25,000 ng•hr/mL.

[0092] The area under the concentration-time curve from pre-dose (time 0) to 24 hours ( $AUC_{0-24}$ ) can be, for example, about 90,000 ng•hr/mL to about 500,000 ng•hr/mL, about 100,000 ng•hr/mL to about 500,000 ng•hr/mL, about 150,000 ng•hr/mL to about 500,000 ng•hr/mL, about 200,000 ng•hr/mL to about 500,000 ng•hr/mL, about 100,000 ng•hr/mL to about 400,000 ng•hr/mL, about 100,000 ng•hr/mL to about 300,000 ng•hr/mL, about 100,000 ng•hr/mL to about 250,000 ng•hr/mL, about 100,000 ng•hr/mL to about 200,000 ng•hr/mL, about 150,000 ng•hr/mL to about 250,000 ng•hr/mL, or about

150,000 ng•hr/mL to about 200,000 ng•hr/mL. In some embodiments, AUC<sub>0-24</sub> is measured in a subject administered a therapeutically-effective amount of the compound once daily. In some embodiments, AUC<sub>0-24</sub> is measured in a subject on the tenth, eleventh, twelfth, thirteenth, fourteenth, or fifteenth day of a treatment regimen, the treatment regimen comprising administering the therapeutically-effective amount of the compound once daily. In some embodiments, AUC<sub>0-24</sub> is measured in a subject on the fifteenth day of the treatment regimen. In some embodiments, the therapeutically-effective amount is 2000 mg. In some embodiments, the therapeutically-effective amount is 3000 mg. In some embodiments, the therapeutically-effective amount is administered orally. In some embodiments, the therapeutically-effective amount is administered with food.

[0093] In some embodiments, the present disclosure provides a pharmaceutical composition comprising a p53-activating compound of the disclosure, wherein after 15 once-daily administrations of an amount of the pharmaceutical composition comprising a therapeutically-effective amount of the compound, a AUC<sub>0-24</sub> about 90,000 ng•hr/mL to about 500,000 ng•hr/mL of the compound is effected in the subject. In some embodiments, the therapeutically-effective amount is 2000 mg. In some embodiments, the therapeutically-effective amount is 3000 mg. In some embodiments, the therapeutically-effective amount is administered orally. In some embodiments, the therapeutically-effective amount is administered with food.

[0094] In some embodiments, a food comprises a high-fat meal. In some embodiments, a high-fat meal comprises a fat content of at least 50% of total caloric content of the meal. In some embodiments, a high-fat meal comprises a fat content at least 500 Kcal from fat. In some embodiments, a high-fat meal comprises at least 55g of fat.

[0095] In some embodiments, a food comprises a medium-fat meal. In some embodiments, a medium-fat meal comprises a fat content of about 25% to about 50% of total caloric content of the meal. In some embodiments, a medium-fat meal comprises a fat content about 125 Kcal to about 500 Kcal from fat. In some embodiments, a medium-fat meal comprises about 14g to about 55g of fat.

[0096] In some embodiments, a food comprises a low-fat meal. In some embodiments, a low-fat meal comprises a fat content of less than 25% of total caloric content of the meal. In some embodiments, a low-fat meal comprises a fat content less than about 125 Kcal from fat. In some embodiments, a low-fat meal comprises less than about 14g of fat.

[0097] In some embodiments, a high-fat meal comprises about 150 calories from protein, about 250 calories from carbohydrate, and about 500 to about 600 calories from fat.

[0098] In some embodiments, a food comprises a high-calorie meal. In some embodiments, a high-calorie meal comprises a calorie content of at least 800 calories. In some embodiments, a high-calorie meal comprises a calorie content of about 800 calories to about 1000 calories.

[0099] In some embodiments, a food comprises a high fat and high-calorie meal. In some embodiments, a high fat and high-calorie meal comprises a fat content of at least 50% of total caloric content of the meal and a calorie content of about 800 calories to about 1000 calories.

**[0100]** In some embodiments, in a method disclosed herein wherein a subject has consumed food within an amount of time prior to administering a compound of the disclosure, the AUC is increased compared to the AUC of the compound administered wherein a subject has not consumed food within an amount of time prior to administering a compound of the disclosure. In some embodiments, the AUC of a compound disclosed herein is increased by about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, or more. In some embodiments the AUC is  $AUC_{0-inf}$ . In some embodiments the AUC is  $AUC_{0-last}$ . In some embodiments the AUC is  $AUC_{0-24}$ .

**[0101]** In some embodiments, the AUC (e.g.,  $AUC_{0-inf}$ ,  $AUC_{0-last}$ ,  $AUC_{0-24}$ ) of a compound disclosed herein is increased by about 40% for a fed subject compared to that of a fasted subject. In some embodiments, the AUC (e.g.,  $AUC_{0-inf}$ ,  $AUC_{0-last}$ ,  $AUC_{0-24}$ ) of a compound disclosed herein is increased by about 45% for a fed subject compared to that of a fasted subject. In some embodiments, the AUC (e.g.,  $AUC_{0-inf}$ ,  $AUC_{0-last}$ ,  $AUC_{0-24}$ ) of a compound disclosed herein is increased by about 79% for a fed subject compared to that of a fasted subject is about 79%.

**[0102]** The plasma apparent total body clearance (CL/F) of a compound disclosed herein can be, for example, about 2 L/h, about 2.5 L/h, about 3 L/h, about 3.5 L/h, about 4 L/h, about 4.5 L/h, about 5 L/h, about 5.5 L/h, about 6 L/h, about 6.5 L/h, about 7 L/h, about 7.5 L/h, about 8 L/h, about 8.5 L/h, about 9 L/h, about 9.5 L/h, about 10 L/h, about 10.5 L/h, about 11 L/h, about 11.5 L/h, about 12 L/h, about 12.5 L/h, about 13 L/h, about 13.5 L/h, about 14 L/h, about 14.5 L/h, about 15 L/h, about 15.5 L/h, about 16 L/h, about 16.5 L/h, about 17 L/h, about 17.5 L/h, about 18 L/h, about 18.5 L/h, about 19 L/h, about 19.5 L/h, or about 20 L/h.

**[0103]** In some embodiments, in a method disclosed herein wherein a subject has consumed food within an amount of time prior to administering a compound of the disclosure, the CL/F is decreased compared to the CL/F of the compound administered wherein a subject has not consumed food within an amount of time prior to administering a compound of the disclosure. In some embodiments, the CL/F of a compound disclosed herein is decreased by about 10%, by about 15%, by about 20%, by about 25%, by about 30%, by about 35%, by about 40%, about 45%, by about 50%, by about 55%, by about 60%, or more.

**[0104]** The plasma apparent volume of distribution ( $V_z/F$ ) of a compound disclosed herein can be, for example, about 90 L, about 100 L, about 110 L, about 120 L, about 130 L, about 135 L, about 140 L, about 145 L, about 150 L, about 155 L, about 160 L, about 165 L, about 170 L, about 175 L, about 180 L, about 185 L, about 190 L, about 195 L, about 200 L, about 225 L, about 250 L, about 275 L, about 300 L, about 325 L, about 350 L, or about 400 L.

**[0105]** In some embodiments, in a method disclosed herein wherein a subject has consumed food within an amount of time prior to administering a compound of the disclosure, the  $V_z/F$  is decreased compared to the  $V_z/F$  of the compound administered wherein a subject has not consumed food within an amount of time prior to administering a compound of the disclosure. In some embodiments, the  $V_z/F$  of a compound

disclosed herein is decreased by about 10%, by about 15%, by about 20%, by about 25%, by about 30%, by about 35%, by about 40%, about 45%, by about 50%, by about 55%, by about 60%, or more.

[0106] The plasma concentration of a compound described herein can be, for example, not less than about 1 ng/mL, not less than about 5 ng/mL, not less than about 10 ng/mL, not less than about 15 ng/mL, not less than about 20 ng/mL, not less than about 25 ng/mL, not less than about 50 ng/mL, not less than about 75 ng/mL, not less than about 100 ng/mL, not less than about 150 ng/mL, not less than about 200 ng/mL, not less than about 300 ng/mL, not less than about 400 ng/mL, not less than about 500 ng/mL, not less than about 600 ng/mL, not less than about 700 ng/mL, not less than about 800 ng/mL, not less than about 900 ng/mL, not less than about 1000 ng/mL, not less than about 1200 ng/mL, or any other plasma concentration of a compound described herein. The plasma concentration can be, for example, about 1 ng/mL to about 2,000 ng/mL; about 1 ng/mL to about 5 ng/mL; about 5 ng/mL to about 10 ng/mL; about 10 ng/mL to about 25 ng/mL; about 25 ng/mL to about 50 ng/mL; about 50 ng/mL to about 75 ng/mL; about 75 ng/mL to about 100 ng/mL; about 100 ng/mL to about 150 ng/mL; about 150 ng/mL to about 200 ng/mL; about 200 ng/mL to about 250 ng/mL; about 250 ng/mL to about 300 ng/mL; about 300 ng/mL to about 350 ng/mL; about 350 ng/mL to about 400 ng/mL; about 400 ng/mL to about 450 ng/mL; about 450 ng/mL to about 500 ng/mL; about 500 ng/mL to about 600 ng/mL; about 600 ng/mL to about 700 ng/mL; about 700 ng/mL to about 800 ng/mL; about 800 ng/mL to about 900 ng/mL; about 900 ng/mL to about 1,000 ng/mL; about 1,000 ng/mL to about 1,100 ng/mL; about 1,100 ng/mL to about 1,200 ng/mL; about 1,200 ng/mL to about 1,300 ng/mL; about 1,300 ng/mL to about 1,400 ng/mL; about 1,400 ng/mL to about 1,500 ng/mL; about 1,500 ng/mL to about 1,600 ng/mL; about 1,600 ng/mL to about 1,700 ng/mL; about 1,700 ng/mL to about 1,800 ng/mL; about 1,800 ng/mL to about 1,900 ng/mL; or about 1,900 ng/mL to about 2,000 ng/mL.

[0107] In some embodiments, the plasma concentration can be about 2,500 ng/mL, about 3,000 ng/mL, about 3,500 ng/mL, about 4,000 ng/mL, about 4,500 ng/mL, about 5,000 ng/mL, about 5,500 ng/mL, about 6,000 ng/mL, about 6,500 ng/mL, about 7,000 ng/mL, about 7,500 ng/mL, about 8,000 ng/mL, about 8,500 ng/mL, about 9,000 ng/mL, about 9,500 ng/mL, or about 10,000 ng/mL. In some embodiments, the plasma concentration can be about 10,000 ng/mL, about 15,000 ng/mL, about 20,000 ng/mL, about 25,000 ng/mL, about 30,000 ng/mL, about 35,000 ng/mL, about 40,000 ng/mL, about 45,000 ng/mL, about 50,000 ng/mL, about 55,000 ng/mL, about 60,000 ng/mL, about 65,000 ng/mL, about 70,000 ng/mL, or about 75,000 ng/mL.

[0108] Additional non-limiting examples of pharmacokinetic parameters that can be used to determine the effect of a treatment of a subject with a composition of the disclosure include: amount of drug excreted unchanged in urine (Ae), renal clearance (CLR), and fraction excreted in urine (fe).

[0109] The pharmacodynamic parameters can be any parameters suitable for describing compositions of the disclosure. For example, the pharmacodynamic profile can exhibit decreases in viability phenotype for the tumor cells or tumor size reduction in tumor cell lines or xenograft studies, for example, about 24 hours, about 48 hours, about 72 hours, or 1 week.

**Food Effect**

[0110] In some embodiments, a compound disclosed herein is administered within 30 minutes after consuming food. In some embodiments, a compound disclosed herein is administered at the same time as consuming food. In some embodiments, administration of a compound disclosed herein with food enhances one or more pharmacokinetic properties compared to administration of the compound under fasting conditions.

[0111] In a method disclosed herein, administering a compound disclosed herein with food increases one or more pharmacokinetic properties of the compound. In a method disclosed herein, administering a compound disclosed herein with a meal enhances one or more pharmacokinetic properties of the compound compared to administering the compound under fasting conditions.

[0112] In some embodiments, the enhanced pharmacokinetic property is clinically significant. In some embodiments, enhancing a pharmacokinetic property is increasing the pharmacokinetic property. For example, in some embodiment,  $C_{max}$  of a compound disclosed herein is increased when administered with food.

[0113] In some embodiments, enhancing a pharmacokinetic property is increasing the pharmacokinetic property. For example, enhancing pharmacokinetic properties comprises an increase in any one, two, three or all of the following parameters: mean plasma concentration,  $C_{max}$ , AUC, AUC(0-t) and/or AUC(inf). In some embodiments, enhancing a pharmacokinetic property is decreasing the pharmacokinetic property.

[0114] In a method disclosed herein, administering a compound disclosed herein with food increases exposure of the compound to a subject. In a method disclosed herein, administering a compound disclosed herein with food increases exposure of the compound compared to administering the compound under fasting conditions. In some embodiments, the increased exposure is clinically significant.

[0115] A fasted subject can be, for example, a subject who has fasted for some period of time prior to administration of a dose of a compound. Non-limiting examples of the period of time include at least about 1 hour, at least about 2 hours, at least about 3 hours, at least about 4 hours, at least about 5 hours, at least about 6 hours, at least about 7 hours, at least about 8 hours, at least about 9 hours, at least about 10 hours, at least about 11 hours, at least about 12 hours, at least about 13 hours, at least about 14 hours, at least about 15 hours, or more. In some embodiments, a subject is eligible to drink water or consume health care products while fasting.

[0116] In some embodiments, a dose is in a single dosage form, or a plurality of dosage forms (e.g., a 600 mg dose can be one 600 mg dosage form, two 300 mg dosage forms, three 200 mg dosage forms, six 100 mg dosage forms, etc.). In some embodiments, a dose comprises a plurality of pills administered simultaneously.

[0117] A fed subject can be, for example, a subject who has consumed food some period of time prior to drug administration. In some embodiments, food was finished some period of time prior to drug administration. In some embodiments, a subject has consumed food within an amount of time prior to administering a compound of the disclosure. In some embodiments, an amount of time is about 5

minutes, about 10 minutes, about 15 minutes, about 20 minutes, about 25 minutes, about 30 minutes, about 35 minutes, about 40 minutes, about 45 minutes, about 50 minutes, about 55 minutes, about 1 hour, about 1.5 hours, or about 2 hours. In some embodiments, a compound disclosed herein is within approximately 0 to 60 minutes, approximately 0 to 30, or 5 to 20 minutes of consuming food. In some embodiments a compound disclosed herein is administered 60 minutes, 30 minutes, 25 minutes, 20 minutes, 15 minutes, 10 minutes, or 5 minutes after consuming food.

[0118] In some embodiments, one or more pharmacokinetic properties is greater for a fed subject compared to that of a fasted subject. In some embodiments, mean plasma concentration is increased for a fed subject compared to that of a fasted subject. In some embodiments,  $C_{\max}$  is increased for a fed subject compared to that of a fasted subject. In some embodiments,  $T_{\max}$  is increased for a fed subject compared to that of a fasted subject. In some embodiments,  $AUC_{0-\text{last}}$  is increased for a fed subject compared to that of a fasted subject. In some embodiments,  $AUC_{0-\text{Inf}}$  is increased for a fed subject compared to that of a fasted subject.

[0119] In some embodiments, one or more pharmacokinetic properties is increased by about 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85% or more, for a fed subject compared to that of a fasted subject.

[0120] In some embodiments, one or more pharmacokinetic properties is greater for a fasted subject compared to that of a fed subject. In some embodiments, plasma CL/F is greater for a fasted subject compared to that of a fed subject. In some embodiments,  $V_z/F$  is greater for a fasted subject compared to that of a fed subject.

[0121] In a method disclosed herein, one or more pharmacokinetic properties of the compound is unchanged for a fasted subject compared to that of a fed subject. In some embodiments, total dose excreted in urine was unchanged over a 96 hour interval for a fasted subject compared to that of a fed subject. In some embodiments,  $CL_R$  was unchanged over a 96 hour interval for a fasted subject compared to that of a fed subject.

[0122] In some embodiments, a subject is of Caucasian ethnicity. In some embodiments, a subject is of non-Asian ethnicity. In some embodiments, a subject is of Asian ethnicity. In some embodiments, a subject is of Japanese ethnicity.

[0123] In some embodiments, one or more pharmacokinetic properties is greater for a subject of Japanese ethnicity compared to that of a subject of Caucasian or non-Asian ethnicity. In some embodiments, mean plasma concentration is greater for a subject of Japanese ethnicity compared to that of a subject of Caucasian or non-Asian ethnicity. In some embodiments,  $C_{\max}$  is greater for a subject of Japanese ethnicity compared to that of a subject of Caucasian or non-Asian ethnicity. In some embodiments,  $T_{\max}$  is greater for a subject of Japanese ethnicity compared to that of a subject of Caucasian or non-Asian ethnicity. In some embodiments,  $AUC_{0-\text{last}}$  is greater for a subject of Japanese ethnicity compared to that of a subject of Caucasian or non-Asian ethnicity. In some embodiments,  $AUC_{0-\text{Inf}}$  is greater for a subject of Japanese ethnicity compared to that of a subject of Caucasian or non-Asian ethnicity.

[0124] In some embodiments, a Japanese subject is a subject that was born in Japan and has parents and grandparents who were both ethnically Japanese and were both born in Japan. In some embodiments, a Japanese subject is a subject that was born in Japan and has parents and grandparents who were both ethnically Japanese. In some embodiments, a Japanese subject is a subject that has parents and grandparents who were both ethnically Japanese.

[0125] In some embodiments, one or more pharmacokinetic properties is greater for a fed subject of Caucasian or non-Asian ethnicity compared to that of a fasted subject of Caucasian or non-Asian ethnicity. In some embodiments, mean plasma concentration is greater for a fed subject of Caucasian or non-Asian ethnicity compared to that of a fasted subject of Caucasian or non-Asian ethnicity. In some embodiments, C<sub>max</sub> is greater for a fed subject of Caucasian or non-Asian ethnicity compared to that of a fasted subject of Caucasian or non-Asian ethnicity. In some embodiments, AUC is greater for a fed subject of Caucasian or non-Asian ethnicity compared to that of a fasted subject of Caucasian or non-Asian ethnicity. In some embodiments, T<sub>max</sub> is prolonged for a fed subject of Caucasian or non-Asian ethnicity compared to that of a fasted subject of Caucasian or non-Asian ethnicity.

[0126] Non-limiting examples of treatment-related adverse events (TRAEs) that can be used to determine the effect of a treatment of a subject with a composition of the disclosure include: Nausea, Vomiting, Blood creatinine increased, Diarrhea, Fatigue, ALT increased, AST increased, Anemia, Decreased appetite, Proteinuria, Rash maculo-papular, Headache, Lipase increased, Platelet count decreased, Amylase increased, and Dehydration.

[0127] In some embodiments, the TRAE is self-reported by the subject. In some embodiments the TRAE is observed by a health practitioner.

[0128] In some embodiments, the TRAE is a gastrointestinal (GI) TRAE. Non-limiting examples of GI TRAEs that can be used to determine the effect of a treatment of a subject with a composition of the disclosure include: nausea, vomiting, and diarrhea. In some embodiments, the GI TRAE is nausea. In some embodiments, the GI TRAE is vomiting. In some embodiments, the GI TRAE is diarrhea.

[0129] In some embodiments, the gastrointestinal adverse event is self-reported by the subject. In some embodiments, the gastrointestinal adverse event is observed by a health practitioner.

[0130] In some embodiments, the rate of one or more TRAEs is less frequent following administration of a compound of the disclosure to a fed subject compared to that of a fasted subject. In some embodiments, the rate of GI TRAEs is less frequent following administration of a compound of the disclosure to a fed subject compared to that of a fasted subject.

[0131] In some embodiments, a compound disclosed herein administered with food led to an improvement in one or more TRAEs (e.g., nausea, vomiting, or diarrhea). In some embodiments, a compound disclosed herein administered with food led to an improvement in one or more gastrointestinal adverse events (e.g., nausea, vomiting, or diarrhea).

[0132] In some embodiments, the incidence of one or more GI TRAEs observed in a fed subject is decreased compared to the incidence observed in a fasted subject. In some embodiments, the incidence of one or more GI TRAEs observed in a fed subject is decreased by about 15%, about 20%, about 25%,

about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, or more, compared to the incidence observed in a fasted subject.

[0133] In some embodiments, the incidence of one or more GI TRAEs observed in a fed subject is decreased by about 15% compared to the incidence observed in a fasted subject. In some embodiments, the incidence of one or more GI TRAEs observed in a fed subject is decreased by about 20% compared to the incidence observed in a fasted subject. In some embodiments, the incidence of one or more GI TRAEs observed in a fed subject is decreased by about 25% compared to the incidence observed in a fasted subject. In some embodiments, the incidence of one or more GI TRAEs observed in a fed subject is decreased by about 30% compared to the incidence observed in a fasted subject. In some embodiments, the incidence of one or more GI TRAEs observed in a fed subject is decreased by about 35% compared to the incidence observed in a fasted subject. In some embodiments, the incidence of one or more GI TRAEs observed in a fed subject is decreased by about 40% compared to the incidence observed in a fasted subject. In some embodiments, the incidence of one or more GI TRAEs observed in a fed subject is decreased by about 45% compared to the incidence observed in a fasted subject. In some embodiments, the incidence of one or more GI TRAEs observed in a fed subject is decreased by about 50% compared to the incidence observed in a fasted subject. In some embodiments, the incidence of one or more GI TRAEs observed in a fed subject is decreased by about 55% compared to the incidence observed in a fasted subject. In some embodiments, the incidence of one or more GI TRAEs observed in a fed subject is decreased by about 60% compared to the incidence observed in a fasted subject. In some embodiments, the incidence of one or more GI TRAEs observed in a fed subject is decreased by about 65% compared to the incidence observed in a fasted subject.

[0134] In some embodiments, a food comprises a high-fat meal. In some embodiments, a high-fat meal comprises a fat content of at least 50% of total caloric content of the meal. In some embodiments, a high-fat meal comprises a fat content at least 500 Kcal from fat. In some embodiments, a high-fat meal comprises at least 55g of fat.

[0135] In some embodiments, a food comprises a medium-fat meal. In some embodiments, a medium-fat meal comprises a fat content of about 25% to about 50% of total caloric content of the meal. In some embodiments, a medium-fat meal comprises a fat content about 125 Kcal to about 500 Kcal from fat. In some embodiments, a medium-fat meal comprises about 14g to about 55g of fat.

[0136] In some embodiments, a food comprises a low-fat meal. In some embodiments, a low-fat meal comprises a fat content of less than 25% of total caloric content of the meal. In some embodiments, a low-fat meal comprises a fat content less than about 125 Kcal from fat. In some embodiments, a low-fat meal comprises less than about 14g of fat.

[0137] In some embodiments, a high-fat meal comprises about 150 calories from protein, about 250 calories from carbohydrate, and about 500 to about 600 calories from fat.



[0138] In some embodiments, a food comprises a high-calorie meal. In some embodiments, a high-calorie meal comprises a calorie content of at least 800 calories. In some embodiments, a high-calorie meal comprises a calorie content of about 800 calories to about 1000 calories.

[0139] In some embodiments, a food comprises a high fat and high-calorie meal. In some embodiments, a high fat and high-calorie meal comprises a fat content of at least 50% of total caloric content of the meal and a calorie content of about 800 calories to about 1000 calories.

[0140] In some embodiments, if the therapeutically-effective amount of the compound is administered to a fasted study subject and the therapeutically-effective amount of the compound is administered to a fed study subject, then a  $C_{\max}$  of the compound in the fed study subject is greater than is the  $C_{\max}$  of the compound in the fasted study subject. In some embodiments, if the therapeutically-effective amount of the compound is administered to a group of fed study subjects and a group of fasted subjects, a value of about 8350 ng/mL to about 12300 ng/mL is observed for  $C_{\max}$  in fed subjects, and a value of 7200 ng/mL to about 11100 ng/mL is observed for  $C_{\max}$  in fasted subjects. In some embodiments, if the therapeutically-effective amount of the compound is administered to a group of fed study subjects and a group of fasted subjects, a value of about 12300 ng/mL to about 28100 ng/mL is observed for  $C_{\max}$  in fed subjects, and a value of 2820 ng/mL to about 20100 ng/mL is observed for  $C_{\max}$  in fasted subjects. In some embodiments, if the therapeutically-effective amount of the compound is administered to a group of fasted subjects, a value of about 6710 ng/mL to about 20600 ng/mL is observed for  $C_{\max}$ .

[0141] In some embodiments, if the therapeutically-effective amount of the compound is administered to a fasted study subject and the therapeutically-effective amount of the compound is administered to a fed study subject, then a  $T_{\max}$  of the compound in the fed study subject is greater than is the  $T_{\max}$  of the compound in the fasted study subject. In some embodiments, if the therapeutically-effective amount of the compound is administered to a group of fed study subjects and a group of fasted subjects, a value of about 1.51 h to about 5.01 h is observed for  $T_{\max}$  in fed subjects, and a value of 1.54 h to about 2.51 h is observed for  $T_{\max}$  in fasted subjects. In some embodiments, if the therapeutically-effective amount of the compound is administered to a group of fed study subjects and a group of fasted subjects, a value of about 2.11 h to about 8.08 h is observed for  $T_{\max}$  in fed subjects, and a value of 1.03 h to about 5.94 h is observed for  $T_{\max}$  in fasted subjects. In some embodiments, if the therapeutically-effective amount of the compound is administered to a group of fasted subjects, a value of about 1.59 h to about 5.06 h is observed for  $T_{\max}$ .

[0142] In some embodiments, if the therapeutically-effective amount of the compound is administered to a fasted study subject and the therapeutically-effective amount of the compound is administered to a fed study subject, then a  $t_{1/2}$  of the compound in the fed study subject is greater than is the  $t_{1/2}$  of the compound in the fasted study subject. In some embodiments, if the therapeutically-effective amount of the compound is administered to a group of fed study subjects and a group of fasted subjects, a value of about 11.9 h to about 21.7 h is observed for  $t_{1/2}$  in fed subjects, and a value of 13.6 h to about 20.8 h is observed for  $t_{1/2}$  in fasted subjects. In some embodiments, if the therapeutically-effective amount of the compound is administered to a group of fasted subjects, a value of about 11.9 h to about 21.7 h is observed for  $t_{1/2}$  in fasted subjects. In some embodiments, if the therapeutically-effective amount of the compound is administered to a group of fed study subjects and a group of fasted subjects, a value of

about 13.5 h to about 38.2 h is observed for  $t_{1/2}$  in fed subjects, and a value of 11.5 h to about 45.8 h is observed for  $t_{1/2}$  in fasted subjects. In some embodiments, if the therapeutically-effective amount of the compound is administered to a group of fasted subjects, a value of about 11.2 h to about 30.0 h is observed for  $t_{1/2}$ .

**[0143]** In some embodiments, if the therapeutically-effective amount of the compound is administered to a fasted study subject and the therapeutically-effective amount of the compound is administered to a fed study subject, then a AUC of the compound in the fed study subject is greater than is the AUC of the compound in the fasted study subject.

**[0144]** In some embodiments, the AUC is  $AUC_{0-inf}$ . In some embodiments, if the therapeutically-effective amount of the compound is administered to a group of fed study subjects and a group of fasted subjects, a value of about 114000 h•ng/mL to about 264000 h•ng/mL is observed for  $AUC_{0-inf}$  in fed subjects, and a value of 98400 h•ng/mL to about 183000 h•ng/mL is observed for  $AUC_{0-inf}$  in fasted subjects. In some embodiments, if the therapeutically-effective amount of the compound is administered to a group of fed study subjects and a group of fasted subjects, a value of about 272000 h•ng/mL to about 839000 h•ng/mL is observed for  $AUC_{0-inf}$  in fed subjects, and a value of 106000 h•ng/mL to about 613000 h•ng/mL is observed for  $AUC_{0-inf}$  in fasted subjects. In some embodiments, if the therapeutically-effective amount of the compound is administered to a group of fasted subjects, a value of about 209000 h•ng/mL to about 487000 h•ng/mL is observed for  $AUC_{0-inf}$ .

