(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
23 July 2020 (23.07.2020)

WIPO | PCT

(10) International Publication Number
WO 2020/150480 A1

(51) International Patent Classification:
C12Q 1/6886 (2018.01) *A61K 31/00* (2006.01)
A61K 31/519 (2006.01)

(21) International Application Number:
PCT/US2020/013889

(22) International Filing Date:
16 January 2020 (16.01.2020)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/793,565 17 January 2019 (17.01.2019) US

(71) Applicant: **BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM** [US/US]; 210 West 7th St., Austin, TX 78701 (US).

(72) Inventors: **HEYMACH, John, V.**; C/o The University Of Texas Md Anderson, Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030 (US). **GALAN-COBO, Ana**; C/o The University Of Texas Md Anderson, Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030 (US).

(74) Agent: **FINDLAY, Geoffrey, S.**; Parker Highlander PLLC, 1120 So. Capital Of Texas Highway, Bldg. One, Suite 200, Austin, TX 78746 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*

WO 2020/150480 A1

(54) Title: METHODS FOR TREATMENT OF LUNG CANCERS

(57) Abstract: Provided are methods and compositions for the treatment of cancers. In some embodiments, NSCLC cancers that have a depletions of or a non-functional mutation in *STK11* /LKB1 and/or KEAP1/NRF2, optionally in combination with depletion of or a non- functional mutation in ATM, may be particularly susceptible to treatment with an ATM inhibitor (ATMi) and/or an ATR inhibitor (ATRi) such as, e.g., AZD0156 or AZD6738, optionally in combination with a PARP inhibitor.

DESCRIPTION

METHODS FOR TREATMENT OF LUNG CANCERS

BACKGROUND OF THE INVENTION

[0001] This application claims the benefit of United States Provisional Patent Application No. 62/793,565, filed January 17, 2019, the entirety of which is incorporated herein by reference. This invention was made with government support under grant numbers CA205150 and CA070907 awarded by the National Institutes of Health. The government has certain rights in the invention.

1. Field of the Invention

[0002] The present invention relates generally to the field of molecular biology and medicine. More particularly, it concerns methods for the treatment of cancers.

2. Description of Related Art

[0003] The serine/threonine kinase *STK11* (LKB1) is the second most commonly altered tumor suppressor in non-small cell lung cancer (NSCLC). Non-functional mutations or loss of LKB1 expression occur more frequently in NSCLC than other genetic alterations; however, there are currently no effective treatment strategies for this subset of tumors. KRAS-mutant LKB1 deficient NSCLC tumors often also have alterations in *KEAP1* or *NRF2* gene, which can activate the KEAP1/NRF2 pathway that can affect antioxidant responses. Unfortunately, however, there are currently no treatment strategies tailored for LKB1-deficient NSCLC.

[0004] Inhibitors of ATM and ATR, two proteins in the DNA damage response (DDR) pathway, are currently undergoing clinical testing. However, there are currently no biomarkers established for identifying which subgroups of patients that may be more likely to benefit from such treatments. Clearly there is a need for new methods of treating cancers.

SUMMARY OF THE INVENTION

[0005] The present invention overcomes limitations in the art by providing methods for the treatment of cancer. The invention is based, in part, on the observation that NSCLC cancers that have a depletion of or non-functional mutations in *STK11*/LKB1 and/or KEAP1/NRF2, optionally in combination with a depletion of or non-functional mutations in ATM (*e.g.*,

depletion of both LKB1 and ATM), may be particularly susceptible to treatment with an ATM inhibitor (ATMi) and/or an ATR inhibitor (ATRi) such as, *e.g.*, AZD0156 or AZD6738. In some aspects, it has been observed that deficiencies in both LKB1 and ATM can render a cancer particularly susceptible to treatment with an ATRi (*e.g.*, AZD6738), and experiments revealed that ATM deficiency in cancerous cells was associated with increased sensitivity to ATRi only when cells also had decreased or loss of functional LKB1. The ATMi or ATRi may optionally be administered in combination with a PARP inhibitor such as, *e.g.*, olaparib, rucaparib, or niraparib.

[0006] As shown in the below examples, alterations of LKB1 or the KEAP1/NRF2 pathway have been observed to be associated with enhanced response to ATM and ATR inhibitors (AMTi and ATRi) and other inhibitors of the DNA damage response (DDR) and may be useful biomarkers for predicting therapeutic response. To investigate the impact of LKB1 loss and KEAP1/NRF2 pathway activation on response to DDR inhibitors (DDRi), *in vitro* activity of ATM inhibitor in NSCLC murine cell lines was tested with or without knock out of LKB1 and/or KEAP1. In these cells, the loss of LKB1 and/or KEAP1 significantly sensitized cells to the ATMi AZD0156. In addition, the activity of the ATRi AZD6738 was evaluated in NSCLC cells with or without knockout of LKB1 and/or KEAP1. Cells deficient in LKB1 (KL) and/or KEAP1 (KLK/KK) were more sensitive to AZD0156 and AZD6738 than cells with intact LKB1 and KEAP1. Experiments were conducted to determine whether the activity of ATR and ATM inhibitors in KL, KK or KLK tumor cells could be enhanced by the addition of a PARP inhibitor (Olaparib). Although all NSCLC cells were partially resistant to the PARP inhibitor olaparib when used as a single agent, treatment of LKB1, KEAP1 or LKB1 plus KEAP1 deficient cells with the combination of olaparib plus ATM or ATR inhibitors (AZD0156 or AZD6738) significantly enhanced the antitumor cell activity of ATM or ATR inhibitors alone. These data were confirmed using an additional panel of LKB1 deficient NSCLC human cell lines (A549, H460 and H2030) treated with a broad spectrum of ATR and ATM inhibitors. In all human cell lines tested, re-expression of LKB1 clearly reduced the sensitivity to ATR inhibition. LKB1 loss was also associated with sensitivity to PARP and ATM inhibitor, although these effects seemed to be less significant compared with ATR inhibitors. *In vivo* experiments using syngeneic, patient derived xenograft (PDX) or GEMMs models also confirmed tumor growth impairment when LKB1 and KEAP1 were lost. To test the impact of ATM loss to DDR inhibitors NSCLC models, the effects of ATR (AZD6738), PARP (olaparib) and Wee1 (AZD1775) inhibitors were tested in ATM deficient (KA) or LKB1

plus ATM deficient (KLA) tumor cell lines. Although ATM has been reported as a marker for ATR inhibitor sensitivity, the data indicated that ATM loss significantly sensitized tumor cells to ATR and PARP inhibitors *in vitro* only when *STK11*/LKB1 was also lost. Likewise, *in vivo* treatment of mice bearing KA or KLA tumors with ATRi, revealed significantly tumor growth impairment only in *STK11*/LKB1 and ATM deficient tumors (KLA), but not in ATM deficient tumors (KA). Tumors with LKB1 deficiency and with KEAP/NRF2 mutations or ATM mutations, are often resistant to standard chemotherapy drugs and immunotherapy. The data indicate that LKB1, KEAP1/NRF2 and ATM loss can significantly enhance the sensitivity to ATR inhibitors, ATM inhibitors, and PARP inhibitors *in vitro,* showing a greater *in vivo* sensitivity when KEAP1 or ATM are co-mutated along with *STK11*/LKB1. Thus, in some embodiments, NSCLC tumors bearing *STK11* or KEAP1/NRF2 mutations, or tumors bearing *STK11*/LKB1 mutation(s) plus KEAP1/NRF2 or ATM mutation(s), can be highly sensitive to ATMi or ATRi. These genes may thus serve as biomarkers for selecting appropriate patients for treatment with an ATMi or ATRi, optionally in combination with an additional anti-cancer therapy such as PARPi, chemotherapy, or immunotherapy.

[0007] An aspect of the present invention relates to a method of treating a cancer in a mammalian subject, comprising administering to the subject a therapeutically effective dose of an ATM inhibitor (ATMi) or an ATR inhibitor (ATRi), wherein the cancer has been determined to have a loss-of-function mutation in, or reduced expression of, *STK11*(LKB1) and/or *KEAP1*(KEAP1). In some embodiments, the cancer has been determined to have a loss-of-function mutation in, or reduced expression of both *STK11*(LKB1) and ATM. In some embodiments, the cancer is a lung cancer, pancreatic cancer, endometrial cancer, breast cancer, or cervical cancer. The cancer may be a non-small cell lung cancer (NSCLC), pancreatic ductal adenocarcinoma, endometrial adenocarcinoma, or cervical cancer. In some embodiments, the lung cancer is a non-small cell lung cancer (NSCLC). In some embodiments, the subject is a human. The cancer may have a loss-of-function mutation or non-functional mutation in *STK11* and/or *KEAP1*. In some embodiments, the cancer has a loss-of-function mutation or non-functional mutation in *STK11*. In some embodiments, the cancer has a loss-of-function mutation or non-functional mutation in *ATM*, or reduced expression of ATM. The method may comprise administering to the subject a therapeutically effective dose of the ATR inhibitor (ATRi). In some embodiments, the cancer has a loss-of-function mutation or non-functional mutation in *KEAP1*. In some embodiments, the cancer has a mutation in *NFE2L2* (NRF2) that results in dysfunctional KEAP1-NRF2 interactions. The method may comprise administering

to the subject a therapeutically effective dose of an ATM inhibitor (ATMi). In some embodiments, the ATMi is AZD0156 AZD1390, Wortmannin, CP-466722, KU-55933, KU-60019, or KU-559403. In some embodiments, the ATMi is AZD0156. The method may further comprise administering to the subject a second anti-cancer therapy such as, *e.g.*, a surgery, an immunotherapy, a radiotherapy, a gene-therapy, or a chemotherapy. In some embodiments, the second anti-cancer therapy is a PARP inhibitor (*e.g.*, olaparib, rucaparib, niraparib, talazoparib (BMN-673), veliparib (ABT-888), olaparib (AZD-2281), Rucaparib (PF-01367338 or AG014699), veliparib (ABT-888), CEP 9722, E7016, or BGB-290). In some embodiments, the PARP inhibitor is olaparib, rucaparib, or niraparib. The method may further comprise administering a radiotherapy to the subject. In some embodiments, the method comprises administering to the subject a therapeutically effective dose of an ATR inhibitor (ATRi). In some embodiments, the ATRi is AZD6738 or BAY1895344, Schisandrin B, NU6027, NVP-BEZ235, VX-803, VX-970, VE-821, VE-822 (M6620), or AZ20. In some embodiments, the ATRi is AZD6738. The AZD6738 may be administered to the subject at a dose of about 240 mg twice daily. The method may further comprise administering to the subject a second anti-cancer therapy such as, *e.g.*, a surgery, an immunotherapy, a radiotherapy, a gene-therapy, or a chemotherapy. The second anti-cancer therapy may be a PARP inhibitor (*e.g.*, olaparib, rucaparib, niraparib, talazoparib (BMN-673), veliparib (ABT-888), olaparib (AZD-2281), Rucaparib (PF-01367338 or AG014699), veliparib (ABT-888), CEP 9722, E7016, or BGB-290). In some embodiments, the PARP inhibitor is olaparib, rucaparib, or niraparib. The method may further comprise administering a radiotherapy to the subject. The method may comprise administering to the subject a therapeutically effective dose of an ATM inhibitor (ATMi) as described herein or above and a therapeutically effective dose of an ATR inhibitor (ATRi) as described herein or above.

[0008] Another aspect of the present invention relates to a use of a composition comprising an effective dose of an ATM inhibitor (ATMi) or an ATR inhibitor (ATRi) for the treatment of a cancer that has been determined to have a mutation in or reduced expression of *STK11* and/or *KEAP1*. The cancer may be a lung cancer, pancreatic cancer, endometrial cancer, breast cancer, or cervical cancer; preferably a lung cancer such as a non-small cell lung cancer (NSCLC), pancreatic ductal adenocarcinoma, endometrial adenocarcinoma, or cervical cancer; or even more preferably a non-small cell lung cancer (NSCLC). The subject may be a human. In some embodiments, the cancer has a loss-of-function mutation or non-functional mutation in in *STK11* and ATM; preferably wherein the cancer has a mutation in *STK11* that results in

dysfunctional protein kinase activity by LKB1; and preferably wherein the cancer has a mutation in *ATM* that results in dysfunctional protein kinase activity by ATM. In some embodiments, the cancer has a loss-of-function mutation or non-functional mutation in in *STK11* and/or *KEAP1;* preferably wherein the cancer has a mutation in *NFE2L2* (NRF2) that results in dysfunctional KEAP1-NRF2 interactions or wherein the cancer has a mutation in *STK11* that results in dysfunctional protein kinase activity by LKB1. The composition may comprise a therapeutically effective dose of an ATM inhibitor (ATMi), such as AZD0156 AZD1390, Wortmannin, CP- 466722, KU-55933, KU-60019, or KU-559403, or preferably AZD0156. The composition may comprise a therapeutically effective dose of an ATR inhibitor (ATRi), such as AZD6738 or BAY1895344, Schisandrin B, NU6027, NVP-BEZ235, VX-803, VX-970, VE-821, VE-822 (M6620), or AZ20, or preferably AZD6738. The AZD6738 may be administered to the subject at a dose of about 240mg twice daily. A second anti-cancer therapy may be administered to the subject, such as a surgery, an immunotherapy, a radiotherapy, a gene-therapy, or a chemotherapy. The second anti-cancer therapy may be a PARP inhibitor, preferably olaparib, rucaparib, niraparib, talazoparib (BMN-673), veliparib (ABT-888), olaparib (AZD-2281), rucaparib (PF-01367338 or AG014699), veliparib (ABT-888), CEP 9722, E7016, or BGB-290; or even more preferably wherein the PARP inhibitor is olaparib, rucaparib, or niraparib. A radiotherapy may be administered to the subject.

[0009] As used herein, a "loss-of-function mutation" refers to a mutation (*e.g.*, a substitution, deletion, truncation, or frameshift mutation) that results in expression of a mutant protein that no longer exhibits wild-type activity (*e.g.*, reduced or eliminated wild-type biological activity or enzymatic activity), results in expression of only a fragment of the protein that no longer exhibits wild-type activity, or results in no expression of the wild-type protein. For example, a loss-of-function mutation affecting the *STK11* gene in a cancerous cell may result in the loss of expression of the LKB1 protein, expression of only a fragment of the LKB1 protein, or expression of a LKB1 protein that exhibits diminished or no enzymatic activity (*e.g.*, no serine/threonine kinase enzymatic activity) in the cancerous cell. Similarly, a loss-of-function mutation affecting the *KEAP1* gene in a cancerous cell may result in the loss of expression of the KEAP1 protein, expression of only a fragment of the KEAP1 protein, or expression of a KEAP1 protein that exhibits diminished or no activity (*e.g.*, inability to interact with or activate NRF2) in the cancerous cell. In some embodiments, the cancer has a loss-of-function mutation in *NFE2L2* (NRF2) that results in dysfunctional KEAP1-NRF2 interactions. Similarly, a loss-of-function mutation affecting the *ATM* gene in a cancerous cell may result in

the loss of expression of the ATM protein, expression of only a fragment of the ATM protein, or expression of a ATM protein that exhibits diminished or no activity (*e.g.*, reduced or eliminated serine/threonine kinase activity by the ATM) in the cancerous cell. A variety of mutations have been observed affecting the *STK11* gene that can result in dysfunctional or defective LKB1, e.g., as described in Launonen, 2005; Zaba *et al.*, 2013; Johnson *et al.*, 2012; and Gill *et al.*, 2011. A variety of mutations have been observed in the *KEAP1* gene that can result in dysfunctional or defective KEAP1, e.g., as described in Alizadeh *et al.,* 2017; Hast *et al.*, 2014; Choi *et al.*, 2017; Hellmann *et al.*, 2018; Zucman-Rossi *et al.*, 2015; Singh *et al.*, 2006; Chu *et al.*, 2018; and Rekhtman *et al.*, 2016. Mutations that can result in dysfunctional or defective ATM include Boultwood, 2001; Yuille *et al.*, 1998; Camacho *et al.*, 2002; Bullrich *et al.*, 1999; Lin 2012; Stankovic *et al.*, 2002; Starostik *et al.*, 1998; Wan and Wu, 2013; Beltran *et al.*, 2013; Ding *et al.*, 2008; Kim *et al.*, 2006; Davies *et al.*, 2005.

