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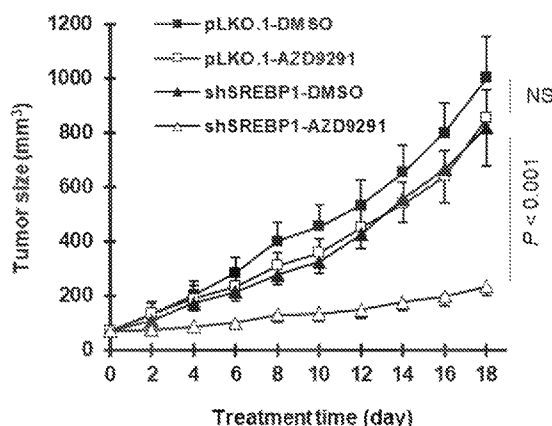


FIG. 1

(57) Abstract: This disclosure relates to combination therapies of epidermal growth factor receptor (EGFR) inhibitors and sterol regulatory element-binding protein (SREBP1) inhibitors for uses in cancer therapies such as lung cancer treatment. In certain embodiments, this disclosure relates to methods of treating cancer comprising administering an effective amount of EGFR inhibitor in combination with a SREBP1 inhibitor to a subject in need thereof. In certain embodiments, the combination has a synergistic effect compared administration of the inhibitors individually.



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**COMBINATIONS OF EPIDERMAL GROWTH FACTOR RECEPTOR INHIBITORS
AND STEROL REGULATORY ELEMENT-BINDING PROTEIN INHIBITORS FOR
USES IN CANCER THERAPIES**

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 63/121,201 filed December 3, 2020. The entirety of this application is hereby incorporated by reference for all purposes.

**STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR
DEVELOPMENT**

This invention was made with government support under CA233259, CA217691, CA223220, and CA245386 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND

Death due to lung cancer is a major public health issue. Non-small cell lung cancer (NSCLC) is the predominant form of the lung cancer, which is typically accompanied by mutations in the epidermal growth factor receptor (EGFR) tyrosine kinase domain encoded by exons 18-24. Exon 19 deletions, e.g., delE746_A750, delS752_I759) and an exon 21 L858R substitution are examples. Therapies with EGFR inhibitors provide extended survival; however, most patients eventually acquire resistance. For example, many patients develop acquired resistance due to a secondary T790M mutation in exon 20 of the EGFR gene. See Stewart et al., Transl Lung Cancer Res. 2015, 4(1): 67-81. Osimertinib is a kinase inhibitor indicated for the treatment of patients with metastatic epidermal growth factor receptor (EGFR) T790M mutation-positive non-small cell lung cancer (NSCLC) who have progressed on or after EGFR tyrosine kinase inhibitor therapy. See Soria et al., N Engl J Med, 2018, 378:113-25. However, some patients still develop resistance. Thus, there is a need to identify improved therapies.

Sterol regulatory element binding proteins (SREBPs) are transcriptional factors that control lipogenesis. There are two SREBP genes in mammals, SREBP-1 and SREBP-2. The SREBP-1 gene transcribes two isoforms SREBP-1a and SREBP-1c encoded from different promoters, which

regulate genes that control fatty acid synthesis. SREBPs are located in the endoplasmic reticulum (ER) membrane in association with SREBPs cleavage-activating protein (SCAP) in which they are retained by insulin-induced gene (Insig) when cellular sterol levels are sufficient. Once sterol levels decrease, SCAP protein dissociates with the Insig protein and escorts SREBPs to the golgi, where they are sequentially cleaved by site-1 and site-2 proteases (S1P and S2P). The mature protein enters into the nucleus resulting in the transcription of lipogenesis genes.

Guo et al., report EGFR signaling through an Akt-SREBP-1-dependent, rapamycin-resistant pathway sensitizes glioblastomas to anti-lipogenic therapy. *Sci Signal*, 2009, 2(101): ra82.

References cited herein are not an admission of prior art.

SUMMARY

This disclosure relates to combination therapies of epidermal growth factor receptor (EGFR) inhibitors and sterol regulatory element-binding protein (SREBP1) inhibitors for uses in cancer therapies such as lung cancer treatment. In certain embodiments, this disclosure relates to methods of treating cancer comprising administering an effective amount of EGFR inhibitor in combination with a SREBP1 inhibitor to a subject in need thereof. In certain embodiments, the combination has a synergistic effect compared administration of the inhibitors individually.

In certain embodiments, epidermal growth factor receptor inhibitor is selected from N-(2-((2-(dimethylamino)ethyl)(methyl)amino)-4-methoxy-5-((4-(1-methyl-1H-indol-3-yl)pyrimidin-2-yl)amino)phenyl)acrylamide (osimertinib), gefitinib, erlotinib, afatinib, and lapatinib, derivative, ester, or salt thereof.

In certain embodiments, the SREBP1 inhibitor is 4-((diethylamino)methyl)-N-(2-methoxyphenethyl)-N-(pyrrolidin-3-yl)benzamide (PF-429242), derivative, ester, or salt thereof.

In certain embodiments, SREBP1 inhibitor is 3a-(hydroxymethyl)-5a,5b,8,8,11a-pentamethyl-1-(prop-1-en-2-yl)icosahydro-1H-cyclopenta[a]chrysen-9-ol (betulin), derivative, ester, or salt thereof.

In certain embodiments, the SREBP1 inhibitor is 2-(2-propylpyridin-4-yl)-4-(p-tolyl)thiazole (fatostatin), derivative, ester, or salt thereof.

In certain embodiments, the cancer is lung cancer. In certain embodiments, the cancer is non-small cell lung cancer. In certain embodiments, a cancer sample of the subject is diagnosed with a T790M mutation in EGFR exon 20.

5 In certain embodiments, this disclosure relates to methods of treating non-small cell lung cancer comprising administering N-(2-((2-(dimethylamino)ethyl)(methyl)amino)-4-methoxy-5-((4-(1-methyl-1H-indol-3-yl)pyrimidin-2-yl)amino)phenyl)acrylamide (osimertinib) or salt thereof in combination with 4-((diethylamino)methyl)-N-(2-methoxyphenethyl)-N-(pyrrolidin-3-yl)benzamide (PF-429242) or salt thereof.

10 In certain embodiments, this disclosure relates to methods of treating non-small cell lung cancer comprising administering N-(2-((2-(dimethylamino)ethyl)(methyl)amino)-4-methoxy-5-((4-(1-methyl-1H-indol-3-yl)pyrimidin-2-yl)amino)phenyl)acrylamide (osimertinib) or salt thereof in combination with 3a-(hydroxymethyl)-5a,5b,8,8,11a-pentamethyl-1-(prop-1-en-2-yl)icosahydro-1H-cyclopenta[a]chrysen-9-ol(betulin) or salt thereof.

15 In certain embodiments, this disclosure relates to methods of treating non-small cell lung cancer comprising administering N-(2-((2-(dimethylamino)ethyl)(methyl)amino)-4-methoxy-5-((4-(1-methyl-1H-indol-3-yl)pyrimidin-2-yl)amino)phenyl)acrylamide (osimertinib) or salt thereof in combination with 2-(2-propylpyridin-4-yl)-4-(p-tolyl)thiazole (fatostatin) or salt thereof.

20 In certain embodiments, it is contemplated that any of the combinations exemplified herein may be used in combinations with additional anticancer agents.

In certain embodiments, this disclosure relates to pharmaceutical compositions comprising a kinase inhibitor of the epidermal growth factor receptor (EGFR) and a SREBP1 inhibitor.

In certain embodiments, this disclosure relates to the use of a kinase inhibitor of the epidermal growth factor receptor (EGFR) and a SREBP1 inhibitor in the treatment of cancer.

25 In certain embodiments, this disclosure relates to the production of a medicament comprising a kinase inhibitor of the epidermal growth factor receptor (EGFR) and a SREBP1 inhibitor for use in treating cancer.

In certain embodiments, a cancer sample of the subject is diagnosed with a L858R activating mutant, an Exon19 deletion activating mutant, or a T790M resistance mutant.

30 In certain embodiments, this disclosure relates to kits or pharmaceutical packaging comprising combinations of agents disclosed herein with instructions for use.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

Figure 1 shows data indicating genetic knockdown of SREBP1 reverses AZD9291 resistance in vivo. PC-9/AR-pLKO.1 and PC-9/AR-shSREBP1 tumors were treated with vehicle or AZD9291 (5mg/kg/day, og) for 3 weeks. Corresponding tumor growth curves were measured at the indicated time points.

Figure 2A shows data where PC-9/AR and HCC827/AR cells were exposed to varied concentrations of AZD9291 alone, PF429242(PF) alone or their respective combinations for 3 days. Cell numbers were the estimated with the SRB assay.

Figure 2B shows data where PC-9/AR and HCC827/AR cells were seeded in 12-well plates treated with 50nM AZD9291, 100nM PF429242 or their combination; these treatments were repeated with fresh medium every 3days. After 10 days, the cells were then fixed and stained with crystal violet dye.

Figure 2C shows data where the indicated cell lines were exposed to 250 nM AZD929 1,5 μ M PF429242 or their combinations for 72h. Apoptosis was detected with flow cytometry for annexin V – positive cells.

Figure 2D shows data indicating chemical inhibition of SREBP1 combined with AZD9291 synergistically decreases survival with enhanced apoptosis of AZD9291-resistant NSCLC cells and overcomes AZD9291 resistance in vivo. PC-9/AR cells grown in NU/NU mice as xenografted tumors were treated with vehicle, AZD9291 alone (5mg/kg/day, og), PF429242 alone (25mg/kg/day, ip) or the combination of AZD9291 with PF429242. Tumor sizes were measured at the indicated time points.

Figure 3A shows data indicating the combination of AZD9291 and betulin synergistically decreases survival, inhibits colony formation and enhances apoptosis of AZD9291-resistant cells. Both PC-9/AR and HCC827/AR cells were exposed to DMSO, 250nM AZD9291 alone, 5 μ M betulin alone or the combination of AZD291 and betulin. After 72hr, the cells were harvested for detection of annexin V-positive cells with flowcytometry.

Figure 3B shows data where PC-9/AR and HCC827/AR cells were seeded in 12-well plates were treated with 50nM AZD9291, 150nM betulin or their combinations. The treatments were repeated every 3 days with fresh medium. After 10 days, the cells were fixed and stained with crystal violet dye.

Figure 3C shows data where PC-9/AR and HCC827/AR cells in 96-well plates were exposed to varied concentrations of AZD9291 alone, betulin alone or their combinations for 3 days. Cell numbers were then estimated with the SRB assay.

DETAILED DISCUSSION

Before the present disclosure is described in greater detail, it is to be understood that this disclosure is not limited to embodiments described, and as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present disclosure, the preferred methods and materials are now described.

All publications and patents cited in this specification are herein incorporated by reference as if each individual publication or patent were specifically and individually indicated to be incorporated by reference and are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited. The citation of any publication is for its disclosure prior to the filing date and should not be construed as an admission that the present disclosure is not entitled to antedate such publication by virtue of prior disclosure. Further, the dates of publication provided could be different from the actual publication dates that may need to be independently confirmed.

As will be apparent to those of skill in the art upon reading this disclosure, each of the individual embodiments described and illustrated herein has discrete components and features which may be readily separated from or combined with the features of any of the other several embodiments without departing from the scope or spirit of the present disclosure. Any recited method can be carried out in the order of events recited or in any other order that is logically possible.