**[0145]** In some embodiments, the AUC is  $AUC_{0-last}$ . In some embodiments, if the therapeutically-effective amount of the compound is administered to a group of fed study subjects and a group of fasted subjects, a value of about 113000 h•ng/mL to about 253000 h•ng/mL is observed for  $AUC_{0-last}$  in fed subjects, and a value of 95900 h•ng/mL to about 182000 h•ng/mL is observed for  $AUC_{0-last}$  in fasted subjects. In some embodiments, if the therapeutically-effective amount of the compound is administered to a group of fed study subjects and a group of fasted subjects, a value of about 269000 h•ng/mL to about 697000 h•ng/mL is observed for  $AUC_{0-last}$  in fed subjects, and a value of 105000 h•ng/mL to about 555000 h•ng/mL is observed for  $AUC_{0-last}$  in fasted subjects. In some embodiments, if the therapeutically-effective amount of the compound is administered to a group of fasted subjects, a value of about 201000 h•ng/mL to about 481000 h•ng/mL is observed for  $AUC_{0-last}$ .

**[0146]** In some embodiments, the AUC is  $AUC_{0-24}$ . In some embodiments, if the therapeutically-effective amount of the compound is administered to a group of fed study subjects and a group of fasted subjects, a value of about 85800 h•ng/mL to about 152000 h•ng/mL is observed for  $AUC_{0-24}$  in fed subjects, and a value of 72900 h•ng/mL to about 121000 h•ng/mL is observed for  $AUC_{0-24}$  in fasted subjects. In some embodiments, if the therapeutically-effective amount of the compound is administered to a group of fed study subjects and a group of fasted subjects, a value of about 178000 h•ng/mL to about 347000 h•ng/mL is observed for  $AUC_{0-24}$  in fed subjects, and a value of 46900 h•ng/mL to about 277000 h•ng/mL is observed for  $AUC_{0-24}$  in fasted subjects. In some embodiments, if the therapeutically-effective amount of the compound is administered to a group of fasted subjects, a value of about 97300 h•ng/mL to about 283000 h•ng/mL is observed for  $AUC_{0-24}$ .

[0147] In some embodiments, the AUC is  $AUC_{0-96}$ . In some embodiments, if the therapeutically-effective amount of the compound is administered to a group of fed study subjects and a group of fasted subjects, a value of about 114000 h•ng/mL to about 253000 h•ng/mL is observed for  $AUC_{0-96}$  in fed subjects, and a value of 97600 h•ng/mL to about 182000 h•ng/mL is observed for  $AUC_{0-96}$  in fasted subjects.

[0148] In some embodiments, the AUC is  $AUC_{\%extrap}$ . In some embodiments, if the therapeutically-effective amount of the compound is administered to a group of fed study subjects and a group of fasted subjects, a value of about 0.884 % to about 4.21 % is observed for  $AUC_{\%extrap}$  in fed subjects, and a value of 0.821 % to about 3.62 % is observed for  $AUC_{\%extrap}$  in fasted subjects. In some embodiments, if the therapeutically-effective amount of the compound is administered to a group of fed study subjects and a group of fasted subjects, a value of about 0.953 % to about 16.8 % is observed for  $AUC_{\%extrap}$  in fed subjects, and a value of 0.901 % to about 9.51 % is observed for  $AUC_{\%extrap}$  in fasted subjects. In some embodiments, if the therapeutically-effective amount of the compound is administered to a group of fasted subjects, a value of about 0.482 % to about 10.5 % is observed for  $AUC_{\%extrap}$ .

[0149] In some embodiments, if the therapeutically-effective amount of the compound is administered to a fasted study subject and the therapeutically-effective amount of the compound is administered to a fed study subject, then a CL/F of the compound in the fasted study subject is greater than is the CL/F of the compound in the fed study subject. In some embodiments, if the therapeutically-effective amount of the compound is administered to a group of fed study subjects and a group of fasted subjects, a value of about 3.79 L/h to about 8.76 L/h is observed for CL/F in fed subjects, and a value of 5.45 L/h to about 10.2 L/h is observed for CL/F in fasted subjects. In some embodiments, if the therapeutically-effective amount of the compound is administered to a group of fed study subjects and a group of fasted subjects, a value of about 2.39 L/h to about 7.36 L/h is observed for CL/F in fed subjects, and a value of 3.27 L/h to about 18.8 L/h is observed for CL/F in fasted subjects. In some embodiments, if the therapeutically-effective amount of the compound is administered to a group of fasted subjects, a value of about 4.11 L/h to about 9.56 L/h is observed for CL/F.

[0150] In some embodiments, if the therapeutically-effective amount of the compound is administered to a fasted study subject and the therapeutically-effective amount of the compound is administered to a fed study subject, then a  $V_z/F$  of the compound in the fasted study subject is greater than is the  $V_z/F$  of the compound in the fed study subject. In some embodiments, if the therapeutically-effective amount of the compound is administered to a group of fed study subjects and a group of fasted subjects, a value of about 99.6 L to about 164 L is observed for  $V_z/F$  in fed subjects, and a value of 107 L to about 199 L is observed for  $V_z/F$  in fasted subjects. In some embodiments, if the therapeutically-effective amount of the compound is administered to a group of fed study subjects and a group of fasted subjects, a value of about 86.8 L to about 216 L is observed for  $V_z/F$  in fed subjects, and a value of 117 L to about 382 L is observed for  $V_z/F$  in fasted subjects. In some embodiments, if the therapeutically-effective amount of the compound is administered to a group of fasted subjects, a value of about 93.8 L/h to about 251 L/h is observed for  $V_z/F$ .

[0151] In some embodiments, if the therapeutically-effective amount of the compound is administered to a fasted study subject and the therapeutically-effective amount of the compound is administered to a fed study subject, then a ratio of mean peak plasma concentration ( $C_{max}$ ) to mean 12-hour plasma concentration ( $C_{12h}$ ) of the compound is greater than is the ratio of mean peak plasma concentration ( $C_{max}$ ) to mean 12-hour plasma concentration ( $C_{12h}$ ) of the compound in a fasted state.

**Pharmaceutically-acceptable Salts.**

[0152] The disclosure provides the use of pharmaceutically-acceptable salts of any therapeutic compound described herein. Pharmaceutically-acceptable salts include, for example, acid-addition salts and base-addition salts. The acid that is added to the compound to form an acid-addition salt can be an organic acid or an inorganic acid. A base that is added to the compound to form a base-addition salt can be an organic base or an inorganic base. In some embodiments, a pharmaceutically-acceptable salt is a metal salt. In some embodiments, a pharmaceutically-acceptable salt is an ammonium salt.

[0153] Metal salts can arise from the addition of an inorganic base to a compound of the disclosure. The inorganic base consists of a metal cation paired with a basic counterion, such as, for example, hydroxide, carbonate, bicarbonate, or phosphate. The metal can be an alkali metal, alkaline earth metal, transition metal, or main group metal. In some embodiments, the metal is lithium, sodium, potassium, cesium, cerium, magnesium, manganese, iron, calcium, strontium, cobalt, titanium, aluminum, copper, cadmium, or zinc.

[0154] In some embodiments, a metal salt is a lithium salt, a sodium salt, a potassium salt, a cesium salt, a cerium salt, a magnesium salt, a manganese salt, an iron salt, a calcium salt, a strontium salt, a cobalt salt, a titanium salt, an aluminum salt, a copper salt, a cadmium salt, or a zinc salt.

[0155] Ammonium salts can arise from the addition of ammonia or an organic amine to a compound of the present disclosure. In some embodiments, the organic amine is triethyl amine, diisopropyl amine, ethanol amine, diethanol amine, triethanol amine, morpholine, N-methylmorpholine, piperidine, N-methylpiperidine, N-ethylpiperidine, dibenzylamine, piperazine, pyridine, pyrazole, imidazole, or pyrazine.

[0156] In some embodiments, an ammonium salt is a triethyl amine salt, a trimethyl amine salt, a diisopropyl amine salt, an ethanol amine salt, a diethanol amine salt, a triethanol amine salt, a morpholine salt, an N-methylmorpholine salt, a piperidine salt, an N-methylpiperidine salt, an N-ethylpiperidine salt, a dibenzylamine salt, a piperazine salt, a pyridine salt, a pyrazole salt, a pyridazine salt, a pyrimidine salt, an imidazole salt, or a pyrazine salt.

[0157] Acid addition salts can arise from the addition of an acid to a compound of the present disclosure. In some embodiments, the acid is organic. In some embodiments, the acid is inorganic. In some embodiments, the acid is hydrochloric acid, hydrobromic acid, hydroiodic acid, nitric acid, nitrous acid, sulfuric acid, sulfurous acid, a phosphoric acid, isonicotinic acid, lactic acid, salicylic acid, tartaric acid, ascorbic acid, gentisic acid, gluconic acid, glucuronic acid, saccharic acid, formic acid, benzoic acid, glutamic acid, pantothenic acid, acetic acid, propionic acid, butyric acid, fumaric acid, succinic acid,

methanesulfonic acid, ethanesulfonic acid, benzenesulfonic acid, p-toluenesulfonic acid, citric acid, oxalic acid, or maleic acid.

In some embodiments, the salt is a hydrochloride salt, a hydrobromide salt, a hydroiodide salt, a nitrate salt, a nitrite salt, a sulfate salt, a sulfite salt, a phosphate salt, isonicotinate salt, a lactate salt, a salicylate salt, a tartrate salt, an ascorbate salt, a gentisate salt, a gluconate salt, a glucuronate salt, a saccharate salt, a formate salt, a benzoate salt, a glutamate salt, a pantothenate salt, an acetate salt, a propionate salt, a butyrate salt, a fumarate salt, a succinate salt, a methanesulfonate salt, an ethanesulfonate salt, a benzenesulfonate salt, a p-toluenesulfonate salt, a citrate salt, an oxalate salt, or a maleate salt.

#### **Pharmaceutical Compositions of the disclosure.**

[0158] A pharmaceutical composition of the disclosure can be used, for example, before, during, or after treatment of a subject with, for example, another pharmaceutical agent.

[0159] Subjects can be, for example, elderly adults, adults, adolescents, pre-adolescents, children, toddlers, infants, neonates, and non-human animals. In some embodiments, a subject is a patient.

[0160] A pharmaceutical composition of the disclosure can be a combination of any pharmaceutical compounds described herein with other chemical components, such as carriers, stabilizers, diluents, dispersing agents, suspending agents, thickening agents, and/or excipients. The pharmaceutical composition facilitates administration of the compound to an organism. Pharmaceutical compositions can be administered in therapeutically-effective amounts as pharmaceutical compositions by various forms and routes including, for example, intravenous, subcutaneous, intramuscular, oral, parenteral, ophthalmic, subcutaneous, transdermal, nasal, vaginal, and topical administration.

[0161] A pharmaceutical composition can be administered in a local manner, for example, via injection of the compound directly into an organ, optionally in a depot or sustained release formulation or implant. Pharmaceutical compositions can be provided in the form of a rapid release formulation, in the form of an extended release formulation, or in the form of an intermediate release formulation. A rapid release form can provide an immediate release. An extended release formulation can provide a controlled release or a sustained delayed release.

[0162] For oral administration, pharmaceutical compositions can be formulated by combining the active compounds with pharmaceutically-acceptable carriers or excipients. Such carriers can be used to formulate liquids, gels, syrups, elixirs, slurries, or suspensions, for oral ingestion by a subject. Non-limiting examples of solvents used in an oral dissolvable formulation can include water, ethanol, isopropanol, saline, physiological saline, DMSO, dimethylformamide, potassium phosphate buffer, phosphate buffer saline (PBS), sodium phosphate buffer, 4-2-hydroxyethyl-1-piperazineethanesulfonic acid buffer (HEPES), 3-(N-morpholino)propanesulfonic acid buffer (MOPS), piperazine-N,N'-bis(2-ethanesulfonic acid) buffer (PIPES), and saline sodium citrate buffer (SSC). Non-limiting examples of co-solvents used in an oral dissolvable formulation can include sucrose, urea, cremaphor, DMSO, and potassium phosphate buffer.

[0163] Pharmaceutical preparations can be formulated for intravenous administration. The pharmaceutical compositions can be in a form suitable for parenteral injection as a sterile suspension,

solution or emulsion in oily or aqueous vehicles, and can contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water soluble form. Suspensions of the active compounds can be prepared as oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. The suspension can also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. Alternatively, the active ingredient can be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

[0164] The active compounds can be administered topically and can be formulated into a variety of topically administrable compositions, such as solutions, suspensions, lotions, gels, pastes, medicated sticks, balms, creams, and ointments. Such pharmaceutical compositions can contain solubilizers, stabilizers, tonicity enhancing agents, buffers and preservatives.

[0165] The compounds of the disclosure can be applied topically to the skin, or a body cavity, for example, oral, vaginal, bladder, cranial, spinal, thoracic, or pelvic cavity of a subject. The compounds of the disclosure can be applied to an accessible body cavity.

[0166] The compounds can also be formulated in rectal compositions such as enemas, rectal gels, rectal foams, rectal aerosols, suppositories, jelly suppositories, or retention enemas, containing conventional suppository bases such as cocoa butter or other glycerides, as well as synthetic polymers such as polyvinylpyrrolidone, and PEG. In suppository forms of the compositions, a low-melting wax such as a mixture of fatty acid glycerides, optionally in combination with cocoa butter, can be melted.

[0167] In practicing the methods of treatment or use provided herein, therapeutically-effective amounts of the compounds described herein are administered in pharmaceutical compositions to a subject having a disease or condition to be treated. In some embodiments, the subject is a mammal such as a human. A therapeutically-effective amount can vary widely depending on the severity of the disease, the age and relative health of the subject, the potency of the compounds used, and other factors. The compounds can be used singly or in combination with one or more therapeutic agents as components of mixtures.

[0168] Pharmaceutical compositions can be formulated using one or more physiologically-acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active compounds into preparations that can be used pharmaceutically. Formulations can be modified depending upon the route of administration chosen. Pharmaceutical compositions comprising a compound described herein can be manufactured, for example, by mixing, dissolving, emulsifying, encapsulating, entrapping, or compression processes.

[0169] The pharmaceutical compositions can include at least one pharmaceutically-acceptable carrier, diluent, or excipient and compounds described herein as free-base or pharmaceutically-acceptable salt form. Pharmaceutical compositions can contain solubilizers, stabilizers, tonicity enhancing agents, buffers and preservatives.

[0170] Methods for the preparation of compositions comprising the compounds described herein include formulating the compounds with one or more inert, pharmaceutically-acceptable excipients or carriers to

form a solid, semi-solid, or liquid composition. Solid compositions include, for example, powders, tablets, dispersible granules, capsules, and cachets. Liquid compositions include, for example, solutions in which a compound is dissolved, emulsions comprising a compound, or a solution containing liposomes, micelles, or nanoparticles comprising a compound as disclosed herein. Semi-solid compositions include, for example, gels, suspensions and creams. The compositions can be in liquid solutions or suspensions, solid forms suitable for solution or suspension in a liquid prior to use, or as emulsions. These compositions can also contain minor amounts of nontoxic, auxiliary substances, such as wetting or emulsifying agents, pH buffering agents, and other pharmaceutically-acceptable additives.

[0171] Non-limiting examples of dosage forms suitable for use in the disclosure include liquid, powder, gel, nanosuspension, nanoparticle, microgel, aqueous or oily suspensions, emulsion, and any combination thereof.

[0172] Non-limiting examples of pharmaceutically-acceptable excipients suitable for use in the disclosure include binding agents, disintegrating agents, anti-adherents, anti-static agents, surfactants, anti-oxidants, coating agents, coloring agents, plasticizers, preservatives, suspending agents, emulsifying agents, anti-microbial agents, spheronization agents, and any combination thereof.

[0173] A composition of the disclosure can be, for example, an immediate release form or a controlled release formulation. An immediate release formulation can be formulated to allow the compounds to act rapidly. Non-limiting examples of immediate release formulations include readily dissolvable formulations. A controlled release formulation can be a pharmaceutical formulation that has been adapted such that release rates and release profiles of the active agent can be matched to physiological and chronotherapeutic requirements or, alternatively, has been formulated to effect release of an active agent at a programmed rate. Non-limiting examples of controlled release formulations include granules, delayed release granules, hydrogels (e.g., of synthetic or natural origin), other gelling agents (e.g., gel-forming dietary fibers), matrix-based formulations (e.g., formulations comprising a polymeric material having at least one active ingredient dispersed through), granules within a matrix, polymeric mixtures, and granular masses.

[0174] In some, a controlled release formulation is a delayed release form. A delayed release form can be formulated to delay a compound's action for an extended period of time. A delayed release form can be formulated to delay the release of an effective dose of one or more compounds, for example, for about 4, about 8, about 12, about 16, or about 24 hours.

[0175] A controlled release formulation can be a sustained release form. A sustained release form can be formulated to sustain, for example, the compound's action over an extended period of time. A sustained release form can be formulated to provide an effective dose of any compound described herein (e.g., provide a physiologically-effective blood profile) over about 4, about 8, about 12, about 16 or about 24 hours.

[0176] Non-limiting examples of pharmaceutically-acceptable excipients can be found, for example, in Remington: The Science and Practice of Pharmacy, Nineteenth Ed (Easton, Pa.: Mack Publishing Company, 1995); Hoover, John E., Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton,

Pennsylvania 1975; Liberman, H.A. and Lachman, L., Eds., *Pharmaceutical Dosage Forms*, Marcel Dekker, New York, N.Y., 1980; and *Pharmaceutical Dosage Forms and Drug Delivery Systems*, Seventh Ed. (Lippincott Williams & Wilkins 1999), each of which is incorporated by reference in its entirety.

[0177] Multiple therapeutic agents can be administered in any order or simultaneously. In some embodiments, a compound of the disclosure is administered in combination with, before, or after treatment with another therapeutic agent. If simultaneously, the multiple therapeutic agents can be provided in a single, unified form, or in multiple forms, for example, as multiple separate pills. The agents can be packed together or separately, in a single package or in a plurality of packages. One or all of the therapeutic agents can be given in multiple doses. If not simultaneous, the timing between the multiple doses can vary to as much as about a month.

[0178] In some embodiments, a compound of the disclosure is administered in combination with, before, or after treatment with an antiemetic agent. Suitable antiemetic agents include, but are not limited to: 5-HT<sub>3</sub> receptor antagonists, such as ondansetron, granisetron, dolasetron, and palonosetron; NK<sub>1</sub> receptor antagonists, such as aprepitant and fosaprepitant; corticosteroids, such as dexamethasone, dopamine receptor antagonists, such as phenothiazines (eg, prochlorperazine), butyrophenones (eg, haloperidol, droperidol), and substituted benzamides (eg, metoclopramide); benzodiazepines, such as lorazepam and alprazolam; olanzapine; and cannabinoids, such as dronabinol and nabilone.

[0179] In some embodiments, a compound of the disclosure is administered in combination with, before, or after treatment with a gastric acid reducing agent. In some embodiments, the gastric acid reducing agent is a H<sub>2</sub>-receptor antagonist. In some embodiments, the H<sub>2</sub>-receptor antagonist is cimetidine, ranitidine, famotidine, nizatidine, roxatidine, lafutidine, lafutidine, lavotidine, niperotidine, or sufolidine. In some embodiments, the H<sub>2</sub>-receptor antagonist is famotidine. In some embodiments, the gastric acid reducing agent is a proton pump inhibitor. In some embodiments, the proton pump inhibitor is omeprazole, esomeprazole, lansoprazole, dexlansoprazole, pantoprazole, or rabeprazole. In some embodiments, the proton pump inhibitor is rabeprazole.

[0180] In some embodiments, the present disclosure provides a method of treating a condition in a subject in need thereof, the method comprising selecting the subject for treatment on the basis that the subject has not been treated with a proton pump inhibitor within 7 days before administration of a p53 activating compound of the present disclosure to the subject, and administering the p53 activating compound to the subject on the basis of the selecting. In some embodiments, the method comprises administering to the subject a H<sub>2</sub>-receptor antagonist within 7 days before the administering the p53 activating compound.

[0181] In some embodiments, the present disclosure provides a method of treating a condition in a subject in need thereof, the method comprising a) selecting the subject for treatment with a p53 activating compound of the present disclosure on the basis that the subject has not been treated with a CYP3A4 modulator, a P-gp inhibitor, or a BCRP inhibitor, and b) administering the p53 activating compound to the subject on the basis of the selecting. CYP3A4 modulators include CYP3A4 inhibitors, such as, for example, clarithromycin, cobicistat, elvitegravir and ritonavir, grapefruit juice, indinavir and ritonavir,



idelalisib, itraconazole, ketoconazole, lopinavir and ritonavir, nefazodone, nelfinavir, paritaprevir/ritonavir/ombitasvir and/or dasabuvir, posaconazole, ritonavir, saquinavir and ritonavir, telaprevir, tipranavir and ritonavir, telithromycin, and voriconazole; and CYP3A4 inducers, such as, for example, apalutamide, carbamazepine, enzalutamide, mitotane, phenytoin, rifampin, and St. John's wort. P-gp inhibitors include, for example, amiodarone, carvedilol, clarithromycin, dronedarone, itraconazole, lopinavir and ritonavir, propafenone, quinidine, ranolazine, ritonavir, saquinavir and ritonavir, tipranavir and ritonavir, and verapamil. BCRP inhibitors include, for example, curcumin, cyclosporine A, and eltrombopag.

[0182] In some embodiments, the present disclosure provides a method of treating a condition in a subject in need thereof, the method comprising transitioning the subject from treatment with a proton pump inhibitor to treatment with a H<sub>2</sub>-receptor antagonist, and administering to the subject a p53 activating compound of the disclosure no earlier than 7 days after the transitioning.

[0183] Therapeutic agents described herein can be administered before, during, or after the occurrence of a disease or condition, and the timing of administering the composition containing a therapeutic agent can vary. For example, the compositions can be used as a prophylactic and can be administered continuously to subjects with a propensity to conditions or diseases in order to lessen a likelihood of the occurrence of the disease or condition. The compositions can be administered to a subject during or as soon as possible after the onset of the symptoms. The administration of the therapeutic agents can be initiated within the first 48 hours of the onset of the symptoms, within the first 24 hours of the onset of the symptoms, within the first 6 hours of the onset of the symptoms, or within 3 hours of the onset of the symptoms. The initial administration can be via any route practical, such as by any route described herein using any formulation described herein.

[0184] A compound can be administered as soon as is practical after the onset of a disease or condition is detected or suspected, and for a length of time necessary for the treatment of the disease, such as, for example, from about 1 month to about 3 months. In some embodiments, the length of time a compound can be administered can be about 1 day, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, about 1 week, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 5 weeks, about 6 weeks, about 7 weeks, about 8 weeks, about 2 months, about 9 weeks, about 10 weeks, about 11 weeks, about 12 weeks, about 3 months, about 13 weeks, about 14 weeks, about 15 weeks, about 16 weeks, about 4 months, about 17 weeks, about 18 weeks, about 19 weeks, about 20 weeks, about 5 months, about 21 weeks, about 22 weeks, about 23 weeks, about 24 weeks, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 1 year, about 13 months, about 14 months, about 15 months, about 16 months, about 17 months, about 18 months, about 19 months, about 20 months, about 21 months, about 22 months about 23 months, about 2 years, about 2.5 years, about 3 years, about 3.5 years, about 4 years, about 4.5 years, about 5 years, about 6 years, about 7 years, about 8 years, about 9 years, or about 10 years. The length of treatment can vary for each subject.

[0185] Pharmaceutical compositions described herein can be in unit dosage forms suitable for single administration of precise dosages. In unit dosage form, the formulation is divided into unit doses

containing appropriate quantities of one or more compounds. The unit dosage can be in the form of a package containing discrete quantities of the formulation. Non-limiting examples are packaged injectables, vials, or ampoules. Aqueous suspension compositions can be packaged in single-dose non-reclosable containers. Multiple-dose reclosable containers can be used, for example, in combination with or without a preservative. Formulations for injection can be presented in unit dosage form, for example, in ampoules, or in multi dose containers with a preservative.

**[0186]** Pharmaceutical compositions provided herein, can be administered in conjunction with other therapies, for example, chemotherapy, radiation, surgery, anti-inflammatory agents, and selected vitamins. The other agents can be administered prior to, after, or concomitantly with the pharmaceutical compositions.

**[0187]** Depending on the intended mode of administration, the pharmaceutical compositions can be in the form of solid, semi solid or liquid dosage forms, such as, for example, tablets, suppositories, pills, capsules, powders, liquids, suspensions, lotions, creams, or gels, for example, in unit dosage form suitable for single administration of a precise dosage.

**[0188]** For solid compositions, nontoxic solid carriers include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talc, cellulose, glucose, sucrose, and magnesium carbonate.

**[0189]** Non-limiting examples of pharmaceutically active agents suitable for combination with compositions of the disclosure include anti-infectives, i.e., aminoglycosides, antiviral agents, antimicrobials, anticholinergics/antispasmodics, antidiabetic agents, antihypertensive agents, antineoplastics, cardiovascular agents, central nervous system agents, coagulation modifiers, hormones, immunologic agents, immunosuppressive agents, and ophthalmic preparations.

**[0190]** Compounds can be delivered via liposomal technology. The use of liposomes as drug carriers can increase the therapeutic index of the compounds. Liposomes are composed of natural phospholipids, and can contain mixed lipid chains with surfactant properties (e.g., egg phosphatidylethanolamine). A liposome design can employ surface ligands for attaching to unhealthy tissue. Non-limiting examples of liposomes include the multilamellar vesicle (MLV), the small unilamellar vesicle (SUV), and the large unilamellar vesicle (LUV). Liposomal physicochemical properties can be modulated to optimize penetration through biological barriers and retention at the site of administration, and to reduce a likelihood of developing premature degradation and toxicity to non-target tissues. Optimal liposomal properties depend on the administration route: large-sized liposomes show good retention upon local injection, small-sized liposomes are better suited to achieve passive targeting. PEGylation reduces the uptake of the liposomes by the liver and spleen, and increases the circulation time, resulting in increased localization at the inflamed site due to the enhanced permeability and retention (EPR) effect. Additionally, liposomal surfaces can be modified to achieve selective delivery of the encapsulated drug to specific target cells. Non-limiting examples of targeting ligands include monoclonal antibodies, vitamins, peptides, and polysaccharides specific for receptors concentrated on the surface of cells associated with the disease.

[0191] Non-limiting examples of dosage forms suitable for use in the disclosure include liquid, elixir, nanosuspension, aqueous or oily suspensions, drops, syrups, and any combination thereof. Non-limiting examples of pharmaceutically-acceptable excipients suitable for use in the disclosure include granulating agents, binding agents, lubricating agents, disintegrating agents, sweetening agents, glidants, anti-adherents, anti-static agents, surfactants, anti-oxidants, gums, coating agents, coloring agents, flavoring agents, coating agents, plasticizers, preservatives, suspending agents, emulsifying agents, plant cellulosic material and spheronization agents, and any combination thereof.

[0192] Compositions of the disclosure can be packaged as a kit. In some embodiments, a kit includes written instructions on the administration/use of the composition. The written material can be, for example, a label. The written material can suggest conditions methods of administration. The instructions provide the subject and the supervising physician with the best guidance for achieving the optimal clinical outcome from the administration of the therapy. The written material can be a label. In some embodiments, the label can be approved by a regulatory agency, for example the U.S. Food and Drug Administration (FDA), the European Medicines Agency (EMA), or other regulatory agencies.