[0010] As used herein the specification, a "non-functional mutation" affecting a gene refers to a mutation (*e.g.*, a substitution, deletion, truncation, or frameshift mutation) that results in expression of a nonfunctional protein that has completely lost all wild-type activity. For example, a non-functional mutation in the *KEAP1* gene would result in expression of a mutant KEAP1 that is completely unable to interact with or activate NRF2. Similarly, a non-functional mutation in the *STK11* gene would result in expression of a mutant LKB1 that has none of the serine/threonine kinase enzymatic activity of wild-type LKB1. In some embodiments, the cancer has a non-functional mutation in *NFE2L2* (NRF2) that results in dysfunctional KEAP1-NRF2 interactions. Non-limiting examples of non-functional mutations in KEAP1 or *STK11* that are specifically contemplated are provided herein (e.g., Launonen (2005), Zaba *et al.* (2013), Johnson *et al.* (2012), Gill *et al.* (2011), Alizadeh *et al.* (2017), Hast *et al.* (2014), Choi *et al.* (2017), Hellmann *et al.* (2018), Zucman-Rossi *et al.* (2015), Singh *et al.* (2006), Chu *et al.* (2018), and/or Rekhtman *et al.* (2016)). Non-functional mutations in ATM include, *e.g.*, those described in Boultwood, 2001; Yuille *et al.*, 1998; Camacho *et al.*, 2002; Bullrich *et al.*, 1999; Lin 2012; Stankovic *et al.*, 2002; Starostik *et al.*, 1998; Wan and Wu, 2013; Beltran *et al.*, 2013; Ding *et al.*, 2008; Kim *et al.*, 2006; Davies *et al.*, 2005.

[0011] As used herein the specification, "a" or "an" may mean one or more. As used herein in the claim(s), when used in conjunction with the word "comprising", the words "a" or "an" may mean one or more than one.

[0012] The use of the term "or" in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or." As used herein "another" may mean at least a second or more.

[0013] Throughout this application, the term "about" is used to indicate that a value includes the inherent variation of error for the device, the method being employed to determine the value, or the variation that exists among the study subjects.

[0014] Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0016] FIGS. 1A-1C: LKB1 and KEAP1 loss drive sensitivity to ATM and ATR inhibitors. NSCLC KRAS mutant (K) with additional knock out of LKB1 (KL) and/or KEAP1 knock out (KLK or KK) cells were treated with decreased concentrations (μM) of PARPi (Olaparib), ATMi (AZD0156) and ATRi (AZD6738). Statistical significance: *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001.

[0017] FIGS. 2A-2D: PARPi improves efficacy of ATMi and ATRi alone. NSCLC cells were treated with decreased concentrations (μM) of PARPi (Olaparib), ATMi (AZD0156) and ATRi (AZD6738).

[0018] FIGS. 3A-3B: Sensitivity to combo PARPi plus ATMi or ATRi. NSCLC cells were treated with decreased concentrations (μM) of PARPi (Olaparib), ATMi (AZD0156) and ATRi (AZD6738). Statistical significance: *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001.

[0019] FIG. 4: ATR inhibition induces apoptosis in a LKB1 and KEAP1 dependent manner. Percentage of live cells quantify by Annexin-V/7AAD staining performed in LKR13 murine isogenic pairs treated with 1μM of PARPi (Olaparib), ATM inhibitor (AZD0156), ATR inhibitor (AZD6738) or combinations for 48 hours. Statistical significance: *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001.

[0020] FIG. 5: LKB1 re-expression reduces sensitivity to PARP, ATR and ATM inhibitors. Control LKB1 deficient and LKB1 re-expressing human cells were treated with decreased concentrations (μM) of PARPi (Olaparib), ATMi (AZD0156 and ATRi (AZD6738) and indicated combinations. Average of fold change in mean IC50 values (LKB1 proficient vs deficient) from 3 independent experiments are graphed.

[0021] FIG. 6: LKB1 re-expression reduces sensitivity to ATR inhibition. LKB1 deficient control and LKB1 re-expressing human cells were treated with decreased concentrations (μM) of the indicated ATR inhibitors. Average of fold change in mean IC50 values (LKB1 proficient vs deficient) from 3 independent experiments are graphed.

[0022] FIG. 7: LKB1 re-expression does not significantly reduce sensitivity to ATM inhibition. LKB1 deficient control (black) and LKB1 re-expressing human cells (grey) were treated with decreased concentrations (μM) of the indicated ATM inhibitors.

[0023] FIG. 8: LKB1 and KEAP1 loss drive sensitivity ATR inhibition. NSCLC KRAS mutant (K) with additional knock out of LKB1 (KL) and/or KEAP1 knock out (KLK or KK) cells were treated with decreased concentrations (μM) of the indicated ATR inhibitors. KK cells displayed the lowest % viability at 0.01 μM of VE-822, VE-821, BAY-1895344, and AZD6738.

[0024] FIG. 9: LKB1 and KEAP1 lost drive sensitivity ATM inhibition. NSCLC KRAS mutant (K) with additional knock out of LKB1 (KL) and/or KEAP1 knock out (KLK or KK) cells were treated with decreased concentrations (μM) of the indicated ATM inhibitors. KK cells displayed the lowest % viability at 0.01 μM of VE-822, VE-821, BAY-1895344, and AZD6738.

[0025] FIGS. 10A-F. LKB1 and KEAP1 loss cooperatively drive sensitivity ATR inhibition *in vivo*. Tumor volume measured in syngeneic models from subcutaneous injection of KL **(FIG. 10A)** and KLK **(FIG. 10B)** cell lines in 129SV immunocompetent mice. Tumor

volume measured in PDX derived from KL **(FIG. 10C)** and KLK **(FIG. 10D)** tumors in NSG immune deficient mice. Survival data from KL **(FIG. 10E)** and KLK **(FIG. 10F)** GEMMs models. Mice were dosed orally with AZD6738 or vehicle at dose schedule indicated for 28 days. All data are presented as mean ± standard error of the mean (error bars) for each group (n = 10-13). Statistical significance: *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001.

[0026] FIGS. 11A-D. LKB1 and ATM loss cooperatively drive sensitivity to PARP and ATR inhibitors *in vitro*. NSCLC KRAS mutant (K) with additional knock out of LKB1 (KL) and/or ATM knock out (KLA or KA) cells were treated with decreased concentrations (μM) of PARPi (Olaparib) **(FIG. 11A)**, WEE1i (AZD1775) **(FIG. 11B)**, ATRi (AZD6738) **(FIG. 11C)** and ATMi (AZD0156) **(FIG. 11D)**.

[0027] FIGS. 12A-B. LKB1 and ATM loss cooperatively drive sensitivity ATR inhibition *in vivo*. Tumor volume measured in syngeneic models from subcutaneous injection of KA **(FIG. 12A)** and KLA **(FIG. 12B)** cell lines in 129SV immunocompetent mice. Mice were dosed orally with AZD6738 or vehicle at dose schedule indicated for 28 days. All data are presented as mean ± standard error of the mean (error bars) for each group (n = 10-13). Statistical significance: *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001.

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

[0028] The present invention is based, in part, on the discovery that cancers such as lung cancers (*e.g.*, NSCLC) that contain one or more mutations in *STK11* and/or *KEAP1*, such as a loss of function mutation, may be particularly susceptible to treatment with an ATM inhibitor (ATMi) or an ATR inhibitor (ATRi). In some embodiments the ATMi and/or ATRi is administered to a mammalian subject, such as a human patient, in combination with a PARP inhibitor. In some aspects, cancers having both, a decrease in functional LKB1 and a decrease in functional ATM may be particularly susceptible to treatment with an ATRi. Since the serine/threonine kinase *STK11* (LKB1), is often altered or mutated in NSCLC, this therapeutic approach may be particularly useful for the treatment of NSCLC. Without wishing to be bound by any theory, the data provided herein supports the idea that *STK11*/LKB1 alterations may be a genomic driver of primary resistance to PD-1 axis inhibitors in *KRAS*-mutant lung adenocarcinoma. LKB1 deficient tumors (KL) are highly enriched in concurrent KEAP1 mutations activating the KEAP1/NRF2 pathway (KLK) and also ATM (KLA).

II. Biomarkers

[0029] In certain embodiments, the present disclosure concerns the detection of a mutation or expression of genes, such as *STK11*, *KEAP1*, and/or *ATM*. These genes can be used to predict response to an ATM inhibitor, an ATR inhibitor, or other inhibitors of the DNA damage response (DDR), such as for the treatment of cancer, specifically lung cancer. The genes may have a non-functional mutation or loss of expression, such as through non-mutational mechanism including genomic loss or promoter methylation. The loss of LKB1 and/or KEAP1, optionally in combination with loss of ATM, can sensitize cells to DDR inhibitors, such as ATM or ATR inhibition.

[0030] Serine/threonine kinase 11 (*STK11*), also named liver kinase B1 (LKB1), whose germline inactivation is responsible of the autosomal dominant Peutz-Jeghers syndrome is a tumor-suppressor gene frequently mutated in NSCLC. *STK11* controls the activity of AMP-activated protein kinase (AMPK) family members, thereby playing a role in various processes such as cell metabolism, cell polarity, apoptosis and DNA damage response. *STK11* acts by phosphorylating the T-loop of AMPK family proteins, thus promoting their activity. The *STK11* gene encodes the LKB1 protein, and is the second most commonly altered tumor suppressor in NSCLC. Non-functional mutations or loss of LKB1 expression (often through non-mutational mechanisms like genomic loss or promoter methylation) occur more frequently in NSCLC than other alterations such as EGFR, ALK, ROS, RET and BRAF combined.

[0031] Nuclear factor erythroid 2-related factor 2 (NRF2), also known as Nuclear factor (erythroid-derived 2)-like 2 (*NFE2L2*) and its natural inhibitor Kelch-like erythroid cell derived protein with CNC homology (ECH)-associated protein 1 (KEAP1) are essential in the regulation of cytoprotective and detoxifying defense systems, including phase I (cytochrome P450s) and phase II (detoxifying, and antioxidant proteins) enzymes. Alterations of genes in the KEAP1– NRF2 pathway are found in lung cancer and to be closely related with tumor progression. KEAP1 mainly contains five functional domains including the NTR (the N-terminal region), BTB (the broad complex, tramtrack, and bric-a-brac), IVR (intervening linker domain), DGR (double glycine/Keclch repeats) and CTR (the C-terminal region). The DC domain (including DGR and CTR) is responsible for binding to Neh2 of NRF2, while the BTB domain is required for the dimerization of two KEAP1molecules.

[0032] The Ataxia-telangiectasia gene (ATM) provides instructions for making the ATM protein that is located primarily in the nucleus of cells, where it can affect the rate of cell

proliferation. ATM can play a role in the development and activity of the nervous system and the immune system. The ATM protein also assists cells in recognizing damaged or broken DNA strands. Mutations in ATM have been observed in cancers such as breast cancers (Jerzak *et al.*, 2018), pancreatic cancer (Biankin *et al.,* 2012), and lung adenocarcinoma (Ding *et al.,* 2008). In colorectal cancer, loss of ATM protein expression is associated with worse prognosis, based on immunohistochemical analysis of stage II/III cancers (Beggs *et al.,* 2012; Biankin *et al.,* 2012*;* Ding *et al.,* 2008.

[0033] The present methods can comprise detecting somatic mutations, loss of heterozygosity, decreased expression, or DNA methylation in the promoter region of *STK11*, *KEAP1* or *ATM*. In some embodiments, inactivating mutations in *STK11* may arise in a variety of sites in a cancer.

[0034] The gene mutation or expression may be analyzed from a patient sample. The patient sample can be any bodily tissue or fluid that includes nucleic acids from the lung cancer in the subject. In certain embodiments, the sample will be a blood sample comprising circulating tumor cells or cell free DNA. In other embodiments, the sample can be a tissue, such as a lung tissue. The lung tissue can be from a tumor tissue and may be fresh frozen or formalin-fixed, paraffin-embedded (FFPE). In certain embodiments, a lung tumor FFPE sample is obtained.

[0035] Samples that are suitable for use in the methods described herein contain genetic material, *e.g.*, genomic DNA (gDNA). Genomic DNA is typically extracted from biological samples such as blood or mucosal scrapings of the lining of the mouth, but can be extracted from other biological samples including urine, tumor, or expectorant. The sample itself will typically include nucleated cells (*e.g.*, blood or buccal cells) or tissue removed from the subject including normal or tumor tissue. Methods and reagents are known in the art for obtaining, processing, and analyzing samples. In some embodiments, the sample is obtained with the assistance of a health care provider, *e.g.*, to draw blood. In some embodiments, the sample is obtained without the assistance of a health care provider, *e.g.*, where the sample is obtained non-invasively, such as a sample comprising buccal cells that is obtained using a buccal swab or brush, or a mouthwash sample.

[0036] In some cases, a biological sample may be processed for DNA isolation. For example, DNA in a cell or tissue sample can be separated from other components of the sample.

Cells can be harvested from a biological sample using standard techniques known in the art. For example, cells can be harvested by centrifuging a cell sample and resuspending the pelleted cells. The cells can be resuspended in a buffered solution such as phosphate-buffered saline (PBS). After centrifuging the cell suspension to obtain a cell pellet, the cells can be lysed to extract DNA, *e.g.*, gDNA. See, *e.g.*, Ausubel *et al.*, 2003. The sample can be concentrated and/or purified to isolate DNA. All samples obtained from a subject, including those subjected to any sort of further processing, are considered to be obtained from the subject. Routine methods can be used to extract genomic DNA from a biological sample, including, for example, phenol extraction. Alternatively, genomic DNA can be extracted with kits such as the QIAamp® Tissue Kit (Qiagen, Chatsworth, Calif.) and the Wizard® Genomic DNA purification kit (Promega). Non-limiting examples of sources of samples include urine, blood, and tissue. The biological sample may comprise or consist of cancerous cells or a tumor.

[0037] The presence or absence of mutations as described herein can be determined using methods known in the art. For example, gel electrophoresis, capillary electrophoresis, size exclusion chromatography, sequencing, and/or arrays can be used to detect the presence or absence of mutations. Amplification of nucleic acids, where desirable, can be accomplished using methods known in the art, *e.g.*, PCR. In one example, a sample (*e.g.*, a sample comprising genomic DNA), is obtained from a subject. The DNA in the sample is then examined to determine the identity of a mutation as described herein. A mutation can be detected by any method described herein, *e.g.*, by sequencing or by hybridization of the gene in the genomic DNA, RNA, or cDNA to a nucleic acid probe, *e.g.*, a DNA probe (which includes cDNA and oligonucleotide probes) or an RNA probe. The nucleic acid probe can be designed to specifically or preferentially hybridize with a particular variant.