Embodiments of the present disclosure will employ, unless otherwise indicated, techniques of medicine, organic chemistry, biochemistry, molecular biology, pharmacology, and the like, which are within the skill of the art. Such techniques are explained fully in the literature.

To the extent that any chemical formulas reported herein contain one or more chiral centers, the formulas are intended to encompass all stable stereoisomers, enantiomers, and diastereomers. It is also understood that formulas encompass all tautomeric forms.

It must be noted that, as used in the specification and the appended claims, the singular
5 forms “a”, “an”, and “the” include plural referents unless the context clearly dictates otherwise.

Sterol regulatory element binding transcription factor 1 (SREBP1 also known as SREBF1) refers to the proteins synthesized as precursors that attaches to the nuclear membrane. Following cleavage, the mature proteins translocate to the nucleus and activates transcription. SREBP1 inhibitors specifically bind parts of nuclear membrane bound precursors or the mature proteins
10 after cleavage. Examples include osimertinib, betulin, fatostatin, and PF-429242. The gene encoding SREBP1 is located on chromosome 17. Homo sapiens sterol regulatory element binding transcription factor 1 (SREBF1), transcript variant 1, mRNA, has a sequence identified by NCBI Reference Sequence: NM_001005291.3.

EGFR (also known as ErbB-1 or HER-1) is a cell surface protein that binds to epidermal
15 growth factor (EGF). EGFR is found on the surface of some cells causing them to divide when epidermal growth factor binds to it. EGFR is sometimes found at abnormally high levels in certain cancer cells. Some types of cancers show mutations in their EGFRs, which may cause unregulated cell division through continual or abnormal activation of the EGFR. Epidermal growth factor receptor (EGFR) inhibitors specifically binds to parts of EGFR. Examples include afatinib
20 gefitinib, erlotinib, lapatinib, cetuximab, osimertinib, panitumumab, neratinib, vandetanib, necitumumab, dacomitinib, saracatinib, and canertinib. EGFR inhibitors can be classified as either tyrosine kinase inhibitors (TKI) (e.g., erlotinib, gefitinib): these bind to the tyrosine kinase domain in the epidermal growth factor receptor; or EGFR monoclonal antibodies (e.g., cetuximab, necitumumab).

25 The term "specific binding" in relation to an inhibitor as an agent refers to a molecule that binds a target molecule with a greater affinity than other random molecules or proteins. Examples of specific binding agents include an antibody that binds an epitope of an antigen or a receptor which binds a ligand. In certain embodiments, "specifically binds" refers to the ability of a specific binding agent to recognize and bind a target molecule or polypeptide, such that its affinity (as
30 determined by, e.g., affinity ELISA, or other assays) is at least 10 times as great, but optionally 50

times as great, 100, 250 or 500 times as great, or even at least 1000 times as great as the affinity of the same agent for any other or other random molecules or polypeptides.

“Subject” refers any animal, preferably a human patient, livestock, mouse model or domestic pet.

5 As used herein, the terms “prevent” and “preventing” include the prevention of the recurrence, spread or onset. It is not intended that the present disclosure be limited to complete prevention. In some embodiments, the onset is delayed, or the severity of the disease is reduced.

As used herein, the terms “treat” and “treating” are not limited to the case where the subject (e.g. patient) is cured and the disease is eradicated. Rather, embodiments, of the present disclosure
10 also contemplate treatment that merely reduces symptoms, and/or delays disease progression.

As used herein, the term “combination with” when used to describe administration with an additional treatment means that the agent may be administered prior to, together with, or after the additional treatment, or a combination thereof.

“Cancer” refers any of various cellular diseases with malignant neoplasms characterized
15 by the proliferation of cells. It is not intended that the diseased cells must actually invade surrounding tissue and metastasize to new body sites. Cancer can involve any tissue of the body and have many different forms in each body area. Within the context of certain embodiments, whether “cancer is reduced” may be identified by a variety of diagnostic manners known to one skill in the art including, but not limited to, observation the reduction in size or number of tumor
20 masses or if an increase of apoptosis of cancer cells observed, e.g., if more than a 5 % increase in apoptosis of cancer cells is observed for a sample compound compared to a control without the compound(s). It may also be identified by a change in relevant biomarker or gene expression profile, such as PSA for prostate cancer, HER2 for breast cancer, or others.

A “chemotherapy agent,” “chemotherapeutic,” “anti-cancer agent,” or the like, refer to
25 molecules that are recognized to aid in the treatment of a cancer. Contemplated examples include the following molecules or derivatives such as abemaciclib, abiraterone acetate, methotrexate, paclitaxel, adriamycin, acalabrutinib, brentuximab vedotin, ado-trastuzumab emtansine, aflibercept, afatinib, netupitant, palonosetron, imiquimod, aldesleukin, alectinib, alemtuzumab, pemetrexed disodium, copanlisib, melphalan, brigatinib, chlorambucil, amifostine, aminolevulinic
30 acid, anastrozole, apalutamide, aprepitant, pamidronate disodium, exemestane, nelarabine, arsenic trioxide, ofatumumab, atezolizumab, bevacizumab, avelumab, axicabtagene ciloleucel, axitinib,

azacitidine, carmustine, belinostat, bendamustine, inotuzumab ozogamicin, bevacizumab, bexarotene, bicalutamide, bleomycin, blinatumomab, bortezomib, bosutinib, brentuximab vedotin, brigatinib, busulfan, irinotecan, capecitabine, fluorouracil, carboplatin, carfilzomib, ceritinib, daunorubicin, cetuximab, cisplatin, cladribine, cyclophosphamide, clofarabine, cobimetinib, cabozantinib-S-malate, dactinomycin, crizotinib, ifosfamide, ramucirumab, cytarabine, dabrafenib, dacarbazine, decitabine, daratumumab, dasatinib, defibrotide, degarelix, denileukin diftitox, denosumab, dexamethasone, dexrazoxane, dinutuximab, docetaxel, doxorubicin, durvalumab, rasburicase, epirubicin, elotuzumab, oxaliplatin, eltrombopag olamine, enasidenib, enzalutamide, eribulin, vismodegib, erlotinib, etoposide, everolimus, raloxifene, toremifene, panobinostat, fulvestrant, letrozole, filgrastim, fludarabine, flutamide, pralatrexate, obinutuzumab, gefitinib, gemcitabine, gemtuzumab ozogamicin, glucarpidase, goserelin, propranolol, trastuzumab, topotecan, palbociclib, ibritumomab tiuxetan, ibrutinib, ponatinib, idarubicin, idelalisib, imatinib, talimogene laherparepvec, ipilimumab, romidepsin, ixabepilone, ixazomib, ruxolitinib, cabazitaxel, palifermin, pembrolizumab, ribociclib, tisagenlecleucel, lanreotide, lapatinib, olaratumab, lenalidomide, lenvatinib, leucovorin, leuprolide, lomustine, trifluridine, olaparib, vincristine, procarbazine, mechlorethamine, megestrol, trametinib, temozolomide, methylalantrexone bromide, midostaurin, mitomycin C, mitoxantrone, plerixafor, vinorelbine, necitumumab, neratinib, sorafenib, nilutamide, nilotinib, niraparib, nivolumab, tamoxifen, romiplostim, sonidegib, omacetaxine, pegaspargase, ondansetron, osimertinib, panitumumab, pazopanib, interferon alfa-2b, pertuzumab, pomalidomide, mercaptopurine, regorafenib, rituximab, rolapitant, rucaparib, siltuximab, sunitinib, thioguanine, temsirolimus, thalidomide, thiotepa, trabectedin, valrubicin, vandetanib, vinblastine, vemurafenib, vorinostat, zoledronic acid, or combinations thereof such as cyclophosphamide, methotrexate, 5-fluorouracil (CMF); doxorubicin, cyclophosphamide (AC); mustine, vincristine, procarbazine, prednisolone (MOPP); adriamycin, bleomycin, vinblastine, dacarbazine (ABVD); cyclophosphamide, doxorubicin, vincristine, prednisolone (CHOP); bleomycin, etoposide, cisplatin (BEP); epirubicin, cisplatin, 5-fluorouracil (ECF); epirubicin, cisplatin, capecitabine (ECX); methotrexate, vincristine, doxorubicin, cisplatin (MVAC).

In certain embodiments, the chemotherapy agent is an anti-PD-1, anti-PD-L1 anti-CTLA4 antibody or combinations thereof, such as an anti-CTLA4 (e.g., ipilimumab, tremelimumab) and

anti-PD1 (e.g., nivolumab, pembrolizumab, cemiplimab) and anti-PD-L1 (e.g., atezolizumab, avelumab, durvalumab).

As used herein, the term “derivative” refers to a structurally similar compound that retains sufficient functional attributes of the identified analogue. The derivative may be structurally similar because it is lacking one or more atoms, substituted, a salt, in different hydration/oxidation states, or because one or more atoms within the molecule are switched, such as, but not limited to, adding a hydroxyl group, replacing an oxygen atom with a sulfur atom, or replacing an amino group with a hydroxyl group, oxidizing a hydroxyl group to a carbonyl group, reducing a carbonyl group to a hydroxyl group, and reducing a carbon-to-carbon double bond to an alkyl group or oxidizing a carbon-to-carbon single bond to a double bond. A derivative optionally has one or more, the same or different, substitutions. Derivatives may be prepared by any variety of synthetic methods or appropriate adaptations presented in synthetic or organic chemistry text books, such as those provided in “March's Advanced Organic Chemistry: Reactions, Mechanisms, and Structure”, Wiley, 6th Edition (2007) Michael B. Smith or “Domino Reactions in Organic Synthesis”, Wiley (2006) Lutz F. Tietze, hereby incorporated by reference.

The term “substituted” refers to a molecule wherein at least one hydrogen atom is replaced with a substituent. When substituted, one or more of the groups are “substituents”. The molecule may be multiply substituted. In the case of an oxo substituent (=O), two hydrogen atoms are replaced. Example substituents within this context may include halogen, hydroxy, alkyl, alkoxy, nitro, cyano, oxo, carbocyclyl, carbocycloalkyl, heterocarbocyclyl, heterocarbocycloalkyl, aryl, arylalkyl, heteroaryl, or heteroarylalkyl,

As used herein, “alkyl” means a noncyclic straight chain or branched, unsaturated or saturated hydrocarbon such as those containing from 1 to 20 carbon atoms. In certain embodiments, any “alkyl” disclosed herein may be a lower alkyl and a higher alkyl or any of the specific alkyl groups reported in this section. A “lower alkyl” refers to unsaturated or saturated hydrocarbons having 1 to 6 carbon atoms or 1 to 4 carbon atoms and a “higher alkyl” refers to unsaturated or saturated hydrocarbon having 6 or more carbon atoms. A “C₁-C₆” refers to an alkyl containing 1 to 6 carbon atoms. Likewise, a “C₆-C₂₂” refers to higher alkyls containing 6 to 22 carbon atoms. Representative saturated straight chain alkyls include methyl, ethyl, n-propyl, n-butyl, n-pentyl, n-hexyl, n-septyl, n-octyl, n-nonyl, hexadecyl, dodecyl, tetradecyl, izosonyl, octadecyl, and the like; while saturated branched alkyls include isopropyl, sec-butyl, isobutyl, tert-

butyl, isopentyl, and the like. Unsaturated alkyls contain at least one double or triple bond between adjacent carbon atoms (referred to as an “alkenyl” or “alkynyl”, respectively). Representative straight chain and branched alkenyls include ethylenyl, propylenyl, 1-butenyl, 2-butenyl, isobutylenyl, 1-pentenyl, 2-pentenyl, 3-methyl-1-butenyl, 2-methyl-2-butenyl, 2,3-dimethyl-2-butenyl, and the like; while representative straight chain and branched alkynyls include acetylenyl, propynyl, 1-butyne, 2-butyne, 1-pentyne, 2-pentyne, 3-methyl-1-butyne, and the like.

Non-aromatic mono or polycyclic alkyls are referred to herein as “carbocycles” or “carbocyclyl” groups. Representative saturated carbocycles include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and the like; while unsaturated carbocycles include cyclopentenyl and cyclohexenyl, and the like. Carbocyclyls include cycloalkyls and cycloalkenyls.

“Heterocarbocycles” or “heterocarbocyclyl” groups are carbocycles which contain from 1 to 4 heteroatoms independently selected from nitrogen, phosphorous, oxygen and sulfur which may be saturated or unsaturated (but not aromatic), monocyclic or polycyclic, and wherein the nitrogen and sulfur heteroatoms may be optionally oxidized (e.g. -S(O)-, -SO₂-, -N(O)-), and the nitrogen heteroatom may be optionally quaternized. Heterocarbocycles include morpholinyl, pyrrolidinonyl, pyrrolidinyl, piperidinyl, hydantoinyl, valerolactamyl, oxiranyl, oxetanyl, tetrahydrofuranyl, tetrahydropyranyl, tetrahydropyridinyl, tetrahydropyrimidinyl, tetrahydrothiophenyl, tetrahydrothiopyranyl, and the like.

As used herein, “heterocycle” or “heterocyclyl” refers to mono- and polycyclic ring systems having 1 to 4 heteroatoms selected from nitrogen, oxygen and sulfur, and containing at least 1 carbon atom. The mono- and polycyclic ring systems may be aromatic, non-aromatic or mixtures of aromatic and non-aromatic rings. Heterocycle includes heterocarbocycles, heteroaryls, and the like.

The terms “cycloalkyl” and “cycloalkenyl” refer to mono-, bi-, or tri homocyclic ring groups of 3 to 15 carbon atoms which are, respectively, fully saturated and partially unsaturated.

The term “aryl” refers to aromatic homocyclic (i.e., hydrocarbon) mono-, bi- or tricyclic ring-containing groups preferably having 6 to 12 members such as phenyl, naphthyl and biphenyl. Phenyl is a preferred aryl group. The term “substituted aryl” refers to aryl groups substituted with one or more groups, preferably selected from alkyl, substituted alkyl, alkenyl (optionally substituted), aryl (optionally substituted), heterocyclyl (optionally substituted), halo, hydroxy, alkoxy (optionally substituted), aryloxy (optionally substituted), alkanoyl (optionally substituted),

aroyl, (optionally substituted), alkylester (optionally substituted), aryylester (optionally substituted), cyano, nitro, amino, substituted amino, amido, lactam, urea, urethane, sulfonyl, and, the like, where optionally one or more pair of substituents together with the atoms to which they are bonded form a 3 to 7 member ring.

5 The term “prodrug” refers to an agent that is converted into a biologically active form in vivo. Prodrugs are often useful because, in some situations, they may be easier to administer than the parent compound. They may, for instance, be bioavailable by oral administration whereas the parent compound is not. The prodrug may also have improved solubility in pharmaceutical compositions over the parent drug. A prodrug may be converted into the parent drug by various
10 mechanisms, including enzymatic processes and metabolic hydrolysis. Typical prodrugs are pharmaceutically acceptable esters. Prodrugs include compounds wherein a hydroxy, amino or mercapto group is bonded to any group that, when the prodrug of the active compound is administered to a subject, cleaves to form a free hydroxy, free amino or free mercapto group, respectively. Examples of prodrugs include, but are not limited to, acetate, formate and benzoate
15 derivatives of an alcohol or acetamide, formamide and benzamide derivatives of an amine functional group in the active compound and the like.

For example, if a disclosed compound or a pharmaceutically acceptable form of the compound contains a carboxylic acid functional group, a prodrug can comprise a pharmaceutically acceptable ester formed by the replacement of the hydrogen atom of the acid group with a group
20 such as (C₁-C₈)alkyl, (C₂-C₁₂)(alkanoyloxy)methyl, 1-(alkanoyloxy)ethyl having from 4 to 9 carbon atoms, 1-methyl-1-(alkanoyloxy)ethyl having from 5 to 10 carbon atoms, (alkoxycarbonyloxy)methyl having from 3 to 6 carbon atoms, 1-(alkoxycarbonyloxy)ethyl having from 4 to 7 carbon atoms, 1-methyl-1-(alkoxycarbonyloxy)ethyl having from 5 to 8 carbon atoms, N-(alkoxycarbonyl)aminomethyl having from 3 to 9 carbon atoms, 1-(N-
25 (alkoxycarbonyl)amino)ethyl having from 4 to 10 carbon atoms, 3-phthalidyl, 4-crotonolactonyl, gamma-butyrolacton-4-yl, di-N,N-(C₁-C₂)alkylamino(C₂-C₃)alkyl (such as beta-dimethylaminoethyl), carbamoyl-(C₁-C₂)alkyl, N,N-di(C₁-C₂)alkylcarbamoyl-(C₁-C₂)alkyl and piperidino-, pyrrolidino- or morpholino(C₂-C₃)alkyl.

If a disclosed compound or a pharmaceutically acceptable form of the compound contains
30 an alcohol functional group, a prodrug can be formed by the replacement of the hydrogen atom of the alcohol group with a group such as (C₁-C₆)(alkanoyloxy)methyl, 1-((C₁-C₆)alkanoyloxy)

ethyl, 1-methyl-1-((C₁-C₆)alkanoyloxy)ethyl (C₁-C₆)(alkoxycarbonyloxy)methyl, N-(C₁-C₆)alkoxycarbonylaminomethyl, succinoyl, (C₁-C₆)alkanoyl, alpha-amino(C₁-C₄)alkanoyl, arylacyl and alpha-aminoacyl, or alpha-aminoacyl-alpha-aminoacyl, where each alpha-aminoacyl group is independently selected from naturally occurring L-amino acids -P(O)(OH)₂, -P(O)(O(C₁-C₆)alkyl)₂, and glycosyl (the radical resulting from the removal of a hydroxyl group of the hemiacetal form of a carbohydrate).

If a disclosed compound or a pharmaceutically acceptable form of the compound incorporates an amine functional group, a prodrug can be formed by the replacement of a hydrogen atom in the amine group with a group such as R-carbonyl, RO-carbonyl, NRR'-carbonyl where R and R' are each independently (C₁-C₁₀)alkyl, (C₃-C₇)cycloalkyl, benzyl, a natural alpha-aminoacyl, -C(OH)C(O)OY₁ wherein Y₁ is H, (C₁-C₆)alkyl or benzyl, -C(OY₂)Y₃ wherein Y₂ is (C₁-C₄) alkyl and Y₃ is (C₁-C₆)alkyl, carboxy(C₁-C₆)alkyl, amino(C₁-C₄)alkyl or mono-Nor di-N,N-(C₁-C₆)alkylaminoalkyl, -C(Y₄)Y₅ wherein Y₄ is H or methyl and Y₅ is mono-N- or di-N,N-(C₁-C₆)alkylamino, morpholino, piperidin-1-yl or pyrrolidin-1-yl.

As used herein, "pharmaceutically acceptable esters" include, but are not limited to, alkyl, alkenyl, alkynyl, aryl, arylalkyl, and cycloalkyl esters of acidic groups, including, but not limited to, carboxylic acids, phosphoric acids, phosphinic acids, sulfonic acids, sulfinic acids, and boronic acids.

As used herein, "pharmaceutically acceptable enol ethers" include, but are not limited to, derivatives of formula -C=C(OR) where R can be selected from alkyl, alkenyl, alkynyl, aryl, aralkyl, and cycloalkyl. Pharmaceutically acceptable enol esters include, but are not limited to, derivatives of formula -C=C(OC(O)R) where R can be selected from hydrogen, alkyl, alkenyl, alkynyl, aryl, aralkyl, and cycloalkyl.

Methods of Use

In certain embodiments, this disclosure relates to methods of treating cancer comprising administering an effective amount of an epidermal growth factor receptor (EGFR) inhibitor in combination with a SREBP1 inhibitor to a subject in need thereof. In certain embodiments, the combination has a synergistic effect compared to the inhibitors individually.

In certain embodiments, epidermal growth factor receptor inhibitor is selected from osimertinib [N-(2-((2-(dimethylamino)ethyl)(methyl)amino)-4-methoxy-5-((4-(1-methyl-1H-

indol-3-yl)pyrimidin-2-yl)amino)phenyl)acrylamide], gefitinib, erlotinib and lapatinib, derivative, ester, or salt thereof.

In certain embodiments, the SREBP1 inhibitor is 4-((diethylamino)methyl)-N-(2-methoxyphenethyl)-N-(pyrrolidin-3-yl)benzamide (PF-429242), derivative, ester, or salt thereof.

5 In certain embodiments, SREBP1 inhibitor is betulin [3a-(hydroxymethyl)-5a,5b,8,8,11a-pentamethyl-1-(prop-1-en-2-yl)icosahydro-1H-cyclopenta[a]chrysen-9-ol], derivative, ester, or salt thereof.

In certain embodiments, the SREBP1 inhibitor is fatostatin [2-(2-propylpyridin-4-yl)-4-(p-tolyl)thiazole], derivative, ester, or salt thereof.

10 In certain embodiments, the cancer is lung cancer. In certain embodiments, the cancer is non-small cell lung cancer. In certain embodiments, a cancer sample of the subject is diagnosed with a T790M mutation in EGFR exon 20. In certain embodiments, a cancer sample of the subject is diagnosed with a L858R activating mutant, an Exon19 deletion activating mutant, and a T790M resistance mutant, a T790M mutation in EGFR exon 20.

15 In certain embodiments, this disclosure relates to methods of treating non-small cell lung cancer comprising administering N-(2-((2-(dimethylamino)ethyl)(methyl)amino)-4-methoxy-5-((4-(1-methyl-1H-indol-3-yl)pyrimidin-2-yl)amino)phenyl)acrylamide (osimertinib) or salt thereof in combination with 4-((diethylamino)methyl)-N-(2-methoxyphenethyl)-N-(pyrrolidin-3-yl)benzamide (PF-429242) or salt thereof.

20 In certain embodiments, this disclosure relates to methods of treating non-small cell lung cancer comprising administering N-(2-((2-(dimethylamino)ethyl)(methyl)amino)-4-methoxy-5-((4-(1-methyl-1H-indol-3-yl)pyrimidin-2-yl)amino)phenyl)acrylamide (osimertinib) or salt thereof in combination with 3a-(hydroxymethyl)-5a,5b,8,8,11a-pentamethyl-1-(prop-1-en-2-yl)icosahydro-1H-cyclopenta[a]chrysen-9-ol(betulin) or salt thereof.

25 In certain embodiments, this disclosure relates to methods of treating non-small cell lung cancer comprising administering N-(2-((2-(dimethylamino)ethyl)(methyl)amino)-4-methoxy-5-((4-(1-methyl-1H-indol-3-yl)pyrimidin-2-yl)amino)phenyl)acrylamide (osimertinib) or salt thereof in combination with 2-(2-propylpyridin-4-yl)-4-(p-tolyl)thiazole (fatostatin) or salt thereof.

30 In certain embodiments, this disclosure relates to methods wherein a subject is diagnosed with activating mutant forms of EGFR such as a L858R EGFR mutant, and/or Exon19 deletions

such as delS752_I759 and/or delE746_A750 EGFR mutants and /or resistant mutant forms of EGFR such as a T790M EGFR mutant, and/or selectivity over other enzyme receptors which may make a combination of inhibitors disclosed herein especially promising for development as therapeutic agents. In certain embodiments, a combination of inhibitors shows a higher inhibition of certain activating or resistance mutant forms of EGFR while at the same time showing relatively low inhibition of WT EGFR. In certain embodiments, the combination of inhibitors are more suitable as therapeutic agents, particularly for the treatment of cancer, lung cancer, or non-small cell lung cancer due to reduction of toxicology associated with WT EGFR inhibition e.g., skin rashes and/or diarrhea.

In certain embodiments, types of cancers which may be susceptible to treatment using the combination of inhibitors disclosed herein, or pharmaceutically acceptable salts thereof, include, but are not limited to, ovarian cancer, cervical cancer, colorectal cancer, breast cancer, pancreatic cancer, glioma, glioblastoma, melanoma, prostate cancer, leukemia, lymphoma, non-Hodgkin's lymphoma, gastric cancer, lung cancer, hepatocellular cancer, gastric cancer, gastrointestinal stromal tumor (GIST), thyroid cancer, bile duct cancer, endometrial cancer, renal cancer, anaplastic large cell lymphoma, acute myeloid leukemia (AML), multiple myeloma, melanoma and mesothelioma.

In certain embodiments, the methods of treatment of cancer mentioned herein, the combination of inhibitors will be administered to a mammal, more particularly a human being. Similarly, for the uses of the combination of inhibitors for the treatment of cancer mentioned herein, it is envisaged that the combination of inhibitors will be administered to a mammal, more particularly a human being.

In certain embodiments, there is therefore provided the combination of inhibitors as defined hereinbefore, or a pharmaceutically acceptable salt thereof, for use as a medicament.

In certain embodiments, there is provided combination of inhibitors as defined hereinbefore, or a pharmaceutically acceptable salt thereof, for use in the treatment of a disease mediated through L858R EGFR mutant and/or T790M EGFR and/or the Exon19 deletion activating mutant. In certain embodiments, said disease mediated through L858R EGFR mutant and/or T790M EGFR mutant and/or the Exon19 deletion activating mutant is cancer.

In certain embodiments, there is provided the use of the combination of inhibitors as defined hereinbefore, or a pharmaceutically acceptable salt thereof, for the manufacture of a

medicament for the treatment of a disease mediated through L858R EGFR mutant and/or T790M EGFR mutant and/or the Exon19 deletion activating mutant. In one embodiment, said disease mediated through L858R EGFR mutant and/or T790M EGFR mutant and/or the Exon19 deletion activating mutant is cancer.

5 In certain embodiments, there is provided the use of combination of inhibitors as defined hereinbefore, or a pharmaceutically acceptable salt thereof, for the manufacture of a medicament for the treatment of cancer.

 In certain embodiments, there is provided a method for producing an anti-cancer effect in a warm-blooded animal, such as man, in need of such treatment, which comprises administering
10 to said animal an effective amount of combination of inhibitors, or a pharmaceutically acceptable salt thereof, as defined herein.

 In certain embodiments, there is provided a method of treating a human suffering from a disease in which inhibition of L858R EGFR mutant and/or T790M EGFR mutant and/or the Exon19 deletion activating mutant is beneficial, comprising the steps of administering to a person
15 in need thereof of a therapeutically effective amount of combination of inhibitors as defined hereinbefore, or a pharmaceutically acceptable salt thereof. In one embodiment of the invention, the disease in which inhibition of L858R EGFR mutant and/or T790M EGFR mutant and/or the Exon19 deletion activating mutant is beneficial is cancer.

 In any of the aspects or embodiments mentioned herein where cancer is mentioned in a
20 general sense, said cancer may be selected from ovarian cancer, cervical cancer, colorectal cancer, breast cancer, pancreatic cancer, glioma, glioblastoma, melanoma, prostate cancer, leukemia, lymphoma, non-Hodgkin's lymphoma, gastric cancer, lung cancer, hepatocellular cancer, gastric cancer, gastrointestinal stromal tumor (GIST), thyroid cancer, bile duct cancer, endometrial cancer, renal cancer, anaplastic large cell lymphoma, acute myeloid leukemia (AML), multiple
25 myeloma, melanoma and mesothelioma.

 The anti-cancer treatment described hereinbefore may be applied as a sole therapy or may involve, in addition to the inhibitors of the invention, conventional surgery or radiotherapy or chemotherapy or immunotherapy. Such chemotherapy could be administered concurrently, simultaneously, sequentially or separately to treatment with combinations of inhibitors disclosed
30 and may include other anti-tumor agents.

Pharmaceutical compositions

In certain embodiments, this disclosure relates to pharmaceutical compositions comprising a kinase inhibitor of the epidermal growth factor receptor (EGFR) and a SREBP1 inhibitor and a pharmaceutically acceptable excipient. In certain embodiments, the pharmaceutically acceptable excipient is selected from a diluent, disintegrant, solubilizing agent, or a lubricant.

In certain embodiments, the pharmaceutically acceptable excipient is a diluent. Examples include microcrystalline cellulose, other diluents may be, for example: calcium carbonate, calcium phosphate, calcium sulfate, cellulose acetate, erythritol, ethylcellulose, fructose, inulin, isomalt, lactitol, lactose, magnesium carbonate, magnesium oxide, maltitol, maltodextrin, maltose, mannitol, polydextrose, polyethylene glycol, pullulan, simethicone, sodium bicarbonate, sodium carbonate, sodium chloride, sorbitol, starch, sucrose, trehalose and xylitol.

In certain embodiments, the pharmaceutically acceptable excipient is a disintegrant. Examples of a disintegrant may be, for example: alginic acid, calcium alginate, carboxymethylcellulose calcium, chitosan, colloidal silicon dioxide, croscarmellose sodium, crospovidone, glycine, guar gum, hydroxypropyl cellulose, low-substituted hydroxypropyl cellulose, magnesium aluminum silicate, methylcellulose, povidone, sodium alginate, sodium carboxymethylcellulose, sodium starch glycolate and starch.

In certain embodiments, the pharmaceutically acceptable excipient is a solubilizing agent. Examples of a solubilizing agent may be, for example: benzalkonium chloride, benzyl benzoate, sulfobutyl ether β -cyclodextrin sodium, cetylpyridinium chloride, cyclodextrins, diethylene glycol monoethyl ether, fumaric acid, hydroxypropyl beta cyclodextrin, hypromellose, lanolin alcohols, lecithin, oleyl alcohol, phospholipids, poloxamer, polyoxyethylene alkyl ethers, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, polyoxyl hydroxystearate, polyoxylglycerides, povidone, pyrrolidone, sodium lauryl sulfate, sorbitan esters (sorbitan fatty acid esters), tricaprylin, triolein and vitamin E polyethylene glycol succinate.

In certain embodiments, the pharmaceutically acceptable excipient is a lubricant. Examples of a lubricant may be, for example calcium stearate, glyceryl behenate, glyceryl dibehenate, glyceryl monostearate, glyceryl palmitostearate, a mixture of behenate esters of glycerin (e.g. a mixture of glyceryl dibehenate, tribehenin and glyceryl behenate), leucine, magnesium stearate, myristic acid, palmitic acid, poloxamer, polyethylene glycol, potassium benzoate, sodium

benzoate, sodium lauryl sulfate, sodium stearate, sodium stearyl fumarate, stearic acid, talc, tribehenin and zinc stearate.

In certain embodiments, the pharmaceutically acceptable excipient is selected from lactose, sucrose, mannitol, triethyl citrate, dextrose, cellulose, methyl cellulose, ethyl cellulose, hydroxyl
5 propyl cellulose, hydroxypropyl methylcellulose, carboxymethylcellulose, croscarmellose sodium, polyvinyl N-pyrrolidone, crospovidone, ethyl cellulose, povidone, methyl and ethyl acrylate copolymer, polyethylene glycol, fatty acid esters of sorbitol, lauryl sulfate, gelatin, glycerin, glyceryl monooleate, silicon dioxide, titanium dioxide, talc, corn starch, carnauba wax, stearic acid, sorbic acid, magnesium stearate, calcium stearate, castor oil, mineral oil, calcium
10 phosphate, starch, carboxymethyl ether of starch, iron oxide, triacetin, acacia gum, esters, or salts thereof.

In certain embodiments, the pharmaceutical composition is in the form of a tablet, pill, capsule, gel, gel capsule or cream. In certain embodiments, the pharmaceutical composition is in the form of a sterilized pH buffered aqueous salt solution or a saline phosphate buffer between a
15 pH of 6 to 8, optionally comprising a saccharide or polysaccharide.

In certain embodiments, the pharmaceutically acceptable form is a pharmaceutically acceptable salt. As used herein, the term "pharmaceutically acceptable salt" refers to those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of subjects without undue toxicity, irritation, allergic response and the like, and are commensurate
20 with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, Berge et al. describes pharmaceutically acceptable salts in detail in J. Pharmaceutical Sciences (1977) 66:1-19. Pharmaceutically acceptable salts of the compounds provided herein include those derived from suitable inorganic and organic acids and bases. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with
25 inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange.

Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate,
30 benzenesulfonate, besylate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate,

glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like. In some embodiments, organic acids from which salts can be derived include, for example, acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid, and the like.

Pharmaceutically acceptable salts derived from appropriate bases include alkali metal, alkaline earth metal, ammonium and $N^+(C_{1-4}alkyl)_4$ salts. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, iron, zinc, copper, manganese, aluminum, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, lower alkyl sulfonate, and aryl sulfonate. Organic bases from which salts can be derived include, for example, primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines, basic ion exchange resins, and the like, such as isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, and ethanolamine. In some embodiments, the pharmaceutically acceptable base addition salt is chosen from ammonium, potassium, sodium, calcium, and magnesium salts.

In certain embodiments, an inhibitor disclosed herein may be used in the “free base form” or as a pharmaceutically acceptable salt, or as any mixture thereof. In one embodiment the inhibitor is in the free base form. It is understood that “free base form” refers to the case where the inhibitor is not in the form of a salt.

In certain embodiments, the EGFR inhibitor is N-(2-((2-(dimethylamino)ethyl)(methyl)amino)-4-methoxy-5-((4-(1-methyl-1H-indol-3-yl)pyrimidin-2-yl)amino)phenyl)acrylamide (osimertinib), derivative, ester, or salt thereof.

In certain embodiments, the inhibitor is N-(2-((2-(dimethylamino)ethyl)(methyl)amino)-4-methoxy-5-((4-(1-methyl-1H-indol-3-yl)pyrimidin-2-yl)amino)phenyl)acrylamide (osimertinib) as a pharmaceutically acceptable salt which is the mesylate salt of AZD9291.

In certain embodiments, the mesylate salt of N-(2-((2-(dimethylamino)ethyl)(methylamino)-4-methoxy-5-((4-(1-methyl-1H-indol-3-yl)pyrimidin-2-yl)amino)phenyl)acrylamide (osimertinib) contains a 1:1 molar ratio with methanesulfonic acid.

5 In certain embodiments, the inhibitor is a pharmaceutically acceptable salt of N-(2-((2-(dimethylamino)ethyl)(methylamino)-4-methoxy-5-((4-(1-methyl-1H-indol-3-yl)pyrimidin-2-yl)amino)phenyl)acrylamide (osimertinib) which is a crystalline mesylate salt.

In certain embodiments, this disclosure relates to the use of a kinase inhibitor of the epidermal growth factor receptor (EGFR) and a SREBP1 inhibitor in the treatment of cancer.

10 In certain embodiments, this disclosure relates to the production of a medicament comprising a kinase inhibitor of the epidermal growth factor receptor (EGFR) and a SREBP1 inhibitor for use in treating cancer.

In certain embodiments, this disclosure relates to kits or pharmaceutical packaging comprising combinations of agents disclosed herein with instructions for use. In certain embodiments, the individual agent may be packaged in a container, e.g., vial, box, syringe, or
15 bottle. In certain embodiments, instructions may be in a pamphlet inside a container or on the outside or inside of the container.

RNA interference

20 In certain embodiments, the SREBP1 inhibitor is by RNA interference, e.g., naked double stranded siRNA, vector encoded expressed short harpin RNA, antisense oligonucleotides that specifically target or bind SREBP1 mRNA.

In certain embodiments, the disclosure relates to compositions comprising isolated antisense nucleobase polymers, interference nucleobase polymers, and RNA-blocking oligonucleotides. In certain embodiments, the nucleobase polymers are 8 to 25 base oligomers
25 that mimic DNA or RNA. Many nucleobase polymers differ from native RNA or DNA in the chemical structure that links the four common bases.

The term “nucleobase polymer” refers to a polymer comprising nitrogen containing aromatic or heterocyclic bases that bind to naturally occurring nucleic acids through hydrogen bonding otherwise known as base pairing. A typical nucleobase polymer is a nucleic acid, RNA,
30 DNA, or chemically modified form thereof. A nucleic acid may be single or double stranded or both, e.g., they may contain overhangs. Nucleobase polymers may contain naturally occurring or

synthetically modified bases and backbones. In certain embodiments, a nucleobase polymer need not be entirely complementary, e.g., may contain one or more insertions, deletions, or be in a hairpin structure provided that there is sufficient specific binding or hybridization.

With regard to the nucleobases, it is contemplated that the term encompasses isobases, otherwise known as modified bases, e.g., are isoelectronic or have other substitutes configured to mimic naturally occurring hydrogen bonding base-pairs, e.g., within any of the sequences herein U may be substituted for T, or T may be substituted for U. Examples of nucleotides with modified adenosine or guanosine include, but are not limited to, hypoxanthine, xanthine, 7-methylguanine. Examples of nucleotides with modified cytidine, thymidine, or uridine include 5,6-dihydrouracil, 5-methylcytosine, 5-hydroxymethylcytosine. Contemplated isobases include 2'-deoxy-5-methylisocytidine (iC) and 2'-deoxy-isoguanosine (iG) (see U.S. Pat. No. 6,001,983; No. 6,037,120; No. 6,617,106; and No. 6,977,161).

Nucleobase polymers may be chemically modified, e.g., within the sugar backbone or on the 5' or 3' ends. The nucleobase polymers can be modified, for example, 2'-amino, 2'-O-allyl, 2'-fluoro, 2'-O-methyl, 2'-methyl, 2'-H of the ribose ring. In certain embodiments, nucleobase polymers disclosed herein may contain monomers of phosphodiester, phosphorothioate, methylphosphonate, phosphorodiamidate, piperazine phosphorodiamidate, ribose, 2'-O-methyl ribose, 2'-O-methoxyethyl ribose, 2'-methyl ribose, 2'-fluororibose, deoxyribose, 1-(hydroxymethyl)-2,5-dioxabicyclo[2.2.1]heptan-7-ol, P-(2-(hydroxymethyl)morpholino)-N,N-dimethylphosphonamidate, morpholin-2-ylmethanol, (2-(hydroxymethyl)morpholino)(piperazin-1-yl)phosphinate, or peptide nucleic acids or combinations thereof.

In certain embodiments, nucleobase polymers are contemplated to comprise peptide nucleic acids (PNAs). One example of a peptide nucleic acid is one that has 2-aminoethyl glycine linkages or similar analogs in place of the regular phosphodiester backbone. In certain embodiments, nucleobase polymers are contemplated to comprise phosphorodiamidate morpholino oligomers (PMO). In certain embodiments, the nucleobase polymer comprises monomers of (2-(hydroxymethyl)morpholino)(piperazin-1-yl)phosphinate.

In certain embodiments, the disclosure relates to composition comprising an isolated antisense nucleobase polymer that specifically binds mRNA of SREBP1. In certain embodiments, the nucleobase polymer is a nucleic acid or nucleic acid mimetic that hybridizes to mRNA of SREBP1. In particular, the instant disclosure features small nucleic acid molecules, such as short

interfering short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), and short hairpin RNA (shRNA) molecules and methods used to modulate the of mRNA of SREBP1.

5 **Anti-SREBP1 antibodies**

In certain embodiments, the SREBP1 inhibitor is an antibody that specifically binds SREBP1 protein.

In certain contexts, an “antibody” refers to a protein based molecule that is naturally produced by animals in response to the presence of a protein or other molecule or that is not
10 recognized by the animal’s immune system to be a “self” molecule, i.e. recognized by the animal to be a foreign molecule, i.e., an antigen to the antibody. The immune system of the animal will create an antibody to specifically bind the antigen, and thereby targeting the antigen for degradation, or any organism attached to the antigen. It is well recognized by skilled artisans that the molecular structure of a natural antibody can be synthesized and altered by laboratory
15 techniques. Recombinant engineering can be used to generate fully synthetic antibodies or fragments thereof providing control over variations of the amino acid sequences of the antibody. Thus, the term “antibody” is intended to include natural antibodies, monoclonal antibody, or non-naturally produced synthetic antibodies, such as specific binding single chain antibodies, bispecific antibodies or fragments thereof. These antibodies may have chemical modifications. The term
20 “monoclonal antibodies” refers to a collection of antibodies encoded by the same nucleic acid molecule that are optionally produced by a single hybridoma (or clone thereof) or other cell line, or by a transgenic mammal such that each monoclonal antibody will typically recognize the same antigen. The term “monoclonal” is not limited to any particular method for making the antibody, nor is the term limited to antibodies produced in a particular species, e.g., mouse, rat, etc.

25 From a structural standpoint, an antibody is a combination of proteins: two heavy chain proteins and two light chain proteins. The heavy chains are longer than the light chains. The two heavy chains typically have the same amino acid sequence. Similarly, the two light chains typically have the same amino acid sequence. Each of the heavy and light chains contain a variable segment that contains amino acid sequences which participate in binding to the antigen. The
30 variable segments of the heavy chain do not have the same amino acid sequences as the light chains. The variable segments are often referred to as the antigen binding domains. The antigen

and the variable regions of the antibody may physically interact with each other at specific smaller segments of an antigen often referred to as the "epitope." Epitopes usually consist of surface groupings of molecules, for example, amino acids or carbohydrates. The terms "variable region," "antigen binding domain," and "antigen binding region" refer to that portion of the antibody molecule which contains the amino acid residues that interact with an antigen and confer on the antibody its specificity and affinity for the antigen. Small binding regions within the antigen-binding domain that typically interact with the epitope are also commonly alternatively referred to as the "complementarity-determining regions, or CDRs."

The term "antibody fragment" refers to a peptide or polypeptide which comprises less than a complete, intact antibody. Complete antibodies comprise two functionally independent parts or fragments: an antigen binding fragment known as "Fab," and a carboxy terminal crystallizable fragment known as the "Fc" fragment. The Fab fragment includes the first constant domain from both the heavy and light chain (CH1 and CL1) together with the variable regions from both the heavy and light chains that bind the specific antigen. Each of the heavy and light chain variable regions includes three complementarity determining regions (CDRs) and framework amino acid residues which separate the individual CDRs. The Fc region comprises the second and third heavy chain constant regions (CH2 and CH3) and is involved in effector functions such as complement activation and attack by phagocytic cells. In some antibodies, the Fc and Fab regions are separated by an antibody "hinge region," and depending on how the full-length antibody is proteolytically cleaved, the hinge region may be associated with either the Fab or Fc fragment. For example, cleavage of an antibody with the protease papain results in the hinge region being associated with the resulting Fc fragment, while cleavage with the protease pepsin provides a fragment wherein the hinge is associated with both Fab fragments simultaneously. Because the two Fab fragments are in fact covalently linked following pepsin cleavage, the resulting fragment is termed the F(ab')₂ fragment.

Targeting SREBP1-mediated lipid metabolism for overcoming acquired resistance of EGFR mutant NSCLC cells to the EGFR inhibitor, (osimertinib, AZD9291)

Although it is not intended that embodiments of this disclosure be limited by any particular mechanism, it is believed that modulation of SREBP1 has a role of in overcoming AZD9291 acquired resistance. Experimental data indicates that AZD9291 facilitated degradation of the

mature form of SREBP1 (mSREBP1) in a GSK3/FBXW7-dependent manner and reduces the levels of its regulated proteins in EGFR mutant NSCLC cells/tumors accompanied with suppression of lipogenesis. Once becoming resistant, EGFR mutant NSCLC cell lines possessed elevated levels of mSREBP1, which were resistant to AZD9291 modulation. Both genetic and pharmacological inhibition of SREBP1 sensitized AZD9291-resistance cells/tumors to AZD9291. The connection between AZD9291 and modulation of SREBP1-mediated lipid metabolism has an impact on overcoming AZD9291 acquired resistance. Thus, combinations of SREBP1 inhibitors and EGFR inhibitors are desirable for cancer treatments.

In certain embodiments, this disclosure relates to methods of overcoming acquired resistance to AZD9291 (osimertinib), a mutation-selective EGFR inhibitor for treating NSCLC patients with activating and resistant EGFR mutations, or other EGFR inhibitors by co-administering SREBP1 inhibitors.

Non-small cell lung cancer (NSCLC) and is a major cause of cancer-related deaths worldwide. NSCLC is typically treated with epidermal growth factor receptor (EGFR) inhibitors [first generation EGFR-tyrosine kinase inhibitors (EGFR-TKIs; e.g., gefitinib and erlotinib) and second-generation EGFR-TKIs (e.g., afatinib)]. Patients receiving these EGFR-TKIs typically progress and develop acquired resistance. The most common mechanism being the development of a T790M mutation in EGFR exon 20. AZD9291 (osimertinib) is a third generation EGFR-TKI that inhibits the activating EGFR mutations and the resistant T790M mutation. However, patients inevitably develop acquired resistance to this treatment. Therefore, there is a need to develop improved therapies.

One of the hallmarks of cancer is lipid metabolism reprogramming. Cancer cells exhibit significant metabolic alterations to support cell proliferation. Unlike normal cells that rely mainly on the uptake of exogenous fatty acids, cancer cells increase the rate of de novo synthesis, lipogenesis. is elevated in human cancers. Lipid uptake and storage is also increased in tumors.

Sterol regulatory element-binding proteins (SREBPs), a family of membrane-bound transcription factors, regulate lipid metabolism. Three forms of SREBPs (SREBP1-a, SREBP1-c and SREBP2) are encoded by the genes SREBF1 and SREBF2. SREBP1 mainly regulates genes that are involved in fatty acid synthesis, phospholipid and triacylglycerol synthesis, while SREBP2 primarily regulates cholesterol synthesis.

A connection between SREBP1 modulation including its mediated lipid metabolism and AZD9291-mediated targeted cancer therapy has been identified. Experiments indicate that AZD9291 dramatically decreases the levels of mSREBP1 and its targeted proteins including fatty-acid synthase (FASN) and acetyl-CoA carboxylase (ACC) in EGFR mutant (EGFRm) NSCLC cell lines by enhancing GSK3/FBXW7-mediated mSREBP1 degradation. AZD9291 losses its ability to decrease the levels of mSREBP1, FASN and ACC and to suppress lipid metabolism in EGFRm NSCLC cell lines with AZD9291 acquired resistance possessing elevated mSREBP1. Targeting SREBP1 with genetic and pharmacological approaches reverses the responses of AZD9291-resistance cells and tumors to in vitro and in vivo, indicating a therapeutic avenue for overcoming acquired resistance to AZD9291 and possibly other EGFR inhibitors.

AZD9291 inhibits mTOR complex 2 (mTORC2) signaling and decreases mSREBP1 levels in EGFRm NSCLC cells.

AZD9291 effectively suppresses MEK/ERK signaling with induction of apoptosis through modulation of Bim and Mcl-1 degradation. Experiments were performed to determine whether AZD9291 affects PI3K/Akt, another important signaling pathway downstream of EGFR, in EGFRm NSCLC cells. AZD9291 effectively decreased the levels of not only p-Akt (S473), but also p-Akt (T450) and p-NDRG1 (T346), which all serve as substrates of mTORC2, in PC-9 and HCC827 cells, suggesting that AZD9291 inhibits mTORC2 signaling.

As mTORC2 stabilizes mSREBP1 via inhibiting its degradation, experiments were performed to determine whether AZD9291 decreases mSREBP1 levels in the sensitive NSCLC cell lines with EGFR activating mutations. Concentration-dependent and time-dependent reduction of mSREBP1, ACC and FASN was observed with minimal decrease of precursor SREBP1 in EGFRm cell lines, but not in cell lines with wild-type (WT) EGFR. Interestingly, other EGFR-TKIs, including the 1st generation EGFR-TKI, erlotinib, the 2nd generation EGFR-TKI, afatinib, and the 3rd generation EGFR-TKIs, EGF816 and CO1686, all effectively decreased the levels of mSREBP1, ACC and FASN in HCC827 and PC-9 cells. Immunocytofluorescence further confirmed that, in both HCC827 and PC-9 cells, AZD9291 significantly reduced FASN and ACC levels. In AZD9291-treated PC-9 xenograft tumors, decreased FASN was also detected in comparison with vehicle-treated tumor tissue. These results together indicate that ADZ9291 and

other EGFR-TKIs effectively decrease the levels of mSREBP1 and its regulated proteins such as FASN and ACC primarily in EGFRm NSCLC cells.

AZD9291 effectively suppresses SREBP1-regulated lipid metabolism in EGFRm NSCLC cells

As mSREBP/ACC/FASN axis regulates lipid metabolism, particularly fatty acid synthesis, phospholipid and triacylglycerol synthesis, experiments were performed to determine whether AZD9291 accordingly alters lipid metabolism in EGFRm NSCLC cells. In both HCC827 and PC-9 cells, AZD9291 treatment substantially reduced lipid droplets as detected by Nile Red staining. In PC-9 xenograft tumors receiving AZD9291 treatment, lipid droplets were also dramatically reduced using both Nile Red staining and Oil Red O staining. Therefore, it appears that AZD9291 inhibits lipid metabolism in EGFRm NSCLC cells and tumors. Furthermore, an untargeted lipidomic analysis was conducted in HCC827 cells treated with DMSO and AZD9291, respectively. Unsupervised hierarchical clustering separated the cells exposed to AZD9291 from the cells of DMSO group, based on the distinct profiles of 148 lipid metabolites. Out of the 148 lipid metabolites, 50 of them were significantly (false discovery rate, FDR < 0.05) altered when treated with AZD9291. Lipid classes of triacylglycerol (TAG), diacylglycerol (DAG), sphingomyelin (SM), ceramide (CER) and phosphatidylethanolamine (PE), especially polyunsaturated fatty acids phosphatidylethanolamine (PUFA PE), were significantly decreased in cells treated with AZD9291. Class phosphatidylcholine (PC) showed diverse but not significant changes when cells were treated with AZD9291. These results thus confirm that AZD9291 effectively suppresses lipid metabolism, particularly those regulated by SREBP1, in EGFRm NSCLC cells.

EGFRm NSCLC cells with acquired resistance to AZD9291 and NSCLC tissues relapsed from EGFR-TKI treatment possess elevated levels of mSREBP1 or FASN, which are resistant to AZD9291 modulation

To explore whether the acquired AZD9291-resistance is related to dysregulation of SREBP1-dependent lipid metabolism, the basal levels of mSREBP1 between PC-9 and HCC827 parental cell lines and their-derived AZD9291-resistant cell lines including PC-9/AR, PC-9/GR/AR, PC-9/3M and HCC827/AR were compared. The basal levels of mSREBP1, ACC and

FASN were higher in these resistant cell lines than their corresponding parental cell lines. Minimal or no reduction of mSREBP1, ACC and FASN in the resistant cell lines when treated with AZD9291 was detected. Moreover, immunofluorescent staining also showed that both FASN levels and lipid droplets were reduced with AZD9291 treatment in PC-9 and HCC827 cells, but not in their corresponding resistant cell lines. Hence, AZD9291 loses its ability to reduce mSREBP1 levels and to inhibit its regulated metabolism in cells with acquired resistance to AZD9291.

The paired EGFRm NSCLC tissues from 46 patients before EGFR-TKI treatment (baseline) and after relapse from the treatment was also analyzed. FASN expression was detected in these tissues with IHC. The FASN levels were significantly increased in relapsed tissues compared with those in tissues before the treatment. Among 46 patients, 38 patients partially responded to EGFR-TKI treatment, whereas 8 patients were not responsive (primary resistance).

To further understand the role of SREBP1/FASN axis in acquired resistance to EGFR-TKIs, the responded patients were divided into two groups: local progression (25/38) and dramatic progression (13/38), based on the responses of patients and progression to treatments (20). In tissues from the dramatic progression patients, 11 of 13 (85%) relapsed tissues showed increased levels of FASN in comparison with the baseline tissues. The overall FASN expression was significantly higher in the relapsed tissues than in baseline tissues. In tissues from the local progression patients, FASN expression was elevated in the majority of relapsed tissues (17/25; 68%) although the overall FASN levels did not show significant difference between pre and post treatment tissues. In tissues from the primary resistant patients, the FASN expression was not significantly altered post relapse; however, the baseline levels of FASN in this group were significantly higher than those in the responded group, suggesting that tumors with highly elevated SREBP1/FASN axis may respond poorly to EGFR-TKI treatment.

AZD9291 reduces mSREBP1 levels through facilitating GSK3/FBXW7-mediated mSREBP1 degradation

Considering that mSREBP1 is an unstable protein undergoing GSK3/FBXW7-mediated proteasomal degradation, experiments were performed to determine whether AZD9291 decreases mSREBP1 levels through modulating its degradation. The presence of MG132, a widely used proteasome inhibitor, not only enhanced the basal levels of mSREBP1, but also rescued mSREBP1

reduction induced by AZD9291 in PC-9, HCC827 and H1975 cells. The cycloheximide (CHX) chase assay showed that, compared with DMSO treatment, mSREBP1 was degraded more rapidly in AZD9291-treated PC-9 and HCC827 cells than in their corresponding DMSO-treated cells. These findings collectively indicate that AZD9291 destabilizes mSREBP1 by promoting proteasomal degradation.

Experiments were performed to determine whether GSK3/FBXW7 is involved in AZD9291-induced mSREBP1 degradation. AZD9291 reduced mSREBP1 levels in the absence of a GSK3 inhibitor (CHIR99021 or SB216763) but did not do so in the presence of these GSK3 inhibitors. In agreement, silencing GSK3 with a specific small interfering RNA (siRNA) also rescued mSREBP1 reduction induced by AZD9291 in PC-9 and HCC827 cells. Furthermore, knockdown of FBXW7 with a specific siRNA or small hairpin RNA (shRNA) enhanced the basal level of mSREBP1 and rescued mSREBP1 reduction induced by AZD9291 in these two cell lines. Taken together, these results indicate that AZD9291 promotes GSK3/FBXW7-mediated mSREBP1 degradation.

Genetic knockdown of SREBP1 reverses AZD9291 resistance in vitro and in vivo

As mSREBP1/FASN was elevated in AZD9291-resistant cell lines, experiments were performed to determine whether this lipid metabolism-modulated axis plays a critical role in mediating acquired resistance to AZD9291. If so, enforced suppression of this axis may resensitize the resistant cells to AZD9291 treatment. Therefore, the impact of genetic knockdown of SREBP1 was tested on sensitivities of AZD9291-resistance cell lines to AZD9291. Western blotting confirmed siRNA-mediated SREBP1 knockdown effectively enhanced the effects of AZD9291 on inducing cleavage of PARP and caspase-3 and on increasing the percentage of annexin-V-positive cells in both PC-9/AR and HCC827/AR cells. Similarly, both transient and stable knockdown of SREBP1 by using a shRNA in both PC-9/AR and HCC827/AR cell lines also enhanced the ability of AZD9291 in reducing FASN levels, in decreasing cell survival, in inducing cleavage of PARP and caspase-3 and in increasing apoptotic cells.

Moreover, an in vivo study was conducted to test the impact of SREBP1 knockdown on the response of AZD9291-resistant tumors to AZD9291. AZD9291 significantly inhibited the growth of PC-9/AR-shSREBP1 tumors, while it had minimal effect on the growth of PC-9/AR-pLKO.1 tumors (Figs. 1). Meanwhile, mice body weights showed no differences between control

and AZD9291 groups. IHC staining showed that both ACC and FASN expression were significantly decreased in the PC-9/AR-shSREBP1 tumors receiving AZD9291 treatment; but not in PC-9/AR-pLKO.1 tumors treated with AZD9291. Beyond, immunofluorescent staining also showed that the amounts of lipid droplets and FASN expression levels were reduced in PC-9/AR-shSREBP1 tumors received AZD9291 treatment, but not in PC-9/AR-pLKO.1 tumors treated with AZD9291.

Therefore, these in vitro and in vivo results indicate that enforced suppression of SREBP1 via genetic gene knockdown re-sensitizes AZD9291-resistant cells and tumors to AZD9291, suggesting a role of dysregulated SREBP1/ACC/FASN-mediated lipid metabolism in emergence of acquired resistance to AZD9291.

Chemical inhibition of SREBP1 combined with AZD9291 synergistically decreases the survival of AZD9291-resistant NSCLC cells with enhanced apoptosis and overcomes AZD9291 resistance in vivo.

Small molecule inhibitors that target SREBP1 were used in combination with AZD9291 to overcome AZD9291 acquired resistance. Two SREBP1 inhibitors, PF429242 and betulin, when combined with AZD9291, respectively, synergistically decreased the survival of AZD9291 resistant cells (PC-9/AR and HCC827/AR) with CIs < 1 (Fig. 2A). The colony formation assay clearly showed that these combinations were significantly more potent than either agent alone in suppressing colony formation and growth of both PC-9/AR and HCC827/AR cells (Fig. 2B). Observation of morphological change showed that the combination of AZD9291 and PF429242 enhanced cell detachment, a typical apoptotic phenotype. Indeed, compared with each single agent treatment, the combination of AZD9291 with either PF429242 or betulin significantly enhanced induction of apoptosis as indicated by increased annexin V-positive cells (Figs. 2C) and cleavage of PARP and caspase-3. Moreover, immunofluorescent staining further showed that the combination of AZD9291 and PF429242 dramatically reduced the lipid droplets (Nile Red staining) and the level of FASN in both PC-9/AR and HCC827/AR cells. Hence, it is clear that combination of AZD9291 with a SREBP1 small molecule inhibitor enhances induction of apoptosis and inhibition of lipid metabolism in AZD9291-resistant cells.

Following these in vitro studies, the ability of the AZD9291 and PF429242 combination to suppress the growth of AZD9291-resistance xenografts was validated in vivo. While treatment

with AZD9291 or PF429242 alone had a minimal effect on inhibiting the growth of PC-9/AR tumors, their combination significantly reduced the growth of these tumors based on both tumor sizes (Figs. 2D) and weights. The combination did not apparently alter mouse body weights. Thus, the combination of AZD9291 and PF429242 effectively inhibits the growth of AZD9291-resistant tumors in vivo with well-tolerated safety, indicating its safeness and efficacy in overcoming acquired resistance to AZD9291.

FASN expression and lipid droplets was also detected using immunofluorescent staining in these tumors and found that both FASN and Nile Red staining (lipid droplets) were reduced in tumors treated with the combination in comparison with single agent-treated tumors. Oil Red staining also generated similar result regarding reduction of lipid droplets. With IHC, both ACC and FASN levels were much lower in the tumors receiving the combination treatment than those in the tumors treated with either agent alone. Hence, the combination effectively also inhibits the SREBP1/ACC/FASN axis and lipid metabolism in AZD9291-resistant tumors.

AZD9291 combined with inhibition of SREBP1 enhances BIM-dependent apoptosis in AZD9291-resistant cells.

Modulation of Bim and Mcl-1 is a mechanism for AZD9291 to induce apoptosis in EGFRm NSCLC cells. Experiments were performed to determine the effects of AZD9291 and PF429242 on modulation of Bim and Mcl-1 in AZD9291-resistant cell lines. The combination of AZD9291 and PF429242 increased the levels of not only Bim, but also Mcl-1 in both PC-9/AR and HCC827/AR cell lines, whereas each single agent alone did not or minimally elevate the levels of these proteins. Consistently, AZD9291 apparently increased the levels of Bim and Mcl-1 in the two resistance cell lines in which SREBP1 was knocked down, but weakly in the corresponding pLKO.1 control cell lined. Thus, it is clear that inhibition of SREBP1 and AZD9291 combination enhances elevation of both Bim and Mcl-1 levels in AZD9291-resistant cell lines. In agreement with these in vitro findings, the increased levels of Bim, Mcl-1 and PARP cleavage were detected with Western blotting in PC-9/AR tumors treated with AZD9291 and PF429242 combination in comparison with tumors receiving single agent treatments and in PC-9/AR-shSREBP1 tumors comparing with PC-9/AR-pLKO.1 tumors receiving AZD9291 treatment. With IHC, the enhanced Bim elevation and PARP cleavage was confirmed in PC-9/AR tumors receiving AZD9291 and PF429242 co-treatment and in PC-9/AR-SREBP1 treated with AZD9291. Thus, inhibition of

SREBP1 combined with AZD9291 enhances Bim elevation with augmented induction of apoptosis in vivo in AZD9291-resistant tumors despite with an elevation of Mcl-1 levels.

Following these findings, experiments were performed to determine whether Bim elevation plays a key role in enhancing apoptosis by AZD9291 and PF429242 combination in AZD9291-resistant cells. To this end, Bim was knocked out in both PC-9/AR and HCC827/AR cell lines with CRISPR/cas9 technique. Its impact was tested on induction of apoptosis by the AZD9291 and PF429242 combination. The combination of AZD9291 and PF429242 significantly enhanced induction of apoptosis in PC-9 parental cell line evident with increased cleavage of caspase-3 and PARP and annexin V-positive populations, but not or minimally in all Bim-KO PC-9 cell lines. Similar results were also generated in HCC827/AR-Bim KO cell lines. Together, these findings indicate that combination of AZD9291 with SREBP1 inhibition enhances Bim-dependent apoptosis in AZD9291-resistant NSCLC cells.

CLAIMS

What is claimed:

1. A method of treating cancer comprising administering an effective amount of a kinase inhibitor of the epidermal growth factor receptor (EGFR) in combination with a SREBP1 inhibitor to a subject in need thereof.
2. The method of claim 1 wherein the EGFR inhibitor is N-(2-((2-(dimethylamino)ethyl)(methyl)amino)-4-methoxy-5-((4-(1-methyl-1H-indol-3-yl)pyrimidin-2-yl)amino)phenyl)acrylamide (osimertinib), derivative, ester, or salt thereof.
3. The method of claim 1 wherein SREBP1 inhibitor is 4-((diethylamino)methyl)-N-(2-methoxyphenethyl)-N-(pyrrolidin-3-yl)benzamide (PF-429242), derivative, ester, or salt thereof.
4. The method of claim 1 wherein SREBP1 inhibitor is 3a-(hydroxymethyl)-5a,5b,8,8,11a-pentamethyl-1-(prop-1-en-2-yl)icosahydro-1H-cyclopenta[a]chrysen-9-ol (betulin), derivative, ester, or salt thereof.
5. The method of claim 1 wherein SREBP1 inhibitor is 2-(2-propylpyridin-4-yl)-4-(p-tolyl)thiazole (fatostatin), derivative, ester, or salt thereof.
6. The method of claim 1 wherein the cancer is lung cancer.
7. The method of claim 1 wherein the cancer is non-small cell lung cancer.
8. A method of treating non-small cell lung cancer comprising administering N-(2-((2-(dimethylamino)ethyl)(methyl)amino)-4-methoxy-5-((4-(1-methyl-1H-indol-3-yl)pyrimidin-2-yl)amino)phenyl)acrylamide (osimertinib) or salt thereof in combination with 4-((diethylamino)methyl)-N-(2-methoxyphenethyl)-N-(pyrrolidin-3-yl)benzamide (PF-429242) or salt thereof.

9. A method of treating non-small cell lung cancer comprising administering N-(2-((2-(dimethylamino)ethyl)(methyl)amino)-4-methoxy-5-((4-(1-methyl-1H-indol-3-yl)pyrimidin-2-yl)amino)phenyl)acrylamide (osimertinib) or salt thereof in combination with 3a-(hydroxymethyl)-5a,5b,8,8,11a-pentamethyl-1-(prop-1-en-2-yl)icosahydro-1H-cyclopenta[a]chrysen-9-ol(betulin) or salt thereof.
10. A method of treating non-small cell lung cancer comprising administering N-(2-((2-(dimethylamino)ethyl)(methyl)amino)-4-methoxy-5-((4-(1-methyl-1H-indol-3-yl)pyrimidin-2-yl)amino)phenyl)acrylamide (osimertinib) or salt thereof in combination with 2-(2-propylpyridin-4-yl)-4-(p-tolyl)thiazole (fatostatin) or salt thereof.
11. A pharmaceutical composition comprising a kinase inhibitor of the epidermal growth factor receptor (EGFR) and a SREBP1 inhibitor.
12. The pharmaceutical composition of claim 12 wherein the EGFR inhibitor is N-(2-((2-(dimethylamino)ethyl)(methyl)amino)-4-methoxy-5-((4-(1-methyl-1H-indol-3-yl)pyrimidin-2-yl)amino)phenyl)acrylamide (osimertinib), derivative, ester, or salt thereof.
13. The pharmaceutical composition of claim 12 wherein the SREBP1 inhibitor is 4-((diethylamino)methyl)-N-(2-methoxyphenethyl)-N-(pyrrolidin-3-yl)benzamide (PF-429242), derivative, ester, or salt thereof.
14. The pharmaceutical composition of claim 12 wherein the SREBP1 inhibitor is 3a-(hydroxymethyl)-5a,5b,8,8,11a-pentamethyl-1-(prop-1-en-2-yl)icosahydro-1H-cyclopenta[a]chrysen-9-ol (betulin), derivative, ester, or salt thereof.
15. The pharmaceutical composition of claim 12 wherein the SREBP1 inhibitor is 2-(2-propylpyridin-4-yl)-4-(p-tolyl)thiazole (fatostatin), derivative, ester, or salt thereof.

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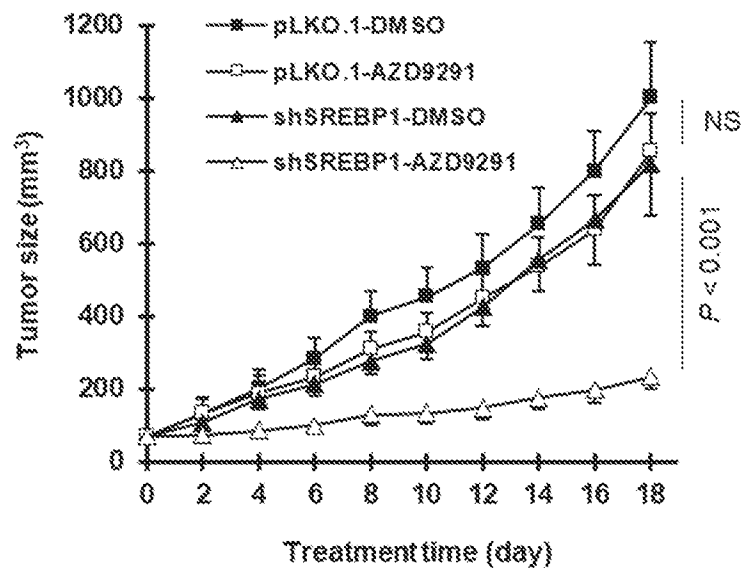


FIG. 1

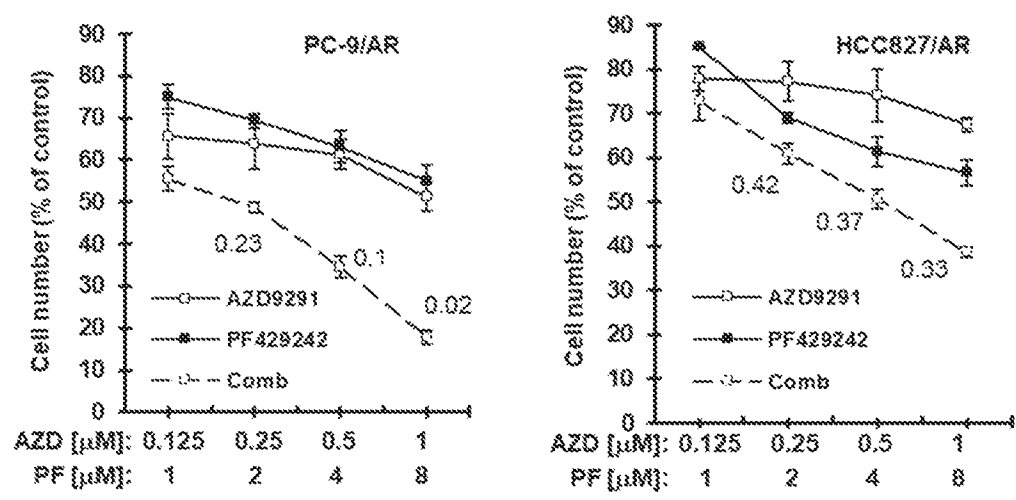


FIG. 2A

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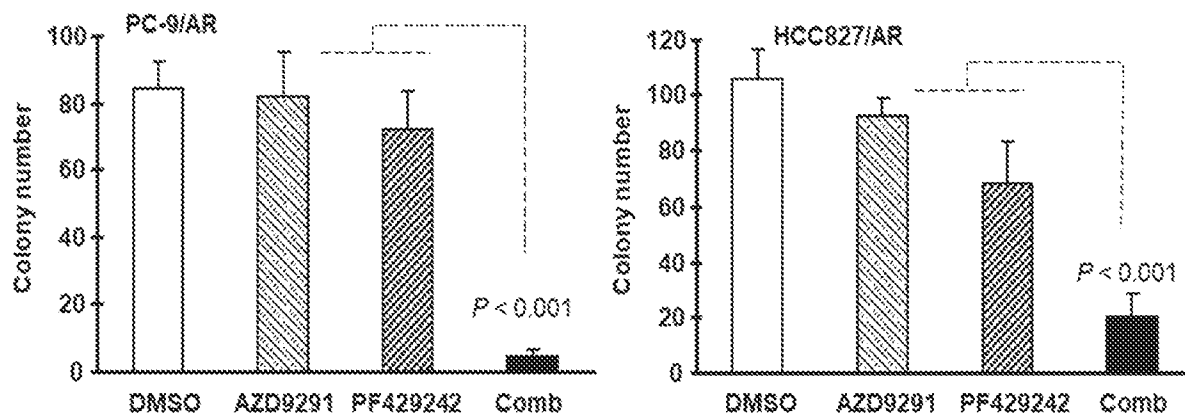


FIG. 2B

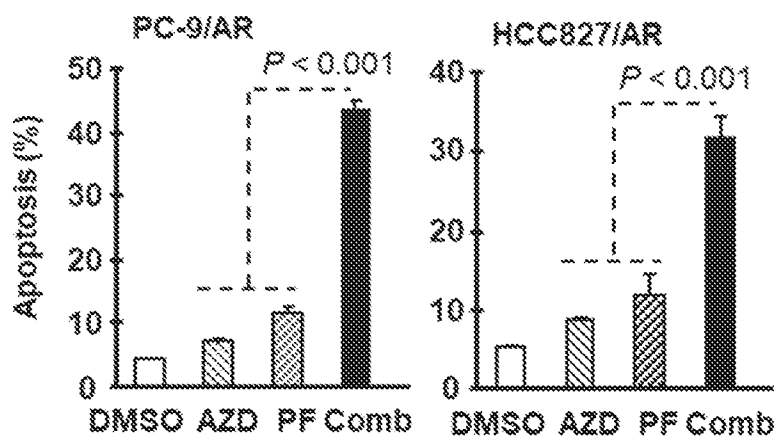


FIG. 2C

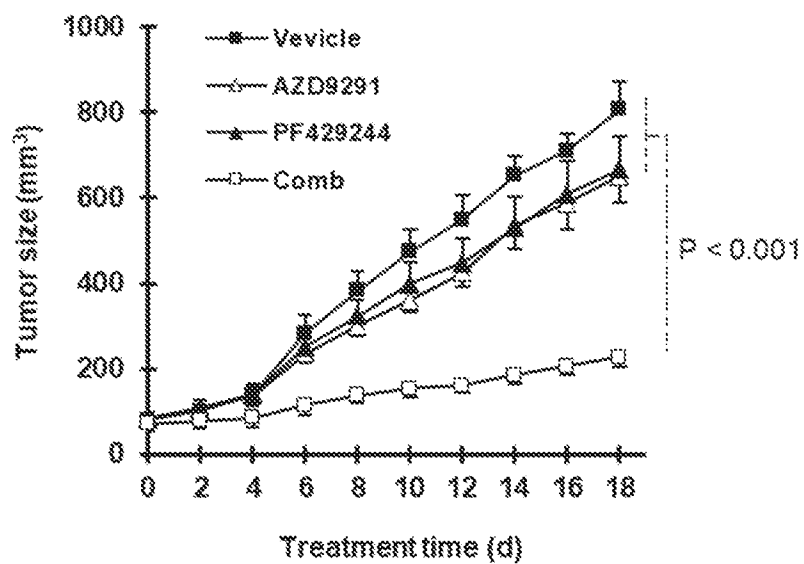


FIG. 2D

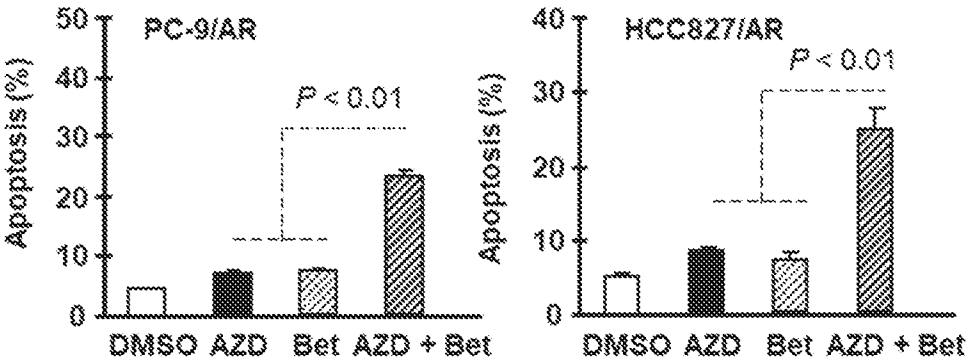


FIG. 3A

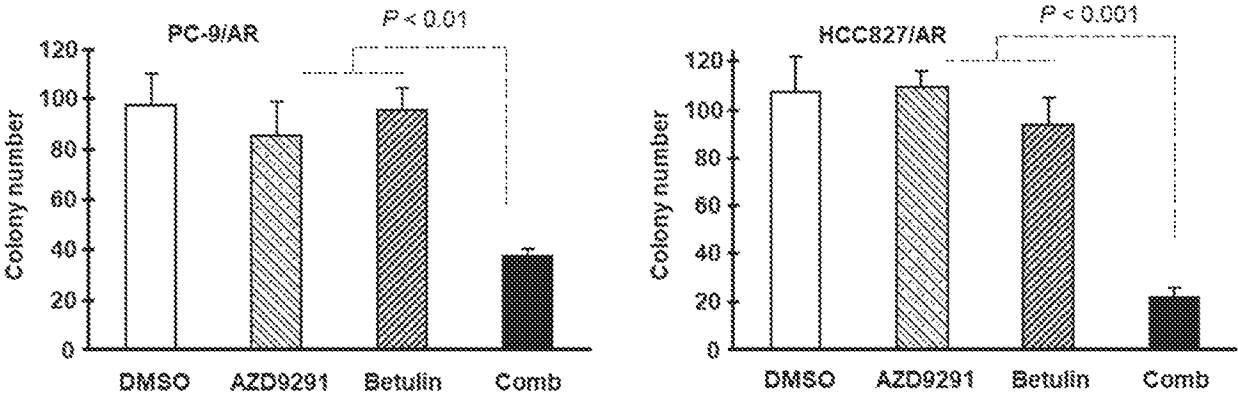


FIG. 3B

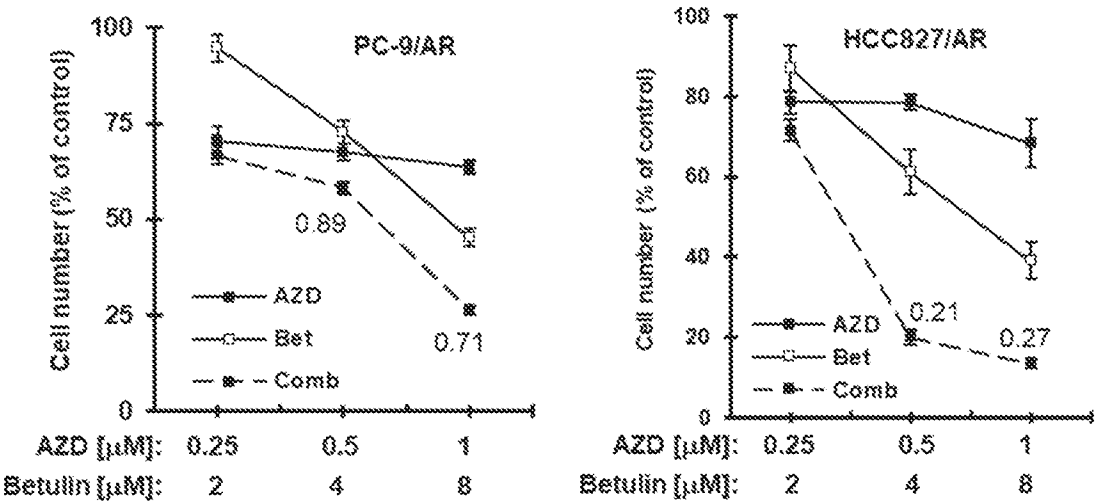


FIG. 3C

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2021/061851

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 31/132; A61K 31/40; A61K 31/4025; A61K 31/403; A61K 31/4439; A61K 31/496 (2022.01)

CPC - A61K 31/132; A61K 31/40; A61K 31/4025; A61K 31/403; A61K 31/4439; A61K 31/496; A61K 31/506; A61K 31/56; A61P 35/00 (2022.02)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

see Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

see Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

see Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	Li et al. Inhibition of SREBP increases gefitinib sensitivity in non-small cell lung cancer cells. Oncotarget 7(32): 52392-52403, 20 July 2016. [retrieved on 02.02.2022]. Retrieved on the Internet < https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5239560/pdf/oncotarget-07-52392.pdf >. entire document	1, 4-7, 11, 14, 15 --- 2, 3, 9, 10, 12, 13
Y	CN 108033950 B (HARBIN MEDICAL UNIVERSITY) 19 March 2019 (19.03.2019) entire document	2, 12
Y	CHENG et al. Lipid metabolism reprogramming and its potential targets in cancer. Cancer Communications 38(1): 27, 21 May 2018. [retrieved on 02.02.2022]. Retrieved from the Internet. < https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5993136/ >. entire document	3
Y	WO 2019/155059 A1 (ASTRAZENECA AB) 15 August 2019 (15.08.2019) entire document	8-10
Y	CHENG et al. SCAP/SREBPs are Central Players in Lipid Metabolism and Novel Metabolic Targets in Cancer Therapy. Current Topics in Medicinal Chemistry 18:1-10, 11 May 2018. [retrieved on 07.02.2022]. Retrieved from the Internet. < https://www.researchgate.net/profile/Xiang-Cheng-11/publication/325322504_SCAPSREBPs_are_Central_Players_in_Lipid_Metabolism_and_Novel_Metabolic_Targets_in_Cancer_Therapy/links/5b80704b299bf1d5a725a0c5/SCAP-SREBPs-are-Central-Players-in-Lipid-Metabolism-and-Novel-Metabolic-Targets-in-Cancer-Therapy.pdf >. entire document	8

☒ Further documents are listed in the continuation of Box C.☐ See patent family annex.

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

03 February 2022

Date of mailing of the international search report

MAR 09 2022

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2021/061851

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2020/077361 A1 (THE GENERAL HOSPITAL CORPORATION et al) 16 April 2020 (16.04.2020) entire document	13
P, X	CHEN et al. Induction of SREBP1 degradation coupled with suppression of SREBP1-mediated lipogenesis impacts the response of EGFR mutant NSCLC cells to osimertinib. Oncogene 40:6653-6665, 11 October 2021. [retrieved on 02.02.2022]. Retrieved from the Internet. < https://www.nature.com/articles/s41388-021-02057-0 >. entire document	1-15
P, X	XU et al. Lipidomics reveals that sustained SREBP-1-dependent lipogenesis is a key mediator of gefitinib-acquired resistance in EGFR-mutant lung cancer. Cell Death Discovery 7:353, 13 November 2021. [retrieved on 03.02.2022]. Retrieved in the Internet. < https://www.nature.com/articles/s41420-021-00744-1.pdf >	1-15