#### **Dosing.**

[0193] Pharmaceutical compositions described herein can be in unit dosage forms suitable for single administration of precise dosages. In unit dosage form, the formulation is divided into unit doses containing appropriate quantities of one or more compounds. The unit dosage can be in the form of a package containing discrete quantities of the formulation. Non-limiting examples are liquids in vials or ampoules. Aqueous suspension compositions can be packaged in single-dose non-reclosable containers. Multiple-dose reclosable containers can be used, for example, in combination with a preservative. Formulations for parenteral injection can be presented in unit dosage form, for example, in ampoules, or in multi dose containers with a preservative.

[0194] A compound described herein can be present in a composition in a range of from about 1 mg to about 3000 mg; from about 10 mg to about 3000 mg; from about 100 mg to about 3000 mg; from about 1000 mg to about 3000 mg; 1 mg to about 2000 mg; from about 10 mg to about 2000 mg; from about 100 mg to about 2000 mg; from about 1000 mg to about 2000 mg; from about 5 mg to about 1000 mg, from about 10 mg to about 500 mg, from about 50 mg to about 250 mg, from about 100 mg to about 200 mg, from about 1 mg to about 50 mg, from about 50 mg to about 100 mg, from about 100 mg to about 150 mg, from about 150 mg to about 200 mg, from about 200 mg to about 250 mg, from about 250 mg to about 300 mg, from about 300 mg to about 350 mg, from about 350 mg to about 400 mg, from about 400 mg to about 450 mg, from about 450 mg to about 500 mg, from about 500 mg to about 550 mg, from about 550 mg to about 600 mg, from about 600 mg to about 650 mg, from about 650 mg to about 700 mg, from about 700 mg to about 750 mg, from about 750 mg to about 800 mg, from about 800 mg to about 850 mg, from about 850 mg to about 900 mg, from about 900 mg to about 950 mg, or from about 950 mg to about 1000 mg.

[0195] A compound described herein can be present in a composition in an amount of about 1 mg, about 2 mg, about 3 mg, about 4 mg, about 5 mg, about 10 mg, about 15 mg, about 20 mg, about 25 mg, about

30 mg, about 35 mg, about 40 mg, about 45 mg, about 50 mg, about 55 mg, about 60 mg, about 65 mg, about 70 mg, about 75 mg, about 80 mg, about 85 mg, about 90 mg, about 95 mg, about 100 mg, about 125 mg, about 150 mg, about 175 mg, about 200 mg, about 250 mg, about 300 mg, about 350 mg, about 400 mg, about 450 mg, about 500 mg, about 550 mg, about 600 mg, about 650 mg, about 700 mg, about 750 mg, about 800 mg, about 850 mg, about 900 mg, about 950 mg, about 1000 mg, about 1050 mg, about 1100 mg, about 1150 mg, about 1200 mg, about 1250 mg, about 1300 mg, about 1350 mg, about 1400 mg, about 1450 mg, about 1500 mg, about 1550 mg, about 1600 mg, about 1650 mg, about 1700 mg, about 1750 mg, about 1800 mg, about 1850 mg, about 1900 mg, about 1950 mg, about 2000 mg, about 2100 mg, about 2200 mg, about 2300 mg, about 2400 mg, about 2500 mg, about 2600 mg, about 2700 mg, about 2800 mg, about 2900 mg, or about 3000 mg.

[0196] In some embodiments, a compound of the disclosure can be present in a composition in an amount of from about 500 mg to about 2000 mg. In some embodiments, a compound of the disclosure can be present in a composition in an amount of from about 1000 mg to about 1500 mg. In some embodiments, a compound of the disclosure can be present in a composition in an amount of about 500 mg. In some embodiments, a compound of the disclosure can be present in a composition in an amount of about 1000 mg. In some embodiments, a compound of the disclosure can be present in a composition in an amount of about 1500 mg. In some embodiments, a compound of the disclosure can be present in a composition in an amount of about 2000 mg.

[0197] In some embodiments, a dose can be expressed in terms of an amount of the drug divided by the mass of the subject, for example, milligrams of drug per kilograms of subject body mass. In some embodiments, a compound is administered in an amount ranging from about 5 mg/kg to about 50 mg/kg, 250 mg/kg to about 2000 mg/kg, about 10 mg/kg to about 800 mg/kg, about 50 mg/kg to about 400 mg/kg, about 100 mg/kg to about 300 mg/kg, or about 150 mg/kg to about 200 mg/kg.

#### **Companion Diagnostic Methods.**

[0198] Disclosed herein are methods of treating a condition in a subject in need thereof, the method comprising administering to the subject a therapeutically-effective amount of a small molecule compound that binds a p53 mutant protein, wherein a mutational status of a KRAS gene in the subject has been previously determined. In some embodiments, the mutational status of the KRAS gene in the subject has previously been determined based on an assay performed on a biological sample of the subject. In some embodiments, the subject has been previously determined to have a mutation in a TP53 gene that encodes a p53 mutant in the subject.

[0199] Further disclosed herein is a method of treating a condition in a subject in need thereof, the method comprising: a) based on a result of an assay performed on a biological sample of the subject, determining a mutational status of a KRAS gene in the subject; and b) based at least in part on the mutational status of the KRAS gene in the subject, administering to the subject a therapeutically-effective amount of a compound that binds a p53 mutant protein. In some embodiments, the method further comprises, based on the result of an assay, determining that the subject harbors a mutation in a TP53 gene that encodes a p53 mutant protein, wherein the administering to the subject the therapeutically-effective

amount of the compound that binds the p53 mutant protein is based at least in part on the determining that the subject harbors the mutation in the TP53 gene that encodes the p53 mutant protein.

[0200] In some embodiments, the method further comprises performing the assay on the biological sample of the subject. In some embodiments, the method further comprises performing the assay on the biological sample of the subject, wherein performing the assay on the biological sample of the subject further comprises detecting the mutation in the TP53 gene that encodes the p53 mutant in the subject.

[0201] In some embodiments, the mutational status of the KRAS gene in the subject comprises absence of a missense mutation at any of codons 12 and 13. In some embodiments, the mutational status of the KRAS gene in the subject comprises absence of a missense mutation at any of codons 12, 13, and 61. In some embodiments, the mutational status of the KRAS gene in the subject comprises absence of a missense mutation at any of codons 12, 13, 59, 61, 117, and 146.

[0202] In some embodiments, the mutational status of the KRAS gene in the subject comprises absence of a mutation that would encode an amino acid other than glycine at codon 12. In some embodiments, the mutational status of the KRAS gene in the subject comprises absence of a mutation that would encode an amino acid other than glycine at codon 13. In some embodiments, the mutational status of the KRAS gene in the subject comprises absence of a mutation that would encode an amino acid other than alanine at codon 59. In some embodiments, the mutational status of the KRAS gene in the subject comprises absence of a mutation that would encode an amino acid other than glutamine at codon 61. In some embodiments, the mutational status of the KRAS gene in the subject comprises absence of a mutation that would encode an amino acid other than lysine at codon 117. In some embodiments, the mutational status of the KRAS gene in the subject comprises absence of a mutation that would encode an amino acid other than alanine at codon 146.

[0203] In some embodiments, the mutational status of the KRAS gene in the subject is a KRAS gene that encodes a wild type K-ras protein. In some embodiments, the wild type K-Ras protein has a sequence according to SEQ ID NO: 1 or SEQ ID NO: 2.

[0204] In some embodiments, the mutational status of the KRAS gene in the subject is a copy number variation status of the KRAS gene. In some embodiments, the mutational status of the KRAS gene in the subject comprises absence of a KRAS copy number variation.

[0205] The mutational status of a gene in a subject can be, for example, a single nucleotide polymorphism (SNP), a mutated gene sequence (MUT), loss of gene expression without gene mutation (LOE), wild-type gene sequence (WT), or an entire gene deletion (NULL).

[0206] In some embodiments, the methods of the disclosure further comprise obtaining the biological sample of the subject. In some embodiments, the methods of the disclosure further comprise performing the assay on the biological sample of the subject to detect a mutation in a gene (e.g. TP53, KRAS, PIK3CA, and BRCA1/2) that encodes a protein in the subject.

[0207] In some embodiments, a mutational status of a BRCA1 or BRCA2 gene in the subject has been previously determined. In some embodiments, the mutational status of the BRCA1 or BRCA2 gene in the subject has previously been determined based on an assay performed on a biological sample of the

subject. In some embodiments, the subject has been previously determined to have a mutation in a TP53 gene that encodes a p53 mutant in the subject.

[0208] Further disclosed herein is a method of treating a condition in a subject in need thereof, the method comprising: based at least in part on a mutational status of a BRCA1 or BRCA2 gene in the subject, administering to the subject a therapeutically-effective amount of a compound that binds a p53 mutant protein. Further disclosed herein is a method of treating a condition in a subject in need thereof, the method comprising: a) determining a mutational status of a BRCA1 or BRCA2 gene in the subject; and b) based at least in part on the mutational status of the BRCA1 or BRCA2 gene in the subject, administering to the subject a therapeutically-effective amount of a compound that binds a p53 mutant protein. Further disclosed herein is a method of treating a condition in a subject in need thereof, the method comprising: a) based on a result of an assay performed on a biological sample of the subject, determining a mutational status of a BRCA1 or BRCA2 gene in the subject; and b) based at least in part on the mutational status of the BRCA1 or BRCA2 gene in the subject, administering to the subject a therapeutically-effective amount of a compound that binds a p53 mutant protein. Further disclosed herein is a method of treating a condition in a subject in need thereof, the method comprising: a) performing an assay on a biological sample of the subject to determine a mutational status of a BRCA1 or BRCA2 gene in the subject; and b) based at least in part on the mutational status of the BRCA1 or BRCA2 gene in the subject, administering to the subject a therapeutically-effective amount of a compound that binds a p53 mutant protein. In some embodiments, the method further comprises, based on the result of an assay, determining that the subject harbors a mutation in a TP53 gene that encodes a p53 mutant protein, wherein the administering to the subject the therapeutically-effective amount of the compound that binds the p53 mutant protein is based at least in part on the determining that the subject harbors the mutation in the TP53 gene that encodes the p53 mutant protein.

[0209] Further disclosed herein is a method of treating a condition in a subject in need thereof, the method comprising: based at least in part on a mutational status of a PIK3CA gene in the subject, administering to the subject a therapeutically-effective amount of a compound that binds a p53 mutant protein. Further disclosed herein is a method of treating a condition in a subject in need thereof, the method comprising: a) determining a mutational status of a PIK3CA gene in the subject; and b) based at least in part on the mutational status of the PIK3CA gene in the subject, administering to the subject a therapeutically-effective amount of a compound that binds a p53 mutant protein. Further disclosed herein is a method of treating a condition in a subject in need thereof, the method comprising: a) based on a result of an assay performed on a biological sample of the subject, determining a mutational status of a PIK3CA gene in the subject; and b) based at least in part on the mutational status of the PIK3CA gene in the subject, administering to the subject a therapeutically-effective amount of a compound that binds a p53 mutant protein. Further disclosed herein is a method of treating a condition in a subject in need thereof, the method comprising: a) performing an assay on a biological sample of the subject to determine a mutational status of a PIK3CA gene in the subject; and b) based at least in part on the mutational status of the PIK3CA gene in the subject, administering to the subject a therapeutically-effective amount of a

compound that binds a p53 mutant protein. In some embodiments, the method further comprises, based on the result of an assay, determining that the subject harbors a mutation in a TP53 gene that encodes a p53 mutant protein, wherein the administering to the subject the therapeutically-effective amount of the compound that binds the p53 mutant protein is based at least in part on the determining that the subject harbors the mutation in the TP53 gene that encodes the p53 mutant protein.

**[0210]** In some embodiments, a homologous recombination deficiency (HRD) status of a cancer in the subject has been previously determined. In some embodiments, the HRD status of the cancer in the subject has previously been determined based on an assay performed on a biological sample of the subject. In some embodiments, the subject has been previously determined to have a mutation in a TP53 gene that encodes a p53 mutant in the subject.

**[0211]** Further disclosed herein is a method of treating a condition in a subject in need thereof, the method comprising: based at least in part on a HRD status of the cancer in the subject, administering to the subject a therapeutically-effective amount of a compound that binds a p53 mutant protein. Further disclosed herein is a method of treating a condition in a subject in need thereof, the method comprising: a) determining a HRD status of a cancer in the subject; and b) based at least in part on the HRD status of the cancer in the subject, administering to the subject a therapeutically-effective amount of a compound that binds a p53 mutant protein. Further disclosed herein is a method of treating a condition in a subject in need thereof, the method comprising: a) based on a result of an assay performed on a biological sample of the subject, determining a HRD status of a cancer in the subject; and b) based at least in part on the HRD status of the cancer in the subject, administering to the subject a therapeutically-effective amount of a compound that binds a p53 mutant protein. Further disclosed herein is a method of treating a condition in a subject in need thereof, the method comprising: a) performing an assay on a biological sample of the subject to determine a HRD status of a cancer in the subject; and b) based at least in part on the HRD status of the cancer in the subject, administering to the subject a therapeutically-effective amount of a compound that binds a p53 mutant protein. In some embodiments, the method further comprises, based on the result of an assay, determining that the subject harbors a mutation in a TP53 gene that encodes a p53 mutant protein, wherein the administering to the subject the therapeutically-effective amount of the compound that binds the p53 mutant protein is based at least in part on the determining that the subject harbors the mutation in the TP53 gene that encodes the p53 mutant protein.

**[0212]** In some embodiments, the administering of the therapeutically-effective amount of the small molecule compound to the subject reduces a likelihood of denaturation of the p53 mutant in the subject. In some embodiments, the administering of the therapeutically-effective amount of the small molecule compound to the subject reactivates a p53 pathway in the subject. In some embodiments, the administering of the therapeutically-effective amount of the small molecule compound to the subject increases anti-cancer activity of the p53 mutant in the subject.

**[0213]** In some embodiments, the small molecule compound increases an ability of the p53 mutant to bind DNA. In some embodiments, the small molecule compound induces a conformational change in the p53 mutant. In some embodiments, the small molecule compound selectively binds the p53 mutant as

compared to a wild type p53. In some embodiments, the small molecule compound increases stability of a biologically-active conformation of the p53 mutant relative to the stability of the biologically-active conformation of the p53 mutant in absence of the small molecule compound.

[0214] In some embodiments, the mutation in the TP53 gene deactivates a p53 pathway in the subject. In some embodiments, the subject has a cancer, wherein the cancer expresses the p53 mutant. In some embodiments, the mutation is at amino acid 220. In some embodiments, the p53 mutation is a p53 Y220C. In some embodiments, the p53 mutant has a reduced ability to bind to DNA in the subject as compared to wild-type p53. In some embodiments, the TP53 gene contains a frameshift mutation. In some embodiments, the TP53 gene contains a splice site mutation. In some embodiments, the TP53 gene contains an insertion mutation. In some embodiments, the TP53 gene contains a deletion mutation. In some embodiments, the TP53 gene contains a substitution mutation. In some embodiments, the TP53 gene contains a copy number variation. In some embodiments, the TP53 gene contains a copy number loss. In some embodiments, the TP53 gene contains a single nucleotide polymorphism. In some embodiments, the mutation in the TP53 gene is c.659A>G.

[0215] Further disclosed herein is a method of treating a condition in a subject in need thereof, the method comprising a) based on a result of an assay performed on a biological sample of the subject, determining a mutational status of a KRAS gene in the subject; and b) based at least in part on the mutational status of the KRAS gene in the subject, determining to not administer to the subject a compound that binds a p53 mutant protein. In some embodiments, the mutational status of the KRAS gene in the subject comprises mutation at codon 12 that encodes cysteine. In some embodiments, the mutational status of the KRAS gene in the subject comprises mutation at codon 12 that encodes arginine. In some embodiments, the mutational status of the KRAS gene in the subject comprises mutation at codon 12 that encodes serine. In some embodiments, the mutational status of the KRAS gene in the subject comprises mutation at codon 12 that encodes alanine. In some embodiments, the mutational status of the KRAS gene in the subject comprises mutation at codon 12 that encodes glutamic acid. In some embodiments, the mutational status of the KRAS gene in the subject comprises mutation at codon 12 that encodes valine.

[0216] In some embodiments, the present disclosure provides a use of a compound herein (e.g., Compound 1) in treatment of cancer in a subject in need thereof, wherein the subject has been previously determined to lack an oncogenic mutation in a KRAS gene based on an assay performed on a biological sample of the subject.

[0217] Further disclosed herein is a method of selecting a subject for treatment with a compound herein (e.g., Compound 1), the method comprising performing an assay on a biological sample of the subject to determine a mutational status of a KRAS gene in the subject, wherein if the KRAS gene in the subject does not comprise an oncogenic mutation, then the subject is selected for treatment with a compound herein (e.g., Compound 1). Further disclosed herein is a method of selecting a subject for treatment with a compound herein, the method comprising based on a result of an assay performed on a biological sample of the subject, determining a mutational status of a KRAS gene in the subject, wherein if the KRAS gene



in the subject does not comprise an oncogenic mutation, then the subject is selected for treatment with a compound herein (e.g., Compound 1).

[0218] In some embodiments, the mutational status of the KRAS gene in the subject comprises mutation at codon 13 that encodes aspartic acid.

[0219] In some embodiments, the mutational status of the KRAS gene in the subject comprises a mutation at codon 59 that encodes glutamic acid. In some embodiments, the mutational status of the KRAS gene in the subject comprises a mutation at codon 59 that encodes glycine. In some embodiments, the mutational status of the KRAS gene in the subject comprises a mutation at codon 59 that encodes threonine.

[0220] In some embodiments, the mutational status of the KRAS gene in the subject comprises a mutation at codon 61 that encodes lysine. In some embodiments, the mutational status of the KRAS gene in the subject comprises a mutation at codon 61 that encodes leucine. In some embodiments, the mutational status of the KRAS gene in the subject comprises a mutation at codon 61 that encodes arginine. In some embodiments, the mutational status of the KRAS gene in the subject comprises a mutation at codon 61 that encodes histidine.

[0221] In some embodiments, the mutational status of the KRAS gene in the subject comprises a mutation at codon 117 that encodes asparagine.

[0222] In some embodiments, the mutational status of the KRAS gene in the subject comprises a mutation at codon 146 that encodes proline. In some embodiments, the mutational status of the KRAS gene in the subject comprises a mutation at codon 146 that encodes threonine. In some embodiments, the mutational status of the KRAS gene in the subject comprises a mutation at codon 146 that encodes valine.

[0223] In some embodiments, the mutational status of the KRAS gene in the subject is a KRAS gene that encodes a mutant K-ras protein. In some embodiments, the mutant K-Ras protein does not have a sequence according to SEQ ID NO: 1 or SEQ ID NO: 2.

[0224] In some embodiments, the mutational status of the KRAS gene in the subject is a copy number variation status of the KRAS gene. In some embodiments, the mutational status of the KRAS gene in the subject is presence of a KRAS copy number variation.

[0225] Further disclosed herein is a method of treating a condition in a subject in need thereof, the method comprising: based at least in part on a mutational status of a BRCA1 or BRCA2 gene in the subject, not administering to the subject a therapeutically-effective amount of a compound that binds a p53 mutant protein. Further disclosed herein is a method of treating a condition in a subject in need thereof, the method comprising: a) determining a mutational status of a BRCA1 or BRCA2 gene in the subject; and b) based at least in part on the mutational status of the BRCA1 or BRCA2 gene in the subject, not administering to the subject a therapeutically-effective amount of a compound that binds a p53 mutant protein. Further disclosed herein is a method of treating a condition in a subject in need thereof, the method comprising: a) based on a result of an assay performed on a biological sample of the subject, determining a mutational status of a BRCA1 or BRCA2 gene in the subject; and b) based at least in part on the mutational status of the BRCA1 or BRCA2 gene in the subject, not administering to the

subject a therapeutically-effective amount of a compound that binds a p53 mutant protein. Further disclosed herein is a method of treating a condition in a subject in need thereof, the method comprising: a) performing an assay on a biological sample of the subject to determine a mutational status of a BRCA1 or BRCA2 gene in the subject; and b) based at least in part on the mutational status of the BRCA1 or BRCA2 gene in the subject, not administering to the subject a therapeutically-effective amount of a compound that binds a p53 mutant protein.

[0226] In some embodiments, the present disclosure provides a use of a compound herein (e.g., Compound 1) in treatment of cancer in a subject in need thereof, wherein the subject has been previously determined to lack an oncogenic mutation in a BRCA1 or BRCA2 gene based on an assay performed on a biological sample of the subject.

[0227] Further disclosed herein is a method of selecting a subject for treatment with a compound herein (e.g., Compound 1), the method comprising performing an assay on a biological sample of the subject to determine a mutational status of a BRCA1 or BRCA2 gene in the subject, wherein if the BRCA1 or BRCA2 gene in the subject does not comprise an oncogenic mutation, then the subject is selected for treatment with a compound herein (e.g., Compound 1). Further disclosed herein is a method of selecting a subject for treatment with a compound herein (e.g., Compound 1), the method comprising based on a result of an assay performed on a biological sample of the subject, determining a mutational status of a BRCA1 or BRCA2 gene in the subject, wherein if the BRCA1 or BRCA2 gene in the subject does not comprise an oncogenic mutation, then the subject is selected for treatment with a compound herein (e.g., Compound 1).

[0228] Further disclosed herein is a method of treating a condition in a subject in need thereof, the method comprising: based at least in part on a mutational status of a PIK3CA gene in the subject, not administering to the subject a therapeutically-effective amount of a compound that binds a p53 mutant protein. Further disclosed herein is a method of treating a condition in a subject in need thereof, the method comprising: a) determining a mutational status of a PIK3CA gene in the subject; and b) based at least in part on the mutational status of the PIK3CA gene in the subject, not administering to the subject a therapeutically-effective amount of a compound that binds a p53 mutant protein. Further disclosed herein is a method of treating a condition in a subject in need thereof, the method comprising: a) based on a result of an assay performed on a biological sample of the subject, determining a mutational status of a PIK3CA gene in the subject; and b) based at least in part on the mutational status of the PIK3CA gene in the subject, not administering to the subject a therapeutically-effective amount of a compound that binds a p53 mutant protein. Further disclosed herein is a method of treating a condition in a subject in need thereof, the method comprising: a) performing an assay on a biological sample of the subject to determine a mutational status of a PIK3CA gene in the subject; and b) based at least in part on the mutational status of the PIK3CA gene in the subject, not administering to the subject a therapeutically-effective amount of a compound that binds a p53 mutant protein.

[0229] In some embodiments, the present disclosure provides a use of a compound herein (e.g., Compound 1) in treatment of cancer in a subject in need thereof, wherein the subject has been previously

determined to lack an oncogenic mutation in a PIK3CA gene based on an assay performed on a biological sample of the subject.

[0230] In some embodiments, the mutational status of the PIK3CA gene in the subject is a PIK3CA gene that does not encode an oncogenic mutation. In some embodiments, the mutational status of the PIK3CA gene in the subject comprises an absence of a missense mutation at any of codons 542, 545, 1047, and 1047. In some embodiments, the mutational status of the PIK3CA gene in the subject comprises absence of a mutation that would encode an amino acid other than glutamic acid at codon 542. In some embodiments, the mutational status of the PIK3CA gene in the subject comprises absence of a mutation that would encode an amino acid other than glutamic acid at codon 545. In some embodiments, the mutational status of the PIK3CA gene in the subject comprises absence of a mutation that would encode an amino acid other than histidine at codon 1047. In some embodiments, the mutational status of the PIK3CA gene in the subject comprises absence of a mutation that would encode an amino acid other than lysine at codon 117. In some embodiments, the mutational status of the PIK3CA gene in the subject is a PIK3CA gene that encodes a wild type p110 $\alpha$  protein. In some embodiments, the mutational status of the PIK3CA gene in the subject is absence of a copy number variation status of the PIK3CA gene.

[0231] Further disclosed herein is a method of selecting a subject for treatment with a compound herein (e.g., Compound 1), the method comprising performing an assay on a biological sample of the subject to determine a mutational status of a PIK3CA gene in the subject, wherein if the PIK3CA gene in the subject does not comprise an oncogenic mutation, then the subject is selected for treatment with a compound herein (e.g., Compound 1). Further disclosed herein is a method of selecting a subject for treatment with a compound herein (e.g., Compound 1), the method comprising based on a result of an assay performed on a biological sample of the subject, determining a mutational status of a PIK3CA gene in the subject, wherein if the PIK3CA gene in the subject does not comprise an oncogenic mutation, then the subject is selected for treatment with a compound herein (e.g., Compound 1).

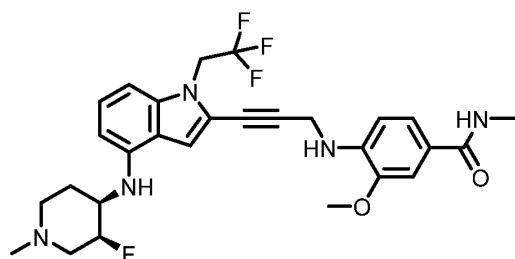
[0232] Further disclosed herein is a method of treating a condition in a subject in need thereof, the method comprising: based at least in part on a HRD status of the cancer in the subject, not administering to the subject a therapeutically-effective amount of a compound that binds a p53 mutant protein. Further disclosed herein is a method of treating a condition in a subject in need thereof, the method comprising: a) determining a HRD status of a cancer in the subject; and b) based at least in part on the HRD status of the cancer in the subject, not administering to the subject a therapeutically-effective amount of a compound that binds a p53 mutant protein. Further disclosed herein is a method of treating a condition in a subject in need thereof, the method comprising: a) based on a result of an assay performed on a biological sample of the subject, determining a HRD status of a cancer in the subject; and b) based at least in part on the HRD status of the cancer in the subject, not administering to the subject a therapeutically-effective amount of a compound that binds a p53 mutant protein. Further disclosed herein is a method of treating a condition in a subject in need thereof, the method comprising: a) performing an assay on a biological sample of the subject to determine a HRD status of a cancer in the subject; and b)

based at least in part on the HRD status of the cancer in the subject, not administering to the subject a therapeutically-effective amount of a compound that binds a p53 mutant protein.

[0233] In some embodiments, the present disclosure provides a use of a compound herein (e.g., Compound 1) in treatment of cancer in a subject in need thereof, wherein a HRD status of the cancer has been previously determined based on an assay performed on a biological sample of the subject, wherein the HRD status is negative.

[0234] Further disclosed herein is a method of selecting a subject for treatment with a compound herein (e.g., Compound 1), the method comprising performing an assay on a biological sample of the subject to determine a HRD status of the cancer in the subject, wherein if the HRD status of the cancer is negative, then the subject is selected for treatment with a compound herein. Further disclosed herein is a method of selecting a subject for treatment with a compound herein (e.g., Compound 1), the method comprising based on a result of an assay performed on a biological sample of the subject, determining a HRD status of the cancer in the subject, wherein if the HRD status of the cancer is negative, then the subject is selected for treatment with a compound herein (e.g., Compound 1).

[0235] Disclosed herein are methods of treating a condition in a subject in need thereof, the method comprising administering to the subject a therapeutically-effective amount of a compound of the formula:



(Compound 1),

or a pharmaceutically-acceptable salt thereof,

wherein, based on an assay performed on a biological sample of the subject, the subject has been determined to have a TP53 gene that encodes a Y220C p53 mutant protein, and a KRAS gene that encodes a wild-type K-ras protein.

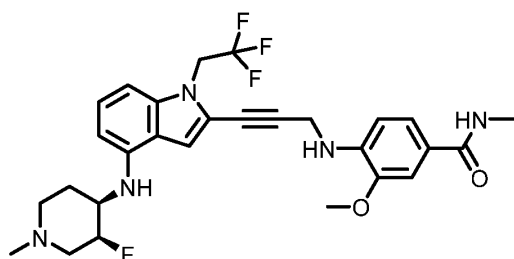
[0236] In some embodiments, the biological sample of the subject has been determined to have the KRAS gene that encodes the wild-type K-ras protein, and the mutation in the TP53 gene that encodes a Y220C p53 mutant protein.

