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(54) Title: METHODS FOR TREATMENT OF CANCERS WITH EGFR ACTIVATING MUTATIONS

(57) Abstract: The present disclosure provides methods for treating cancer in a patient determined to have an EGFR activating mutation by administering a CDK inhibitor and/or SAC component inhibitor.



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DESCRIPTION

METHODS FOR TREATMENT OF CANCERS WITH EGFR ACTIVATING MUTATIONS

[0001] This application claims the benefit of United States Provisional Patent
5 Application No. 62/642,472, filed March 13, 2018, which is incorporated herein by reference
in its entirety.

BACKGROUND

[0002] This invention was made with government support under grant number
CA190628 awarded by the National Institutes of Health. The government has certain rights in
10 the invention.

1. Field

[0003] The present invention relates generally to the fields of cancer biology and
medicine. More particularly, it concerns methods for treating cancer with activating EGFR
mutations.

15 2. Description of Related Art

[0004] While EGFR mutant NSCLC patients are initially responsive to EGFR targeted
therapies, resistant disease inevitably emerges. In nearly half of the resistant cases, tumors lack
secondary EGFR mutations such as T790M and are refractory to 2nd and 3rd generation EGFR
tyrosine kinase inhibitors (TKIs). The identification of treatment regimens with efficacy
20 against cancers resistant to TKIs, such as T790M-negative resistance, remains a major clinical
challenge.

SUMMARY

[0005] In some embodiments, the present disclosure provides a method of treating
cancer in a subject comprising administering an effective amount of a cyclin dependent kinase
25 (CDK) inhibitor and/or a spindle assembly checkpoint (SAC) component inhibitor to the
subject, wherein the subject is determined to have one or more EGFR activating mutations. In
some aspects, the subject is determined to have 2, 3, or 4 EGFR activating mutations. In
particular aspects, the subject is human.

[0006] In some aspects, the one or more EGFR activating mutations are selected from the group consisting of L858R, an exon 19 deletion, and an exon 20 insertion. In certain aspects, the exon 19 deletion is an in-frame deletion between L747 and L749, such as an E746-A750 deletion, L747-E749 deletion, or A750P. In particular aspects, the exon 20 insertion is N771Del
5 Ins FH. In some aspects, the EGFR mutations are G719S, G719A, S768I, E709A, R776H, or L861Q.

[0007] In certain aspects, the subject is determined to have an EGFR activating mutation by analyzing a genomic sample from the patient. In some aspects, the genomic sample is isolated from saliva, blood, urine, normal tissue, or tumor tissue. In some aspects, the
10 presence of an EGFR activating mutation is determined by nucleic acid sequencing or PCR analyses.

[0008] In some aspects, the CDK inhibitor is further defined as a CDK2 inhibitor, CDK5 inhibitor, CDK1 inhibitor, or CDK9 inhibitor. In certain aspects, the CDK inhibitor is dinaciclib (SCH727965), alvocidib (flavopiridol), roscovitine (seliciclib, CYC202), SNS-032
15 (BMS-387032), LY2857785, ADZ5438, BMS-265246, NU6027, LDC000067, wogonin, or RO-3306. In certain aspects, the CDK inhibitor is MSC2530818, senexin A, LDC4297 (LDC044297), PHA-793887, BS-181 HCl, PHA-767491, THX1 2HCl, or XL413. In some aspects, the CDK inhibitor is dinaciclib or alvocidib. In certain aspects, the CDK inhibitor is not a CDK4 inhibitor and/or CDK6 inhibitor. In particular aspects, the CDK inhibitor is not
20 palociclib (PD0332991), abemaciclib (LY2835219), or ribociclob.

[0009] In certain aspects, the SAC component inhibitor is further defined as a polo-like kinase 1 (PLK1) inhibitor, Aurora kinase inhibitor, survivin, and/or KSP inhibitor. In some aspects, the PLK1 inhibitor is BI 2536, volasertib, GSK461364, ON-01910, GW 843682X, or HMN-214. In some aspects, the PLK1 inhibitor is volasertib or ON-01910. In other aspects,
25 the SAC inhibitor is not a PLK1 inhibitor. In some aspects, the Aurora kinase inhibitor is a Pan-Aurora inhibitor, Aurora A/B inhibitor, or an Aurora A inhibitor. In certain aspects, the Aurora kinase inhibitor is AMG 900, alisertib, PF-03814735, Tozasertib, MLN8054, or SNS-314 Mesylate. In some aspects, the Aurora kinase inhibitor is AMG-900 or alisertib. In some aspects, the KSP inhibitor is ispinesib or SB743921. In some aspects, the survivin inhibitor is
30 YM155.

[0010] In some aspects, the treatment results in accumulation of cells in the G2/M phase, enlarged nuclear size, and/or polyploidy.

[0011] In certain aspects, the cancer is resistant to one or more tyrosine kinase inhibitors (TKIs). In some aspects, the one or more TKIs are selected from the group consisting of osimertinib, erlotinib, gefitinib, afatinib, poziotinib, dacomitinib, and CO-1686. In some aspects, the cancer has acquired broad spectrum drug resistance. In some aspects, the cancer is resistant to pemetrexed, irinotecan, vinblastine, and/or gemcitabine. In certain aspects, the cancer has acquired mutations for poziotinib and/or other TKIs. In some aspects, the acquired mutation for poziotinib comprises an EGFR exon 20 insertion. In some aspects, the cancer has undergone epithelial to mesenchymal transition (EMT). In some aspects, EMT is demonstrated by decreased E-cadherin expression, increased expression of vimentin and/or Axl, and/or an increased invasive phenotype.

[0012] In additional aspects, the subject is further determined to comprise a secondary mutation. In some aspects, the secondary mutation is a T790M resistance mutation. In other aspects, the subject is determined to not have a secondary mutation. In some aspects, the subject is determined to not have a T790M resistance mutation.

[0013] In further aspects, the method further comprises administering at least one additional anti-cancer therapy. In some aspects, the at least one additional anti-cancer therapy is chemotherapy, radiotherapy, gene therapy, surgery, hormonal therapy, anti-angiogenic therapy or immunotherapy. In some aspects, the at least one additional anti-cancer therapy is a TKI and/or chemotherapy. In particular aspects, the TKI is osimertinib, erlotinib, gefitinib, afatinib, dacomitinib, or CO-1686. In certain aspects, the chemotherapy is pemetrexed, irinotecan, vinblastine, or gemcitabine.

[0014] In some aspects, the CDK inhibitor, SAC component inhibitor, and/or anti-cancer therapy are administered intravenously, subcutaneously, intraosseously, orally, transdermally, in sustained release, in controlled release, in delayed release, as a suppository, or sublingually. In some aspects, administering the CDK inhibitor, SAC component inhibitor, and/or anti-cancer therapy comprises local, regional or systemic administration. In certain aspects, the CDK inhibitor, SAC component inhibitor, and/or anti-cancer therapy are administered two or more times.

[0015] In some aspects, the cancer is oral cancer, oropharyngeal cancer, nasopharyngeal cancer, respiratory cancer, urogenital cancer, gastrointestinal cancer, central or peripheral nervous system tissue cancer, an endocrine or neuroendocrine cancer or hematopoietic cancer, glioma, sarcoma, carcinoma, lymphoma, melanoma, fibroma, meningioma, brain cancer, oropharyngeal cancer, nasopharyngeal cancer, renal cancer, biliary cancer, pheochromocytoma, pancreatic islet cell cancer, Li-Fraumeni tumors, thyroid cancer, parathyroid cancer, pituitary tumors, adrenal gland tumors, osteogenic sarcoma tumors, multiple neuroendocrine type I and type II tumors, breast cancer, lung cancer, head and neck cancer, prostate cancer, esophageal cancer, tracheal cancer, liver cancer, bladder cancer, stomach cancer, pancreatic cancer, ovarian cancer, uterine cancer, cervical cancer, testicular cancer, colon cancer, rectal cancer or skin cancer. In particular aspects, the cancer is non-small cell lung cancer.

[0016] In another embodiment, there is provided a pharmaceutical composition comprising a CDK inhibitor and/or SAC component inhibitor for use in a subject determined to have one or more EGFR activating mutations.

[0017] In some aspects, the one or more EGFR activating mutations are selected from the group consisting of L858R, an exon 19 deletion, and an exon 20 insertion. In certain aspects, the exon 19 deletion is an in-frame deletion between L747 and L749. In particular aspects, the exon 20 insertion is N771Del Ins FH.

[0018] In certain aspects, the subject was determined to have an EGFR activating mutation by analyzing a genomic sample from the patient. In some aspects, the genomic sample is isolated from saliva, blood, urine, normal tissue, or tumor tissue. In some aspects, the presence of an EGFR activating mutation is determined by nucleic acid sequencing or PCR analyses.

[0019] In some aspects, the CDK inhibitor is further defined as a CDK2 inhibitor, CDK5 inhibitor, CDK1 inhibitor, or CDK 9 inhibitor. In certain aspects, the CDK inhibitor is dinaciclib (SCH727965), alvocidib (flavopiridol), roscovitine (seliciclib, CYC202), SNS-032 (BMS-387032), LY2857785, ADZ5438, BMS-265246, NU6027, LDC000067, wogonin, or RO-3306. In certain aspects, the CDK inhibitor is MSC2530818, senexin A, LDC4297 (LDC044297), PHA-793887, BS-181 HCl, PHA-767491, THX1 2HCl, or XL413. In some aspects, the CDK inhibitor is dinaciclib or alvocidib. In certain aspects, the CDK inhibitor is

not a CDK4 inhibitor and/or CDK6 inhibitor. In particular aspects, the CDK inhibitor is not palociclib (PD0332991), abemaciclib (LY2835219), or ribociclob.

[0020] In certain aspects, the SAC component inhibitor is further defined as a polo-like kinase 1 (PLK1) inhibitor, Aurora kinase inhibitor, survivin, and/or KSP inhibitor. In some aspect, the PLK1 inhibitor is BI 2536, volasertib, GSK461364, ON-01910, GW 843682X, or HMN-214. In some aspects, the PLK1 inhibitor is volasertib or ON-01910. In other aspects, the SAC inhibitor is not a PLK1 inhibitor. In some aspects, the Aurora kinase inhibitor is a Pan-Aurora inhibitor, Aurora A/B inhibitor, or an Aurora A inhibitor. In certain aspects, the Aurora kinase inhibitor is AMG 900, alisertib, PF-03814735, Tozasertib, MLN8054, or SNS-10 314 Mesylate. In some aspects, the Aurora kinase inhibitor is AMG-900 or alisertib. In some aspects, the KSP inhibitor is ispinesib or SB743921. In some aspects, the survivin inhibitor is YM155.

[0021] In certain aspects, the cancer is resistant to one or more tyrosine kinase inhibitors (TKIs). In some aspects, the one or more TKIs are selected from the group consisting of osimertinib, erlotinib, gefitinib, afatinib, poziotinib, dacomitinib, and CO-1686. In some 15 aspects, the cancer has acquired broad spectrum drug resistance. In some aspects, the cancer is resistant to pemetrexed, irinotecan, vinblastine, and/or gemcitabine. In certain aspects, the cancer has acquired mutations for poziotinib and other TKIs. In some aspects, the acquired mutation for poziotinib comprises an EGFR exon 20 insertion. In some aspects, the cancer has 20 undergone epithelial to mesenchymal transition (EMT). In some aspects, EMT is demonstrated by decreased E-cadherin expression, increased expression of vimentin and/or Axl, and/or an increased invasive phenotype.

[0022] In additional aspects, the subject is further determined to comprise a secondary mutation. In some aspects, the secondary mutation is a T790M resistance mutation, C797S 25 resistance mutation or L792H resistance mutation. In other aspects, the subject is determined to not have a secondary mutation. In some aspects, the subject is determined to not have a T790M resistance mutation.

[0023] In further aspects, the composition further comprises at least one additional anti-cancer therapy. In some aspects, the at least one additional anti-cancer therapy is 30 chemotherapy, radiotherapy, gene therapy, surgery, hormonal therapy, anti-angiogenic therapy or immunotherapy. In some aspects, the at least one additional anti-cancer therapy is a TKI

and/or chemotherapy. In particular aspects, the TKI is osimertinib, erlotinib, gefitinib, afatinib, dacomitinib, or CO-1686. In certain aspects, the chemotherapy is pemetrexed, irinotecan, vinblastine, or gemcitabine.

[0024] In some aspects, the cancer is oral cancer, oropharyngeal cancer, nasopharyngeal cancer, respiratory cancer, urogenital cancer, gastrointestinal cancer, central or peripheral nervous system tissue cancer, an endocrine or neuroendocrine cancer or hematopoietic cancer, glioma, sarcoma, carcinoma, lymphoma, melanoma, fibroma, meningioma, brain cancer, oropharyngeal cancer, nasopharyngeal cancer, renal cancer, biliary cancer, pheochromocytoma, pancreatic islet cell cancer, Li-Fraumeni tumors, thyroid cancer, parathyroid cancer, pituitary tumors, adrenal gland tumors, osteogenic sarcoma tumors, multiple neuroendocrine type I and type II tumors, breast cancer, lung cancer, head and neck cancer, prostate cancer, esophageal cancer, tracheal cancer, liver cancer, bladder cancer, stomach cancer, pancreatic cancer, ovarian cancer, uterine cancer, cervical cancer, testicular cancer, colon cancer, rectal cancer or skin cancer. In particular aspects, the cancer is non-small cell lung cancer.

[0025] In another embodiment, there is provided a method of predicting a response to a CDK inhibitor and/or SAC component inhibitor alone or in combination with a second anti-cancer therapy in a subject having a cancer comprising detecting an EGFR activating mutation in a genomic sample obtained from said patient, wherein if the sample is positive for the presence of the EGFR activating mutation, then the patient is predicted to have a favorable response to the CDK inhibitor and/or SAC component inhibitor alone or in combination with an anti-cancer therapy.

[0026] In some aspects, a favorable response to CDK inhibitor and/or SAC component inhibitor alone or in combination with an anti-cancer therapy comprises reduction in tumor size or burden, blocking of tumor growth, reduction in tumor-associated pain, reduction in cancer associated pathology, reduction in cancer associated symptoms, cancer non-progression, increased disease free interval, increased time to progression, induction of remission, reduction of metastasis, or increased patient survival.

[0027] In some aspects, the one or more EGFR activating mutations are selected from the group consisting of L858R, an exon 19 deletion, and an exon 20 insertion. In certain aspects,

the exon 19 deletion is an in-frame deletion between L747 and L749. In particular aspects, the exon 20 insertion is N771Del Ins FH.

[0028] In certain aspects, the subject was determined to have an EGFR activating mutation by analyzing a genomic sample from the patient. In some aspects, the genomic sample is isolated from saliva, blood, urine, normal tissue, or tumor tissue. In some aspects, the presence of an EGFR activating mutation is determined by nucleic acid sequencing or PCR analyses.

[0029] In some aspects, the CDK inhibitor is further defined as a CDK2 inhibitor, CDK5 inhibitor, CDK1 inhibitor, or CDK 9 inhibitor. In certain aspects, the CDK inhibitor is dinaciclib (SCH727965), alvociclib (flavopiridol), roscovitine (seliciclib, CYC202), SNS-032 (BMS-387032), LY2857785, ADZ5438, BMS-265246, NU6027, LDC000067, wogonin, or RO-3306. In certain aspects, the CDK inhibitor is MSC2530818, senexin A, LDC4297 (LDC044297), PHA-793887, BS-181 HCl, PHA-767491, THX1 2HCl, or XL413. In some aspects, the CDK inhibitor is dinaciclib or alvociclib. In certain aspects, the CDK inhibitor is not a CDK4 inhibitor and/or CDK6 inhibitor. In particular aspects, the CDK inhibitor is not palociclib (PD0332991), abemaciclib (LY2835219), or ribociclob.

[0030] In certain aspects, the SAC component inhibitor is further defined as a polo-like kinase 1 (PLK1) inhibitor, Aurora kinase inhibitor, survivin, and/or KSP inhibitor. In some aspect, the PLK1 inhibitor is BI 2536, volasertib, GSK461364, ON-01910, GW 843682X, or HMN-214. In some aspects, the PLK1 inhibitor is volasertib or ON-01910. In other aspects, the SAC inhibitor is not a PLK1 inhibitor. In some aspects, the Aurora kinase inhibitor is a Pan-Aurora inhibitor, Aurora A/B inhibitor, or an Aurora A inhibitor. In certain aspects, the Aurora kinase inhibitor is AMG 900, alisertib, PF-03814735, Tozasertib, MLN8054, or SNS-314 Mesylate. In some aspects, the Aurora kinase inhibitor is AMG-900 or alisertib. In some aspects, the KSP inhibitor is ispinesib or SB743921. In some aspects, the survivin inhibitor is YM155.

[0031] In certain aspects, the cancer is resistant to one or more tyrosine kinase inhibitors (TKIs). In some aspects, the one or more TKIs are selected from the group consisting of osimertinib, erlotinib, gefitinib, afatinib, poziotinib, dacomitinib, and CO-1686. In some aspects, the cancer has acquired broad spectrum drug resistance. In some aspects, the cancer is resistant to pemetrexed, irinotecan, vinblastine, and/or gemcitabine. In certain aspects, the

cancer has acquired mutations for poziotinib and other TKIs. In some aspects, the acquired mutation for poziotinib comprises an EGFR exon 20 insertion. In some aspects, the cancer has undergone epithelial to mesenchymal transition (EMT). In some aspects, EMT is demonstrated by decreased E-cadherin expression, increased expression of vimentin and/or Axl, and/or an increased invasive phenotype.

[0032] In additional aspects, the subject is further determined to comprise a secondary mutation. In some aspects, the secondary mutation is a T790M resistance mutation. In other aspects, the subject is determined to not have a secondary mutation. In some aspects, the subject is determined to not have a T790M resistance mutation.

[0033] In additional aspects, the method further comprises administering CDK inhibitor and/or SAC component inhibitor alone or in combination with a second anti-cancer therapy to said patient predicted to have a favorable response. In some aspects, the at least one additional anti-cancer therapy is chemotherapy, radiotherapy, gene therapy, surgery, hormonal therapy, anti-angiogenic therapy or immunotherapy. In some aspects, the at least one additional anti-cancer therapy is a TKI and/or chemotherapy. In particular aspects, the TKI is osimertinib, erlotinib, gefitinib, afatinib, dacomitinib, or CO-1686. In certain aspects, the chemotherapy is pemetrexed, irinotecan, vinblastine, or gemcitabine.

[0034] In some aspects, the CDK inhibitor, SAC component inhibitor, and/or anti-cancer therapy are administered intravenously, subcutaneously, intraosseously, orally, transdermally, in sustained release, in controlled release, in delayed release, as a suppository, or sublingually. In some aspects, administering the CDK inhibitor, SAC component inhibitor, and/or anti-cancer therapy comprises local, regional or systemic administration. In certain aspects, the CDK inhibitor, SAC component inhibitor, and/or anti-cancer therapy are administered two or more times.

[0035] In some aspects, the cancer is oral cancer, oropharyngeal cancer, nasopharyngeal cancer, respiratory cancer, urogenital cancer, gastrointestinal cancer, central or peripheral nervous system tissue cancer, an endocrine or neuroendocrine cancer or hematopoietic cancer, glioma, sarcoma, carcinoma, lymphoma, melanoma, fibroma, meningioma, brain cancer, oropharyngeal cancer, nasopharyngeal cancer, renal cancer, biliary cancer, pheochromocytoma, pancreatic islet cell cancer, Li-Fraumeni tumors, thyroid cancer, parathyroid cancer, pituitary tumors, adrenal gland tumors, osteogenic sarcoma tumors,

multiple neuroendocrine type I and type II tumors, breast cancer, lung cancer, head and neck cancer, prostate cancer, esophageal cancer, tracheal cancer, liver cancer, bladder cancer, stomach cancer, pancreatic cancer, ovarian cancer, uterine cancer, cervical cancer, testicular cancer, colon cancer, rectal cancer or skin cancer. In particular aspects, the cancer is non-small
5 cell lung cancer.

[0036] 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
10 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

[0037] 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
15 understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0038] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawings(s) will be provided by the Office upon request and payment of the necessary fee.

20 [0039] **FIG. 1:** HCC827 and HCC4006 ER cells were negative for T790M secondary EGFR mutations. HCC827 ER cells are resistant to multiple EGFR inhibitors including erlotinib, gefitinib, afatinib, C0-1686, and osimertinib.

[0040] **FIGS. 2A-2D:** (A) HCC827 erlotinib resistant (ER) and HCC4006 ER variants have undergone an epithelial to mesenchymal transition (EMT) as demonstrated by a loss of
25 E-cadherin and increased expression of vimentin and Axl. (B) HCC827 ER and HCC4006 ER cells display a significantly increased invasive capacity as determined by Boyden chamber assay. (C) RPPA proteomic analysis revealed alteration in EMT-related proteins and signal transduction pathways in ER cells compared to parental NSCLC cell lines. (D) RPPA proteomic profiling reveals heterogeneity between ER clones derived from the same parental
30 cell lines.

[0041] **FIGS. 3A-3C:** High throughput drug screening was performed to test the efficacy of 1,321 compounds. EGFR TKI resistant cells that have undergone EMT have acquired broad spectrum drug resistance to chemotherapeutic agents (**A**), serine/threonine kinase inhibitors (**B**) and tyrosine kinase inhibitors (**C**).

5 [0042] **FIGS. 4A-4H:** (**A-B**) High throughput drug screen analysis revealed that EGFR TKI resistant cells exhibit sustained sensitivity to agents targeting cyclin dependent kinases (CDK), Aurora kinase, polo-like kinase 1 (PLK1), Bcl, KSP and survivin. (**C**) Protein expression of PLK1, Aurora-A, Survivin and KSP in EGFR TKI resistant cells as determined by Western blotting. (**D**) EGFR TKI resistant cells were treated with increasing concentrations
10 of inhibitors of Aurora kinase (alisertib, AMG900), PLK1 (volasertib), or KSP (ispinesib) for two weeks and viability was evaluated by clonogenic assay. (**E-H**) Quantification of clonogenic assays reveals that tumor cell viability is significantly inhibited following treatment with inhibitors targeting Aurora kinase, PLK1, and KSP.