[0038] A set of probes typically refers to a set of primers, usually primer pairs, and/or detectably-labeled probes that are used to detect the target genetic variations used in the actionable treatment recommendations of the present disclosure. The primer pairs are used in an amplification reaction to define an amplicon that spans a region for a target genetic variation for each of the aforementioned genes. The set of amplicons are detected by a set of matched probes. In an exemplary embodiment, the present methods may use TaqMan™ (Roche Molecular Systems, Pleasanton, Calif.) assays that are used to detect a set of target genetic variations. In one embodiment, the set of probes are a set of primers used to generate amplicons that are detected by a nucleic acid sequencing reaction, such as a next generation sequencing

reaction. In these embodiments, for example, AmpliSEQ™ (Life Technologies/Ion Torrent, Carlsbad, Calif.) or TruSEQ™ (Illumina, San Diego, Calif.) technology can be employed.

[0039] Analysis of nucleic acid markers can be performed using techniques known in the art including, without limitation, sequence analysis, and electrophoretic analysis. Non-limiting examples of sequence analysis include Maxam-Gilbert sequencing, Sanger sequencing, capillary array DNA sequencing, thermal cycle sequencing (Sears *et al.,* 1992), solid-phase sequencing (Zimmerman *et al.*, 1992), sequencing with mass spectrometry such as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS; Fu *et al.*, 1998), and sequencing by hybridization (Chee *et al.*, 1996; Drmanac *et al.*, 1993; Drmanac *et al.*, 1998). Non-limiting examples of electrophoretic analysis include slab gel electrophoresis such as agarose or polyacrylamide gel electrophoresis, capillary electrophoresis, and denaturing gradient gel electrophoresis. Additionally, next generation sequencing methods can be performed using commercially available kits and instruments from companies such as the Life Technologies/Ion Torrent PGM or Proton, the Illumina HiSEQ or MiSEQ, and the Roche/454 next generation sequencing system.

[0040] Other methods of nucleic acid analysis can include direct manual sequencing (Church and Gilbert, 1988; Sanger *et al.*, 1977; U.S. Patent No. 5,288,644); automated fluorescent sequencing; single-stranded conformation polymorphism assays (SSCP) (Schafer *et al.*, 1995); clamped denaturing gel electrophoresis (CDGE); two-dimensional gel electrophoresis (2DGE or TDGE); conformational sensitive gel electrophoresis (CSGE); denaturing gradient gel electrophoresis (DGGE) (Sheffield *et al.*, 1989); denaturing high performance liquid chromatography (DHPLC, Underhill *et al.*, 1997); infrared matrix-assisted laser desorption/ionization (IR-MALDI) mass spectrometry (WO 99/57318); mobility shift analysis; restriction enzyme analysis (Flavell *et al.*, 1978; Geever *et al.*, 1981); quantitative real-time PCR (Raca *et al.*, 2004); heteroduplex analysis; chemical mismatch cleavage (CMC) (Cotton *et al.*, 1985); RNase protection assays (Myers *et al.*, 1985); use of polypeptides that recognize nucleotide mismatches, *e.g.*, *E. coli* mutS protein; allele-specific PCR, and combinations of such methods. See, *e.g.*, U.S. Patent Publication No. 2004/0014095, which is incorporated herein by reference in its entirety.

[0041] In one example, a method of identifying a mutation in a sample comprises contacting a nucleic acid from said sample with a nucleic acid probe that is capable of specifically hybridizing to nucleic acid encoding a mutated protein, or fragment thereof

incorporating a mutation, and detecting said hybridization. In a particular embodiment, said probe is detectably labeled such as with a radioisotope (^{3}H, ^{32}P, or ^{33}P), a fluorescent agent (rhodamine, or fluorescein) or a chromogenic agent. In a particular embodiment, the probe is an antisense oligomer, for example PNA, morpholino-phosphoramidates, LNA or 2′-alkoxyalkoxy. The probe may be from about 8 nucleotides to about 100 nucleotides, or about 10 to about 75, or about 15 to about 50, or about 20 to about 30. In another aspect, said probes of the present disclosure are provided in a kit for identifying mutations in a sample, said kit comprising an oligonucleotide that specifically hybridizes to or adjacent to a site of mutation in the *STK11, KEAP1*, or *ATM* gene. The kit may further comprise instructions for treating patients having tumors that contain *STK11*, *KEAP1*, or *ATM* mutations with a DDR inhibitor based on the result of a hybridization test using the kit.

[0042] In another aspect, a method for detecting *STK11*, *KEAP1*, or *ATM* mutation in a sample comprises amplifying from said sample nucleic acids corresponding to *STK11*, *KEAP1*or *ATM* or a fragment thereof suspected of containing a mutation, and comparing the electrophoretic mobility of the amplified nucleic acid to the electrophoretic mobility of corresponding wild-type *STK11*, *KEAP1*, or *ATM* gene or fragment thereof. A difference in the mobility indicates the presence of a mutation in the amplified nucleic acid sequence. Electrophoretic mobility may be determined on polyacrylamide gel.

[0043] Alternatively, nucleic acids may be analyzed for detection of mutations using Enzymatic Mutation Detection (EMD) (Del Tito *et al.*, 1998). EMD uses the bacteriophage resolvase T_4 endonuclease VII, which scans along double-stranded DNA until it detects and cleaves structural distortions caused by base pair mismatches resulting from point mutations, insertions and deletions. Detection of two short fragments formed by resolvase cleavage, for example by gel electrophoresis, indicates the presence of a mutation. Benefits of the EMD method are a single protocol to identify point mutations, deletions, and insertions assayed directly from PCR reactions eliminating the need for sample purification, shortening the hybridization time, and increasing the signal-to-noise ratio. Mixed samples containing up to a 20-fold excess of normal DNA and fragments up to 4 kb in size can been assayed. However, EMD scanning does not identify particular base changes that occur in mutation positive samples requiring additional sequencing procedures to identity of the mutation if necessary. CEL I enzyme can be used similarly to resolvase T_4 endonuclease VII as demonstrated in U.S. Patent No. 5,869,245.

III. Methods of Treatment

[0044] Further provided herein are methods for treating or delaying progression of cancer in an individual comprising administering to the individual an effective amount of a DDR inhibitor (*e.g.*, an ATMi and/or ATRi), optionally in combination with a PARP inhibitor, a chemotherapy, or immunotherapy, to a subject determined to have decrease expression of or a mutation (*e.g.*, loss-of-function mutation, null mutation, *etc.*) of *STK11, KEAP1,* and/or *ATM* in a cancer such as, *e.g.*, a lung cancer, NSCLC, *etc.* The cancer in the subject may have more than one *STK11, KEAP1,* and/or *ATM* mutations.

[0045] Examples of cancers contemplated for treatment include lung cancer, head and neck cancer, breast cancer, pancreatic cancer, prostate cancer, renal cancer, bone cancer, testicular cancer, cervical cancer, gastrointestinal cancer, lymphomas, pre-neoplastic lesions in the lung, colon cancer, melanoma, and bladder cancer. In some embodiments, the cancer is lung cancer, and the lung cancer may be, *e.g.*, NSCLC.

[0046] NSCLC patients bearing *STK11*/LKB1 and/or KEAP1/NRF2 and/or ATM alteration(s) could be administered treatment with an ATM inhibitor (e.g., AZD0156) and/or ATR inhibitor (e.g., AZD6738), used as monotherapy or in combination with other treatments, for example immunotherapy, chemotherapy or other DDR inhibitors, such as a PARP inhibitor (e.g., Olaparib).

[0047] ATR inhibitors (ATRi) are highly suitable DNA repair inhibitors for use in the various aspects of the present invention. ATR inhibitors are believed to target the ATR kinase, which is a key protein in late G2 phase arrest and DNA repair. It is activated by DNA damage and will further activate the downstream protein Chk1 by phosphorylation, resulting in arrest and initiation of repair. As most cancer cells are defect in G1 phase of the cell cycle they are often dependent on G2 arrest for the repair of DNA. When G2 arrest is suppressed the cell will continue with mitosis without repair of damage, which may eventually lead to mitotic catastrophe. ATR inhibitors that may be administered include but are not limited to AZD6738, BAY1895344, Schisandrin B, NU6027, NVP-BEZ235, VX-803, VX-970 (also referred to as VE-822 or M6620), VE-821, and AZ20.

[0048] ATM serine/threonine kinase (ATM, also called ataxia-telangiectasia mutated kinase) is a serine/threonine kinase that is recruited and activated by DNA double-strand breaks. It can phosphorylate several proteins that can initiate activation of the DNA damage

checkpoint, leading to cell cycle arrest, DNA repair or apoptosis. ATM inhibitors that may be administered include but are not limited to AZD0156, AZD1390, Wortmannin, CP- 466722, KU-55933, KU-60019, and KU-559403.

[0049] The term "subject" or "patient" as used herein refers to any mammalian subject or patient to which the subject methods are performed. Generally, the patient is human, although as will be appreciated by those in the art, the patient may be an animal. Thus, other animals, including mammals such as rodents (including mice, rats, hamsters and guinea pigs), cats, dogs, rabbits, farm animals including cows, horses, goats, sheep, pigs, *etc.,* and primates (including monkeys, chimpanzees, orangutans and gorillas) are included within the definition of patient.

[0050] "Treatment" and "treating" refer to administration or application of a therapeutic agent to a subject or performance of a procedure or modality on a subject for the purpose of obtaining a therapeutic benefit of a disease or health-related condition. For example, a treatment may include administration chemotherapy, immunotherapy, radiotherapy, performance of surgery, or any combination thereof.

A. Parenteral Compositions and Formulations

[0051] In further embodiments, a compound of the present disclosure may be administered via a parenteral route. As used herein, the term "parenteral" includes routes that bypass the alimentary tract. Specifically, the pharmaceutical compositions disclosed herein may be administered for example, but not limited to intravenously, intradermally, intramuscularly, intraarterially, intrathecally, subcutaneous, or intraperitoneally U.S. Pat. Nos. 6,613,308, 5,466,468, 5,543,158; 5,641,515; and 5,399,363 (each specifically incorporated herein by reference in its entirety).

[0052] Solutions of the active compounds as free base or pharmacologically acceptable salts may be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions may also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms. The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions (U.S. Patent 5,466,468, specifically incorporated herein by reference in its

entirety). In all cases the form must be sterile and must be fluid to the extent that easy injectability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (*e.g.*, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and/or vegetable oils. Proper fluidity may be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

[0053] For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous, and intraperitoneal administration. In this connection, sterile aqueous media that can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage may be dissolved in isotonic NaCl solution and either added hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-1580). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject. Moreover, for human administration, preparations should meet sterility, pyrogenicity, general safety and purity standards as required by FDA Office of Biologics standards.

[0054] Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the

preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. A powdered composition is combined with a liquid carrier such as, e.g., water or a saline solution, with or without a stabilizing agent.

[0055] In some embodiments, the ATMi and/or ATRi is administered orally. For example, olaparib, AZD0156 and AZD6738 may be administered orally to a subject.

B. Combination Therapies

[0001] The methods and compositions, including combination therapies, enhance the therapeutic or protective effect, and/or increase the therapeutic effect of another anti-cancer or anti-hyperproliferative therapy. Therapeutic and prophylactic methods and compositions can be provided in a combined amount effective to achieve the desired effect, such as the killing of a cancer cell and/or the inhibition of cellular hyperproliferation. A tissue, tumor, or cell can be contacted with one or more ATMi and/or ATRi therapy in conjunction with a radiotherapy, chemotherapy, surgical therapy, or immunotherapy. For example, in some embodiments, the ATMi and/or ATRi therapy is administered to a subject with a lung cancer (*e.g.*, NSCLC) in combination with an immune checkpoint inhibitor or checkpoint inhibitor such as, *e.g.*, a PARP inhibitor (*e.g.*, olaparib (AZD-2281), rucaparib (PF-01367338), niraparib (MK-4827), talazoparib (BMN-673), veliparib (ABT-888), *etc.*). PARP inhibitors can cause increased DNA damage, particularly in tumors with existing defects in DNA repair.

[0002] Administration in combination can include simultaneous administration of two or more agents in the same dosage form, simultaneous administration in separate dosage forms, and separate administration. That is, the subject therapeutic composition and another therapeutic agent can be formulated together in the same dosage form and administered simultaneously. Alternatively, subject therapeutic composition and another therapeutic agent can be simultaneously administered, wherein both the agents are present in separate formulations. In another alternative, the therapeutic agent can be administered just followed by the other therapeutic agent or vice versa. In the separate administration protocol, the subject therapeutic composition and another therapeutic agent may be administered a few minutes apart, or a few hours apart, or a few days apart.

[0003] An anti-cancer first treatment may be administered before, during, after, or in various combinations relative to a second anti-cancer treatment. The administrations may be in

intervals ranging from concurrently to minutes to days to weeks. In embodiments where the first treatment is provided to a patient separately from the second treatment, one would generally ensure that a significant period of time did not expire between the time of each delivery, such that the two compounds would still be able to exert an advantageously combined effect on the patient. In such instances, it is contemplated that one may provide a patient with the first therapy and the second therapy within about 12 to 24 or 72 h of each other and, more particularly, within about 6-12 h of each other. In some situations, it may be desirable to extend the time period for treatment significantly where several days (2, 3, 4, 5, 6, or 7) to several weeks (1, 2, 3, 4, 5, 6, 7, or 8) lapse between respective administrations.

[0004] In certain embodiments, a course of treatment will last 1-90 days or more (this such range includes intervening days). It is contemplated that one agent may be given on any day of day 1 to day 90 (this such range includes intervening days) or any combination thereof, and another agent is given on any day of day 1 to day 90 (this such range includes intervening days) or any combination thereof. Within a single day (24-hour period), the patient may be given one or multiple administrations of the agent(s). Moreover, after a course of treatment, it is contemplated that there is a period of time at which no anti-cancer treatment is administered. This time period may last 1-7 days, and/or 1-5 weeks, and/or 1-12 months or more (this such range includes intervening days), depending on the condition of the patient, such as their prognosis, strength, health, *etc.* It is expected that the treatment cycles would be repeated as necessary.

[0005] The combination therapy may comprise other DNA damage response (DDR) inhibitors, such as PARP inhibitors. PARP1 has a role in repair of single-stranded DNA (ssDNA) breaks. PARP1 works by modifying nuclear proteins by poly ADP-ribosylation. It also works in conjunction with BRCA, which acts on double strands; members of the PARP family act on single strands; or, when BRCA fails, PARP can takes over those jobs as well (in a DNA repair context). PARP inhibitors include but are not limited to olaparib, rucaparib, niraparib, talazoparib (BMN-673), veliparib (ABT-888), olaparib (AZD-2281), Rucaparib (PF-01367338 or AG014699), veliparib (ABT-888), CEP 9722, E7016, and BGB-290.

[0006] The subject may be administered an immunotherapy in combination with the ATM or ATR inhibitor. In some embodiments, the immunotherapy may be an immune checkpoint inhibitor. Inhibitory immune checkpoints that may be targeted by immune checkpoint blockade include adenosine A2A receptor (A2AR), B7-H3 (also known as CD276),

B and T lymphocyte attenuator (BTLA), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4, also known as CD152), indoleamine 2,3-dioxygenase (IDO), killer-cell immunoglobulin (KIR), lymphocyte activation gene-3 (LAG3), programmed death 1 (PD-1), T-cell immunoglobulin domain and mucin domain 3 (TIM-3) and V-domain Ig suppressor of T cell activation (VISTA). In particular, the immune checkpoint inhibitors target the PD-1 axis and/or CTLA-4.