[0237] In some embodiments, the present disclosure provides a method of treating a condition in a subject in need thereof, the method comprising:

- a) based on a result of an assay performed on a biological sample of the subject,
  - i) determining that the subject harbors a KRAS gene that encodes a wild-type K-ras protein, and
  - ii) determining that the subject harbors a mutation in a TP53 gene that encodes a Y220C p53 mutant protein; and
- b) based at least in part on

- i) the determining that the subject harbors the KRAS gene that encodes the wild-type K-ras protein, and
- ii) the determining that the subject harbors a mutation in the TP53 gene that encodes the p53 mutant protein,

administering to the subject a therapeutically-effective amount of a compound of the formula:



(Compound 1),

or a pharmaceutically-acceptable salt thereof.

[0238] In some embodiments, the method further comprises determining that the biological sample harbors the KRAS gene that encodes the wild-type K-ras protein, and the mutation in the TP53 gene that encodes a Y220C p53 mutant protein.

[0239] In some embodiments, the disclosure provides a method comprising: a) administering to a subject a dose of a compound provided herein (e.g., Compound 1), wherein the subject has a cancer, wherein the subject is on a therapeutic regimen of the compound; b) observing that the subject does not exhibit an improvement in the cancer; c) based at least in part on the observing that the subject does not exhibit the improvement in the cancer, performing an assay on a tissue of the subject to determine a mutational status of a KRAS gene in the subject; (d) determining that the mutational status of the KRAS gene in the subject is that the KRAS gene is non-wild type; and (e) based at least in part on the mutational status of the KRAS gene, determining to discontinue the therapeutic regimen of the compound. In some embodiments, the non-wild type gene comprises any mutation described herein.

[0240] In some embodiments, the present disclosure provides a method of treatment where a recommendation is made based on observation of a physiological state of the patient. A health care practitioner can recommend administration of a therapy, such as a pharmaceutical composition of the disclosure by, for example, consultation with the subject or other healthcare providers, or by entering the recommendation into a medical record.

[0241] In some embodiments, the present disclosure provides a method of treating cancer in a subject, the method comprising: (a) administering to the subject a first course of a compound that binds a p53 mutant; (b) after (a), determining that said subject exhibits a gastrointestinal adverse event; (c) after (b), recommending that the compound is administered to the subject with food, wherein the recommendation is based on at least the determination that the subject has exhibited the gastrointestinal adverse event; and (d) administering a second course of the compound to the subject with food in accordance with the recommendation.

[0242] In some embodiments, the present disclosure provides a method of treating cancer in a subject, the method comprising: (a) administering to the subject a first course of a compound that binds a p53

mutant; (b) after (a), observing that said subject exhibits a gastrointestinal adverse event; (c) after (b), determining that administration of the compound with food is warranted based on at least the observation that subject has exhibited the gastrointestinal adverse event; and (d) administering a second course of the compound to the subject with food in accordance with the determination.

[0243] In some embodiments, the present disclosure provides a method of treating cancer, the method comprising: (a) identifying a subject that: (i) has been treated with a first course of a compound that binds a p53 mutant, and (ii) has exhibited a gastrointestinal adverse event after treatment with the first course of the compound; (b) recommending that the compound is administered to the subject with food, wherein the recommendation is based on at least the basis that the subject has exhibited the gastrointestinal adverse event; and (c) administering a second course of the compound to the subject with food in accordance with the recommendation.

#### **Clinical Trials.**

[0244] To determine the suitability of a compound disclosed herein for treatment of humans, clinical trials are performed. For example, patients diagnosed with cancer and in need of treatment are selected and separated in treatment and one or more groups, wherein the treatment group is administered a compound disclosed herein, while the control groups receive a placebo or a known anti-cancer drug. The treatment safety and efficacy of the compound disclosed herein can thus be evaluated by performing comparisons of the patient groups with respect to factors such as survival and quality-of-life. In this example, the patient group treated with a compound disclosed herein show improved long-term survival compared to a patient control group treated with a placebo.

[0245] Results from genetic and biomarker tests and additional tests of the biological samples for biomarkers relevant to the safety and efficacy of the compounds disclosed herein can be investigated for possible correlation with patient outcome.

[0246] For example, clinical activity or response can be evaluated by standard imaging assessments, such as computed tomography (CT), magnetic resonance imaging (MRI), and bone scans. In addition, [18F]-fluorodeoxyglucose and [18F]-fluorothymidine positron emission tomography (FDG-PET and FLT-PET, respectively), or other techniques considered clinically appropriate for the patient's specific disease type can be used. CT-imaging can be performed, for example, at the end of Cycle 2, and every 2 cycles (e.g., Cycles 4 and 6) thereafter for DR-A and after the last infusion in Cycle 3 and every 3 cycles (e.g., Cycles 6 and 9) thereafter in DR-B. Anti-cancer cell activity can be assessed using IWG (2014) (Appendix H) criteria for patients with lymphomas. Additionally, for patients with an FDG-avid lymphoma, FDG-PET imaging can be performed at baseline and post-baseline as outlined in IWG 2014. FLT-PET imaging can be performed at baseline for patients with cancer cell commonly showing sufficient uptake of FLT tracer, e.g., patients with lymphoma. For example, DR-A assigned patients who demonstrate a standard uptake value (SUV) of  $>5$  at baseline can have a repeat FLT image one day after their last infusion of study medication in Cycle 1, i.e., Day 16. For example, DR-B patients who demonstrate a standard uptake value (SUV) of  $>5$  at baseline can have a repeat FLT image one day after their last infusion of study medication in Cycle 1, i.e., Day 12.

**Biological Samples.**

[0247] A biological sample can be a fluid or other material derived from the body of a normal or diseased subject, such as blood, serum, plasma, lymph, urine, saliva, tears, cerebrospinal fluid, milk, amniotic fluid, bile, ascites fluid, pus, and the like. In some embodiments, a biological sample is an organ or tissue extract and culture fluid in which any cells or tissue preparation from a subject has been incubated. The biological samples can be any samples from which genetic material can be obtained. Biological samples can also include solid or liquid cancer cell samples or specimens. The cancer cell sample can be a cancer cell tissue sample. The cancer cell sample can be a primary cancer sample, a metastatic cancer sample, or a recurrent cancer sample. In some embodiments, the cancer cell tissue sample can be obtained from surgically excised tissue. Exemplary sources of biological samples include fine needle aspiration, core needle biopsy, vacuum assisted biopsy, incisional biopsy, excisional biopsy, punch biopsy, shave biopsy or skin biopsy. In some cases, the biological samples comprise fine needle aspiration samples. In some embodiments, the biological samples comprise tissue samples, including, for example, excisional biopsy, incisional biopsy, or other biopsy. The biological samples can comprise a mixture of two or more sources; for example, fine needle aspirates and tissue samples. Tissue samples and cellular samples can also be obtained without invasive surgery, for example by punctuating the chest wall or the abdominal wall or from masses of breast, thyroid or other sites with a fine needle and withdrawing cellular material (fine needle aspiration biopsy). In some embodiments, a biological sample is a bone marrow aspirate sample. A biological sample can be obtained by methods known in the art such as the biopsy methods provided herein, swabbing, scraping, phlebotomy, or any other suitable method.

[0248] The biological samples obtained can be used in fresh, frozen, or fixed (e.g., formaldehyde fixed-paraffin embedded) form, depending on the nature of the sample, the assay used, and the convenience of the practitioner. Although fresh, frozen and fixed materials are suitable for various RNA and protein assays, generally, fresh tissues can be preferred for ex vivo measurements of activity.

[0249] Fixed tissue samples can also be employed. Tissue obtained by biopsy is often fixed, usually by formalin, formaldehyde, or glutaraldehyde, for example, or by alcohol immersion. Fixed biological samples are often dehydrated and embedded in paraffin or other solid supports. Non-embedded, fixed tissue, as well as fixed and embedded tissue, can be used in the present methods. Solid supports for embedding fixed tissue can be removed with organic solvents to allow subsequent rehydration of preserved tissue.

[0250] In some cases, an assay described herein includes a step of cell or tissue culture. For example, cells from a biopsy can be disaggregated using enzymes (such as collagenase and hyaluronidase) and or physical disruption (e.g., repeated passage through a 25-gauge needle) to dissociate the cells, collected by centrifugation, and resuspended in desired buffer or culture medium for culture, immediate analysis, or further processing.

**Subject/Patient Population.**

[0251] In some embodiments, a subject treated for cancer in accordance with the methods provided herein is a human, who has or is diagnosed with a cancer. In other embodiments, a subject treated for

cancer using methods provided herein is a human, predisposed or susceptible to a cancer. In some embodiments, a subject treated for using methods provided herein is a human, at risk of developing a cancer.

[0252] In some embodiments, a subject treated for cancer in accordance with the methods provided herein is a human, who has or is diagnosed with a cancer, determined to carry a p53 deactivating mutation and/or lacking wild-type p53. In other embodiments, a subject treated for cancer in accordance with the methods provided herein is a human, predisposed or susceptible to a cancer, determined to carry a p53 deactivating mutation and/or lacking wild-type p53. In some embodiments, a subject treated for cancer using methods provided herein is a human, at risk of developing a cancer, determined to carry a p53 deactivating mutation and/or lacking wild-type p53.

[0253] A p53 deactivating mutation is a mutation that leads to loss of (or a decrease in) the in vitro apoptotic activity of p53. Non-limiting examples of p53 deactivating mutations include mutations at Val143, His168, Arg175, Tyr220, Gly245, Arg248, Arg249, Phe270, Arg273, and Arg282. For example, Y220C, Y220S, Y220H, R248Q, R248W, R273C, and R273H. Additional non-limiting examples of p53 deactivating mutations include E62\_W91del, V122X, C135S, V143A, Q144P, W146X, V157F, R158H, Y163N, H168Y, V173L, R175H, R175L, R175P, R175Q, R175S, P219H, Y234C, Y234H, M237I, S240R, G245C, G245S, M246I, R249S, I255N, V272M, R273L, V274F, G279E, R280K, D281H, R282W, R306P, P300\_L308del, P300\_Y327del, D324\_I332del, R337C, and L344P, wherein X is any amino acid.

[0254] Accordingly, in some embodiments, a subject treated for cancer using methods provided herein is a human, at risk of developing, who has, or is diagnosed with a cancer that is determined to harbor a p53 deactivating mutation.

[0255] In some embodiments, a subject treated for cancer in accordance with the methods provided herein is a human, who has or is diagnosed with a cancer, determined to lack a dominant p53 deactivating mutation. Dominant p53 deactivating mutation or dominant negative mutation, as used herein, is a mutation wherein the mutated p53 inhibits or disrupt the activity of the wild-type p53 gene.

[0256] In some embodiments, a subject treated for cancer in accordance with the methods provided herein is a refractory patient. In a certain embodiment, a refractory patient is a patient refractory to a standard therapy (e.g., surgery, radiation, anti-androgen therapy and/or drug therapy such as chemotherapy). In certain embodiments, a patient with the cancer is refractory to a therapy when the cancer has not significantly been eradicated and/or the one or more symptoms have not been significantly alleviated. The determination of whether a patient is refractory can be made either in vivo or in vitro by any method effective for assaying the effectiveness of a treatment of cancer. In various embodiments, a patient with cancer is refractory when the number of CTCs or MNBCs associated with the cancer has not decreased or has increased. In various embodiments, a patient with cancer is refractory when one or more cancer cells metastasize and/or spread to another organ.

[0257] In some embodiments, a subject treated for cancer using methods provided herein is a human that has proven refractory to therapies other than treatment with a compound disclosed herein, but is no longer



on these therapies. In certain embodiments, a subject treated for cancer in accordance with the methods provided herein is a human already receiving one or more conventional anti-cancer therapies, such as surgery, drug therapy such as chemotherapy, anti-androgen therapy or radiation. Among these patients are refractory patients, patients who are too young for conventional therapies, and patients with recurring cancer cells despite treatment with existing therapies.

[0258] In some embodiments, the subject is a human who has had at least one unsuccessful prior treatment and/or therapy of the cancer.

**Methods of detecting wild-type proteins and/or mutations.**

[0259] In some embodiments, a subject with a cancer that does not comprise a KRAS gene containing an oncogenic mutation is a candidate for cancer treatment with a compound of the disclosure. In some embodiments, the cancer comprises a p53-deactivating mutation. Cancer cells from patient groups can be assayed to determine mutations and/or expression of wild-type proteins (e.g., p53 and/or K-ras) prior to treatment with a compound of the disclosure.

[0260] In some embodiments, a subject is selected for treatment with a p53-activating compound of the present disclosure at least in part on the basis of the mutational status of one or more genes. In cases where a subject is selected for treatment on the basis of the mutational status of more than one gene, the selection can be made, at least in part, on the basis of a result of a single assay or the results of more than one assay. For example, a biological sample of the subject (e.g., a solid tumor sample) can be subjected to a single assay that can provide a result, wherein the result describes the mutational status of two or more genes (e.g., TP53, KRAS, BRCA1, and/or BRCA2).

[0261] In some embodiments, the assay is DNA sequencing. In some embodiments, the assay is next-generation DNA sequencing. In some embodiments, the assay is RNA sequencing. In some embodiment, the assay is real-time PCR.

[0262] In some embodiments, the biological sample is a liquid biopsy. In some embodiments, the biological sample is a blood sample. In some embodiments, the biological sample is circulating tumor DNA. In some embodiments, the biological sample is cell free DNA. In some embodiments, the biological sample is a solid tumor sample.

[0263] In some embodiments, the selection for treatment is made, at least in part, on the basis of results from more than one assay. The assays can be same type of assay or different assays, and can be conducted simultaneously or subsequently by the same party or different parties. Each assay can be conducted on the same biological sample or different biological samples. For example, a biological sample of the subject can be subjected to an assay that determines a mutational status of at least one gene, optionally stored for a period of time, and subjected to another assay that determines the mutational status of another gene. Alternatively or in addition, a first biological sample (e.g., a solid tumor sample) can be subjected to an assay that determines the mutational status of one gene (e.g., TP53), and a second biological sample (e.g., circulating tumor DNA) can be subjected to a second assay that determines the status of another gene (e.g., KRAS, BRCA1, and/or BRCA2).

[0264] The activity of the p53 pathway can be determined by the mutational status of genes involved in the p53 pathways, including, for example, AKT1, AKT2, AKT3, ALK, BRAF, CDK4, CDKN2A, DDR2, EGFR, ERBB2 (HER2), FGFR1, FGFR3, GNA11, GNQ, GNAS, KDR, KIT, KRAS, MAP2K1 (MEK1), MET, HRAS, NOTCH1, NRAS, NTRK2, PIK3CA, NF1, PTEN, RAC1, RB1, NTRK3, STK11, PIK3R1, TSC1, TSC2, RET, TP53, and VHL. Genes that modulate the activity of p53 can also be assessed, including, for example, kinases: ABL1, JAK1, JAK2, JAK3; receptor tyrosine kinases: FLT3 and KIT; receptors: CSF3R, IL7R, MPL, and NOTCH1; transcription factors: BCOR, CEBPA, CREBBP, ETV6, GATA1, GATA2, MLL, KZF1, PAX5, RUNX1, STAT3, WT1, and TP53; epigenetic factors: ASXL1, DNMT3A, EZH2, KDM6A (UTX), SUZ12, TET2, PTPN11, SF3B1, SRSF2, U2AF35, and ZRSR2; RAS proteins: HRAS, KRAS, and NRAS; adaptors CBL and CBL-B; FBXW7, IDH1, IDH2, and NPM1.

[0265] Cancer cell samples can be obtained, for example, from solid or liquid tumors via primary or metastatic tumor resection (e.g., pneumonectomy, lobectomy, wedge resection, and craniotomy) primary or metastatic disease biopsy (e.g., transbronchial or needle core), pleural or ascites fluid (e.g., FFPE cell pellet), bone marrow aspirate, bone marrow clot, and bone marrow biopsy, or macro-dissection of tumor rich areas (solid tumors).

[0266] To detect mutational status of KRAS, TP53, BRCA1 and/or BRCA2 in a tissue, cancerous tissue can be isolated from surrounding normal tissues. For example, the tissue can be isolated from paraffin or cryostat sections. Cancer cells can also be separated from normal cells by flow cytometry. If the cancer cells tissue is highly contaminated with normal cells, detection of mutations can be more difficult.

[0267] Various methods and assays for analyzing mutations are suitable for use in the disclosure. Non-limiting examples of assays include polymerase chain reaction (PCR), quantitative PCR (qPCR), real-time PCR (RT-PCR), Sanger sequencing, restriction fragment length polymorphism (RFLP), microarray, Southern Blot, northern blot, western blot, eastern Blot, H&E staining, microscopic assessment of tumors, massively parallel sequencing (MPS), next-generation DNA sequencing (NGS) (e.g., extraction, purification, quantitation, and amplification of DNA, library preparation), immunohistochemistry (IHC), protein quantification, chromogenic in situ hybridization (CISH), and fluorescent in situ hybridization (FISH).

[0268] A microarray allows a researcher to investigate multiple DNA sequences attached to a surface, for example, a DNA chip made of glass or silicon, or a polymeric bead or resin. The DNA sequences are hybridized with fluorescent or luminescent probes. The microarray can indicate the presence of oligonucleotide sequences in a sample based on hybridization of sample sequences to the probes, followed by washing and subsequent detection of the probes. Quantification of the fluorescent or luminescent signal indicates the presence of known oligonucleotide sequences in the sample.

[0269] PCR allows rapid amplification of DNA oligomers, and can be used to identify an oligonucleotide sequence in a sample. PCR experiments involve contacting an oligonucleotide sample with a PCR mixture containing primers complementary to a target sequence, one or more DNA polymerase enzymes, deoxynucleotide triphosphate (dNTP) building blocks, including dATP, dGTP,

dTTP, and dCTP, suitable buffers, salts, and additives. If a sample contains an oligonucleotide sequence complementary to a pair of primers, the experiment amplifies the sample sequence, which can be collected and identified.

[0270] In some embodiments, an assay comprises amplifying a biomolecule from the cancer sample. The biomolecule can be a nucleic acid molecule, such as DNA or RNA. In some embodiments, the assay comprises circularization of a nucleic acid molecule, followed by digestion of the circularized nucleic acid molecule.

[0271] In some embodiments, the assay comprises contacting an organism, or a biochemical sample collected from an organism, such as a nucleic acid sample, with a library of oligonucleotides, such as PCR primers. The library can contain any number of oligonucleotide molecules. The oligonucleotide molecules can bind individual DNA or RNA motifs, or any combination of motifs described herein. The motifs can be any distance apart, and the distance can be known or unknown. In some embodiments, two or more oligonucleotides in the same library bind motifs a known distance apart in a parent nucleic acid sequence. Binding of the primers to the parent sequence can take place based on the complementarity of the primers to the parent sequence. Binding can take place, for example, under annealing, or under stringent conditions.

[0272] In some embodiments, the results of an assay are used to design a new oligonucleotide sequence for future use. In some embodiments, the results of an assay are used to design a new oligonucleotide library for future use. In some embodiments, the results of an assay are used to revise, refine, or update an existing oligonucleotide library for future use. For example, an assay can reveal that a previously undocumented nucleic acid sequence is associated with the presence of a target material. This information can be used to design or redesign nucleic acid molecules and libraries.

[0273] In some embodiments, one or more nucleic acid molecules in a library comprise a barcode tag. In some embodiments, one or more of the nucleic acid molecules in a library comprise type I or type II restriction sites suitable for circularization and cutting an amplified sample nucleic acid sequence. Such primers can be used to circularize a PCR product and cut the PCR product to provide a product nucleic acid sequence with a sequence that is organized differently from the nucleic acid sequence native to the sample organism.

[0274] After a PCR experiment, the presence of an amplified sequence can be verified. Non-limiting examples of methods for finding an amplified sequence include DNA sequencing, whole transcriptome shotgun sequencing (WTSS, or RNA-seq), mass spectrometry (MS), microarray, pyrosequencing, column purification analysis, polyacrylamide gel electrophoresis, and index tag sequencing of a PCR product generated from an index-tagged primer.

[0275] In some embodiments, more than one nucleic acid sequence in the sample organism is amplified. Non-limiting examples of methods of separating different nucleic acid sequences in a PCR product mixture include column purification, high performance liquid chromatography (HPLC), HPLC/MS, polyacrylamide gel electrophoresis, and size exclusion chromatography.

[0276] The amplified nucleic acid molecules can be identified by sequencing. Nucleic acid sequencing can be performed on automated instrumentation. Sequencing experiments can be done in parallel to analyze tens, hundreds, or thousands of sequences simultaneously. Non-limiting examples of sequencing techniques follow.

[0277] In pyrosequencing, DNA is amplified within a water droplet containing a single DNA template bound to a primer-coated bead in an oil solution. Nucleotides are added to a growing sequence, and the addition of each base is evidenced by visual light.

[0278] Ion semiconductor sequencing detects the addition of a nucleic acid residue as an electrical signal associated with a hydrogen ion liberated during synthesis. A reaction well containing a template is flooded with the four types of nucleotide building blocks, one at a time. The timing of the electrical signal identifies which building block was added, and identifies the corresponding residue in the template.

[0279] DNA nanoball uses rolling circle replication to amplify DNA into nanoballs. Unchained sequencing by ligation of the nanoballs reveals the DNA sequence.

[0280] In a reversible dyes approach, nucleic acid molecules are annealed to primers on a slide and amplified. Four types of fluorescent dye residues, each complementary to a native nucleobase, are added, the residue complementary to the next base in the nucleic acid sequence is added, and unincorporated dyes are rinsed from the slide. Four types of reversible terminator bases (RT-bases) are added, and non-incorporated nucleotides are washed away. Fluorescence indicates the addition of a dye residue, thus identifying the complementary base in the template sequence. The dye residue is chemically removed, and the cycle repeats.

[0281] Detection of point mutations can be accomplished by molecular cloning of the KRAS, PIK3CA, TP53, BRCA1 and/or BRCA2 allele(s) present in the cancer cell tissue and sequencing that allele(s). Alternatively, PCR can be used to amplify KRAS, PIK3CA, TP53, BRCA1 and/or BRCA2 gene sequences directly from a genomic DNA preparation from the cancer cell tissue. The DNA sequence of the amplified sequences can then be determined. Specific deletions of KRAS, PIK3CA, TP53, BRCA1 and/or BRCA2 genes can also be detected. For example, restriction fragment length polymorphism (RFLP) probes for the KRAS, PIK3CA, TP53, BRCA1 and/or BRCA2 gene or surrounding marker genes can be used to score loss of a KRAS, TP53, PIK3CA, BRCA1 and/or BRCA2 allele.

[0282] Loss of wild-type KRAS, TP53, PIK3CA, BRCA1 and/or BRCA2 genes can also be detected on the basis of the loss of a wild-type expression product of the KRAS, TP53, PIK3CA, BRCA1 and/or BRCA2 genes. Such expression products include both the mRNA as well as the p53, BRCA1, BRCA2, and/or K-ras protein products. Point mutations can be detected by sequencing the mRNA directly or via molecular cloning of cDNA made from the mRNA. The sequence of the cloned cDNA can be determined using DNA sequencing techniques. The cDNA can also be sequenced via PCR.

[0283] Alternatively, mismatch detection can be used to detect point mutations in the KRAS, TP53, PIK3CA, BRCA1 and/or BRCA2 genes or the mRNA products. The method can involve the use of a labeled riboprobes complementary to the human wild-type KRAS, TP53, PIK3CA, BRCA1 and/or

BRCA2 genes. The riboprobes and either mRNA or DNA isolated from the cancer cell tissue are annealed (hybridized) together and subsequently digested with the enzyme RNase A which is able to detect some mismatches in a duplex RNA structure. If a mismatch is detected by RNase A, the enzyme cleaves at the site of the mismatch. Thus, when the annealed RNA preparation is separated on an electrophoretic gel matrix, if a mismatch has been detected and cleaved by RNase A, an RNA product is seen that is smaller than the full-length duplex RNA for the riboprobe and the KRAS, TP53, PIK3CA, BRCA1 and/or BRCA2 mRNA or DNA. The riboprobe need not be the full length of the KRAS, TP53, PIK3CA, BRCA1 and/or BRCA2 mRNA or genes but can be a segment of either. For example, if the riboprobe comprises only a segment of KRAS, TP53, PIK3CA, BRCA1 and/or BRCA2 mRNA or genes, a number of these probes can be used to screen the whole mRNA sequence for mismatches.

[0284] In similar fashion, DNA probes can be used to detect mismatches, through enzymatic or chemical cleavage. Alternatively, mismatches can be detected by shifts in the electrophoretic mobility of mismatched duplexes relative to matched duplexes. With either riboprobes or DNA probes, the cellular mRNA or DNA which might contain a mutation can be amplified using PCR (see below) before hybridization.

[0285] DNA sequences of the KRAS, TP53, PIK3CA, BRCA1 and/or BRCA2 genes from the cancer cell tissue which have been amplified by use of PCR can also be screened using allele-specific probes. These probes are nucleic acid oligomers, each of which contains a region of the KRAS, TP53, PIK3CA, BRCA1 and/or BRCA2 gene sequences harboring a known mutation. For example, one oligomer can be about 30 nucleotides in length, corresponding to certain portion(s) of the KRAS, TP53, PIK3CA, BRCA1 and/or BRCA2 gene sequence(s). At the position coding for the 175th codon of p53 gene the oligomer encodes an alanine, rather than the wild-type codon valine. By use of a battery of such allele-specific probes, the PCR amplification products can be screened to identify the presence of previously identified mutations in KRAS, TP53, PIK3CA, BRCA1 and/or BRCA2 genes. Hybridization of allele-specific probes with amplified KRAS, TP53, PIK3CA, BRCA1 and/or BRCA2 sequences can be performed, for example, on a nylon filter. Hybridization to a particular probe indicates the presence of the same mutation in the cancer cell tissue as in the allele-specific probe.

[0286] The identification of KRAS, TP53, PIK3CA, BRCA1 and/or BRCA2 gene structural changes in cancer cells can be facilitated through the application of a diverse series of high resolution, high throughput microarray platforms. Essentially two types of array include those that carry PCR products from cloned nucleic acids (e.g., cDNA, BACs, cosmids) and those that use oligonucleotides. The methods can provide a way to survey genome wide DNA copy number abnormalities and expression levels to allow correlations between losses, gains, and amplifications in cancer cells with genes that are over- and under- expressed in the same samples. The gene expression arrays that provide estimates of mRNA levels in cancer cells have given rise to exon-specific arrays that can identify both gene expression levels, alternative splicing events and mRNA processing alterations.

[0287] Oligonucleotide arrays can be used to interrogate single nucleotide polymorphisms (SNPs) throughout the genome for linkage and association studies and these have been adapted to quantify copy

number abnormalities and loss of heterozygosity events. DNA sequencing arrays can allow resequencing of chromosome regions and whole genomes.

**[0288]** SNP-based arrays or other gene arrays or chips can determine the presence of wild-type TP53 and/or KRAS alleles and the structure of mutations. A single nucleotide polymorphism (SNP), a variation at a single site in DNA, is the most frequent type of variation in the genome. For example, there are an estimated 5-10 million SNPs in the human genome. SNPs can be synonymous or nonsynonymous substitutions. Synonymous SNP substitutions do not result in a change of amino acid in the protein due to the degeneracy of the genetic code, but can affect function in other ways. For example, a seemingly silent mutation in gene that codes for a membrane transport protein can slow down translation, allowing the peptide chain to misfold and produce a less functional mutant membrane transport protein.