15 [0043] **FIGS. 5A-5D:** (**A**) Treatment of EGFR TKI resistant cells with CDK inhibitors dinaciclib or alvocidib significantly increases the percentage of sub-G1 cells as determined by PI staining and flow cytometry. (**B**) Inhibition of PLK1, KSP, or Aurora kinase results in G2/M arrest, increased sub-G1 populations, and increased percentage of polyploid cells. (**C-D**) Inhibition of CDK, PLK1, KSP, or Aurora kinase results in enhanced nuclear size.

20 [0044] **FIGS. 6A-6D:** The patient-derived NSCLC cell line, MDA-011, expresses EGFR^{L858R} (**A**) and markers indicating a mesenchymal phenotype (**B**), and is resistant to erlotinib and osimertinib (**C**). (**D**) MDA-11 cells are sensitive to inhibitors of CDK (dinaciclib, alvocidib), PLK1 (volasertib, ON-01910), KSP (ispinesib, SB743921) and Aurora A (AMG900, Alisertib).

25 [0045] **FIG. 7:** EGFR mutant H1975 cells are highly sensitive to the EGFR inhibitor osimertinib. H.1975 cells were cultured continuously in osimertinib until resistant cells emerged (H1975 OR5 and H1975 OR16). Both parental H1975 and H1975 OR5 and H1975 OR16 (osimertinib resistant) cells were sensitive to SAC inhibitors including drugs targeting PLK1 (volasertib), KSP (ispinasib) and aurora kinases (AMG900 and Alisertib).

30 [0046] **FIG. 8:** YUL0019 cells which harbor an EGFR exon 20 insertion mutation are highly sensitive to the EGFR inhibitor poziotinib. YUL0019 cells were cultured continuously in poziotinib until resistant cells emerged (YUL0019 PR8). Both parental YUL0019 and

YUL0019 PR8 (poziotinib resistant) cells were sensitive to SAC inhibitors including drugs targeting PLK1 (volasertib), KSP (ispinasib) and aurora kinases (AMG900 and Alisertib).

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

[0047] To address the unmet need for treatment regimens for TKI-resistant cancer, a panel of NSCLC cell lines with acquired resistance to the EGFR TKI, erlotinib, were derived in the present studies. A subset of EGFR TKI resistant variants was negative for secondary EGFR mutations, were resistant to 2nd and 3rd generation EGFR TKIs including osimertinib, afatinib, and dacomitinib, and had undergone epithelial to mesenchymal transition (EMT) as demonstrated by loss of E-cadherin, enhanced expression of N-cadherin and Axl and an increased invasive phenotype as determined by Boyden chamber assay. Proteomic profiling revealed that although EGFR TKI resistant cells displayed similar mesenchymal and invasive phenotypes, there was significant heterogeneity in protein expression and pathway activation among resistant variants derived from the same parental cell line.

[0048] To identify therapeutic agents with activity against EMT-associated EGFR TKI resistance, a high-throughput drug screening was performed to test the efficacy of 1,321 compounds. EMT-associated EGFR TKI resistance was accompanied by the acquisition of broad-spectrum drug resistance. Compared to parental cells, mesenchymal EGFR TKI resistant cells were significantly more resistant to chemotherapeutic agents used to treat NSCLC including pemetrexed, irinotecan, vinblastine, and gemcitabine. EGFR TKI resistant cells displayed acquired resistance to 147 other tyrosine kinase and serine/threonine kinase inhibitors. In contrast, both parental cells and mesenchymal EGFR TKI resistant variants were highly sensitive to CDK inhibitors and agents targeting spindle assembly checkpoint (SAC) components including PLK1, Aurora, KSP, and survivin. These findings were validated by MTS and clonogenic assays. Treatment with SAC inhibitors induced the accumulation of cells in G2/M phase, enlarged nuclear size, and polyploidy.

[0049] To clinically validate these findings, a cell line (MDA-011) was established from the pleural effusion of an EGFR mutant NSCLC patient with T790M-negative resistance to erlotinib. *In vitro*, MDA-011 cells were resistant to erlotinib and osimertinib. MDA-011 cells were highly sensitive to CDK inhibitors and SAC inhibitors as determined by MTS and clonogenic assays. These data indicated that EMT-associated resistance to EGFR TKIs is

associated with broad spectrum drug resistance but vulnerabilities to CDK and SAC inhibition can potentially be exploited to overcome resistant disease in NSCLC patients.

[0050] Accordingly, in certain embodiments, the present disclosure provides methods for treating cancers with activating EGFR mutations, such as NSCLC, with inhibitor of CDK and/or inhibitor of SAC components. The EGFR mutant NSCLC cells may or may not have acquired resistance to EGFR TKIs. Exemplary CDK inhibitors include dinaciclib and alvocidib, and exemplary agents targeting spindle assembly checkpoint (SAC) components include PLK1 (volasertib), Aurora A (AMG-900 and alisertib), and KSP (ispinesib). In some aspects, the cancer, such as NSCLC, cells express EGFR activating mutations such as L858R, exon 19 deletions, and exon 20 insertions. The cancer cells may have acquired resistance to EGFR TKIs and/or broad-spectrum drug resistance, such as due to T790M mutation and/or epithelial to mesenchymal transition.

I. Definitions

[0051] An “EGFR activating mutation” is referred to herein as somatic mutation(s) that can lead to the development of cancer, such as lung cancer, and are found in exons 18-21 of EGFR. Exemplary EGFR activating mutations include single-nucleotide substitutions, such as L858R, V765A, T783, or G719 change to serine, alanine, or cysteine, exon 19 deletion(s) (*e.g.*, in-frame deletions of exon 19 between L747 to L749), and exon 20 insertion(s) and/or duplication(s) (*e.g.*, D770_N771 (ins NPG), D770_(ins SVQ) and D770_(ins G) N771T). Other “resistance mutations” may be acquired, such as T790M.

[0052] 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.

[0053] 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. The terms “about”, “substantially” and “approximately” mean, in general, the stated value plus or minus 5%.

[0054] “Treatment” or “treating” includes (1) inhibiting a disease in a subject or patient experiencing or displaying the pathology or symptomatology of the disease (*e.g.*,

arresting further development of the pathology and/or symptomatology), (2) ameliorating a disease in a subject or patient that is experiencing or displaying the pathology or symptomatology of the disease (*e.g.*, reversing the pathology and/or symptomatology), and/or (3) effecting any measurable decrease in a disease in a subject or patient that is experiencing
5 or displaying the pathology or symptomatology of the disease.

[0055] "Prevention" or "preventing" includes: (1) inhibiting the onset of a disease in a subject or patient which may be at risk and/or predisposed to the disease but does not yet experience or display any or all of the pathology or symptomatology of the disease, and/or (2) slowing the onset of the pathology or symptomatology of a disease in a subject or patient which
10 may be at risk and/or predisposed to the disease but does not yet experience or display any or all of the pathology or symptomatology of the disease.

[0056] As used herein, the term "patient" or "subject" refers to a living mammalian organism, such as a human, monkey, cow, sheep, goat, dog, cat, mouse, rat, guinea pig, or transgenic species thereof. In certain embodiments, the patient or subject is a primate. Non-
15 limiting examples of human patients are adults, juveniles, infants and fetuses.

[0057] The term "effective," as that term is used in the specification and/or claims, means adequate to accomplish a desired, expected, or intended result. "Effective amount," "therapeutically effective amount" or "pharmaceutically effective amount" when used in the context of treating a patient or subject with a compound means that amount of the compound
20 which, when administered to a subject or patient for treating or preventing a disease, is an amount sufficient to affect such treatment or prevention of the disease.

[0058] As used herein, the term " IC_{50} " refers to an inhibitory dose which is 50% of the maximum response obtained. This quantitative measure indicates how much of a particular drug or other substance (inhibitor) is needed to inhibit a given biological, biochemical or
25 chemical process (or component of a process, *i.e.* an enzyme, cell, cell receptor or microorganism) by half.

[0059] An "anti-cancer" agent is capable of negatively affecting a cancer cell/tumor in a subject, for example, by promoting killing of cancer cells, inducing apoptosis in cancer cells, reducing the growth rate of cancer cells, reducing the incidence or number of metastases,
30 reducing tumor size, inhibiting tumor growth, reducing the blood supply to a tumor or cancer

cells, promoting an immune response against cancer cells or a tumor, preventing or inhibiting the progression of cancer, or increasing the lifespan of a subject with cancer.

[0060] The term “insertion(s)” or “insertion mutation(s)” refers to the addition of one or more nucleotide base pairs into a DNA sequence. For example, an insertion mutation of
5 exon 20 of EGFR can occur between amino acids 767 to 774, of about 2-21 base pairs.

[0061] “Detection,” “detectable” and grammatical equivalents thereof refer to ways of determining the presence and/or quantity and/or identity of a target nucleic acid sequence. In some embodiments, detection occurs amplifying the target nucleic acid sequence. In other
10 embodiments, sequencing of the target nucleic acid can be characterized as “detecting” the target nucleic acid. A label attached to the probe can include any of a variety of different labels known in the art that can be detected by, for example, chemical or physical means. Labels that can be attached to probes may include, for example, fluorescent and luminescence materials.

[0062] “Amplifying,” “amplification,” and grammatical equivalents thereof refers to any method by which at least a part of a target nucleic acid sequence is reproduced in a
15 template-dependent manner, including without limitation, a broad range of techniques for amplifying nucleic acid sequences, either linearly or exponentially. Exemplary means for performing an amplifying step include ligase chain reaction (LCR), ligase detection reaction (LDR), ligation followed by Q-replicative amplification, PCR, primer extension, strand displacement amplification (SDA), hyperbranched strand displacement amplification, multiple
20 displacement amplification (MDA), nucleic acid strand-based amplification (NASBA), two-step multiplexed amplifications, rolling circle amplification (RCA), recombinase-polymerase amplification (RPA) (TwistDx, Cambridge, UK), and self-sustained sequence replication (3SR), including multiplex versions or combinations thereof, for example but not limited to, OLA/PCR, PCR/OLA, LDR/PCR, PCR/PCR/LDR, PCR/LDR, LCR/PCR, PCR/LCR (also
25 known as combined chain reaction-CCR), and the like. Descriptions of such techniques can be found in, among other places, Sambrook *et al.* Molecular Cloning, 3rd Edition).

[0063] As generally used herein “pharmaceutically acceptable” refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound
30 medical judgment, suitable for use in contact with the tissues, organs, and/or bodily fluids of human beings and animals without excessive toxicity, irritation, allergic response, or other problems or complications commensurate with a reasonable benefit/risk ratio.

[0064] "Pharmaceutically acceptable salts" means salts of compounds of the present invention which are pharmaceutically acceptable, as defined above, and which possess the desired pharmacological activity. Non-limiting examples of such salts include acid addition salts formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, and phosphoric acid; or with organic acids such as 1,2-ethanedisulfonic acid, 2-hydroxyethanesulfonic acid, 2-naphthalenesulfonic acid, 3-phenylpropionic acid, 4,4'-methylenebis(3-hydroxy-2-ene-1-carboxylic acid), 4-methylbicyclo[2.2.2]oct-2-ene-1-carboxylic acid, acetic acid, aliphatic mono- and dicarboxylic acids, aliphatic sulfuric acids, aromatic sulfuric acids, benzenesulfonic acid, benzoic acid, camphorsulfonic acid, carbonic acid, cinnamic acid, citric acid, cyclopentanepropionic acid, ethanesulfonic acid, fumaric acid, glucoheptonic acid, gluconic acid, glutamic acid, glycolic acid, heptanoic acid, hexanoic acid, hydroxynaphthoic acid, lactic acid, laurylsulfuric acid, maleic acid, malic acid, malonic acid, mandelic acid, methanesulfonic acid, muconic acid, *o*-(4-hydroxybenzoyl)benzoic acid, oxalic acid, *p*-chlorobenzenesulfonic acid, phenyl-substituted alkanoic acids, propionic acid, *p*-toluenesulfonic acid, pyruvic acid, salicylic acid, stearic acid, succinic acid, tartaric acid, tertiarybutylacetic acid, and trimethylacetic acid. Pharmaceutically acceptable salts also include base addition salts which may be formed when acidic protons present are capable of reacting with inorganic or organic bases. Acceptable inorganic bases include sodium hydroxide, sodium carbonate, potassium hydroxide, aluminum hydroxide and calcium hydroxide. Non-limiting examples of acceptable organic bases include ethanolamine, diethanolamine, triethanolamine, tromethamine, and *N*-methylglucamine. It should be recognized that the particular anion or cation forming a part of any salt of this invention is not critical, so long as the salt, as a whole, is pharmacologically acceptable. Additional examples of pharmaceutically acceptable salts and their methods of preparation and use are presented in *Handbook of Pharmaceutical Salts: Properties, and Use* (P. H. Stahl & C. G. Wermuth eds., Verlag Helvetica Chimica Acta, 2002).

II. EGFR Activating Mutations

[0065] Certain embodiments of the present disclosure concern determining if a subject has one or more EGFR activating mutations, such as L858R, exon 19 deletion(s), or exon 20 mutation(s), such as an insertion mutation, particularly one or more insertion mutations. The subject may have 2, 3, 4, or more activating EGFR mutations. Mutation detection methods are known the art including PCR analyses and nucleic acid sequencing as well as FISH and CGH.

In particular aspects, the exon 20 mutations are detected by DNA sequencing, such as from a tumor or circulating free DNA from plasma.

[0066] The EGFR exon 19 mutation(s) may comprise one or more point mutations, insertions, and/or deletions of 3-18 nucleotides between amino acids in-frame deletions of exon 19 between 747-749.

[0067] The EGFR exon 20 mutation(s) may comprise one or more point mutations, insertions, and/or deletions of 3-18 nucleotides between amino acids 763-778. The one or more EGFR exon 20 mutations may be located at one or more residues selected from the group consisting of A763, A767, S768, V769, D770, N771, P772, and H773.

[0068] EGFR exon 20 insertions may include H773_V774insH, A767_v769ASV, N771_P772insH, D770_N771insG, H779_V774insH, N771delinsHH, S768_D770dupDVD, A767_V769dupASV, A767_V769dupASV, P772_H773dup, N771_H773dupNPH, S768_D770dupSVD, N771delinsGY, S768_D770delinsSVD, D770_D770delinsGY, A767_V769dupASV, and/or H773dup. In particular aspects, the exon 20 mutations are A763insFQEA, A767insASV, S768dupSVD, V769insASV, D770insSVD, D770insNPG, H773insNPH, N771del insGY, N771del insFH and/or N771dupNPH.

[0069] 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.

[0070] 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.

[0071] 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.

5 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
10 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
15 purification kit (Promega). Non-limiting examples of sources of samples include urine, blood, and tissue.

[0072] The presence or absence of EGFR activating 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
20 detect the presence or absence of insertion 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 an insertion mutation as described herein. An insertion mutation can be detected by any method described herein, *e.g.*, by sequencing or
25 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.

[0073] A set of probes typically refers to a set of primers, usually primer pairs, and/or
30 detectably-labeled probes that are used to detect the target genetic variations (*e.g.*, EGFR activating mutations) 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, such as EGFR activating mutations. In one
5 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.

[0074] Analysis of nucleic acid markers can be performed using techniques known in
10 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-
15 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
20 companies such as the Life Technologies/Ion Torrent PGM or Proton, the Illumina HiSEQ or MiSEQ, and the Roche/454 next generation sequencing system.

[0075] 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
25 *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
30 analysis (Orita *et al.*, 1989); 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.

[0076] In one example, a method of identifying an EGFR activating 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 EGFR 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 (^3H , ^{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 EGFR activating mutations in a sample, said kit comprising an oligonucleotide that specifically hybridizes to or adjacent to a site of mutation in the EGFR gene. The kit may further comprise instructions for treating patients having tumors that contain EGFR activating mutations with a CDK inhibitor and/or SAC inhibitor based on the result of a hybridization test using the kit.

[0077] In another aspect, a method for detecting an exon 20 mutation in a sample comprises amplifying from said sample nucleic acids corresponding to exon 20 of said EGFR gene, 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 EGFR 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.

[0078] 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 T₄ 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 be 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.

5 CEL I enzyme can be used similarly to resolvase T₄ endonuclease VII as demonstrated in U.S. Patent No. 5,869,245.

III. Methods of Treatment

[0079] 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

10 CDK inhibitor, SAC component inhibitor, or a structurally similar inhibitor, to a subject determined to have an EGFR activating mutation, such as a L858R mutation, exon 20 deletion, and/or exon 20 insertion. The subject may have more than one EGFR activating mutation.

[0080] Examples of cancers contemplated for treatment include lung cancer, head and neck cancer, breast cancer, pancreatic cancer, prostate cancer, renal cancer, bone cancer,

15 testicular cancer, cervical cancer, gastrointestinal cancer, lymphomas, pre-neoplastic lesions in the lung, colon cancer, melanoma, and bladder cancer. In particular aspects, the cancer is non-small cell lung cancer.

[0081] In some embodiments, the subject is a mammal, *e.g.*, a primate, preferably a higher primate, *e.g.*, a human (*e.g.*, a patient having, or at risk of having, a disorder described

20 herein). In one embodiment, the subject is in need of enhancing an immune response. In certain embodiments, the subject is, or is at risk of being, immunocompromised. For example, the subject is undergoing or has undergone a chemotherapeutic treatment and/or radiation therapy. Alternatively, or in combination, the subject is, or is at risk of being, immunocompromised as a result of an infection.

[0082] CDK inhibitors may be a CDK2 inhibitor, CDK5 inhibitor, CDK1 inhibitor, or CDK 9 inhibitor. The CDK inhibitor may be the CDK inhibitor is dinaciclib (SCH727965), alvocidib (flavopiridol), roscovitine (seliciclib, CYC202), SNS-032 (BMS-387032), LY2857785, ADZ5438, BMS-265246, NU6027, LDC000067, wogonin, or RO-3306. Additional exemplary CDK inhibitors include MSC2530818, senexin A, LDC4297

25 (LDC044297), PHA-793887, BS-181 HCl, PHA-767491, THX1 2HCl, or XL413. In particular

30 aspects, the CDK inhibitor may be dinaciclib or alvocidib

[0083] SAC component inhibitors may be a polo-like kinase 1 (PLK1) inhibitor, Aurora kinase inhibitor, survivin, and/or KSP inhibitor. The PLK1 inhibitor may be BI 2536, volasertib, GSK461364, ON-01910, GW 843682X, or HMN-214. The Aurora kinase inhibitor may be a Pan-Aurora inhibitor, Aurora A/B inhibitor, or an Aurora A inhibitor. The Aurora kinase inhibitor may be AMG 900, alisertib, PF-03814735, Tozasertib, MLN8054, or SNS-314 Mesylate. The KSP inhibitor may be ispinesib or SB743921. The survivin inhibitor may be YM155. In some aspects, the SAC component inhibitor is not a MAD2 inhibitor.

[0084] Certain embodiments concern the administration of a TKI in combination with the CDK inhibitor and/or SAC component inhibitor. The TKI may be osimertinib, erlotinib, gefitinib, afatinib, poziotinib, dacomitinib, and CO-1686 or other TKIs known in the art. In some aspects, the TKI is poziotinib (also known as HM781-36B, HM781-36, and 1-[4-[4-(3,4-dichloro-2-fluoroanilino)-7-methoxyquinazolin-6-yl]oxypiperidin-1-yl]prop-2-en-1-one) is administered. Poziotinib is a quinazoline-based pan-HER inhibitor that irreversibly blocks signaling through the HER family of tyrosine-kinase receptors including HER1, HER2, and HER4. Poziotinib or structurally similar compounds (*e.g.*, U.S. Patent No. 8,188,102 and U.S. Patent Publication No. 20130071452; incorporated herein by reference) may be used in the present methods.

A. Pharmaceutical Compositions

[0085] Also provided herein are pharmaceutical compositions and formulations comprising a CDK inhibitor and/or a SAC component inhibitor, and a pharmaceutically acceptable carrier for subjects determined to have an EGFR activating mutation, such as a L858 mutation, exon 19 deletion, or exon 20 insertion.

[0086] Pharmaceutical compositions and formulations as described herein can be prepared by mixing the active ingredients (such as an antibody or a polypeptide) having the desired degree of purity with one or more optional pharmaceutically acceptable carriers (Remington's Pharmaceutical Sciences 22nd edition, 2012), in the form of lyophilized formulations or aqueous solutions. Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol;

cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.* Zn- protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG). Exemplary pharmaceutically acceptable carriers herein further include interstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX[®], Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including rHuPH20, are described in U.S. Patent Publication Nos. 2005/0260186 and 2006/0104968. In one aspect, a sHASEGP is combined with one or more additional glycosaminoglycanases such as chondroitinases.

B. Combination Therapies

[0087] In certain embodiments, the compositions and methods of the present embodiments involve CDK inhibition and/or SAC component inhibition in combination with at least one additional therapy. The additional therapy may be radiation therapy, surgery (*e.g.*, lumpectomy and a mastectomy), chemotherapy, gene therapy, DNA therapy, viral therapy, RNA therapy, immunotherapy, bone marrow transplantation, nanotherapy, monoclonal antibody therapy, or a combination of the foregoing. The additional therapy may be in the form of adjuvant or neoadjuvant therapy.

[0088] In some embodiments, the additional therapy is the administration of small molecule enzymatic inhibitor or anti-metastatic agent. In some embodiments, the additional therapy is the administration of side-effect limiting agents (*e.g.*, agents intended to lessen the occurrence and/or severity of side effects of treatment, such as anti-nausea agents, *etc.*). In some embodiments, the additional therapy is radiation therapy. In some embodiments, the additional therapy is surgery. In some embodiments, the additional therapy is a combination of radiation therapy and surgery. In some embodiments, the additional therapy is gamma irradiation. In some embodiments, the additional therapy is therapy targeting PBK/AKT/mTOR pathway, HSP90 inhibitor, tubulin inhibitor, apoptosis inhibitor, and/or

chemopreventative agent. The additional therapy may be one or more of the chemotherapeutic agents known in the art.

[0089] The inhibitors may be administered before, during, after, or in various combinations relative to an additional cancer therapy, such as immune checkpoint therapy. The administrations may be in intervals ranging from concurrently to minutes to days to weeks. In embodiments where the inhibitors is provided to a patient separately from an additional therapeutic agent, 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 antibody therapy and the anti-cancer 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.

[0090] Various combinations may be employed. For the example below a CDK inhibitor and/or SAC component inhibitor is “A” and an anti-cancer therapy is “B”:

A/B/A	B/A/B	B/B/A	A/A/B	A/B/B	B/A/A	A/B/B/B	B/A/B/B
B/B/B/A	B/B/A/B	A/A/B/B	A/B/A/B	A/B/B/A	B/B/A/A		
B/A/B/A	B/A/A/B	A/A/A/B	B/A/A/A	A/B/A/A	A/A/B/A		

[0091] Administration of any compound or therapy of the present embodiments to a patient will follow general protocols for the administration of such compounds, taking into account the toxicity, if any, of the agents. Therefore, in some embodiments there is a step of monitoring toxicity that is attributable to combination therapy.

1. Chemotherapy

[0092] A wide variety of chemotherapeutic agents may be used in accordance with the present embodiments. The term “chemotherapy” refers to the use of drugs to treat cancer. A “chemotherapeutic agent” is used to connote a compound or composition that is administered in the treatment of cancer. These agents or drugs are categorized by their mode of activity within a cell, for example, whether and at what stage they affect the cell cycle. Alternatively, an agent may be characterized based on its ability to directly cross-link DNA, to intercalate

into DNA, or to induce chromosomal and mitotic aberrations by affecting nucleic acid synthesis.

[0093] Examples of chemotherapeutic agents include alkylating agents, such as thiotepa and cyclophosphamide; alkyl sulfonates, such as busulfan, improsulfan, and pposulfan; aziridines, such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines, including altretamine, triethylenemelamine, trietylenephosphoramidate, triethylenethiophosphoramidate, and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analogue topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratiostatine; a sarcodictyin; spongistatin; nitrogen mustards, such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, and uracil mustard; nitrosureas, such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimustine; antibiotics, such as the enediyne antibiotics (*e.g.*, calicheamicin, especially calicheamicin gammaII and calicheamicin omegaII); dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores, aclacinomysins, actinomycin, authrarnycin, azaserine, bleomycins, cactinomycin, carabacin, carminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, and zorubicin; anti-metabolites, such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues, such as denopterin, pteropterin, and trimetrexate; purine analogs, such as fludarabine, 6-mercaptopurine, thiamiprine, and thioguanine; pyrimidine analogs, such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, and floxuridine; androgens, such as calusterone, dromostanolone propionate, epitostanol, mepitostane, and testolactone; anti-adrenals, such as mitotane and trilostane; folic acid replenisher, such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic

acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids, such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSKpolysaccharide complex; razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2''-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; taxoids, *e.g.*, paclitaxel and docetaxel gemcitabine; 6-thioguanine; mercaptopurine; platinum coordination complexes, such as cisplatin, oxaliplatin, and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; irinotecan (*e.g.*, CPT-11); topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids, such as retinoic acid; capecitabine; carboplatin, procarbazine, plicomycin, gemcitabine, navelbine, farnesyl-protein transferase inhibitors, transplatinum, and pharmaceutically acceptable salts, acids, or derivatives of any of the above.

2. Radiotherapy

[0094] Other factors that cause DNA damage and have been used extensively include what are commonly known as γ -rays, X-rays, and/or the directed delivery of radioisotopes to tumor cells. Other forms of DNA damaging factors are also contemplated, such as microwaves, proton beam irradiation (U.S. Patents 5,760,395 and 4,870,287), and UV-irradiation. It is most likely that all of these factors affect a broad range of damage on DNA, on the precursors of DNA, on the replication and repair of DNA, and on the assembly and maintenance of chromosomes. Dosage ranges for X-rays range from daily doses of 50 to 200 roentgens for prolonged periods of time (3 to 4 wk), to single doses of 2000 to 6000 roentgens. Dosage ranges for radioisotopes vary widely, and depend on the half-life of the isotope, the strength and type of radiation emitted, and the uptake by the neoplastic cells.

3. Immunotherapy

[0095] The skilled artisan will understand that additional immunotherapies may be used in combination or in conjunction with methods of the embodiments. In the context of cancer treatment, immunotherapeutics, generally, rely on the use of immune effector cells and

molecules to target and destroy cancer cells. Rituximab (RITUXAN®) is such an example. The immune effector may be, for example, an antibody specific for some marker on the surface of a tumor cell. The antibody alone may serve as an effector of therapy or it may recruit other cells to actually affect cell killing. The antibody also may be conjugated to a drug or toxin
5 (chemotherapeutic, radionuclide, ricin A chain, cholera toxin, pertussis toxin, etc.) and serve as a targeting agent. Alternatively, the effector may be a lymphocyte carrying a surface molecule that interacts, either directly or indirectly, with a tumor cell target. Various effector cells include cytotoxic T cells and NK cells

[0096] Antibody-drug conjugates have emerged as a breakthrough approach to the
10 development of cancer therapeutics. Cancer is one of the leading causes of deaths in the world. Antibody-drug conjugates (ADCs) comprise monoclonal antibodies (MAbs) that are covalently linked to cell-killing drugs. This approach combines the high specificity of MAbs against their antigen targets with highly potent cytotoxic drugs, resulting in “armed” MAbs that deliver the payload (drug) to tumor cells with enriched levels of the antigen. Targeted delivery
15 of the drug also minimizes its exposure in normal tissues, resulting in decreased toxicity and improved therapeutic index. The approval of two ADC drugs, ADCETRIS® (brentuximab vedotin) in 2011 and KADCYLA® (trastuzumab emtansine or T-DM1) in 2013 by FDA validated the approach. There are currently more than 30 ADC drug candidates in various stages of clinical trials for cancer treatment (Leal *et al.*, 2014). As antibody engineering and
20 linker-payload optimization are becoming more and more mature, the discovery and development of new ADCs are increasingly dependent on the identification and validation of new targets that are suitable to this approach and the generation of targeting MAbs. Two criteria for ADC targets are upregulated/high levels of expression in tumor cells and robust internalization.

[0097] In one aspect of immunotherapy, the tumor cell must bear some marker that is
25 amenable to targeting, *i.e.*, is not present on the majority of other cells. Many tumor markers exist and any of these may be suitable for targeting in the context of the present embodiments. Common tumor markers include CD20, carcinoembryonic antigen, tyrosinase (p97), gp68, TAG-72, HMFG, Sialyl Lewis Antigen, MucA, MucB, PLAP, laminin receptor, erb B, and
30 p155. An alternative aspect of immunotherapy is to combine anticancer effects with immune stimulatory effects. Immune stimulating molecules also exist including: cytokines, such as IL-

2, IL-4, IL-12, GM-CSF, gamma-IFN, chemokines, such as MIP-1, MCP-1, IL-8, and growth factors, such as FLT3 ligand.

[0098] Examples of immunotherapies include immune adjuvants, *e.g.*, *Mycobacterium bovis*, *Plasmodium falciparum*, dinitrochlorobenzene, and aromatic compounds (U.S. Patents 5,801,005 and 5,739,169; Hui and Hashimoto, 1998; Christodoulides *et al.*, 1998); cytokine therapy, *e.g.*, interferons α , β , and γ , IL-1, GM-CSF, and TNF (Bukowski *et al.*, 1998; Davidson *et al.*, 1998; Hellstrand *et al.*, 1998); gene therapy, *e.g.*, TNF, IL-1, IL-2, and p53 (Qin *et al.*, 1998; Austin-Ward and Villaseca, 1998; U.S. Patents 5,830,880 and 5,846,945); and monoclonal antibodies, *e.g.*, anti-CD20, anti-ganglioside GM2, and anti-p185 (Hollander, 2012; Hanibuchi *et al.*, 1998; U.S. Patent 5,824,311). It is contemplated that one or more anti-cancer therapies may be employed with the antibody therapies described herein.

[0099] In some embodiments, the immunotherapy may be an immune checkpoint inhibitor. Immune checkpoints either turn up a signal (*e.g.*, co-stimulatory molecules) or turn down a signal. 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.

[00100] 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, *Nat Rev Cancer*, 12(4): 252-64, 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.

[00101] 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.

[00102] 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.

[00103] 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
5 increased expression of CTLA-4, an inhibitory receptor for B7 molecules.

[00104] 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.

[00105] Anti-human-CTLA-4 antibodies (or VH and/or VL domains derived
10 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 01/14424, WO 98/42752, and WO 00/37504 (CP675,206, also known as tremelimumab; formerly ticilimumab); U.S. Patent No. 6,207,156; Hurwitz *et al.*, 1998;
15 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.
20 8,017,114; all incorporated herein by reference.

[00106] 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 01/14424). In other embodiments, the antibody comprises the heavy and light chain CDRs or VRs of ipilimumab. Accordingly, in one embodiment, the antibody comprises
25 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
30 90%, 95%, or 99% variable region identity with ipilimumab).

[00107] 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
5 reference.

4. Surgery

[00108] Approximately 60% of persons with cancer will undergo surgery of some type, which includes preventative, diagnostic or staging, curative, and palliative surgery. Curative surgery includes resection in which all or part of cancerous tissue is physically
10 removed, excised, and/or destroyed and may be used in conjunction with other therapies, such as the treatment of the present embodiments, chemotherapy, radiotherapy, hormonal therapy, gene therapy, immunotherapy, and/or alternative therapies. Tumor resection refers to physical removal of at least part of a tumor. In addition to tumor resection, treatment by surgery includes laser surgery, cryosurgery, electrosurgery, and microscopically-controlled surgery (Mohs'
15 surgery).

[00109] Upon excision of part or all of cancerous cells, tissue, or tumor, a cavity may be formed in the body. Treatment may be accomplished by perfusion, direct injection, or local application of the area with an additional anti-cancer therapy. Such treatment may be repeated, for example, every 1, 2, 3, 4, 5, 6, or 7 days, or every 1, 2, 3, 4, and 5 weeks or every
20 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months. These treatments may be of varying dosages as well.

5. Other Agents

[00110] It is contemplated that other agents may be used in combination with certain aspects of the present embodiments to improve the therapeutic efficacy of treatment.
25 These additional agents include agents that affect the upregulation of cell surface receptors and GAP junctions, cytostatic and differentiation agents, inhibitors of cell adhesion, agents that increase the sensitivity of the hyperproliferative cells to apoptotic inducers, or other biological agents. Increases in intercellular signaling by elevating the number of GAP junctions would increase the anti-hyperproliferative effects on the neighboring hyperproliferative cell
30 population. In other embodiments, cytostatic or differentiation agents can be used in combination with certain aspects of the present embodiments to improve the anti-

hyperproliferative efficacy of the treatments. Inhibitors of cell adhesion are contemplated to improve the efficacy of the present embodiments. Examples of cell adhesion inhibitors are focal adhesion kinase (FAKs) inhibitors and Lovastatin. It is further contemplated that other agents that increase the sensitivity of a hyperproliferative cell to apoptosis, such as the antibody c225, could be used in combination with certain aspects of the present embodiments to improve the treatment efficacy.

IV. Kit

[00111] Also within the scope of the present disclosure are kits for detecting EGFR activating mutations, such as those disclosed herein. An example of such a kit may include a set of L858R, exon 19 deletion, and/or exon 20 mutation-specific primer. The kit may further comprise instructions for use of the primers to detect the presence or absence of the specific EGFR activating mutations described herein. The kit may further comprise instructions for diagnostic purposes, indicating that a positive identification of EGFR activating mutations described herein in a sample from a cancer patient indicates sensitivity to the CDK inhibitor and/or SAC component inhibitor. The kit may further comprise instructions that indicate that a positive identification of EGFR activating mutations described herein in a sample from a cancer patient indicates that a patient should be treated with a CDK inhibitor and/or SAC component inhibitor.

V. Examples

[00112] 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 – Identification of Methods for Treating Cancers with EGFR Activating Mutations

[00113] While EGFR mutant NSCLC patients are initially responsive to EGFR targeted therapies, resistant disease inevitably emerges and in nearly half of resistance cases, tumors lack secondary EGFR mutations such as T790M and are refractory to 2nd and 3rd generation EGFR tyrosine kinase inhibitors (TKI). The identification of treatment regimens with efficacy against T790M-negative resistance remains a major clinical challenge. To address this unmet need, a panel of NSCLC cell lines with acquired resistance to the EGFR TKI, erlotinib, gefitinib, osimertinib or poziotinib were derived.

[00114] Proteomic profiling of resistant cells was performed by reverse phase protein array (RPPA) and Western blotting for markers of epithelial to mesenchymal transition (EMT). Cell Titer glow assay in a high-throughput 384 well plate system was utilized for drug screening. Long-term effect of SAC and CDK inhibitors was evaluated by J 4 day clonogenic assay.

[00115] Using HCC827 and HCC4006 (EGFR mutant NSCLC cells), EGFR TKI resistant variants were derived in vitro by continuously culturing cells in increasing concentrations of the TKI, such as erlotinib. Erlotinib resistant cells (ER) were negative for secondary EGFR mutations and were resistant to 2nd and 3rd generation EGFR TKIs including osimertinib, afatinib, and dacomitinib (FIG. 1) and had undergone epithelial to mesenchymal transition (EMT) as demonstrated by loss of E-cadherin, enhanced expression of N-cadherin and Axl and an increased invasive phenotype as determined by Boyden chamber assay. Proteomic profiling revealed that although EGFR TKI resistant cells displayed similar mesenchymal and invasive phenotypes, there was significant heterogeneity in protein expression and pathway activation among resistant variants derived from the same parental cell line.

[00116] To identify therapeutic agents with activity against EMT-associated EGFR TKI resistance, a high-throughput drug screening was performed to test the efficacy of 1,321 compounds. EMT-associated EGFR TKI resistance was accompanied by the acquisition of broad-spectrum drug resistance. Compared to parental cells, mesenchymal EGFR TKI resistant cells were significantly more resistant to chemotherapeutic agents used to treat NSCLC including pemetrexed, irinotecan, vinblastine, and gemcitabine. EGFR TKI resistant

cells displayed acquired resistance to 147 other tyrosine kinase and serine/threonine kinase inhibitors. In contrast, both parental cells and mesenchymal EGFR TKI resistant variants were highly sensitive to CDK inhibitors and agents targeting spindle assembly checkpoint (SAC) components including PLK1, Aurora, KSP, and survivin. These finding were validated by MTS and clonogenic assays. Treatment with SAC inhibitors induced the accumulation of cells in G2/M phase, enlarged nuclear size, and polyploidy.

[00117] To clinically validate these findings, a cell line (MDA-011) was established from the pleural effusion of an EGFR mutant NSCLC patient with T790M-negative resistance to erlotinib. *In vitro*, MDA-011 cells were resistant to erlotinib and osimertinib. MDA-011 cells were highly sensitive to CDK inhibitors and SAC inhibitors as determined by MTS and clonogenic assays. These data indicated that EMT-associated resistance to EGFR TKIs is associated with broad spectrum drug resistance but have vulnerabilities to CDK and SAC inhibition which can be exploited to overcome resistant disease in NSCLC patients.

[00118] Table 1: IC₅₀ for SAC inhibitor for 5 day Cell Titer Glo Assay.

Drug	Target	HCC4006 GIC ₅₀ (μM)	HCC4006 ER GIC ₅₀ (μM)
BI 2538	PLK1	0.01	0.01
BI6727 (Volasertib)	PLK1	0.01	0.01
GSK461364	PLK1	0.01	0.01
ON-01910	PLK1	0.02	0.02
GW 843682X	PLK1	0.32	0.30
HMN-214	PLK1	0.55	0.64
AMG 900	Pan-Aurora	0.01	0.01
MLN8237 (Alisertib)	Aurora A	0.02	0.02
PF-03814735	Aurora A/B	0.02	0.02
TOZASERTIB	Aurora A	0.05	0.06
MLN8054	Pan-Aurora	0.16	0.18

SNS-314 Mesylate	Pan-Aurora	0.01	0.01
Ispinesib	KSP	0.01	0.01
SB 743921	KSP	0.01	0.01
YM155	Survivin	0.01	0.01

[00119] Table 2: IC50 for SAC inhibitor for 5 day Cell Titer Glo Assay.

Drug	Target	HCC4006 GIC ₅₀ (μM)	HCC4006 ER GIC ₅₀ (μM)
BI 2538	PLK1	0.01	0.07
BI6727 (Volasertib)	PLK1	0.01	0.54
GSK461364	PLK1	0.01	0.01
ON-01910	PLK1	0.02	0.02
GW 843682X	PLK1	0.17	0.22
HMN-214	PLK1	0.70	0.69
ON-01910	PLK1	0.01	0.02
AMG 900	Pan-Aurora	0.01	0.01
MLN8054	Pan-Aurora	0.25	0.08
MLN8237 (Aisertib)	Aurora A	0.03	0.02
PF-03814735	Aurora A/B	0.02	0.02
SNS-314 Mesylate	Pan-Aurora	0.05	0.03
TOZASERTIB	Aurora A	0.02	0.04
Ispinesib (SB-713992)	KSP	0.01	0.03
SB 743921	KSP	0.01	0.03
YM155	Survivin	0.01	0.03

[00120] Table 3: IC₅₀ for CDK inhibitor for 5 day Cell Titer Glow Assay.

Drug	Target	HCC4006 GIC ₅₀ (μM)	HCC4006 ER GIC ₅₀ (μM)
AZD5438	CDK	0.18	0.28
Dinaciclib	CDK	0.01	0.01
Alvocidib	CDK	0.05	0.04
Abemaciclib	CDK	0.03	0.30
Pelbociclib	CDK	0.04	0.04
Drug	Target	HCC4006 GIC ₅₀ (μM)	HCC4006 ER GIC ₅₀ (μM)
AZD5438	CDK	0.16	0.11
Dinaciclib	CDK	0.01	0.01
Alvocidib	CDK	0.03	0.03
Abemaciclib	CDK	0.06	0.11
Pelbociclib	CDK	0.40	0.88

[00121] Next, H1975 cells (EGFR mutation positive with T790M) were cultured in osimertinib until resistant variants emerged. Osimertinib resistant cells (H1975 OR5 and H1975 OR16) had undergone EMT and were sensitive to CDK inhibitors and agents targeting SAC components including PLK1 (volasertib), Aurora (AMG-900 and alisertib), KSP (ispinesib) (FIG. 7).

[00122] In addition, the CDK and SAC inhibitors were investigated in the setting of acquired resistance to inhibitors with activity against EGFR exon 20 insertion mutations. YUL-0019 cells harbor an EGFR exon 20 insertion mutation and are highly sensitive to the EGFR inhibitor, poziotinib. YUL-0019 cells were continuously cultured in poziotinib until resistant cells emerged. YUL-0019 cells and YUL-0019 PR8 (poziotinib resistant) cells were highly sensitive to CDK inhibitors (including dinaciclib and alvocidib) SAC inhibitors including PLK1 (volasertib), Aurora (AMG-900 and alisertib), and KSP (ispinesib) (FIG. 8).

These data indicate that EMT-associated resistance to EGFR TKIs is associated with broad

spectrum drug resistance but have vulnerabilities to CDK and SAC inhibition which can be exploited to overcome resistant disease in NSCLC patients.

* * *

5 **[00123]** 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
10 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.

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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.

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- International Patent Publication No. WO2009/114335
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International Patent Publication No. WO2011/066342
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- 5 International Patent Publication No. WO01/14424
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- 20 U.S. Patent No. 5,824,311
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U.S. Patent No. 5,846,945
U.S. Patent No. 5,869,245
- 25 U.S. Patent No. 5,885,796
U.S. Patent No. 6,207,156
U.S. Patent No. 8,008,449
U.S. Patent No. 8,017,114
U.S. Patent No. 8,119,129
- 30 U.S. Patent No. 8,188,102
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CLAIMS

1. A method of treating cancer in a subject comprising administering an effective amount of a cyclin dependent kinase (CDK) inhibitor and/or a spindle assembly checkpoint (SAC) component inhibitor to the subject, wherein the subject is determined to have one or more EGFR activating mutations.
5
2. The method of claim 1, wherein the one or more EGFR activating mutations are selected from the group consisting of L858R, an exon 19 deletion, and an exon 20 insertion.
3. The method of claim 2, wherein the exon 19 deletion is E746-A750 deletion, L747-E749 deletion, or A750P.
10
4. The method of claim 2, wherein the exon 20 insertion is N771Del Ins FH.
5. The method of any one of claims 1-4, wherein the subject is determined to have 2, 3, or 4 EGFR activating mutations.
6. The method of claim 1, wherein the subject was determined to have an EGFR activating mutation by analyzing a genomic sample from the subject.
15
7. The method of claim 6, wherein the genomic sample is isolated from saliva, blood, urine, normal tissue, or tumor tissue.
8. The method of claim 6, wherein the presence of an EGFR activating mutation is determined by nucleic acid sequencing or PCR analyses.
- 20 9. The method of claim 1, wherein the CDK inhibitor is further defined as a CDK2 inhibitor, CDK5 inhibitor, CDK1 inhibitor, or CDK 9 inhibitor.

10. The method of claim 1, wherein the CDK inhibitor is dinaciclib (SCH727965), alvocidib (flavopiridol), roscovitine (seliciclib, CYC202), SNS-032 (BMS-387032), LY2857785, ADZ5438, BMS-265246, NU6027, LDC000067, wogonin, or RO-3306.
11. The method of claim 1, wherein the CDK inhibitor is MSC2530818, senexin A,
5 LDC4297 (LDC044297), PHA-793887, BS-181 HCl, PHA-767491, THX1 2HCl, or XL413.
12. The method of claim 1, wherein the CDK inhibitor is dinaciclib or alvocidib.
13. The method of claim 1, wherein the CDK inhibitor is not a CDK4 inhibitor and/or CDK6 inhibitor.
- 10 14. The method of claim 1, wherein the CDK inhibitor is not palociclib (PD0332991), abemaciclib (LY2835219), or ribociclob.
15. The method of claim 1, wherein the SAC component inhibitor is further defined as a polo-like kinase 1 (PLK1) inhibitor, Aurora kinase inhibitor, survivin, and/or KSP inhibitor.
- 15 16. The method of claim 15, wherein the PLK1 inhibitor is BI 2536, volasertib, GSK461364, ON-01910, GW 843682X, or HMN-214.
17. The method of claim 15, wherein the PLK1 inhibitor is volasertib or ON-01910.
18. The method of claim 1, wherein the SAC inhibitor is not a PLK1 inhibitor.
19. The method of claim 15, wherein the Aurora kinase inhibitor is a Pan-Aurora inhibitor,
20 Aurora A/B inhibitor, or an Aurora A inhibitor.
20. The method of claim 15, wherein the Aurora kinase inhibitor is AMG 900, alisertib, PF-03814735, Tozasertib, MLN8054, or SNS-314 Mesylate.
21. The method of claim 1, wherein the Aurora kinase inhibitor is AMG-900 or alisertib.

22. The method of claim 15, wherein the KSP inhibitor is ispinesib or SB743921.
23. The method of claim 15, wherein the survivin inhibitor is YM155.
24. The method of any one of claims 1-23, wherein the treatment results in accumulation of cells in G2/M phase, enlarged nuclear size, and/or polyploidy.
- 5 25. The method of claim 1, wherein the cancer is resistant to one or more tyrosine kinase inhibitors (TKIs).
26. The method of claim 25, wherein the one or more TKIs are selected from the group consisting of osimertinib, erlotinib, gefitinib, afatinib, poziotinib, dacomitinib, and CO-1686.
- 10 27. The method of claim 1, wherein the cancer has acquired broad spectrum drug resistance.
28. The method of claim 1, wherein the cancer is resistant to pemetrexed, irinotecan, vinblastine, and/or gemcitabine.
29. The method of claim 1, wherein the cancer has acquired mutations for poziotinib, erlotinib, or osimertinib.
- 15 30. The method of claim 29, wherein the acquired mutation for poziotinib, erlotinib, or osimertinib comprises an EGFR exon 20 insertion.
31. The method of claim 1, wherein the cancer has undergone epithelial to mesenchymal transition (EMT).
32. The method of claim 31, wherein EMT is demonstrated by decreased E-cadherin
20 expression, increased expression of vimentin and/or Axl, and/or an increased invasive phenotype.
33. The method of claim 1, wherein the subject is further determined to comprise a secondary mutation.

34. The method of claim 33, wherein the secondary mutation is a T790M resistance mutation, C797S resistance mutation or L792H resistance mutation.
35. The method of claim 1, wherein the cancer does not comprise a secondary mutation.
36. The method of claim 1, wherein the cancer does not comprise a T790M resistance
5 mutation.
37. The method of any one of claims 1-36, further comprising administering at least one additional anti-cancer therapy.
38. The method of claim 37, wherein the at least one additional anti-cancer therapy is chemotherapy, radiotherapy, gene therapy, surgery, hormonal therapy, anti-angiogenic
10 therapy or immunotherapy.
39. The method of claim 37, wherein the at least one additional anti-cancer therapy is a TKI and/or chemotherapy.
40. The method of claim 39, wherein the TKI is osimertinib, erlotinib, gefitinib, afatinib, dacomitinib, or CO-1686.
- 15 41. The method of claim 39, wherein the chemotherapy is pemetrexed, irinotecan, vinblastine, or gemcitabine.
42. The method of claim 39, wherein the CDK inhibitor, SAC component inhibitor, and/or anti-cancer therapy are administered intravenously, subcutaneously, intraosseously, orally, transdermally, in sustained release, in controlled release, in delayed release, as
20 a suppository, or sublingually.
43. The method of claim 39, wherein administering the CDK inhibitor, SAC component inhibitor, and/or anti-cancer therapy comprises local, regional or systemic administration.

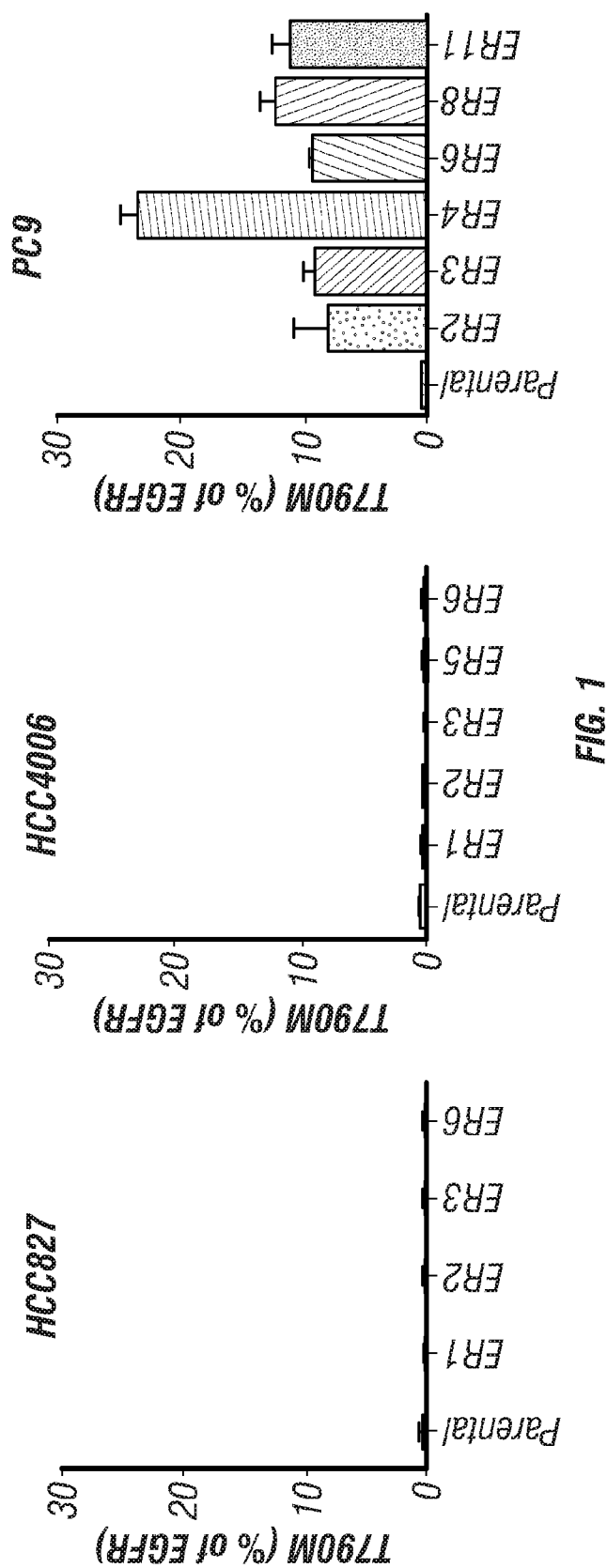
44. The method of claim 39, wherein the CDK inhibitor, SAC component inhibitor, and/or anti-cancer therapy are administered two or more times.
45. The method of claim 1, wherein the subject is human.
46. The method of claim 45, wherein the subject has cancer.
- 5 47. The method of claim 46, wherein the cancer is oral cancer, oropharyngeal cancer, nasopharyngeal cancer, respiratory cancer, urogenital cancer, gastrointestinal cancer, central or peripheral nervous system tissue cancer, an endocrine or neuroendocrine cancer or hematopoietic cancer, glioma, sarcoma, carcinoma, lymphoma, melanoma, fibroma, meningioma, brain cancer, oropharyngeal cancer, nasopharyngeal cancer, 10 renal cancer, biliary cancer, pheochromocytoma, pancreatic islet cell cancer, Li-Fraumeni tumors, thyroid cancer, parathyroid cancer, pituitary tumors, adrenal gland tumors, osteogenic sarcoma tumors, multiple neuroendocrine type I and type II tumors, breast cancer, lung cancer, head and neck cancer, prostate cancer, esophageal cancer, tracheal cancer, liver cancer, bladder cancer, stomach cancer, pancreatic cancer, ovarian 15 cancer, uterine cancer, cervical cancer, testicular cancer, colon cancer, rectal cancer or skin cancer.
48. The method of claim 46, wherein the cancer is non-small cell lung cancer.
49. A pharmaceutical composition comprising a CDK inhibitor and/or SAC component inhibitor for use in a subject determined to have one or more EGFR activating 20 mutations.
50. The composition of claim 49, wherein the one or more EGFR activating mutations are selected from the group consisting of L858R, an exon 19 deletion, and an exon 20 insertion.

51. The composition of claim 50, wherein the exon 19 deletion is E746-A750 deletion, L747-E749 deletion, or A750P.
52. The composition of claim 50, wherein the exon 20 insertion is N771Del Ins FH.
53. The composition of claim 49, wherein the subject is determined to have 2, 3, or 4 EGFR
5 activating mutations.
54. The composition of claim 49, wherein the CDK inhibitor is further defined as a CDK2 inhibitor, CDK5 inhibitor, CDK1 inhibitor, or CDK 9 inhibitor.
55. The composition of claim 49, wherein the CDK inhibitor is dinaciclib (SCH727965), alvocidib (flavopiridol), roscovitine (seliciclib, CYC202), SNS-032 (BMS-387032),
10 LY2857785, ADZ5438, BMS-265246, NU6027, LDC000067, wogonin, or RO-3306.
56. The composition of claim 49, wherein the CDK inhibitor is MSC2530818, senexin A, LDC4297 (LDC044297), PHA-793887, BS-181 HCl, PHA-767491, THX1 2HCl, or XL413.
57. The composition of claim 49, wherein the CDK inhibitor is dinaciclib or alvocidib.
- 15 58. The composition of claim 49, wherein the CDK inhibitor is not a CDK4 inhibitor and/or CDK6 inhibitor.
59. The composition of claim 49, wherein the CDK inhibitor is not palociclib (PD0332991), abemaciclib (LY2835219), or ribociclob.
60. The composition of claim 49, wherein the SAC component inhibitor is further defined
20 as a polo-like kinase 1 (PLK1) inhibitor, Aurora kinase inhibitor, survivin, and/or KSP inhibitor.
61. The composition of claim 60, wherein the PLK1 inhibitor is BI 2536, volasertib, GSK461364, ON-01910, GW 843682X, or HMN-214.

62. The composition of claim 60, wherein the PLK1 inhibitor is volasertib or ON-01910.
63. The composition of claim 49, wherein the SAC inhibitor is not a PLK1 inhibitor.
64. The composition of claim 60, wherein the Aurora kinase inhibitor is a Pan-Aurora inhibitor, Aurora A/B inhibitor, or an Aurora A inhibitor.
- 5 65. The composition of claim 60, wherein the Aurora kinase inhibitor is AMG 900, alisertib, PF-03814735, Tozasertib, MLN8054, or SNS-314 Mesylate.
66. The composition of claim 49, wherein the Aurora kinase inhibitor is AMG-900 or alisertib.
67. The composition of claim 60, wherein the KSP inhibitor is ispinesib or SB743921.
- 10 68. The composition of claim 60, wherein the survivin inhibitor is YM155.
69. The composition of claim 49, wherein the cancer does not comprise a T790M resistance mutation.
70. The composition of claim 49, further comprising administering at least one additional anti-cancer therapy.
- 15 71. The composition of claim 70, wherein the at least one additional anti-cancer therapy is chemotherapy, radiotherapy, gene therapy, surgery, hormonal therapy, anti-angiogenic therapy or immunotherapy.
72. The composition of claim 70, wherein the at least one additional anti-cancer therapy is a TKI and/or chemotherapy.
- 20 73. The composition of claim 72, wherein the TKI is osimertinib, erlotinib, gefitinib, afatinib, dacomitinib, or CO-1686.
74. The composition of claim 72, wherein the chemotherapy is pemetrexed, irinotecan, vinblastine, or gemcitabine.

75. The composition of claim 49, wherein the cancer is non-small cell lung cancer.
76. The composition of claim 49, wherein the subject is human.
77. A method of predicting a response to a CDK inhibitor and/or SAC component inhibitor alone or in combination with a second anti-cancer therapy in a subject having a cancer comprising detecting an EGFR activating mutation in a genomic sample obtained from said subject, wherein if the sample is positive for the presence of the EGFR activating mutation, then the subject is predicted to have a favorable response to the CDK inhibitor and/or SAC component inhibitor alone or in combination with an anti-cancer therapy.
78. The method of claim 77, wherein the anti-cancer therapy is a TKI and/or chemotherapy.
79. The method of claim 77, wherein a favorable response to CDK inhibitor and/or SAC component inhibitor alone or in combination with an anti-cancer therapy comprises reduction in tumor size or burden, blocking of tumor growth, reduction in tumor-associated pain, reduction in cancer associated pathology, reduction in cancer associated symptoms, cancer non-progression, increased disease free interval, increased time to progression, induction of remission, reduction of metastasis, or increased subject survival.
80. The method of claim 77, further comprising administering CDK inhibitor and/or SAC component inhibitor alone or in combination with a second anti-cancer therapy to said subject predicted to have a favorable response.
81. The method of claim 80, wherein the second anti-cancer therapy is a TKI or chemotherapy.

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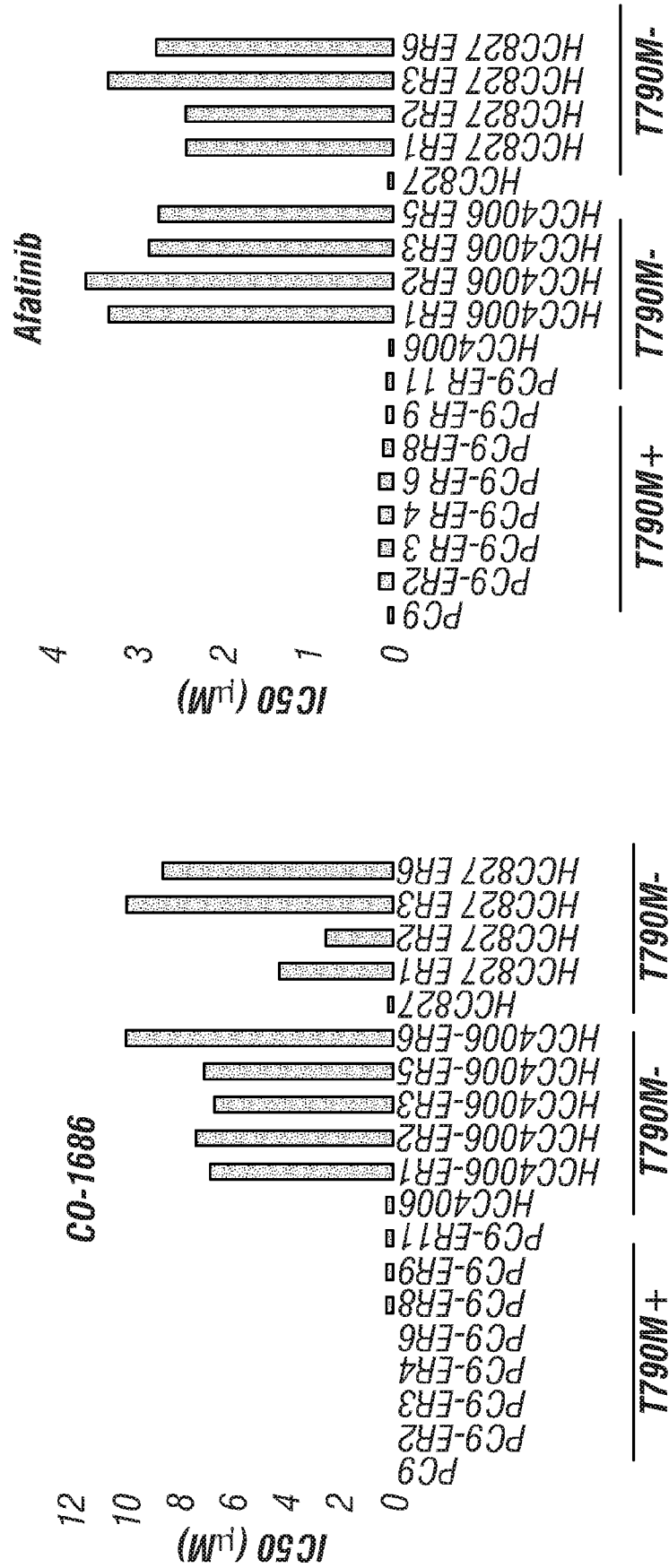


FIG. 1
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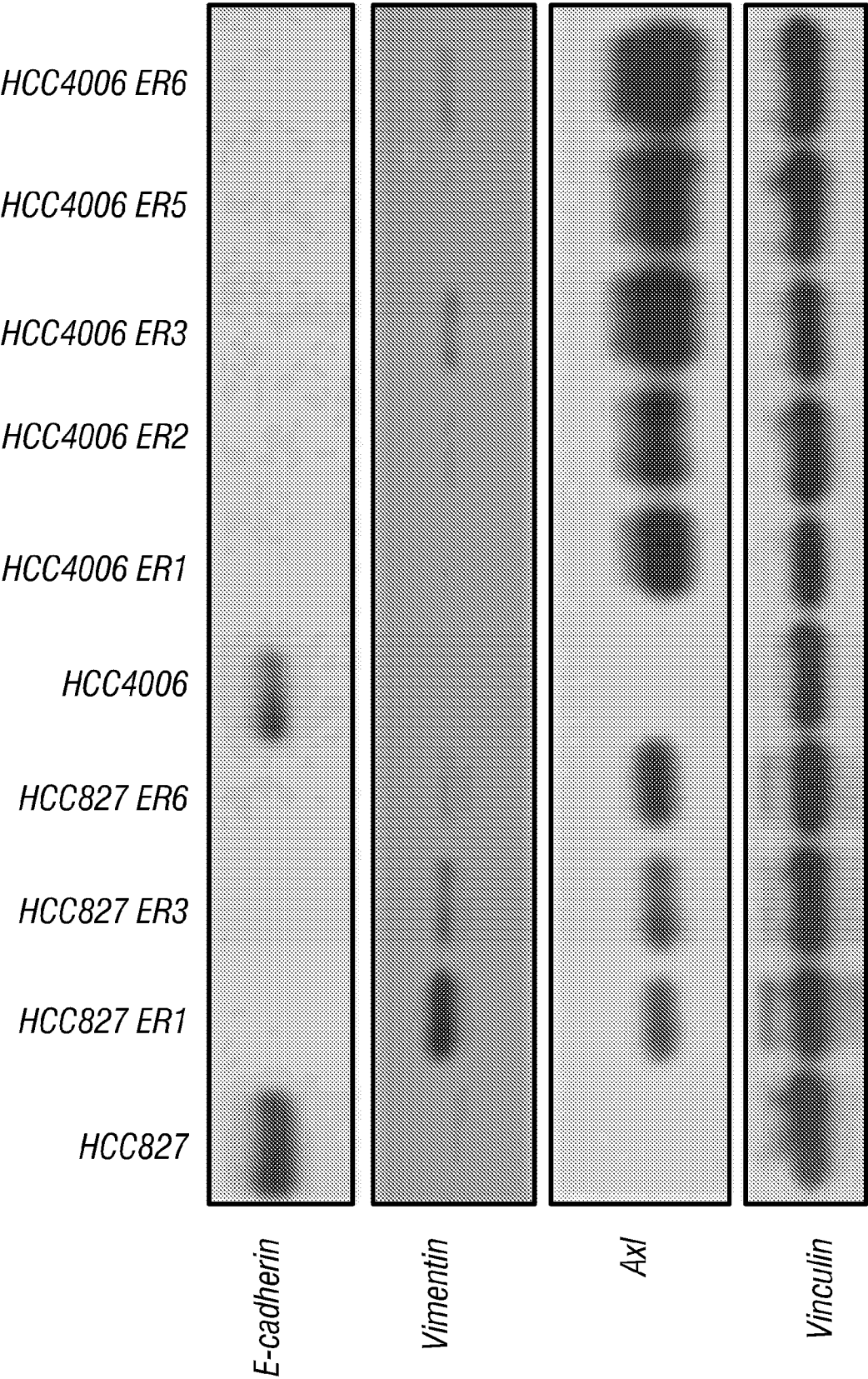
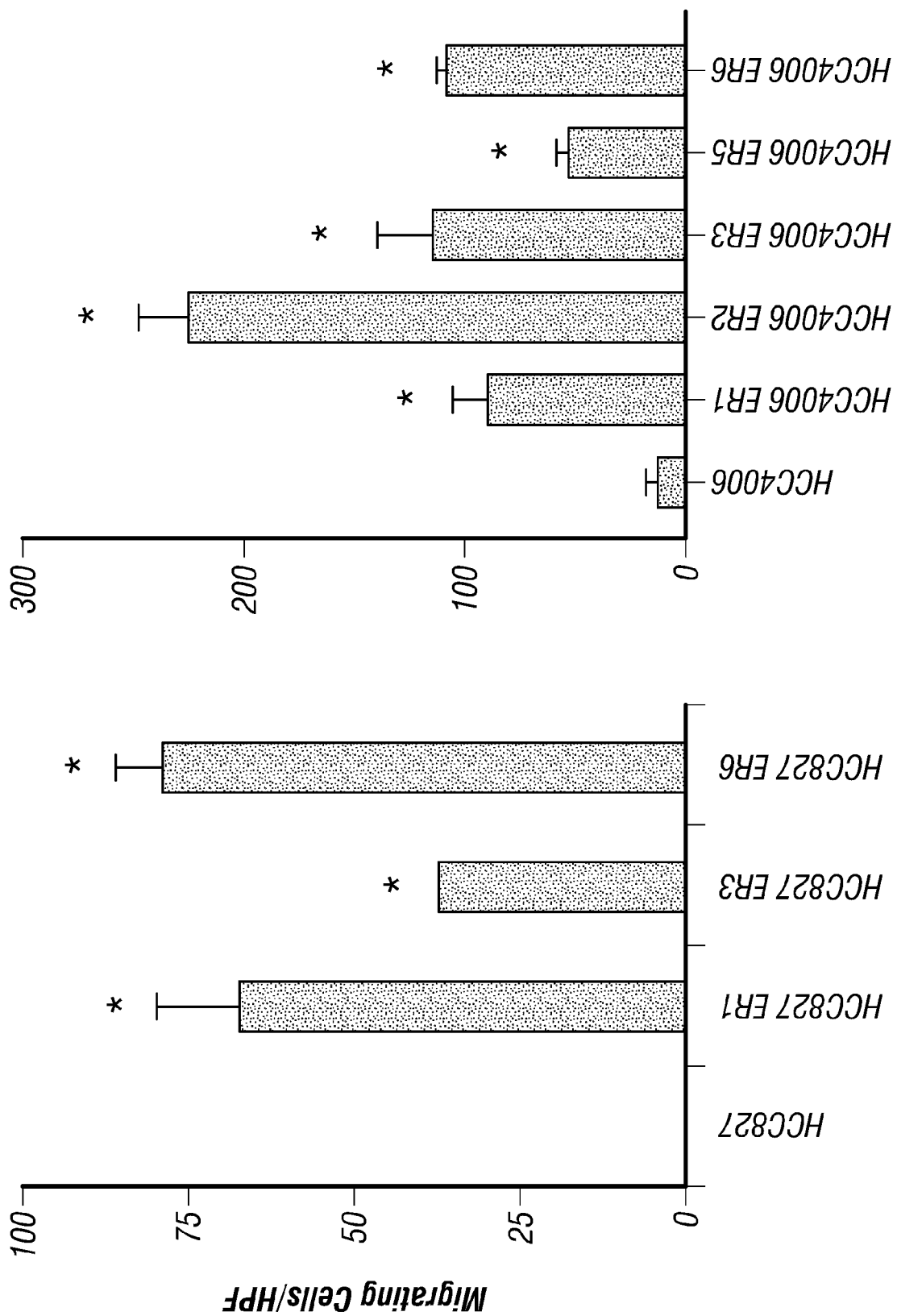


FIG. 2A

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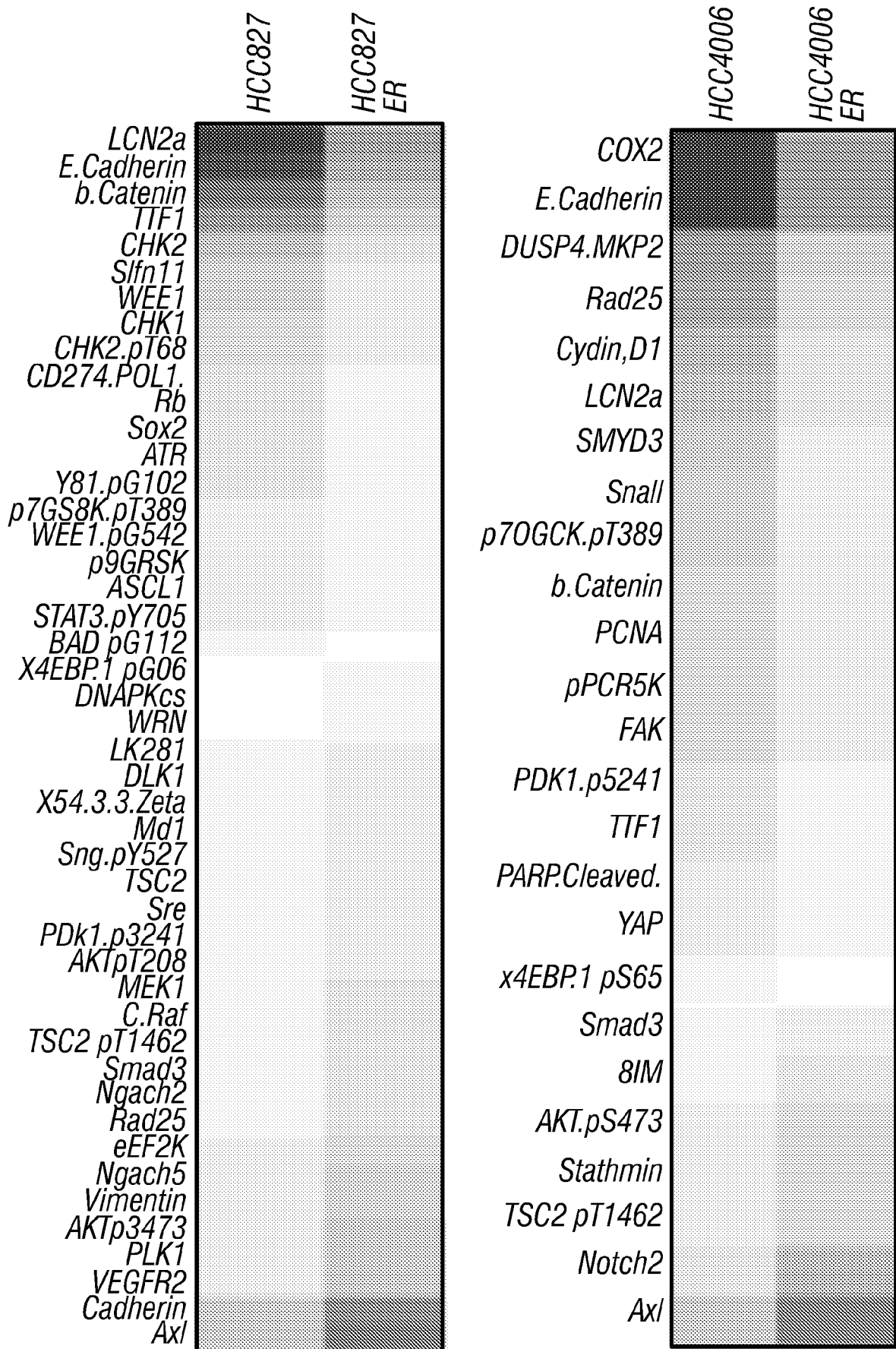
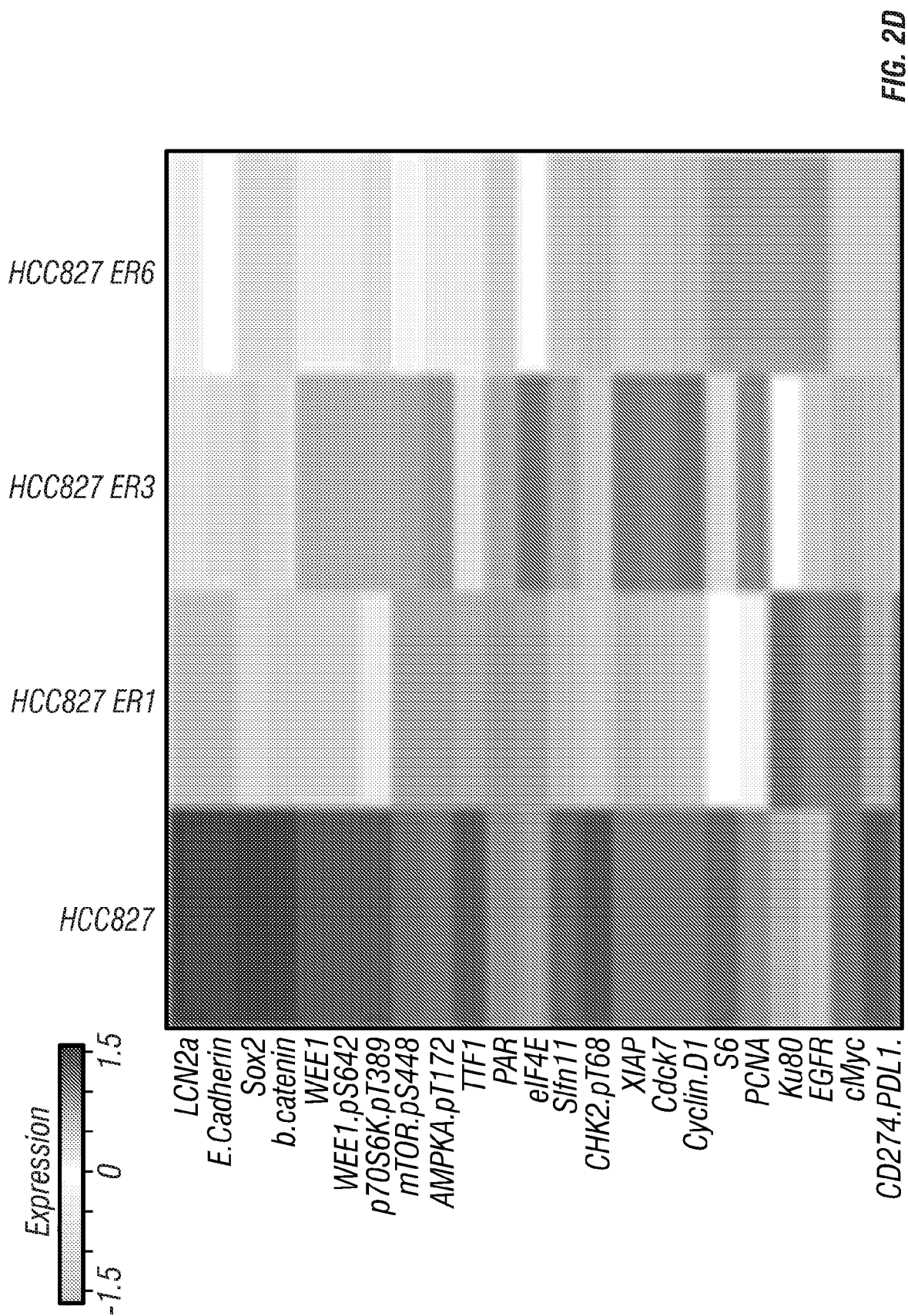


FIG. 2C

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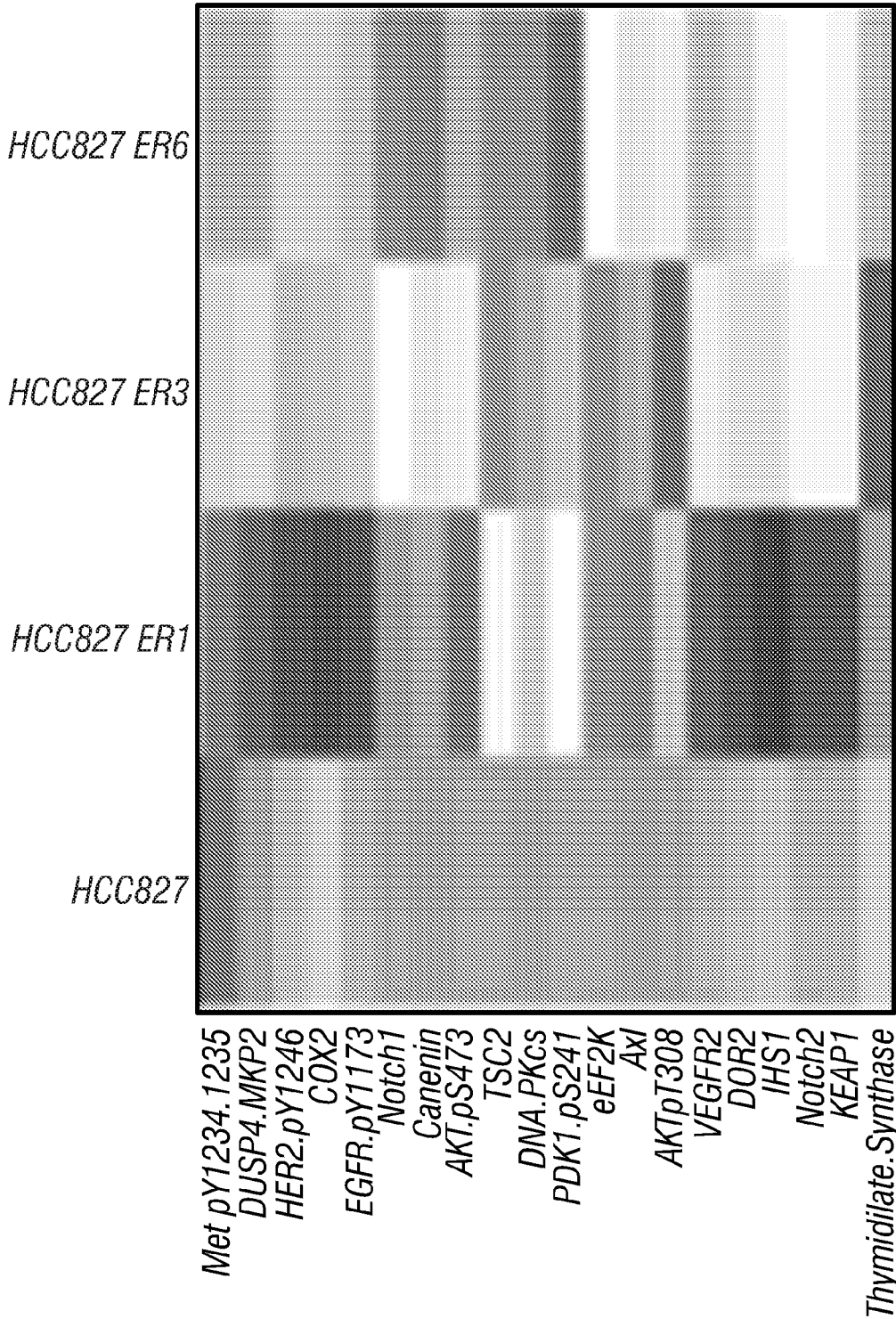


FIG. 2D
(Cont'd)

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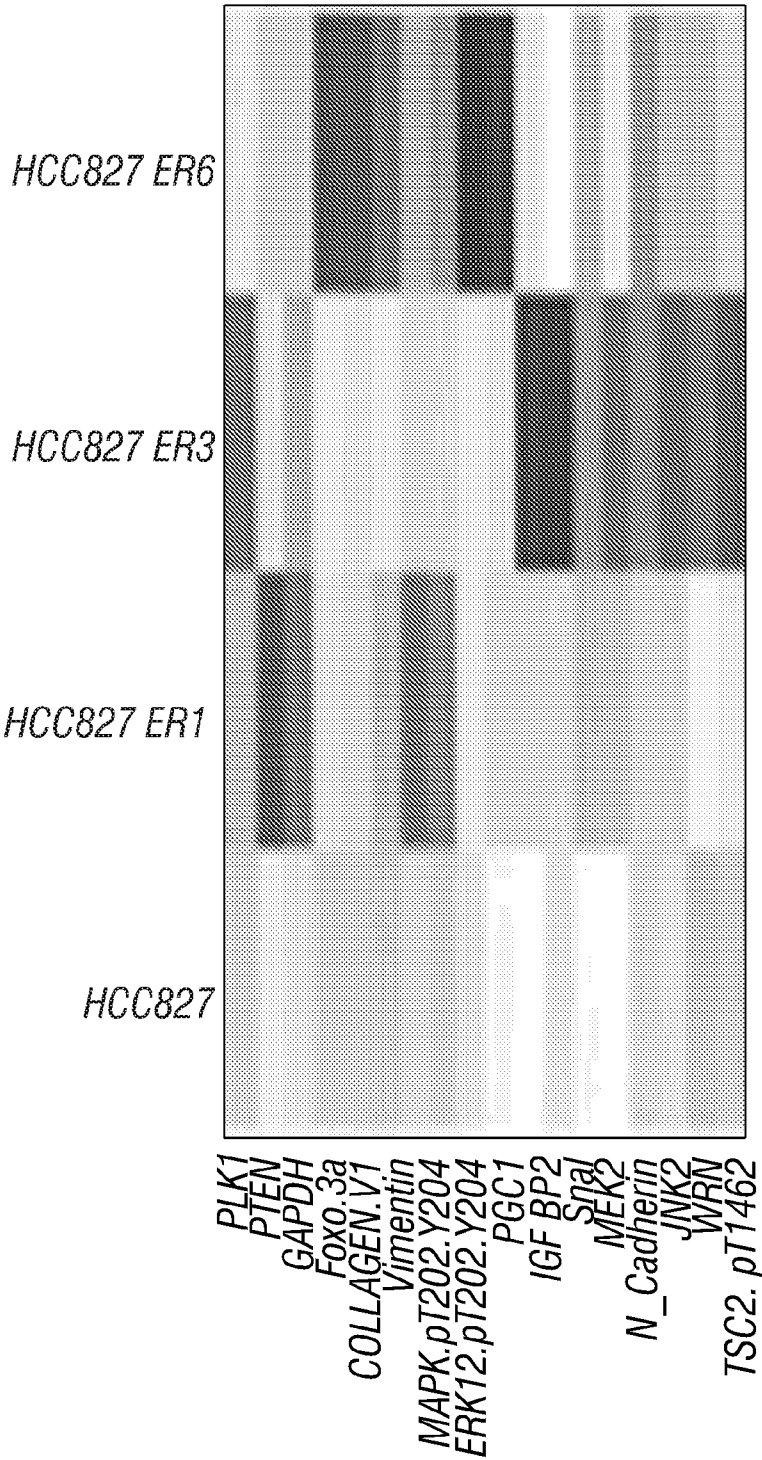
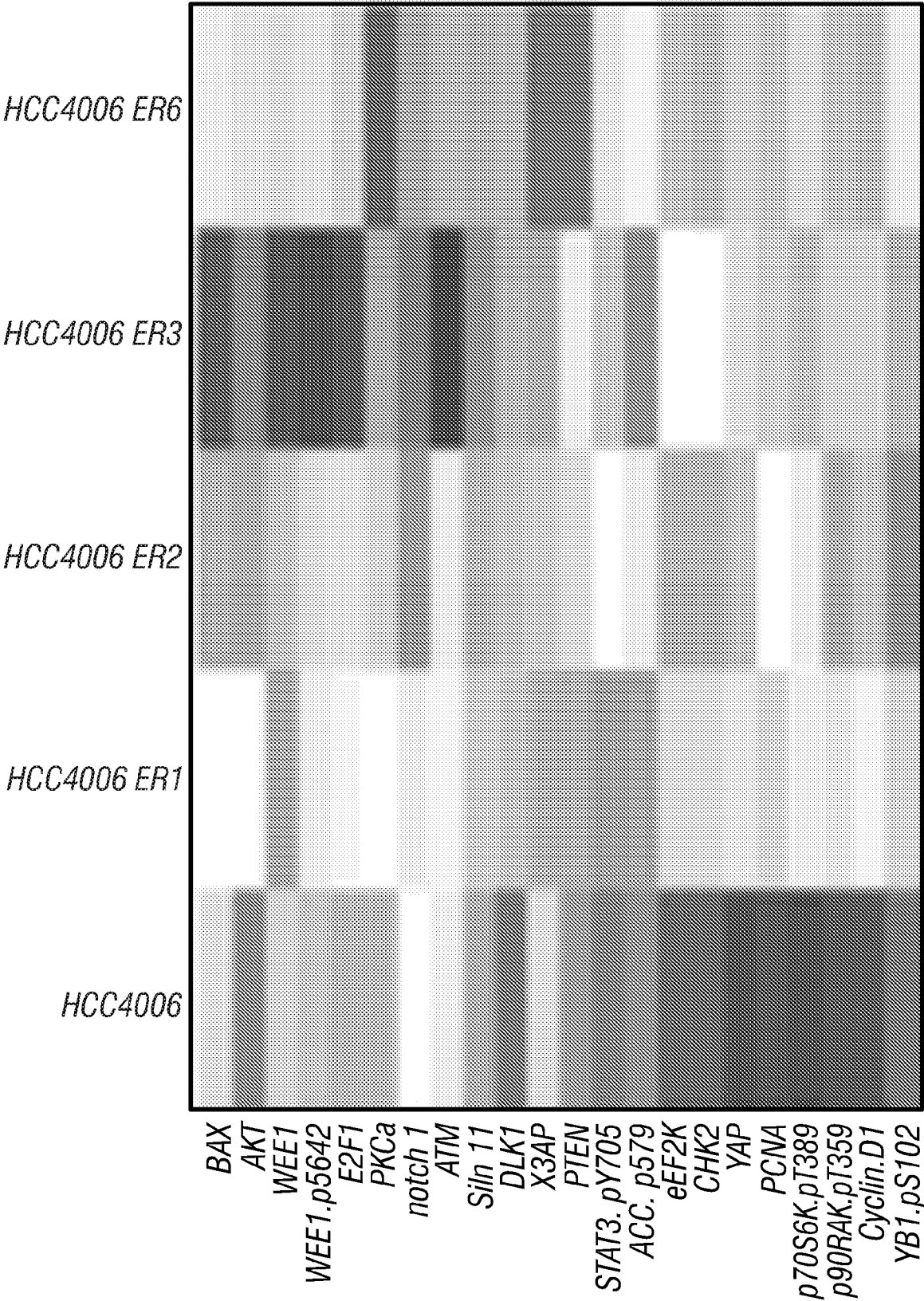


FIG. 2D
(Cont'd)

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FIG. 2D
(Cont'd)



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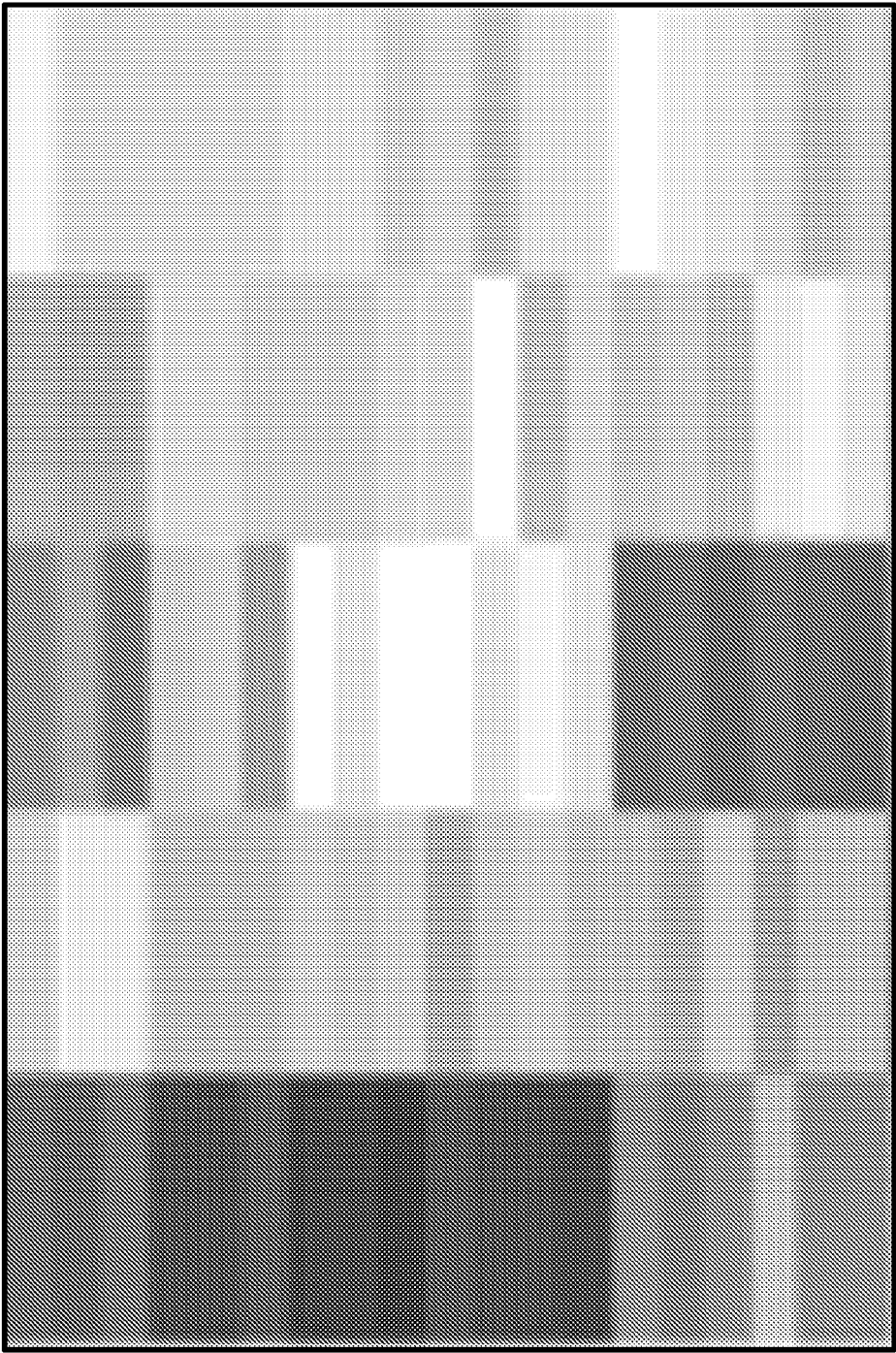


FIG. 2D
(Cont'd)

EGFR
S6. pS235. 236
Met
TTF1
FAK
E. Cadherin
OUSP4. MKP2
X4EBP.1p865
COX2
p9QRSK
b.cathenin
SnaI
SMYD3
MEK1.2p217 221
HER2.Py1245
MetpY1234. 1235
Sre.pY416
MOM2.pS166
EGFR.pY1173

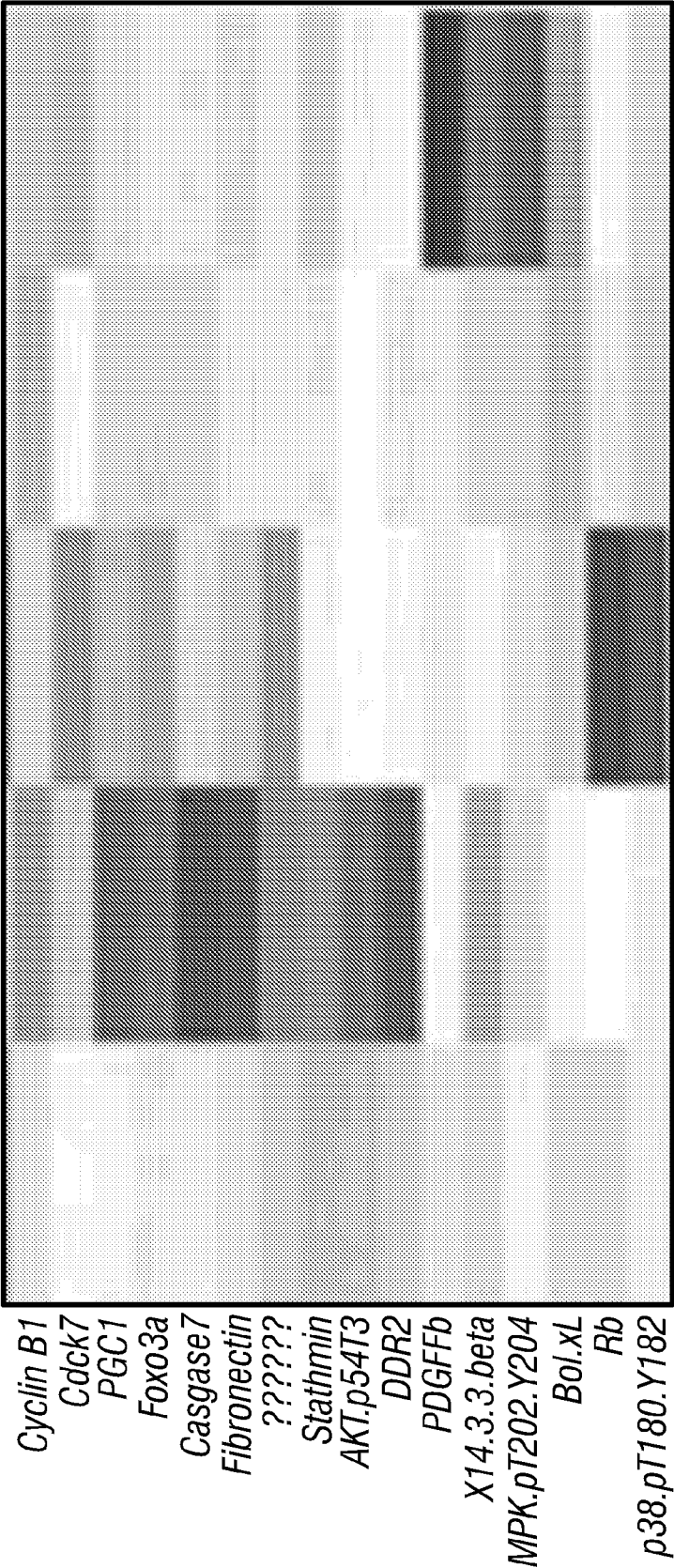


FIG. 2D
(Cont'd)

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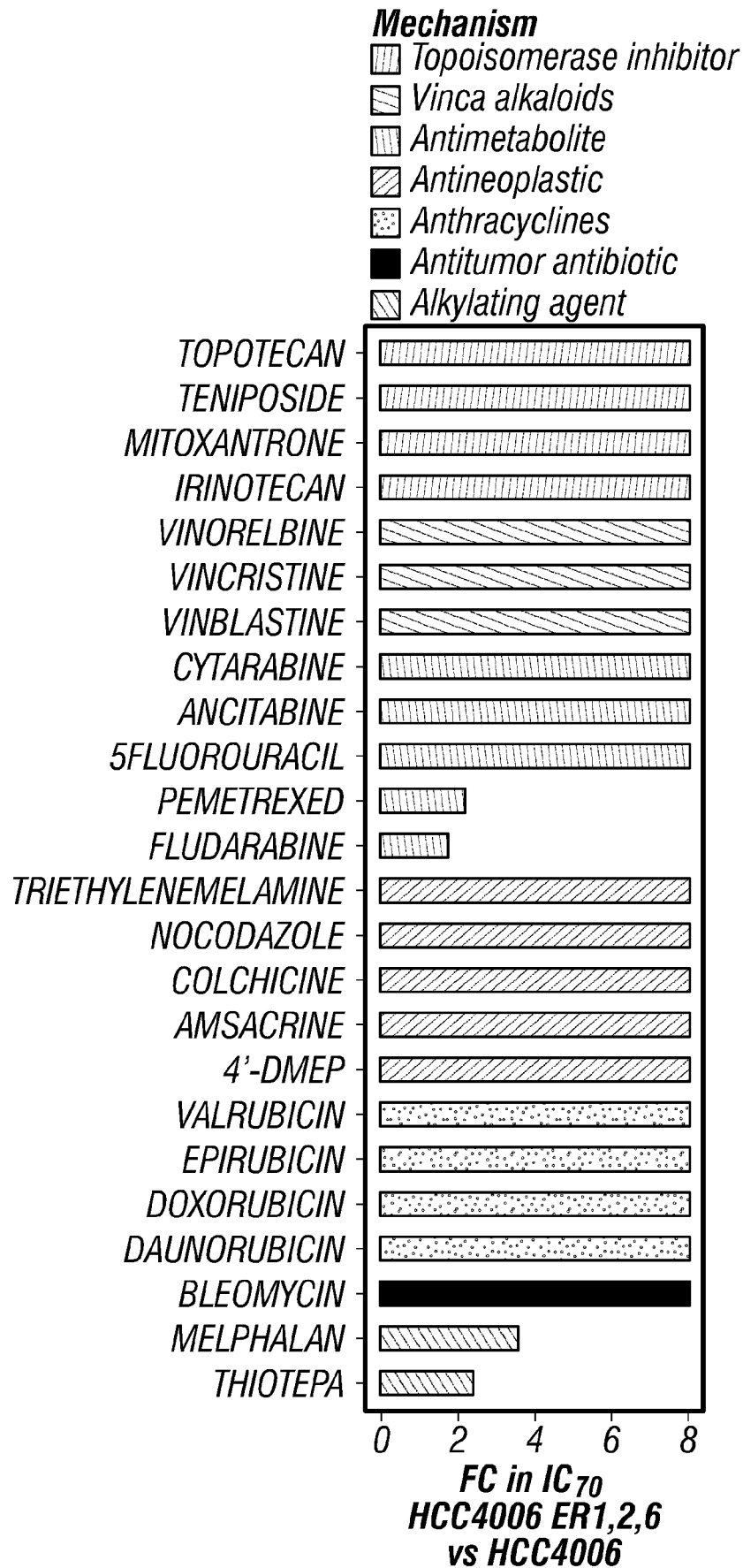


FIG. 3A

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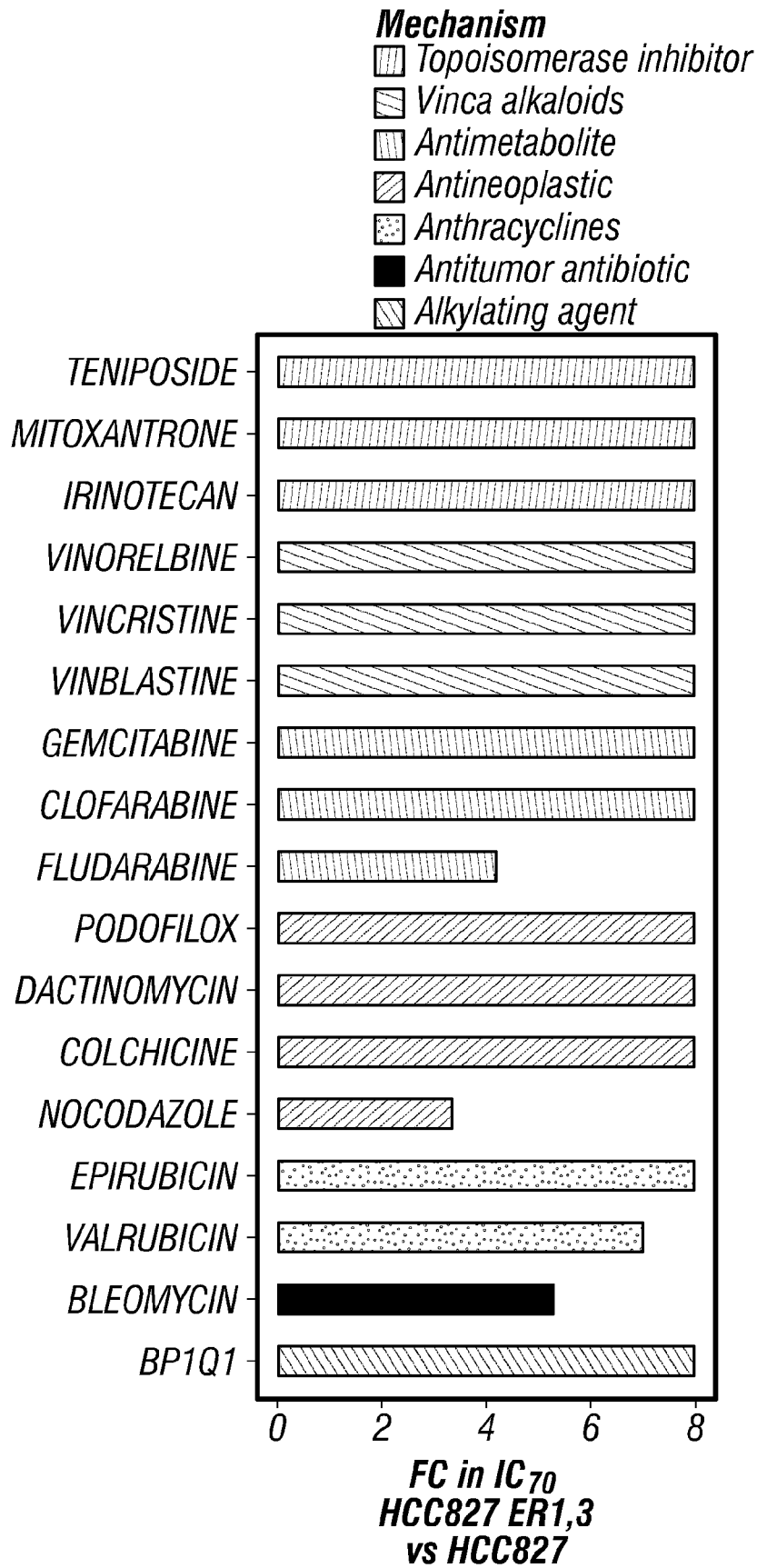
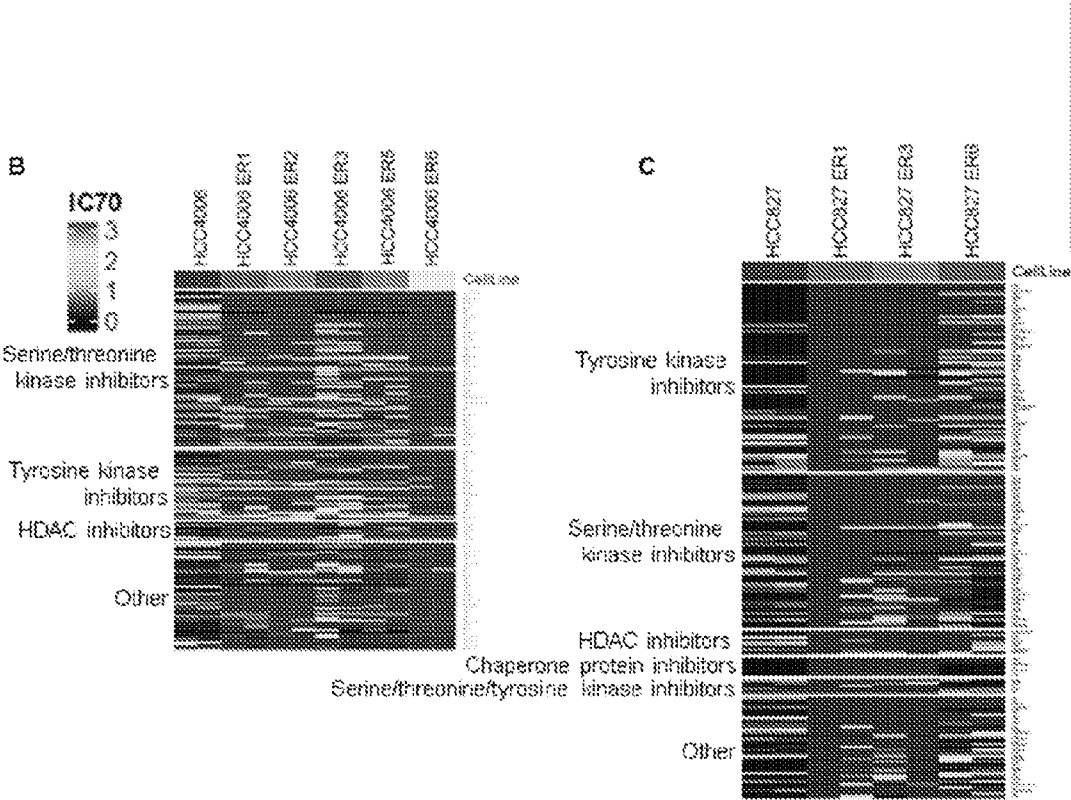
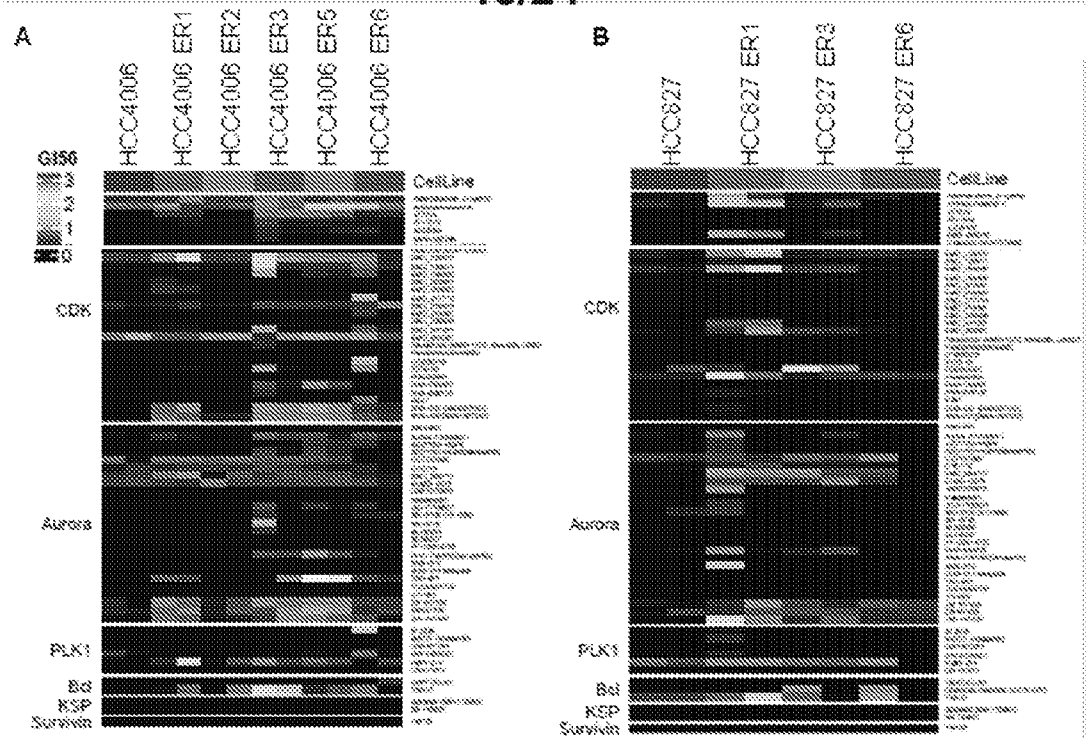


FIG. 3A
(Cont'd)



FIGS. 3B-3C



FIGS. 4A-4B

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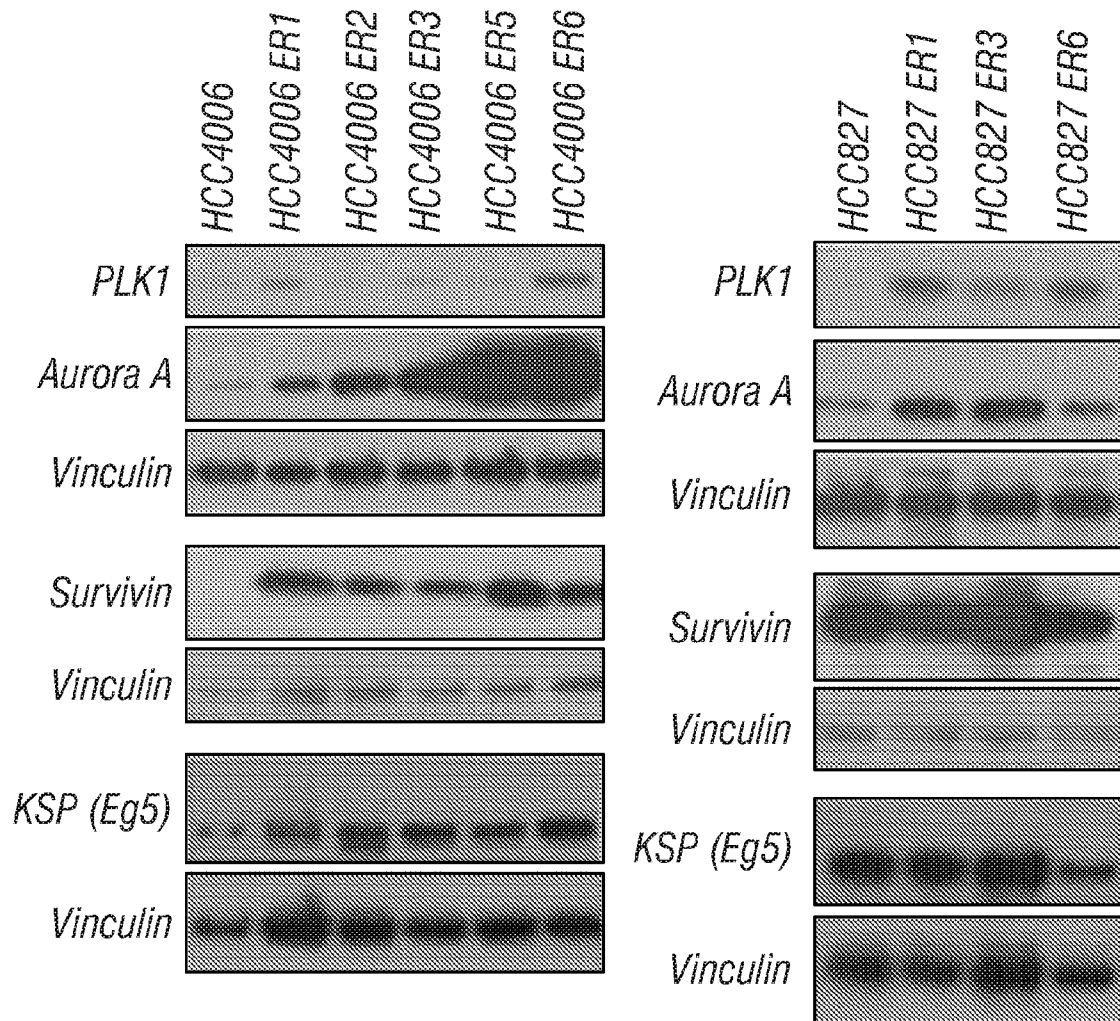


FIG. 4C

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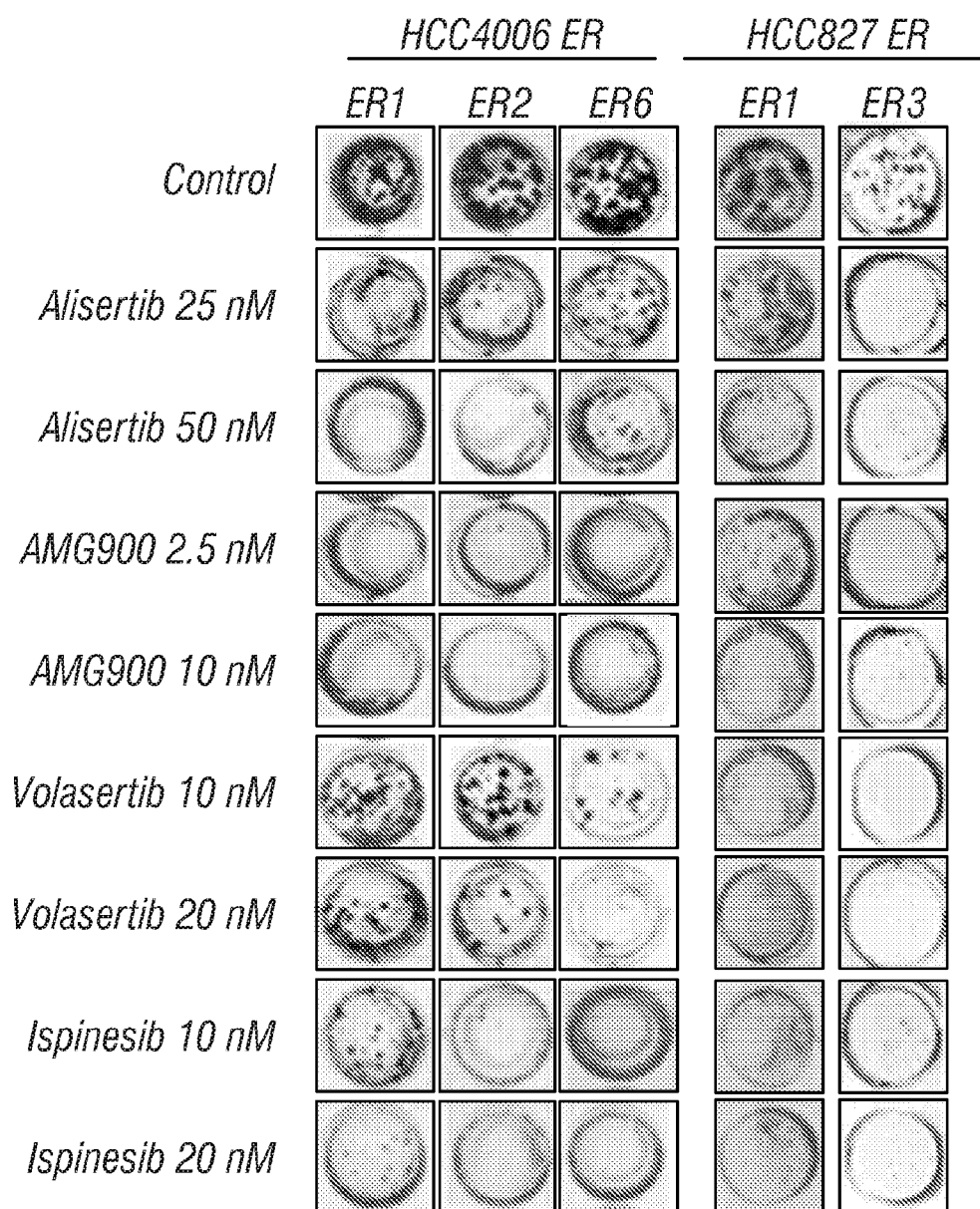


FIG. 4D

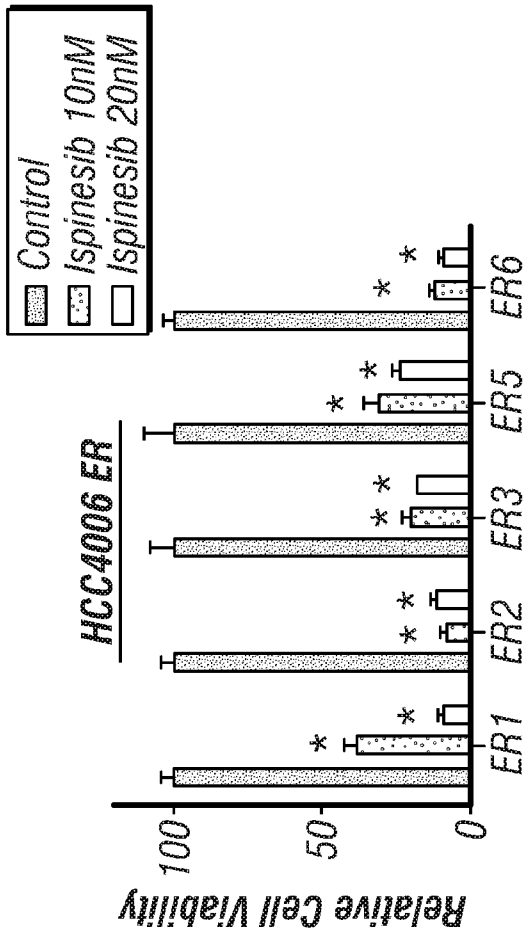


FIG. 4F

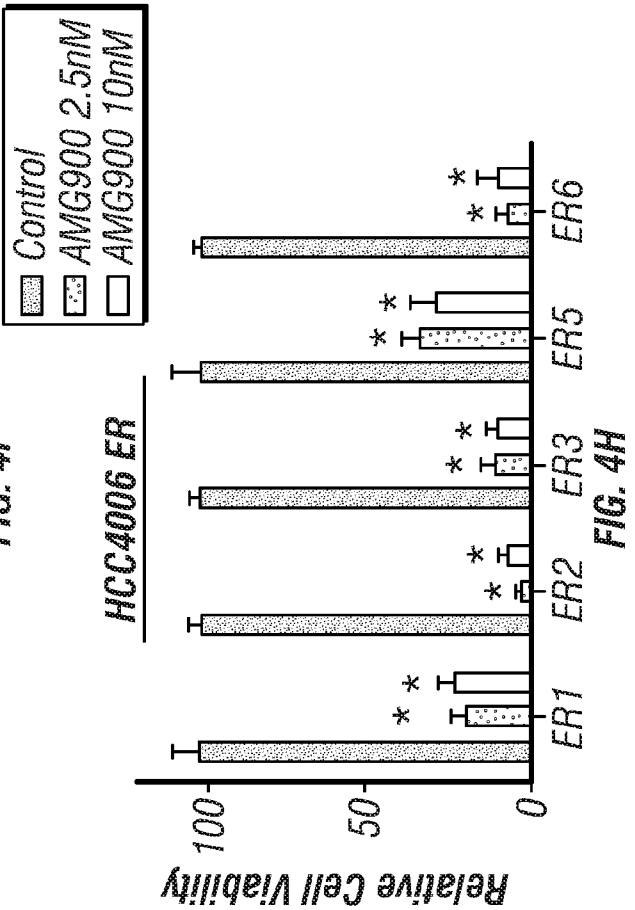


FIG. 4H

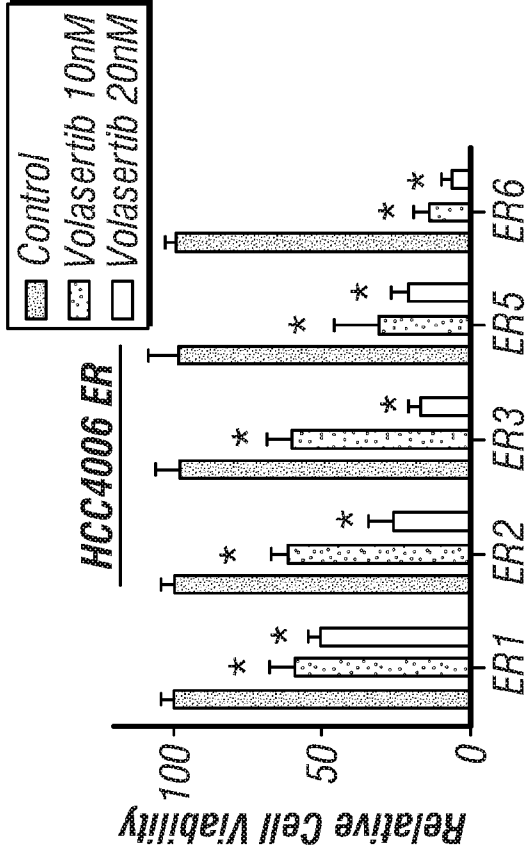


FIG. 4E

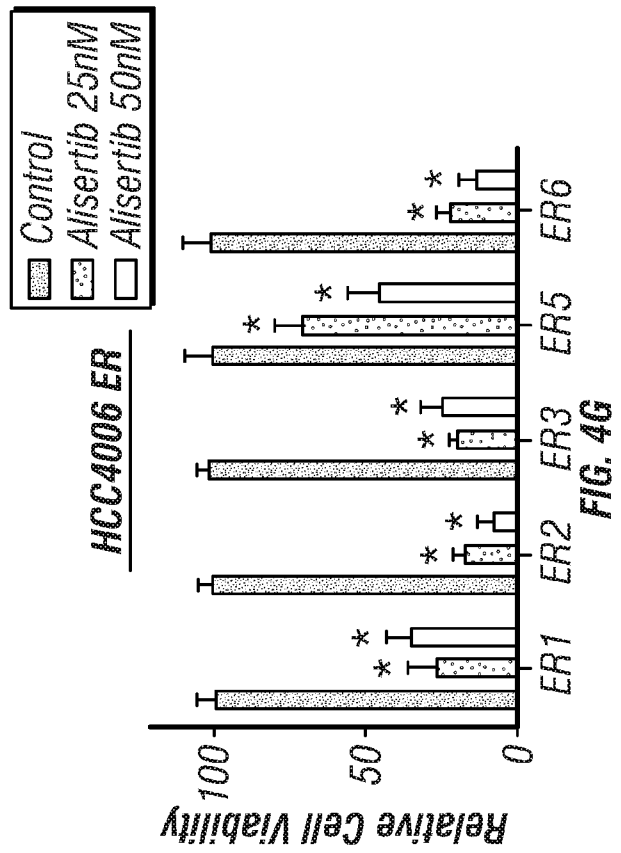


FIG. 4G

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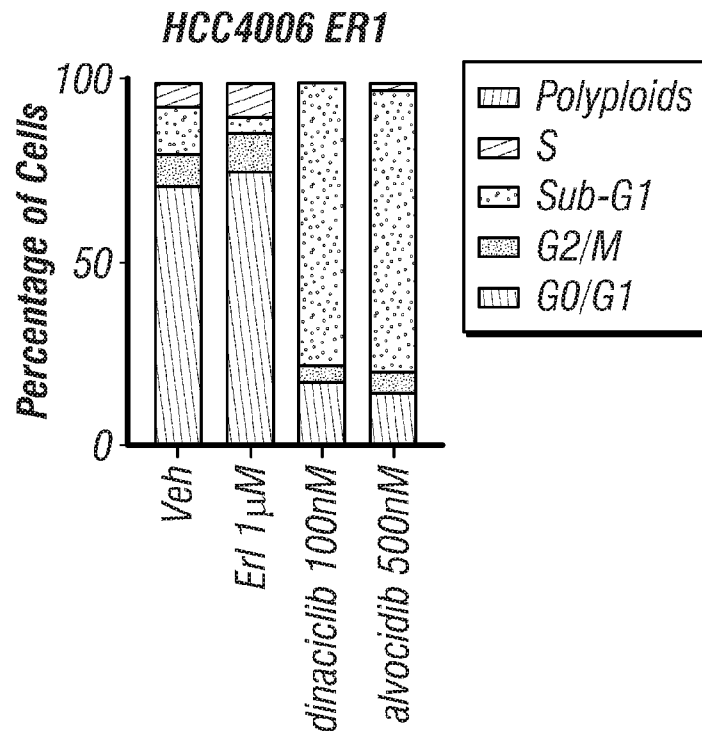


FIG. 5A

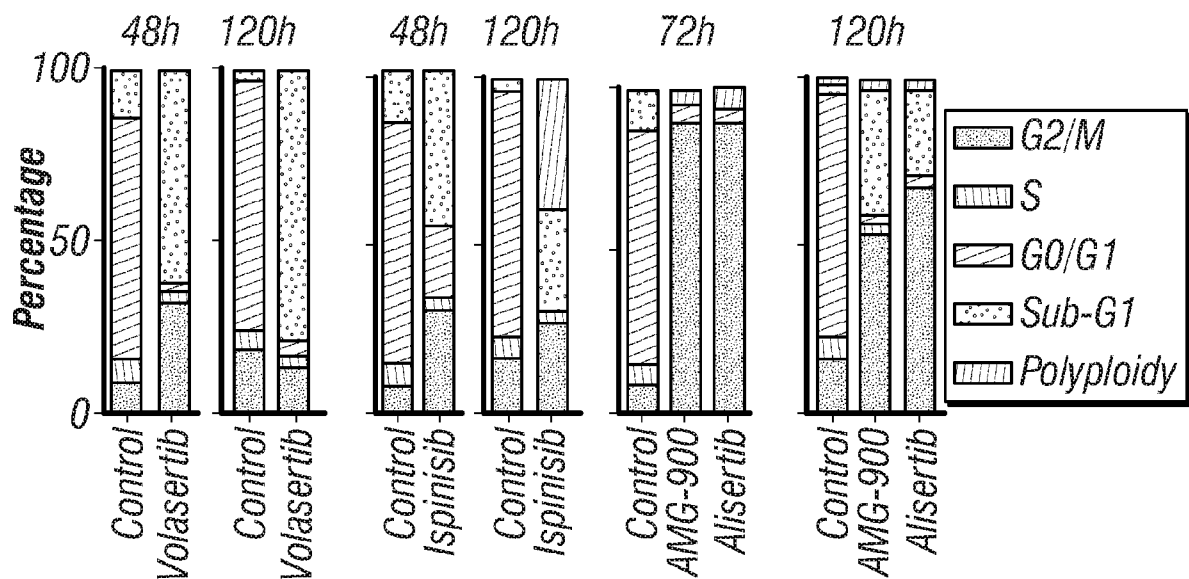


FIG. 5B

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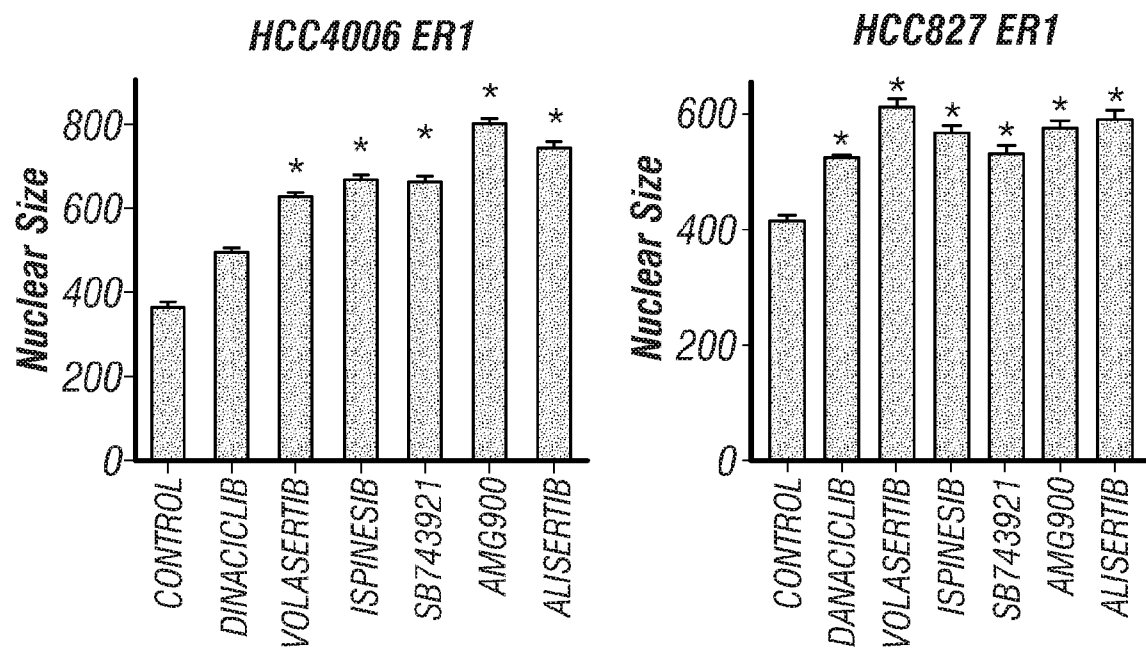


FIG. 5C

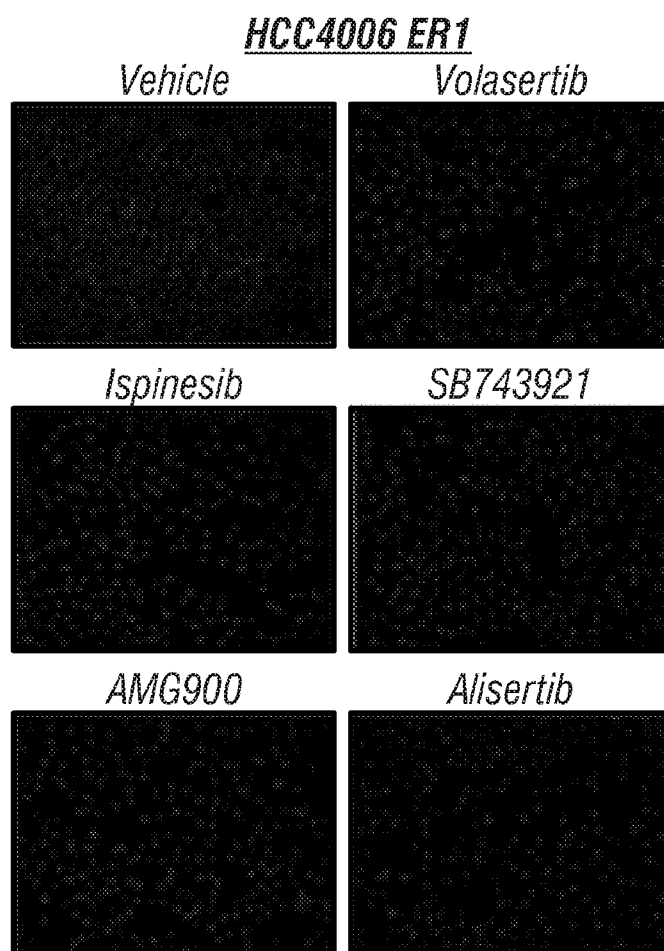


FIG. 5D

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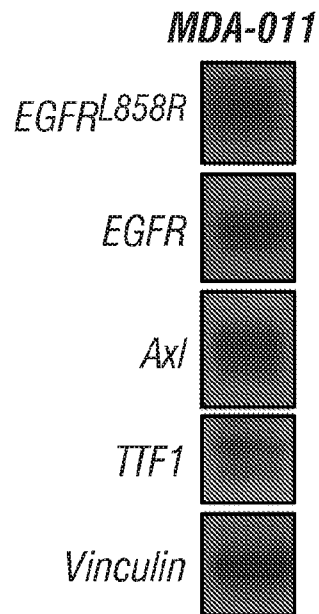


FIG. 6A

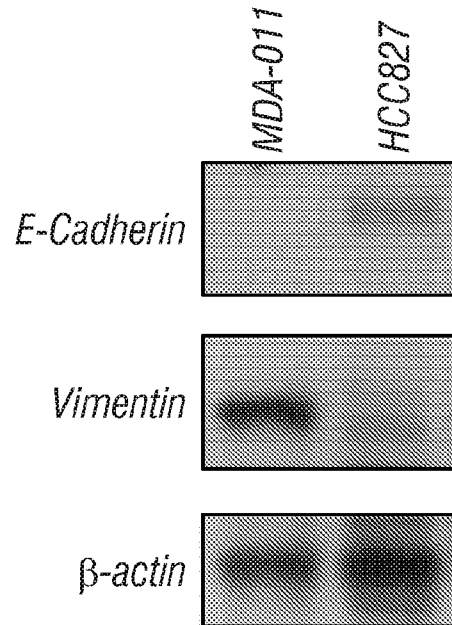


FIG. 6B

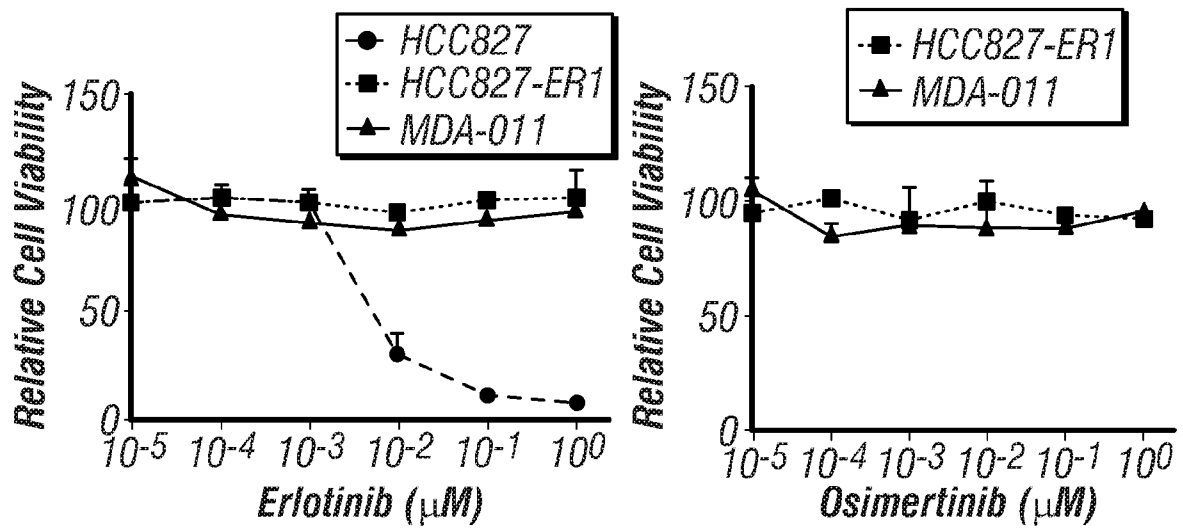


FIG. 6C

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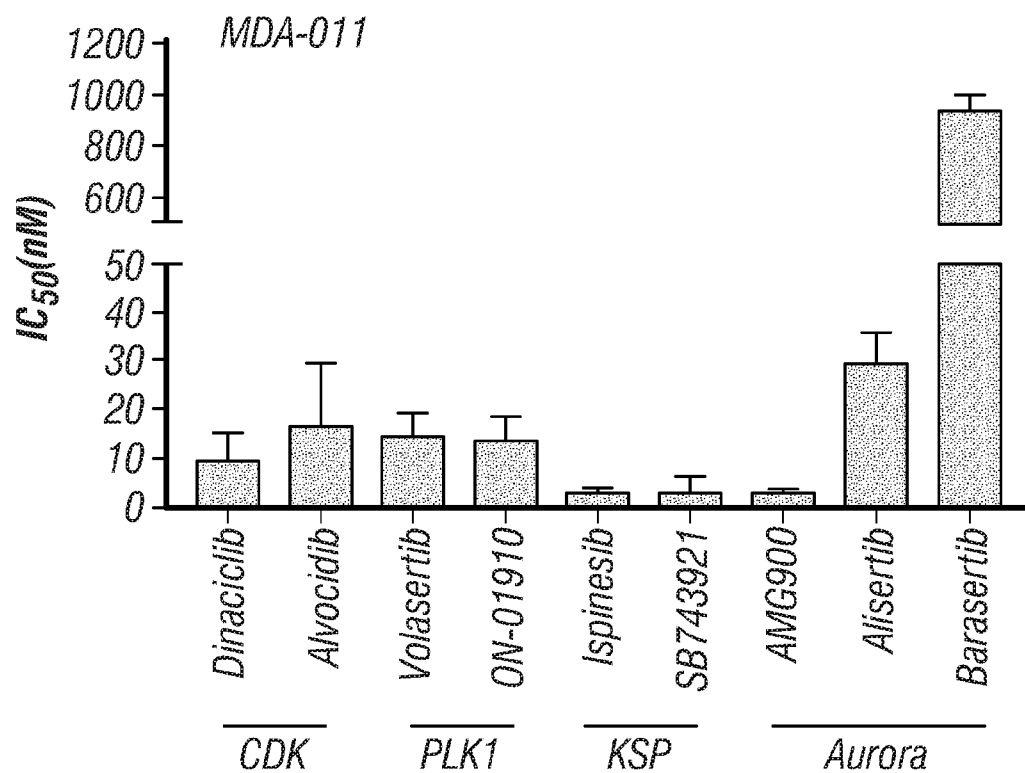


FIG. 6D

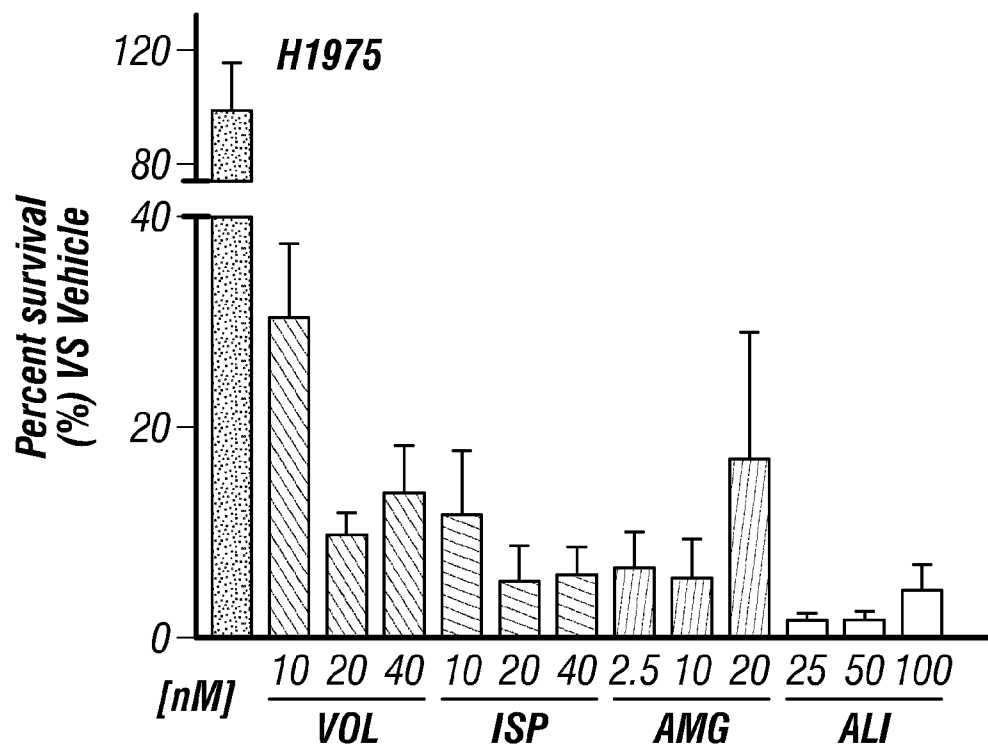


FIG. 7

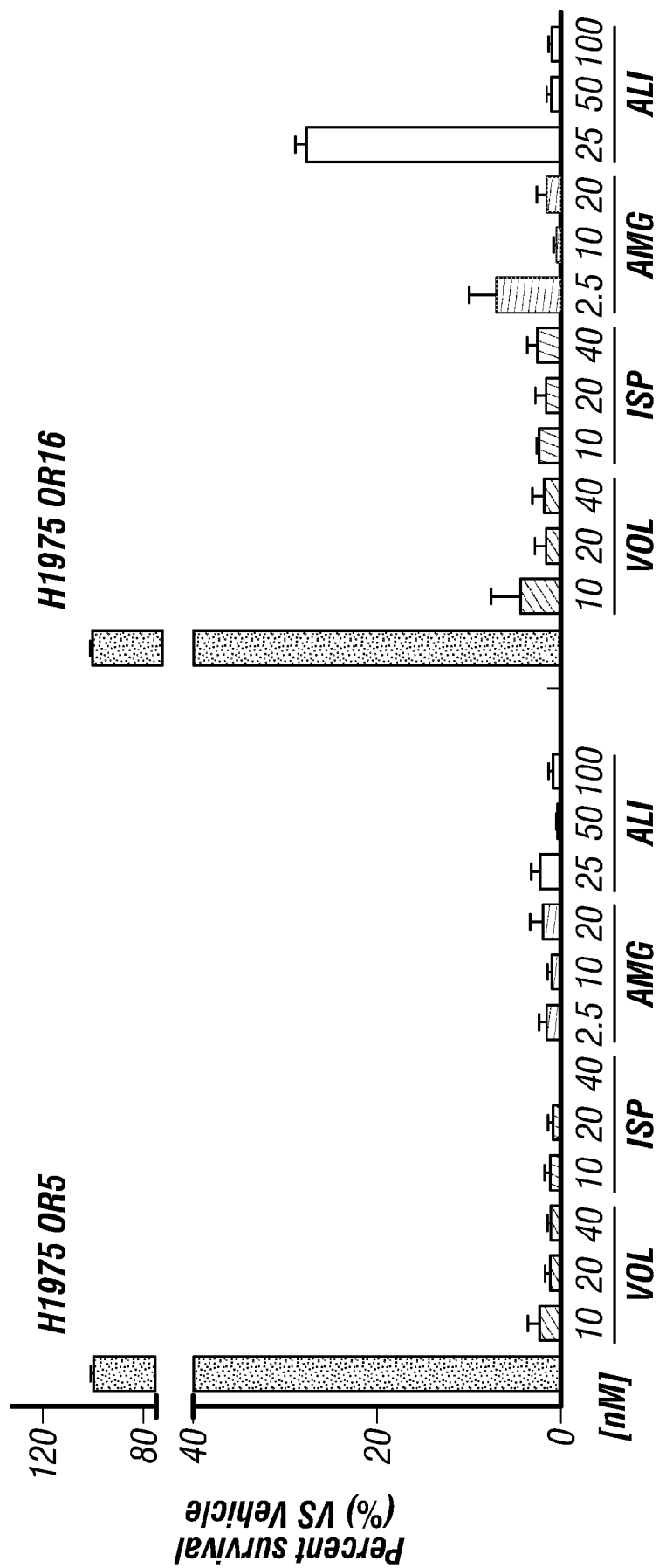


FIG. 7
(Cont'd)

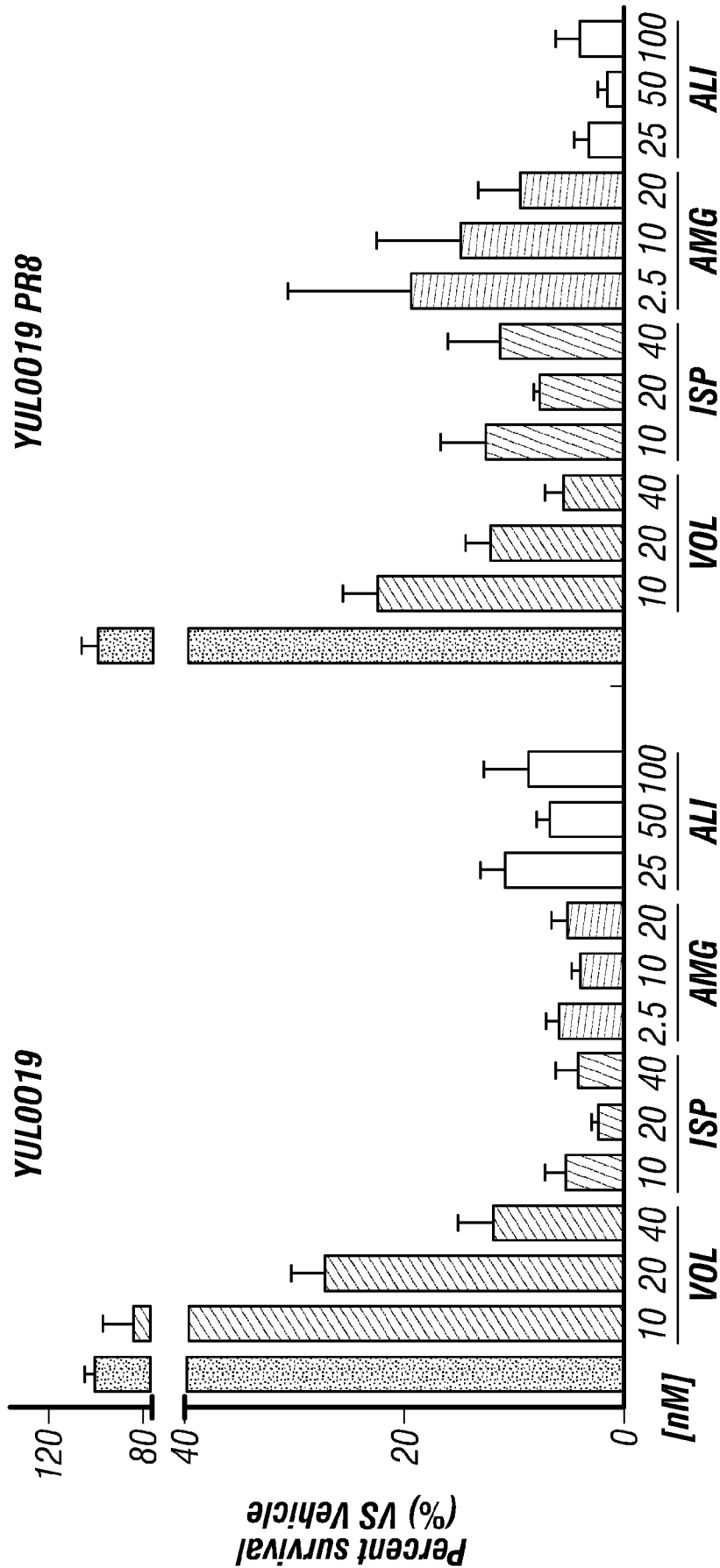


FIG. 8

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 19/22067

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 31/44, A61K 38/17, A61K 31/4709 (2019.01)

CPC - C12Q 1/6886, G01N 33/57484, C12Q 2600/106, C12Q 2600/118, C12Q 2600/156, G01N 2800/52, A61K 31/517, A61K 31/4706, A61K 31/506

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	US 2015/0099737 A1 (BRAIN et al.) 9 April 2015 (09.04.2015) Abstract; para [0005]; para [0139]	1, 2, 6-14, 25-28, 31-36, 45-48
Y	US 2016/0310482 A1 (WYETH LLC et al.) 27 October 2016 (27.10.2016) Abstract; para [0004]; para [0010]; para [0018]; para [0021-0022]; para [0029]; para [0095-0096]	1, 2, 6-14, 25-28, 31-36, 45-48
Y	US 2017/0112809 A1 (PIRAMAL ENTERPRISES LIMITED) 27 April 2017 (27.04.2017) Abstract; Claim 7; para [0067]	9, 10, 12
Y	US 2016/0346240 A1 (QUADRIGA BIOSCIENCES, INC.) 1 December 2016 (01.12.2016) Abstract; para [0130]	11, 13, 14
Y	US 2011/0301209 A1 (ZAKNOEN et al.) 8 December 2011 (08.12.2011) Abstract; Claim 19; para [0060]	28
Y	US 2014/0155397 A1 (HEYMACH et al.) 5 June 2014 (05.06.2014) Abstract; para [0126]	31

☐ 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

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Date of the actual completion of the international search

8 July 2019

Date of mailing of the international search report

06 AUG 2019

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 19/22067

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.: 24, 37-44
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:

----Please see continuation in first extra sheet -----

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.:
1, 2, 6-14, 25-28, 31-36, 45-48

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.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 19/22067

Continuation of Box No. III. Observations where unity of invention is lacking.

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I+: Claims 1-23, 25-36, 45-48, directed to a method of treating cancer in a subject. The method will be searched to the extent that the method encompasses administering an effective amount of a cyclin dependent kinase (CDK) inhibitor to the subject wherein the subject is determined to have an EGFR activating mutation comprising L858R.

It is believed that claims 1, 2, 6-14, 25-28, 31-36, 45-48, encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass a cyclin dependent kinase (CDK) inhibitor and wherein the subject is determined to have an EGFR activating mutation comprising L858R.

Additional treatment methods encompassing additional agents, will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected treatment method/s. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be a method encompassing administering an effective amount of a SAC component inhibitor to the subject wherein the subject is determined to have an EGFR activating mutation comprising an exon 19 deletion (Claims 1-3, 6-8, 15-23, 25-28, 31-36, 45-48).

Group II: Claims 49-76, directed to a pharmaceutical composition comprising a CDK inhibitor and/or SAC component inhibitor. The composition will be searched upon payment of additional fees and election as such.

Group III: Claims 77-81, directed to a method of predicting a response to a CDK inhibitor and/or SAC component inhibitor alone or in combination with a second anti-cancer therapy in a subject having a cancer. The method will be searched upon payment of additional fees and election as such.

The inventions listed as Group I+, Group II and Group III do not relate to a single special technical feature under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special technical features

The inventions of Group I+ require a method of treating cancer in a subject, not required by Groups II and III.

Group II requires a pharmaceutical composition that is not required by Groups I+ and III.

Group III requires a method of predicting a response to a cancer inhibitor, not required by Groups I+ and II.

Additionally, the inventions of Group I+ comprise the special technical feature of one or more distinct activating mutations of EGFR, and treatments comprising distinct CDK inhibitors and/or SAC component inhibitors. Each type of EGFR activating mutation and inhibitor treatment is considered a distinct and unique feature, not required by any of the other inventions.

Common technical features

The inventions of Groups I+, II and III share the common technical feature of use of a composition comprising a CDK inhibitor and/or SAC component inhibitor for treating cancer in a subject, wherein the subject has one or more EGFR activating mutations. However, these common technical features are previously made obvious by US 2015/0099737 A1 to Brain et al., (hereinafter 'Brain') in view of US 2013/0230511 A1 to The University of Texas System (hereinafter 'Univ Texas').

Brain teaches a CDK inhibitor for treating cancer in a subject, wherein the subject has an aberrantly activated EGFR pathway such as due to EGFR amplification (Abstract - 'The invention is directed to novel pyrrolopyrimidine compounds of formula (1) wherein R_{sup.1}, R_{sup.2y}, R_{sup.4}, R_{sup.8-R}, A and L are defined herein and to salts, including pharmaceutically acceptable salts thereof. The compounds of the present invention are CDK4/6 inhibitors and could be useful in the treatment of diseases and disorders mediated by CDK4/6, such as cancer, including mantle cell lymphoma, liposarcoma, non small cell lung cancer, melanoma, squamous cell esophageal cancer and breast cancer.'; para [0005] - 'In addition to these genetic defects directly related to the D-cyclin-CDK4/6-INK4a-pRb pathway, the activity of the CDK4/6 kinases can also be enhanced by oncogenic aberrations of the mitogen pathways that increase D-cyclin expression. The examples here include EGFR amplifications in non small cell lung cancer (NSCLC)'; para [0139] - 'The compounds of the present invention may be useful in the treatment of cancers that have genetic aberrations in the upstream regulators of D-cyclins, where the defect results in an increase of D-cyclins abundance, can also be considered for treatment. These include, but are not limited to non small cell lung cancers with activating aberrations of EGFR pathway'). Brain does not expressly teach activating aberrations of EGFR pathway are due to specific EGFR mutations.

Univ Texas teaches EGFR activating mutations in lung cancer (para [0047] - 'Mutations of EGFR or amplification can lead to its constant activation, resulting in uncontrolled cell division, a predisposition of cancer. Consequently, mutations and amplifications of EGFR have been identified in several types of cancer, including lung cancer, glioblastoma multiforme, and renal cancer'; para [0040] - 'FIG. 13 shows that VEGF increased tumor cell survival in the presence of erlotinib and axitinib reversed the effect. HCC827 cells, which harbor the EGFR activating mutation, were treated with VEGF and with or without the VEGFR TKI axitinib. After 24 hours, increasing concentrations of erlotinib were added to the cells.'). It would have been obvious to one of ordinary skill in the art that the CDK inhibitor of Brain could be used to treat lung cancer with EGFR activating mutations according to Univ Texas, because Univ Texas teaches that aberrant activation of EGFR pathway in lung cancer is due to EGFR mutations.

---please see continuation on next extra sheet-----

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 19/22067

Continuation of Box No. III. Observations where unity of invention is lacking.

contd. from previous sheet

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

Therefore, Group I+, Group II and Group III inventions lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.

NOTE, continuation of Item 4 above: claims 24, 37-44 are held unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

NOTE, Claim 21 as drafted is objected to for lack of antecedent basis because Claim 1 does not recite an "Aurora kinase inhibitor". For the purposes of this application, claim 21 is construed as dependent from claim 15 that recites an "Aurora kinase inhibitor".