[0007] The immune checkpoint inhibitors may be drugs such as small molecules, recombinant forms of ligand or receptors, or, in particular, are antibodies, such as human antibodies (*e.g.,* International Patent Publication WO2015016718; Pardoll, 2012; both incorporated herein by reference). Known inhibitors of the immune checkpoint proteins or analogs thereof may be used, in particular chimerized, humanized or human forms of antibodies may be used. As the skilled person will know, alternative and/or equivalent names may be in use for certain antibodies mentioned in the present disclosure. Such alternative and/or equivalent names are interchangeable in the context of the present invention. For example, it is known that lambrolizumab is also known under the alternative and equivalent names MK-3475 and pembrolizumab.

[0008] In some embodiments, the PD-1 binding antagonist is a molecule that inhibits the binding of PD-1 to its ligand binding partners. In a specific aspect, the PD-1 ligand binding partners are PDL1 and/or PDL2. In another embodiment, a PDL1 binding antagonist is a molecule that inhibits the binding of PDL1 to its binding partners. In a specific aspect, PDL1 binding partners are PD-1 and/or B7-1. In another embodiment, the PDL2 binding antagonist is a molecule that inhibits the binding of PDL2 to its binding partners. In a specific aspect, a PDL2 binding partner is PD-1. The antagonist may be an antibody, an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or oligopeptide. Exemplary antibodies are described in U.S. Patent Nos. 8,735,553, 8,354,509, and 8,008,449, all incorporated herein by reference. Other PD-1 axis antagonists for use in the methods provided herein are known in the art such as described in U.S. Patent Publication Nos. US20140294898, US2014022021, and US20110008369, all incorporated herein by reference.

[0009] In some embodiments, the PD-1 binding antagonist is an anti-PD-1 antibody (*e.g.*, a human antibody, a humanized antibody, or a chimeric antibody). In some embodiments, the anti-PD-1 antibody is selected from the group consisting of nivolumab, pembrolizumab, and CT-011. In some embodiments, the PD-1 binding antagonist is an immunoadhesin (*e.g.,*

an immunoadhesin comprising an extracellular or PD-1 binding portion of PDL1 or PDL2 fused to a constant region (*e.g.*, an Fc region of an immunoglobulin sequence). In some embodiments, the PD-1 binding antagonist is AMP- 224. Nivolumab, also known as MDX-1106-04, MDX-1106, ONO-4538, BMS-936558, and OPDIVO®, is an anti-PD-1 antibody described in WO2006/121168. Pembrolizumab, also known as MK-3475, Merck 3475, lambrolizumab, KEYTRUDA®, and SCH-900475, is an anti-PD-1 antibody described in WO2009/114335. CT-011, also known as hBAT or hBAT-1, is an anti-PD-1 antibody described in WO2009/101611. AMP-224, also known as B7-DCIg, is a PDL2-Fc fusion soluble receptor described in WO2010/027827 and WO2011/066342.

[0010] Another immune checkpoint that can be targeted in the methods provided herein is the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), also known as CD152. The complete cDNA sequence of human CTLA-4 has the Genbank accession number L15006. CTLA-4 is found on the surface of T cells and acts as an "off" switch when bound to CD80 or CD86 on the surface of antigen-presenting cells. CTLA4 is a member of the immunoglobulin superfamily that is expressed on the surface of Helper T cells and transmits an inhibitory signal to T cells. CTLA4 is similar to the T-cell co-stimulatory protein, CD28, and both molecules bind to CD80 and CD86, also called B7-1 and B7-2 respectively, on antigen-presenting cells. CTLA4 transmits an inhibitory signal to T cells, whereas CD28 transmits a stimulatory signal. Intracellular CTLA4 is also found in regulatory T cells and may be important to their function. T cell activation through the T cell receptor and CD28 leads to increased expression of CTLA-4, an inhibitory receptor for B7 molecules.

[0011] In some embodiments, the immune checkpoint inhibitor is an anti-CTLA-4 antibody (*e.g.*, a human antibody, a humanized antibody, or a chimeric antibody), an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or oligopeptide.

[0012] Anti-human-CTLA-4 antibodies (or VH and/or VL domains derived therefrom) suitable for use in the present methods can be generated using methods well known in the art. Alternatively, art recognized anti-CTLA-4 antibodies can be used. For example, the anti-CTLA-4 antibodies disclosed in: U.S. Patent No. 8,119,129; International Patent Publication Nos. WO 01/014424, WO 98/42752, and WO 00/037504 (CP675,206, also known as tremelimumab; formerly ticilimumab); U.S. Patent No. 6,207,156; Hurwitz *et al.*, 1998; Camacho *et al.*, 2004; and Mokyr *et al.*, 1998 can be used in the methods disclosed herein. The teachings of each of the aforementioned publications are hereby incorporated by reference.

Antibodies that compete with any of these art-recognized antibodies for binding to CTLA-4 also can be used. For example, a humanized CTLA-4 antibody is described in International Patent Application Nos. WO2001014424, and WO2000037504, and U.S. Patent No. 8,017,114; all incorporated herein by reference.

[0013] An exemplary anti-CTLA-4 antibody is ipilimumab (also known as 10D1, MDX- 010, MDX- 101, and Yervoy®) or antigen binding fragments and variants thereof (see, *e.g.*, WO 01/014424). In other embodiments, the antibody comprises the heavy and light chain CDRs or VRs of ipilimumab. Accordingly, in one embodiment, the antibody comprises the CDR1, CDR2, and CDR3 domains of the VH region of ipilimumab, and the CDR1, CDR2 and CDR3 domains of the VL region of ipilimumab. In another embodiment, the antibody competes for binding with and/or binds to the same epitope on CTLA-4 as the above- mentioned antibodies. In another embodiment, the antibody has at least about 90% variable region amino acid sequence identity with the above-mentioned antibodies (*e.g.*, at least about 90%, 95%, or 99% variable region identity with ipilimumab).

[0014] Other molecules for modulating CTLA-4 include CTLA-4 ligands and receptors such as described in U.S. Patent Nos. 5,844,905, 5,885,796 and International Patent Application Nos. WO1995001994 and WO1998042752; all incorporated herein by reference, and immunoadhesins such as described in U.S. Patent No. 8,329,867, incorporated herein by reference.

IV. Examples

[0015] The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

EXAMPLE 1

LKB1 deficiency and KEAP1/NRF2 pathway alterations as biomarkers of response for ATR and ATM inhibitors and other inhibitors of DNA damage response (DDR) in in NSCLC

[0016] As described below, lung cancer cell lines were used to generate mutant cancerous cells that were knockout mutants for LKB1 and/or KEAP1. Knockout of LKB1 and/or KEAP1 resulted in enhanced sensitivity to ATMi and ATRi. While PARP inhibition produced little or no effect on viability of the cancer cells by itself, when the cancer cells were exposed to a PARP inhibitor in combination with the ATMi or ATRi, the PARP inhibitor further reduced viability of the cancer cells beyond the level of inhibition of viability of the cancer cells observed with the ATMi or ATRi alone. ATM loss was also observed to significantly increase PARPi sensitivity and ATRi sensitivity in a LKB1 dependent manner.

Methods

[0017] Using CRISPR Cas9 technology different isogenic pairs were made by knocking out expression of LKB1 ("KL" cells), KEAP1 ("KK" cells), both LKB1 and KEAP1 ("KLK" cells), ATM (KA), or both LKB1 and ATM (KLA) in a KRAS mutant murine primary cell line derived from lung adenocarcinoma. Drug screening and apoptosis assays were performed using different DNA damage response (DDR) inhibitors alone or in combination. To further test the impact of LKB1 on DDR, additional isogenic pairs were made by overexpressing LKB1 in a set of four LKB1 deficient human NSCLC cells (A549, H460 and H2030). Drug screening was performed using different DDR inhibitors. *In vivo* experiments were performed using syngeneic, PDX and GEMM models treated with ATRi AZD6738.

Results

[0018] *In vitro* drug screening assays were performed using murine NSCLC cell lines with or without additional knockout of LKB1 and/or KEAP1. NSCLC murine cells were partially resistant to PARP inhibition by olaparib, although LKB1 and KEAP1 loss significantly increased sensitivity (FIG. 1A). Knockout of LKB1 and/or KEAP1, induced enhanced sensitivity to ATM and ATR inhibitors, AZD0156 and AZD6738, respectively (FIG. 1B, C). Although all murine cells were partially resistant to PARP inhibition, this PARP inhibition by olaparib significantly enhanced sensitivity to ATM and ATR inhibition (FIG. 2A-D). Thus, knockout of LKB1 and/or KEAP1 resulted in enhanced sensitivity to ATM and ATR

inhibitors. Further, while PARP inhibition by olaparib produced little or no inhibition of the NSCLC murine cells by itself, this PARP inhibition further significantly enhanced the cancer cells sensitivity to ATM and ATR inhibition. Additionally, ATM loss was observed to significantly increase PARPi sensitivity and ATRi sensitivity in a LKB1 dependent manner.

[0019] The combination of PARPi+ATMi appeared most effective in KL cells followed by KLK and KK cells (FIG. 3A). Olaparib also enhanced the anti-tumor cell activity of the ATR inhibitor AZD6738 with greatest efficacy observed in KLK cells followed by KK and KL cells (FIG. 3B). Apoptosis assays were performed in LKR13 murine isogenic pairs exposed to 1μM of Olaparib, AZD6738 and AZD0156, or combinations for 48 hours. Cell death induction was higher after ATR inhibition alone or in combination with PARP inhibitor, compared with ATMi or PARPi used as single treatments. Interestingly, both LKB1 and KEAP1 loss was shown to enhance apoptosis induction after ATRi or PARPi+ATRi treatments, and consistently, cells lacking both genes displayed the greatest sensitivity (FIG. 4). Drug screening experiments were also performed using human isogenic pairs treated with the PARPi Olaparib and a variety of ATR and ATM inhibitors. In all the human isogenic pairs, re-expression of LKB1 reduced the sensitivity to Olaparib, ATRi (AZD6738), and ATMi (AZD0156), as well as to combinations (FIG. 5), to all ATR inhibitors tested (FIG. 6), and also to the ATMi AZD0156 (FIG. 7), being more pronounced and consistent with the differences found with all ATR inhibitors. Similar results were found using the murine NSCLC cells LKR13 (FIG. 8, FIG. 9). In addition to LKB1, KEAP1 loss was shown to drive sensitivity to ATR inhibitors as well as to ATM inhibitors.

[0020] *In vivo* experiments performed in syngeneic (FIGS. 10A-B), PDX (FIGS. 10C-D) and GEMMs (FIGS. 10E-F) models also confirmed greater sensitivity to ATR inhibition in tumors with both LKB1 and KEAP1 deficiency. On the other hand, single knock-out of ATM did not enhance sensitivity to DDR inhibitors Olaparib (PARPi) or AZD6738 (ATRi). Conversely, ATM loss significantly increased sensitivity to PARPi and ATRi in a LKB1 dependent manner (FIGS. 11 A-C). ATM loss did not raise sensitivity to Wee1i (AZD1775) (FIG. 11B). Treatments with ATMi (AZD0156) did not show any effects on those tumor cells lacking ATM expression across multiple experiments (Figure 11D). In contrast to reports that ATM deficiency may function as a biomarker for ATR inhibition (Min *et al.*, 2017), this data indicates that ATM deficiency only increases sensitivity to ATRi when functional LKB1 is

also lost in cancerous cells. Consistent with the *in vitro* data, *in vivo* administration of ATRi significantly impaired tumor growth in KLA but not in KA syngeneic models (FIGS. 12A-B).

* * *

[0021] All of the methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

REFERENCES

The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

U.S. Patent No. 5,288,644
U.S. Patent No. 5,399,363
U.S. Patent No. 5,466,468
U.S. Patent No. 5,543,158
U.S. Patent No. 5,641,515
U.S. Patent No. 5,844,905
U.S. Patent No. 5,869,245
U.S. Patent No. 5,885,796
U.S. Patent No. 6,207,156
U.S. Patent No. 6,613,308
U.S. Patent No. 8,008,449
U.S. Patent No. 8,017,114
U.S. Patent No. 8,119,129
U.S. Patent No. 8,329,867
U.S. Patent No. 8,354,509
U.S. Patent No. 8,735,553
U.S. Patent Publication No. 2004-0014095
U.S. Patent Publication No. 2011-0008369
U.S. Patent Publication No. 2014-0022021
U.S. Patent Publication No. 2014-0294898

International Patent Publication No. WO 1995/001994
International Patent Publication No. WO 1998/042752
International Patent Publication No. WO 1999/057318
International Patent Publication No. WO 2000/037504
International Patent Publication No. WO 2001/014424
International Patent Publication No. WO 2006/121168
International Patent Publication No. WO 2009/101611
International Patent Publication No. WO 2009/114335
International Patent Publication No. WO 2010/027827

International Patent Publication No. WO 2011/066342
International Patent Publication No. WO 2015/016718

Alizadeh *et al. Cancer Discov.*, Jan;7(1):86-101, 2017.
Ausubel *et al.*, Current Protocols in Molecular Biology, John Wiley & Sons, New York, NY, 2003.
Beggs *et al., Oncotarget*;3:1348–55, 2012.
Beltran *et al., Eur Urol*;63:920–6, 2013.
Biankin *et al., Nature*;491:399–405, 2012.
Boultwood., *J Clin Pathol.*;54:512–6, 2001.
Bullrich *et al., Cancer Res*;59:24–7, 1999.
Camacho *et al.*, *J Clin Oncology* 22(145): Abstract No. 2505 (antibody CP-675206), 2004.
Camacho E, *et al., Blood*;99:238–44, 2002.
Chee *et al.*, *Science,* 274:610-614, 1996.
Choi *et al.* Oncogene;36(37):5285-5295., 2017/
Chu *et al. Eur Rev Med Pharmacol Sci*; 22(14):4458-4466, 2018.
Church and Gilbert, *Proc. Natl. Acad. Sci. USA* 81:1991-1995, 1988.
Cotton *et al.*, *Proc. Natl. Acad. Sci. USA* 85:4397-4401, 1985.
Davies. *et al., Cancer Res.*; 65, 7591–7595, 2005.
Del Tito *et al.*, *Clinical Chemistry* 44:731-739, 1998.
Ding *et al., Nature*;455:1069–75, 2008.
Drmanac et al., Nat. Biotechnol., 16:54-58, 1998.
Drmanac et al., Science, 260:1649-1652, 1993.
Flavell *et al.*, *Cell* 15:25, 1978.
Fu *et al.*, *Nat. Biotechnol.,* 16:381-384, 1998.
Geever *et al.*, *Proc. Natl. Acad. Sci. USA* 78:5081, 1981.
Genbank accession number L15006.
Gill *et al. Oncogene* ; Sep 1;30(35):3784-91, 2011.
Hast *et al. Cancer Res.* ; Feb 1;74(3):808-17, 2014.
Hellmann *et al. Clin Cancer Res.* ; Jan 15;24(2):334-340, 2018.
Hurwitz *et al.*, *Proc Natl Acad Sci USA* 95(17): 10067-10071, 1998.
Jerzak *et al.*, *Curr Oncol.* Apr;25(2):e176-e180, 2018.
Johnson *et al., Biochem Res Int.* 2012:940405, 2012.
Kim *et al., Hum. Mol. Genet.*; 15, 1181–1186, 2006.
Launonen, *Hum Mutat.* Oct;26(4):291-7, 2005.
Lin *et al., Clin Cancer Res*;18:4191–200, 2012.
Min *et al.*, *Molecular Cancer Therapeutics* 16:566-577, 2017.

Mokyr *et al.*, *Cancer Res* 58:5301-5304, 1998.

Myers *et al.*, *Science* 230:1242, 1985.

Pardoll, *Nat Rev Cancer,* 12(4): 252-64, 2012.

Raca *et al.*, *Genet Test* 8(4):387-94, 2004.

Rekhtman *et al. Clin Cancer Res.* ; Jul 15;22(14):3618-29, 2016.

Remington's Pharmaceutical Sciences 15th Edition.

Sanger *et al.*, *Proc. Natl. Acad. Sci. USA* 74:5463-5467, 1977.

Schafer *et al.*, *Nat. Biotechnol.* 15:33-39, 1995.

Sears *et al., Biotechniques,* 13:626-633, 1992.

Sheffield *et al.*, *Proc. Natl. Acad. Sci. USA* 86:232-236, 1989.

Singh *et al. PLoS Med.* ; Oct;3(10):e420, 2006.

Stankovic *et al., Blood*;99:300–9, 2002.

Starostik *et al., Cancer Res*;58:4552–7, 1998.

Underhill *et al.*, *Genome Res.* 7:996-1005, 1997.

Wan and Wu. (*Blood*;121:4627–34, 2013.

Yuille *et al., Oncogene*;16:789–96, 1998.

Zaba *et al. Lung Cancer.*; Nov;82(2):368-9, 2013.

Zimmerman *et al., Methods Mol. Cell. Biol.,* 3:39-42, 1992.

Zucman-Rossi *et al. Gastroenterology.* ; Oct;149(5):1226-1239.e4, 2015.

CLAIMS

1. A method of treating a cancer in a mammalian subject, comprising administering to the subject a therapeutically effective dose of an ATM inhibitor (ATMi) or an ATR inhibitor (ATRi), wherein the cancer has been determined to have a loss-of-function mutation in, or reduced expression of, *STK11*(LKB1) and/or *KEAP1*(KEAP1).

2. The method of claim 1, wherein the cancer is a lung cancer, pancreatic cancer, endometrial cancer, breast cancer, or cervical cancer.

3. The method of claim 2, wherein the cancer is a non-small cell lung cancer (NSCLC), pancreatic ductal adenocarcinoma, endometrial adenocarcinoma, or cervical cancer.

4. The method of claim 3, wherein the lung cancer is a non-small cell lung cancer (NSCLC).

5. The method of claim 1, wherein the subject is a human.

6. The method of claim 1, wherein the cancer has a loss-of-function mutation or non-functional mutation in *STK11* and/or *KEAP1*.

7. The method of claim 1, wherein the cancer has a loss-of-function mutation or non-functional mutation in *STK11*.

8. The method of any one of claims 6-7, wherein the cancer a loss-of-function mutation or non-functional mutation in *ATM*, or reduced expression of ATM.

9. The method of claim 8, wherein the method comprises administering to the subject a therapeutically effective dose of the ATR inhibitor (ATRi).

10. The method of claim 1, wherein the cancer has a loss-of-function mutation or non-functional mutation in *KEAP1*.

11. The method of claim 1, wherein the cancer has a mutation in *NFE2L2* (NRF2) that results in dysfunctional KEAP1-NRF2 interactions.

12. The method of any one of claims 1-11, wherein the method comprises administering to the subject a therapeutically effective dose of an ATM inhibitor (ATMi).

13. The method of claim 12, wherein the ATMi is AZD0156 AZD1390, Wortmannin, CP-466722, KU-55933, KU-60019, or KU-559403.

14. The method of claim 13, wherein the ATMi is AZD0156.

15. The method of claim 12, wherein the method further comprises administering to the subject a second anti-cancer therapy.

16. The method of claim 15, wherein the second anti-cancer therapy is a surgery, an immunotherapy, a radiotherapy, a gene-therapy, or a chemotherapy.

17. The method of claim 16, wherein the second anti-cancer therapy is a PARP inhibitor.

18. The method of claim 17, wherein the PARP inhibitor is olaparib, rucaparib, niraparib, talazoparib (BMN-673), veliparib (ABT-888), olaparib (AZD-2281), Rucaparib (PF-01367338 or AG014699), veliparib (ABT-888), CEP 9722, E7016, or BGB-290.

19. The method of claim 18, wherein the PARP inhibitor is olaparib, rucaparib, or niraparib.

20. The method of claim 17, wherein the method further comprises administering a radiotherapy to the subject.

21. The method of any one of claims 1-11, wherein the method comprises administering to the subject a therapeutically effective dose of an ATR inhibitor (ATRi).

22. The method of claim 21, wherein the ATRi is AZD6738 or BAY1895344, Schisandrin B, NU6027, NVP-BEZ235, VX-803, VX-970, VE-821, VE-822 (M6620), or AZ20.

23. The method of claim 23, wherein the ATRi is AZD6738.

24. The method of claim 14, wherein the AZD6738 is administered to the subject at a dose of about 240 mg twice daily.

25. The method of claim 21, wherein the method further comprises administering to the subject a second anti-cancer therapy.

26. The method of claim 25, wherein the second anti-cancer therapy is a surgery, an immunotherapy, a radiotherapy, a gene-therapy, or a chemotherapy.

27. The method of claim 26, wherein the second anti-cancer therapy is a PARP inhibitor.

28. The method of claim 27, wherein the PARP inhibitor is olaparib, rucaparib, niraparib, talazoparib (BMN-673), veliparib (ABT-888), olaparib (AZD-2281), Rucaparib (PF-01367338 or AG014699), veliparib (ABT-888), CEP 9722, E7016, or BGB-290.

29. The method of claim 28, wherein the PARP inhibitor is olaparib, rucaparib, or niraparib.

30. The method of claim 27, wherein the method further comprises administering a radiotherapy to the subject.

31. The method of any one of claims 1-11, wherein the method comprises administering to the subject a therapeutically effective dose of an ATM inhibitor (ATMi) as described in any one of claims 13-20 and a therapeutically effective dose of an ATR inhibitor (ATRi) as described in any one of claims 22-30.

32. Use of a composition comprising an effective dose of an ATM inhibitor (ATMi) or an ATR inhibitor (ATRi) for the treatment of a cancer that has been determined to have a mutation in or reduced expression of *STK11* and/or *KEAP1*.

33. The use of claim 32, wherein the cancer is a lung cancer, pancreatic cancer, endometrial cancer, breast cancer, or cervical cancer; preferably a lung cancer such as a non-small cell lung cancer (NSCLC), pancreatic ductal adenocarcinoma, endometrial adenocarcinoma, or cervical cancer; or even more preferably a non-small cell lung cancer (NSCLC).

34. The use of any one of claims 32-33, wherein the subject is a human.

35. The use of any one of claims 32-34, wherein the cancer has a loss-of-function mutation or non-functional mutation in in *STK11* and *ATM;* preferably wherein the cancer has a mutation in *STK11* that results in dysfunctional protein kinase activity by LKB1; and preferably wherein the cancer has a mutation in *ATM* that results in dysfunctional protein kinase activity by ATM.

36. The use of any one of claims 32-34, wherein the cancer has a loss-of-function mutation or non-functional mutation in in *STK11* and/or *KEAP1;* preferably wherein the cancer has a mutation in *NFE2L2* (NRF2) that results in dysfunctional KEAP1-NRF2 interactions or wherein the cancer has a mutation in *STK11* that results in dysfunctional protein kinase activity by LKB1.

37. The use of any one of claims 32-36, wherein the composition comprises a therapeutically effective dose of an ATM inhibitor (ATMi), such as AZD0156 AZD1390, Wortmannin, CP- 466722, KU-55933, KU-60019, or KU-559403, or preferably AZD0156.

38. The use of any one of claims 32-37, wherein the composition comprises a therapeutically effective dose of an ATR inhibitor (ATRi), such as AZD6738 or BAY1895344, Schisandrin B, NU6027, NVP-BEZ235, VX-803, VX-970, VE-821, VE-822 (M6620), or AZ20, or preferably AZD6738.

39. The use of claim 38, wherein AZD6738 is administered to the subject at a dose of about 240mg twice daily.

40. The use of any one of claims 32-39, wherein a second anti-cancer therapy is administered to the subject, such as a surgery, an immunotherapy, a radiotherapy, a gene-therapy, or a chemotherapy.

41. The use of claim 40, wherein the second anti-cancer therapy is a PARP inhibitor, preferably olaparib, rucaparib, niraparib, talazoparib (BMN-673), veliparib (ABT-888), olaparib (AZD-2281), Rucaparib (PF-01367338 or AG014699), veliparib (ABT-888), CEP 9722, E7016, or BGB-290; or even more preferably wherein the PARP inhibitor is olaparib, rucaparib, or niraparib.

42. The use of any one of claims 32-41, wherein a radiotherapy is administered to the subject.


Olaparib (PARPi)
IC50 (μM)
150
100
50
0
***
**
**
**
ns
Mutant
Knock-out
K
KL
KK
KLK
Kras
Stk11/Lkb1
Keap1
AZD0156 (ATMi)
IC50 (μM)
4
3
2
1
0
***
***
**
*
Mutant
Knock-out
K
KL
KK
KLK
Kras
Stk11/Lkb1
Keap1

FIGS. 1A-B

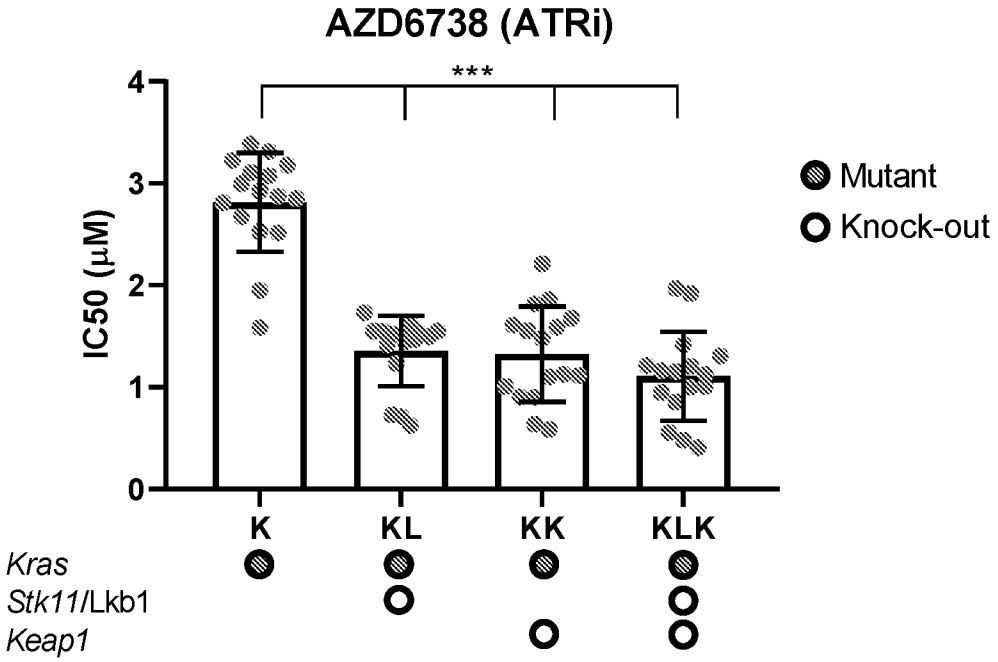
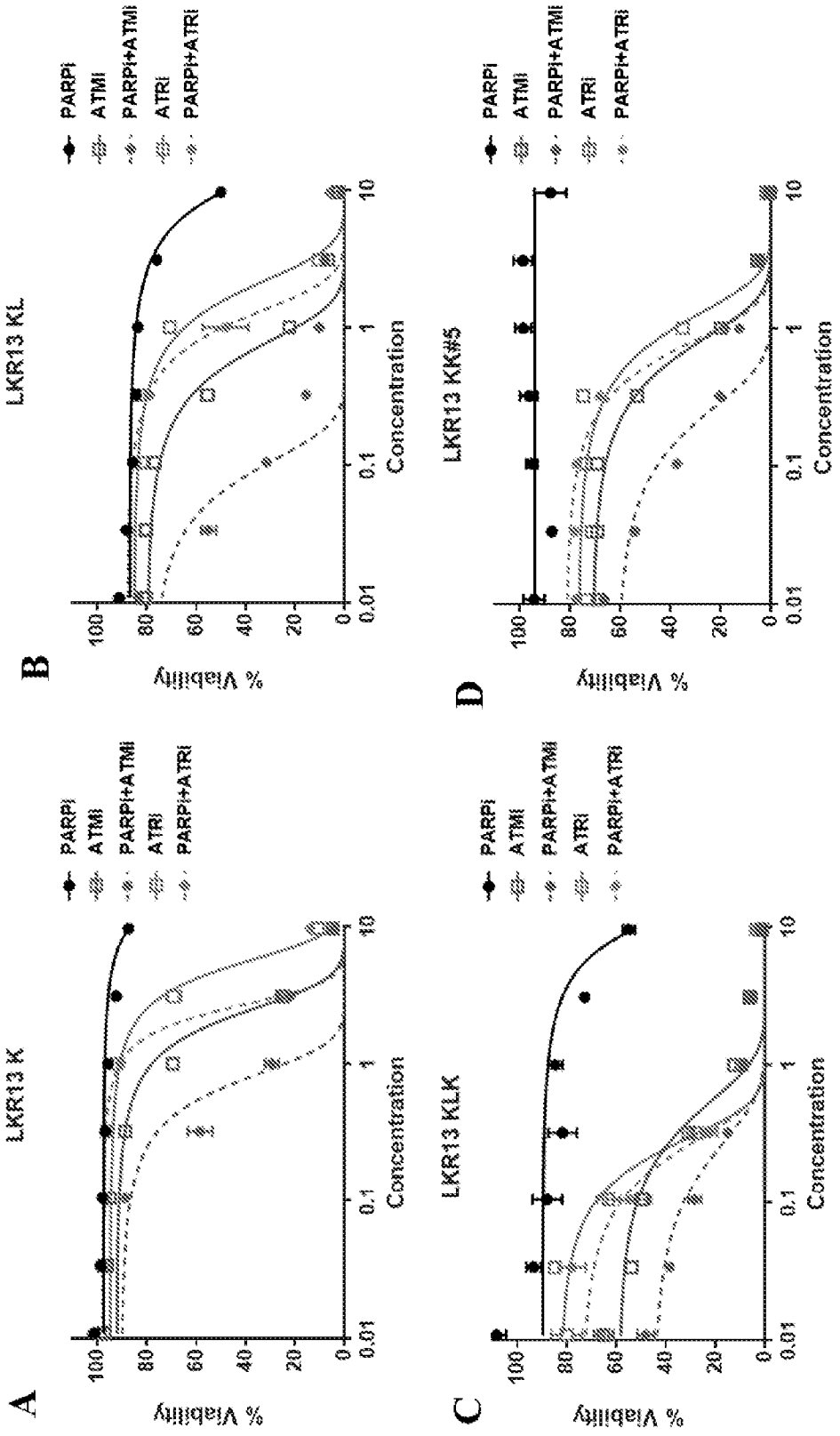
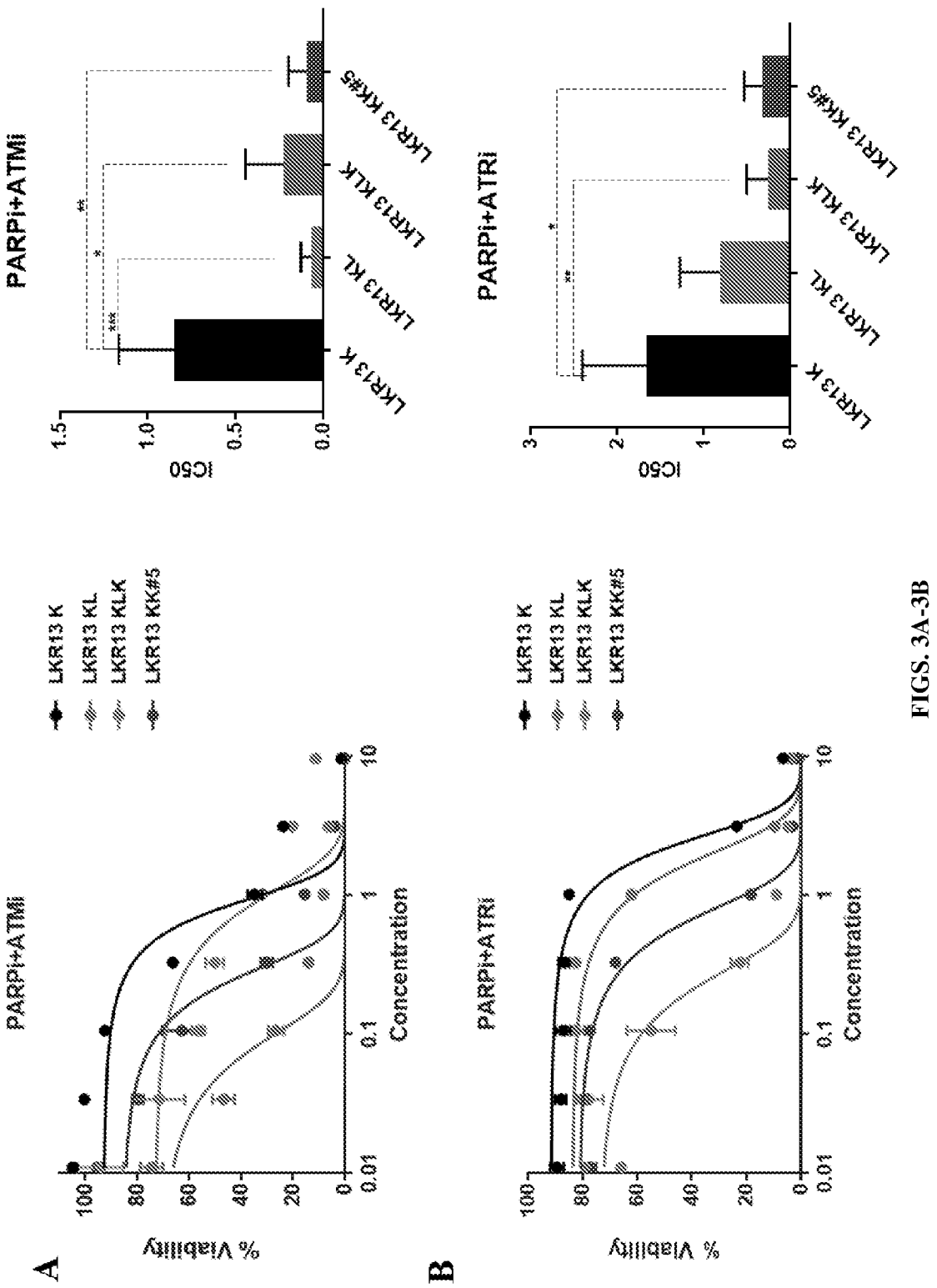