Nonsynonymous SNP substitutions can be missense substitutions or nonsense substitutions. Missense substitutions occur when a single base change results in change in amino acid sequence of the protein and malfunction thereof leads to disease. Nonsense substitutions occur when a point mutation results in a premature stop codon, or a nonsense codon in the transcribed mRNA, which results in a truncated and usually, nonfunctional, protein product. As SNPs are highly conserved throughout evolution and within a population, the map of SNPs serves as an excellent genotypic marker for research. SNP array is a useful tool to study the whole genome.

**[0289]** In addition, SNP array can be used for studying the Loss Of Heterozygosity (LOH). LOH is a form of allelic imbalance that can result from the complete loss of an allele or from an increase in copy number of one allele relative to the other. While other chip-based methods (e.g., comparative genomic hybridization can detect only genomic gains or deletions), SNP array has the additional advantage of detecting copy number neutral LOH due to uniparental disomy (UPD). In UPD, one allele or whole chromosome from one parent are missing leading to reduplication of the other parental allele (uniparental = from one parent, disomy = duplicated). In a disease setting this occurrence can be pathologic when the wild-type allele (e.g., from the mother) is missing and instead two copies of the heterozygous allele (e.g., from the father) are present. This usage of SNP array has a huge potential in cancer diagnostics as LOH is a prominent characteristic of most human cancers. SNP array technology have shown that cancers (e.g., gastric cancer, liver cancer, etc.) and hematologic malignancies (ALL, MDS, CML, etc.) have a high rate of LOH due to genomic deletions or UPD and genomic gains. In the present disclosure, using high density SNP array to detect LOH allows identification of pattern of allelic imbalance to determine the presence of wild-type p53 allele.

**[0290]** Examples of p53 gene sequence and SNP arrays include p53 Gene Chip (Affymetrix, Santa Clara, CA), Ampli-Chip® p53 microarray (Roche Molecular Systems, Pleasanton, CA), GeneChip Mapping arrays (Affymetrix, Santa Clara, CA), SNP Array 6.0 (Affymetrix, Santa Clara, CA), BeadArrays (Illumina, San Diego, CA), etc.

**[0291]** Nonlimiting examples of assays suitable for determination of KRAS mutational status include the Therascreen KRAS RGQ PCR Kit (QIAGEN, Hilden, Germany), KRAS XL StripAssay® (ViennaLab Diagnostics, Wien, Austria), SNaPshot Multiplex PCR, cobas® KRAS Mutation Test (Roche

Diagnostics, Florham Park, NJ), ddPCR™ *KRAS G12/G13* Screening Kit (Bio-Rad Laboratories, Philadelphia, PA), OncoBEAM® RAS CRC Kit (Sysmex Inostics, Baltimore, MD), and Idylla™ *KRAS* Mutation Test (Biocartis, Mechelen, Belgium).

[0292] Nonlimiting examples of assays suitable for determination of BRCA1/2 mutational status include BRACANALYSIS CDX® Germline Companion Diagnostic Test (Myriad Genetics, Salt Lake City, UT), CleanPlex® BRCA1 & BRCA2 Kit v3 (Paragon Genomics, Inc., Fremont, CA), Guardant360® CDx (Guardant, Palo Alto, CA), and BRCAssure® (Laboratory Corporation of America, Burlington, NC).

[0293] Nonlimiting examples of assays suitable for determination of PIK3CA mutational status include Agena PIK3CA Breast Panel (Agena Bioscience, San Diego, CA), Invitae PIK3CA Diagnostic (Invitae, San Francisco, CA), *therascreen* PIK3CA RGQ PCR Kit (QIAGEN, Hilden, Germany), PIK3CA Mutation Analysis Kit for Real-Time PCR (EnteroGen, Woodland Hills, CA), FoundationOne® CDx (Cambridge, MA), Caris® Life Science Caris Molecular Intelligence® Tumor Profiling (Irving, TX), Illumina® TruSight™ Oncology 500 (San Diego, CA), NeoGenomics NeoTYPE® (Fort Meyers, FL), Tempus xT (Chicago, IL), and Thermo Fisher OncoPrint™ Dx Target Test (Waltham, MA).

[0294] Mutations of wild-type *KRAS*, *TP53*, *PIK3CA*, *BRCA1* and/or *BRCA2* genes can also be detected on the basis of the mutation of a wild-type expression product of the *KRAS*, *TP53*, *PIK3CA*, *BRCA1* and/or *BRCA2* genes. Such expression products include both the mRNA as well as the p110α, p53, *BRCA1*, *BRCA2* and/or K-ras protein products themselves. Point mutations can be detected by sequencing the mRNA directly or via molecular cloning of cDNA made from the mRNA. The sequence of the cloned cDNA can be determined using DNA sequencing techniques. The cDNA can also be sequenced via the polymerase chain reaction (PCR). A panel of monoclonal antibodies could be used in which each of the epitopes involved in p53, *BRCA1*, *BRCA2*, and/or K-ras functions are represented by monoclonal antibodies. Loss or perturbation of binding of a monoclonal antibody in the panel can indicate mutational alteration of the p53 and/or K-ras proteins and thus of the *KRAS*, *TP53*, *PIK3CA*, *BRCA1* and/or *BRCA2* genes themselves. Mutant *TP53* and/or *KRAS* genes or gene products can also be detected in body samples, including, for example, serum, stool, urine, and sputum. The same techniques discussed above for detection of mutant *KRAS*, *TP53*, *PIK3CA*, *BRCA1* and/or *BRCA2* genes or gene products in tissues can be applied to other body samples.

[0295] Loss of wild-type *KRAS*, *TP53*, *PIK3CA*, *BRCA1* and/or *BRCA2* genes can also be detected by screening for loss of wild-type p53, *PIK3CA*, *BRCA1*, *BRCA2*, and/or K-ras protein function. Although all of the functions which the p53 protein undoubtedly possesses have yet to be elucidated, at least two specific functions are known. Protein p53 binds to the SV40 large T antigen as well as to the adenovirus E1B antigen. Loss of the ability of the p53 protein to bind to either or both of these antigens indicates a mutational alteration in the protein which reflects a mutational alteration of the gene itself. Alternatively, a panel of monoclonal antibodies could be used in which each of the epitopes involved in p53 functions are represented by a monoclonal antibody. Loss or perturbation of binding of a monoclonal antibody in the panel would indicate mutational alteration of the p53 protein and thus of the p53 gene itself. Any method for detecting an altered p53 protein can be used to detect loss of wild-type p53 genes.

[0296] Determination of a p53 deactivating mutation and/or lack of or reduced expression of wild-type p53 in the subject with cancer can be performed before, during, or after the administration of a compound disclosed herein. Determination of a KRAS mutation and/or lack expression of wild-type K-ras in the subject with cancer can be performed before, during, or after the administration of a compound disclosed herein. Determination of a BRCA1 and/or BRCA2 mutation and/or lack expression of wild-type BRCA1 and/or BRCA2 in the subject with cancer can be performed before, during, or after the administration of a compound disclosed herein. Determination of a PIK3CA mutation and/or lack expression of wild-type p110 $\alpha$  in the subject with cancer can be performed before, during, or after the administration of a compound disclosed herein. In some embodiments, the determination of a lack of a KRAS mutation, and/or expression of wild-type K-ras is performed before the first administration of the compound to the subject, for example about 5 years – about 1 month, about 4 years – about 1 month, about 3 years – 1 month, about 2 years – about 1 month, about 1 years – about 1 month, about 5 years – about 1 week, about 4 years – about 1 week, about 3 years – about 1 month, about 2 years – about 1 week, about 1 year – about 1 week, about 5 years – about 1 day, about 4 years – about 1 day, about 3 years – about 1 day, about 2 years – about 1 day, about 1 year – about 1 day, about 15 months – about 1 month, about 15 months – about 1 week, about 15 months – about 1 day, about 12 months – about 1 month, about 12 months – about 1 week, about 12 months – about 1 day, about 6 months – 1 about month, about 6 months – about 1 week, about 6 months – about 1 day, about 3 months – 1 about month, about 3 months – about 1 week, or about 3 months – about 1 day prior to the first administration of the compound to the subject. In some examples, the confirmation of the lack of the KRAS mutation and/or expression of wild-type K-ras is performed up to 6 years, 5 years, 4 years, 3 years, 24 months, 23 months, 22 months, 21 months, 20 months, 19 months, 18 months, 17 months, 16 months, 15 months, 14 months, 13 months, 12 months, 11 months, 10 months, 9 months, 8 months, 7 months, 6 months, 5 months, 4 months, 3 months, 2 months, 1 months, 4 weeks (28 days), 3 weeks (21 days), 2 weeks (14 days), 1 week (7 days), 6 days, 5 days, 4 days, 3 days, 2 days, or 1 day before the first administration of the compound to the subject. In some examples the confirmation of the lack of the KRAS mutation is performed within 1 month of the first administration of the compound to the subject. In some examples the confirmation of the lack of the KRAS mutation is performed within 21 days of the first administration of the compound to the subject.

## EXAMPLES

### EXAMPLE 1: Retrospective confirmatory testing for a p53 Y220C mutation.

[0297] NGS is used to perform retrospective confirmatory testing for a p53 Y220C mutation in blood or solid tissue. If a patient does not have a p53 Y220C mutation detected at the pre-screening step, the patient may be enrolled prospectively.

[0298] DNA is obtained from a cancer sample of a patient. The DNA is sequenced using NGS to determine the entire p53 coding region, including TP53 exons, introns, and splice sites. Dysfunctional p53 is inferred from the identification of substitutions, indels, frameshift mutations, splice site mutation, insertions or deletions, copy number variants, large deletions, or polymorphisms. Minimum tumor



content is 20%. Average read depth is about 750 reads/amplicon. The lower limit of detection is 5% mutant allele at an average read depth of  $\geq 450$  reads per amplicon. When the average read depth is  $< 450$  reads per amplicon, the limit of detection is 15% mutant allele.

[0299] Detection of TP53 gene copy number is based on the number of TP53 amplicon reads from a tumor compared with the average number reads across 14 normal DNA samples. Limits of the assay: the loss of one or more alleles is determined if tumor content is  $> 60\%$  (99% sensitivity); the loss of two alleles is determined if tumor sample content is  $> 30\%$  (99% sensitivity). More than 10% of tumors with wild-type TP53 have a copy number less than 0.5 ( $< 0.5$ ).

[0300] A patient with a confirmed Y220C mutation is given a regimen comprising a therapeutically-effective amount of a compound of the disclosure or a pharmaceutically-acceptable salt thereof.

**EXAMPLE 2: Phase 1 clinical trial for patients with advanced solid tumor harboring a p53 Y220C mutation.**

[0301] Compound 1 (4-((3-(4-(((3S,4R)-3-fluoro-1-methylpiperidin-4-yl)amino)-1-(2,2,2-trifluoroethyl)-1H-indol-2-yl)prop-2-yn-1-yl)amino)-3-methoxy-N-methylbenzamide) is a selective p53 reactivator that stabilizes the p53 Y220C mutant protein. The single amino acid change creates a small crevice in the p53 protein, making the protein thermally unstable at physiological temperatures and unable to interact effectively with DNA to activate transcription of target genes. By selectively binding the crevice, Compound 1 stabilizes the mutant protein structure in the wild type (WT) conformation, thereby restoring the function of the mutant p53.

[0302] Compound 1 was supplied as 50, 150, and 500 mg tablets. Study drug was provided in appropriately labeled bottles. Patients received respective supplies of study drug with dosing instructions based on the prescribed dose. Compound 1 was administered orally on 21-day continuous dosing cycles. The initial dosing schedule was once daily but was adjusted as needed to a more or less frequent schedule during the course of the study. When administered, all Compound 1 doses were taken orally at approximately the same time each morning with food restricted to 2 hours prior to, and one hour after dosing. Whenever possible, all doses of Compound 1 were taken with approximately 240 mL of water. Patients continued to receive Compound 1 for as long as clinical benefits are demonstrated or until disease progression or other treatment discontinuation criteria were met.

[0303] The study selectively enrolled advanced cancer patients with tumors harboring a p53 Y220C mutation confirmed (either historically or prospectively) prior to a patient signing the main study informed consent. Patients had demonstrated solid malignancy with a p53 Y220C mutation identified by a molecular test performed by a Clinical Laboratory Improvement Amendments (CLIA) certified lab. The p53 Y220C mutation was derived from the tumor, and was not a result of suspected clonal hematopoiesis of indeterminate potential (CHIP).

[0304] CHIP is a common age-related phenomenon in which hematopoietic stem cells or other early blood cell progenitors contribute to the formation of a genetically distinct subpopulation of blood cells. CHIP can account for up to 25% to 40% of TP53 variants detected in saliva and blood during germline testing and can require additional confirmatory testing. Among TP53 mutations, Y220C is one of the

most commonly observed in CHIP. A liquid assay detects p53 Y220C that is derived from CHIP rather than tumor if the assay does not assess for CHIP.

**[0305] p53 Y220C Testing:** Eligible patients had a blood sample collected during screening for retrospective germline sequencing to determine the presence of a p53 Y220C mutation using a molecular test. Germline results were not an entry requirement for the study, and all patients began treatment with Compound 1 prior to results being available. Patients with a positive or indeterminate germline test were offered genetic counseling and additional confirmatory testing using tissue from a skin biopsy. Germline testing was exploratory and provided the basis for understanding whether a germline p53 Y220C mutation increases a patient's sensitivity to Compound 1 toxicities due to systemic reactivation of p53. All patients were monitored closely for toxicities by standard safety assessments during frequent clinic visits. If toxicities were observed, appropriate action was taken including interruption and/or discontinuation of treatment.

**[0306]** Acceptable patient identification assays included: 1) a solid tissue assay conducted in a CLIA certified laboratory or 2) a ctDNA assay that includes a matched normal buffy coat sample conducted in a CLIA certified laboratory. Patients who had an identified p53 Y220C mutation in tumor were eligible for screening. Patients with a p53 Y220C mutations identified by a different assay (e.g., confirmed via a p53 Y220C Identification form) required a prescreening step. Patients were identified using a Resolution ctDX Lung™ blood-based test or a FoundationOne® CDx tissue test to prospectively confirm the presence of a p53 Y220C mutation. The Resolution ctDX Lung™ test included sequencing of both the plasma and the matched normal buffy coat layer to determine whether the p53 Y220C mutation originated from the tumor. The tissue-based CTA based on FoundationOne® CDx test used a fresh biopsy or archival tissue. The decision to perform a prescreening biopsy was based on the patient providing prescreening written informed consent and on the Investigator assessing the procedure to be in line with standard of care (i.e., of low risk and the tumor of sufficient size to be feasible to perform). After provision of informed consent for the main study, patients had a blood sample collected during screening for retrospective testing to confirm the presence of a p53 Y220C mutation using the Resolution ctDX Lung™ test. Patients who had available archival tumor tissue had tissue sent for retrospective confirmatory testing with the CTA based on FoundationOne® CDx test. If archival tumor tissue was not available, a fresh tumor biopsy was required for the retrospective confirmatory testing, if a biopsy procedure was in line with standard of care (i.e., of low risk and the tumor is of sufficient size to be biopsied). Tissue biopsies and/or archival tumor tissue collected during prescreening were used if available in place of samples for retrospective confirmatory testing.

**[0307]** To explore whether the presence of additional p53 mutations or non-p53 mutations are associated with clinical outcome, the pre-treatment specimen was analyzed by the 324-gene panel included in the CTA based on FoundationOne® CDx molecular test. Additional genomic analysis included loss of heterozygosity (LOH), somatic-germline zygosity (SGZ), microsatellite instability (MSI), and tumor mutational burden (TMB).

[0308] The Resolution ctDX Lung™ test contains a large tumor gene panel, including p53. In addition to the use of the Resolution ctDX Lung™ test for confirmatory p53 Y220C testing, the test was also used to explore the potential relationship of additional p53 (and non-p53) mutations and response to treatment with Compound 1. In addition, blood was collected during Compound 1 treatment and analyzed by the Resolution ctDX Lung™ test to explore whether circulating p53 Y220C levels can be used as a surrogate biomarker of efficacy or disease progression. The ctDNA analysis also explored whether emerging p53 (and non-p53) mutations altered the response to treatment with Compound 1.

[0309] Patients with advanced solid tumors harboring p53 Y220C mutations were enrolled in a phase 1 clinical trial for administration of Compound 1. The patients were 12 years of older in age, and had locally advanced or metastatic solid tumors harboring a p53 Y220C mutation. Up to 60 patients were enrolled in Phase 1 to determine the maximum tolerated dose (MTD) and to identify a recommended Phase 2 dose (RP2D). The primary objective of the study was to determine the maximum tolerated dose (MTD), select a recommended phase 2 dose (RP2D), and to evaluate the safety and tolerability of compound 1.

[0310] Endpoints for the primary objectives included incidence of dose-limiting toxicities (DLTs), adverse events (AE), serious adverse events (SAE), and changes between baseline and post-baseline laboratory assessments, electrocardiograms (ECG), vital signs, and physical exams. Toxicities were graded using the Common Terminology Criteria for Adverse Events (CTCAE) v5.0. The secondary objectives were to characterize the pharmacokinetics (PK) of Compound 1 when administered orally; describe the concentration of Compound 1 when administered orally; and evaluate the preliminary efficacy of Compound 1 using tumor response criteria. Secondary endpoints were evaluated using plasma PK parameters including but not limited to: maximum observed plasma concentration ( $C_{max}$ ), time of maximum observed plasma concentration ( $T_{max}$ ), area under the concentration-time curve from time zero to time of last quantifiable concentration or in one dosing interval ( $AUC_{0-T}$ ,  $AUC_{tau}$ ), trough observed concentrations ( $C_{trough}/C_{tau}$ ); Compound 1 concentration; and overall response rates (complete response [CR] + partial response [PR]; ORR) and Time to response (CR + PR; TTR), Duration of response (DoR), Disease control rate (CR + PR + stable disease [SD]; DCR) at 18 weeks, Progression-free survival (PFS) as determined by RECIST v1.1 as assessed by blinded independent central review (BICR), Prostate Cancer Working Group 3 (PCWG3) modified RECIST v1.1 as assessed by BICR. A partial response was a response with at least a 30% reduction in tumor size. TTR is defined as the time from the initiation of study treatment to the first documentation of response (CR + PR), which is subsequently confirmed. DoR is defined as the time from documentation of objective tumor response (CR or PR, whichever occurs first) to the first occurrence of radiographic disease progression or death from any cause. Confirmation of CR or PR is required at  $\geq 4$  weeks; however, the date of documented objective tumor response is based on the date the criteria were met prior to the confirmation. DCR is defined as the percentage of patients with a complete or partial response or stable disease (CR + PR + SD) at 6, 12, 18, and 24 weeks if the minimum duration of SD is 6 weeks ( $\pm 1$  week) from drug initiation. PFS is defined as the time from the

initiation of study treatment to the first occurrence of radiographic disease progression or death from any cause, whichever occurs first.

**[0311]** Exploratory endpoints included evaluating the effects of Compound 1 on various pharmacodynamic (PDc) markers of Compound 1 activity in blood and/or tumor tissue; exploring exposure-response relationships between Compound 1 plasma exposure and safety, PDc markers, and preliminary efficacy of Compound 1; and exploring PK metabolites of Compound 1 in plasma. Exploratory endpoints included circulating tumor DNA (ctDNA) (e.g., Y220C, other p53 mutations, non-p53 mutations), p53 target gene and protein expression (e.g., total p53, p21, macrophage inhibitory cytokine-1 [MIC-1], and various markers of immune cell activation) in patients' blood, circulating tumor cells (CTC), and tumor samples collected before and during Compound 1 treatment; and presence or concentrations of additional pharmacokinetic metabolites.

**[0312]** Enrollment in Phase 1 was restricted to advanced solid tumor patients whose cancers had a p53 Y220C mutation identified by NGS. Phase 1 included an accelerated titration design in the initial dose cohorts, followed by a modified toxicity probability interval (mTPI) design in subsequent dose cohorts. The approach minimized the number of patients treated at potentially sub-therapeutic dose levels. Adolescent patients, 12 to 17 years of age and  $\geq 40$  kg, were eligible to enroll in the mTPI portion of Phase 1 upon SRC approval. The safety data and available PK data were reviewed by the SRC prior to enrollment of the adolescent patient.

**[0313]** The dose levels for Phase 1 are summarized in **TABLE 3**. The starting dose of Compound 1 was 150 mg once daily (QD). If the first patient did not tolerate 150 mg, then the patient was offered the opportunity to reduce dosage to 100 mg after recovery if dose modification criteria were met. The maximum permitted dose of Compound 1 was 5,000 mg daily due to the presence of methanol as a residual solvent in the drug product. The maximum permitted dose was within permissible daily exposure limits set by ICH Q3C guidance for Class 2 solvents such as methanol. Based on allometric scaling of data from nonclinical studies, pharmacological activity in humans was projected to be observed starting at a Compound 1 dose level of approximately 570 mg. Accordingly, the maximum allowable dose of 5,000 mg of Compound 1 was well above the projected efficacious dose of Compound 1. **FIG. 1** illustrates the design of phase 1 and phase 2 studies of administering Compound 1 in patients with advanced solid tumors harboring a p53 Y220C mutation (BICR: blinded independent central review; BID: twice daily; CTC: circulating tumor cells; ctDNA: circulating tumor DNA; DCR: disease control rate; DoR: duration of response; MTD: maximum tolerated dose; mTPI: modified toxicity probability interval design; ORR: objective response rate by RECIST (Response Evaluation Criteria in Solid Tumors)1.1; OS, overall survival; PFS: progression-free survival; PK: pharmacokinetics; QD: once daily; RP2D: recommended Phase 2 dose; TTR: time-to-response). OS is defined as the time from the initiation of study treatment to death from any cause. For those patients who do not die before the end of the study or were lost to follow-up, OS is censored on the last day the patient was known to be alive or at the data cut-off date, whichever occurs first.

TABLE 3

| Dose Level | Total Daily Dose (mg) | % Escalation from Prior Dose Level |
|------------|-----------------------|------------------------------------|
| 1          | 150                   | Starting Dose                      |
| 2          | 300                   | 100%                               |
| 3          | 600                   | 100%                               |
| 4          | 1000                  | 67%                                |
| 5          | 1500                  | 50%                                |
| 6          | 2000                  | 33%                                |
| 7          | 2500                  | 25%                                |

**[0314] Tumor molecular profiling for KRAS status and treatment efficacy:** Molecular profiling was performed on tumors from phase 1 patients to assess the impact of *KRAS* mutation status on response. *KRAS* mutations were defined as single nucleotide variants (SNV). The *TP53* Y220C/*KRAS* wild-type (WT) efficacy evaluable analysis set included patients with measurable disease (without *KRAS* SNV) at baseline with  $\geq 1$  post-baseline tumor assessment within the efficacious dose range.

**[0315]** 67 patients were treated in the efficacious dose range (1150 mg QD to 1500 mg BID). Of patients in the efficacious dose range, the median age was 63 (range 32–84) years, 61% were female, 76% were white, 67% had an ECOG performance status of 1 and the median number of prior lines of systemic therapy was 3 (range 1–9) (TABLE 4).

TABLE 4

|                                          |            |
|------------------------------------------|------------|
| <b>Age, years</b>                        |            |
| Median (min–max)                         | 63 (32–84) |
| <b>Sex, n (%)</b>                        |            |
| Female                                   | 41 (61)    |
| Male                                     | 26 (39)    |
| <b>Race, n (%)</b>                       |            |
| White                                    | 51 (76)    |
| Asian                                    | 5 (7)      |
| Black or African American                | 6 (9)      |
| Other                                    | 1 (1)      |
| Not reported/unknown                     | 4 (6)      |
| <b>ECOG status, n (%)</b>                |            |
| 0                                        | 22 (33)    |
| 1                                        | 45 (67)    |
| <b>Prior systemic therapies, n (%)</b>   |            |
| 1                                        | 6 (9)      |
| 2                                        | 19 (28)    |
| $\geq 3$                                 | 37 (55)    |
| Not reported                             | 5 (6)      |
| Median (min–max)                         | 3 (1–9)    |
| <b>Germline <i>TP53</i> Y220C, n (%)</b> |            |
| Negative                                 | 66 (99)    |
| Positive                                 | 1 (1)      |
| <b>KRAS mutation status</b>              |            |
| Wild-type                                | 50 (75)    |
| KRAS SNV                                 | 17 (25)    |
| <b>Cancer type, n (%)</b>                |            |
| Ovary                                    | 22 (33)    |

|                 |         |
|-----------------|---------|
| Pancreas        | 12 (18) |
| Breast          | 9 (13)  |
| Colon           | 5 (7)   |
| Prostate        | 6 (9)   |
| Head and neck   | 2 (3)   |
| Endometrial     | 1 (1)   |
| Small cell lung | 2 (3)   |
| Other*          | 8 (12)  |

\*Includes sarcoma, cholangiocarcinoma, esophageal cancer, gastro-esophageal cancer, germ cell tumor, pleomorphic rhabdomyosarcoma, small intestine cancer, urothelial cancer.

ECOG, Eastern Cooperative Oncology Group; SNV, single nucleotide variant.

**[0316] Accelerated Titration:** To minimize the number of patients treated at potentially subtherapeutic dose levels, the initial portion of Phase 1 followed an accelerated titration design whereby each cohort includes a minimum of 1 and no more than 2 patients. During the accelerated titration portion of Phase 1, at least one patient had completed Cycle 1 (C1) and had been assessed for safety and DLT for at least 3 weeks (including C2D1 pre-dose assessments) before enrollment of the next cohort began. A second patient was eligible to be enrolled within the same cohort if the patient could begin treatment within 2 weeks of the first patient's treatment initiation within the same cohort. A sentinel approach was employed in Cohort 1, whereby the first patient must have completed at least one week of treatment and have reported safety assessments on C1D8 before a second Cohort 1 patient began treatment.

**[0317]** If a second patient was treated in any dose cohort, the safety of both patients was fully assessed before a subsequent cohort is opened. The accelerated titration portion continued until a  $\geq$  Grade 2 drug-related toxicity was observed in  $\geq 1$  patient during the 21-day DLT observation period (C1). Once a  $\geq$  Grade 2 drug-related toxicity was observed, the accelerated titration portion ended, and the mTPI portion began at a dose level no higher than the level where the  $\geq$  Grade 2 toxicity was observed. Data from the accelerated titration portion of the study was included in dose escalation decisions associated with the mTPI design if the dose level was the same.

**[0318]** If preliminary efficacy was observed during the accelerated titration portion of the study in the absence of  $\geq$  Grade 2 drug-related toxicity during the 21-day DLT observation period (C1), the study transitioned to the mTPI portion of the study starting with the next highest dose cohort to gather additional safety, PK, PDc and preliminary efficacy data in subsequent cohorts.

**[0319] mTPI:** The mTPI is a rule-based method using Bayesian modeling to define dosing decisions. The mTPI portion of Phase 1 guides the selection of dose levels until an MTD is determined based on the rules of the mTPI, the dose escalation portion of the study is stopped, or the maximum allowable dose of Compound 1 is reached. If  $\geq$  Grade 2 drug-related toxicity was observed during the accelerated titration phase, the first dose cohort of the mTPI portion employed a dose that was no higher than was the highest dose cohort employed during the accelerated titration phase. Initially, each mTPI dose cohort enrolled 3 to 4 patients. A fourth patient was enrolled in a cohort if the patient could begin treatment within 2 weeks of the third patient starting treatment. To establish a dose level as safe and tolerable, a minimum of 3 patients treated at that dose level were required to have completed the 21-day DLT period (C1), as well as

pre- dose assessments on C2D1. If a fourth patient was treated in any dose cohort, the safety of all 4 patients was fully assessed before a future dosing decision is made.

**[0320]** During the mTPI period, prespecified rules associated with dose decisions based on statistical modeling using all safety data guides selection of dose levels to be tested. Dosing decisions (i.e., escalation, de-escalation or stay) are based on the observed toxicity outcomes and a predefined set of operating characteristics. In addition, PK and PDc data are reviewed, when available, to help inform dose selection decisions. At the end of Phase 1, the MTD is estimated as the highest dose with an estimated toxicity less than or equal to the target toxicity equivalence interval (e.g.,  $\leq 35\%$ ) among the tested doses. The mTPI design is eligible to be stopped and the MTD determined if a minimum of 9 patients are enrolled and evaluated at a given dose cohort and the mTPI decision is “stay.” A maximum number of 12 patients are enrolled in a single dose cohort. During the mTPI period, if the current cohort under evaluation is filled and another patient is identified who might be eligible for the study, the potential patient undergoes screening to be enrolled in the next lowest dose cohort, provided enrollment in the lower dose cohort has not exceeded 12 patients, and that any lower dose cohort has shown preliminary evidence of PDc activity and/or preliminary efficacy.

**[0321]** Any DLTs experienced by patients enrolled in a previously cleared cohort are not included in subsequent dose escalation decisions or in the definition of MTD. However, data from the patients are reviewed and are taken into consideration for assessments of safety and tolerability and in the selection of the RP2D. Dosing may also be stopped prior to establishing the MTD if safety concerns emerge, or if the clinical and PDc data indicate that an optimal biologically active dose has been reached, or if no further increase in exposure is likely to be achieved with further increase in dose.

**[0322]** Serial blood samples for PK assessments were collected from all patients, including adolescents. Serial blood samples from all patients were also collected for PDc assessment of response to Compound 1. Pre- and on-treatment tumor biopsies for PDc assessment were optional. The relationship between plasma Compound 1 concentrations and pharmacodynamic biomarkers of response were explored. When the MTD was established, this cohort was expanded up to 12 patients. At least one additional cohort at a total daily dose below the MTD and considered to be active were expanded up to 12 patients. These expanded cohorts provided additional information about the relationship between dose, safety/tolerability, and efficacy to inform the selection of RP2D. Data from Phase 1 are reviewed to assess the benefit-risk for each dose level when selecting RP2D. The RP2D is determined as the dose resulting in the best therapeutic window for Compound 1 based on the review of all available safety, PK, PDc, and preliminary efficacy data. The RP2D can be the same as the MTD or below the MTD.

**[0323] Inpatient dose escalation:** During Phase 1, inpatient dose escalation was permitted if a patient tolerated Compound 1 well (i.e., has not experienced any drug-related  $\geq$  Grade 2 adverse events) and was on treatment for at least 2 cycles. A patient’s dose was increased to a dose that has been established as safe and tolerable. Whenever a patient underwent dose escalation, the patient was required to have weekly safety assessments at the new dose level during the first 2 cycles as outlined for Cycles 1 and 2.

**[0324] Phase 1 dose escalation:** Phase 1 employed an accelerated titration design in the initial dose escalation cohorts until a  $\geq$  Grade 2 drug-related toxicity occurred. In subsequent dose escalation cohorts, an mTPI design is employed, starting at a dose level no higher than that at which the Grade 2 toxicity occurred. The mTPI design described above is used to determine the MTD. The mTPI design employs a simple beta-binomial hierarchical model where decision rules are based on calculating the unit probability mass (UPM) of 3 intervals corresponding to underdosing, proper dosing, and overdosing in terms of toxicity. The 3 dosing intervals were defined by an equivalence interval that reflects uncertainty around the true target toxicity, and were associated with 3 different dose level decisions. The underdosing interval corresponded to a dose escalation, overdosing corresponded to a dose de-escalation, and proper dosing corresponded to staying at the current dose. After each cohort of patients, the mTPI design calculated the UPMs for the 3 dosing intervals, and the interval with the largest UPM defined the corresponding dose-finding decision. The dose escalation portion of the study stops when either the lowest dose is above MTD or a prespecified maximum sample size is reached. Up to 60 patients are included in the mTPI portion of Phase 1, with target DLT rate of 30%, an equivalence interval of  $\pm 5\%$ , and cohort size of 3 or 4. Due to the rarity of the p53 Y220C mutation, the mTPI design is eligible to be stopped and an MTD selected if the mTPI design rules call for more than 9 patients to be enrolled at a single dose.

**[0325]** Compound 1 concentration data were summarized by dose level and Study Day using nominal time points. Individual and mean concentrations versus nominal time plots were presented by dose level. Pharmacokinetic parameter values were derived by non-compartmental methods by a validated pharmacokinetic analysis program. Actual times were used for all formal analyses. PK parameters were summarized by dose level. Individual values were listed for each PK parameter (e.g., C<sub>max</sub>, T<sub>max</sub>, AUC<sub>0-T</sub>, AUC<sub>tau</sub>, C<sub>trough</sub>/C<sub>tau</sub>, accumulation ratios) by dose level, and the following (non-model-based) descriptive statistics were provided: N (number of patients with non-missing data), arithmetic mean, standard deviation, arithmetic percent coefficient of variation (CV) (calculated as  $100 \times \text{standard deviation}/\text{arithmetic mean}$ ), median, minimum, maximum, geometric mean, and geometric percent CV (calculated as:

$$100 \times \sqrt{\exp(s^2) - 1},$$

where  $s^2$  is the observed variance on the natural log-scale).

**[0326]** The operating characteristics associated with the mTPI design under varying maximum sample sizes are evaluated when (i) all dose levels have true toxicity rates equal to 10%; and (ii) dose levels have monotonically increasing true toxicity rates. Operating characteristics of the mTPI design are evaluated based on the mTPI portion of the study having 5 dose levels.

**[0327]** Most frequent treatment-related adverse events (TRAEs) ( $>20\%$ ) were nausea, vomiting, and increased blood creatinine. Compound 1 administered with food led to improvement in gastrointestinal toxicities (nausea, vomiting, and diarrhea), with similar safety profiles observed across the efficacious range. A 3% rate of drug discontinuation due to a TRAE was observed. **TABLE 5** summarizes the incidence of TRAEs in patients administered 1150 mg QD to 1500 mg BID Compound 1.



TABLE 5

| All TRAEs, n (%)           |              | Max CTCAE |           |                        |                      |
|----------------------------|--------------|-----------|-----------|------------------------|----------------------|
| Preferred Term             | Overall n=67 | 1         | 2         | 3                      | 4                    |
| Any TRAE                   | 60 (89.6)    | 16 (23.9) | 27 (40.3) | 16 <sup>†</sup> (23.9) | 1 <sup>‡</sup> (1.5) |
| Nausea                     | 34 (50.7)    | 22 (32.8) | 11 (16.4) | 1 (1.5)                | -                    |
| Vomiting                   | 29 (43.3)    | 16 (23.9) | 12 (17.9) | 1 (1.5)                | -                    |
| Blood creatinine increased | 18 (26.9)    | 10 (14.9) | 8 (11.9)  | -                      | -                    |
| Diarrhea                   | 13 (19.4)    | 12 (17.9) | -         | 1 (1.5)                | -                    |
| Fatigue                    | 13 (19.4)    | 8 (11.9)  | 5 (7.5)   | -                      | -                    |
| ALT increased              | 12 (17.9)    | 4 (6.0)   | 5 (7.5)   | 3 (4.5)                | -                    |
| AST increased              | 11 (16.4)    | 7 (10.4)  | 2 (3.0)   | 2 (3.0)                | -                    |
| Anemia                     | 10 (14.9)    | 1 (1.5)   | 6 (9.0)   | 3 (4.5)                | -                    |
| Decreased appetite         | 7 (10.4)     | 2 (3.0)   | 4 (6.0)   | 1 (1.5)                | -                    |
| Proteinuria                | 6 (9.0)      | 1 (1.5)   | 5 (7.5)   |                        | -                    |
| Rash maculo-papular        | 6 (9.0)      | 1 (1.5)   | 3 (4.5)   | 2 (3.0)                | -                    |
| Headache                   | 5 (7.5)      | 4 (6.0)   | 1 (1.5)   |                        | -                    |
| Lipase increased           | 5 (7.5)      | 4 (6.0)   | -         | 1 (1.5)                | -                    |
| Platelet count decreased   | 4 (6.0)      | 1 (1.5)   | 1 (1.5)   | 2 (3.0)                | -                    |
| Amylase increased          | 4 (6.0)      | 3 (4.5)   | 1 (1.5)   | -                      | -                    |
| Dehydration                | 4 (6.0)      | -         | 4 (6.0)   | -                      | -                    |

<sup>†</sup>Includes five additional grade 3 treatment-related adverse events: neutrophil count decreased, acute kidney injury, pancreatitis, pneumonitis, and rash erythematous. Note that a patient could have multiple grade 3 events.

<sup>‡</sup>Includes one patient with grade 4 immune thrombocytopenia.

Data cut-off: 05 Sept 2023. ALT, alanine aminotransferase; AST, aspartate aminotransferase; BID, twice daily; CTCAE, Common Terminology Criteria for Adverse Events; QD, once daily; TRAE, treatment-related adverse event.

**[0328]** Compound 1 demonstrated dose-proportionality and linear PK from 150 mg to 2500 mg QD at steady state (**FIGURE 2**). **FIGURE 2** depicts day 15 (steady state) exposures of Compound 1. The median half-life of Compound 1 was 19 hours at steady state (day 15) across doses (AUC, area under the curve; SD, standard deviation).

**[0329]** Clinical efficacy was achieved in heavily pre-treated patients across multiple tumor types. Within safety population (N=67), 51 patients had measurable disease at baseline and a post baseline tumor assessment. Of those patients: 38 patients were included in the TP53 Y220C/KRAS WT efficacy evaluable population, and 13 patients had tumors harboring KRAS mutations. Among patients with measurable disease included in the efficacy evaluable population (N=51), tumor shrinkage was observed across multiple tumor types, including patients whose tumor was KRAS wild-type (**FIGURE 3**).

[0330] **FIGURE 3** includes patients with measurable disease at baseline and  $\geq 1$  post-baseline assessment. One patient without tumor measurement at first scan is not represented. “Other” tumor types include sarcoma, esophageal cancer, germ cell tumor, pleomorphic rhabdomyosarcoma, and urothelial cancer. BID, twice daily; PD, progressive disease; PR, partial response; QD, once daily; SD, stable disease; WT, wildtype.

[0331] Of patients with a TP53 Y220C mutation and KRAS WT disease, a total of 13 confirmed partial responses (PRs) (overall response rate [ORR] = 34%) were observed across multiple tumor types, including ovarian, breast, small-cell lung, and endometrial cancers (**TABLE 6**).

TABLE 6

|                        | 2000 mg QD<br>N=16 | 1150 mg QD-1500 mg BID<br>N=38 |
|------------------------|--------------------|--------------------------------|
|                        | ORR, n (%)         | ORR, n (%)                     |
| <b>Overall</b>         | 6 (38)             | 13 (34)                        |
| <b>PR*</b>             | 6                  | 13                             |
| <b>SD</b>              | 8                  | 20                             |
| <b>PD</b>              | 2                  | 5                              |
| <b>Ovary</b>           | 2 (40)             | 7 (47)                         |
| <b>Breast</b>          | 2 (67)             | 3 (38)                         |
| <b>Small cell lung</b> | 0 (0)              | 1 (50)                         |
| <b>Endometrial</b>     | 1 (100)            | 1 (100)                        |
| <b>Other</b>           | 1 (33)             | 1 (9)                          |

\*All partial responses were confirmed. Only tumor types that had at least one responder were included in the analysis of overall responses across tumor types. KRAS WT efficacy evaluable: All treated patients with measurable disease at baseline, KRAS WT defined as no KRAS SNVs, and  $\geq 1$  post-baseline assessment. BID, twice daily; ORR, objective response rate; PD, progressive disease; PR, partial response; QD, once daily; SD, stable disease; SNV, single nucleotide variant; WT, wild-type.

[0332] At 2000 mg QD, the ORR was 38% in patients with TP53 Y220C and KRAS WT tumors.

[0333] In patients with a KRAS mutation, clinical activity was observed but there were no confirmed responses. Clinical activity or clinical efficacy is activity found at various trial endpoints or tumor response criteria, including ORR, time to response (TTR), duration of response (DoR), disease control rate (DCR) at 6, 12, 18, and 24 weeks, and progression-free survival (PFS) per BICR and overall survival (OS).

[0334] Among all responders, median time to response and median duration of response were 1.5 months and 7 months, respectively (**FIGURE 4**). **FIGURE 4** summarizes target lesion reduction across tumor types (1150 mg QD to 1500 mg BID) in the TP53 Y220C/KRAS WT efficacy evaluable population, and includes all patients with measurable disease at baseline, KRAS WT and  $\geq 1$  post-baseline assessment (n=38). “Other” tumor types included 1 patient with germ cell tumor, 2 patients with sarcoma, 1 patient with urothelial cancer and 1 patient with esophageal cancer.

[0335] Among the 51 patients treated with Compound 1 in the efficacious dose range and who were TP53 Y220C/KRAS WT, 40 patients had a ctDNA TP53 Y220C variant allele frequency (VAF) available at baseline (**FIGURE 5**). **FIGURE 5** shows percentage change in TP53 Y220C VAF at Week 3 from

baseline. CFBL, change from baseline; ctDNA, circulating tumor DNA; NGS, next-generation sequencing; PR, partial response; QD, once daily; VAF, variant allele frequency. 80% (n=32) and 42.5% (n=17) of patients had a change in TP53 Y220C VAF from baseline of  $\geq 50\%$  and  $\geq 95\%$  respectively. 92.5% (n=37) of patients had a reduction in TP53 Y220C VAF, suggesting on target activity. In addition, all patients experiencing a PR had a reduction in TP53 Y220C VAF.

*Case study.*

**[0336]** A 51-year-old woman presented with metastatic triple negative breast cancer (TNBC). The patient had previously undergone neoadjuvant therapy (carboplatin, paclitaxel and pembrolizumab followed by cyclophosphamide, doxorubicin and pembrolizumab), bilateral mastectomy followed by pembrolizumab maintenance, radiotherapy, and breast reconstruction, and treatment with pegylated liposomal doxorubicin for disease recurrence. Progressive disease was observed in axilla with extensive skin lesions on adjacent breast and arm, limiting mobility. TP53 Y220C was detected by next-generation sequencing. Compound 1 2000 mg QD was started. Rapid, visible reduction in arm swelling and improved mobility of arm and fingers was observed within the first week. PR was observed at 6 weeks (41% reduction in axilla lesion), and confirmed at 12 weeks and ongoing. **FIGURE 6** provides patient images of triple negative breast cancer at baseline and post-cycle 3 of treatment with Compound 1.

*Conclusions.*

**[0337]** Compound 1 demonstrated a favorable safety profile in the efficacious dose range, with improvement in gastrointestinal adverse events when Compound 1 was taken with food. Single agent clinical efficacy was achieved in heavily pre-treated patients across multiple tumor types.

**EXAMPLE 3: Compound 1 Activity in Ovarian Cancer**

**[0338]** In a 22 patient ovarian cancer subset of individuals treated in the phase 1 clinical trial detailed in **EXAMPLE 3**, preliminary efficacy (ORR) per investigator-assessed RECIST v1.1 and serum CA-125 response (defined as  $>50\%$  decrease at 2 separate timepoints, 4 weeks apart) were analyzed. Safety across tumor types was also evaluated within the efficacious dose range. This subgroup analysis investigated the efficacy of Compound 1 in patients with advanced ovarian cancer treated across the efficacious dose range (1150 mg daily to 1500 mg twice daily).

**[0339]** The median age of patients with ovarian cancer (N=22) was 66 years (range 49 – 81 years). At baseline, 20 patients had high-grade serous ovarian cancer and two had endometrioid cancer; two had a BRCA2 mutation and 20 lacked a BRCA1/2 mutation; six were homologous recombination deficiency (HRD) positive, 11 were HRD negative and five had unknown HRD status. All were KRAS WT. The median number of prior lines of systemic therapy was four (range 1 – 9). At study entry, 19 patients were platinum resistant, two were platinum refractory, and one was platinum sensitive. Other patient demographics and disease characteristics are summarized in **TABLE 7**.

TABLE 7

|                                                                                              |                                                 |
|----------------------------------------------------------------------------------------------|-------------------------------------------------|
| <b>Age, years</b><br>Median (min–max)                                                        | 66 (49–81)                                      |
| <b>Race, n (%)</b><br>White<br>Asian<br>Black or African American<br>Not reported/unknown    | 15 (68)<br>3 (14)<br>2 (9)<br>2 (9)             |
| <b>ECOG status, n (%)</b><br>0<br>1                                                          | 6 (27)<br>16 (73)                               |
| <b>Prior systemic therapies, n (%)</b><br>1<br>2<br>≥3<br>Not reported<br>Median (min–max)   | 1 (5)<br>4 (18)<br>14 (64)<br>3 (14)<br>4 (1–9) |
| <b>Platinum status</b><br>Platinum Sensitive<br>Platinum Resistant*<br>Platinum Refractory** | 2 (9)<br>19 (86)<br>1 (5)                       |
| <b>Histology</b><br>HGSOC<br>Endometrioid                                                    | 20 (91)<br>2 (9)                                |
| <b>Measurable disease at baseline, n (%)</b><br>Yes<br>No                                    | 20 (91)<br>2 (9)                                |
| <b>Germline <i>TP53</i> Y220C, n (%)</b><br>Negative<br>Positive                             | 22 (100)<br>0 (0)                               |
| <b>Somatic <i>BRCA1/2</i> mutation status, n (%)</b><br><i>BRCA1</i><br><i>BRCA2</i>         | 0 (0)<br>2 (9)                                  |
| <b>Germline <i>BRCA1/2</i> mutation status, n (%)</b><br>No<br>Unknown                       | 13 (59)<br>9 (41)                               |
| <b>HRD status</b><br>Positive<br>Negative<br>Unknown                                         | 6 (27)<br>11 (50)<br>5 (23)                     |
| <b><i>KRAS</i> status, n (%)</b><br>Wild type <sup>†</sup>                                   | 22 (100)                                        |

\*Patients with disease progressing within 6 months of platinum-based chemotherapy. \*\*Patients with disease progressing during platinum-based therapy or within 4 weeks after last dose. <sup>†</sup>Defined as no *KRAS* single-nucleotide variant. ECOG, Eastern Cooperative Oncology Group; HGSOC, high-grade serous ovarian cancer; HRD, homologous recombination deficiency.

[0340] Results showed that of the 15 patients with measurable disease at baseline and  $\geq 1$  post-baseline tumor assessment (efficacy evaluable population), seven patients achieved a confirmed partial response (PR), seven had stable disease (SD), and one had progressive disease. FIG. 7 is a chart summarizing best percent change in sum of tumor target lesions among the 15 patients in the ovarian subgroup with measurable disease. In FIG. 7, \* denotes a patient who experienced an initial, unconfirmed, partial response that was not subsequently confirmed. NE, not evaluable; ORR, overall response rate; PD, progressive disease; PR, partial response; SD, stable disease.

[0341] The median duration of response was seven months. Of 15 patients with measurable serum CA-125 at baseline, six had a CA-125 response. Among these, five patients achieved radiographic PR and one had SD. FIG. 8 is a chart summarizing the best percent change in CA-125 levels of the 15 patients in the ovarian subgroup with measurable CA-125 at baseline. \*A CA-125 response is defined as a decrease in CA-125 by 50% from baseline at two separate timepoints, 4 weeks apart.

[0342] FIG. 9 is a chart summarizing the time to response and duration of treatment for patients in the ovarian subgroup with measurable disease at baseline at  $\geq 1$  post baseline tumor assessment. The median duration of response was 7 months.

[0343] In the overall population of 67 patients assessed in the efficacious dose range ( $\geq 1150$ mg QD), including this subset of patients with ovarian cancer, treatment-related adverse events (TRAEs) were mostly grade 1 and 2 and the most TRAEs were nausea (51%), vomiting (43%), and increased blood creatinine (27%). The frequency and severity of TRAEs were similar in the ovarian cancer population compared with the overall population. Compound 1 administration with food led to an improvement in nausea and vomiting.

#### **EXAMPLE 4: Compound 1 pharmacokinetics in fasted and fed subjects.**

[0344] Compound 1 pharmacokinetics were analyzed in healthy subjects administered Compound 1 in a fasted state. A subject was considered fasted if the subject fasted overnight for at least 10 hours and water was permitted ad libitum except for 1 hour before and after drug administration. For fasted subjects, Compound 1 was administered with at least 8 ounces (240 mL) of water. Compound 1 pharmacokinetics were also analyzed in healthy subjects administered Compound 1 in a fed state. A subject was considered fed after consuming a high-fat meal 30 minutes prior to drug administration, and the meal was finished at least 5 minutes prior to drug administration. For both fasted and fed subjects, no food was permitted for at least 4 hours post-dose and water was permitted starting at 1 hour post-dose.

[0345] Subjects were healthy, non-smoking male and female Caucasian/non-Asian or Japanese participants 18 to 55 years of age with a BMI between 18.5 and 30.0 kg/m<sup>3</sup>.

#### *Part 1 Study Design (1000 mg Compound 1)*

[0346] In Part 1 of the study, analysis was performed to compare 1000 mg Compound 1 in fed and fasted states. A dose of 1000 mg of Compound 1 consisted of two 500 mg tablets administered as a single

oral dose. The analysis of Part 1 was performed at two separate time points in two different schemes (1A and 1B).

[0347] In scheme 1A, at period 1, Compound 1 analyzed in healthy subjects administered 1000 mg Compound 1 (n=3) in a fed state. Following a washout period of 7-9 days, these subjects were then administered 1000 mg Compound 1 in a fasted state.

[0348] Separately, in scheme 1B, at period 1, Compound 1 was analyzed in healthy subjects administered 1000 mg Compound 1 (n=3) in a fasted state. Following a washout period of 7-9 days, these subjects were then administered 1000 mg Compound 1 in a fed state.

*Part 2 Study Design (2000 mg Compound 1)*

[0349] In Part 2 of the study, analysis was performed to compare 2000 mg Compound 1 in fed and fasted states and also to compared ethnicity of Caucasian/non-Asian subjects and Japanese subjects. A dose of 2000 mg of Compound 1 consisted of four 500 mg tablets administered as a single oral dose. A participant was classified as Japanese if (i) the participant was born in Japan, (ii) had parents and grandparents who were ethnically Japanese and were born in Japan, (iii) had lived less than 10 years outside of Japan, and (iv) underwent no significant change in lifestyle since leaving Japan.

[0350] For Caucasian/non-Asian subjects (n = 20) the analysis was performed at two separate time points in two different schemes (2A and 2B). During scheme 2A, at period 1, Compound 1 was analyzed in subjects administered 2000 mg Compound 1 (n=10) in a fed state. Following a washout period of 7-9 days, at period 2, these subjects were then administered 2000 mg Compound 1 in a fasted state. Separately in scheme 2B, at period 1, Compound 1 was analyzed in subjects administered 2000 mg Compound 1 (n=10) in a fasted state. Following a washout period of 7-9 days, at period 2, these subjects were then administered 2000 mg Compound 1 in a fed state.

[0351] For Japanese subjects (n=6), Compound 1 pharmacokinetics were analyzed in subjects administered 2000 mg Compound 1 in a fasted state.

*Pharmacokinetic (PK) Analysis – Blood Samples*

[0352] Blood for the analysis of study drug (Compound 1) and metabolite (M1) were collected.

[0353] Arithmetic mean ( $\pm$  SD) Compound 1 plasma concentration-time plots were grouped in Part 1 of the study by fed/fasted condition (linear and semi-logarithmic scales) (FIGs. 10-11) and in Part 2 of the study by ethnicity and fed/fasted condition (FIGs. 12-13).

[0354] Following administration of single oral doses of Compound 1 in Part 1 and Part 2, plasma concentrations of Compound 1 reached the maximum peak rapidly and declined roughly in a bi-exponential manner. Mean Compound 1 concentrations throughout the PK profile were higher for Caucasian/non-Asian participants under the fed condition compared to the fasted condition, for both the 1000 mg and 2000 mg doses (FIGs. 10-13).

[0355] Mean Compound 1 plasma concentrations were higher in Japanese participants compared to Caucasian/non-Asian participants after administration of a 2000 mg dose in the fasted condition (FIGs. 12-13).

[0356] Various pharmacokinetic (PK) parameters were determined for each subject. (Tables 11-14 and Figures 14-19). For example, terminal elimination rate constant ( $\lambda_z$ ); percentage of AUC<sub>0-inf</sub> that is due to extrapolation beyond t<sub>last</sub> (AUC%<sub>extrap</sub>); area under the concentration-time curve from pre-dose (time 0) to 24 hours (AUC<sub>0-24</sub>); area under the concentration-time curve from pre-dose (time 0) to 96 hours (AUC<sub>0-96</sub>); area under the concentration-time curve from pre-dose (time 0) extrapolated to infinity (AUC<sub>0-inf</sub>); area under the concentration-time curve from pre-dose (time 0) to t<sub>last</sub> (AUC<sub>0-last</sub>); apparent total body clearance (CL/F); maximum observed plasma concentration (C<sub>max</sub>); apparent terminal elimination half-life (t<sub>1/2</sub>); time prior to the first measurable (non-zero) concentration (t<sub>lag</sub>); time of last quantifiable concentration (t<sub>last</sub>); time corresponding to the occurrence of C<sub>max</sub> (T<sub>max</sub>); and apparent volume of distribution (V<sub>z</sub>/F).

[0357] Statistics of Compound 1 plasma PK parameters were evaluated by fed/fasted condition in Part 1 of the study (TABLE 8) and by ethnicity and fed/fasted condition in Part 2 of the study (TABLE 9).