AZD6738 (ATRi)
***
IC50 (μM)
0
1
2
3
4
K
KL
KK
KLK
Kras
Stk11/Lkb1
Keap1
Mutant
Knock-out

FIG. 1C


A
LKR13 K
B
LKR13 KL
C
LKR13 KLK
D
LKR13 KK#5
PARPi
ATMi
PARPi+ATMi
ATRi
PARPi+ATRi
% Viability
Concentration
0
20
40
60
80
100
0.01
0.1
1
10

FIGS. 2A-2D


A
PARPi+ATMi
% Viability
Concentration
LKR13 K
LKR13 KL
LKR13 KLK
LKR13 KK#5
B
PARPi+ATRi
% Viability
Concentration
LKR13 K
LKR13 KL
LKR13 KLK
LKR13 KK#5
PARPi+ATMi
IC50
LKR13 K
LKR13 KL
LKR13 KLK
LKR13 KK#5
PARPi+ATRi
IC50
LKR13 K
LKR13 KL
LKR13 KLK
LKR13 KK#5

FIGS. 3A-3B

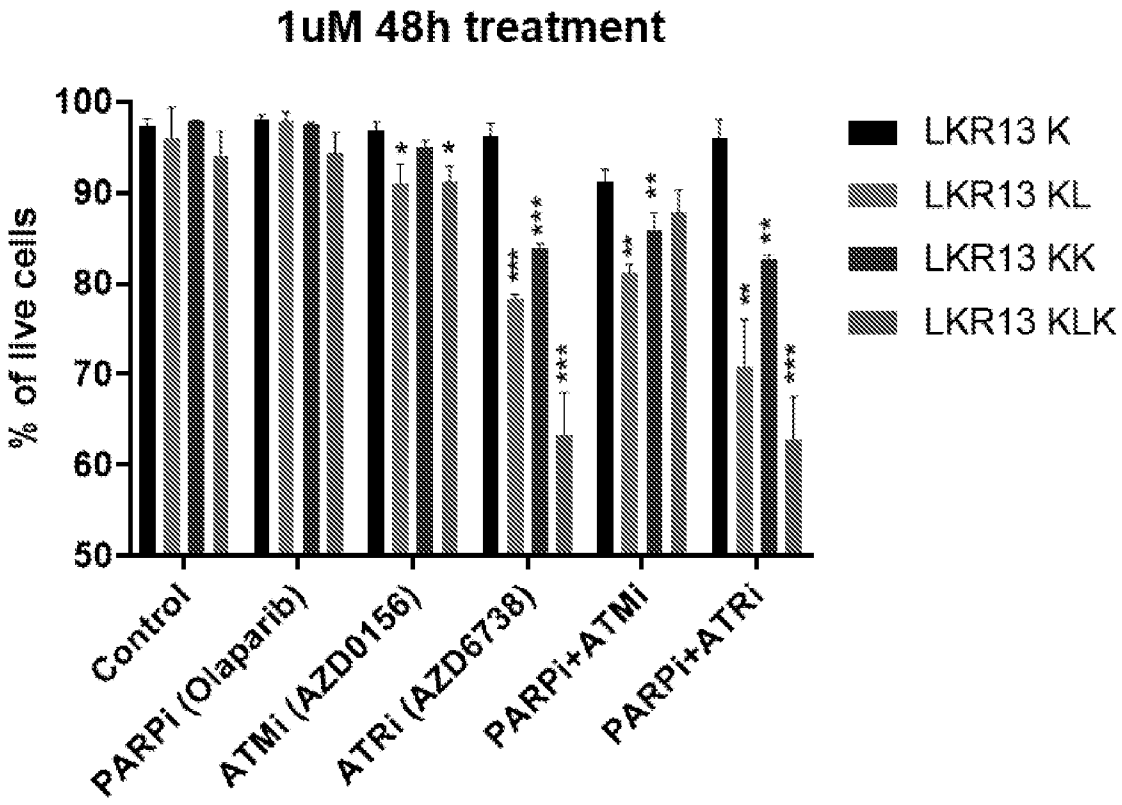

1uM 48h treatment
% of live cells
100
90
80
70
60
50
LKR13 K
LKR13 KL
LKR13 KK
LKR13 KLK
Control
PARPi (Olaparib)
ATMi (AZD0156)
ATRi (AZD6738)
PARPi+ATMi
PARPi+ATRi

FIG. 4

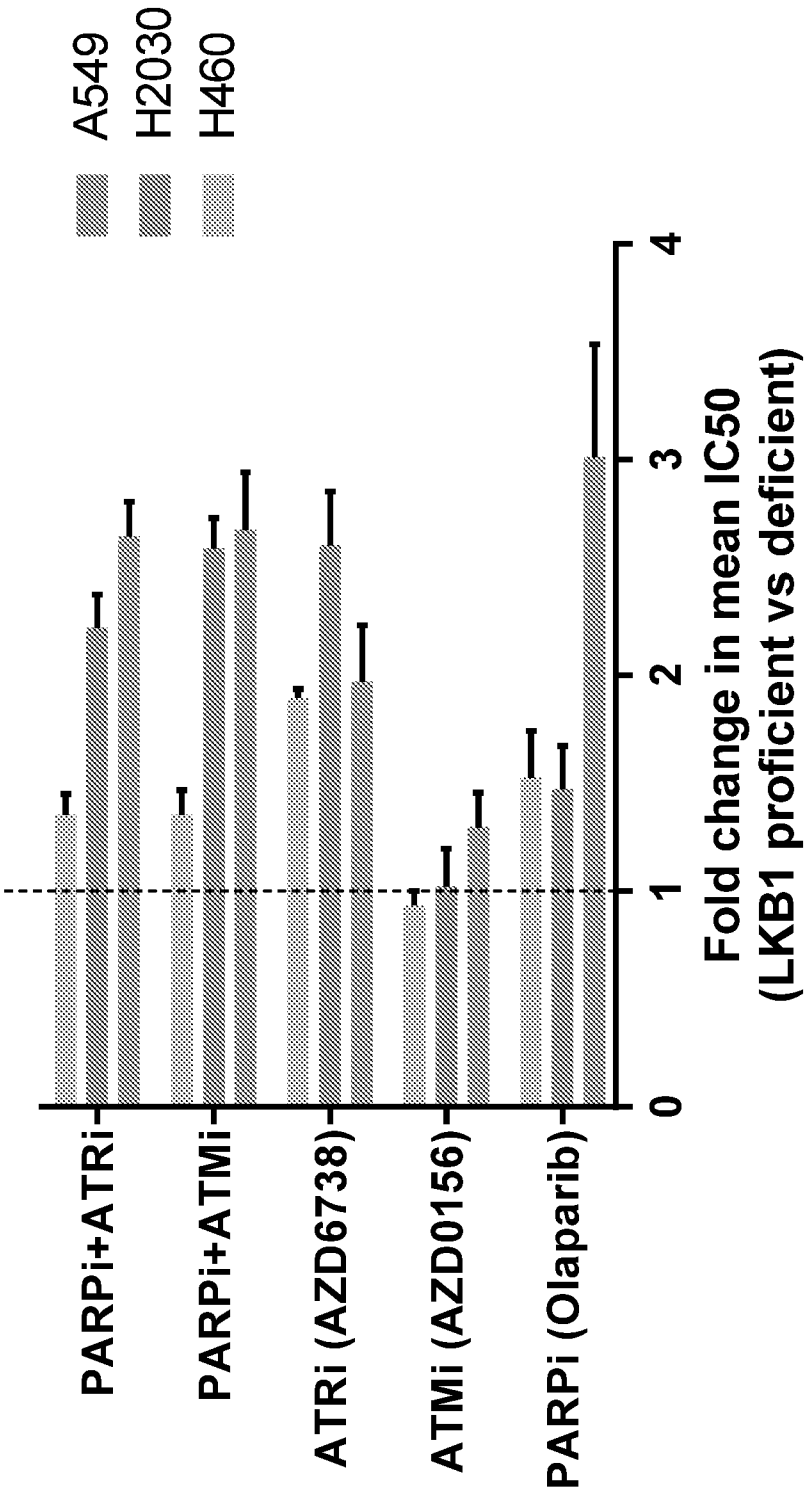

A549
H2030
H460
PARPi+ATRi
PARPi+ATMi
ATRi (AZD6738)
ATMi (AZD0156)
PARPi (Olaparib)
0
1
2
3
4
Fold change in mean IC50
(LKB1 proficient vs deficient)

FIG. 5

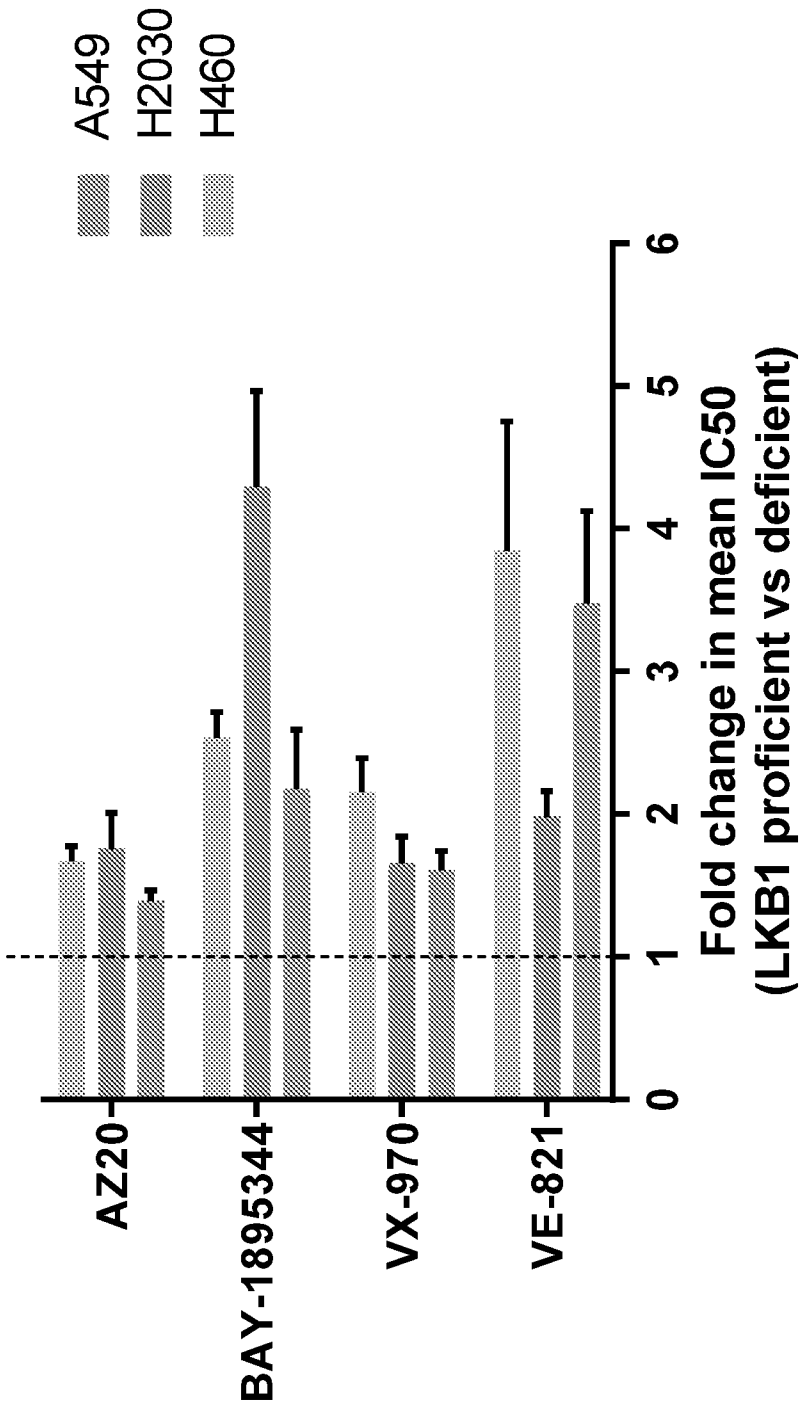

A549
H2030
H460
Fold change in mean IC50
(LKB1 proficient vs deficient)
0
1
2
3
4
5
6
AZ20
BAY-1895344
VX-970
VE-821

FIG. 6

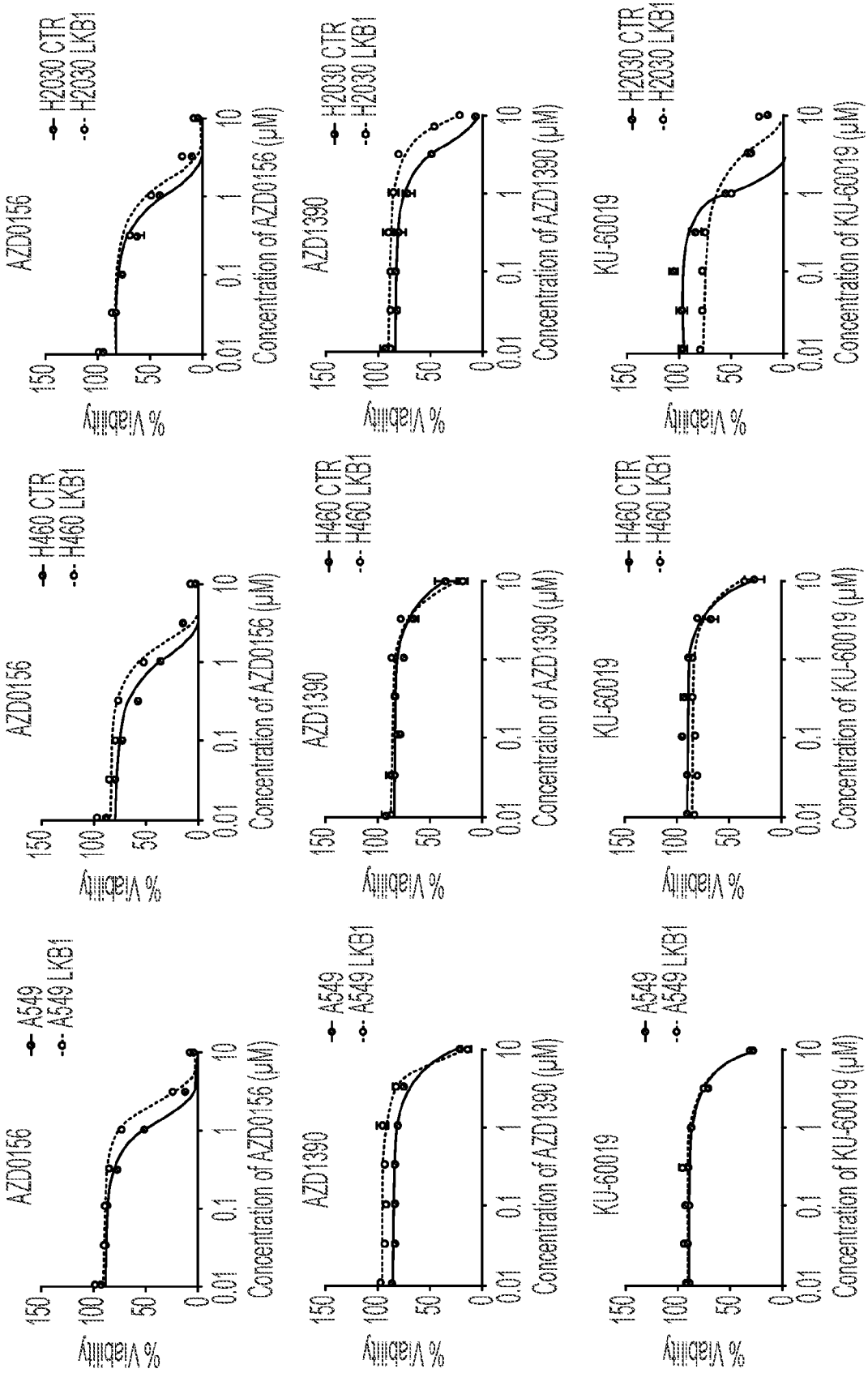

AZD0156
H2030 CTR
H2030 LKB1
% Viability
Concentration of AZD0156 (μM)
AZD1390
Concentration of AZD1390 (μM)
KU-60019
Concentration of KU-60019 (μM)
H460 CTR
H460 LKB1
A549
A549 LKB1
0
50
100
150
0.01
0.1
1
10

FIG. 7

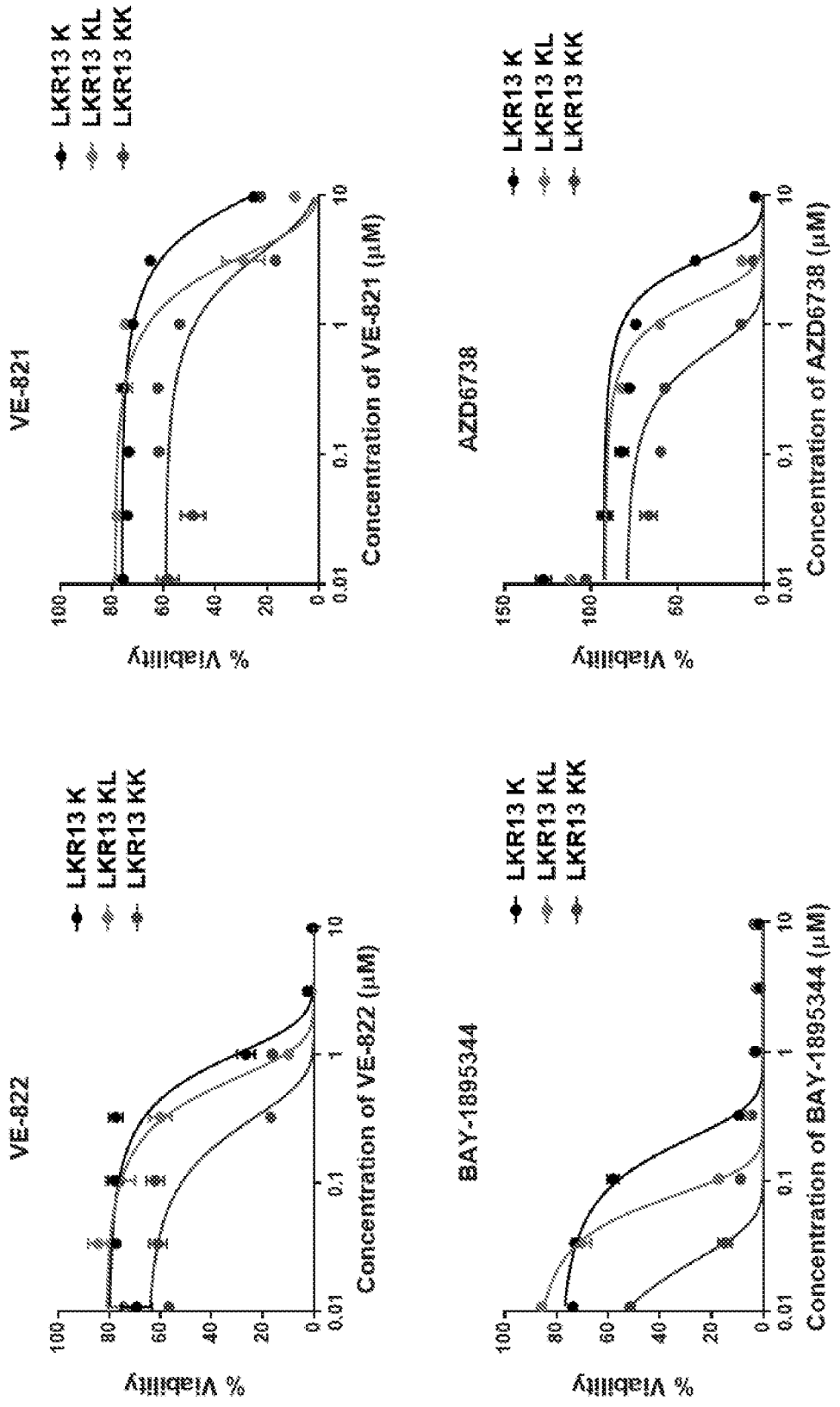

VE-821
LKR13 K
LKR13 KL
LKR13 KK
% Viability
Concentration of VE-821 (μM)
VE-822
LKR13 K
LKR13 KL
LKR13 KK
% Viability
Concentration of VE-822 (μM)
AZD6738
LKR13 K
LKR13 KL
LKR13 KK
% Viability
Concentration of AZD6738 (μM)
BAY-1895344
LKR13 K
LKR13 KL
LKR13 KK
% Viability
Concentration of BAY-1895344 (μM)

FIG. 8

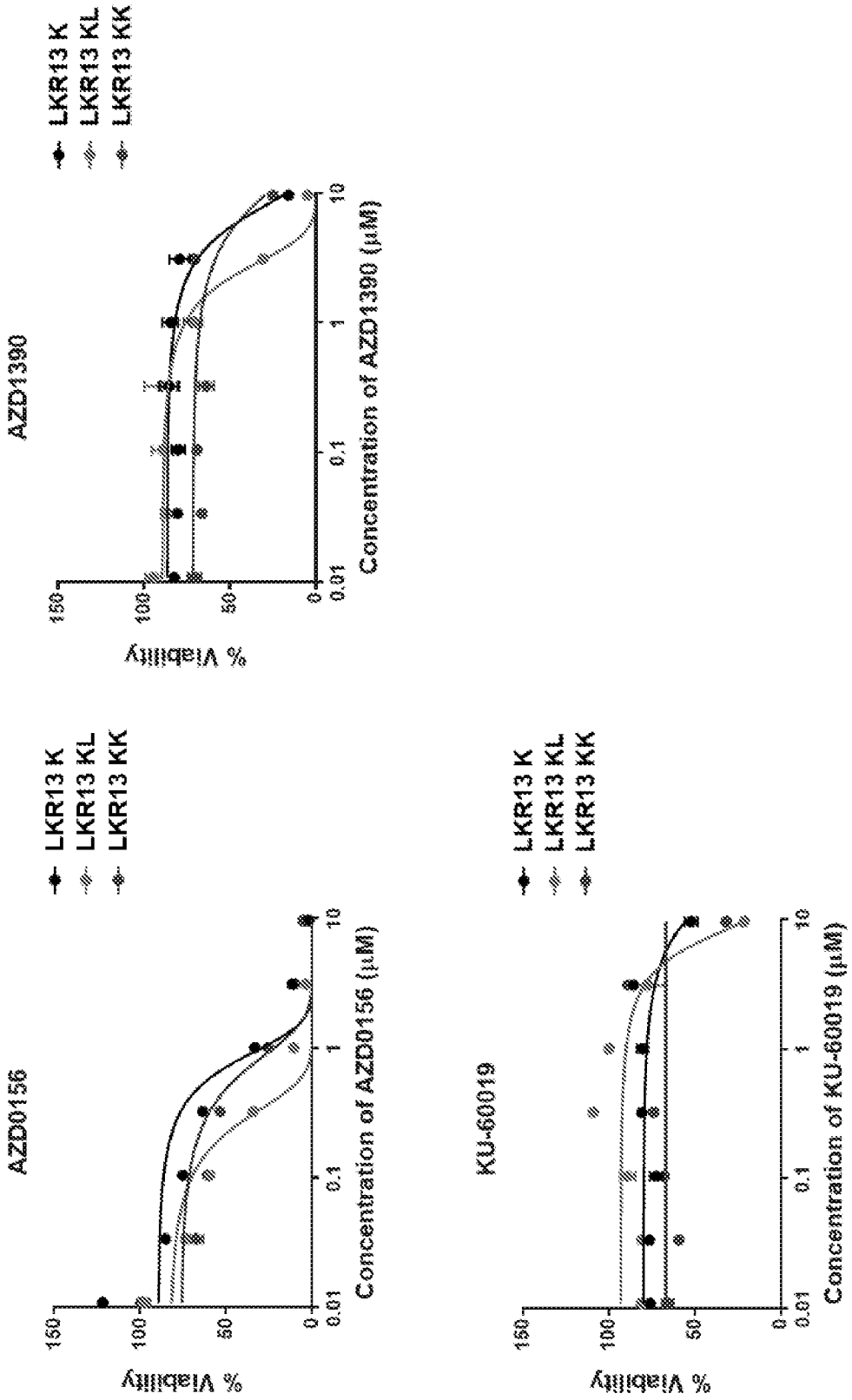

AZD1390
LKR13 K
LKR13 KL
LKR13 KK
% Viability
Concentration of AZD1390 (μM)
AZD0156
LKR13 K
LKR13 KL
LKR13 KK
% Viability
Concentration of AZD0156 (μM)
KU-60019
LKR13 K
LKR13 KL
LKR13 KK
% Viability
Concentration of KU-60019 (μM)

FIG. 9

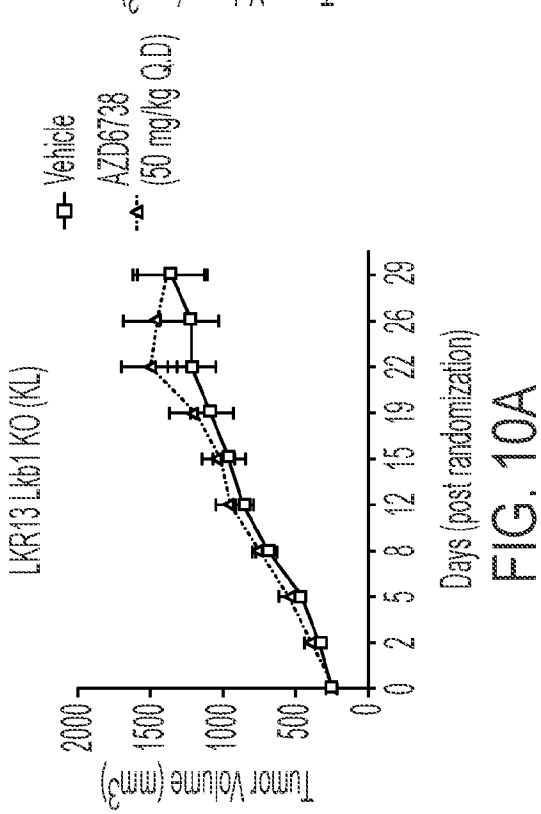


FIG. 10A

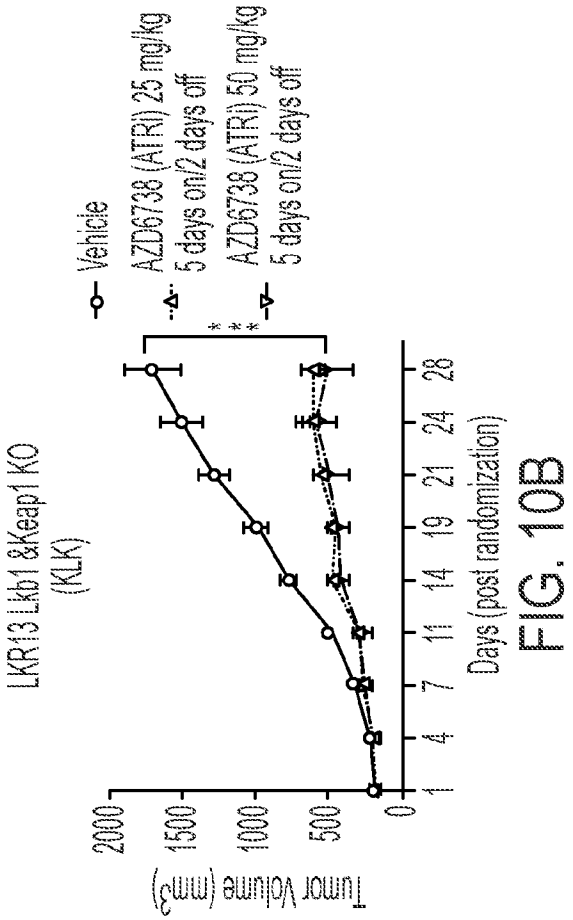


FIG. 10B

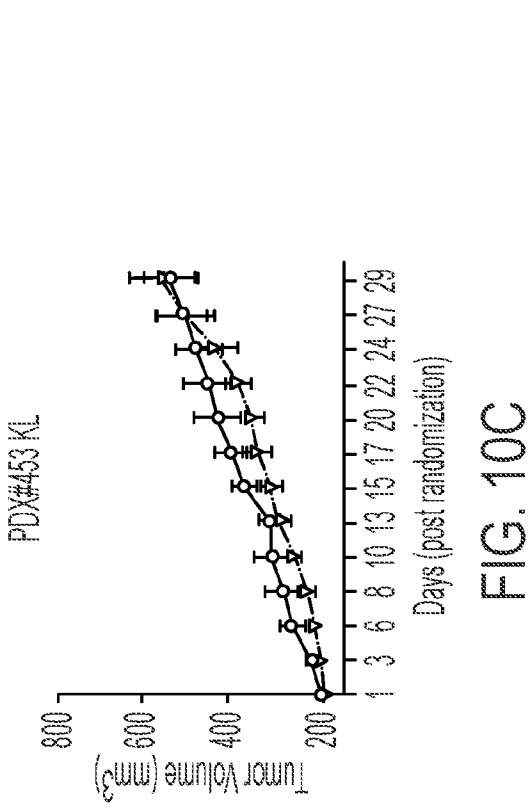


FIG. 10C

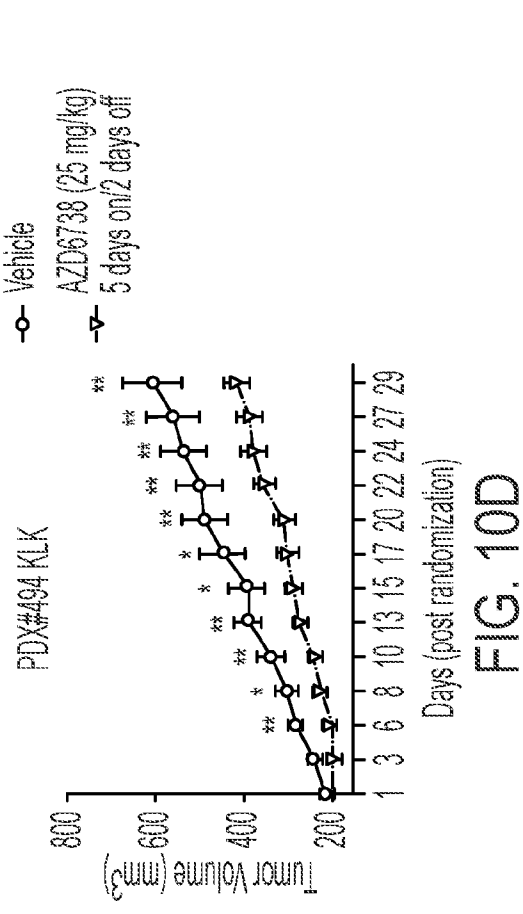
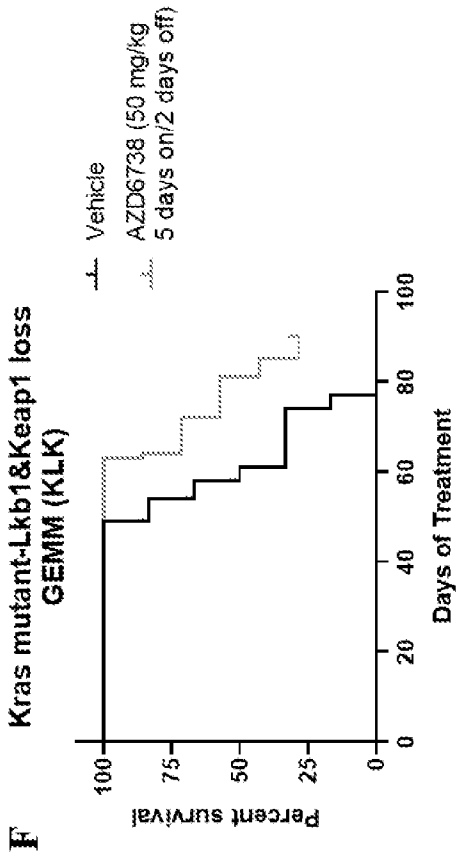
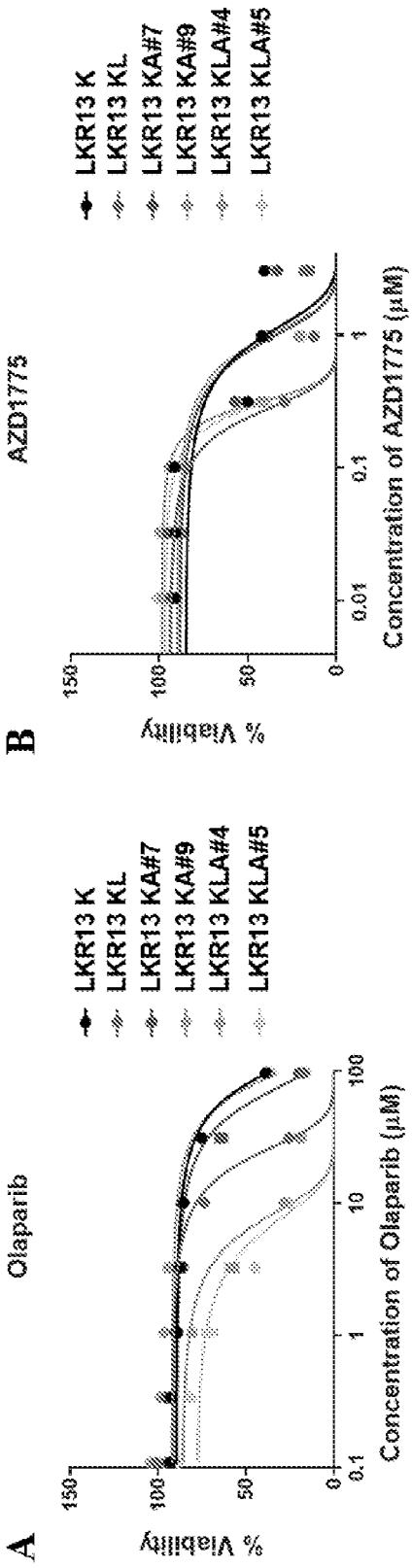


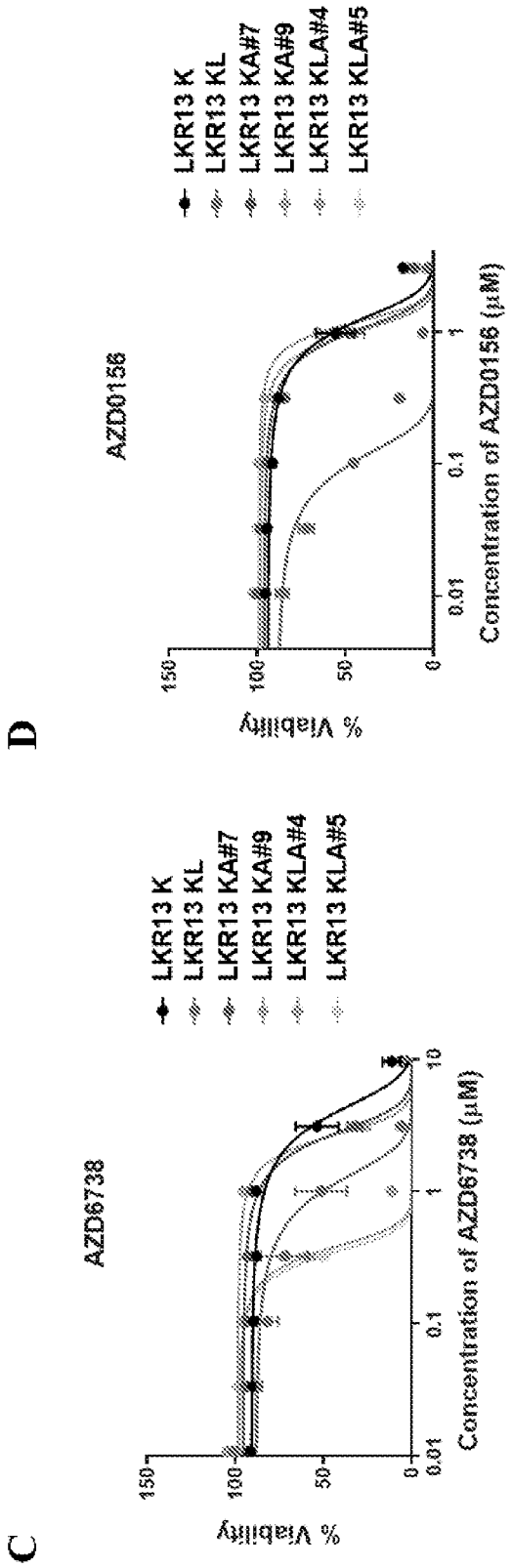
FIG. 10D



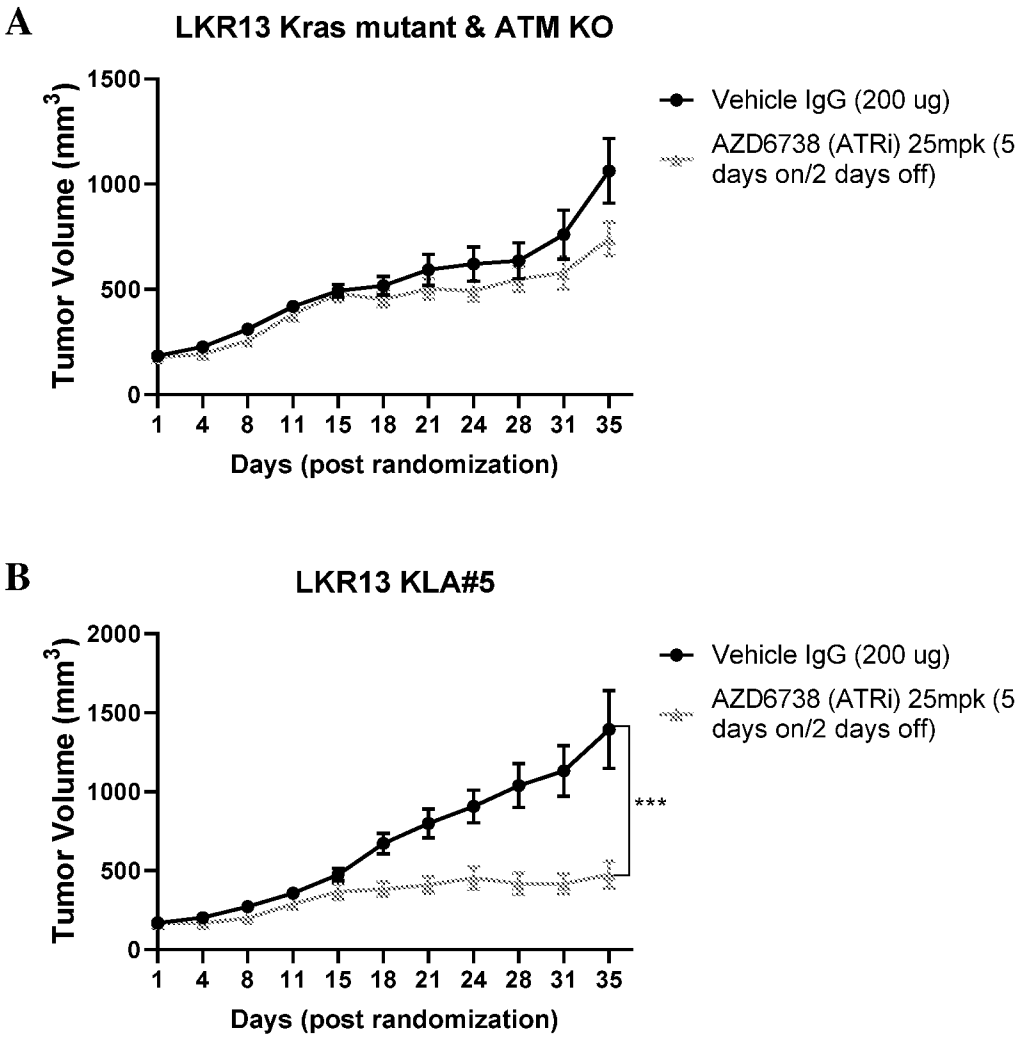
FIGS. 10 E-F



FIGS. 11 A-B


C
AZD6738
% Viability
150
100
50
0
0.01
0.1
1
10
Concentration of AZD6738 (μM)
LKR13 K
LKR13 KL
LKR13 KA#7
LKR13 KA#9
LKR13 KLA#4
LKR13 KLA#5
D
AZD0156
% Viability
150
100
50
0
0.01
0.1
1
Concentration of AZD0156 (μM)
LKR13 K
LKR13 KL
LKR13 KA#7
LKR13 KA#9
LKR13 KLA#4
LKR13 KLA#5

FIGS. 11 C-D


A
LKR13 Kras mutant & ATM KO
Tumor Volume (mm3)
1500
1000
500
0
1 4 8 11 15 18 21 24 28 31 35
Days (post randomization)
Vehicle IgG (200 ug)
AZD6738 (ATRi) 25mpk (5 days on/2 days off)
B
LKR13 KLA#5
Tumor Volume (mm3)
2000
1500
1000
500
0
1 4 8 11 15 18 21 24 28 31 35
Days (post randomization)
***
Vehicle IgG (200 ug)
AZD6738 (ATRi) 25mpk (5 days on/2 days off)

FIGS. 12A-B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 20/13889

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C12Q 1/6886, A61K 31/519, A61K 31/00 (2020.01)

CPC - A61K 31/4196, A61K 31/02, G01N 33/502, C12Q 2600/106, C12Q 1/6883

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.
Y	WO 2015/118338 A1 (MISSION THERAPEUTICS LIMITED) 13 August 2015 (13.08.2015); pg 17, ln 11-20; pg 17, ln 21-29; pg 26, ln 9	1-11, 32-34
Y	SHERMAN et al., AID-Induced Genotoxic Stress Promotes B Cell Differentiation in the Germinal Center via ATM and LKB1 Signaling, Molecular Cell, 24 September 2010, Vol 39, No 6, pg 873-885; abstract; pg 874, col 2, para 2; pg 880, col 1, para 1; pg 881, col 2, para 2	1-11, 32-34
Y	US 9,994,843 B2 (NATIONAL UNIVERSITY CORPORATION TOKYO MEDICAL AND DENTAL UNIVERSITY) 12 June 2018 (12.06.2018); col 2, ln 52- col 3, ln 4; col 20, ln 39- 44	10-11

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

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"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
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"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	Date of mailing of the international search report
10 March 2020	16 APR 2020

Name and mailing address of the ISA/US	Authorized officer
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Lee Young Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 20/13889

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 12-31 and 35-42
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.