TABLE 8

| Pharmacokinetic Plasma Parameter (unit) | Statistic        | Compound 1 Fed 1000 mg (N=6) | Compound 1 Fasted 1000 mg (N=6) |
|-----------------------------------------|------------------|------------------------------|---------------------------------|
| AUC(h•ng/mL)                            | n                | 6                            | 6                               |
|                                         | GeoMean          | 180000                       | 150000                          |
|                                         | gCV%             | 33.4                         | 23.2                            |
|                                         | Minimum, Maximum | 114000, 264000               | 98400, 183000                   |
| AUC(h•ng/mL)                            | n                | 6                            | 6                               |
|                                         | GeoMean          | 177000                       | 147000                          |
|                                         | gCV%             | 32.6                         | 23.4                            |
|                                         | Minimum, Maximum | 113000, 253000               | 95900, 182000                   |
| C(ng/mL)                                | n                | 6                            | 6                               |
|                                         | GeoMean          | 10600                        | 8690                            |
|                                         | gCV%             | 18.2                         | 19.0                            |
|                                         | Minimum, Maximum | 8350, 12300                  | 7200, 11100                     |
| T(h)                                    | n                | 6                            | 6                               |
|                                         | Median           | 3.25                         | 2.27                            |
|                                         | Minimum, Maximum | 1.51, 5.01                   | 1.54, 2.51                      |
| t(h)                                    | n                | 6                            | 6                               |
|                                         | GeoMean          | NC                           | NC                              |
|                                         | gCV%             | NC                           | NC                              |
|                                         | Minimum, Maximum | 0.00, 0.713                  | 0.00, 0.00                      |
| AUC(h•ng/mL)                            | n                | 6                            | 6                               |
|                                         | GeoMean          | 119000                       | 97900                           |
|                                         | gCV%             | 25.6                         | 18.6                            |
|                                         | Minimum, Maximum | 85800, 152000                | 72900, 121000                   |
| AUC(h•ng/mL)                            | n                | 6                            | 6                               |
|                                         | GeoMean          | 177000                       | 148000                          |
|                                         | gCV%             | 32.2                         | 22.7                            |
|                                         | Minimum, Maximum | 114000, 253000               | 97600, 182000                   |
| AUC(%)                                  | n                | 6                            | 6                               |
|                                         | GeoMean          | 1.61                         | 1.61                            |
|                                         | gCV%             | 65.5                         | 64.1                            |
|                                         | Minimum, Maximum | 0.884, 4.21                  | 0.821, 3.62                     |
| t <sup>1</sup> (h)                      | n                | 6                            | 6                               |

|                   |                  |                |                |
|-------------------|------------------|----------------|----------------|
|                   | GeoMean          | 15.8           | 15.4           |
|                   | gCV%             | 21.0           | 17.3           |
|                   | Minimum, Maximum | 11.9, 21.7     | 13.6, 20.8     |
| $\lambda_z$ (1/h) | n                | 6              | 6              |
|                   | GeoMean          | 0.0438         | 0.0450         |
|                   | gCV%             | 21.0           | 17.3           |
|                   | Minimum, Maximum | 0.0320, 0.0580 | 0.0334, 0.0511 |
| CL/F (L/h)        | n                | 6              | 6              |
|                   | GeoMean          | 5.55           | 6.67           |
|                   | gCV%             | 33.4           | 23.2           |
|                   | Minimum, Maximum | 3.79, 8.76     | 5.45, 10.2     |
| $V_z/F$ (L)       | n                | 6              | 6              |
|                   | GeoMean          | 127            | 148            |
|                   | gCV%             | 19.5           | 23.5           |
|                   | Minimum, Maximum | 99.6, 164      | 107, 199       |

TABLE 9

| Pharmacokinetic<br>Plasma<br>Parameter (unit) | Statistic           | Caucasian/Non-Asian                 |                                           | Japanese                                 |
|-----------------------------------------------|---------------------|-------------------------------------|-------------------------------------------|------------------------------------------|
|                                               |                     | Compound 1 Fed<br>2000 mg<br>(N=22) | Compound 1<br>Fasted<br>2000 mg<br>(N=21) | Compound 1<br>Fasted<br>2000 mg<br>(N=6) |
| AUC(h•ng/mL)                                  | Minimum,<br>Maximum | 269000, 697000                      | 106000, 555000                            | 201000, 481000                           |
|                                               | n                   | 20                                  | 20                                        | 6                                        |
|                                               | GeoMean             | 479000                              | 259000                                    | 338000                                   |
|                                               | gCV%                | 29.5                                | 55.5                                      | 35.5                                     |
| AUC(h•ng/mL)                                  | Minimum,<br>Maximum | 272000, 839000                      | 106000, 613000                            | 209000, 487000                           |
|                                               | n                   | 21                                  | 21                                        | 6                                        |
|                                               | GeoMean             | 448000                              | 254000                                    | 326000                                   |
|                                               | gCV%                | 26.4                                | 53.1                                      | 34.5                                     |
| C(ng/mL)                                      | Minimum,<br>Maximum | 269000, 697000                      | 105000, 555000                            | 201000, 481000                           |
|                                               | n                   | 21                                  | 21                                        | 6                                        |
|                                               | GeoMean             | 19000                               | 10300                                     | 14700                                    |
|                                               | gCV%                | 20.7                                | 52.2                                      | 45.1                                     |
| T(h)                                          | Minimum,<br>Maximum | 12300, 28100                        | 2820, 20100                               | 6710, 20600                              |
|                                               | n                   | 21                                  | 21                                        | 6                                        |
|                                               | Median              | 4.03                                | 2.52                                      | 3.94                                     |
|                                               | Minimum,<br>Maximum | 2.11, 8.08                          | 1.03, 5.94                                | 1.59, 5.06                               |
| t(h)                                          | n                   | 22                                  | 21                                        | 6                                        |
|                                               | GeoMean             | NC                                  | NC                                        | NC                                       |
|                                               | gCV%                | NC                                  | NC                                        | NC                                       |
|                                               | Minimum,<br>Maximum | 0.00, 1.03                          | 0.00, 0.00                                | 0.00, 0.00                               |
| AUC(h•ng/mL)                                  | n                   | 21                                  | 21                                        | 6                                        |
|                                               | GeoMean             | 250000                              | 138000                                    | 193000                                   |
|                                               | gCV%                | 20.9                                | 50.6                                      | 39.6                                     |
|                                               | Minimum,<br>Maximum | 178000, 347000                      | 46900, 277000                             | 97300, 283000                            |
| AUC(h•ng/mL)                                  | n                   | 21                                  | 21                                        | 6                                        |



|                       |                  |                |                |                |
|-----------------------|------------------|----------------|----------------|----------------|
|                       | GeoMean          | 448000         | 255000         | 326000         |
|                       | gCV%             | 26.4           | 52.9           | 34.5           |
| AUC(%)                | n                | 20             | 20             | 6              |
|                       | GeoMean          | 4.74           | 3.42           | 2.13           |
|                       | gCV%             | 90.6           | 80.8           | 154.1          |
|                       | Minimum, Maximum | 0.953, 16.8    | 0.901, 9.51    | 0.482, 10.5    |
| t <sup>1</sup> (h)    | n                | 20             | 21             | 6              |
|                       | GeoMean          | 22.7           | 19.9           | 17.4           |
|                       | gCV%             | 26.6           | 35.1           | 33.9           |
|                       | Minimum, Maximum | 13.5, 38.2     | 11.5, 45.8     | 11.2, 30.0     |
| $\lambda_z$ (1/h)     | n                | 20             | 21             | 6              |
|                       | GeoMean          | 0.0306         | 0.0348         | 0.0398         |
|                       | gCV%             | 26.6           | 35.1           | 33.9           |
|                       | Minimum, Maximum | 0.0181, 0.0513 | 0.0151, 0.0602 | 0.0231, 0.0617 |
| CL/F (L/h)            | n                | 20             | 20             | 6              |
|                       | GeoMean          | 4.18           | 7.71           | 5.92           |
|                       | gCV%             | 29.5           | 55.5           | 35.5           |
|                       | Minimum, Maximum | 2.39, 7.36     | 3.27, 18.8     | 4.11, 9.56     |
| V <sub>z</sub> /F (L) | n                | 20             | 20             | 6              |
|                       | GeoMean          | 137            | 212            | 149            |
|                       | gCV%             | 22.0           | 38.0           | 39.3           |
|                       | Minimum, Maximum | 86.8, 216      | 117, 382       | 93.8, 251      |

**[0358]** For Part 1, after administration of a single 1000 mg oral dose of Compound 1, geometric mean (gCV%) C<sub>max</sub> values were 8690 ng/mL (19.0%) and 10600 ng/mL (18.2%) under fasted and fed conditions, respectively. Median T<sub>max</sub> values were 2.27 hours (range: 1.54 to 2.51 hours) and 3.25 hours (range: 1.51 to 5.01 hours) under fasted and fed conditions, respectively. Geometric mean (gCV%) AUC<sub>0</sub>-last values were 147000 h•ng/mL (23.4%) and 177000 h•ng/mL (32.6%) under fasted and fed conditions, respectively.

**[0359]** Geometric mean (gCV%) AUC<sub>0</sub>-inf values were 150000 h•ng/mL (23.2%) and 180000 h•ng/mL (33.4%) under fasted and fed conditions, respectively, with the percentage obtained through extrapolation (AUC%extrap) less than 5% for both fed and fasted conditions. Low variability was observed for the primary plasma PK parameters for both groups (lower than 23.4% and 33.4% for fasted and fed conditions, respectively). Plasma CL/F and V<sub>z</sub>/F were slightly higher in the fasted condition.

**[0360]** For Part 2, after administration of a single 2000 mg oral dose of Compound 1, geometric mean (gCV%) C<sub>max</sub> values were 10300 ng/mL (52.2%) and 19000 ng/mL (20.7%) under fasted and fed conditions, respectively, for Caucasian/non-Asian participants. Median T<sub>max</sub> values were 2.52 hours (range: 1.03 to 5.94 hours) and 4.03 hours (range: 2.11 to 8.08 hours) under fasted and fed conditions, respectively, for Caucasian/non-Asian participants. Geometric mean (gCV%) AUC<sub>0</sub>-last values were 254000 h•ng/mL (53.1%) and 448000 h•ng/mL (26.4%) under fasted and fed conditions, respectively, for Caucasian/non-Asian participants.

[0361] Geometric mean (gCV%) AUC<sub>0-inf</sub> values were 259000 h•ng/mL (55.5%) and 479000 h•ng/mL (29.5%) under fasted and fed conditions, respectively, for Caucasian/non-Asian participants with the percentage obtained through extrapolation (AUC%<sub>extrap</sub>) less than 17% for both fed and fasted conditions. Low to moderate variability was observed for the primary plasma PK parameters for both groups (lower than 55.5% and 29.5% for fasted and fed conditions, respectively). Plasma CL/F and VzF were slightly higher in the fasted condition.

[0362] For Caucasian/non-Asian participants, numerically Compound 1 maximum exposure (C<sub>max</sub>) and systemic exposure (AUC) was higher under fed conditions (high-fat meal) compared to fasted conditions. In addition, a slight delay was observed in absorption with a prolonged T<sub>max</sub> under fed conditions (approximately 1.00 hours and 1.50 hours delay for Part 1 and Part 2, respectively).

[0363] For Japanese participants under fasted conditions, Compound 1 peak exposure (C<sub>max</sub>) and systemic exposure (AUC) were numerically higher in Japanese participants compared to Caucasian/non-Asian participants under fasted conditions. No formal statistical comparison was performed during the study.

#### *Analysis of Food Effect.*

[0364] Statistical analysis of Compound 1 plasma PK parameters were evaluated for food effects for Part 1 (TABLE 10) and Part 2 (TABLE 11). The log-transformed PK parameters were analyzed using an analysis of variance model with treatment (fed/fasted condition) and period as fixed effects and participant as a random effect.

TABLE 10

| Comparison<br>(Fed/Fasted) | Parameter<br>(Unit) | N | Geometric Mean |           | % Ratio<br>(Fed/Fasted) | 90%<br>Confidence<br>Interval<br>of Ratio | Intra-<br>subject<br>CV% |
|----------------------------|---------------------|---|----------------|-----------|-------------------------|-------------------------------------------|--------------------------|
|                            |                     |   | Fed            | Fasted    |                         |                                           |                          |
| Fed vs<br>Fasted           | AUC(h•ng/mL)        | 6 | 180315.79      | 149952.06 | 120.25                  | (100.89,<br>143.33)                       | 14.34                    |
|                            | AUC(h•ng/mL)        | 6 | 176887.42      | 147154.57 | 120.21                  | (101.26,<br>142.69)                       | 14.00                    |
|                            | C(ng/mL)            | 6 | 10585.20       | 8694.08   | 121.75                  | (105.55,<br>140.44)                       | 11.64                    |

TABLE 11

| Comparison<br>(Fed/Fasted) | Parameter<br>(Unit) | N  | Geometric Mean |           | % Ratio<br>(Fed/Fasted) | 90%<br>Confidence<br>Interval<br>of Ratio | Intra-<br>subject<br>CV% |
|----------------------------|---------------------|----|----------------|-----------|-------------------------|-------------------------------------------|--------------------------|
|                            |                     |    | Fed            | Fasted    |                         |                                           |                          |
| Fed vs<br>Fasted           | AUC(h•ng/mL)        | 18 | 464526.09      | 259891.02 | 178.74                  | (150.88,<br>211.74)                       | 29.74                    |
|                            | AUC(h•ng/mL)        | 20 | 448599.57      | 259397.05 | 172.94                  | (148.55,<br>201.33)                       | 28.26                    |
|                            | C(ng/mL)            | 20 | 19085.52       | 10382.97  | 183.82                  | (153.27,<br>220.46)                       | 34.08                    |

[0365] Data were also assessed in ladder plots of Compound 1 plasma PK parameters (AUC<sub>0-inf</sub>, AUC<sub>0-last</sub>, and C<sub>max</sub>) by fed/fasted condition (FIGs. 14-16) and by ethnicity and fed/fasted condition (FIGs. 17-19).

[0366] Compound 1 was rapidly absorbed following single oral doses administered under fasted conditions with median T<sub>max</sub> ranging from 1.54 to 2.51 hours (Part 1) and 1.03 to 5.94 hours (Part 2). Compound 1 plasma concentrations declined in a bi-exponential manner.

[0367] For Part 1, the geometric mean ratios for fed/fasted conditions were 120.25% (90% CI: 100.89, 143.33), 120.21% (90% CI: 101.26, 142.69), and 121.75% (90% CI: 105.55, 140.44) for AUC<sub>0-inf</sub>, AUC<sub>0-last</sub>, and C<sub>max</sub>, respectively (TABLE 10). For Part 2, the geometric mean ratios for fed/fasted conditions were 178.74% (90% CI: 150.88, 211.74), 172.94% (90% CI: 148.55, 201.33), 183.82% (90% CI: 153.27, 220.46) for AUC<sub>0-inf</sub>, AUC<sub>0-last</sub>, and C<sub>max</sub>, respectively (TABLE 11).

[0368] For Part 1, the administration of a single dose of 1000 mg of Compound 1 with food (high-fat meal) increased the peak exposure (C<sub>max</sub>) of Compound 1 by approximately 22% compared to the fasted state, and the systemic exposure (AUC) by approximately 20% in terms of both AUC<sub>0-inf</sub> and AUC<sub>0-last</sub>. A prolonged T<sub>max</sub> (3.25 hours vs 2.27 hours) was also observed under fed condition (high-fat meal).

[0369] For part 2, the administration of a single dose of 2000 mg of Compound 1 with food (high-fat meal) increased the peak exposure (C<sub>max</sub>) of Compound 1 by approximately 84% compared with the fasted state, and the systemic exposure (AUC) by 79% and 73% in terms of AUC<sub>0-inf</sub> and AUC<sub>0-last</sub>, respectively. A prolonged T<sub>max</sub> (4.03 hours vs 2.52 hours) was also observed under fed condition (high-fat meal).

#### *Analysis for the Effect of Ethnicity*

[0370] Between Caucasian/non-Asian and Japanese participants in Part 2 of the study, after administration of a single dose of 2000 mg of Compound 1 under fasted conditions, Compound 1 peak exposure (C<sub>max</sub>) and systemic exposure (AUC) were found to be numerically higher in Japanese participants compared to Caucasian/non-Asian participants.

[0371] After administration of a single dose of 2000 mg of Compound 1 under fasted condition, Compound 1 peak exposure (geometric mean) C<sub>max</sub> was 10300 ng/mL for Caucasian/non-Asian participants and 14700 ng/mL for Japanese participants, occurring at a median time of 2.52 and 3.94 hours, respectively. Geometric mean AUC<sub>0-inf</sub> was 259000 and 338000 h•ng/mL and geometric mean AUC<sub>0-last</sub> was 254000 and 326000 h•ng/mL for Caucasian/non-Asian and Japanese participants, respectively.

[0372] In addition, a slight delay in absorption was observed for Japanese participants, with a prolonged T<sub>max</sub> compared to Caucasian/non-Asian participants (3.94 hours vs 2.52 hours). Plasma CL/F and V<sub>z</sub>/F were numerically lower for Japanese participants compared to Caucasian/non-Asian participants.

*Pharmacokinetic (PK) Analysis – Urine Samples*

[0373] Urine for the analysis of study drug was also collected. Participants were asked to void at the end of each collection period to ensure complete collection of urine during each interval. The entire volume of urine voided during each interval was collected and recorded, and a small volume stored for analysis of study drug concentrations.

[0374] Various pharmacokinetic (PK) parameters were determined for each subject. (Figures 20-22). For example, amount of drug excreted unchanged in urine ( $A_e$ ), renal clearance ( $CL_R$ ), and fraction excreted in urine ( $f_e$ ).

[0375] Arithmetic mean ( $\pm$  SD) Compound 1 urinary cumulative excretion fraction (%) versus midpoint of collection interval timepoint grouped by fed/fasted condition was assessed in Part 1 of the study (FIG. 20), fed/fasted condition for Caucasian/non-Asian participants in Part 2 of the study (FIG. 21), and fasted condition in Japanese participants in Part 2 of the study (FIG. 22).

[0376] Following administration of single doses of Compound 1, less than 4% of the dose was excreted in urine as unchanged Compound 1 over a 96-hour interval, under fasted and fed conditions for all participants (Caucasian/non-Asian and Japanese participants).

[0377] Following administration of a single dose of 1000 mg Compound 1, 2.53% and 3.29% of the total dose administered was excreted in urine as unchanged Compound 1 over a 96-hour interval under fasted and fed conditions, respectively. Following administration of a single dose of 2000 mg Compound 1, 1.90% and 3.66% of the total dose administered was excreted in urine as Compound 1 over a 96-hour interval for Caucasian/non-Asian participants under fasted and fed conditions, respectively. In addition, 2.28% of the total dose administered was excreted in urine as unchanged Compound 1 over a 96-hour interval under fasted conditions for Japanese participants.

[0378] For Part 1, geometric mean of renal clearance ( $CL_R$ ) was 0.172 L/h and 0.186 L/h under fasted and fed conditions, respectively. In Part 2, geometric mean of  $CL_R$  was 0.152 L/h and 0.164 L/h under fasted and fed conditions, respectively for Caucasian/non-Asian participants. On the other hand, geometric mean  $CL_R$  was 0.140 L/h under fasted conditions for Japanese participants.

*Pharmacokinetic Parameter Conclusions.*

[0379] Compound 1 was rapidly absorbed following single oral doses administered under fasted conditions with median  $T_{max}$  ranging from 1.54 to 2.51 hours (Part 1) to 1.03 to 5.94 hours (Part 2). Compound 1 plasma concentrations declined roughly in a bi-exponential manner.

[0380] For Caucasian/non-Asian participants, the administration of a single dose of 1000 mg of Compound 1 with food (high-fat meal) increased the peak exposure ( $C_{max}$ ) of Compound 1 by approximately 22% compared to the fasted state, and the systemic exposure (AUC) by approximately 20% in terms of both AUC<sub>0-inf</sub> and AUC<sub>0-last</sub>. A prolonged  $T_{max}$  (3.25 hours vs 2.27 hours) was also observed under fed condition (high-fat meal). Administration of a single dose of 2000 mg Compound 1 with food (high-fat meal) increased the peak exposure of Compound 1 by approximately 84% compared with the fasted state, and the systemic exposure by 79% and 73% in terms of AUC<sub>0-inf</sub> and AUC<sub>0-last</sub>,

respectively. Furthermore, a prolonged  $T_{max}$  (4.03 hours vs 2.52 hours) was also observed under fed conditions (high-fat meal).

[0381] After administration of a single dose of 2000 mg of Compound 1 under fasted condition, Compound 1 peak exposure (geometric mean)  $C_{max}$  was 10300 ng/mL for Caucasian/non-Asian participants and 14700 ng/mL for Japanese participants, occurring at a median time of 2.52 and 3.94 hours, respectively. Geometric mean  $AUC_{0-inf}$  was 259000 and 338000 h•ng/mL and geometric mean  $AUC_{0-last}$  was 254000 and 326000 h•ng/mL for Caucasian/non-Asian and Japanese participants, respectively.

[0382] Following administration of single doses of Compound, less than 4% of the dose was excreted in urine as unchanged Compound 1 over a 96-hour interval, under fasted and fed conditions for all.

**[0383] EXAMPLE 5: Effect of Food on the Pharmacokinetic and Safety Profile of Compound 1 in Healthy Subjects and Patients with Solid Tumors.**

[0384] Physiologically based pharmacokinetic (PBPK) modeling and pharmacokinetic (PK) studies were conducted in healthy volunteers and patients with solid tumors in fasted and fed states to assess potential food effects on Compound 1 PK and safety.

[0385] **Description of Methods & Materials:** A PBPK model was developed using in vitro and clinical PK data to predict the effect of a standard meal on Compound 1 PKs in healthy volunteers and patients with solid tumors. The model was verified using PK data obtained following single and repeat doses of Compound 1 (150–2500 mg) in a study of patients with solid tumors harboring a TP53 Y220C mutation; most predictions fell within 1.5-fold of observed. A healthy volunteer study was also conducted in healthy volunteers receiving a single 2000 mg dose of Compound 1 (n=22) PO with or without a high-fat meal, with PK samples collected over 96h post-dose. Patients in the study of patients with advanced solid tumors harboring a TP53 Y220 mutation receiving Compound 1 (2000 mg, PO, once daily) starting in the fasted (n=13) or fed state (n=12;  $\geq 100$  kcal, 3 g fat) were also assessed, with PK samples collected over 24h post-dose on Day 1 and at steady state.

[0386] **Data & Results:** Using the PBPK model, 10 virtual trials of 10 healthy volunteers (50%/50% male/female; aged 20–50 years) and 10 virtual trials of 10 patients with solid tumors (50%/50% male/female; aged 30–75 years), receiving one dose of Compound 1 2000 mg PO under a fasted or fed state were generated. Food intake in healthy volunteers was predicted to increase area under the curve (AUC) from 0–infinity ( $AUC_{0-inf}$ ) by 45% and maximum concentration ( $C_{max}$ ) by 28%. For patients with solid tumors, food intake was predicted to increase  $AUC_{0-inf}$  by 47% and  $C_{max}$  by 35%. Exposure predictions for multiple oral doses showed similar results.

[0387] In the healthy volunteer study, healthy volunteers were administered a single dose of Compound 1 2000 mg PO with or without a high-fat meal.  $AUC_{0-inf}$  and  $C_{max}$  were 79% and 84% higher, respectively, in the fed versus fasted state.

[0388] In the study of patients with advanced solid tumors harboring a TP53 Y220 mutation, AUC from 0–24h (AUC<sub>0-24</sub>) and C<sub>max</sub> were 40% and 42% higher (at steady state), respectively, in patients with solid tumors administered Compound 1 2000 mg PO with versus without food (TABLE 12).

[0389] In the study of patients with advanced solid tumors harboring a TP53 Y220 mutation, rates of treatment-related adverse events (TRAEs) were similar for Compound 1 2000 mg administered with versus without food; gastrointestinal (GI) TRAEs were less frequent with food (TABLE 12).

TABLE 12

|                                                                   | Compound 1 2000 mg QD PO      |                              |
|-------------------------------------------------------------------|-------------------------------|------------------------------|
|                                                                   | Fasted                        | Fed                          |
| <b>PK analysis</b>                                                |                               |                              |
| <b>GeoMean AUC<sub>0-24</sub> at steady state, h·ng/mL (gCV%)</b> | <b>n=12</b><br>245,956 (59.2) | <b>n=6</b><br>343,830 (28.7) |
| <b>GeoMean C<sub>max</sub> at steady state, ng/mL (gCV%)</b>      | <b>n=13</b><br>14,963 (55.4)  | <b>n=6</b><br>21,268 (27.1)  |
| <b>GI TRAEs, n (%)</b>                                            | <b>n=13</b>                   | <b>n=16</b>                  |
| <b>Nausea</b>                                                     | 9 (69.2)                      | 3 (18.8)                     |
| <b>Vomiting</b>                                                   | 8 (61.5)                      | 5 (31.2)                     |
| <b>Diarrhea</b>                                                   | 4 (30.8)                      | 1 (6.2)                      |

#### EXAMPLE 6: Analysis of Patient Subgroup with Advanced Breast Cancer

[0390] Patients with locally advanced or metastatic breast cancer with a TP53 Y220C mutation were eligible to receive Compound 1 orally on a continuous schedule across the efficacious dose range (1150 mg once daily [QD] to 1500 mg twice daily). Safety and preliminary efficacy (per investigator assessed RECIST v1.1) were assessed. Local next generation sequencing was used to determine *TP53* Y220C, *BRCA*, *PIK3CA* and *KRAS* tumor mutation status.

[0391] Nine patients with breast cancer (HR+/HER2- n=3; HR+/HER2+ n=1; HR-/HER2+ n=1; TNBC n=3; unknown n=1) received Compound 1 in the Phase 1 trial. Median age was 53 years (range 32–65 years); ECOG performance score was 0 (n=3) or 1 (n=6). Two patients had a somatic BRCA2 mutation, no patient had a confirmed BRCA1 mutation (two patient had unknown BRCA1 status; one had unknown BRCA2 status), 2 patients had a PIK3CA mutation and all patients were KRAS WT. The median number of prior lines of systemic therapy was four (range: 2–9). Of the eight patients with measurable disease at baseline, three achieved a confirmed partial response

(PR; overall response rate 37.5%), four had stable disease (SD), and one had progressive disease (PD) as best objective response. Reductions in target lesions were reported in all patients, with a maximum change in tumor volume from baseline ranging from -2.4% to -55.2%. One patient (51 years of age) with TNBC had a PR at 6 weeks (41% reduction in axilla lesion) confirmed at 12 weeks and ongoing; improvement in symptoms (arm swelling, mobility in arm and fingers) was seen in the first week of treatment. In the overall Phase 1 population (N=67 pts with solid tumors), the most frequent treatment-related adverse events (TRAEs) were nausea (51%), vomiting (43%), and

increased blood creatinine (27%) which were mostly grade 1/2. In the overall population, administration of Compound 1 with food led to an improvement in gastrointestinal (GI) adverse events including nausea and vomiting.

## EMBODIMENTS

[0392] The following non-limiting embodiments provide illustrative examples of the invention, but do not limit the scope of the invention.

[0393] Embodiment 1. A method of treating a condition in a subject in need thereof, the method comprising administering to the subject a therapeutically-effective amount of a small molecule compound that binds a p53 mutant protein, wherein a mutational status of a KRAS gene in the subject has been previously determined.

[0394] Embodiment 2. The method of embodiment 1, wherein the mutational status of the KRAS gene in the subject has previously been determined based on an assay performed on a biological sample of the subject.

[0395] Embodiment 3. The method of embodiment 1 or 2, wherein the subject has been previously determined to have a mutation in a TP53 gene that encodes a p53 mutant in the subject.

[0396] Embodiment 4. A method of treating a condition in a subject in need thereof, the method comprising: a) based on a result of an assay performed on a biological sample of the subject, determining a mutational status of a KRAS gene in the subject; and b) based at least in part on the mutational status of the KRAS gene in the subject, administering to the subject a therapeutically-effective amount of a compound that binds a p53 mutant protein.

[0397] Embodiment 5. The method of embodiment 4, wherein the method further comprises, based on the result of an assay, determining that the subject harbors a mutation in a TP53 gene that encodes a p53 mutant protein, wherein the administering to the subject the therapeutically-effective amount of the compound that binds the p53 mutant protein is based at least in part on the determining that the subject harbors the mutation in the TP53 gene that encodes the p53 mutant protein.

[0398] Embodiment 6. The method of any one of embodiments 2, 4, and 5, further comprising performing the assay on the biological sample of the subject.

[0399] Embodiment 7. The method of embodiment 5, further comprising performing the assay on the biological sample of the subject, wherein performing the assay on the biological sample of the subject further comprises detecting the mutation in the TP53 gene that encodes the p53 mutant in the subject.

[0400] Embodiment 7A. The method of any one of embodiments 1-7, wherein the gene is a KRAS gene.

[0401] Embodiment 8. The method of embodiment 7A, wherein the mutational status of the KRAS gene in the subject comprises an absence of a missense mutation at any of codons 12, 13, 59, 61, 117, and 146.

[0402] Embodiment 9. The method of embodiment 7A or 8, wherein the mutational status of the KRAS gene in the subject comprises absence of a mutation that would encode an amino acid other than glycine at codon 12.



[0403] Embodiment 10. The method of embodiment 7A, 8, or 9 wherein the mutational status of the KRAS gene in the subject comprises absence of a mutation that would encode an amino acid other than glycine at codon 13.

[0404] Embodiment 11. The method of any one of embodiments 7A and 9-10, wherein the mutational status of the KRAS gene in the subject comprises absence of a mutation that would encode an amino acid other than alanine at codon 59.

[0405] Embodiment 12. The method of any one of embodiments 7A and 9-11, wherein the mutational status of the KRAS gene in the subject comprises absence of a mutation that would encode an amino acid other than glutamine at codon 61.

[0406] Embodiment 13. The method of any one of embodiments 7A and 9-12, wherein the mutational status of the KRAS gene in the subject comprises absence of a mutation that would encode an amino acid other than lysine at codon 117.

[0407] Embodiment 14. The method of any one of embodiments 7A and 9-13, wherein the mutational status of the KRAS gene in the subject comprises absence of a mutation that would encode an amino acid other than alanine at codon 146.

[0408] Embodiment 15. The method of any one of embodiments 7A and 9-14, wherein the mutational status of the KRAS gene in the subject is a KRAS gene that encodes a wild type K-ras protein.

[0409] Embodiment 16. The method of embodiment 15, wherein the wild type K-Ras protein has a sequence according to SEQ ID NO: 1 or SEQ ID NO: 2.

[0410] Embodiment 17. The method of any one of embodiments 7A and 9-16, wherein the mutational status of the KRAS gene in the subject is a copy number variation status of the KRAS gene.

[0411] Embodiment 18. The method of embodiment 17, wherein the mutational status of the KRAS gene in the subject is absence of a KRAS copy number variation.

[0412] Embodiment A1. The method of any one of embodiments 1-7, wherein the gene is BRCA1.

[0413] Embodiment A2. The method of embodiment A1, wherein the mutational status of the BRCA1 gene in the subject is a BRCA1 gene that encodes a wild type breast cancer type 2 susceptibility protein.

[0414] Embodiment A3. The method of embodiment A1 or A2, wherein the mutational status of the BRCA1 gene in the subject is a BRCA1 gene that does not encode an oncogenic mutation.

[0415] Embodiment A4. The method of any one of embodiments A1-A3, wherein the mutational status of the BRCA1 gene in the subject is absence of a copy number variation status of the BRCA1 gene.

[0416] Embodiment A5. The method of any one of embodiments 1-7, wherein the gene is BRCA2.

[0417] Embodiment A6. The method of embodiment A5, wherein the mutational status of the BRCA2 gene in the subject is a BRCA2 gene that encodes a wild type breast cancer type 2 susceptibility protein.

[0418] Embodiment A7. The method of embodiment A5 or A6, wherein the mutational status of the BRCA2 gene in the subject is a BRCA2 gene that does not encode an oncogenic mutation.

- [0419] Embodiment A8. The method of any one of embodiments A5-A7, wherein the mutational status of the BRCA2 gene in the subject is absence of a copy number variation status of the BRCA2 gene.
- [0420] Embodiment A9. The method of any one of embodiments 1-7, wherein the gene is PIK3CA.
- [0421] Embodiment A10. The method of embodiment A9, wherein the mutational status of the PIK3CA gene in the subject is a PIK3CA gene that does not encode an oncogenic mutation.
- [0422] Embodiment A11. The method of embodiment A9 or A10, wherein the mutational status of the PIK3CA gene in the subject comprises an absence of a missense mutation at any of codons 542, 545, 1047, and 1047.
- [0423] Embodiment A12. The method of any one of embodiments A9-A11, wherein the mutational status of the PIK3CA gene in the subject comprises absence of a mutation that would encode an amino acid other than glutamic acid at codon 542.
- [0424] Embodiment A13. The method of any one of embodiments A9-A12, wherein the mutational status of the PIK3CA gene in the subject comprises absence of a mutation that would encode an amino acid other than glutamic acid at codon 545.
- [0425] Embodiment A14. The method of any one of embodiments A9-A13, wherein the mutational status of the PIK3CA gene in the subject comprises absence of a mutation that would encode an amino acid other than histidine at codon 1047.
- [0426] Embodiment A15. The method of any one of embodiments A9-A14, wherein the mutational status of the PIK3CA gene in the subject comprises absence of a mutation that would encode an amino acid other than lysine at codon 117.
- [0427] Embodiment A16. The method of any one of embodiments A9-A15, wherein the mutational status of the PIK3CA gene in the subject is a PIK3CA gene that encodes a wild type p110 $\alpha$  protein.
- [0428] Embodiment A17. The method of any one of embodiments A9-A16, wherein the mutational status of the PIK3CA gene in the subject is absence of a copy number variation status of the PIK3CA gene.
- [0429] Embodiment 19. The method of any one of embodiments 9-17 and A1-A17, wherein the compound increases an ability of the p53 mutant to bind DNA.
- [0430] Embodiment 20. The method of any one of embodiments 9-18 and A1-A17, wherein the compound induces a conformational change in the p53 mutant.
- [0431] Embodiment 21. The method of any one of embodiments 3-7 and A1-A17, wherein the compound selectively binds the p53 mutant as compared to a wild type p53.
- [0432] Embodiment 22. The method of any one of embodiments 3, 5, 7, and A1-A17, wherein the mutation in the TP53 gene deactivates a p53 pathway in the subject.
- [0433] Embodiment 23. The method of any one of embodiments 3, 5, 7, 22, and A1-A17 wherein the mutation in the TP53 gene is c.659A>G.
- [0434] Embodiment 24. The method of any one of embodiments 3, 5, 7, 22, 23, and A1-A17, wherein the p53 mutant has a sequence according to SEQ ID NO: 3.

- [0435] Embodiment 25. The method of any one of embodiments 3, 5, 7, 22-24, and A1-A17, wherein the mutation in the TP53 gene is at amino acid 220.
- [0436] Embodiment 26. The method of any one of embodiments 3, 5, 7, 22-25, and A1-A17, wherein the TP53 gene contains a single nucleotide polymorphism.
- [0437] Embodiment 27. The method of any one of embodiments 1-26 and A1-A17, wherein the p53 mutant is a p53 Y220C.
- [0438] Embodiment 28. The method of any one of embodiments 1-27 and A1-A17, wherein the p53 mutant has a reduced ability to bind to DNA in the subject as compared to wild-type p53.
- [0439] Embodiment 29. The method of any one of embodiments 1-28 and A1-A17, wherein the therapeutically effective amount is from about 50 mg to about 3000 mg.
- [0440] Embodiment 30. The method of any one of embodiments 1-29 and A1-A17, wherein the administering is once daily.
- [0441] Embodiment 31. The method of any one of embodiments 2, 4, 5-7 and A1-A17, wherein the assay is DNA sequencing.
- [0442] Embodiment 32. The method of any one of embodiments 2, 4, 5-7, and A1-A17, wherein the assay is next-generation DNA sequencing.
- [0443] Embodiment 33. The method of any one of embodiments 2, 4, 5-7, and A1-A17, wherein the assay is RNA sequencing.
- [0444] Embodiment 34. The method of any one of embodiments 2, 4, 5-7, and A1-A17, wherein the assay is a real time polymerase chain reaction assay.
- [0445] Embodiment 35. The method of any one of embodiments 2, 4, 5-7, 31-34, and A1-A17, wherein the biological sample is a liquid biopsy.
- [0446] Embodiment 36. The method of any one of embodiments 2, 4, 5-7, 31-34, and A1-A17, wherein the biological sample is a blood sample.
- [0447] Embodiment 37. The method of any one of embodiments 2, 4, 5-7, 31-34, and A1-A17 wherein the biological sample is circulating tumor DNA.
- [0448] Embodiment 38. The method of any one of embodiments 2, 4, 5-7, 31-34, and A1-A17, wherein the biological sample is cell free DNA.
- [0449] Embodiment 39. The method of any one of embodiments 2, 4, 5-7, 31-34, and A1-A17, wherein the biological sample is a solid tumor sample.
- [0450] Embodiment 40. The method of any one of embodiments 1-39 and A1-A17, wherein the condition is a cancer.
- [0451] Embodiment 41. The method of any one of embodiments 1-40 and A1-A17, wherein the condition is a cancer, wherein the cancer expresses the p53 mutant.
- [0452] Embodiment 42. The method of any one of embodiments 1-41 and A1-A17, wherein the condition is ovarian cancer.
- [0453] Embodiment 43. The method of any one of embodiments 1-41 and A1-A17, wherein the condition is breast cancer.

[0454] Embodiment 43A. The method of embodiment 43, wherein the breast cancer is triple negative breast cancer.

[0455] Embodiment 44. The method of any one of embodiments 1-41 and A1-A17, wherein the condition is lung cancer.

[0456] Embodiment 44A. The method of any one of embodiments 40-44 and 43A, wherein the cancer is HRD-negative.

[0457] Embodiment 45. The method of any one of embodiments 1-44, 43A, 44A, and A1-A17, wherein the administering is oral.

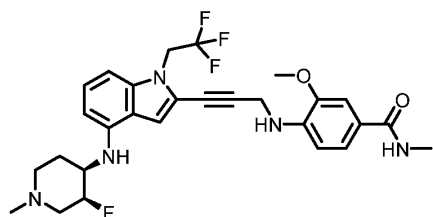
[0458] Embodiment 46. The method of any one of embodiments 1-44, 43A, 44A, and A1-A17, wherein the administering is intravenous.

[0459] Embodiment 47. The method of any one of embodiments 1-44, 43A, 44A, and A1-A17, wherein the administering is subcutaneous.

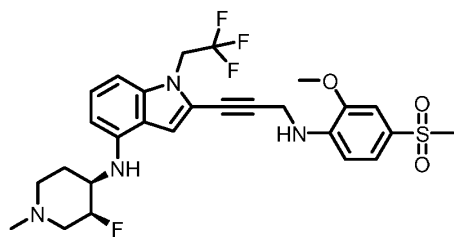
[0460] Embodiment 48. The method of any one of embodiments 1-44, 43A, 44A, and A1-A17, wherein the administering is topical.

[0461] Embodiment 49. The method of any one of embodiments 1-48, 43A, 44A, and A1-A17, wherein the subject is human.

[0462] Embodiment 50. The method of any one of embodiments 1-79, 43A, 44A, and A1-A17, wherein the compound is:



[0463] Embodiment 51. The method of any one of embodiments 1-79, 43A, 44A, and A1-A17, wherein the compound is:



[0464] Embodiment 52. A method of treating cancer in a subject in need thereof, the method comprising:

- (a) administering to the subject a therapeutically-effective first course of a compound that binds a p53 mutant;
- (b) after (a), determining that the subject exhibits a gastrointestinal adverse event; and

- (c) administering a therapeutically-effective second course of the compound to the subject with food based at least in part on the determining that the subject exhibited the gastrointestinal adverse event.

[0465] Embodiment 53. The method of embodiment 52, wherein the compound increases an ability of the p53 mutant to bind DNA.

[0466] Embodiment 84. The method of embodiment 52 or 53, wherein the compound induces a conformational change in the p53 mutant.

[0467] Embodiment 55. The method of any one of embodiments 52-54, wherein the compound selectively binds the p53 mutant as compared to a wild type p53.

[0468] Embodiment 56. The method of any one of embodiments 52-55, wherein the compound is a compound of any one of embodiments 50-81.

[0469] Embodiment 57. The method of any one of embodiments 52-56, wherein the cancer expresses the p53 mutant.

[0470] Embodiment 58. The method of any one of embodiments 52-57, wherein the p53 mutant is p53 Y220C.

[0471] Embodiment 59. The method of any one of embodiments 52-58, wherein the cancer is ovarian cancer.

[0472] Embodiment 60. The method of any one of embodiments 52-58, wherein the cancer is breast cancer.

[0473] Embodiment 61. The method of any one of embodiments 52-58, wherein the cancer is lung cancer.

[0474] Embodiment 62. The method of any one of embodiments 52-61, wherein the administering of the first course is orally and the administering of the second course is orally.

[0475] Embodiment 63. The method of any one of embodiments 52-62, wherein the gastrointestinal adverse event comprises nausea.

[0476] Embodiment 64. The method of any one of embodiments 52-62, wherein the gastrointestinal adverse event comprises diarrhea.

[0477] Embodiment 65. The method of any one of embodiments 52-62, wherein the gastrointestinal adverse event comprises vomiting.

[0478] Embodiment 66. The method of any one of embodiments 52-65, wherein the gastrointestinal adverse event is self-reported by the subject.

[0479] Embodiment 67. The method of any one of embodiments 52-66, wherein the gastrointestinal adverse event is observed by a health practitioner.

[0480] Embodiment 68. The method of any one of embodiments 52-67, wherein the administering the second course of the compound to the subject with food comprises feeding the subject prior to providing the compound to the subject.

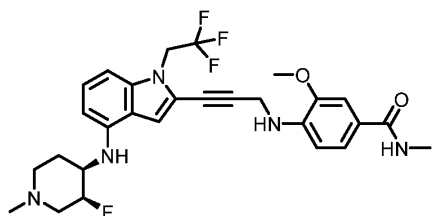
- [0481] Embodiment 69. The method of any one of embodiments 52-68, wherein the administering the second course of the compound to the subject with food comprises feeding the subject within about 5 minutes prior to providing the compound to the subject.
- [0482] Embodiment 70. The method of any one of embodiments 52-67, wherein the administering the second course of the compound to the subject with food comprises feeding the subject after providing the compound to the subject.
- [0483] Embodiment 71. The method of any one of embodiments 52-67 and 70, wherein the administering the second course of the compound to the subject with food comprises feeding the subject within about 60 minutes after providing the compound to the subject.
- [0484] Embodiment 72. The method of any one of embodiments 52-67 and 70, wherein the administering the second course of the compound to the subject with food comprises feeding the subject within about 30 minutes after providing the compound to the subject.
- [0485] Embodiment 73. The method of any one of embodiments 52-67 and 70, wherein the administering the second course of the compound to the subject with food comprises feeding the subject within about 15 minutes after providing the compound to the subject.
- [0486] Embodiment 74. The method of any one of embodiments 52-67 and 70, wherein the administering the second course of the compound to the subject with food comprises feeding the subject within about 5 minutes after providing the compound to the subject.
- [0487] Embodiment 75. The method of any one of embodiments 52-74, wherein the food is a high-fat food.
- [0488] Embodiment 76. The method of any one of embodiments 52-75, wherein the food comprises a fat content of at least 50% of total caloric content.
- [0489] Embodiment 77. The method of any one of embodiments 52-76, wherein the food comprises a fat content of at least 500 Kcal from fat.
- [0490] Embodiment 78. The method of any one of embodiments 52-74, wherein the food is a medium-fat food.
- [0491] Embodiment 79. The method of any one of embodiments 52-74 and 78, wherein the food comprises a fat content of about 25% to about 50% of total caloric content.
- [0492] Embodiment 80. The method of any one of embodiments 52-74 and 78-79, wherein the food comprises a fat content about 125 Kcal to about 500 Kcal from fat.
- [0493] Embodiment 81. The method of any one of embodiments 52-80, wherein the food is a high-calorie food.
- [0494] Embodiment 82. The method of any one of embodiments 52-81, wherein the food comprises a calorie content of at least 800 calories.
- [0495] Embodiment 83. The method of any one of embodiments 52-82, wherein the first course comprises doses of about 50 mg to about 3000 mg of the compound, and the second course comprises doses of about 50 mg to about 3000 mg of the compound.

- [0496] Embodiment 84. The method of any one of embodiments 52-82, wherein the first course comprises doses of about 1000 mg of the compound, and the second course comprises doses of about 1000 mg of the compound.
- [0497] Embodiment 85. The method of any one of embodiments 52-82, wherein the first course comprises doses of about 2000 mg of the compound, and the second course comprises doses of about 2000 mg of the compound.
- [0498] Embodiment 86. The method of any one of embodiments 52-82, wherein the first course comprises doses of about 3000 mg of the compound, and the second course comprises doses of about 3000 mg of the compound.
- [0499] Embodiment 87. A method of treating cancer, the method comprising:  
identifying a subject that: (i) has been treated with a therapeutically-effective first course of a compound that binds a p53 mutant, and (ii) has exhibited a gastrointestinal adverse event after treatment with the first course of the compound;  
recommending that the compound is administered to the subject with food, wherein the recommendation is based on at least the basis that the subject has exhibited the gastrointestinal adverse event; and  
administering a therapeutically-effective second course of the compound to the subject with food in accordance with the recommendation.
- [0500] Embodiment 88. The method of embodiment 87, wherein the compound increases an ability of the p53 mutant to bind DNA.
- [0501] Embodiment 89. The method of any one of embodiments 87-88, wherein the compound induces a conformational change in the p53 mutant.
- [0502] Embodiment 90. The method of any one of embodiments 87-89, wherein the compound selectively binds the p53 mutant as compared to a wild type p53.
- [0503] Embodiment 91. The method of any one of embodiments 87-90, wherein the compound is a compound of any one of embodiments 50-81.
- [0504] Embodiment 92. The method of any one of embodiments 87-91, wherein the cancer expresses the p53 mutant.
- [0505] Embodiment 93. The method of any one of embodiments 87-92, wherein the p53 mutant is p53 Y220C.
- [0506] Embodiment 94. The method of any one of embodiments 87-93, wherein the cancer is ovarian cancer.
- [0507] Embodiment 95. The method of any one of embodiments 87-93, wherein the cancer is breast cancer.
- [0508] Embodiment 96. The method of any one of embodiments 87-93, wherein the cancer is lung cancer.
- [0509] Embodiment 97. The method of any one of embodiments 87-96, wherein the administering of the first course is orally and the administering of the second course is orally.

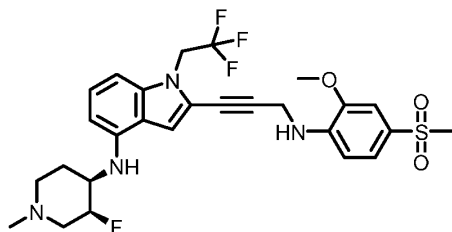
- [0510] Embodiment 98. The method of any one of embodiments 87-97, wherein the gastrointestinal adverse event comprises nausea.
- [0511] Embodiment 99. The method of any one of embodiments 87-97, wherein the gastrointestinal adverse event comprises diarrhea.
- [0512] Embodiment 100. The method of any one of embodiments 87-97, wherein the gastrointestinal adverse event comprises vomiting.
- [0513] Embodiment 101. The method of any one of embodiments 87-100, wherein the gastrointestinal adverse event is self-reported by the subject.
- [0514] Embodiment 102. The method of any one of embodiments 87-101, wherein the gastrointestinal adverse event is observed by a health practitioner.
- [0515] Embodiment 103. The method of any one of embodiments 87-102, wherein the administering the second course of the compound to the subject with food comprises feeding the subject prior to providing the compound to the subject.
- [0516] Embodiment 104. The method of any one of embodiments 87-103, wherein the administering the second course of the compound to the subject with food comprises feeding the subject within about 5 minutes prior to providing the compound to the subject.
- [0517] Embodiment 105. The method of any one of embodiments 87-102, wherein the administering the second course of the compound to the subject with food comprises feeding the subject after providing the compound to the subject.
- [0518] Embodiment 106. The method of any one of embodiments 87-102 and 105, wherein the administering the second course of the compound to the subject with food comprises feeding the subject within about 60 minutes after providing the compound to the subject.
- [0519] Embodiment 107. The method of any one of embodiments 87-102 and 105, wherein the administering the second course of the compound to the subject with food comprises feeding the subject within about 30 minutes after providing the compound to the subject.
- [0520] Embodiment 108. The method of any one of embodiments 87-102 and 105, wherein the administering the second course of the compound to the subject with food comprises feeding the subject within about 15 minutes after providing the compound to the subject.
- [0521] Embodiment 109. The method of any one of embodiments 87-102 and 105, wherein the administering the second course of the compound to the subject with food comprises feeding the subject within about 5 minutes after providing the compound to the subject.
- [0522] Embodiment 110. The method of any one of embodiments 87-109, wherein the food is a high-fat food.
- [0523] Embodiment 111. The method of any one of embodiments 87-110, wherein the food comprises a fat content of at least 50% of total caloric content.
- [0524] Embodiment 112. The method of any one of embodiments 87-111, wherein the food comprises a fat content of at least 500 Kcal from fat.



- [0525] Embodiment 113. The method of any one of embodiments 87-109, wherein the food is a medium-fat food.
- [0526] Embodiment 114. The method of any one of embodiments 87-109 and 113, wherein the food comprises a fat content of about 25% to about 50% of total caloric content.
- [0527] Embodiment 115. The method of any one of embodiments 87-109 and 113-114, wherein the food comprises a fat content about 125 Kcal to about 500 Kcal from fat.
- [0528] Embodiment 116. The method of any one of embodiments 87-115, wherein the food is a high-calorie food.
- [0529] Embodiment 117. The method of any one of embodiments 87-116, wherein the food comprises a calorie content of at least 800 calories.
- [0530] Embodiment 118. The method of any one of embodiments 87-117, wherein the first course comprises doses of about 50 mg to about 3000 mg of the compound, and the second course comprises doses of about 50 mg to about 3000 mg of the compound.
- [0531] Embodiment 119. The method of any one of embodiments 87-117, wherein the first course comprises doses of about 1000 mg of the compound, and the second course comprises doses of about 1000 mg of the compound.
- [0532] Embodiment 120. The method of any one of embodiments 87-117, wherein the first course comprises doses of about 2000 mg of the compound, and the second course comprises doses of about 2000 mg of the compound.
- [0533] Embodiment 121. The method of any one of embodiments 87-117, wherein the first course comprises doses of about 3000 mg of the compound, and the second course comprises doses of about 3000 mg of the compound.
- [0534] Embodiment 122. The method of any one of embodiments 52-121, wherein the compound is:



- [0535] Embodiment 123. The method of any one of embodiments 52-121, wherein the compound is:



## CLAIMS

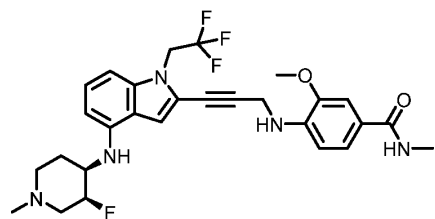
## WHAT IS CLAIMED IS:

1. A method of treating a condition in a subject in need thereof, the method comprising administering to the subject a therapeutically-effective amount of a small molecule compound that binds a p53 mutant protein, wherein a mutational status of a KRAS gene in the subject has been previously determined.
2. The method of claim 1, wherein the mutational status of the KRAS gene in the subject has previously been determined based on an assay performed on a biological sample of the subject.
3. The method of claim 1 or claim 2, wherein the subject has been previously determined to have a mutation in a TP53 gene that encodes a p53 mutant in the subject.
4. A method of treating a condition in a subject in need thereof, the method comprising: a) based on a result of an assay performed on a biological sample of the subject, determining a mutational status of a KRAS gene in the subject; and b) based at least in part on the mutational status of the KRAS gene in the subject, administering to the subject a therapeutically-effective amount of a compound that binds a p53 mutant protein.
5. The method of claim 4, wherein the method further comprises, based on the result of an assay, determining that the subject harbors a mutation in a TP53 gene that encodes a p53 mutant protein, wherein the administering to the subject the therapeutically-effective amount of the compound that binds the p53 mutant protein is based at least in part on the determining that the subject harbors the mutation in the TP53 gene that encodes the p53 mutant protein.
6. The method of any one of claims 2, 4, and 5, further comprising performing the assay on the biological sample of the subject.
7. The method of claim 5, further comprising performing the assay on the biological sample of the subject, wherein performing the assay on the biological sample of the subject further comprises detecting the mutation in the TP53 gene that encodes the p53 mutant in the subject.

8. The method of any one of claims 1-7, wherein the mutational status of the KRAS gene in the subject comprises an absence of a missense mutation at any of codons 12, 13, 59, 61, 117, and 146.
9. The method of any one of claims 1-8, wherein the mutational status of the KRAS gene in the subject comprises absence of a mutation that would encode an amino acid other than glycine at codon 12.
10. The method of any one of claims 1-9, wherein the mutational status of the KRAS gene in the subject comprises absence of a mutation that would encode an amino acid other than glycine at codon 13.
11. The method of any one of claims 1-10, wherein the mutational status of the KRAS gene in the subject comprises absence of a mutation that would encode an amino acid other than alanine at codon 59.
12. The method of any one of claims 1-11, wherein the mutational status of the KRAS gene in the subject comprises absence of a mutation that would encode an amino acid other than glutamine at codon 61.
13. The method of any one of claims 1-12, wherein the mutational status of the KRAS gene in the subject comprises absence of a mutation that would encode an amino acid other than lysine at codon 117.
14. The method of any one of claims 1-13, wherein the mutational status of the KRAS gene in the subject comprises absence of a mutation that would encode an amino acid other than alanine at codon 146.
15. The method of any one of claims 1-14, wherein the mutational status of the KRAS gene in the subject is a KRAS gene that encodes a wild type K-ras protein.
16. The method of claim 15, wherein the wild type K-Ras protein has a sequence according to SEQ ID NO: 1 or SEQ ID NO: 2.
17. The method of any one of claims 1-16, wherein the mutational status of the KRAS gene in the subject is a copy number variation status of the KRAS gene.
18. The method of claim 17, wherein the mutational status of the KRAS gene in the subject is absence of a KRAS copy number variation.

19. The method of any one of claims 1-17, wherein the compound increases an ability of the p53 mutant to bind DNA.
20. The method of any one of claims 1-18, wherein the compound induces a conformational change in the p53 mutant.
21. The method of any one of claims 3-7, wherein the compound selectively binds the p53 mutant as compared to a wild type p53.
22. The method of any one of claims 3, 5, and 7, wherein the mutation in the TP53 gene deactivates a p53 pathway in the subject.
23. The method of any one of claims 3, 5, 7, and 22, wherein the mutation in the TP53 gene is c.659A>G.
24. The method of any one of claims 3, 5, 7, 22, and 23, wherein the p53 mutant has a sequence according to SEQ ID NO: 3.
25. The method of any one of claims 3, 5, 7, and 22-24, wherein the mutation in the TP53 gene is at amino acid 220.
26. The method of any one of claims 3, 5, 7, and 22-25, wherein the TP53 gene contains a single nucleotide polymorphism.
27. The method of any one of claims 1-26, wherein the p53 mutant is a p53 Y220C.
28. The method of any one of claims 1-27, wherein the p53 mutant has a reduced ability to bind to DNA in the subject as compared to wild-type p53.
29. The method of any one of claims 1-28, wherein the therapeutically effective amount is from about 50 mg to about 3000 mg.
30. The method of any one of claims 1-29, wherein the administering is once daily.
31. The method of any one of claims 2, 4, and 5-7, wherein the assay is DNA sequencing.
32. The method of any one of claims 2, 4, and 5-7, wherein the assay is next-generation DNA sequencing.
33. The method of any one of claims 2, 4, and 5-7, wherein the assay is RNA sequencing.

34. The method of any one of claims 2, 4, and 5-7, wherein the assay is a real time polymerase chain reaction assay.
35. The method of any one of claims 2, 4, 5-7, and 31-34, wherein the biological sample is a liquid biopsy.
36. The method of any one of claims 2, 4, 5-7, and 31-34, wherein the biological sample is a blood sample.
37. The method of any one of claims 2, 4, 5-7, and 31-34, wherein the biological sample is circulating tumor DNA.
38. The method of any one of claims 2, 4, 5-7, and 31-34, wherein the biological sample is cell free DNA.
39. The method of any one of claims 2, 4, 5-7, and 31-34, wherein the biological sample is a solid tumor sample.
40. The method of any one of claims 1-39, wherein the condition is a cancer.
41. The method of any one of claims 1-40, wherein the condition is a cancer, wherein the cancer expresses the p53 mutant.
42. The method of any one of claims 1-41, wherein the condition is ovarian cancer.
43. The method of any one of claims 1-41, wherein the condition is breast cancer.
44. The method of any one of claims 1-41, wherein the condition is lung cancer.
45. The method of any one of claims 1-44, wherein the administering is oral.
46. The method of any one of claims 1-44, wherein the administering is intravenous.
47. The method of any one of claims 1-44, wherein the administering is subcutaneous.
48. The method of any one of claims 1-44, wherein the administering is topical.
49. The method of any one of claims 1-48, wherein the subject is human.
50. The method of any one of claims 1-49, wherein the compound is:



51. The method of any one of claims 1-49, wherein the compound is:

