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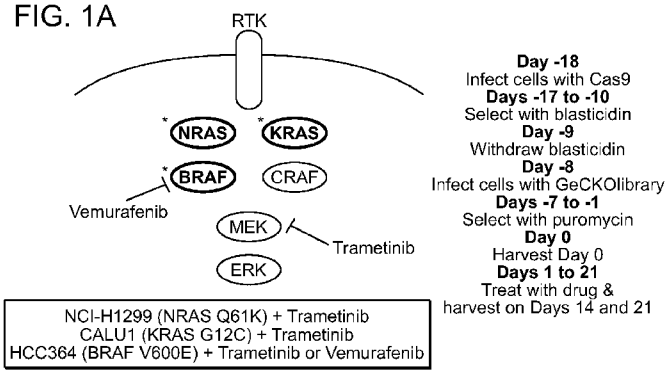
(54) **Title**: COMPOSITIONS AND METHODS FOR TREATING RAS/MAPK MUTANT LUNG CANCER

FIG. 1A



(57) **Abstract**: The present invention features compositions and methods for typing an ALK-, BRAF-, EGFR-, NRAS-, or KRAS- or mutant lung cancer in a subject as a cancer sensitive or resistant to an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor, and related methods of treating such cancers. In particular embodiments, the present invention features compositions and methods for typing an ALK-, BRAF-, EGFR-, NRAS-, or KRAS-mutant lung cancer, determining whether a subject having an ALK-, BRAF-, EGFR-, NRAS-, or KRAS-mutant lung cancer is eligible for entry into a clinical trial for an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor, and monitoring effectiveness of treatment of an ALK-, BRAF-, EGFR-, NRAS-, or KRAS-mutant lung cancer. In some embodiments, the methods comprise measuring a level, copy number, or sequence of KEAP1 or NRF2 polynucleotide in a biological sample obtained from the subject relative to a reference level or sequence. The present invention also features compositions and methods for increasing sensitivity to an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor and treating an ALK-, BRAF-, EGFR-, NRAS-, or KRAS-mutant lung cancer.

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COMPOSITIONS AND METHODS FOR TREATING RAS/MAPK MUTANT LUNG CANCER

CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit of the following U.S. Provisional Application No.: 62/267,017, filed December 14, 2015, the entire content of which is incorporated herein by reference.

STATEMENT OF RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH

This invention was made with government support under Grant Nos. F32 CA189306 and U01 CA176058 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

The receptor tyrosine kinase (RTK)/ mitogen-activated protein kinase (MAPK) pathway plays an important role in the development of lung and other cancers. Alterations in the RTK/Ras/MAPK pathway, such as mutations or copy number alterations in multiple nodes of this pathway are common in many types of cancer, including lung cancer. EGFR inhibitors can elicit dramatic responses in EGFR-mutant lung cancer, but resistance inevitably occurs. Likewise, while BRAF inhibitors have shown promising results in BRAF-mutant lung cancer in recent trials, resistance will likely occur, as is seen in BRAF-mutant melanoma. Furthermore, ALK inhibitors can elicit dramatic responses in ALK-mutant lung cancer, but resistance often occurs. In addition to this acquired resistance, intrinsic resistance may explain why single-agent MEK inhibition has had limited success in lung cancer. Accordingly, there is an urgent need for new, improved compositions and methods for identifying and treating patients having a RTK/Ras/MAPK mutant lung cancer who are resistant to or who develop resistance to ALK, MEK, BRAF, or EGFR inhibitors.

SUMMARY OF THE INVENTION

As described below, the present invention features compositions and methods for typing an ALK-, BRAF-, EGFR-, NRAS-, or KRAS- or mutant lung cancer in a subject as a cancer sensitive or resistant to an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor, and related methods of treating such cancers.

In one aspect, the invention features a method of treating a selected subject having lung cancer, the method involving increasing KEAP1 level or activity or decreasing activity of a MAP kinase pathway in the subject, where the subject is selected by (i) detecting a mutation in a MAP kinase pathway protein and resistance to an inhibitor of MAP kinase pathway signaling and (ii) detecting decreased KEAP1 levels and/or increased activity of NRF2 in a biological sample of the subject relative to a reference sequence or level.

In another aspect, the invention features a method of treating a subject having lung cancer, the method involving characterizing the lung cancer by detecting in a biological sample of the subject (i) a mutation in a MAP kinase pathway protein and resistance to an inhibitor of MAP kinase pathway signaling and (ii) detecting decreased KEAP1 levels and/or increased activity of NRF2 in a biological sample of the subject relative to a reference sequence or level; and increasing KEAP1 levels or activity or decreasing activity of a MAP kinase pathway in the subject. In one embodiment, the activity of the MAP kinase pathway is decreased by administering to the subject an effective amount of a MAP kinase pathway inhibitor (e.g., an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor). In particular embodiments of the above aspects, the MEK inhibitor is trametinib, selumetinib, or MEK 162; the BRAF inhibitor is vemurafenib or dabrafenib; the EGFR inhibitor is erlotinib, afatinib, or cetuximab; and the ALK inhibitor is ASP-3026, alectinib, brigatinib, ceritinib, CEP-28122, CEP-37440, crizotinib, entrectinib, PF-06463922, TSR-011, X-376, or X-396.

In another aspect, the invention features a method of treating a subject having an ALK-, BRAF-, EGFR-, NRAS-, or KRAS-mutant lung cancer, the method involving detecting a wild-type KEAP1 polynucleotide, or detecting wild-type copy number or wild-type level of NRF2 polynucleotide in a biological sample of the subject relative to a reference sequence or level; and administering to the subject an effective amount of an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor.

In another aspect, the invention features a method of treating a subject having an ALK-, BRAF-, EGFR-, NRAS-, or KRAS-mutant lung cancer, the method involving administering to a selected subject an effective amount of an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor, where the subject is selected by detecting a wild-type KEAP1 polynucleotide, or detecting wild-type copy number or wild-type level of NRF2 polynucleotide in a biological sample of the subject relative to a reference sequence or level.

In another aspect, the invention features a method for typing lung cancer in a subject as sensitive or resistant to an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor, the method involving detecting a level or sequence of KEAP1 polynucleotide or a

level or copy number of NRF2 polynucleotide in a biological sample obtained from a subject characterized as having an ALK-, BRAF-, EGFR-, NRAS-, or KRAS-mutant lung cancer relative to a reference level or sequence, where the cancer is typed as resistant to an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor if a decrease in the level of or a mutation in KEAP1 polynucleotide or an increase in level or copy number of NRF2 polynucleotide is detected.

In another aspect, the invention features a method for determining whether a subject having lung cancer is eligible for entry into a clinical trial for treating lung cancer with an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor, the method involving detecting a level or sequence of KEAP1 or a level or copy number of NRF2 polynucleotide in a biological sample obtained from the subject characterized as having an ALK-, BRAF-, EGFR-, NRAS-, or KRAS-mutant lung cancer relative to a reference level or sequence, where failure to detect a mutation in KEAP1 polynucleotide or failure to detect an increase in copy number or level of NRF2 polynucleotide indicates the subject is eligible for entry. In one embodiment, the subject is entered into the clinical trial.

In another aspect, the invention features a method of identifying a subject with lung cancer that would benefit from treatment with an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor, the method involving detecting a level or sequence of KEAP1 polynucleotide or a level or copy number of NRF2 polynucleotide in a biological sample obtained from a subject characterized as having an ALK-, BRAF-, EGFR-, NRAS-, or KRAS-mutant relative to a reference level or sequence, where the subject is identified as a subject that would benefit from treatment with a an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor if a mutation in KEAP1 polynucleotide or an increase in copy number or level of NRF2 polynucleotide is not detected.

In particular embodiments, the invention further includes the step of administering an effective amount of an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor to the subject if a mutation in KEAP1 polynucleotide or an increase in level or copy number of NRF2 polynucleotide is not detected.

In another aspect, the invention features a method of monitoring effectiveness of lung cancer treatment in a subject, the method involving administering to the subject an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor; and detecting a level or sequence of KEAP1 or NRF2 polynucleotide in a biological sample obtained from the subject relative to a reference level or sequence, where detection of a mutation in the sequence of a KEAP1 polynucleotide or an increase in copy number or level of NRF2 polynucleotide

indicates the lung cancer is resistant to an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor.

In another aspect, the invention features a method of increasing sensitivity of a subject having an ALK-, BRAF-, NRAS-, or KRAS-mutant lung cancer to an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor, the method involving administering to the subject an effective amount of a KEAP1 polynucleotide or a NRF2 inhibitor and an effective amount of an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor, thereby increasing sensitivity of the subject to the inhibitor.

In another aspect, the invention features a method of treating a subject having an ALK-, BRAF-, EGFR-, NRAS-, or KRAS-mutant lung cancer, the method involving administering to a subject an effective amount of a KEAP1 polynucleotide or a NRF2 inhibitor and an effective amount of an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor.

In another aspect, the invention features a therapeutic composition for increasing sensitivity of a subject having an ALK-, BRAF-, NRAS-, or KRAS-mutant lung cancer to an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor, the composition involving a KEAP1 polynucleotide in a pharmaceutically acceptable carrier. In one embodiment, the composition contains a NRF2 inhibitor, an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor.

In another aspect, the invention features a kit for typing lung cancer, the kit containing a capture reagent that specifically binds to a KEAP1 polynucleotide and a capture reagent that specifically binds a polynucleotide that is any one or more of ALK, BRAF, EGFR, NRAS, and KRAS.

In another aspect, the invention features a kit for treating an ALK-, BRAF-, EGFR-, NRAS-, or KRAS-ALK-, BRAF-, EGFR-, NRAS-, or KRAS-mutant lung cancer, the kit containing a capture reagent that specifically binds to a KEAP1 polynucleotide and an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor. In one embodiment, the capture reagent specifically binds to a NRF2 polynucleotide. In one embodiment, the capture reagent is a primer or hybridization probe that specifically binds to a KEAP1 polynucleotide. In one embodiment, the capture reagent is a primer or hybridization probe that specifically binds to a NRF2 polynucleotide. In one embodiment, the capture reagent detects a mutation in a KEAP1 polynucleotide.

In various embodiments of any of the above-aspects or any other aspect of the invention delineated herein, an effective amount of KEAP1 polynucleotide, a NRF2 inhibitor

and an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor is administered. In particular embodiments, the MEK inhibitor is trametinib, selumetinib, or MEK 162. In particular embodiments, the BRAF inhibitor is vemurafenib or dabrafenib. In particular embodiments, the EGFR inhibitor is erlotinib, afatinib, or cetuximab. In particular embodiments, the ALK inhibitor is ASP-3026, alectinib, brigatinib, ceritinib, CEP-28122, CEP-37440, crizotinib, entrectinib, PF-06463922, TSR-011, X-376, or X-396. In particular embodiments, the NRF2 inhibitor is an inhibitory polynucleotide that reduces expression of NRF2, retinoic acid, 6-hydroxy-1-methylindole-3-acetonitrile (6-HMA), luteolin, bleomycin, brusatol, or AEM1. In various embodiments of any of the above-aspects, the subject is identified as having a decrease in KEAP1 polynucleotide, or a mutation in KEAP1 polynucleotide in a biological sample of the subject relative to a reference sequence or level. In various embodiments of any of the above-aspects or any other aspect of the invention delineated herein, the subject is identified as having an increase in copy number or level of NRF2 polynucleotide in a biological sample of the subject relative to a reference sequence or level. In various embodiments of any of the above-aspects or any other aspect of the invention delineated herein, the mutation in KEAP1 polynucleotide is a loss-of-function mutation. In various embodiments of any of the above-aspects or any other aspect of the invention delineated herein, the mutation in KEAP1 polynucleotide or the increase in copy number of level NRF2 polynucleotide does not re-activate a MAPK pathway. In various embodiments of any of the above-aspects or any other aspect of the invention delineated herein, the biological sample is blood. In various embodiments of any of the above-aspects or any other aspect of the invention delineated herein, the subject is human.

Compositions and articles defined by the invention were isolated or otherwise manufactured in connection with the examples provided below. Other features and advantages of the invention will be apparent from the detailed description, and from the claims.

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person of ordinary skill in the art to which this invention belongs. The following references provide one of skill with a general definition of many of the terms used in this invention: Singleton et al., Dictionary of Microbiology and Molecular Biology (2nd ed. 1994); The Cambridge Dictionary of Science and Technology (Walker ed., 1988); The Glossary of Genetics, 5th Ed., R. Rieger et al. (eds.), Springer Verlag (1991); and

Hale & Marham, The Harper Collins Dictionary of Biology (1991). As used herein, the following terms have the meanings ascribed to them below, unless specified otherwise.

By "agent" is meant any small molecule chemical compound, antibody, nucleic acid molecule, or polypeptide, or fragments thereof.

By “ameliorate” is meant decrease, suppress, attenuate, diminish, arrest, or stabilize the development or progression of a disease.

By "alteration" is meant a change (increase or decrease) in the expression levels or activity of a gene or polypeptide as detected by standard art known methods such as those described herein. As used herein, an alteration includes a 10% change in expression levels, preferably a 25% change, more preferably a 40% change, and most preferably a 50% or greater change in expression levels.

“Amplification” refers to any means by which a polynucleotide sequence is copied and thus expanded into a larger number of polynucleotide sequences. “Amplification of a gene” refers to any means by which copy number of the gene in a genome of an organism is increased, e.g., by gene duplication. In some embodiments herein, “amplification of NRF2” or “amplification of a NRF2 polynucleotide” refers to an increase in copy number of polynucleotide sequences encoding a NRF2 polypeptide in a genome of an organism.

By "analog" is meant a molecule that is not identical, but has analogous functional or structural features. For example, a polypeptide analog retains the biological activity of a corresponding naturally-occurring polypeptide, while having certain biochemical modifications that enhance the analog's function relative to a naturally occurring polypeptide. Such biochemical modifications could increase the analog's protease resistance, membrane permeability, or half-life, without altering, for example, ligand binding. An analog may include an unnatural amino acid.

In this disclosure, "comprises," "comprising," "containing" and "having" and the like can have the meaning ascribed to them in U.S. Patent law and can mean " includes," "including," and the like; "consisting essentially of" or "consists essentially" likewise has the meaning ascribed in U.S. Patent law and the term is open-ended, allowing for the presence of more than that which is recited so long as basic or novel characteristics of that which is recited is not changed by the presence of more than that which is recited, but excludes prior art embodiments.

By “MAP Kinase Pathway” is meant a conserved signal transduction pathway in which activated Ras induces a kinase cascade that activates MAP kinase. Proteins within the MAP kinase pathway include, for example, ALK, RAF, EGFR, RAS, and MEK. The MAP

Kinase Pathway is described, for example, by Lodish et al., Molecular Cell Biology, 4th edition, New York; W.Hl. Freeman, 2000, at section 20.5 Map Kinase Pathways, which is incorporated herin by reference.

By "MAP Kinase Pathway Inhibitor" is meant any agent that inhibits the activity of the Map kinase pathway. Exemplary MAPK pathway inhibitors include ALK inhibitors, MEK inhibitors, BRAF inhibitors, or EGFR inhibitors, as specified herein.

By "ALK inhibitor" is meant an agent that reduces or eliminate a biological function or activity of an ALK polypeptide (e.g., anaplastic lymphoma kinase). Exemplary biological activities or functions of an ALK polypeptide include receptor tyrosine protein kinase activity. Examples of an ALK inhibitor include, without limitation ASP-3026, alectinib (ALECENSA), brigatinib (AP26113), ceritinib (ZYKADIA), CEP-28122, CEP-37440, crizotinib (XALKORI), entrectinib (e.g., NMS-E628, RXDX-101), PF-06463922, TSR-011, X-376 and X-396.

By "ALK (anaplastic lymphoma kinase) polypeptide" is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to GenBank Accession No. AAB71619.1 and having tyrosine kinase activity. The sequence at GenBank Accession No. AAB71619.1 is shown below.

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   1 mgaigllwll plllstaavg sgmgtgqrag spaagsplqp replsysrlq rkslavdfvv
  61 pslfrvyard lllppsssel kagrpeargs laldcapllr llgpapgvsw tagspapaea
 121 rtlsrvlkgg svrklrrakq lvlelgeeai legcvgppge aavgllqfnl selfswwirq
 181 gegrlrirlm pekkasevgr egrlsaaira sqprllfqif gtghsslesp tnmpspspdy
 241 ftwnltwimk dsfpflshrs ryglecsfdf pceleysppl hdlrnqswsw rripseeasq
 301 mdlldgpgae rskemprgsf lllntsadsk htilspwmrs ssehctlavs vhrhlqpsgr
 361 yiaqllphne aareillmpt pgkhgwtvlq grigrpdnpf rvaleyissg nrslsavdff
 421 alkncsegts pgskmalqss ftcwngtvlq lgqacdfhqd caqgedesqm crklpvgfyc
 481 nfedgfcgwt qgtlsphtpq wqvrtlkdar fqdhqdhall lsttdvpase satvtsatfp
 541 apiksspcel rmswlirgvl rgnvslvlve nktgkeqgrm vwhvaayegl slwqwmvlpl
 601 ldvsdrfwlq mvawwgqgsr aivafdnisi sldcyltisg edkilqntap ksrnlfernp
 661 nkelkpgens prqtpifdpt vhwlfttcga sgphgptqaq cnnayqnsnl svevgsegpl
 721 kgiqiwkvpa tdtysisgyg aaggkggknt mmrshgvsvl gifnlekddm lyilvgqqge
 781 dacpstnqli qkvcigennv ieeeirvnrs vhewaggggg gggatyvfkm kdgvpvplii
 841 aaggggrayg aktdtfhper lennssvlgl ngnsgaaggg ggwndntsll wagkslqega
 901 tgghscpqam kkwgwetrgg fgggggcss ggggggyigg naasnndpem dgedgvsfis
 961 plgilytpal kvmeghgevn ikhylncshc evdechmdpe shkvicfcdh gtvlaedgvs
1021 civsptpeph lplslilsvv tsalvaalvl afsgimivyr rkhqelqamq melqspeykl
1081 sklrtstimt dynpnycfag ktssisdlke vprknitlir glghgafgev yegqvsgmpn
1141 dpsplqvavk tlpevcseqd eldflmeali iskfnhqniv rcigvslqsl prfillelma
1201 ggdlksflre trprpsqpss lamldllhva rdiacgcqyl eenhfihrdi aarnclltcp
1261 gpgrvakigd fgmardiyra syyrkggcam lpvkwmppea fmegiftskt dtwsfgvllw
1321 eifslgympy psksnqevle fvtsggrmdp pkncpgpvyr imtqcwqhqp edrpnfaiil
1381 erieyctqdp dvintalpie ygplveeeek vpvrpkdpeg vppllvsqqa kreeerspaa
1441 ppplpttssg kaakkptaae vsvrvprgpa vegghvnmaf sqsnppselh kvhgsrnkpt
1501 slwnptygsw ftekptkknn piakkephdr gnlglegsct vppnvatgrl pgaslleps
1561 sltanmkevp lfrlrhfpcg nvnygyqqqg lpleaatapg aghyedtilk sknsmnqpgp
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By "ALK polynucleotide" is meant a polynucleotide encoding an ALK polypeptide. An exemplary ALK polynucleotide sequence is provided at NCBI Accession No. NM_004304.4, which sequence is provided below:

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   1 agctgcaagt ggcgggcgcc caggcagatg cgatccagcg gctctggggg cggcagcggt
  61 ggtagcagct ggtacctccc gccgcctctg ttcggagggt cgcggggcac cgaggtgctt
 121 tccggccgcc ctctggtcgg ccacccaaag ccgcgggcgc tgatgatggg tgaggagggg
 181 gcggcaagat ttcgggcgcc cctgccctga acgccctcag ctgctgccgc cggggccgct
 241 ccagtgcctg cgaactctga ggagccgagg cgccggtgag agcaaggacg ctgcaaactt
 301 gcgcagcgcg ggggctggga ttcacgccca gaagttcagc aggcagacag tccgaagcct
 361 tcccgcagcg gagagatagc ttgagggtgc gcaagacggc agcctccgcc ctcggttccc
 421 gcccagaccg ggcagaagag cttggaggag ccaaaaggaa cgcaaaaggc ggccaggaca
 481 gcgtgcagca gctgggagcc gccgttctca gccttaaaag ttgcagagat tggaggctgc
 541 cccgagaggg gacagacccc agctccgact gcgggggcga ggagaggacg gtacccaact
 601 gccacctccc ttcaaccata gtagttcctc tgtaccgagc gcagcgagct acagacgggg
 661 gcgcggcact cggcgcggag agcgggaggc tcaaggtccc agccagtgag cccagtgtgc
 721 ttgagtgtct ctggactcgc ccctgagctt ccaggtctgt ttcatttaga ctcctgctcg
 781 cctccgtgca gttgggggaa agcaagagac ttgcgcgcac gcacagtcct ctggagatca
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1081 actcagctac tcgcgcctgc agaggaagag tctggcagtt gacttcgtgg tgccctcgct
1141 cttccgtgtc tacgcccggg acctactgct gccaccatcc tcctcggagc tgaaggctgg
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1261 gccggcgccg ggggtctcct ggaccgccgg ttcaccagcc ccggcagagg cccggacgct
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1381 ggagctgggc gaggaggcga tcttggaggg ttgcgtcggg ccccccgggg aggcggctgt
1441 ggggctgctc cagttcaatc tcagcgagct gttcagttgg tggattcgcc aaggcgaagg
1501 gcgactgagg atccgcctga tgcccgagaa gaaggcgtcg gaagtgggca gagagggaag
1561 gctgtccgcg gcaattcgcg cctcccagcc ccgccttctc ttccagatct tcgggactgg
1621 tcatagctcc ttggaatcac caacaaacat gccttctcct tctcctgatt attttacatg
1681 gaatctcacc tggataatga aagactcctt ccctttcctg tctcatcgca gccgatatgg
1741 tctggagtgc agctttgact tcccctgtga gctggagtat tcccctccac tgcatgacct
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1861 gctggatggg cctggggcag agcgttctaa ggagatgccc agaggctcct ttctccttct
1921 caacacctca gctgactcca agcacaccat cctgagtccg tggatgagga gcagcagtga
1981 gcactgcaca ctggccgtct cggtgcacag gcacctgcag ccctctggaa ggtacattgc
2041 ccagctgctg ccccacaacg aggctgcaag agagatcctc ctgatgccca ctccagggaa
2101 gcatggttgg acagtgctcc agggaagaat cgggcgtcca gacaacccat ttcgagtggc
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2221 gaactgcagt gaaggaacat ccccaggctc caagatggcc ctgcagagct ccttcacttg
2281 ttggaatggg acagtcctcc agcttgggca ggcctgtgac ttccaccagg actgtgccca
2341 gggagaagat gagagccaga tgtgccggaa actgcctgtg ggtttttact gcaactttga
2401 agatggcttc tgtggctgga cccaaggcac actgtcaccc cacactcctc aatggcaggt
2461 caggacccta aaggatgccc ggttccagga ccaccaagac catgctctat tgctcagtac
2521 cactgatgtc cccgcttctg aaagtgctac agtgaccagt gctacgtttc ctgcaccgat
2581 caagagctct ccatgtgagc tccgaatgtc ctggctcatt cgtggagtct tgaggggaaa
2641 cgtgtccttg gtgctagtgg agaacaaaac cgggaaggag caaggcagga tggtctggca
2701 tgtcgccgcc tatgaaggct tgagcctgtg gcagtggatg gtgttgcctc tcctcgatgt
2761 gtctgacagg ttctggctgc agatggtcgc atggtgggga caaggatcca gagccatcgt
2821 ggcttttgac aatatctcca tcagcctgga ctgctacctc accattagcg gagaggacaa
2881 gatcctgcag aatacagcac ccaaatcaag aaacctgttt gagagaaacc caaacaagga
2941 gctgaaaccc ggggaaaatt caccaagaca gacccccatc tttgacccta cagttcattg
3001 gctgttcacc acatgtgggg ccagcgggcc ccatggcccc acccaggcac agtgcaacaa
3061 cgcctaccag aactccaacc tgagcgtgga ggtggggagc gagggccccc tgaaaggcat
3121 ccagatctgg aaggtgccag ccaccgacac ctacagcatc tcgggctacg gagctgctgg
3181 cgggaaaggc gggaagaaca ccatgatgcg gtcccacggc gtgtctgtgc tgggcatctt
3241 caacctggag aaggatgaca tgctgtacat cctggttggg cagcagggag aggacgcctg
3301 ccccagtaca aaccagttaa tccagaaagt ctgcattgga gagaacaatg tgatagaaga
```

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3361 agaaatccgt gtgaacagaa gcgtgcatga gtgggcagga ggcggaggag gaggggtgg
3421 agccacctac gtatttaaga tgaaggatgg agtgccggtg cccctgatca ttgcagccgg
3481 aggtggtggc agggcctacg gggccaagac agacacgttc cacccagaga gactggagaa
3541 taactcctcg gttctagggc taaacggcaa ttccggagcc gcaggtggtg gaggtggctg
3601 gaatgataac acttccttgc tctgggccgg aaaatctttg caggagggtg ccaccggagg
3661 acattcctgc ccccaggcca tgaagaagtg ggggtgggag acaagagggg gtttcggagg
3721 gggtggaggg gggtgctcct caggtggagg aggcggagga tatataggcg gcaatgcagc
3781 ctcaaacaat gaccccgaaa tggatgggga agatggggtt tccttcatca gtccactggg
3841 catcctgtac accccagctt taaaagtgat ggaaggccac ggggaagtga atattaagca
3901 ttatctaaac tgcagtcact gtgaggtaga cgaatgtcac atggaccctg aaagccacaa
3961 ggtcatctgc ttctgtgacc acgggacggt gctggctgag gatggcgtct cctgcattgt
4021 gtcacccacc ccggagccac acctgccact ctcgctgatc ctctctgtgg tgacctctgc
4081 cctcgtggcc gccctggtcc tggctttctc cggcatcatg attgtgtacc gccggaagca
4141 ccaggagctg caagccatgc agatggagct gcagagccct gagtacaagc tgagcaagct
4201 ccgcacctcg accatcatga ccgactacaa ccccaactac tgctttgctg gcaagacctc
4261 ctccatcagt gacctgaagg aggtgccgcg gaaaaacatc accctcattc ggggtctggg
4321 ccatggcgcc tttggggagg tgtatgaagg ccaggtgtcc ggaatgccca acgacccaag
4381 ccccctgcaa gtggctgtga agacgctgcc tgaagtgtgc tctgaacagg acgaactgga
4441 tttcctcatg gaagccctga tcatcagcaa attcaaccac cagaacattg ttcgctgcat
4501 tggggtgagc ctgcaatccc tgccccggtt catcctgctg gagctcatgg cgggggggaga
4561 cctcaagtcc ttcctccgag agacccgccc tcgcccgagc cagccctcct ccctggccat
4621 gctggacctt ctgcacgtgg ctcgggacat tgcctgtggc tgtcagtatt tggaggaaaa
4681 ccacttcatc caccgagaca ttgctgccag aaactgcctc ttgacctgtc caggccctgg
4741 aagagtggcc aagattggag acttcgggat ggcccgagac atctacaggg cgagctacta
4801 tagaaaggga ggctgtgcca tgctgccagt taagtggatg cccccagagg ccttcatgga
4861 aggaatattc acttctaaaa cagacacatg gtcctttgga gtgctgctat gggaaatctt
4921 ttctcttgga tatatgccat accccagcaa aagcaaccag gaagttctgg agtttgtcac
4981 cagtggaggc cggatggacc cacccaagaa ctgccctggg cctgtatacc ggataatgac
5041 tcagtgctgg caacatcagc ctgaagacag gcccaacttt gccatcattt tggagaggat
5101 tgaatactgc acccaggacc cggatgtaat caacaccgct ttgccgatag aatatggtcc
5161 acttgtggaa gaggaagaga aagtgcctgt gaggcccaag gaccctgagg gggttcctcc
5221 tctcctggtc tctcaacagg caaaacggga ggaggagcgc agcccagctg ccccaccacc
5281 tctgcctacc acctcctctg gcaaggctgc aaagaaaccc acagctgcag agatctctgt
5341 tcgagtccct agagggccgg ccgtggaagg gggacacgtg aatatggcat tctctcagtc
5401 caaccctcct tcggagttgc acaaggtcca cggatccaga aacaagccca ccagcttgtg
5461 gaacccaacg tacggctcct ggtttacaga gaaacccacc aaaaagaata atcctatagc
5521 aaagaaggag ccacacgaca ggggtaacct ggggctggag ggaagctgta ctgtcccacc
5581 taacgttgca actgggagac ttccgggggc ctcactgctc ctagagccct cttcgctgac
5641 tgccaatatg aaggaggtac ctctgttcag gctacgtcac ttcccttgtg ggaatgtcaa
5701 ttacggctac cagcaacagg gcttgccctt agaagccgct actgcccctg gagctggtca
5761 ttacgaggat accattctga aaagcaagaa tagcatgaac cagcctgggc cctgagctcg
5821 gtcgcacact cacttctctt ccttgggatc cctaagaccg tggaggagag agaggcaatg
5881 gctccttcac aaaccagaga ccaaatgtca cgttttgttt tgtgccaacc tattttgaag
5941 taccaccaaa aaagctgtat tttgaaaatg ctttagaaag gttttgagca tgggttcatc
6001 ctattctttc gaaagaagaa aatatcataa aaatgagtga taaatacaag gcccagatgt
6061 ggttgcataa ggtttttatg catgtttgtt gtatacttcc ttatgcttct ttcaaattgt
6121 gtgtgctctg cttcaatgta gtcagaatta gctgcttcta tgtttcatag ttggggtcat
6181 agatgtttcc ttgccttgtt gatgtggaca tgagccattt gaggggagag ggaacggaaa
6241 taaaggagtt atttgtaatg actaaaa
```

By an "ALK-mutant lung cancer" is meant a lung cancer characterized by or associated with a mutation in an ALK polynucleotide or polypeptide. In some embodiments, the ALK mutation results in an alteration in receptor tyrosine kinase activity in a cell.

By "BRAF inhibitor" is meant an agent that reduces or eliminates a biological function or activity of a BRAF polypeptide (e.g., B-Raf proto-oncogene). Exemplary biological activities or functions of a BRAF polypeptide include serine/threonine protein

kinase activity and regulation of MAP kinase/ERKs (extracellular signal-regulated kinases) signaling pathways. Examples of a BRAF inhibitor include, without limitation, vemurafenib and dabrafenib. In particular embodiments, the BRAF inhibitor is vemurafenib.

By "BRAF polypeptide" is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Accession No. NP_004324.2 and having serine/threonine protein kinase activity. The sequence at NCBI Accession No. NP_004324.2 is shown below:

```
   1 maalsggggg gaepgqalfn gdmepeagag agaaassaad paipeevwni kqmikltqeh
  61 iealldkfgg ehnppsiyle ayeeytskld alqqreqqll eslgngtdfs vsssasmdtv
 121 tsssssslsv lpsslsvfqn ptdvarsnpk spqkpivrvf lpnkqrtvvp arcgvtvrds
 181 lkkalmmrgl ipeccavyri qdgekkpigw dtdiswltge elhvevlenv pltthnfvrk
 241 tfftlafcdf crkllfqgfr cqtcgykfhq rcstevplmc vnydqldllf vskffehhpi
 301 pqeeaslaet altsgsspsa pasdsigpqi ltspspsksi pipqpfrpad edhrnqfgqr
 361 drsssapnvh intiepvnid dlirdqgfrg dggsttglsa tppaslpgsl tnvkalqksp
 421 gpqrerksss ssedrnrmkt lgrrdssddw eipdgqitvg qrigsgsfgt vykgkwhgdv
 481 avkmlnvtap tpqqlqafkn evgvlrktrh vnillfmgys tkpqlaivtq wcegsslyhh
 541 lhiietkfem iklidiarqt aqgmdylhak siihrdlksn niflhedltv kigdfglatv
 601 ksrwsgshqf eqlsgsilwm apevirmqdk npysfqsdvy afgivlyelm tgqlpysnin
 661 nrdqiifmvg rgylspdlsk vrsncpkamk rlmaeclkkk rderplfpqi lasiellars
 721 lpkihrsase pslnragfqt edfslyacas pktpiqaggy gafpvh
```

By "BRAF polynucleotide" is meant a polynucleotide encoding a BRAF polypeptide. An exemplary BRAF polynucleotide sequence is provided at NCBI Accession No. NM_004333.4. The sequence is provided below:

```
   1 cgcctccctt ccccctcccc gcccgacagc ggccgctcgg gccccggctc tcggttataa
  61 gatggcggcg ctgagcggtg gcggtggtgg cggcgcggag ccgggccagg ctctgttcaa
 121 cggggacatg gagcccgagg ccggcgccgg cgccggcgcc gcggcctctt cggctgcgga
 181 ccctgccatt ccggaggagg tgtggaatat caaacaaatg attaagttga cacaggaaca
 241 tatagaggcc ctattggaca aatttggtgg ggagcataat ccaccatcaa tatatctgga
 301 ggcctatgaa gaatacacca gcaagctaga tgcactccaa caaagagaac aacagttatt
 361 ggaatctctg gggaacggaa ctgatttttc tgtttctagc tctgcatcaa tggataccgt
 421 tacatcttct tcctcttcta gcctttcagt gctaccttca tctctttcag tttttcaaaa
 481 tcccacagat gtggcacgga gcaacccccaa gtcaccacaa aaacctatcg ttagagtctt
 541 cctgcccaac aaacagagga cagtggtacc tgcaaggtgt ggagttacag tccgagacag
 601 tctaaagaaa gcactgatga tgagaggtct aatcccagag tgctgtgctg tttacagaat
 661 tcaggatgga gagaagaaac caattggttg ggacactgat atttcctggc ttactggaga
 721 agaattgcat gtggaagtgt tggagaatgt tccacttaca acacacaact ttgtacgaaa
 781 aacgtttttc accttagcat tttgtgactt ttgtcgaaag ctgcttttcc agggtttccg
 841 ctgtcaaaca tgtggttata aatttcacca gcgttgtagt acagaagttc cactgatgtg
 901 tgttaattat gaccaacttg atttgctgtt tgtctccaag ttctttgaac accacccaat
 961 accacaggaa gaggcgtcct tagcagagac tgccctaaca tctggatcat ccccttccgc
1021 acccgcctcg gactctattg ggccccaaat tctcaccagt ccgtctcctt caaaatccat
1081 tccaattcca cagcccttcc gaccagcaga tgaagatcat cgaaatcaat ttgggcaacg
1141 agaccgatcc tcatcagctc ccaatgtgca tataaacaca atagaacctg tcaatattga
1201 tgacttgatt agagaccaag gatttcgtgg tgatggagga tcaaccacag gtttgtctgc
1261 taccccccct gcctcattac ctggctcact aactaacgtg aaagccttac agaaatctcc
1321 aggacctcag cgagaaagga agtcatcttc atcctcagaa gacaggaatc gaatgaaaac
1381 acttggtaga cgggactcga gtgatgattg ggagattcct gatgggcaga ttacagtggg
1441 acaaagaatt ggatctggat catttggaac agtctacaag ggaaagtggc atggtgatgt
1501 ggcagtgaaa atgttgaatg tgacagcacc tacacctcag cagttacaag ccttcaaaaa
1561 tgaagtagga gtactcagga aaacacgaca tgtgaatatc ctactcttca tgggctattc
1621 cacaaagcca caactggcta ttgttaccca gtggtgtgag ggctccagct tgtatcacca
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1681 tctccatatc attgagacca aatttgagat gatcaaactt atagatattg cacgacagac
1741 tgcacagggc atggattact tacacgccaa gtcaatcatc cacagagacc tcaagagtaa
1801 taatatattt cttcatgaag acctcacagt aaaaataggt gattttggtc tagctacagt
1861 gaaatctcga tggagtgggt cccatcagtt tgaacagttg tctggatcca ttttgtggat
1921 ggcaccagaa gtcatcagaa tgcaagataa aaatccatac agctttcagt cagatgtata
1981 tgcatttgga attgttctgt atgaattgat gactggacag ttaccttatt caaacatcaa
2041 caacagggac cagataattt ttatggtggg acgaggatac ctgtctccag atctcagtaa
2101 ggtacggagt aactgtccaa aagccatgaa gagattaatg gcagagtgcc tcaaaaagaa
2161 aagagatgag agaccactct ttccccaaat tctcgcctct attgagctgc tggcccgctc
2221 attgccaaaa attcaccgca gtgcatcaga accctccttg aatcgggctg gtttccaaac
2281 agaggatttt agtctatatg cttgtgcttc tccaaaaaca cccatccagg caggggata
2341 tggtgcgttt cctgtccact gaaacaaatg agtgagagag ttcaggagag tagcaacaaa
2401 aggaaaataa atgaacatat gtttgcttat atgttaaatt gaataaaata ctctcttttt
2461 ttttaaggtg aaccaaagaa cacttgtgtg gttaaagact agatataatt tttccccaaa
2521 ctaaaattta tacttaacat tggattttta acatccaagg gttaaaatac atagacattg
2581 ctaaaaattg gcagagcctc ttctagaggc tttacttttct gttccgggtt tgtatcattc
2641 acttggttat tttaagtagt aaacttcagt ttctcatgca acttttgttg ccagctatca
2701 catgtccact agggactcca gaagaagacc ctacctatgc ctgtgtttgc aggtgagaag
2761 ttggcagtcg gttagcctgg gttagataag gcaaactgaa cagatctaat ttaggaagtc
2821 agtagaattt aataattcta ttattattct taataatttt tctataacta tttctttta
2881 taacaatttg gaaaatgtgg atgtctttta tttccttgaa gcaataaact aagtttcttt
2941 ttataaaaa
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By a “BRAF-mutant lung cancer” is meant a lung cancer characterized by or associated with a mutation in a BRAF polynucleotide or polypeptide. In some embodiments, the BRAF mutation results in an alteration in a tyrosine kinase (RTK)/ mitogen-activated protein kinase (MAPK) pathway in a cell.

“Detect” refers to identifying the presence, absence or amount of the analyte to be detected.

By "detectable label" is meant a composition that when linked to a molecule of interest renders the latter detectable, via spectroscopic, photochemical, biochemical, immunochemical, or chemical means. For example, useful labels include radioactive isotopes, magnetic beads, metallic beads, colloidal particles, fluorescent dyes, electron-dense reagents, enzymes (for example, as commonly used in an ELISA), biotin, digoxigenin, or haptens.

By “disease” is meant any condition or disorder that damages or interferes with the normal function of a cell, tissue, or organ. Examples of diseases include lung cancer, such as an ALK-, BRAF-, EGFR-, NRAS-, or KRAS-mutant lung cancer.

By "effective amount" is meant the amount of a required to ameliorate the symptoms of a disease relative to an untreated patient. The effective amount of therapeutic agent(s) used to practice the present invention for therapeutic treatment of a disease varies depending upon the manner of administration, the age, body weight, and general health of the subject. Ultimately, the attending physician or veterinarian will decide the appropriate amount and dosage regimen. Such amount is referred to as an "effective" amount.

By “EGFR inhibitor” is meant an agent that reduces or eliminates a biological function or activity of an EGFR polypeptide (e.g., epidermal growth factor receptor). Exemplary biological activities or functions of an EGFR polypeptide include ligand binding activity, tyrosine autophosphorylation, and regulation or activation of various downstream signaling cascades, such as the RAS-RAF-MEK-ERK and PI3 kinase-AKT modules. Examples of an EGFR inhibitor include, without limitation, erlotinib, afatinib, and cetuximab.

By "EGFR (Epidermal Growth Factor Receptor) polypeptide" is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Accession No. NP_005219.2, NP_958439.1, NP_958440.1, or NP_958441.1 (different isoforms) and having a biological activity or function of an EGFR polypeptide. Exemplary biological activities or functions of a EGFR polypeptide include ligand binding activity, tyrosine autophosphorylation, and regulation or activation of various downstream signaling cascades, such as the RAS-RAF-MEK-ERK and PI3 kinase-AKT modules The sequence at NCBI Accession No. NP_005219.2 is shown below:

```
   1 mrpsgtagaa llallaalcp asraleekkv cqgtsnkltq lgtfedhfls lqrmfnncev
  61 vlgnleityv qrnydlsflk tiqevagyvl ialntverip lenlqiirgn myyensyala
 121 vlsnydankt glkelpmrnl qeilhgavrf snnpalcnve siqwrdivss dflsnmsmdf
 181 qnhlgscqkc dpscpngscw gageencqkl tkiicaqqcs grcrgkspsd cchnqcaagc
 241 tgpresdclv crkfrdeatc kdtcpplmly npttyqmdvn pegkysfgat cvkkcprnyv
 301 vtdhgscvra cgadsyemee dgvrkckkce gpcrkvcngi gigefkdsls inatnikhfk
 361 nctsisgdlh ilpvafrgds fthtppldpq eldilktvke itgflliqaw penrtdlhaf
 421 enleiirgrt kqhgqfslav vslnitslgl rslkeisdgd viisgnknlc yantinwkkl
 481 fgtsgqktki isnrgensck atgqvchalc spegcwgpep rdcvscrnvs rgrecvdkcn
 541 llegeprefv enseciqchp eclpqamnit ctgrgpdnci qcahyidgph cvktcpagvm
 601 genntlvwky adaghvchlc hpnctygctg pglegcptng pkipsiatgm vgallllvv
 661 algiglfmrr rhivrkrtlr rllqerelve pltpsgeapn qallrilket efkkikvlgs
 721 gafgtvykgl wipegekvki pvaikelrea tspkankeil deayvmasvd nphvcrllgi
 781 cltstvqlit qlmpfgclld yvrehkdnig sqyllnwcvq iakgmnyled rrlvhrdlaa
 841 rnvlvktpqh vkitdfglak llgaeekeyh aeggkvpikw malesilhri ythqsdvwsy
 901 gvtvwelmtf gskpydgipa seissilekg erlpqppict idvymimvkc wmidadsrpk
 961 freliiefsk mardpqrylv iqgdermhlp sptdsnfyra lmdeedmddv vdadeylipq
1021 qgffsspsts rtpllsslsa tsnnstvaci drnglqscpi kedsflqrys sdptgalted
1081 siddtflpvp eyinqsvpkr pagsvqnpvy hnqplnpaps rdphyqdphs tavgnpeyln
1141 tvqptcvnst fdspahwaqk gshqisldnp dyqqdffpke akpngifkgs taenaeylrv
1201 apqssefiga
```

By "EGFR polynucleotide" is meant a polynucleotide encoding an EGFR polypeptide. An exemplary EGFR polynucleotide sequence is provided at NCBI Accession No. NM_005228.3. The sequence is provided below:

```
   1 ccccggcgca gcgcggccgc agcagcctcc gccccccgca cggtgtgagc gcccgacgcg
  61 gccgaggcgg ccggagtccc gagctagccc cggcggccgc cgccgcccag accggacgac
 121 aggccacctc gtcggcgtcc gcccgagtcc ccgcctcgcc gccaacgcca caaccaccgc
 181 gcacggcccc ctgactccgt ccagtattga tcgggagagc cggagcgagc tcttcgggga
 241 gcagcgatgc gaccctccgg gacggccggg gcagcgctcc tggcgctgct ggctgcgctc
 301 tgcccggcga gtcgggctct ggaggaaaag aaagtttgcc aaggcacgag taacaagctc
```

```
 361 acgcagttgg gcacttttga agatcatttt ctcagcctcc agaggatgtt caataactgt
 421 gaggtggtcc ttgggaattt ggaaattacc tatgtgcaga ggaattatga tctttccttc
 481 ttaaagacca tccaggaggt ggctggttat gtcctcattg ccctcaacac agtggagcga
 541 attccctttgg aaaacctgca gatcatcaga ggaaatatgt actacgaaaa ttcctatgcc
 601 ttagcagtct tatctaacta tgatgcaaat aaaaccggac tgaaggagct gcccatgaga
 661 aatttacagg aaatcctgca tggcgccgtg cggttcagca acaaccctgc cctgtgcaac
 721 gtggagagca tccagtggcg ggacatagtc agcagtgact ttctcagcaa catgtcgatg
 781 gacttccaga accacctggg cagctgccaa aagtgtgatc caagctgtcc caatgggagc
 841 tgctggggtg caggagagga gaactgccag aaactgacca aaatcatctg tgcccagcag
 901 tgctccgggc gctgccgtgg caagtccccc agtgactgct gccacaacca gtgtgctgca
 961 ggctgcacag gcccccggga gagcgactgc ctggtctgcc gcaaattccg agacgaagcc
1021 acgtgcaagg acacctgccc cccactcatg ctctacaacc ccaccacgta ccagatggat
1081 gtgaaccccg agggcaaata cagctttggt gccacctgcg tgaagaagtg tccccgtaat
1141 tatgtggtga cagatcacgg ctcgtgcgtc cgagcctgtg gggccgacag ctatgagatg
1201 gaggaagacg gcgtccgcaa gtgtaagaag tgcgaagggc cttgccgcaa agtgtgtaac
1261 ggaataggta ttggtgaatt taaagactca ctctccataa atgctacgaa tattaaacac
1321 ttcaaaaact gcacctccat cagtggcgat ctccacatcc tgccggtggc atttaggggt
1381 gactccttca cacatactcc tcctctggat ccacaggaac tggatattct gaaaaccgta
1441 aaggaaatca cagggttttt gctgattcag gcttggcctg aaaacaggac ggacctccat
1501 gcctttgaga acctagaaat catacgcggc aggaccaagc aacatggtca gttttctctt
1561 gcagtcgtca gcctgaacat aacatccttg ggattacgct ccctcaagga gataagtgat
1621 ggagatgtga taatttcagg aaacaaaaat ttgtgctatg caaatacaat aaactggaaa
1681 aaactgtttg ggacctccgg tcagaaaacc aaaattataa gcaacagagg tgaaaacagc
1741 tgcaaggcca caggccaggt ctgccatgcc ttgtgctccc ccgagggctg ctggggcccg
1801 gagcccaggg actgcgtctc ttgccggaat gtcagccgag gcagggaatg cgtggacaag
1861 tgcaaccttc tggagggtga gccaagggag tttgtggaga actctgagtg catacagtgc
1921 cacccagagt gcctgcctca ggccatgaac atcacctgca caggacgggg accagacaac
1981 tgtatccagt gtgcccacta cattgacggc ccccactgcg tcaagacctg cccggcagga
2041 gtcatgggag aaaacaacac cctggtctgg aagtacgcag acgccggcca tgtgtgccac
2101 ctgtgccatc caaactgcac ctacggatgc actgggccag gtcttgaagg ctgtccaacg
2161 aatgggccta agatcccgtc catcgccact gggatggtgg gggccctcct cttgctgctg
2221 gtggtggccc tggggatcgg cctcttcatg cgaaggcgcc acatcgttcg gaagcgcacg
2281 ctgcggaggc tgctgcagga gagggagctt gtggagcctc ttacacccag tggagaagct
2341 cccaaccaag ctctcttgag gatcttgaag gaaactgaat tcaaaaagat caaagtgctg
2401 ggctccggtg cgttcggcac ggtgtataag ggactctgga tcccagaagg tgagaaagtt
2461 aaaattcccg tcgctatcaa ggaattaaga gaagcaacat ctccgaaagc caacaaggaa
2521 atcctcgatg aagcctacgt gatggccagc gtggacaacc cccacgtgtg ccgcctgctg
2581 ggcatctgcc tcacctccac cgtgcagctc atcacgcagc tcatgccctt cggctgcctc
2641 ctggactatg tccgggaaca caaagacaat attggctccc agtacctgct caactggtgt
2701 gtgcagatcg caaagggcat gaactacttg gaggaccgtc gcttggtgca ccgcgacctg
2761 gcagccagga acgtactggt gaaaacaccg cagcatgtca agatcacaga ttttgggctg
2821 gccaaactgc tgggtgcgga agagaaagaa taccatgcag aaggaggcaa agtgcctatc
2881 aagtggatgg cattggaatc aattttacac agaatctata cccaccagag tgatgtctgg
2941 agctacgggg tgaccgtttg ggagttgatg acctttggat ccaagccata tgacggaatc
3001 cctgccagcg agatctcctc catcctggag aaaggagaac gcctccctca gccacccata
3061 tgtaccatcg atgtctacat gatcatggtc aagtgctgga tgatagacgc agatagtcgc
3121 ccaaagttcc gtgagttgat catcgaattc tccaaaatgg cccgagaccc ccagcgctac
3181 cttgtcattc agggggatga aagaatgcat ttgccaagtc ctacagactc caacttctac
3241 cgtgccctga tggatgaaga agacatggac gacgtggtgg atgccgacga gtacctcatc
3301 ccacagcagg gcttcttcag cagccctcc acgtcacgga ctccctcct gagctctctg
3361 agtgcaacca gcaacaattc caccgtggct tgcattgata gaaatgggct gcaaagctgt
3421 cccatcaagg aagacagctt cttgcagcga tacagctcag accccacagg cgccttgact
3481 gaggacagca tagacgacac cttcctccca gtgcctgaat acataaacca gtccgttccc
3541 aaaaggcccg ctggctctgt gcagaatcct gtctatcaca atcagcctct gaaccccgcg
3601 cccagcagag acccacacta ccaggacccc cacagcactg cagtgggcaa ccccgagtat
3661 ctcaacactg tccagcccac ctgtgtcaac agcacattcg acagccctgc ccactgggcc
3721 cagaaaggca gccaccaaat tagcctggac aaccctgact accagcagga cttctttccc
3781 aaggaagcca agccaaatgg catctttaag ggctccacag ctgaaaatgc agaataccta
3841 agggtcgcgc cacaaagcag tgaatttatt ggagcatgac cacggaggat agtatgagcc
3901 ctaaaaatcc agactctttc gatacccagg accaagccac agcaggtcct ccatcccaac
3961 agccatgccc gcattagctc ttagacccac agactggttt tgcaacgttt acaccgacta
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4021 gccaggaagt acttccacct cgggcacatt ttgggaagtt gcattccttt gtcttcaaac
4081 tgtgaagcat ttacagaaac gcatccagca agaatattgt ccctttgagc agaaatttat
4141 ctttcaaaga ggtatatttg aaaaaaaaaa aaagtatatg tgaggatttt tattgattgg
4201 ggatcttgga gtttttcatt gtcgctattg atttttactt caatgggctc ttccaacaag
4261 gaagaagctt gctggtagca cttgctaccc tgagttcatc caggcccaac tgtgagcaag
4321 gagcacaagc cacaagtctt ccagaggatg cttgattcca gtggttctgc ttcaaggctt
4381 ccactgcaaa acactaaaga tccaagaagg ccttcatggc cccagcaggc cggatcggta
4441 ctgtatcaag tcatggcagg tacagtagga taagccactc tgtcccttcc tgggcaaaga
4501 agaaacggag gggatggaat tcttccttag acttactttt gtaaaaatgt ccccacggta
4561 cttactcccc actgatggac cagtggtttc cagtcatgag cgttagactg acttgtttgt
4621 cttccattcc attgttttga aactcagtat gctgcccctg tcttgctgtc atgaaatcag
4681 caagagagga tgacacatca aataataact cggattccag cccacattgg attcatcagc
4741 atttggacca atagcccaca gctgagaatg tggaatacct aaggatagca ccgcttttgt
4801 tctcgcaaaa acgtatctcc taatttgagg ctcagatgaa atgcatcagg tcctttgggg
4861 catagatcag aagactacaa aaatgaagct gctctgaaat ctcctttagc catcacccca
4921 acccccccaaa attagtttgt gttacttatg gaagatagtt ttctcctttt acttcacttc
4981 aaaagctttt tactcaaaga gtatatgttc cctccaggtc agctgccccc aaacccctc
5041 cttacgcttt gtcacacaaa aagtgtctct gccttgagtc atctattcaa gcacttacag
5101 ctctggccac aacagggcat tttacaggtg cgaatgacag tagcattatg agtagtgtgg
5161 aattcaggta gtaaatatga aactagggtt tgaaattgat aatgctttca caacatttgc
5221 agatgtttta gaaggaaaaa agttccttcc taaaataatt tctctacaat tggaagattg
5281 gaagattcag ctagttagga gcccaccttt tttcctaatc tgtgtgtgcc ctgtaacctg
5341 actggttaac agcagtcctt tgtaaacagt gttttaaact ctcctagtca atatccaccc
5401 catccaattt atcaaggaag aaatggttca gaaaatattt tcagcctaca gttatgttca
5461 gtcacacaca catacaaaat gttccttttg cttttaaagt aatttttgac tcccagatca
5521 gtcagagccc ctacagcatt gttaagaaag tatttgattt ttgtctcaat gaaaataaaa
5581 ctatattcat ttccactcta aaaaaaaaaa aaaaaa
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By "fragment" is meant a portion of a polypeptide or nucleic acid molecule. This portion contains, preferably, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the entire length of the reference nucleic acid molecule or polypeptide. A fragment may contain 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 nucleotides or amino acids.

"Hybridization" means hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleobases. For example, adenine and thymine are complementary nucleobases that pair through the formation of hydrogen bonds.

By "inhibitory nucleic acid" or “inhibitory polynucleotide” is meant a double-stranded RNA, siRNA, shRNA, or antisense RNA, or a portion thereof, or a mimetic thereof, that when administered to a mammalian cell results in a decrease (e.g., by 10%, 25%, 50%, 75%, or even 90-100%) in the expression of a target gene. Typically, a nucleic acid inhibitor comprises at least a portion of a target nucleic acid molecule, or an ortholog thereof, or comprises at least a portion of the complementary strand of a target nucleic acid molecule. For example, an inhibitory nucleic acid molecule comprises at least a portion of any or all of the nucleic acids delineated herein.

The terms "isolated," "purified," or "biologically pure" refer to material that is free to varying degrees from components which normally accompany it as found in its native state. "Isolate" denotes a degree of separation from original source or surroundings. "Purify" denotes a degree of separation that is higher than isolation. A "purified" or "biologically pure" protein is sufficiently free of other materials such that any impurities do not materially affect the biological properties of the protein or cause other adverse consequences. That is, a nucleic acid or peptide of this invention is purified if it is substantially free of cellular material, viral material, or culture medium when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized. Purity and homogeneity are typically determined using analytical chemistry techniques, for example, polyacrylamide gel electrophoresis or high performance liquid chromatography. The term "purified" can denote that a nucleic acid or protein gives rise to essentially one band in an electrophoretic gel. For a protein that can be subjected to modifications, for example, phosphorylation or glycosylation, different modifications may give rise to different isolated proteins, which can be separately purified.

By "isolated polynucleotide" is meant a nucleic acid (e.g., a DNA) that is free of the genes which, in the naturally-occurring genome of the organism from which the nucleic acid molecule of the invention is derived, flank the gene. The term therefore includes, for example, a recombinant DNA that is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote; or that exists as a separate molecule (for example, a cDNA or a genomic or cDNA fragment produced by PCR or restriction endonuclease digestion) independent of other sequences. In addition, the term includes an RNA molecule that is transcribed from a DNA molecule, as well as a recombinant DNA that is part of a hybrid gene encoding additional polypeptide sequence.

By an "isolated polypeptide" is meant a polypeptide of the invention that has been separated from components that naturally accompany it. Typically, the polypeptide is isolated when it is at least 60%, by weight, free from the proteins and naturally-occurring organic molecules with which it is naturally associated. Preferably, the preparation is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight, a polypeptide of the invention. An isolated polypeptide of the invention may be obtained, for example, by extraction from a natural source, by expression of a recombinant nucleic acid encoding such a polypeptide; or by chemically synthesizing the protein. Purity can be

measured by any appropriate method, for example, column chromatography, polyacrylamide gel electrophoresis, or by HPLC analysis.

By "KEAP1 polypeptide" is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Accession No. NP_987096.1 and having a biological activity or function of a KEAP1 polypeptide. Biological activities or functions of KEAP1 include, without limitation, targeting NRF2/NFE2L2 for ubiquitination and proteasomal degradation. The sequence at NCBI Accession No. NP_987096.1 is shown below:

```
  1 mqpdprpsga gaccrflplq sqcpegagda vmyastecka evtpsqhgnr tfsytledht
 61 kqafgimnel rlsqqlcdvt lqvkyqdapa aqfmahkvvl assspvfkam ftnglreqgm
121 evvsiegihp kvmerliefa ytasismgek cvlhvmngav myqidsvvra csdflvqqld
181 psnaigianf aeqigcvelh qrareyiymh fgevakqeef fnlshcqlvt lisrddlnvr
241 cesevfhaci nwvkydceqr rfyvqallra vrchsltpnf lqmqlqkcei lqsdsrckdy
301 lvkifeeltl hkptqvmpcr apkvgrliyt aggyfrqsls yleaynpsdg twlrladlqv
361 prsglagcvv ggllyavggr nnspdgntds saldcynpmt nqwspcapms vprnrigvgv
421 idghiyavgg shgcihhnsv eryeperdew hlvapmltrr igvgvavlnr llyavggfdg
481 tnrlnsaecy ypernewrmi tamntirsga gvcvlhnciy aaggydgqdq lnsverydve
541 tetwtfvapm khrrsalgit vhqgriyvlg gydghtflds vecydpdtdt wsevtrmtsg
601 rsgvgvavtm epcrkqidqq nctc
```

By "KEAP1 polynucleotide" is meant a polynucleotide encoding a KEAP1 polypeptide. An exemplary KEAP1 polynucleotide sequence is provided at NCBI Accession No. NM_203500.1. The sequence is provided below:

```
   1 ctttccgccc tctccccgcc tccttttcgg gcgtcccgag gccgctcccc aaccgacaac
  61 caagaccccg caggccacgc agccctggag ccgaggcccc ccgacggcgg aggcgcccgc
 121 gggtccccta cagccaaggt ccctgagtgc cagaggtggt ggtgttgctt atcttctgga
 181 accccatgca gccagatccc aggcctagcg gggctggggc ctgctgccga ttcctgcccc
 241 tgcagtcaca gtgccctgag ggggcagggg acgcggtgat gtacgcctcc actgagtgca
 301 aggcggaggt gacgccctcc cagcatggca accgcacctt cagctacacc ctggaggatc
 361 ataccaagca ggcctttggc atcatgaacg agctgcggct cagccagcag ctgtgtgacg
 421 tcacactgca ggtcaagtac caggatgcac cggccgccca gttcatggcc cacaaggtgg
 481 tgctggcctc atccagccct gtcttcaagg ccatgttcac caacgggctg cgggagcagg
 541 gcatggaggt ggtgtccatt gagggtatcc accccaaggt catggagcgc ctcattgaat
 601 tcgcctacac ggcctccatc tccatgggcg agaagtgtgt cctccacgtc atgaacggtg
 661 ctgtcatgta ccagatcgac agcgttgtcc gtgcctgcag tgacttcctg gtgcagcagc
 721 tggaccccag caatgccatc ggcatcgcca acttcgctga gcagattggc tgtgtggagt
 781 tgcaccagcg tgcccgggag tacatctaca tgcattttgg ggaggtggcc aagcaagagg
 841 agttcttcaa cctgtcccac tgccaactgg tgaccctcat cagccgggac gacctgaacg
 901 tgcgctgcga gtccgaggtc ttccacgcct gcatcaactg ggtcaagtac gactgcgaac
 961 agcgacggtt ctacgtccag gcgctgctgc gggccgtgcg ctgccactcg ttgacgccga
1021 acttcctgca gatgcagctg cagaagtgcg agatcctgca gtccgactcc cgctgcaagg
1081 actacctggt caagatcttc gaggagctca ccctgcacaa gcccacgcag gtgatgccct
1141 gccgggcgcc caaggtgggc cgcctgatct acaccgcggg cggctacttc cgacagtcgc
1201 tcagctacct ggaggcttac aaccccagtg acggcacctg gctccggttg gcggacctgc
1261 aggtgccgcg gagcggcctg gccggctgcg tggtgggcgg gctgttgtac gccgtgggcg
1321 gcaggaacaa ctcgcccgac ggcaacaccg actccagcgc cctggactgt tacaacccca
1381 tgaccaatca gtggtcgccc tgcgcccccа tgagcgtgcc ccgtaaccgc atcggggtgg
1441 gggtcatcga tggccacatc tatgccgtcg gcggctccca cggctgcatc caccacaaca
1501 gtgtggagag gtatgagcca gagcgggatg agtggcactt ggtggcccca atgctgacac
1561 gaaggatcgg ggtgggcgtg gctgtcctca atcgtctcct ttatgccgtg gggggctttg
1621 acgggacaaa ccgccttaat tcagctgagt gttactaccc agagaggaac gagtggcgaa
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1681 tgatcacagc aatgaacacc atccgaagcg gggcaggcgt ctgcgtcctg cacaactgta
1741 tctatgctgc tgggggctat gatggtcagg accagctgaa cagcgtggag cgctacgatg
1801 tggaaacaga gacgtggact ttcgtagccc ccatgaagca ccggcgaagt gccctgggga
1861 tcactgtcca ccaggggaga atctacgtcc ttggaggcta tgatggtcac acgttcctgg
1921 acagtgtgga gtgttacgac ccagatacag acacctggag cgaggtgacc cgaatgacat
1981 cgggccggag tggggtgggc gtggctgtca ccatggagcc ctgccggaag cagattgacc
2041 agcagaactg tacctgttga ggcacttttg tttcttgggc aaaaatacag tccaatgggg
2101 agtatcattg tttttgtaca aaaaccggga ctaaaagaaa agacagcact gcaaataacc
2161 catcttccgg gaagggaggc caggatgcct cagtgttaaa atgacatctc aaaagaagtc
2221 caaagcggga atcatgtgcc cctcagcgga gccccgggag tgtccaagac agcctggctg
2281 ggaaaggggg tgtggaaaga gcaggcttcc aggagagagg cccccaaacc ctctggccgg
2341 gtaataggcc tgggtcccac tcacccatgc cggcagctgt caccatgtga tttattcttg
2401 gatacctggg agggggccaa tgggggcctc agggggaggc cccctctgga aatgtggttc
2461 ccagggatgg gcctgtacat agaagccacc ggatggcact tccccaccgg atggacagtt
2521 attttgttga taagtaaccc tgtaattttc caaggaaaat aaagaacaga ctaactagtg
2581 tctttcaccc tgaaaaaaaa aaaaaa
```

By "KRAS polypeptide" is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Accession No. NP_203524.1 or NP_004976.2 (different isoforms) and having GTPase activity. The sequence at NCBI Accession No. NP_203524.1 is shown below:

```
  1 mteyklvvvg aggvgksalt iqliqnhfvd eydptiedsy rkqvvidget clldildtag
 61 qeeysamrdq ymrtgegflc vfainntksf edihhyreqi krvkdsedvp mvlvgnkcdl
121 psrtvdtkqa qdlarsygip fietsaktrq rvedafytlv reirqyrlkk iskeektpgc
181 vkikkciim
```

By "KRAS polynucleotide" is meant a polynucleotide encoding a KRAS polypeptide. An exemplary NRAS polynucleotide sequence is provided at NCBI Accession No. NM_033360.3. The sequence is provided below:

```
  1 tcctaggcgg cggccgcggc ggcggaggca gcagcggcgg cggcagtggc
ggcggcgaag
 61 gtggcggcgg ctcggccagt actcccggcc cccgccattt cggactggga gcgagcgcgg
121 cgcaggcact gaaggcggcg gcggggccag aggctcagcg gctcccaggt gcgggagaga
181 ggcctgctga aaatgactga atataaactt gtggtagttg gagctggtgg cgtaggcaag
241 agtgccttga cgatacagct aattcagaat cattttgtgg acgaatatga tccaacaata
301 gaggattcct acaggaagca agtagtaatt gatggagaaa cctgtctctt ggatattctc
361 gacacagcag gtcaagagga gtacagtgca atgagggacc agtacatgag gactggggag
421 ggctttcttt gtgtatttgc cataaataat actaaatcat ttgaagatat tcaccattat
481 agagaacaaa ttaaaagagt taaggactct gaagatgtac ctatggtcct agtaggaaat
541 aaatgtgatt tgccttctag aacagtagac acaaaacagg ctcaggactt agcaagaagt
601 tatggaattc cttttattga aacatcagca aagacaagac agagagtgga ggatgctttt
661 tatacattgg tgagggagat ccgacaatac agattgaaaa aaatcagcaa agaagaaaag
721 actcctggct gtgtgaaaat taaaaaatgc attataatgt aatctgggtg ttgatgatgc
781 cttctataca ttagttcgag aaattcgaaa acataaagaa aagatgagca aagatggtaa
841 aaagaagaaa aagaagtcaa agacaaagtg tgtaattatg taaatacaat ttgtacttttt
901 ttcttaaggc atactagtac aagtggtaat ttttgtacat tacactaaat tattagcatt
961 tgttttagca ttacctaatt ttttttcctgc tccatgcaga ctgttagctt ttaccttaaa
1021 tgcttatttt aaaatgacag tggaagtttt ttttttcctct aagtgccagt attcccagag
1081 ttttggtttt tgaactagca atgcctgtga aaaagaaact gaatacctaa gatttctgtc
1141 ttggggtttt tggtgcatgc agttgattac ttcttatttt tcttaccaat tgtgaatgtt
1201 ggtgtgaaac aaattaatga agcttttgaa tcatccctat tctgtgtttt atctagtcac
1261 ataaatggat taattactaa tttcagttga gaccttctaa ttggttttta ctgaaacatt
1321 gagggaacac aaatttatgg gcttcctgat gatgattctt ctaggcatca tgtcctatag
1381 tttgtcatcc ctgatgaatg taaagttaca ctgttcacaa aggttttgtc tcctttccac
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1441 tgctattagt catggtcact ctccccaaaa tattatattt tttctataaa aagaaaaaaa
1501 tggaaaaaaa ttacaaggca atggaaacta ttataaggcc atttccttttt cacattagat
1561 aaattactat aaagactcct aatagctttt cctgttaagg cagacccagt atgaaatggg
1621 gattattata gcaaccattt tggggctata tttacatgct actaaatttt tataataatt
1681 gaaaagattt taacaagtat aaaaaattct cataggaatt aaatgtagtc tccctgtgtc
1741 agactgctct ttcatagtat aactttaaat cttttcttca acttgagtct ttgaagatag
1801 ttttaattct gcttgtgaca ttaaaagatt atttgggcca gttatagctt attaggtgtt
1861 gaagagacca aggttgcaag gccaggccct gtgtgaacct ttgagctttc atagagagtt
1921 tcacagcatg gactgtgtcc ccacggtcat ccagtgttgt catgcattgg ttagtcaaaa
1981 tggggaggga ctagggcagt ttggatagct caacaagata caatctcact ctgtggtggt
2041 cctgctgaca aatcaagagc attgcttttg tttcttaaga aaacaaactc ttttttaaaa
2101 attacttttа aatattaact caaaagttga gattttgggg tggtggtgtg ccaagacatt
2161 aatttttttt ttaaacaatg aagtgaaaaa gttttacaat ctctaggttt ggctagttct
2221 cttaacactg gttaaattaa cattgcataa acacttttca agtctgatcc atatttaata
2281 atgctttaaa ataaaaataa aaacaatcct tttgataaat ttaaaatgtt acttatttta
2341 aaataaatga agtgagatgg catggtgagg tgaaagtatc actggactag gaagaaggtg
2401 acttaggttc tagataggtg tcttttagga ctctgatttt gaggacatca cttactatcc
2461 atttcttcat gttaaaagaa gtcatctcaa actcttagtt ttttttttтt acaactatgt
2521 aatttatatt ccatttacat aaggatacac ttatttgtca agctcagcac aatctgtaaa
2581 tttttaacct atgttacacc atcttcagtg ccagtcttgg gcaaaattgt gcaagaggtg
2641 aagtttatat ttgaatatcc attctcgttt taggactctt cttccatatt agtgtcatct
2701 tgcctcccta ccttccacat gccccatgac ttgatgcagt tttaatactt gtaattcccc
2761 taaccataag atttactgct gctgtggata tctccatgaa gttttcccac tgagtcacat
2821 cagaaatgcc ctacatctta tttcctcagg gctcaagaga atctgacaga taccataaag
2881 ggatttgacc taatcactaa ttttcaggtg gtggctgatg ctttgaacat ctctttgctg
2941 cccaatccat tagcgacagt aggatttttc aaacctggta tgaatagaca gaacсctatc
3001 cagtggaagg agaatttaat aaagatagtg ctgaaagaat tccttaggta atctataact
3061 aggactactc ctggtaacag taatacattc cattgtttta gtaaccagaa atcttcatgc
3121 aatgaaaaat actttaattc atgaagctta ctttttttтt ttggtgtcag agtctcgctc
3181 ttgtcaccca ggctggaatg cagtggcgcc atctcagctc actgcaacct ccatctccca
3241 ggttcaagcg attctcgtgc ctcggcctcc tgagtagctg ggattacagg cgtgtgccac
3301 tacactcaac taatttttgt atttttagga gagacggggt ttcaccctgt tggccaggct
3361 ggtctcgaac tcctgacctc aagtgattca cccaccttgg cctcataaac ctgttttgca
3421 gaactcattt attcagcaaa tatttattga gtgcctacca gatgccagtc accgcacaag
3481 gcactgggta tatggtatcc ccaaacaaga gacataatcc cggtccttag gtagtgctag
3541 tgtggtctgt aatatcttac taaggccttt ggtatacgac ccagagataa cacgatgcgt
3601 attttagttt tgcaaagaag gggtttggtc tctgtgccag ctctataatt gttttgctac
3661 gattccactg aaactcttcg atcaagctac tttatgtaaa tcacttcatt gttttaaagg
3721 aataaacttg attatattgt ttttttattt ggcataactg tgattctttt aggacaatta
3781 ctgtacacat taaggtgtat gtcagatatt catattgacc caaatgtgta atattccagt
3841 tttctctgca taagtaatta aaatatactt aaaaattaat agttttatct gggtacaaat
3901 aaacaggtgc ctgaactagt tcacagacaa ggaaacttct atgtaaaaat cactatgatt
3961 tctgaattgc tatgtgaaac tacagatctt tggaacactg tttaggtagg gtgttaagac
4021 ttacacagta cctcgtttct acacagagaa agaaatggcc atacttcagg aactgcagtg
4081 cttatgaggg gatatttagg cctcttgaat ttttgatgta gatgggcatt ttttaaggt
4141 agtggttaat tacctttatg tgaactttga atggtttaac aaaagatttg tttttgtaga
4201 gattttaaag ggggagaatt ctagaaataa atgttaccta attattacag ccttaaagac
4261 aaaaatcctt gttgaagttt ttttaaaaaa agctaaatta catagactta ggcattaaca
4321 tgtttgtgga agaatatagc agacgtatat tgtatcattt gagtgaatgt tcccaagtag
4381 gcattctagg ctctatttaa ctgagtcaca ctgcatagga atttagaacc taacttttat
4441 aggttatcaa aactgttgtc accattgcac aattttgtcc taatatatac atagaaactt
4501 tgtggggcat gttaagttac agtttgcaca agttcatctc atttgtattc cattgatttt
4561 ttttttcttc taaacatttt ttcttcaaac agtatataac ttttttttagg ggatttttтt
4621 ttagacagca aaaactatct gaagatttcc atttgtcaaa aagtaatgat ttcttgataa
4681 ttgtgtagta atgtttttta gaacccagca gttaccttaa agctgaattt atatttagta
4741 acttctgtgt taatactgga tagcatgaat tctgcattga gaaactgaat agctgtcata
4801 aaatgaaact ttctttctaa agaaagatac tcacatgagt tcttgaagaa tagtcataac
4861 tagattaaga tctgtgtttt agtttaatag tttgaagtgc ctgtttggga taatgatagg
4921 taatttagat gaatttaggg gaaaaaaaag ttatctgcag atatgttgag ggcccatctc
4981 tccccccaca cccccacaga gctaactggg ttacagtgtt ttatccgaaa gtttccaatt
5041 ccactgtctt gtgttttcat gttgaaaata cttttgcatt tttcctttga gtgccaattt
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5101 cttactagta ctatttctta atgtaacatg tttacctgga atgtatttta actatttttg
5161 tatagtgtaa actgaaacat gcacattttg tacattgtgc tttcttttgt gggacatatg
5221 cagtgtgatc cagttgtttt ccatcatttg gttgcgctga cctaggaatg ttggtcatat
5281 caaacattaa aaatgaccac tcttttaatt gaaattaact tttaaatgtt tataggagta
5341 tgtgctgtga agtgatctaa aatttgtaat atttttgtca tgaactgtac tactcctaat
5401 tattgtaatg taataaaaat agttacagtg actatgagtg tgtatttatt catgaaattt
5461 gaactgtttg ccccgaaatg gatatggaat actttataag ccatagacac tatagtatac
5521 cagtgaatct tttatgcagc ttgttagaag tatcctttat ttctaaaagg tgctgtggat
5581 attatgtaaa ggcgtgtttg cttaaactta aaaccatatt tagaagtaga tgcaaaacaa
5641 atctgccttt atgacaaaaa aataggataa cattatttat ttatttcctt ttatcaaaga
5701 aggtaattga tacacaacag gtgacttggt tttaggccca aaggtagcag cagcaacatt
5761 aataatggaa ataattgaat agttagttat gtatgttaat gccagtcacc agcaggctat
5821 ttcaaggtca gaagtaatga ctccatacat attatttatt tctataacta catttaaatc
5881 attaccagg
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By a "KRAS-mutant lung cancer" is meant a lung cancer characterized by or associated with a mutation in a KRAS polynucleotide or polypeptide. In some embodiments, the KRAS mutation results in an alteration in a tyrosine kinase (RTK)/ mitogen-activated protein kinase (MAPK) pathway in cells.

By "marker" is meant any protein or polynucleotide having an alteration in expression level or activity that is associated with a disease or disorder.

By "MEK inhibitor" is meant an agent that reduces or eliminate a biological function or activity of a MEK polypeptide. MEK polypeptides include a MEK1 (Mitogen-Activated Protein Kinase Kinase 1) polypeptide and a MEK2 (Mitogen-Activated Protein Kinase Kinase 2). Exemplary biological activities of MEK1 and MEK2 include phosphorylation/activation of MAP kinases. As components of the MAP kinase signal transduction pathway, MEK polypeptides are involved in many cellular processes such as proliferation, differentiation, transcription regulation, and development. Examples of a MEK inhibitor include, without limitation, trametinib, selumetinib, and MEK162. In particular embodiments, the MEK inhibitor is trametinib.

By "mutation" is meant a change in a polypeptide or polynucleotide sequence relative to a wild-type reference sequence. Exemplary mutations include point mutations, missense mutations, amino acid substitutions, and frameshift mutations. In some embodiments, a mutation in KEAP1 is a loss-of-function mutation, which confers resistance to an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor, in ALK-, BRAF-, EGFR-, NRAS-, or KRAS-mutant lung cancer. A "loss-of-function mutation" is a mutation that decreases or abolishes an activity or function of a polypeptide. A "gain-of-function mutation" is a mutation that enhances or increases an activity or function of a polypeptide.

By "NRAS polypeptide" is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Accession No. NP_002515.1 and having GTPase activity. The sequence at NCBI Accession No. NP_002515.1 is shown below:

```
  1 mteyklvvvg aggvgksalt iqliqnhfvd eydptiedsy rkqvvidget clldildtag
 61 qeeysamrdq ymrtgegflc vfainnsksf adinlyreqi krvkdsddvp mvlvgnkcdl
121 ptrtvdtkqa helaksygip fietsaktrq gvedafytlv reirqyrmkk lnssddgtqg
181 cmglpcvvm
```

By "NRAS polynucleotide" is meant a polynucleotide encoding a NRAS polypeptide. An exemplary NRAS polynucleotide sequence is provided at NCBI Accession No. NM_002524.4. The sequence is provided below:

```
   1 gaaacgtccc gtgtgggagg ggcgggtctg ggtgcggcct gccgcatgac tcgtggttcg
  61 gaggcccacg tggccggggc ggggactcag gcgcctgggg cgccgactga ttacgtagcg
 121 ggcggggccg gaagtgccgc tccttggtgg gggctgttca tggcggttcc ggggtctcca
 181 acatttttcc cggctgtggt cctaaatctg tccaaagcag aggcagtgga gcttgaggtt
 241 cttgctggtg tgaaatgact gagtacaaac tggtggtggt tggagcaggt ggtgttggga
 301 aaagcgcact gacaatccag ctaatccaga accactttgt agatgaatat gatcccacca
 361 tagaggattc ttacagaaaa caagtggtta tagatggtga aacctgtttg ttggacatac
 421 tggatacagc tggacaagaa gagtacagtg ccatgagaga ccaatacatg aggacaggcg
 481 aaggcttcct ctgtgtattt gccatcaata atagcaagtc atttgcggat attaacctct
 541 acagggagca gattaagcga gtaaaagact cggatgatgt acctatggtg ctagtgggaa
 601 acaagtgtga tttgccaaca aggacagttg atacaaaaca agcccacgaa ctggccaaga
 661 gttacgggat tccattcatt gaaacctcag ccaagaccag acagggtgtt gaagatgctt
 721 tttacacact ggtaagagaa atacgccagt accgaatgaa aaaactcaac agcagtgatg
 781 atgggactca gggttgtatg ggattgccat gtgtggtgat gtaacaagat acttttaaag
 841 ttttgtcaga aaagagccac tttcaagctg cactgacacc ctggtcctga cttccctgga
 901 ggagaagtat tcctgttgct gtcttcagtc tcacagagaa gctcctgcta cttccccagc
 961 tctcagtagt ttagtacaat aatctctatt tgagaagttc tcagaataac tacctcctca
1021 cttggctgtc tgaccagaga atgcacctct tgttactccc tgttattttt ctgccctggg
1081 ttcttccaca gcacaaacac acctctgcca ccccaggttt ttcatctgaa aagcagttca
1141 tgtctgaaac agagaaccaa accgcaaacg tgaaattcta ttgaaaacag tgtcttgagc
1201 tctaaagtag caactgctgg tgatttttt tttcttttta ctgttgaact tagaactatg
1261 ctaatttttg gagaaatgtc ataaattact gttttgccaa gaatatagtt attattgctg
1321 tttggtttgt ttataatgtt atcggctcta ttctctaaac tggcatctgc tctagattca
1381 taaatacaaa aatgaatact gaattttgag tctatcctag tcttcacaac tttgacgtaa
1441 ttaaatccaa ctttcacagt gaagtgcctt tttcctagaa gtggtttgta gacttccttt
1501 ataatatttc agtggaatag atgtctcaaa aatccttatg catgaaatga atgtctgaga
1561 tacgtctgtg acttatctac cattgaagga aagctatatc tatttgagag cagatgccat
1621 tttgtacatg tatgaaattg gttttccaga ggcctgtttt ggggctttcc caggagaaag
1681 atgaaactga aagcacatga ataatttcac ttaataattt ttacctaatc tccacttttt
1741 tcataggtta ctacctatac aatgtatgta atttgtttcc cctagcttac tgataaacct
1801 aatattcaat gaacttccat ttgtattcaa atttgtgtca taccagaaag ctctacattt
1861 gcagatgttc aaatattgta aaactttggt gcattgttat ttaatagctg tgatcagtga
1921 ttttcaaacc tcaaatatag tatattaaca aattacattt tcactgtata tcatggtatc
1981 ttaatgatgt atataattgc cttcaatccc cttctcaccc caccctctac agcttccccc
2041 acagcaatag gggcttgatt atttcagttg agtaaagcat ggtgctaatg gaccagggtc
2101 acagtttcaa aacttgaaca atccagttag catcacagag aaagaaattc ttctgcattt
2161 gctcattgca ccagtaactc cagctagtaa ttttgctagg tagctgcagt tagccctgca
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2221 aggaaagaag aggtcagtta gcacaaaccc tttaccatga ctggaaaact cagtatcacg
2281 tatttaaaca ttttttttc ttttagccat gtagaaactc taaattaagc caatattctc
2341 atttgagaat gaggatgtct cagctgagaa acgttttaaa ttctctttat tcataatgtt
2401 ctttgaaggg tttaaaacaa gatgttgata aatctaagct gatgagtttg ctcaaaacag
2461 gaagttgaaa ttgttgagac aggaatggaa aatataatta attgatacct atgaggattt
2521 ggaggcttgg cattttaatt tgcagataat accctggtaa ttctcatgaa aaatagactt
2581 ggataacttt tgataaaaga ctaattccaa aatggccact ttgttcctgt ctttaatatc
2641 taaatactta ctgaggtcct ccatcttcta tattatgaat tttcatttat taagcaaatg
2701 tcatattacc ttgaaattca gaagagaaga aacatatact gtgtccagag tataatgaac
2761 ctgcagagtt gtgcttctta ctgctaattc tgggagcttt cacagtactg tcatcatttg
2821 taaatggaaa ttctgctttt ctgtttctgc tccttctgga gcagtgctac tctgtaattt
2881 tcctgaggct tatcacctca gtcatttctt ttttaaatgt ctgtgactgg cagtgattct
2941 ttttcttaaa aatctattaa atttgatgtc aaattaggga gaaagatagt tactcatctt
3001 gggctcttgt gccaatagcc cttgtatgta tgtacttaga gttttccaag tatgttctaa
3061 gcacagaagt ttctaaatgg ggccaaaatt cagacttgag tatgttcttt gaatacctta
3121 agaagttaca attagccggg catggtggcc cgtgcctgta gtcccagcta cttgagaggc
3181 tgaggcagga gaatcacttc aacccaggag gtggaggtta cagtgagcag agatcgtgcc
3241 actgcactcc agcctgggtg acaagagaga cttgtctcca aaaaaaaagt tacacctagg
3301 tgtgaatttt ggcacaaagg agtgacaaac ttatagttaa aagctgaata acttcagtgt
3361 ggtataaaac gtggttttta ggctatgttt gtgattgctg aaaagaattc tagtttacct
3421 caaaatcctt ctctttcccc aaattaagtg cctggccagc tgtcataaat tacatattcc
3481 ttttggtttt tttaaaggtt acatgttcaa gagtgaaaat aagatgttct gtctgaaggc
3541 taccatgccg gatctgtaaa tgaacctgtt aaatgctgta tttgctccaa cggcttacta
3601 tagaatgtta cttaatacaa tatcatactt attacaattt ttactatagg agtgtaatag
3661 gtaaaattaa tctctatttt agtgggccca tgtttagtct ttcaccatcc tttaaactgc
3721 tgtgaatttt tttgtcatga cttgaaagca aggatagaga aacactttag agatatgtgg
3781 ggtttttta ccattccaga gcttgtgagc ataatcatat ttgctttata tttatagtca
3841 tgaactccta agttggcagc tacaaccaag aaccaaaaaa tggtgcgttc tgcttcttgt
3901 aattcatctc tgctaataaa ttataagaag caaggaaaat tagggaaaat attttatttg
3961 gatggtttct ataaacaagg gactataatt cttgtacatt atttttcatc tttgctgttt
4021 ctttgagcag tctaatgtgc cacacaatta tctaaggtat ttgttttcta taagaattgt
4081 tttaaaagta ttcttgttac cagagtagtt gtattatatt tcaaaacgta agatgatttt
4141 taaaagcctg agtactgacc taagatggaa ttgtatgaac tctgctctgg agggagggga
4201 ggatgtccgt ggaagttgta agacttttat ttttttgtgc catcaaatat aggtaaaaat
4261 aattgtgcaa ttctgctgtt taaacaggaa ctattggcct ccttggccct aaatggaagg
4321 gccgatattt taagttgatt attttattgt aaattaatcc aacctagttc tttttaattt
4381 ggttgaatgt ttttttcttgt taaatgatgt ttaaaaaata aaaactggaa gttcttggct
4441 tagtcataat tctt
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By a "NRAS-mutant lung cancer" is meant a lung cancer characterized by or associated with a mutation in a NRAS polynucleotide or polypeptide. In some embodiments, the NRAS mutation results in an alteration in a tyrosine kinase (RTK)/ mitogen-activated protein kinase (MAPK) pathway in cells.

By "NRF2 inhibitor" is meant an agent that reduces or eliminate a biological function or activity of a NRF2 polypeptide. Exemplary biological activities or functions of NRF2 include transcription factor activity. In some embodiments, the NRF2 inhibitor is an inhibitory polynucleotide that reduces expression of NRF2. In some other embodiments, the NRF2 inhibitor is a small molecule that reduces expression or activity of NRF2. Exemplary NRF2 inhibitors include, without limitation, retinoic acid, 6-hydroxy-1-methylindole-3-acetonitrile (6-HMA), luteolin, bleomycin, and brusatol. Another exemplary NRF2 inhibitor is AEM1, described in Bollong, M. J., Yun, H., Sherwood, L., Woods, A. K., Lairson, L. L. et al. A Small Molecule Inhibits Deregulated NRF2 Transcriptional Activity in Cancer. ACS chemical biology 10, 2193-2198, doi:10.1021/acschembio.5b00448 (2015).

By "NRF2 polypeptide" or "NFE2L2 polypeptide" is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Accession No. NP_006155.2, NP_001138884.1, NP_001138885.1, NP_001300831.1, NP_001300832.1, or NP_001300833.1 (different isoforms) and having transcription factor activity. "NRF2" and "NFE2L2" are used interchangeably herein. The sequence at NCBI Accession No. NP_006155.2 is shown below:

```
  1 mmdlelpppg lpsqqdmdli dilwrqdidl gvsrevfdfs qrrkeyelek qkklekerqe
 61 qlqkeqekaf faqlqldeet geflpiqpaq hiqsetsgsa nysqvahipk sdalyfddcm
121 qllaqtfpfv ddnevssatf qslvpdipgh iespvfiatn qaqspetsva qvapvdldgm
181 qqdieqvwee llsipelqcl niendklvet tmvpspeakl tevdnyhfys sipsmekevg
241 ncsphflnaf edsfssilst edpnqltvns lnsdatvntd fgdefysafi aepsisnsmp
301 spatlshsls ellngpidvs dlslckafnq nhpestaefn dsdsgislnt spsvaspehs
361 vesssygdtl lglsdsevee ldsapgsvkq ngpktpvhss gdmvqplsps qgqsthvhda
421 qcentpekel pvspghrktp ftkdkhssrl eahltrdelr akalhipfpv ekiinlpvvd
481 fnemmskeqf neaqlalird irrrgknkva aqncrkrkle niveleqdld hlkdekekll
541 kekgendksl hllkkqlstl ylevfsmlrd edgkpyspse yslqqtrdgn vflvpkskkp
601 dvkkn
```

By "NRF2 polynucleotide" or "NFE2L2 poynucleotide" is meant a polynucleotide encoding a NRF2 polypeptide. An exemplary NRF2 polynucleotide sequence is provided at NCBI Accession No. NM_006164.4. The sequence is provided below:

```
  1 aaatcaggga ggcgcagctc ctacaccaac gcctttccgg ggctccgggt gtgtttgttc
 61 caactgttta aactgtttca aagcgtccga actccagcga ccttcgcaaa caactcttta
121 tctcgcgggc gagagcgctg cccttatttg cgggggaggg caaactgaac gccggcaccg
181 gggagctaac ggagacctcc tctaggtccc ccgcctgctg ggaccccagc tggcagtccc
241 ttcccgcccc cggaccgcga gcttcttgcg tcagccccgg cgcgggtggg ggattttcgg
301 aagctcagcc cgcgcggccg gcgggggaag gaagggcccg gactcttgcc ccgcccttgt
361 ggggcgggag gcggagcggg gcaggggccc gccggcgtgt agccgattac cgagtgccgg
421 ggagcccgga ggagccgccg acgcagccgc caccgccgcc gccgccgcca ccagagccgc
481 cctgtccgcg ccgcgcctcg gcagccggaa cagggccgcc gtcggggagc cccaacacac
541 ggtccacagc tcatcatgat ggacttggag ctgccgccgc cgggactccc gtcccagcag
601 gacatggatt tgattgacat actttggagg caagatatag atcttggagt aagtcgagaa
661 gtatttgact tcagtcagcg acggaaagag tatgagctgg aaaaacagaa aaaacttgaa
721 aaggaaagac aagaacaact ccaaaaggag caagagaaag ccttttcgc tcagttacaa
781 ctagatgaag agacaggtga atttctccca attcagccag cccagcacat ccagtcagaa
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 841 accagtggat ctgccaacta ctcccaggtt gcccacattc ccaaatcaga tgctttgtac
 901 tttgatgact gcatgcagct tttggcgcag acattcccgt ttgtagatga caatgaggtt
 961 tcttcggcta cgtttcagtc acttgttcct gatattcccg gtcacatcga gagcccagtc
1021 ttcattgcta ctaatcaggc tcagtcacct gaaacttctg ttgctcaggt agcccctgtt
1081 gatttagacg gtatgcaaca ggacattgag caagtttggg aggagctatt atccattcct
1141 gagttacagt gtcttaatat tgaaaatgac aagctggttg agactaccat ggttccaagt
1201 ccagaagcca aactgacaga agttgacaat tatcattttt actcatctat accctcaatg
1261 gaaaaagaag taggtaactg tagtccacat tttcttaatg cttttgagga ttccttcagc
1321 agcatcctct ccacagaaga ccccaaccag ttgacagtga actcattaaa ttcagatgcc
1381 acagtcaaca cagattttgg tgatgaattt tattctgctt tcatagctga gcccagtatc
1441 agcaacagca tgccctcacc tgctacttta agccattcac tctctgaact tctaaatggg
1501 cccattgatg tttctgatct atcactttgc aaagctttca accaaaacca ccctgaaagc
1561 acagcagaat tcaatgattc tgactccggc atttcactaa acacaagtcc cagtgtggca
1621 tcaccagaac actcagtgga atcttccagc tatggagaca cactacttgg cctcagtgat
1681 tctgaagtgg aagagctaga tagtgcccct ggaagtgtca aacagaatgg tcctaaaaca
1741 ccagtacatt cttctgggga tatggtacaa cccttgtcac catctcaggg gcagagcact
1801 cacgtgcatg atgcccaatg tgagaacaca ccagagaaag aattgcctgt aagtcctggt
1861 catcggaaaa ccccattcac aaaagacaaa cattcaagcc gcttggaggc tcatctcaca
1921 agagatgaac ttagggcaaa agctctccat atcccattcc ctgtagaaaa aatcattaac
1981 ctccctgttg ttgacttcaa cgaaatgatg tccaaagagc agttcaatga agctcaactt
2041 gcattaattc gggatatacg taggaggggt aagaataaag tggctgctca gaattgcaga
2101 aaaagaaaac tggaaaatat agtagaacta gagcaagatt tagatcattt gaaagatgaa
2161 aaagaaaaat tgctcaaaga aaaaggagaa aatgacaaaa gccttcacct actgaaaaaa
2221 caactcagca ccttatatct cgaagttttc agcatgctac gtgatgaaga tggaaaacct
2281 tattctccta gtgaatactc cctgcagcaa acaagagatg gcaatgtttt ccttgttccc
2341 aaaagtaaga agccagatgt taagaaaaac tagatttagg aggatttgac cttttctgag
2401 ctagtttttt tgtactatta tactaaaagc tcctactgtg atgtgaaatg ctcatacttt
2461 ataagtaatt ctatgcaaaa tcatagccaa aactagtata gaaaataata cgaaacttta
2521 aaaagcattg gagtgtcagt atgttgaatc agtagtttca ctttaactgt aaacaatttc
2581 ttaggacacc atttgggcta gtttctgtgt aagtgtaaat actacaaaaa cttatttata
2641 ctgttcttat gtcatttgtt atattcatag atttatatga tgatatgaca tctggctaaa
2701 aagaaattat tgcaaaacta accactatgt actttttat aaatactgta tggacaaaaa
2761 atggcatttt ttatattaaa ttgtttagct ctggcaaaaa aaaaaaattt taagagctgg
2821 tactaataaa ggattattat gactgttaaa ttattaaaa
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As used herein, “obtaining” as in “obtaining an agent” includes synthesizing, purchasing, or otherwise acquiring the agent.

By “reduces” is meant a negative alteration of at least 10%, 25%, 50%, 75%, or 100%.

By “reference” is meant a standard or control condition.

A "reference sequence" is a defined sequence used as a basis for sequence comparison. A reference sequence may be a subset of or the entirety of a specified sequence; for example, a segment of a full-length cDNA or gene sequence, or the complete cDNA or gene sequence. For polypeptides, the length of the reference polypeptide sequence will generally be at least about 16 amino acids, preferably at least about 20 amino acids, more preferably at least about 25 amino acids, and even more preferably about 35 amino acids, about 50 amino acids, or about 100 amino acids. For nucleic acids, the length of the reference nucleic acid sequence will generally be at least about 50 nucleotides, preferably at least about 60 nucleotides, more preferably at least about 75 nucleotides, and even more

preferably about 100 nucleotides or about 300 nucleotides or any integer thereabout or therebetween.

By "resistance to an inhibitor" or "resistance to an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor" is meant that a cell or subject having a disease has acquired an alteration that allows it to escape an anti-disease effect of the inhibitor (e.g., ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor). For example, a cell resistant to an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor may be a neoplastic cell (e.g., a lung cancer cell having a mutation in ALK, BRAF, EGFR, KRAS, or NRAS) that has acquired an alteration that allows it to escape an anti-neoplastic effect of an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor. Exemplary anti-neoplastic effects include, but are not limited to, any effect that reduces proliferation, reduces survival, and/or increases cell death (e.g., increases apoptosis).

By "sensitivity to an inhibitor" (e.g. "sensitivity to an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor") is meant that at least one symptom of a disease or condition (e.g., ALK-, BRAF-, EGFR-, KRAS-, or NRAS-mutant lung cancer) is ameliorated by treatment with the inhibitor (e.g., ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor).

"Sample" or "biological sample" as used herein means a biological material isolated from a subject, including any tissue, cell, fluid, or other material obtained or derived from the subject (e.g., a human). The biological sample may contain any biological material suitable for detecting the desired analytes, and may comprise cellular and/or non-cellular material obtained from the subject.

By "siRNA" is meant a double stranded RNA. Optimally, a siRNA is 18, 19, 20, 21, 22, 23 or 24 nucleotides in length and has a 2 base overhang at its 3' end. These dsRNAs can be introduced to an individual cell or to a whole animal; for example, they may be introduced systemically via the bloodstream. Such siRNAs are used to downregulate mRNA levels or promoter activity.

By "specifically binds" is meant a compound or antibody that recognizes and binds a polypeptide of the invention, but which does not substantially recognize and bind other molecules in a sample, for example, a biological sample, which naturally includes a polypeptide of the invention.

Nucleic acid molecules useful in the methods of the invention include any nucleic acid molecule that encodes a polypeptide of the invention or a fragment thereof. Such nucleic acid molecules need not be 100% identical with an endogenous nucleic acid

sequence, but will typically exhibit substantial identity. Polynucleotides having "substantial identity" to an endogenous sequence are typically capable of hybridizing with at least one strand of a double-stranded nucleic acid molecule. Nucleic acid molecules useful in the methods of the invention include any nucleic acid molecule that encodes a polypeptide of the invention or a fragment thereof. Such nucleic acid molecules need not be 100% identical with an endogenous nucleic acid sequence, but will typically exhibit substantial identity. Polynucleotides having "substantial identity" to an endogenous sequence are typically capable of hybridizing with at least one strand of a double-stranded nucleic acid molecule. By "hybridize" is meant pair to form a double-stranded molecule between complementary polynucleotide sequences (e.g., a gene described herein), or portions thereof, under various conditions of stringency. (See, e.g., Wahl, G. M. and S. L. Berger (1987) Methods Enzymol. 152:399; Kimmel, A. R. (1987) Methods Enzymol. 152:507).

For example, stringent salt concentration will ordinarily be less than about 750 mM NaCl and 75 mM trisodium citrate, preferably less than about 500 mM NaCl and 50 mM trisodium citrate, and more preferably less than about 250 mM NaCl and 25 mM trisodium citrate. Low stringency hybridization can be obtained in the absence of organic solvent, e.g., formamide, while high stringency hybridization can be obtained in the presence of at least about 35% formamide, and more preferably at least about 50% formamide. Stringent temperature conditions will ordinarily include temperatures of at least about 30° C, more preferably of at least about 37° C, and most preferably of at least about 42° C. Varying additional parameters, such as hybridization time, the concentration of detergent, e.g., sodium dodecyl sulfate (SDS), and the inclusion or exclusion of carrier DNA, are well known to those of ordinary skill in the art. Various levels of stringency are accomplished by combining these various conditions as needed. In a preferred: embodiment, hybridization will occur at 30° C in 750 mM NaCl, 75 mM trisodium citrate, and 1% SDS. In a more preferred embodiment, hybridization will occur at 37° C in 500 mM NaCl, 50 mM trisodium citrate, 1% SDS, 35% formamide, and 100 μg/ml denatured salmon sperm DNA (ssDNA). In a most preferred embodiment, hybridization will occur at 42° C in 250 mM NaCl, 25 mM trisodium citrate, 1% SDS, 50% formamide, and 200 μg/ml ssDNA. Useful variations on these conditions will be readily apparent to those of ordinary skill in the art.

For most applications, washing steps that follow hybridization will also vary in stringency. Wash stringency conditions can be defined by salt concentration and by temperature. As above, wash stringency can be increased by decreasing salt concentration or by increasing temperature. For example, stringent salt concentration for the wash steps will

preferably be less than about 30 mM NaCl and 3 mM trisodium citrate, and most preferably less than about 15 mM NaCl and 1.5 mM trisodium citrate. Stringent temperature conditions for the wash steps will ordinarily include a temperature of at least about 25° C, more preferably of at least about 42° C, and even more preferably of at least about 68° C. In a preferred embodiment, wash steps will occur at 25° C in 30 mM NaCl, 3 mM trisodium citrate, and 0.1% SDS. In a more preferred embodiment, wash steps will occur at 42 C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. In a more preferred embodiment, wash steps will occur at 68° C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. Additional variations on these conditions will be readily apparent to those of ordinary skill in the art. Hybridization techniques are well known to those of ordinary skill and are described, for example, in Benton and Davis (Science 196:180, 1977); Grunstein and Hogness (Proc. Natl. Acad. Sci., USA 72:3961, 1975); Ausubel et al. (Current Protocols in Molecular Biology, Wiley Interscience, New York, 2001); Berger and Kimmel (Guide to Molecular Cloning Techniques, 1987, Academic Press, New York); and Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York.

By "substantially identical" is meant a polypeptide or nucleic acid molecule exhibiting at least 50% identity to a reference amino acid sequence (for example, any one of the amino acid sequences described herein) or nucleic acid sequence (for example, any one of the nucleic acid sequences described herein). Preferably, such a sequence is at least 60%, more preferably 80% or 85%, and more preferably 90%, 95% or even 99% identical at the amino acid level or nucleic acid to the sequence used for comparison.

Sequence identity is typically measured using sequence analysis software (for example, Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, Wis. 53705, BLAST, BESTFIT, GAP, or PILEUP/PRETTYBOX programs). Such software matches identical or similar sequences by assigning degrees of homology to various substitutions, deletions, and/or other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. In an exemplary approach to determining the degree of identity, a BLAST program may be used, with a probability score between e^{-3} and e^{-100} indicating a closely related sequence.

By "subject" is meant a mammal, including, but not limited to, a human or non-human mammal, such as a bovine, equine, canine, ovine, or feline.

Ranges provided herein are understood to be shorthand for all of the values within the range. For example, a range of 1 to 50 is understood to include any number, combination of numbers, or sub-range from the group consisting 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50.

As used herein, the terms “treat,” treating,” “treatment,” and the like refer to reducing or ameliorating a disorder and/or symptoms associated therewith. It will be appreciated that, although not precluded, treating a disorder or condition does not require that the disorder, condition or symptoms associated therewith be completely eliminated.

Unless specifically stated or obvious from context, as used herein, the term "or" is understood to be inclusive. Unless specifically stated or obvious from context, as used herein, the terms "a", "an", and "the" are understood to be singular or plural.

Unless specifically stated or obvious from context, as used herein, the term “about” is understood as within a range of normal tolerance in the art, for example within 2 standard deviations of the mean. About can be understood as within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value. Unless otherwise clear from context, all numerical values provided herein are modified by the term about.

The recitation of a listing of chemical groups in any definition of a variable herein includes definitions of that variable as any single group or combination of listed groups. The recitation of an embodiment for a variable or aspect herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

Any compositions or methods provided herein can be combined with one or more of any of the other compositions and methods provided herein.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A-1F provides a set of graphs and schematics showing CRISPR-Cas9 genome-scale drug resistance screens and validation that $KEAP1^{KO}$ confers resistance. FIG. 1A shows a pathway schematic and screening timeline. FIG. 1B provides a graph showing enrichment of the top 4 KEAP1 single guide (sg)RNAs compared to all sgRNAs in the library. Error bars represent the standard deviation of the mean. FIGS. 1C-1F provide graphs showing quantification of Crystal violet colony formation assays. Cells were seeded in 24-well plates. In FIG. 1C, 5000 CALU1 cells were treated with 50 nM trametinib for 17 days. 2000 HCC364 cells were treated with 25 nM trametinib or 6.25 uM vemurafenib for 21 days. In FIG. 1D, 5000 HCC827 cells were treated with 100 nM erlotinib for 10 days. 1000 H1975

cells were treated with 100 nM afatinib for 10 days. In Figure 1F, 1000 H3122 cells were treated with 300 nm Crizotinib for 14 days. Error bars represent the standard deviation of the mean of triplicate wells. FIG. 1E shows expression of wildtype KEAP1 resensitized A549 cells to trametinib. Expression of wildtype KEAP1 or KEAP1 G333C was restored in KEAP1-null A549 cells. 5000 cells were seeded in 24-well plates and treated with 25 nM trametinib for 12 days. Error bars represent the standard deviation of six wells. FIG. 1F provides a graph showing that KEAP1^{KO} confers resistance to ALK inhibition in ALK-mutant lung cancer.

FIGS. 2A-2C provide a sereis of graphs and images showing data indicating KEAP1^{KO} does not reactivate ERK but does increase NRF2 levels, and NRF2 also confers resistance. FIG. 2A provides a graph showing whole cell lysates of HCC364-Cas9 cells with the indicated sgRNAs treated with DMSO or trametinib for 48 hours. FIG. 2B a series of images showing immuno blots of nuclear and cytoplasmic fractions of HCC364 cells. FIG. 2C provides a series of graphs showing Crystal violet colony formation assays. 10,000 CALU1 cells expressing the indicated ORFs were seeded in 24-well plates and treated with DMSO for 8 days or trametinib for 10 Days. 10,000 HCC364 cells expressing the indicated ORFs were seeded in 12-well plates and treated with DMSO for 10 days or trametinib/vemurafenib for 21 days. Error bars represent the mean of triplicate wells.

FIGS. 3A-3B provide a series of graphs and images showing trametinib treatment and KEAP1^{KO} increase NRF2 activity. FIG. 3A provides a graph showing the expression of NFE2L2/NRF2 mRNA and NRF2 target genes in HCC364 treated with DMSO or trametinib for 72 hours. Error bars represent the standard deviation of the mean of three biological replicates. FIG. 3B provides a graph showing HCC364 cells treated with DMSO or trametinib for 72 hours. "TRAM" refers to trametinib.

FIGS. 4A-4E provide a series of graphs showing KEAP1^{KO} reduces trametinib-induced ROS and alters expression of metabolic genes. FIG. 4A provides a graph showing HCC364 or CALU1 cells treated with DMSO or trametinib for 72 hr. ROS was measured by DCFDA fluorescence. Error bars represent the standard deviation of the mean of two replicates. FIG. 4B provides a graph showing CALU1 cells treated with DMSO or 50 nM trametinib and the indicated concentration of NAC for 16 days. Population doublings of trametinib-treated cells compared to DMSO-treated cells are shown. Error bars represent the standard deviation of the mean of two replicates. In FIG. 4C, 20,000 CALU1 cells were seeded in 24-well plates and treated with DMSO and BSO for 7 days or 10 nM trametinib and BSO for 12 days. Error bars represent the standard deviation of the mean of triplicate

wells. FIG. 4D and FIG. 4E provide a series of graphs showing expression of NRF2 metabolic target genes in CALU1 treated with DMSO or trametinib for 72 hours. Error bars represent the standard deviation of the mean of three biological replicates.

FIG. 5 provides a series of graphs and images showing optimization of screening conditions. Cells were treated with the indicated concentration of drug. Cells were passaged or fresh media containing drug was added every 3-4 days. Cells were counted at each passage, and the number of population doublings is shown. In parallel, cells were treated with the indicated concentrations of drug for 90 min. Cell lysates were blotted with p-ERK antibody as a marker of BRAF/MEK inhibition. For the CRISPR-Cas9 screens, HCC364 cells were treated with 24 nM trametinib or 6.25 μM vemurafenib, H1299 cells were treated with 1.5 μM trametinib, and CALU1 cells were treated with 50 nM trametinib.

FIGS. 6A-6E provide a series of graphs immunoblots showing confirmation of KEAP1 knockout, KEAP1 overexpression, and NRF2 overexpression. FIG. 6A provides an immunoblot showing deletion of KEAP1 by sgRNAs in HCC364. FIG. 6B provides an immunoblot showing deletion of KEAP1 and increase in NRF2 in CALU1. FIG. 6C provides an immunoblot showing deletion of KEAP1 by sgRNAs in HCC827 and H1975. FIG. 6D is an immunoblot showing KEAP1 expression in A549 cells. FIG. 6E provides an immunoblot showing NRF2 expression in CALU1 and HCC364.

FIG. 7 provides a series of graphs showing $KEAP1^{KO}$ also confers resistance to some chemotherapeutics. 5,000 CALU1 cells were seeded in 24-well plates and treated with DMSO, 5-FU, or cisplatin for 12 days and etoposide, paclitaxel or trametinib for 18 days. Error bars represent the standard deviation of triplicate wells.

FIGS. 8A-8C provide graphs showing that Trametinib treatment increases NRF2 activity in CALU1 cells, which is further increased by $KEAP1^{KO}$. FIG. 8A provides a graph showing the expression of NFE2L2/NRF2 mRNA. FIG. 8B provides a graph showing the expression of NRF2 target genes in CALU1 cells treated with DMSO or trametinib for 72 hours. Error bars represent the standard deviation of biological triplicates. FIG. 8C provides a graph showing CALU1 cells treated with DMSO or trametinib for 72 hours. "D" is DMSO; "T" is Trametinib.

FIGS. 9A-9H provide graphs showing $KEAP1^{KO}$ reduces ROS and increases viability in the presence of BSO. FIG. 9A provides a graph showing trametinib does not affect GSH/GSSG ratio. CALU1 cells were treated for 72 hr. Error bars represent standard deviation of three replicates. FIG. 9B provides a graph showing NADPH and NADP+ levels in CALU1 treated with DMSO or trametinib for 72 hours. Error bars represent the standard

deviation of the mean of six wells. FIG. 9C provides a graph showing showing NRF2 overexpression reduces trametinib-induced ROS. CALU1 cells were treated for 72 hr. Error bars represent the standard deviation of two replicates. FIG 9D provides a graph showing N-acetyl cysteine (NAC) treatment reduces ROS in CALU1 cells. CALU1 cells were treated for 16 days. Error bars represent standard deviation of two replicates. FIG. 9E provides a graph showing trametinib and BSO induce ROS in KEAP1-intact cells. $KEAP1^{KO}$ reduces ROS. CALU1 cells were treated for 72 hr. FIGS. 9F- 9G show $KEAP1^{KO}$ reduces trametinib- and BSO-induced ROS and increases cell viability. FIG. 9F provides a graph showing cells were treated for 72 hr. Error bars represent the standard deviation of two replicates. FIG. 9G provides a graph showing cells were treated with DMSO plus BSO for 6 days or trametinib plus BSO for 10 days. Error bars represent the standard deviation of triplicate wells. FIG. 9H provides a graph showing expression of WT KEAP1 but not G333C in KEAP1-null A549 cells increases trametinib- and BSO-induced ROS. Cells were treated for 72 hr. Error bars represent the standard deviation of two replicates.

FIGS. 10A-10B provides a series of graphs showing $KEAP1^{KO}$ alters cell metabolism in HCC364 cells and the expression of NRF2 metabolic target genes in HCC364 treated with DMSO or trametinib for 72 hours. Error bars represent the standard deviation of the mean of three biological replicates.

FIG 11. provides a schematic showng a model of how KEAP1 loss confers resistance. The schematic on the left shows trametinib treatment inhibits MAPK signaling and induces ROS, which activates NRF2 to low levels. Theschematic on the right shows loss of KEAP1 leads to increased NRF2 activity, which reduces ROS levels and alters cellular metabolism, allowing cells to proliferate in the absence of MAPK signaling.

DETAILED DESCRIPTION OF THE INVENTION

The invention features compositions and methods that are useful for identifying a subject with an ALK-, BRAF-, EGFR-, NRAS-, or KRAS-mutant lung cancer that would benefit from treatment with an ALK inhibitor, MEK inhibitor, RAF inhibitor (e.g., a BRAF inhibitor), RAS inhibitor, ERK inhibitor, EGFR inhibitor, or other RTK inhibitor (such as a MET inhibitor). In some aspects, the methods comprise measuring a level, copy number, or sequence of KEAP1 and/or NRF2 polynucleotide in a biological sample obtained from the subject relative to a reference level or sequence.

The invention is based, at least in part, on the discovery that loss of KEAP1, which targets NFE2L2/NRF2 for ubiquitination and proteasomal degradation, conferred resistance

to ALK, MEK, BRAF, and EGFR inhibition in ALK-, BRAF-, EGFR-, NRAS-, and KRAS-mutant lung cancer. Inhibitors that target components of the receptor tyrosine kinase (RTK)/Ras/mitogen-activated protein kinase (MAPK) pathway have led to clinical responses in lung and other cancers, but resistance inevitably occurs (Balak et al., *Clinical cancer research : an official journal of the American Association for Cancer Research* 12, 6494-6501, (2006); Kosaka *et al., Clinical cancer research* 12, 5764-5769, (2006); Rudin et al. *Journal of thoracic oncology*, e41-42, (2013); Wagle et al., *Journal of clinical oncology* 29, 3085-3096, (2011)). To understand intrinsic and acquired resistance to inhibition of MAPK signaling, genome-scale CRISPR-Cas9 gene deletion screens in the setting of MEK, ALK, and BRAF inhibitors were performed. Loss of *KEAP1*, which targets NFE2L2/NRF2 for ubiquitination and proteasomal degradation, conferred resistance to ALK, BRAF, MEK, and EGFR inhibition in ALK-, BRAF-, NRAS-, KRAS-, and EGFR-mutant lung cancer cells. Loss of KEAP1 increased NRF2 expression without reactivating the MAPK pathway, and overexpression of NRF2 also conferred resistance to these drugs. Treatment with the MEK inhibitor trametinib increased reactive oxygen species (ROS) in cells with intact KEAP1, and loss of KEAP1 or overexpression of NRF2 prevented this increase. In addition, the increased activity of NRF2 upon KEAP1 knockout and trametinib treatment led to an increase in the expression of metabolic genes. Together these observations demonstrate that KEAP1 loss confers resistance to MAPK pathway inhibition by decreasing ROS and altering cell metabolism to allow cells to proliferate in the absence of MAPK signaling. Without being bound by theory, these results indicate that patients with KEAP1/NRF2 pathway alterations may not respond to ALK, BRAF, MEK or EGFR inhibitors. The studies described herein have increased current understanding of the potential resistance mechanisms to inhibition of the Ras/MAPK pathway, and the results will inform patient treatment in the clinic.

RTK/MAPK pathway in cancer

The receptor tyrosine kinase (RTK)/ mitogen-activated protein kinase (MAPK) pathway plays an important role in the development of lung and other cancers, with the frequent occurrence of mutations or copy number alterations in multiple nodes of this pathway (Ding et al. (2008), *Nature*, 455(7216), 1069-75; Imielinski et al. (2012), *Cell*, 150(6), 1107-20). However, single-agent therapy targeting this pathway has had limited clinical success. While BRAF and EGFR inhibitors can produce dramatic responses temporarily, acquired resistance inevitably occurs in lung and other cancers (Wagle et al. (2011), *J Clin Oncol*, 29(22), 3085-96; Balak et al. (2006), *Clin Cancer Res*, 12(21), 6494-

501; Kosaka et al. (2006), *Clin Cancer Res*, 12(19), 5764-9; Rudin et al. (2013), *J Thorac Oncol*, 8(5), e41-2). In addition to this acquired resistance, many tumors also exhibit intrinsic resistance to these inhibitors (Corcoran et al. (2012), *Cancer Discov*, 2(3), 227-35; Prahallad et al., (2012), *Nature*, 483(7387), 100-3), as well as to MEK inhibitors (Sun et al. (2014), *Cell Rep*, 7(1), 86-93). Several studies have now shown that a general theme of resistance to these targeted therapies is activation of the RTK/MAPK pathway by alternative mechanisms (Corcoran et al. (2012), *Cancer Discov*, 2(3), 227-35; Prahallad et al. (2012), *Nature*, 483(7387), 100-3; Johannessen et al. (2010), *Nature*, 468(7326), 968-72; Nazarian et al. (2010), *Nature*, 468(7326), 973-7). In lung cancer, transcriptional induction of ERBB3 causes intrinsic resistance to MEK inhibition in KRAS-mutant cancers (Sun et al. (2014), *Cell Rep*, 7(1), 86-93), and acquired resistance to EGFR inhibitors was found to result from amplification of MET (Engelman et al. (2007), *Science*, 316(5827), 1039-43).

These findings highlight the importance of maintaining RTK/MAPK signaling in lung and other cancers and also suggest some redundancy between different genetic alterations in this pathway. Due to the many ways that cancers can acquire resistance to single therapies targeting the RTK/MAPK pathway, combination therapy may hold more promise for treating tumors with alterations in this pathway. Unfortunately, different tumor types may acquire different mechanisms of reactivating the pathway, and within a single tumor type, multiple mechanisms of resistance may be possible. It will therefore be important to comprehensively catalogue modes of resistance, in order to choose the most promising combination therapy for each cancer. Alternatively, combination therapies targeting vulnerabilities distinct from this pathway may delay or prevent the onset of resistance.

Genome-scale gain-of-function and loss-of-function screens have previously been used to identify mechanisms of resistance to targeted therapeutics (Johannessen et al. (2013), *Nature*, 504(7478), 138-42; Whittaker et al. (2013), *Cancer Discov*, 3(3), 350-62; Berns et al. (2007), *Cancer Cell*, 12(4), 395-402), and CRISPR-Cas9 knockout screens have also recently been used to identify mechanisms of resistance (Shalem et al. (2014), *Science*, 343(6166), 84-7; Wang et al. (2014), *Science*, 343(6166), 80-4). Each of these studies has focused on therapeutics targeting a single alteration. The studies described herein have expanded this approach to explore the hypothesis that resistance to different targeted therapies may result from novel shared mechanisms. In this regard, genome-scale CRISPR drug resistance screens in multiple lung cancer cell lines with different alterations in the Ras/MAPK pathway to identify novel genes whose deletion promotes resistance to two targeted therapeutics in different genetic contexts were performed. Four genome-scale CRISPR-Cas9 screens to

identify mechanisms of resistance to inhibition of MEK or BRAF in lung cancer: NCIH1299 ($NRAS^{Q61K}$) and CALU1 ($KRAS^{G12C}$) cells treated with the MEK inhibitor trametinib, and HCC364 ($BRAF^{V600E}$) cells treated with trametinib or with the BRAF inhibitor vemurafenib were performed. A number of genes were identified whose deletion confers drug resistance in these contexts. Studies herein focused on KEAP1, whose loss conferred resistance in multiple contexts.

KEAP1/NRF2 mediated resistance

It was found that KEAP1 loss confers resistance to inhibition of ALK, BRAF, MEK, or EGFR in lung cancer cell lines with ALK, BRAF, KRAS, NRAS, or EGFR mutations. Importantly, unlike previously reported mechanisms of resistance, the mechanism described here does not involve reactivation of the MAPK pathway. KEAP1 loss or NRF2 overexpression is sufficient to restore cell proliferation in the absence of MAPK signaling. NRF2 has recently been found to be a transforming oncogene. The results herein indicate that increased expression of NRF2 upon KEAP1 loss can confer resistance to MAPK pathway inhibition by reducing ROS and altering cell metabolism.

A recent vemurafenib $BRAF^{V600E}$ basket trial showed that 42% of lung cancers with the BRAF V600E mutation responded to vemurafenib (Hyman et al. *The New England journal of medicine* 373, 726-736, (2015)). As seen with vemurafenib treatment in melanoma or with EGFR inhibitors in lung cancer, acquired resistance will likely arise. Furthermore, while MEK inhibitors only elicit responses in a small number of lung cancer patients (Blumenschein, et al., *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* 26, 894-901, (2015), these responders are also likely to develop resistance. Predicting how resistance may arise in these patients will be important for developing combination therapies. In addition, for those patients that do not initially respond, intrinsic resistance in a subset of these patients may be explained by the mechanisms we describe here. The KEAP1/NRF2 pathway is genetically altered in approximately 30% of lung squamous cell carcinomas and approximately 20% of lung adenocarcinomas (Cerami, et al., *Cancer discovery* 2, 401-404, (2012); Gao et al., *Science signaling* 6, pl1, (2013)). Loss of KEAP1 or gain of NRF2 may therefore be a clinically relevant mechanism of acquired and intrinsic resistance to RTK/Ras/MAPK-targeted therapies in lung cancer. Stratifying patients for treatment based on these findings is important for evaluating the efficacy of these inhibitors in clinical trials.

Without being bound by theory, loss of KEAP1 may be a clinically relevant mechanism of acquired and intrinsic resistance to trametinib, vemurafenib, erlotinib, and afatinib in lung cancer. Stratifying patients for treatment based on these findings will be important for evaluating the efficacy of ALK, MEK, EGFR, and BRAF inhibitors in clinical trials. Thus, in some aspects, the invention provides a method of identifying a subject with an ALK-, BRAF-, EGFR-, NRAS-, or KRAS- mutant lung cancer that would benefit from treatment with an ALK inhibitor, MEK inhibitor, RAF inhibitor (e.g., a BRAF inhibitor), RAS inhibitor, ERK inhibitor, EGFR inhibitor, or other RTK inhibitor (such as a MET inhibitor). In other aspects, the invention provides a method for determining whether a subject is eligible for entry into a clinical trial for treating a lung cancer with an ALK inhibitor, MEK inhibitor, RAF inhibitor (e.g., a BRAF inhibitor), RAS inhibitor, ERK inhibitor, EGFR inhibitor, or other RTK inhibitor (such as a MET inhibitor), as well as methods for monitoring effectiveness of treatment of an ALK-, BRAF-, NRAS-, EGFR-, or KRAS-mutant lung cancer in a subject with a MEK inhibitor, RAF inhibitor (e.g., a BRAF inhibitor), RAS inhibitor, ERK inhibitor, EGFR inhibitor, ALK inhibitor, or other RTK inhibitor (such as a MET inhibitor). In some embodiments, the methods comprise measuring a level or sequence of KEAP1 and/or NRF2 polynucleotide in a biological sample obtained from the subject relative to a reference level or sequence. In certain embodiments, detection of a mutation in the sequence of KEAP1 polynucleotide or an increase in copy number or level of NRF2 polynucleotide indicates the lung cancer is resistant to an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor. In some other embodiments, failure to detect a mutation in the sequence of KEAP1 polynucleotide or failure to detect an increase in the copy number or level of NRF2 polynucleotide indicates the lung cancer is sensitive to an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor.

Targeting the KEAP1/NRF2 axis may also be a promising therapeutic strategy. For example, findings described herein suggest that combination of a Ras/Raf/RTK inhibitor and a NRF2/KEAP1 therapeutic would benefit patients with alterations in the NRF2/KEAP1 pathway. Thus, in some aspects, the invention provides a method of treating a subject having an ALK-, BRAF-, EGFR-, NRAS-, KRAS-mutant lung cancer, the method comprising administering to a selected subject an effective amount of a KEAP1 polynucleotide and an effective amount of an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor, wherein the subject is selected by detecting a decrease in KEAP1 polynucleotide, a mutation in KEAP1 polynucleotide, or an increase in NRF2 polynucleotide in a biological sample of the subject relative to a reference sequence or level.

Methods of treatment

The present invention provides methods of treating a lung cancer (in particular, an ALK-, BRAF-, EGFR-, NRAS-, or KRAS-mutant lung cancer) and/or disorders or symptoms thereof which comprise administering a therapeutically effective amount of a pharmaceutical composition comprising a therapeutic agent (e.g., an ALK inhibitor, MEK inhibitor, RAF inhibitor (e.g., a BRAF inhibitor), RAS inhibitor, ERK inhibitor, RTK inhibitor (such as a MET or EGFR inhibitor), a KEAP1 polynucleotide, or a NRF2 inhibitor, or any combination thereof) to a subject (e.g., a mammal such as a human). Thus, one embodiment is a method of treating a subject suffering from or susceptible to an ALK-, BRAF-, EGFR-, NRAS-, or KRAS-mutant lung cancer or disorder or symptom thereof. The method includes the step of administering to the mammal a therapeutic amount of an amount of a therapeutic agent described herein sufficient to treat the ALK-, BRAF-, EGFR-, NRAS-, or KRAS-mutant lung cancer or symptom thereof, under conditions such that the lung cancer is treated.

The methods herein include administering to the subject (including a subject identified as in need of such treatment) an effective amount of a therapeutic agent described herein (e.g., an ALK inhibitor, MEK inhibitor, RAF inhibitor (e.g., a BRAF inhibitor), RAS inhibitor, ERK inhibitor, RTK inhibitor (such as a MET or EGFR inhibitor), a KEAP1 polynucleotide, or a NRF2 inhibitor, or any combination thereof), or a composition described herein to produce such effect. Identifying a subject in need of such treatment can be in the judgment of a subject or a health care professional and can be subjective (e.g. opinion) or objective (e.g. measurable by a test or diagnostic method).

As used herein, the terms "treat," treating," "treatment," and the like refer to reducing or ameliorating a disorder and/or symptoms associated therewith. It will be appreciated that, although not precluded, treating a disorder or condition does not require that the disorder, condition or symptoms associated therewith be completely eliminated.

As used herein, the terms "prevent," "preventing," "prevention," "prophylactic treatment" and the like refer to reducing the probability of developing a disorder or condition in a subject, who does not have, but is at risk of or susceptible to developing a disorder or condition.

The therapeutic methods of the invention (which include prophylactic treatment) in general comprise administration of a therapeutically effective amount of the therapeutic agents herein, such as an ALK inhibitor, MEK inhibitor, RAF inhibitor (e.g., a BRAF inhibitor), RAS inhibitor, ERK inhibitor, RTK inhibitor (e.g., a MET or EGFR inhibitor), a

KEAP1 polynucleotide, or a NRF2 inhibitor, or any combination thereof, to a subject (e.g., animal, human) in need thereof, including a mammal, particularly a human. Such treatment will be suitably administered to subjects, particularly humans, suffering from, having, susceptible to, or at risk for lung cancer (particularly, ALK-, BRAF-, EGFR-, NRAS-, or KRAS-mutant lung cancer), or a disorder, or symptom thereof. Determination of those subjects "at risk" can be made by any objective or subjective determination by a diagnostic test or opinion of a subject or health care provider (e.g., genetic test, enzyme or protein marker, marker such as a KEAP1 and/or NRF2 polynucleotide or polypeptide, family history, and the like). The compounds herein may be also used in the treatment of any other disorders in which ALK-, BRAF-, EGFR-, NRAS-, or KRAS-mutant lung cancer may be implicated.

In one embodiment, the invention provides a method of monitoring treatment progress. The method includes the step of determining a level of diagnostic marker (e.g., a level, sequence, or copy number of a polynucleotide or polypeptide of KEAP1 and/or NRF2) or diagnostic measurement (e.g., screen, assay) in a subject suffering from or susceptible to a lung cancer associated with mutations in Ras/MAPK pathway (e.g., mutations in ALK-, BRAF-, EGFR-, NRAS-, or KRAS), or disorder or symptoms thereof, in which the subject has been administered a therapeutic or effective amount of a therapeutic agent described herein sufficient to treat the lung cancer or symptoms thereof. The level, sequence, or copy number of a polynucleotide or polypeptide of KEAP1 and/or NRF2 determined in the method can be compared to known levels, sequences, or copy numbers of a polynucleotide or polypeptide of KEAP1 and/or NRF2 in either healthy normal controls or in other afflicted patients to establish the subject's disease status. In preferred embodiments, a second level, sequence, or copy number of a polynucleotide or polypeptide of KEAP1 and/or NRF2 in the subject is determined at a time point later than the determination of the first level, sequence, or copy number, and the two levels, sequences, or copy numbers are compared to monitor the course of disease or the efficacy of the therapy. In certain preferred embodiments, a pre-treatment level, sequence, or copy number of a polynucleotide or polypeptide of KEAP1 and/or NRF2 in the subject is determined prior to beginning treatment according to this invention; this pre-treatment level, sequence, or copy number of a polynucleotide or polypeptide of KEAP1 and/or NRF2 can then be compared to the level, sequence, or copy number of a polynucleotide or polypeptide of KEAP1 and/or NRF2 in the subject after the treatment commences, to determine the efficacy of the treatment.

Pharmaceutical compositions

The present invention features compositions useful for treating a lung cancer, particularly ALK-, BRAF-, EGFR-, NRAS-, or KRAS-mutant lung cancer, in a subject. In some embodiments, the composition comprises one or more of a therapeutic agent as described herein (e.g., ALK inhibitor, MEK inhibitor, RAF inhibitor (e.g., a BRAF inhibitor), RAS inhibitor, ERK inhibitor, RTK inhibitor (e.g., a MET or EGFR inhibitor), a polynucleotide encoding a KEAP1 polypeptide, or a NRF2 inhibitor, or any combination thereof). In particular embodiments, the composition further comprises a vehicle for intracellular delivery of a polypeptide or polynucleotide (e.g., a liposome).

The administration of a composition comprising a therapeutic agent herein for the treatment of an ALK-, BRAF-, EGFR-, NRAS-, or KRAS-mutant lung cancer may be by any suitable means that results in a concentration of the therapeutic that, combined with other components, is effective in ameliorating, reducing, or stabilizing a lung cancer in a subject. The composition may be administered systemically, for example, formulated in a pharmaceutically-acceptable buffer such as physiological saline. Preferable routes of administration include, for example, subcutaneous, intravenous, interperitoneally, intramuscular, or intradermal injections that provide continuous, sustained levels of the agent in the patient. The amount of the therapeutic agent to be administered varies depending upon the manner of administration, the age and body weight of the patient, and with the clinical symptoms of the cancer. Generally, amounts will be in the range of those used for other agents used in the treatment of cancers such as ALK-, BRAF-, EGFR-, NRAS-, or KRAS-mutant lung cancer, although in certain instances lower amounts will be needed because of the increased specificity of the agent. A composition is administered at a dosage that decreases effects or symptoms of lung cancer as determined by a method known to one of ordinary skill in the art.

The therapeutic agent (e.g., ALK inhibitor, MEK inhibitor, RAF inhibitor (e.g., a BRAF inhibitor), RAS inhibitor, ERK inhibitor, RTK inhibitor (e.g., a MET or EGFR inhibitor), polynucleotide encoding a KEAP1 polypeptide, or a NRF2 inhibitor, or any combination thereof) may be contained in any appropriate amount in any suitable carrier substance, and is generally present in an amount of 1-95% by weight of the total weight of the composition. The composition may be provided in a dosage form that is suitable for parenteral (e.g., subcutaneously, intravenously, intramuscularly, or intraperitoneally) administration route. The pharmaceutical compositions may be formulated according to

conventional pharmaceutical practice (see, e.g., Remington: The Science and Practice of Pharmacy (20th ed.), ed. A. R. Gennaro, Lippincott Williams & Wilkins, 2000 and Encyclopedia of Pharmaceutical Technology, eds. J. Swarbrick and J. C. Boylan, 1988-1999, Marcel Dekker, New York).

Pharmaceutical compositions according to the invention may be formulated to release the active agent substantially immediately upon administration or at any predetermined time or time period after administration. The latter types of compositions are generally known as controlled release formulations, which include (i) formulations that create a substantially constant concentration of the drug within the body over an extended period of time; (ii) formulations that after a predetermined lag time create a substantially constant concentration of the drug within the body over an extended period of time; (iii) formulations that sustain action during a predetermined time period by maintaining a relatively, constant, effective level in the body with concomitant minimization of undesirable side effects associated with fluctuations in the plasma level of the active substance (sawtooth kinetic pattern); (iv) formulations that localize action by, e.g., spatial placement of a controlled release composition adjacent to or in contact with an organ, such as the liver; (v) formulations that allow for convenient dosing, such that doses are administered, for example, once every one or two weeks; and (vi) formulations that target a cancer using carriers or chemical derivatives to deliver the therapeutic agent to a particular cell type (e.g., liver cell). For some applications, controlled release formulations obviate the need for frequent dosing during the day to sustain the plasma level at a therapeutic level.

Any of a number of strategies can be pursued in order to obtain controlled release in which the rate of release outweighs the rate of metabolism of the agent in question. In one example, controlled release is obtained by appropriate selection of various formulation parameters and ingredients, including, e.g., various types of controlled release compositions and coatings. Thus, the therapeutic is formulated with appropriate excipients into a pharmaceutical composition that, upon administration, releases the therapeutic in a controlled manner. Examples include single or multiple unit tablet or capsule compositions, oil solutions, suspensions, emulsions, microcapsules, microspheres, molecular complexes, nanoparticles, patches, and liposomes.

The pharmaceutical composition may be administered parenterally by injection, infusion or implantation (subcutaneous, intravenous, intramuscular, intraperitoneal, or the like) in dosage forms, formulations, or via suitable delivery devices or implants containing conventional, non-toxic pharmaceutically acceptable carriers and adjuvants. The formulation

and preparation of such compositions are well known those of ordinary skill in the art of pharmaceutical formulation. Formulations can be found in Remington: The Science and Practice of Pharmacy, supra.

Compositions for parenteral use may be provided in unit dosage forms (e.g., in single-dose ampoules), or in vials containing several doses and in which a suitable preservative may be added (see below). The composition may be in the form of a solution, a suspension, an emulsion, an infusion device, or a delivery device for implantation, or it may be presented as a dry powder to be reconstituted with water or another suitable vehicle before use. Apart from the active agent that reduces or ameliorates a lung cancer, the composition may include suitable parenterally acceptable carriers and/or excipients. The active therapeutic agent(s) (e.g., ALK inhibitor, MEK inhibitor, RAF inhibitor (e.g., a BRAF inhibitor), RAS inhibitor, ERK inhibitor, RTK inhibitor (e.g., a MET or EGFR inhibitor), polynucleotide encoding a KEAP1 polypeptide, or a NRF2 inhibitor, or any combination thereof, as described herein) may be incorporated into microspheres, microcapsules, nanoparticles, liposomes, or the like for controlled release. Furthermore, the composition may include suspending, solubilizing, stabilizing, pH-adjusting agents, tonicity adjusting agents, and/or dispersing, agents.

In some embodiments, the composition comprising the active therapeutic (e.g., ALK inhibitor, MEK inhibitor, RAF inhibitor (e.g., a BRAF inhibitor), RAS inhibitor, ERK inhibitor, RTK inhibitor (e.g., a MET or EGFR inhibitor), polynucleotide encoding a KEAP1 polypeptide, or a NRF2 inhibitor, or any combination thereof, as described herein) is formulated for intravenous delivery. As indicated above, the pharmaceutical compositions according to the invention may be in the form suitable for sterile injection. To prepare such a composition, the suitable therapeutic(s) are dissolved or suspended in a parenterally acceptable liquid vehicle. Among acceptable vehicles and solvents that may be employed are water, water adjusted to a suitable pH by addition of an appropriate amount of hydrochloric acid, sodium hydroxide or a suitable buffer, 1,3-butanediol, Ringer's solution, and isotonic sodium chloride solution and dextrose solution. The aqueous formulation may also contain one or more preservatives (e.g., methyl, ethyl or n-propyl p-hydroxybenzoate). In cases where one of the agents is only sparingly or slightly soluble in water, a dissolution enhancing or solubilizing agent can be added, or the solvent may include 10-60% w/w of propylene glycol or the like.

Polynucleotide therapy

Another therapeutic approach for treating an ALK-, BRAF-, EGFR-, NRAS-, or KRAS-, mutant lung cancer is polynucleotide therapy using a polynucleotide encoding a KEAP1 polypeptide, or fragment thereof, or a NRF2 inhibitor, such as an inhibitory polynucleotides that reduces NRF2 expression. In the studies described herein, it was found that restoring KEAP1 expression in cells which were both KRAS mutant and KEAP1-null increased their sensitivity to trametinib. Further, without being bound by theory, it is believed that elevated NRF2 levels in KEAP1 knockout ($KEAP1^{KO}$) cells mediated resistance. Accordingly, in some aspects, the invention provides a therapeutic composition comprising a KEAP1 polynucleotide and/or a NRF2 inhibitor. In other aspects, the invention provides a method of increasing sensitivity of a subject having an ALK-, BRAF-, EGFR-, NRAS-, or KRAS-, mutant lung cancer to an ALK inhibitor, MEK inhibitor, RAF inhibitor (e.g., a BRAF inhibitor), RAS inhibitor, ERK inhibitor, or RTK inhibitor (e.g., a MET or EGFR inhibitor), the method comprising administering to the subject a KEAP1 polynucleotide and/or a NRF2 inhibitor. In still other aspects, the invention provides a method of treating an ALK-, BRAF-, EGFR-, NRAS-, or KRAS-mutant lung cancer in a subject comprising administering to the subject a KEAP1 polynucleotide and/or a NRF2 inhibitor, in combination with an ALK inhibitor, MEK inhibitor, RAF inhibitor (e.g., a BRAF inhibitor), RAS inhibitor, ERK inhibitor, or RTK inhibitor (e.g., a MET or EGFR inhibitor).

Thus, provided herein are isolated polynucleotides encoding a KEAP polypeptide of the invention, or a fragment thereof. Also provided herein are inhibitory polynucleotides that reduce NRF2 expression. Delivery or expression of such polynucleotides or nucleic acid molecules in a cell or organism is expected to increase sensitivity to inhibition of ALK, MEK, BRAF, or EGFR to treat cancer (particularly, ALK-, BRAF-, EGFR-, NRAS-, or KRAS-mutant lung cancer) in the subject. Such polynucleotides are also expected to increase sensitivity of the subject to other inhibitors of MAPK/RTK pathway components (e.g., RAF, RAS, ERK, or other RTK inhibitors (such as MET inhibitors). Such nucleic acid molecules can be delivered to cells of a subject having an ALK-, BRAF-, EGFR-, NRAS-, or KRAS-mutant lung cancer (in particular, subjects additionally having a KEAP1 mutation and/or NRF2 amplification or overexpression). The nucleic acid molecules must be delivered to the cells of a subject in a form in which they can be taken up so that therapeutically effective levels of the KEAP1 polypeptide, or fragment thereof, can be produced, and/or expression levels of NRF2 in the cells are effectively reduced.

Transducing viral (e.g., retroviral, adenoviral, and adeno-associated viral) vectors can be used for somatic cell gene therapy, especially because of their high efficiency of infection and stable integration and expression (see, e.g., Cayouette et al., Human Gene Therapy 8:423-430, 1997; Kido et al., Current Eye Research 15:833-844, 1996; Bloomer et al., Journal of Virology 71:6641-6649, 1997; Naldini et al., Science 272:263-267, 1996; and Miyoshi et al., Proc. Natl. Acad. Sci. U.S.A. 94:10319, 1997). For example, a polynucleotide encoding a KEAP1 polypeptide of the invention, or a fragment thereof, or an inhibitory polynucleotide that reduces NRF2 expression, can be cloned into a retroviral vector and expression can be driven from its endogenous promoter, from the retroviral long terminal repeat, or from a promoter specific for a target cell type of interest. Other viral vectors that can be used include, for example, a vaccinia virus, a bovine papilloma virus, or a herpes virus, such as Epstein-Barr Virus (also see, for example, the vectors of Miller, Human Gene Therapy 15-14, 1990; Friedman, Science 244:1275-1281, 1989; Eglitis et al., BioTechniques 6:608-614, 1988; Tolstoshev et al., Current Opinion in Biotechnology 1:55-61, 1990; Sharp, The Lancet 337:1277-1278, 1991; Cornetta et al., Nucleic Acid Research and Molecular Biology 36:311-322, 1987; Anderson, Science 226:401-409, 1984; Moen, Blood Cells 17:407-416, 1991; Miller et al., Biotechnology 7:980-990, 1989; Le Gal La Salle et al., Science 259:988-990, 1993; and Johnson, Chest 107:77S-83S, 1995). Retroviral vectors are particularly well developed and have been used in clinical settings (Rosenberg et al., N. Engl. J. Med 323:370, 1990; Anderson et al., U.S. Pat. No. 5,399,346). In some embodiments, a viral vector is used to administer an inhibitory polynucleotide that reduces NRF2 expression or a polynucleotide encoding a KEAP1 polypeptide (or fragment thereof) systemically.

Non-viral approaches can also be employed for the introduction of the therapeutic to a cell of a patient requiring treatment of a cancer (particularly, an ALK-, BRAF-, EGFR-, NRAS-, or KRAS-mutant lung cancer). For example, a nucleic acid molecule can be introduced into a cell by administering the nucleic acid in the presence of lipofection (Feigner et al., Proc. Natl. Acad. Sci. U.S.A. 84:7413, 1987; Ono et al., Neuroscience Letters 17:259, 1990; Brigham et al., Am. J. Med. Sci. 298:278, 1989; Staubinger et al., Methods in Enzymology 101:512, 1983), asialoorosomucoid-polylysine conjugation (Wu et al., Journal of Biological Chemistry 263:14621, 1988; Wu et al., Journal of Biological Chemistry 264:16985, 1989), or by micro-injection under surgical conditions (Wolff et al., Science 247:1465, 1990). Preferably the nucleic acids are administered in combination with a liposome and protamine.

Gene transfer can also be achieved using non-viral means involving transfection in *vitro*. Such methods include the use of calcium phosphate, DEAE dextran, electroporation, and protoplast fusion. Liposomes can also be potentially beneficial for delivery of DNA into a cell. Transplantation of genes encoding KEAP1 polypeptides into the affected tissues of a patient can also be accomplished by transferring a nucleic acid encoding KEAP1 polypeptide into a cultivatable cell type *ex vivo* (e.g., an autologous or heterologous primary cell or progeny thereof), after which the cell (or its descendants) are injected into a targeted tissue.

cDNA expression for use in polynucleotide therapy methods can be directed from any suitable promoter (e.g., the human cytomegalovirus (CMV), simian virus 40 (SV40), or metallothionein promoters), and regulated by any appropriate mammalian regulatory element. For example, if desired, enhancers known to preferentially direct gene expression in specific cell types can be used to direct the expression of a nucleic acid. The enhancers used can include, without limitation, those that are characterized as tissue- or cell-specific enhancers. Alternatively, if a genomic clone is used as a therapeutic construct, regulation can be mediated by the cognate regulatory sequences or, if desired, by regulatory sequences derived from a heterologous source, including any of the promoters or regulatory elements described above.

Delivery of polynucleotides of the invention may also include or be performed in combination with gene or genome editing methods, such as CRISPR-Cas systems, to introduce polynucleotides encoding KEAP1 polypeptides or to introduce or restore wild-type KEAP1 expression in cells. Gene or genome editing methods such as CRISPR-Cas systems are further described in for example, Sander et al. (2014), Nature Biotechnology 32, 347-355; Hsu et al. (2014), Cell 157(6): 1262-1278.

Stratifying Patient Population and Monitoring Effectiveness of MEK/BRAF/EGFR Inhibitor Therapies

In the studies described herein, loss of KEAP1 or amplification of NFE2L2/NRF2 was found to confer resistance to treatment with the BRAF inhibitor vemurafenib in BRAF-mutant lung cancer, the MEK inhibitor trametinib in BRAF-, NRAS-, or KRAS-mutant lung cancer, the ALK inhibitor Crizotinib in lung cancers that had lost KEAP1, and the EGFR inhibitors afatinib and erlotinib in EGFR-mutant lung cancer. Without intending to be bound by theory, it is believed these alterations will also confer resistance to other Raf inhibitors as well as to Receptor Tyrosine Kinase (RTK) inhibitors, such as MET inhibitors.

Thus, information on KEAP1 and/or NRF2 status in an ALK-, BRAF-, EGFR-, NRAS-, or KRAS-mutant lung cancer may predict clinical response of the cancer to inhibitors of components of the MAPK/RTK signaling pathway (e.g., ALK, MEK, RAF, BRAF, RAS, ERK, EGFR, or MET). Accordingly, in one aspect, the invention provides a method of identifying a subject with an ALK-, BRAF-, EGFR-, NRAS-, or KRAS-mutant lung cancer that would benefit from treatment with an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor. In another aspect, the invention provides a method of typing an ALK-, BRAF-, EGFR-, NRAS-, or KRAS-mutant lung cancer as a cancer that is resistant to or sensitive to an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor.

Stratifying patients for treatment based on resistance or sensitivity to ALK, MEK, BRAF, or EGFR inhibitors will be important for evaluating the efficacy of these inhibitors in clinical trials. Therefore, in another aspect, the invention provides a method for determining whether a subject is eligible for entry into a clinical trial for treating a lung cancer with an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor. Subjects identified as having an ALK-, BRAF-, EGFR-, NRAS-, or KRAS-mutant lung cancer that is sensitive to an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor are eligible for entry.

Diagnostic analysis of KEAP1 and NRF2 status should be performed in lung cancer patients with ALK-, NRAS-, KRAS-, BRAF-, or EGFR-mutations who are candidates for ALK, BRAF, MEK, or EGFR inhibitors, as well as other Raf inhibitors and future Ras inhibitors. The anaylsis includes all types of diagnostics, including nucleic acid, antibody, and protein. Thus, in various embodiments of any of the aspects delineated herein, alterations in a polynucleotide or polypeptide of KEAP1 and/or NRF2 (e.g., sequence, copy number, level, post-transcriptional modification, biological activity) are analyzed. In some embodiments, the method includes the step of measuring or detecting a level, copy number, or sequence of KEAP1 and/or NRF2 polynucleotide in a biological sample obtained from the subject relative to a reference level, copy number, or sequence. In particular embodiments, DNA sequencing and copy number analysis are performed on KEAP1 and NFE2L2 in lung cancer patients with ALK-, EGFR-, NRAS-, KRAS-, or BRAF-mutations who are candidates for trametinib or vemurafenib treatment.

The detection of a mutation in the sequence of KEAP1 polynucleotide, a decrease in the level or activity of KEAP1 polynucleotide or polypeptide, or an increase in copy number, level, or activity of NRF2 polynucleotide or polypeptide indicates the lung cancer is resistant to an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor. Failure to detect a mutation in the sequence of KEAP1 polynucleotide or failure to detect an increase in the

copy number or level of NRF2 polynucleotide indicates the lung cancer is sensitive to an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor. Thus, in some embodiments, a subject is identified as sensitive to an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor or as having a lung cancer sensitive to an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor if a mutation in the sequence of KEAP1 polynucleotide or an increase in the copy number or level of NRF2 polynucleotide is not detected in the biological sample obtained from the subject, relative to a reference level, copy number, or sequence. In other embodiments, a subject is identified as resistant to an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor if a decrease in the level of KEAP1 polynucleotide, a mutation in the sequence of KEAP1 polynucleotide or an increase in the copy number or level of NRF2 polynucleotide detected in the biological sample obtained from the subject, relative to a reference level, copy number, or sequence. In some embodiments, the mutation in KEAP1 is a loss-of-function mutation. In some other embodiments, the mutation in KEAP1 is KEAP1 G333C. In some embodiments, if a mutation in the sequence of KEAP1 polynucleotide and/or an increase in the copy number or level of NRF2 polynucleotide is not detected, a sequence, level, or activity of one or more RTK/Ras/MAPK pathway genes (e.g., an ALK polypeptide, BRAF polypeptide, KRAS polypeptide, or NRAS polypeptide) is further measured.

In still another aspect, the invention provides a method of monitoring effectiveness of treatment of an ALK-, BRAF-, EGFR-, NRAS-, or KRAS-mutant lung cancer in a subject with an ALK inhibitor, MEK inhibitor, RAF inhibitor (e.g., BRAF inhibitor), RAS inhibitor, ERK inhibitor, EGFR inhibitor, or other RTK inhibitor (such as a MET inhibitor). In some embodiments, an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor, or any combination thereof, is administered to a subject having an ALK-, BRAF-, EGFR-, NRAS-, or KRAS-mutant lung cancer. Over time, many patients treated with any one or more of these inhibitors acquire resistance to the therapeutic effects of the inhibitor. The early identification of resistance to an ALK inhibitor, MEK inhibitor, RAF inhibitor (e.g., a BRAF inhibitor), RAS inhibitor, ERK inhibitor, or RTK inhibitor (such as a MET or EGFR inhibitor) in a lung cancer patient is important to patient survival because it allows for the selection of alternate therapies. Subjects identified as having an ALK-, BRAF-, EGFR-, NRAS-, or KRAS-mutant lung cancer resistant to any one or more of these inhibitors are identified as in need of alternative treatment. Subjects identified as having an ALK-, BRAF-, EGFR-, NRAS-, or KRAS-mutant lung cancer resistant to one or more of these inhibitors, may be treated for example, with a therapeutic composition comprising a KEAP1

polynucleotide and/or a NRF2 inhibitor, in combination with an ALK inhibitor, MEK inhibitor, RAF inhibitor (e.g., a BRAF inhibitor), RAS inhibitor, ERK inhibitor, RTK inhibitor (such as a MET or EGFR inhibitor). As described elsewhere herein, administering a KEAP1 polynucleotide and/or a NRF2 inhibitor to a subject resistant to an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor may increase sensitivity to one or more of these inhibitors.

Methods of monitoring the sensitivity to an ALK inhibitor, MEK inhibitor, RAF inhibitor (e.g., a BRAF inhibitor), RAS inhibitor, ERK inhibitor, or RTK inhibitor (such as a MET or EGFR inhibitor) of a subject having a lung cancer (particularly, ALK-, BRAF-, EGFR-, NRAS-, or KRAS-mutant lung cancer) are useful in managing subject treatment. Thus, in some embodiments, alterations in a polynucleotide or polypeptide of KEAP1 and/or NRF2 (e.g, sequence, level, post-transcriptional modification, biological activity) are analyzed before and again after subject management or treatment. In these cases, the methods are used to monitor the status of sensitivity to an ALK inhibitor, MEK inhibitor, RAF inhibitor (e.g., BRAF inhibitor), RAS inhibitor, ERK inhibitor, EGFR inhibitor, or MET inhibitor (e.g., response to treatment with the inhibitors, resistance to the inhibitors, amelioration of the disease, or progression of the disease).

For example, polypeptides or polynucleotides of KEAP1 and/or NRF2 be used to monitor a subject's response to certain treatments of a disease (e.g., an ALK inhibitor, MEK inhibitor, RAF inhibitor (e.g., a BRAF inhibitor), RAS inhibitor, ERK inhibitor, RTK inhibitor (such as a MET or EGFR inhibitor) for treating an ALK-, BRAF-, EGFR-, NRAS-, or KRAS-mutant lung cancer). The level, copy number, biological activity, sequence, post-transcriptional modification of a polypeptide or polynucleotide of KEAP1 and/or NRF2 may be assayed before treatment, during treatment, or following the conclusion of a treatment regimen. In some embodiments, multiple assays (e.g., 2, 3, 4, and 5) are made at one or more of those times to assay resistance to an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor.

Alterations in polynucleotides or polypeptides of KEAP1 and/or NRF2 (e.g, sequence, copy number, level, post-transcriptional modification, biological activity) are detected in a biological sample obtained from a patient that has or has a propensity to develop a cancer, such as an ALK-, NRAS-, EGFR-, KRAS-, or BRAF-mutant lung cancer. Biological samples include tissue samples (e.g., cell samples, biopsy samples), such as lung tissue. Biological samples that are used to evaluate the herein disclosed markers include

without limitation tumor cells, blood, serum, plasma, urine. In one embodiment, the biological sample is blood.

The sequence, level, or copy number of a polypeptide or polynucleotide of KEAP1 and/or NRF2 detected in the method can be compared to a reference sequence. The reference sequence, level, or copy number may be a known sequence, level, or copy number of the gene in healthy normal controls. In some embodiments, the sequence of KEAP1 and/or NRF2 in the subject is determined at a time point later than the initial determination of the sequence, and the sequences are compared to monitor the efficacy of the therapy. In other embodiments, a pre-treatment sequence of a polypeptide or polynucleotide of KEAP1 and/or NRF2 in the subject is determined prior to beginning treatment according to this invention; this pre-treatment sequence of a polypeptide or polynucleotide of KEAP1 and/or NRF2 can then be compared to the sequence of the polypeptide or polynucleotide of KEAP1 and/or NRF2 in the subject after the treatment commences, to determine the efficacy of the treatment.

While the examples provided below describe specific methods of detecting levels of polynucleotides or polypeptides of the markers KEAP1 and NRF2, one of ordinary skill appreciates that the invention is not limited to such methods. The biomarkers of this invention can be detected or quantified by any suitable method. For example, methods include, but are not limited to real-time PCR, Southern blot, PCR, mass spectroscopy, and/or antibody binding. Methods for detecting a mutation or amplification of the invention include immunoassay, direct sequencing, and probe hybridization to a polynucleotide encoding the mutant polypeptide. In particular embodiments, a sequence and/or copy number of the markers is detected by DNA sequencing and/or copy number analysis.

Combination Therapies

Also provided herein are methods of increasing sensitivity to an ALK inhibitor, MEK inhibitor, RAF inhibitor (e.g., a BRAF inhibitor), RAS inhibitor, ERK inhibitor, EGFR inhibitor, or other RTK inhibitor (such as a MET inhibitor) in a subject having an ALK-, BRAF-, EGFR-, KRAS-, or NRAS- mutant lung cancer. The findings herein suggest that combination of a Ras/Raf/RTK inhibitor and a NRF2/KEAP1 therapeutic would benefit patients with alterations in the NRF2/KEAP1 pathway. Without being bound by theory, it is believed that administering a KEAP1 polypeptide or polynucleotide and/or a NRF2 inhibitor increases sensitivity to an ALK inhibitor, MEK inhibitor, RAF inhibitor (e.g., a BRAF inhibitor), RAS inhibitor, ERK inhibitor, EGFR inhibitor, or other RTK inhibitor (such as a

MET inhibitor), particularly in a subject having loss of KEAP1 and/or overexpression or amplification of NRF2.

Thus, in some embodiments, a therapeutic composition comprising an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor may be administered to a subject having an ALK-, BRAF-, EGFR-, KRAS-, or NRAS- mutant lung cancer, in combination with a composition comprising a KEAP1 polypeptide or polynucleotide and/or a NRF2 inhibitor. In particular embodiments, the subject is identified as resistant to an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor (e.g., the subject has an alteration in a level, copy number, sequence, or activity of a polynucleotide or polypeptide of KEAP1 and/or NRF2). A KEAP1 polynucleotide and/or NRF2 inhibitor (e.g., an inhibitory polynucleotide that reduces NRF2 expression or small molecule that reduces expression or activity of NRF2) is administered to a subject identified as resistant to an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor to increase sensitivity of the subject to any one of these inhibitors.

In some embodiments, an EGFR inhibitor is administered to a subject having an EGFR-mutant lung cancer in combination with a composition comprising a KEAP1 polypeptide or polynucleotide and/or a NRF2 inhibitor. In some other embodiments, a MEK inhibitor is administered to a subject having a MEK-mutant lung cancer in combination with a composition comprising a KEAP1 polypeptide or polynucleotide and/or a NRF2 inhibitor. In still other embodiments, a BRAF inhibitor is administered to a subject having a BRAF-mutant lung cancer in combination with a composition comprising a KEAP1 polypeptide or polynucleotide and/or a NRF2 inhibitor. In still other embodiments, an ALK inhibitor is administered to a subject having an ALK-mutant lung cancer in combination with a composition comprising a KEAP1 polypeptide or polynucleotide and/or a NRF2 inhibitor.

Results of studies described herein further indicate that a combination of trametinib or vemurafenib plus BSO/NRF2 inhibitor may be beneficial in patients with RAS/BRAF/EGFR mutations and intact KEAP1. Thus, in particular embodiments, a combination of buthionine sulfoximine (BSO) and/or a NRF inhibitor and an ALK inhibitor, MEK inhibitor, EGFR inhibitor, or BRAF inhibitor is administered to a subject having a RAS/BRAF/EGFR mutation and intact KEAP1. In some embodiments, the MEK inhibitor is trametinib. In some other embodiments, the BRAF inhibitor is vemurafenib. In some embodiments, the EGFR inhibitor is erlotinib, afatinib, or cetuximab. In some embodiments, the ALK inhibitor can be ASP-3026, alectinib (ALECENSA), brigatinib (AP26113), ceritinib (ZYKADIA), CEP-28122, CEP-37440, crizotinib (XALKORI), entrectinib (e.g., NMS-E628,

RXDX-101), PF-06463922, TSR-011, X-376 and X-396. In other embodiments, the therapeutic agents described herein (e.g., an ALK inhibitor, MEK inhibitor, RAF inhibitor (e.g., a BRAF inhibitor), RAS inhibitor, ERK inhibitor, RTK inhibitor (such as a MET or EGFR inhibitor), KEAP1 polynucleotide, NRF2 inhibitor (such as an inhibitory polynucleotide that reduces NRF2 expression), or any combination thereof) may be administered to a subject in further combination with standard therapies for cancer (particularly, lung cancer). Such standard therapies include, without limitation, surgery, radiation therapy, or administering chemotherapeutic agent(s) to the subject. Chemotherapeutic agents suitable for treating lung cancer (particularly, non small cell lung cancer) include, without limitation, gemcitabine, 5-fluorouracil, irinotecan, oxaliplatin, paclitaxel, capecitabine, cisplatin, and docetaxel.

Kits

The invention provides kits for treating an ALK-, BRAF-, EGFR-, NRAS-, or KRAS-mutant lung cancer in a subject and/or identifying resistance or sensitivity to an ALK inhibitor, MEK inhibitor, RAF inhibitor (e.g., a BRAF inhibitor), RAS inhibitor, ERK inhibitor, EGFR inhibitor, or other RTK inhibitor (such as a MET inhibitor) in a subject having an ALK-, BRAF-, EGFR-, NRAS-, or KRAS-mutant lung cancer. A kit of the invention provides a capture reagent (e.g., a primer or hybridization probe specifically binding to a KEAP1 or NRF2 polynucleotide) for measuring relative expression level, copy number, activity, and/or a sequence of a marker (e.g., KEAP 1 or NRF2). In other embodiments, the kit further includes reagents suitable for DNA sequencing or copy number analysis of KEAP1 and/or NRF2.

In one embodiment, the kit includes a diagnostic composition comprising a capture reagent detecting at least one marker selected from the group consisting of a KEAP1 polynucleotide or polypeptide and a NRF2 polynucleotide or polypeptide. In one embodiment, the capture reagent detecting a polynucleotide of KEAP 1 or NRF2 is a primer or hybridization probe that specifically binds to a KEAP 1 or NRF2 polynucleotide. In another embodiment, the kit further comprises a capture reagent detecting at least one gene selected from the group consisting of ALK, BRAF, EGFR, NRAS, or KRAS.

The kits may further comprise a therapeutic composition comprising an ALK inhibitor, MEK inhibitor, RAF inhibitor (e.g., a BRAF inhibitor), RAS inhibitor, ERK inhibitor, EGFR inhibitor, or other RTK inhibitor (such as a MET inhibitor). In some embodiments, the MEK inhibitor is trametinib, selumetinib, or MEK 162. In some other

embodiments, the BRAF inhibitor is vemurafenib or dabrafenib. In still other embodiments, the EGFR inhibitor is erlotinib, afatinib, or cetuximab. In some embodiments, the RAF inhibitor is RAF265, XL281/BMS -908662, or sorafenib. In some embodiments, the ALK inhibitor can be ASP-3026, alectinib (ALECENSA), brigatinib (AP26113), ceritinib (ZYKADIA), CEP-28122, CEP-37440, crizotinib (XALKORI), entrectinib (e.g., NMS-E628, RXDX-101), PF-06463922, TSR-011, X-376 and X-396.

The kits may also further comprise a therapeutic composition comprising a polynucleotide encoding a KEAP1 polypeptide and/or a NRF2 inhibitor (e.g., an inhibitory polynucleotide that reduces NRF2 expression). The kits may be in combination with a chemotherapeutic agent suitable for treating lung cancer. In certain embodiments, the kit includes a diagnostic composition (e.g., a capture reagent detecting a polynucleotide of ALK, KEAP1, NRF2, BRAF, EGFR, NRAS, or KRAS) and a therapeutic composition comprising an ALK inhibitor, MEK inhibitor, RAF inhibitor (e.g., a BRAF inhibitor), RAS inhibitor, ERK inhibitor, RTK inhibitor (e.g., EGFR inhibitor, MET inhibitor), a KEAP1 polynucleotide, a NRF2 inhibitor (e.g., an inhibitory polynucleotide that reduces NRF2 expression), other chemotherapeutic agent(s), or any combination thereof.

In some embodiments, the kit comprises a sterile container which contains a therapeutic composition; such containers can be boxes, ampoules, bottles, vials, tubes, bags, pouches, blister-packs, or other suitable container forms known in the art. Such containers can be made of plastic, glass, laminated paper, metal foil, or other materials suitable for holding medicaments.

If desired, the kit further comprises instructions for administering the therapeutic combinations of the invention. In particular embodiments, the instructions include at least one of the following: description of the therapeutic agent; dosage schedule and administration for enhancing anti-tumor activity; precautions; warnings; indications; counter-indications; over dosage information; adverse reactions; animal pharmacology; clinical studies; and/or references. The instructions may be printed directly on the container (when present), or as a label applied to the container, or as a separate sheet, pamphlet, card, or folder supplied in or with the container.

The practice of the present invention employs, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are well within the purview of one of ordinary skill in the art. Such techniques are explained fully in the literature, such as, “Molecular Cloning: A Laboratory Manual”, second edition (Sambrook, 1989);

"Oligonucleotide Synthesis" (Gait, 1984); "Animal Cell Culture" (Freshney, 1987); "Methods in Enzymology" "Handbook of Experimental Immunology" (Weir, 1996); "Gene Transfer Vectors for Mammalian Cells" (Miller and Calos, 1987); "Current Protocols in Molecular Biology" (Ausubel, 1987); "PCR: The Polymerase Chain Reaction", (Mullis, 1994); "Current Protocols in Immunology" (Coligan, 1991). These techniques are applicable to the production of the polynucleotides and polypeptides of the invention, and, as such, may be considered in making and practicing the invention. Particularly useful techniques for particular embodiments will be discussed in the sections that follow.

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the assay, screening, and therapeutic methods of the invention, and are not intended to limit the scope of what the inventors regard as their invention.

EXAMPLES

Example 1: Genome-scale CRISPR loss-of-function screens to identify mechanisms of resistance to BRAF and MEK inhibition

To identify mechanisms of resistance to ALK, MEK and BRAF inhibition in different contexts, four genome-scale CRISPR-Cas9 knockout screens were performed (FIG. 1A). Three screens with the MEK inhibitor trametinib in the NRAS-mutant lung cancer cell line H1299 ($NRAS^{Q61K}$), the BRAF-mutant lung cancer cell line HCC364 ($BRAF^{V600E}$), and the KRAS-mutant lung cancer cell line CALU1 ($KRAS^{G12C}$) were performed. One additional screen was performed in HCC364 cells treated with the BRAF inhibitor vemurafenib. The lowest concentration of drug that inhibited ERK phosphorylation and resulted in proliferative arrest or death was used (FIG. 5). To perform the genome-scale screens, the GeCKO v2 library (Shalem et al., *Science* 343, 84-87 (2014)) was introduced into Cas9-expressing cells, selected cells that incorporated the sgRNAs and allowed genome editing to occur over one week. Cells were then harvested for the Day 0 time point or passaged in the presence of trametinib or vemurafenib (FIG. 1A). Genomic DNA was isolated on days 14 and 21 and sgRNAs were counted by sequencing. sgRNAs that were enriched in the Day 14 and Day 21 samples compared to the Day 0 samples were then identified, using a cutoff of log2 fold change of at least 2 (Table 1). Several of the genes that scored in these screens also scored in a previous vemurafenib resistance screen in BRAF-mutant melanoma library (Shalem et al., *Science* 343, 84-87 (2014)).

The functions of each of the genes that scored in these screens were annotated to determine if particular functional categories scored repeatedly. As expected, several genes in the MAPK pathway scored, including NF1, a negative regulator of Ras/MAPK signaling, and DUSP1, a dual-specificity phosphatase that inhibits ERK. It was also found that several positive regulators of p38/JNK MAPK signaling, suggesting that these other MAPK pathways may play a pro-apoptotic or anti-proliferative role in these cells. PTEN, a negative regulator of PI3K/AKT signaling, and TSC1 and TSC2, negative regulators of mTOR signaling, also scored, suggesting that increased signaling through the PI3K/AKT/mTOR pathway compensates for loss of Ras/MAPK signaling. In addition to these expected pathways, several of the genes that scored are components of histone acetyltransferase (HAT) complexes or of the Mediator complex. There are also several genes whose products are components of E3 ubiquitin ligase complexes. Multiple transcription factors scored, as well as general transcription machinery genes. Other functional categories for which multiple genes scored include Rho signaling and histidine post-translational modifications (Table 1). It was noted that KEAP1, a substrate adaptor protein that targets NFE2L2/NRF2 for ubiquitination and proteasomal degradation, scored in all four screens (Table 1 and FIG. 1B). Experiments described herein below focused on KEAP1.

Table 1: Number of sgRNAs scoring in each screen

Gene	H1299 + Tram	CALU1 + Tram	HCC364 + Tram	HCC364 + Vem
KEAP1	2	4	5	3
CIC	3	5	3	
PPP4R2	3	2		
CCDC101		2		2
TAF5L		2		4
USP22		3		2
TADA1			2	3
TADA2B			2	4
MAPKAPK2	2			
INSM2	2			
MNT	3			
CDKN1B	4			
KAT6A		2		
MED23		2		
MED24		2		
MED10		2		
DUSP1		2		
ERF		2		
BTAF1		2		
CTDSPL2		2		
MLLT1		2		
TAF11		2		

CNOT4	2		
RHOA	2		
DPH2	2		
MYL6	2		
TIPRL	2		
MED12	3		
MED15	3		
ROCK2	3		
DNAJC24	3		
DPH1	3		
TSC1	3		
PTEN	3		
DAPK3	3		
RNF7	4		
TSC2	4		
PAWR	4		
GATA6	5		
ROCK1	5		
PDCD10		2	
IRF2		2	
TADA3			2
ATXN7			2
SUPT20H			2
NF1			3

Example 2: Loss of KEAP1 conferred resistance to ALK, MEK, EGFR or BRAF inhibition in lung cancer with NRAS, BRAF, or KRAS mutation

To validate KEAP1, HCC364 ($BRAF^{V600E}$) and CALU1 ($KRAS^{G12C}$) cells were infected with sgRNAs targeting KEAP1 or GFP (FIGS. 6A-6B). Cells were then seeded at low density in 24-well plates and treated with DMSO, trametinib, or vemurafenib. Cell viability was assessed by crystal violet staining (FIG. 1C). Deletion of KEAP1 ($KEAP1^{KO}$) conferred resistance to trametinib in both cell lines and to vemurafenib in HCC364 cells (FIG. 1C). Because EGFR mutation is common in lung cancer, the ability of KEAP1 loss to confer resistance to EGFR inhibition was also tested. $KEAP1^{KO}$ conferred resistance to erlotinib treatment in HCC827 ($EGFR^{\Delta 746\text{-}750}$) cells and to afatinib treatment in NCI-H1975 ($EGFR^{L858R/T790M}$) cells (FIG. 1D and FIG. 6C). It was also found that restoring wildtype KEAP1 expression in A549 cells, which are KRAS mutant and KEAP1-null, increased their sensitivity to trametinib. In contrast, expression of the $KEAP1^{G333C}$ mutant, which does not regulate NRF2, failed to alter trametinib sensitivity (FIG. 1E and FIG. 6D).

The ability of KEAP1 loss to confer resistance to anaplastic lymphoma kinase (ALK) inhibition was also tested. The loss of KEAP1 (sgKEAP1-1 and sgKEAP1-2) confers

resistance to ALK inhibition by 300 nM crizotinib in comparison to control (sgGFP) in ALK-mutant lung cancer (FIG. 1F).

Example 3: KEAP1^{KO} did not activate the MAPK pathway and conferred resistance via increased NRF2 levels.

Unlike other reported BRAF and MEK inhibitor resistance mechanisms (Rudin, *Journal of thoracic oncology* 8, e41-42, (2013); Wagle, et al. *Journal of clinical oncology* 29, 3085-3096, (2011); Corcoran, et al., *Cancer discovery* 2, 227-235 (2012); Johannessen et al., *Nature* 468, 968-972,(2010); Nazarian et al., *Nature* 468, 973-977, (2010); Prahallad et al., *Nature* 483, 100-103, (2012); Sun et al., *Cell reports* 7, 86-93, (2014)), it was found herein that KEAP1^{KO} did not restore ERK activation (FIG. 2A), indicating that KEAP1^{KO} does not confer resistance by reactivating the MAPK pathway. KEAP1 serves as a substrate adaptor protein that recruits the CUL3 ubiquitin ligase to NRF2, targeting it for proteasomal degradation. As expected, it was found that KEAP1^{KO} led to increased NRF2 protein levels (FIG. 2B) and that overexpression of wildtype NRF2 or NRF2^{G31R}, which contains a mutation in the KEAP1 binding domain, also conferred resistance to trametinib and vemurafenib (FIG. 2C and FIG. 6E), suggesting that elevated NRF2 levels in KEAP1^{KO} cells mediates resistance. Although CALU1 cells have a KEAP1^{P128L} mutation, this mutation has not been reported in cBioPortal or COSMIC (Cerami et al., *Cancer discovery* 2, 401-404, (2012); Gao et al., *Science signaling* 6, pl1, doi:10.1126/scisignal.2004088 (2013); Forbes, et al., *Nucleic acids research* 43, D805-811, (2015)) and NRF2 levels increased upon KEAP1 knockout (FIG. 6B), suggesting that the regulation of NRF2 by KEAP1 is intact in these cells. KEAP1^{KO} also conferred resistance to several chemotherapeutics (FIG. 7), as has been previously reported (Ohta, et al., *Cancer research* 68, 1303-1309, (2008); Shibata et al., *Gastroenterology* 135, 1358-1368, 1368 e1351-1354, (2008); Wang, *et al.*, *Carcinogenesis* 29, 1235-1243, (2008); Zhang, et al., *Molecular cancer therapeutics* 9, 336-346, (2010). However, previous work has shown mechanistic links between KEAP1/NRF2 and the MAPK pathway (DeNicola et al., *Nature* 475, 106-109, (2011); Sun et al., *PloS one* 4, e6588, doi:10.1371/journal.pone.0006588 (2009)).

Example 4: Trametinib treatment activated NRF2.

To further explore the mechanism by which KEAP1^{KO} confers resistance to trametinib, we investigated whether trametinib treatment affected the KEAP1/NRF2 signaling axis. Prior reports demonstrated that Ras/MAPK/Jun signaling increased expression

of NRF2 mRNA and NRF2 target genes (DeNicola et al., *Nature* 475, 106-109, (2011), so it was hypothesized that trametinib treatment would decrease expression of NRF2 mRNA and NRF2 target genes. Surprisingly, it was found that trametinib treatment increased rather than decreased expression of NRF2 mRNA and NRF2 target genes (FIG. 3A and FIGS. 8A-8B) in HCC364 and CALU1 cells. As expected, $KEAP1^{KO}$ also increased NRF2 target gene expression. Trametinib treatment also increased NRF2 protein levels and caused a shift in the migration of NRF2 protein on SDS-PAGE, whereas $KEAP1^{KO}$ maintained the higher molecular weight form of NRF2 (FIG. 3B and FIG. 8C).

The KEAP1/NRF2 axis responds to oxidative and electrophilic stress by scavenging reactive oxygen species (ROS), by regulating expression of drug efflux pumps, and by altering cell metabolism (Hayes et al., *Trends in biochemical sciences* 39, 199-218, (2014)). It was investigated whether each of these functions was involved in resistance to trametinib treatment. Since MAPK pathway inhibition was maintained in $KEAP1^{KO}$ cells (FIG. 2A), drug efflux likely does not explain resistance.

Example 5: Trametinib induced ROS.

It was found that trametinib treatment induced ROS in KEAP1-intact cells (FIG. 4A) but did not affect glutathione levels (FIG. 9A) or the NADPH/NADP+ ratio (FIG. 9B). Trametinib-induced ROS was dramatically decreased in $KEAP1^{KO}$ cells or NRF2 overexpressing cells (FIG. 4A and FIG. 9C), suggesting that $KEAP1^{KO}$ may confer resistance by reducing ROS levels. Reducing ROS with N-acetyl cysteine (NAC) in KEAP1-intact cells treated with trametinib conferred resistance (FIG. 4B and FIG. 9D), suggesting that ROS reduction by $KEAP1^{KO}$ is important for resistance. To investigate whether ROS reduction was important for resistance, cells were treated with trametinib and buthionine sulfoximine (BSO), which induces ROS. The combination of BSO and trametinib greatly decreased viability in control cells expressing sgGFP, while $KEAP1^{KO}$ prevented the BSO-induced decrease in viability (FIG. 4C and FIGS. 9E-9G). Furthermore, combined treatment with BSO and trametinib dramatically increased ROS levels in A549 cells in which wildtype KEAP1 expression had been restored, but not in the parental cells or cells expressing $KEAP1^{G333C}$ (FIG. 9H). Together these observations indicate that trametinib treatment induces ROS, which activates NRF2 to levels that are not sufficient for resistance. Loss of KEAP1 led to further activation of NRF2, which conferred resistance in part by reducing ROS.

In addition to regulating ROS, NRF2 has been reported to regulate the expression of metabolic genes (DeNicola et al. *Nature genetics*, (2015); Mitsuishi et al., *Cancer cell* **22**, 66-79, (2012)). It was found that trametinib induced expression of genes involved in the pentose phosphate pathway, de novo nucleotide synthesis, and NADPH synthesis. $KEAP1^{KO}$ also increased expression of some of these genes, similar to what was seen with other NRF2 targets (FIG. 4D and FIG. 10A). In contrast, expression of genes involved in serine biosynthesis decreased upon trametinib treatment, and $KEAP1^{KO}$ maintained higher expression (FIG. 4E and FIG. 10B). Together these results support a model in which trametinib treatment inhibits MAPK signaling and induces ROS, which activates NRF2 to low levels. KEAP1 loss increases NRF2 activity, which reduces ROS and alters cell metabolism, allowing cells to proliferate in the absence of MAPK signaling (FIG. 11).

The results herein were obtained by the following materials and methods.

Cell lines and reagents

Cells were maintained in RPMI-1640 (NCI-H1299, HCC364, NCI-H1975, and HCC827; Corning) or McCoy's 5A (CALU1; Gibco) supplemented with 2 mM glutamine, 50 U/mL penicillin, 50 U/mL of streptomycin (Gibco), and 10% fetal bovine serum (Sigma), and incubated at 37°C in 5% CO_2. Trametinib, vemurafenib, erlotinib, afatinib, cisplatin, 5-FU, etoposide, and paclitaxel were purchased from Selleck Chemicals.

Screen Optimization

Blasticidin and puromycin concentrations were optimized for each cell line by treating with different concentrations of drug for 3 days (puromycin) or 7 days (blasticidin). The lowest concentration of drug that killed all cells was used in the screens.

To produce Cas9-expressing cell lines, 200,000-400,000 cells were seeded in one well of a 6-well plate. The following day, cells were infected with 3 mL of pLX311-Cas9 virus with a final concentration of 4 μg/mL polybrene. Cells were spun for 2 hrs at 2000 rpm at 30 degrees. 24 hours after infection, cells were selected with blasticidin for 7 days.

To determine Cas9 activity, parental cell lines and Cas9-expressing cell lines were infected with pXPR_011, a Cas9 activity reporter which expresses eGFP as well as a guide RNA targeting eGFP (Doench et al., *Nature biotechnology* 32, 1262-1267, (2014)). 200,000-400,000 cells were seeded in six wells of a 6-well plate and were infected with 25-100 μL virus with a final concentration of 4 μg/mL polybrene. Cells were spun 2 hrs at 2000 rpm at 30 degrees. 24 hours after infection, each well was split into 2 wells, one of which was

selected with puromycin. After 2-3 days of puromycin selection, cells were counted and those with 30-40% infection efficiency were kept for the Cas9 activity assay. After 7 days of puromycin selection, cells were analyzed on an LSRII flow cytometer to determine the amount of GFP-positive cells. Parental cells not expressing Cas9 or pXPR_011 were used as a negative control. Cells expressing pXPR_011 but not Cas9 were used as a positive control.

To optimize inhibitor concentrations, Cas9-expressing cells were infected with different amounts of empty T virus (to mimic sgRNA infection) and were selected with puromycin. After 3 days of puromycin selection, cells were counted and those with 30-40% infection efficiency were used to optimize inhibitor concentration. Cells were kept in puromycin selection for one week prior to optimizing inhibitor concentration.

To determine the optimal drug concentration for the screens, cells expressing Cas9 and empty T virus were treated with different concentrations of drug for 3 weeks. Cells were passaged or fresh drug-containing media was added every 3-4 days. Cells were counted at each passage. The lowest concentration of drug that resulted in death or proliferative arrest was used in the screen (FIG. 5). In parallel, cells were treated with different concentrations of inhibitor for 24 hours and then lysed in RIPA buffer. Immunoblots were performed with total and phospho-ERK antibodies to determine the concentration of inhibitor that blocked ERK phosphorylation.

To titer the GeCKO v2 library in Cas9-expressing cells, $3x10^6$ cells were seeded per well in a 12-well plate and were infected with different amounts of virus (0, 50, 100, 150, 200, 400 μL), with a final concentration of 4 ug/mL polybrene. Cells were spun for 2 hrs at 2000 rpm at 30 degrees. Approximately 6 hours after infection, cells were split into 6-well plates. For each amount of virus, 100,000 cells per well were plated in two wells. 24 hours after infection, one well was treated with puromycin and one with media alone. After 2-3 days of selection, cells were counted to determine the amount of virus that resulted in 30-40% infection efficiency, and this amount of virus was used in the screen.

GeCKO v2 library construction

See Sanjana *et al. Nature methods* 11, 783-784, doi:10.1038/nmeth.3047 (2014).

Genome-scale CRISPR knockout drug resistance screens with GeCKO v2 library

For each screen, two infection replicates were performed. 150 x 10^6 cells were infected per replicate with 40% infection efficiency, in order to obtain 500 cells per sgRNA after selection (60 x 10^6 surviving cells containing 120,000 sgRNAs). 3x10^6 cells per well

were seeded in 12-well plates and were infected with the amount of virus determined during optimization, with a final polybrene concentration of 4 μg/mL. Plates were spun for 2 hrs at 2000 rpm at 30 degrees. Approximately 6 hours after infection, all wells within a replicate were pooled and were split into T225 flasks. 24 hours after infection, cells were selected in puromycin for 1 week and were passaged as necessary. After one week of puromycin selection, 60 x 10^6 cells were harvested for the Day 0 time point, and 60 x 10^6 cells were treated with drug. HCC364 cells were treated with 24 nM trametinib or 6.25 μM vemurafenib; H1299 cells were treated with 1.5 μM trametinib; and CALU1 cells were treated with 50 nM trametinib. Cells were passaged or fresh drug-containing media was added every 3-4 days. Drug-treated cells were harvested on Day 14 and Day 21 of drug treatment. To harvest cells, cells were trypsinized, spun down, washed with PBS, and the cell pellets were frozen at -80 degrees.

Genomic DNA was extracted using the Qiagen Blood and Cell Culture DNA Maxi Kit according to the manufacturer's protocol.

Vectors

Cas9 in the pLX311 backbone (pXPR_BRD111) and sgRNAs in the pXPR_BRD003 backbone were obtained from the Genetic Perturbation Platform at the Broad Institute.

sgKEAP1 arrayed infection

500,000 cells per well were seeded in 48-well plates in 250 μL media with 4 μg/mL polybrene. 25 μL virus (sgKEAP1 or sgEGFP) was added per well and plates were spun 2 hrs at 2000 rpm at 30 degrees C. 6 hours later, each well was split into a 6cm dish. 24 hours after infection, cells were selected with puromycin for one week.

Crystal Violet assays

2,000-10,000 cells were seeded in 12-well or 24-well plates in the indicated drug conditions. Media containing fresh drug was replaced every 3-4 days. After the indicated number of days, cells were washed in PBS, fixed in 10% formalin for 15 minutes, and stained with 0.1% crystal violet in 10% ethanol for 20 minutes. After acquiring images, crystal violet was extracted in 10% acetic acid for 20 minutes. The absorbance at 565 nm was determined using a Spectramax plate reader.

qRT-PCR

RNA was harvested using a Qiagen RNeasy Kit and was reverse transcribed into cDNA using SuperScriptIII according to the manufacturer's recommendations.

Cytoplasmic/nuclear fractionation

5x10^5 cells were seeded in 10 cm dishes. The following day, cells were treated with trametinib (25 nM for HCC364 or 50 nM for CALU1) or DMSO. After 72 hours of drug treatment, cells were lysed and fractionated using NE-PER Nuclear and Cytoplasmic Extraction Reagents (Pierce Biotechnology) according to the manufacturer's recommendations.

Immunoblotting

Cells were lysed in RIPA buffer containing protease and phosphatase inhibitors and were cleared by centrifugation. Protein was quantified using the Pierce BCA assay, and lysate concentrations were normalized. Lysates were run on SDS-PAGE gels and were transferred to nitrocellulose membranes using the Invitrogen iBlot system. Membranes were blocked for one hour in 5% milk in Tris-buffered saline with 0.1% Tween (TBS-T). Membranes were incubated overnight at 4 degrees C with primary antibodies in 5% BSA in TBS-T. Membranes were washed three times in TBST-T then incubated 1 hour at room temperature with secondary antibodies in 5% BSA in TBS-T. Membranes were washed in TBS-T and imaged on a Li-Cor Odyssey Infrared Imaging System. Primary antibodies were total ERK (Cell Signaling #9102), phospho-ERK (Cell Signaling #4370), total AKT (Cell Signaling #9272), phospho-AKT (Cell Signaling #4060), GAPDH (Cell Signaling #5174), LAMIN A/C (Cell Signaling #4777), KEAP1 (Proteintech 10503-2-AP), and NRF2 (Santa Cruz Biotechnology sc-13032).

ORF expression

293T cells were seeded in DMEM + 10% FBS + 0.1% Pen/Strep in 6 cm dishes. 24 hours later, cells were transfected with 100 ng VSVG, 900 ng delta8.9, and 1 µg pLX317-ORF plasmid using OptiMEM and Mirus TransIT. 16 hours after transfection, media was changed to DMEM + 30% FBS + 1% Pen/Strep. Virus was harvested 24 hours later. Cell lines were seeded in 6-well plates and were infected the following day with 1:5 dilution of virus containing 4 µg/mL polybrene. 24 hours after infection, cells were selected with puromycin.

DCFDA assays to measure ROS

Unless otherwise indicated, cells were treated with drug for 3 days. Cells were trypsinized and resuspended in media with 10 μM DCFDA (Sigma D6883) and incubated at 37°C for 90 minutes in the dark. For a positive control, parental cells were treated with 20 μM tert-butyl hydroperoxide (Sigma Aldrich 458139) during incubation. For a negative control, parental cells were incubated in media without DCFDA. DCFDA fluorescence was detected by flow cytometry, using the FITC channel on an LSRII flow cytometer (BD Biosciences).

GSH/GSSG assays

Cells were seeded into 96-well white-walled opaque-bottom plates (Costar 3917; 5000 cells in 100 μL media per well) and allowed to adhere overnight. The following day, cells were treated with 50 μL of media containing DMSO or drug (3x desired final concentration). At the indicated amount of time after treatment, the ratio of reduced and total glutathione was determined using the GSH/GSSG-Glo Assay (Promega V6612) according to the manufacturer's protocol for adherent mammalian cells. A GSH standard curve was included to confirm that experimental readouts were within the linear range of assay detection.

NADPH/NADP+ assays

5000 cells were seeded into 96-well white-walled opaque-bottom plates in 100 μL media per well and allowed to adhere overnight. The following day, cells were treated with 25 μL of media containing 4X trametinib or DMSO. 72 hours later, NADPH and NADP+ levels were determined using the NADP/NADPH-Glo Assay (Promega G9082) according to the manufacturer's protocol for measuring NADPH and NADP+ individually.

Primers and sgRNA sequences

The sequences of primers used in the experiments described herein are provided in Table 2. The sequences of sgRNA used in the experiments described herein are provided in Table 3.

Table 2: Primer Sequences

Gene	Forward Primer	Reverse Primer
NFE2L2	TCCAGTCAGAAACCAGTGGAT	GAATGTCTGCGCCAAAAGCTG
GCLC	GTGTTTCCTGGACTGATCCCA	TCCCTCATCCATCTGGCAAC
GCLM	CATTTACAGCCTTACTGGGAGG	ATGCAGTCAAATCTGGTGGCA
HO1	CTTTCAGAAGGGCCAGGTGA	GTAGACAGGGGCGAAGACTG
NQO1	CTCACCGAGAGCCTAGTTCC	CGTCCTCTCTGAGTGAGCCA
MRP1	CTCTATCTCTCCCGACATGACC	AGCAGACGATCCACAGCAAAA
TKT	GCTGAACCTGAGGAAGATCA	TGTCGAAGTATTTGCCGGTG
TALDO1	GTCATCAACCTGGGAAGGAA	CAACAAATGGGGAGATGAGG
PGD	ATATAGGGACACCACAAGACGG	GCATGAGCGATGGGCCATA
MTHFD2	TGTCCTCAACAAAACCAGGG	TTCCTCTGAAATTGAAGCTGG
ME1	CTGCCTGTCATTCTGGATGT	ACCTCTTACTCTTCTCTGCC
IDH1	CACTACCGCATGTACCAGAAAGG	TCTGGTCCAGGCAAAAATGG
G6PD	TGACCTGGCCAAGAAGAAGA	CAAAGAAGTCCTCCAGCTTG
PHGDH	ATCTCTCACGGGGGTTGTG	AGGCTCGCATCAGTGTCC
SHMT1	TGAACACTGCCATGTGGTGACC	TCTTTGCCAGTCTTGGGATCC
SHMT2	GCCTCATTGACTACAACCAGCTG	ATGTCTGCCAGCAGGTGTGCTT
ACTIN	CAACCGCGAGAAGATGACC	ATCACGATGCCAGTGGTACG

Table 3: sgRNA Sequences

Name	Target Sequence
sgGFP	GGCGAGGGCGATGCCACCTA
sgKEAP1-1	CTTGTGGGCCATGAACTGGG
sgKEAP1-2	TGTGTCCTCCACGTCATGAA
sgLACZ-1	AACGGCGGATTGACCGTAAT
sgLACZ-2	CTAACGCCTGGGTCGAACGC

Other Embodiments

From the foregoing description, it will be apparent that variations and modifications may be made to the invention described herein to adopt it to various usages and conditions. Such embodiments are also within the scope of the following claims.

The recitation of a listing of elements in any definition of a variable herein includes definitions of that variable as any single element or combination (or subcombination) of listed elements. The recitation of an embodiment herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

All patents and publications mentioned in this specification are herein incorporated by reference to the same extent as if each independent patent and publication was specifically and individually indicated to be incorporated by reference.

What is claimed is:

1. A method of treating a selected subject having lung cancer, the method comprising increasing KEAP1 level or activity or decreasing activity of a MAP kinase pathway in the subject, wherein the subject is selected by (i) detecting a mutation in a MAP kinase pathway protein and resistance to an inhibitor of MAP kinase pathway signaling and (ii) detecting decreased KEAP1 levels and/or increased activity of NRF2 in a biological sample of the subject relative to a reference sequence or level.

2. A method of treating a subject having lung cancer, the method comprising

(a) characterizing the lung cancer by detecting in a biological sample of the subject (i) a mutation in a MAP kinase pathway protein and resistance to an inhibitor of MAP kinase pathway signaling and (ii) detecting decreased KEAP1 levels and/or increased activity of NRF2 in a biological sample of the subject relative to a reference sequence or level; and

(b) increasing KEAP1 levels or activity or decreasing activity of a MAP kinase pathway in the subject.

3. The method of claim 1 or 2, wherein the activity of the MAP kinase pathway is decreased by administering to the subject an effective amount of a MAP kinase pathway inhibitor.

4. The method of claim 3, wherein the MAP kinase pathway inhibitor is an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor.

5. The method of claim 4, wherein the MEK inhibitor is trametinib, selumetinib, or MEK 162; the BRAF inhibitor is vemurafenib or dabrafenib; the EGFR inhibitor is erlotinib, afatinib, or cetuximab; and the ALK inhibitor is ASP-3026, alectinib, brigatinib, ceritinib, CEP-28122, CEP-37440, crizotinib, entrectinib, PF-06463922, TSR-011, X-376, or X-396.

6. A method of treating a subject having an ALK-, BRAF-, EGFR-, NRAS-, or KRAS-mutant lung cancer, the method comprising:

(a) detecting a wild-type KEAP1 polynucleotide, or detecting wild-type copy number or wild-type level of NRF2 polynucleotide in a biological sample of the subject relative to a reference sequence or level; and

(b) administering to the subject an effective amount of an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor.

7. A method of treating a subject having an ALK-, BRAF-, EGFR-, NRAS-, or KRAS-mutant lung cancer, the method comprising administering to a selected subject an effective amount of an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor, wherein the subject is selected by detecting a wild-type KEAP1 polynucleotide, or detecting wild-type copy number or wild-type level of NRF2 polynucleotide in a biological sample of the subject relative to a reference sequence or level.

8. A method for typing lung cancer in a subject as sensitive or resistant to an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor, the method comprising:

detecting a level or sequence of KEAP1 polynucleotide or a level or copy number of NRF2 polynucleotide in a biological sample obtained from a subject characterized as having an ALK-, BRAF-, EGFR-, NRAS-, or KRAS-mutant lung cancer relative to a reference level or sequence,

wherein the cancer is typed as resistant to an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor if a decrease in the level of or a mutation in KEAP1 polynucleotide or an increase in level or copy number of NRF2 polynucleotide is detected.

9. A method of identifying a subject with lung cancer that would benefit from treatment with an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor, the method comprising:

detecting a level or sequence of KEAP1 polynucleotide or a level or copy number of NRF2 polynucleotide in a biological sample obtained from a subject characterized as having an ALK-, BRAF-, EGFR-, NRAS-, or KRAS-mutant relative to a reference level or sequence,

wherein the subject is identified as a subject that would benefit from treatment with a an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor if a mutation in KEAP1 polynucleotide or an increase in copy number or level of NRF2 polynucleotide is not detected.

10. The method of any one of claims 8-9, further comprising the step of administering an effective amount of an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor to

the subject if a mutation in KEAP1 polynucleotide or an increase in level or copy number of NRF2 polynucleotide is not detected.

11. A method of monitoring effectiveness of lung cancer treatment in a subject, the method comprising:

(a) administering to the subject an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor; and

(b) detecting a level or sequence of KEAP1 or NRF2 polynucleotide in a biological sample obtained from the subject relative to a reference level or sequence,

wherein detection of a mutation in the sequence of a KEAP1 polynucleotide or an increase in copy number or level of NRF2 polynucleotide indicates the lung cancer is resistant to an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor.

12. A method of increasing sensitivity of a subject having an ALK-, BRAF-, NRAS-, or KRAS-mutant lung cancer to an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor, the method comprising administering to the subject an effective amount of a KEAP1 polynucleotide or a NRF2 inhibitor and an effective amount of an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor, thereby increasing sensitivity of the subject to the inhibitor.

13. A method of treating a subject having an ALK-, BRAF-, EGFR-, NRAS-, or KRAS-mutant lung cancer, the method comprising administering to a subject an effective amount of a KEAP1 polynucleotide or a NRF2 inhibitor and an effective amount of an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor.

14. The method of claim 13, wherein an effective amount of KEAP1 polynucleotide, a NRF2 inhibitor and an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor is administered.

15. The method of any one of claims 6-14, wherein the MEK inhibitor is trametinib, selumetinib, or MEK 162.

16. The method of any one of claims 6-14, wherein the BRAF inhibitor is vemurafenib or dabrafenib.

17. The method of any one of claims 6-14, wherein the EGFR inhibitor is erlotinib, afatinib, or cetuximab.

18. The method of any one of claims 6-14, wherein the ALK inhibitor is ASP-3026, alectinib, brigatinib, ceritinib, CEP-28122, CEP-37440, crizotinib, entrectinib, PF-06463922, TSR-011, X-376, or X-396.

19. The method of any one of claims 6-14, wherein the NRF2 inhibitor is an inhibitory polynucleotide that reduces expression of NRF2, retinoic acid, 6-hydroxy-1-methylindole-3-acetonitrile (6-HMA), luteolin, bleomycin, brusatol, or AEM1.

20. The method of any one of claims 1-15, wherein the subject is identified as having a decrease in KEAP1 polynucleotide, or a mutation in KEAP1 polynucleotide in a biological sample of the subject relative to a reference sequence or level.

21. The method of any one of claims 1-20, wherein the subject is identified as having an increase in copy number or level of NRF2 polynucleotide in a biological sample of the subject relative to a reference sequence or level.

22. The method of any one of claims 1-21, wherein the mutation in KEAP1 polynucleotide is a loss-of-function mutation.

23. The method of any one of claims 1-21, wherein the mutation in KEAP1 polynucleotide or the increase in copy number of level NRF2 polynucleotide does not re-activate a MAPK pathway.

24. The method of any one of claims 1-21, wherein the biological sample is blood.

25. The method of any one of claims 1-21, wherein the subject is human.

26. A therapeutic composition for increasing sensitivity of a subject having an ALK-, BRAF-, NRAS-, or KRAS-mutant lung cancer to an ALK inhibitor, MEK inhibitor, BRAF

inhibitor, or EGFR inhibitor, the composition comprising a KEAP1 polynucleotide in a pharmaceutically acceptable carrier.

27. The therapeutic composition of claim 26, further comprising a NRF2 inhibitor.

28. The therapeutic composition of claim 25 or 26, further comprising an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor.

29. The therapeutic composition of claim 28, wherein the MEK inhibitor is trametinib, selumetinib, or MEK 162.

30. The therapeutic composition of claim 28, wherein the BRAF inhibitor is vemurafenib or dabrafenib.

31. The therapeutic composition of claim 28, wherein the EGFR inhibitor is erlotinib, afatinib, or cetuximab.

32. The therapeutic composition of claim 28, wherein the ALK inhibitor is ASP-3026, alectinib, brigatinib, ceritinib, CEP-28122, CEP-37440, crizotinib, entrectinib, PF-06463922, TSR-011, X-376, or X-396.

33. The therapeutic composition of claim 23, wherein the NRF2 inhibitor is an inhibitory polynucleotide that reduces expression of NRF2, retinoic acid, 6-hydroxy-1-methylindole-3-acetonitrile (6-HMA), luteolin, bleomycin, brusatol, or AEM1.

34. A kit for typing lung cancer, the kit comprising a capture reagent that specifically binds to a KEAP1 polynucleotide and a capture reagent that specifically binds a polynucleotide selected from the group consisting of ALK, BRAF, EGFR, NRAS, and KRAS.

35. A kit for treating an ALK-, BRAF-, EGFR-, NRAS-, or KRAS-ALK-, BRAF-, EGFR-, NRAS-, or KRAS-mutant lung cancer, the kit comprising a capture reagent that specifically binds to a KEAP1 polynucleotide and an ALK inhibitor, MEK inhibitor, BRAF inhibitor, or EGFR inhibitor.

36. The kit of claim 34 or 35, further comprising a capture reagent that specifically binds to a NRF2 polynucleotide.

37. The kit of any one of claims 34-36, wherein the capture reagent is a primer or hybridization probe that specifically binds to a KEAP1 polynucleotide.

38. The kit of claim 34 or 35, wherein the capture reagent is a primer or hybridization probe that specifically binds to a NRF2 polynucleotide.

39. The kit of any one of claims 34-38, wherein the MEK inhibitor is trametinib, selumetinib, or MEK 162.

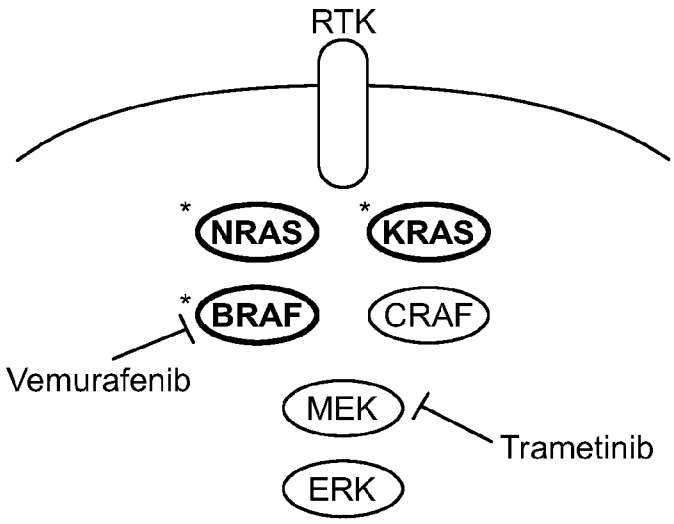
40. The kit of any one of claims 34-38, wherein the BRAF inhibitor is vemurafenib or dabrafenib.

41. The kit of any one of claims 34-38, wherein the EGFR inhibitor is erlotinib, afatinib, or cetuximab.

42. The kit of any one of claims 34-38, wherein the ALK inhibitor is ASP-3026, alectinib, brigatinib, ceritinib, CEP-28122, CEP-37440, crizotinib, entrectinib, PF-06463922, TSR-011, X-376, or X-396.

43. The kit of any one of claims 34-38, wherein the capture reagent detects a mutation in a KEAP1 polynucleotide.

FIG. 1A



Day -18
Infect cells with Cas9
Days -17 to -10
Select with blasticidin
Day -9
Withdraw blasticidin
Day -8
Infect cells with GeCKOlibrary
Days -7 to -1
Select with puromycin
Day 0
Harvest Day 0
Days 1 to 21
Treat with drug &
harvest on Days 14 and 21

NCI-H1299 (NRAS Q61K) + Trametinib
CALU1 (KRAS G12C) + Trametinib
HCC364 (BRAF V600E) + Trametinib or Vemurafenib

FIG. 1B

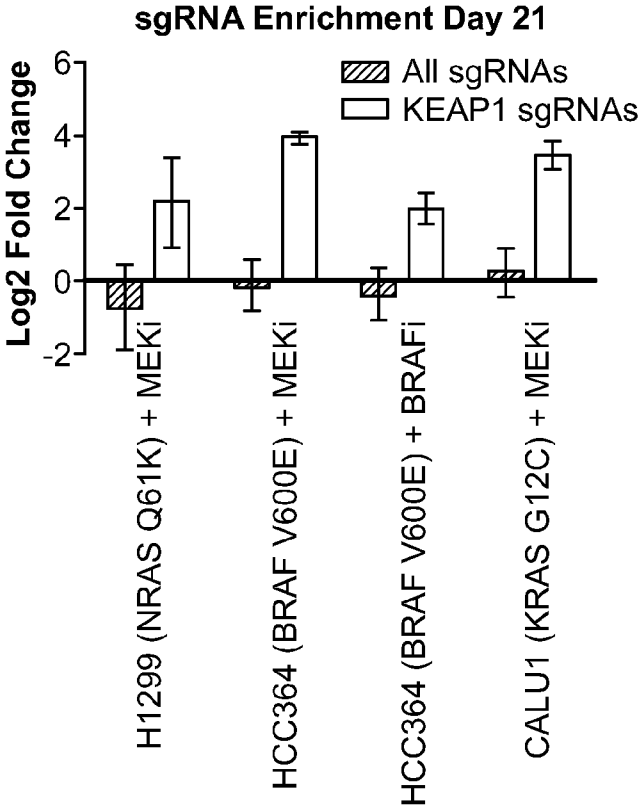


FIG. 1C

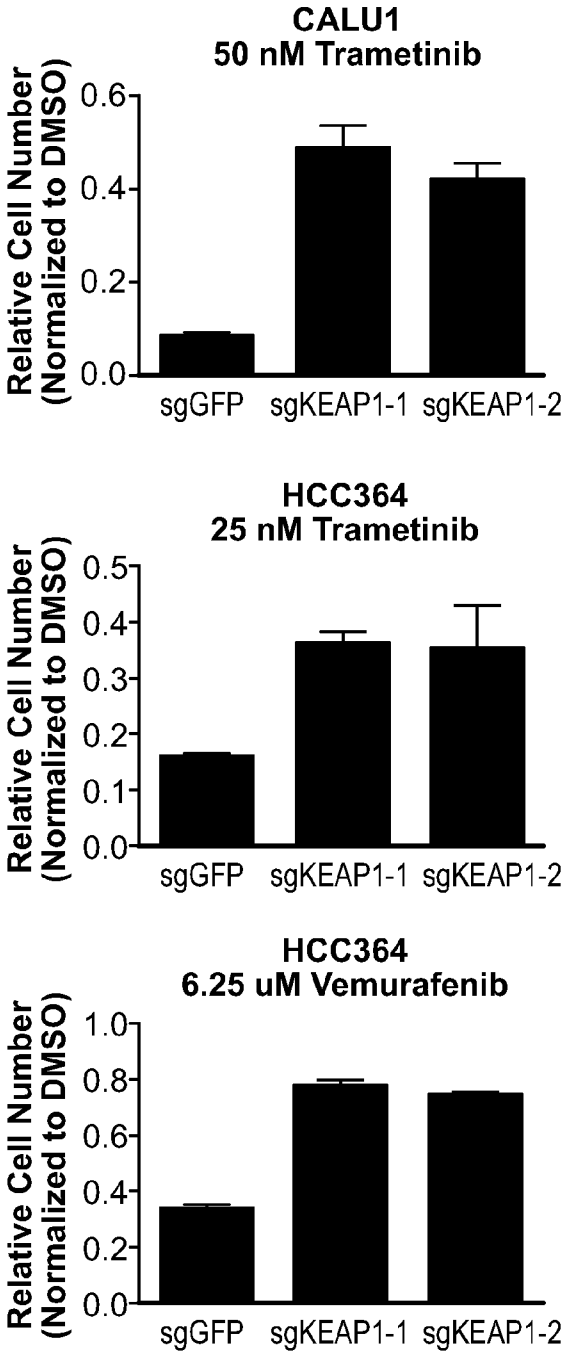

CALU1
50 nM Trametinib
Relative Cell Number
(Normalized to DMSO)
0.6
0.4
0.2
0.0
sgGFP
sgKEAP1-1
sgKEAP1-2
HCC364
25 nM Trametinib
Relative Cell Number
(Normalized to DMSO)
0.5
0.4
0.3
0.2
0.1
0.0
sgGFP
sgKEAP1-1
sgKEAP1-2
HCC364
6.25 uM Vemurafenib
Relative Cell Number
(Normalized to DMSO)
1.0
0.8
0.6
0.4
0.2
0.0
sgGFP
sgKEAP1-1
sgKEAP1-2

FIG. 1D

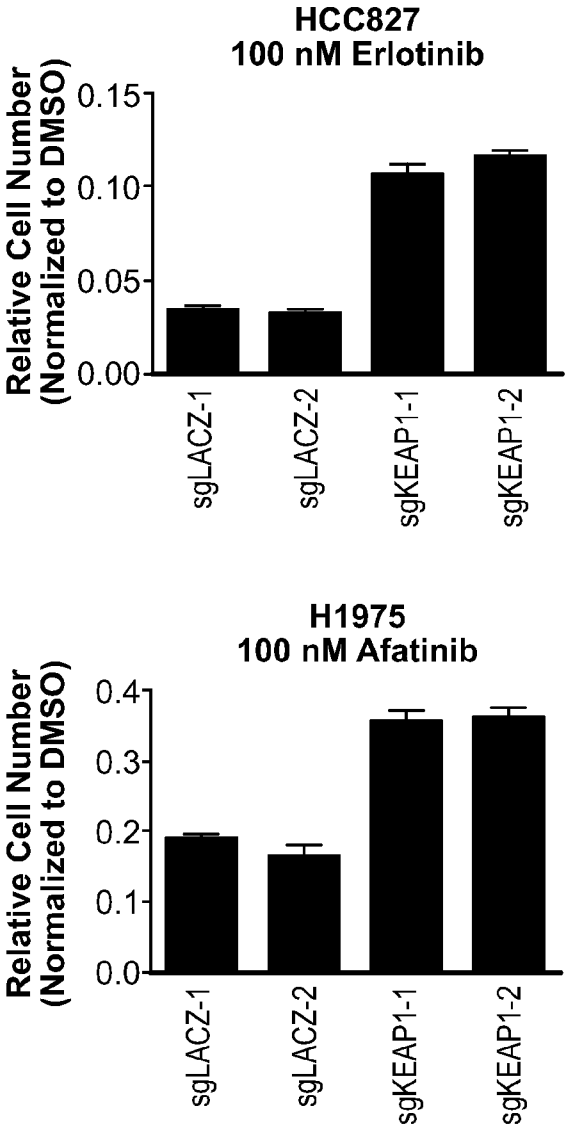

HCC827
100 nM Erlotinib
Relative Cell Number
(Normalized to DMSO)
0.15
0.10
0.05
0.00
sgLACZ-1
sgLACZ-2
sgKEAP1-1
sgKEAP1-2
H1975
100 nM Afatinib
Relative Cell Number
(Normalized to DMSO)
0.4
0.3
0.2
0.1
0.0
sgLACZ-1
sgLACZ-2
sgKEAP1-1
sgKEAP1-2

FIG. 1E

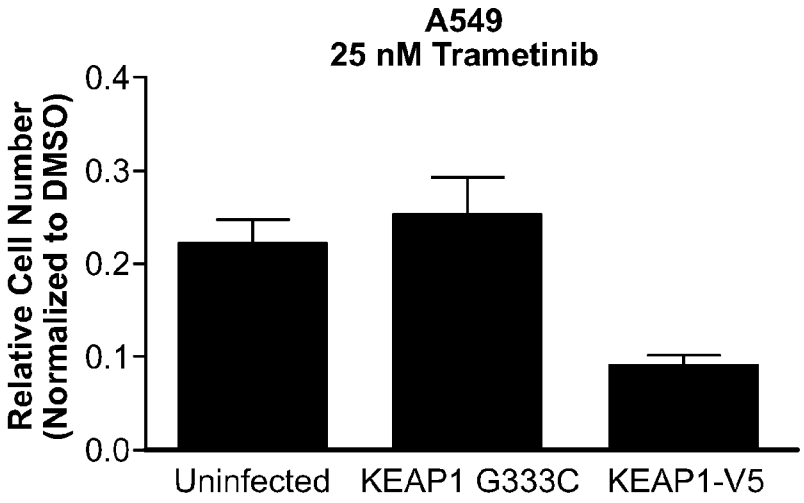

A549
25 nM Trametinib
Relative Cell Number
(Normalized to DMSO)
0.4
0.3
0.2
0.1
0.0
Uninfected
KEAP1 G333C
KEAP1-V5

FIG. 1F

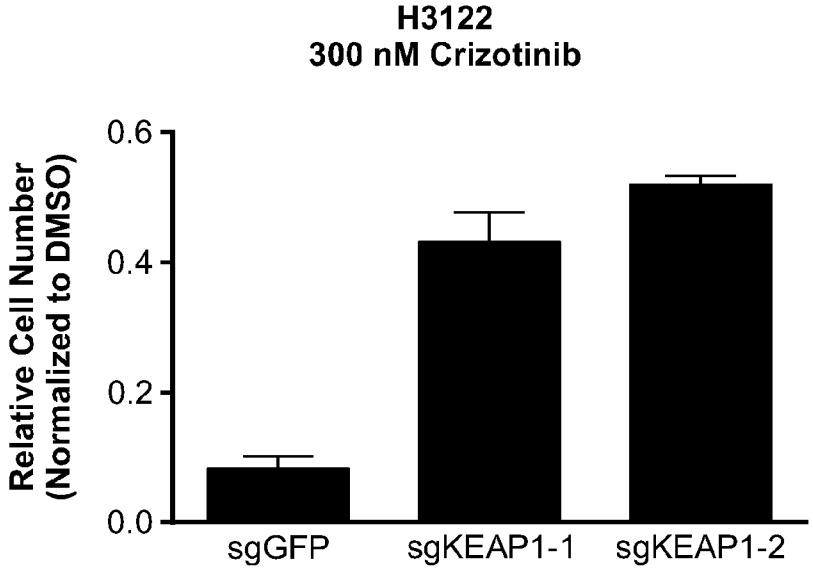

H3122
300 nM Crizotinib
Relative Cell Number
(Normalized to DMSO)
0.6
0.4
0.2
0.0
sgGFP
sgKEAP1-1
sgKEAP1-2

FIG. 2A

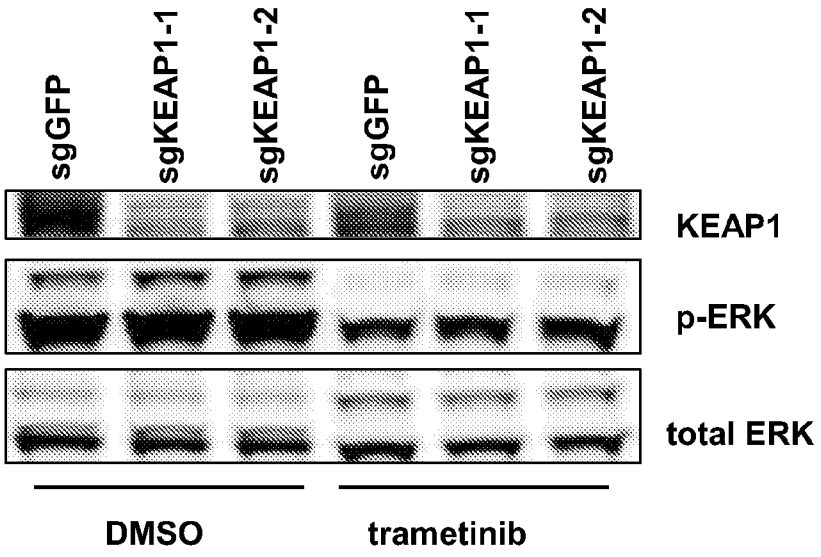

sgGFP
sgKEAP1-1
sgKEAP1-2
sgGFP
sgKEAP1-1
sgKEAP1-2
KEAP1
p-ERK
total ERK
DMSO
trametinib

FIG. 2B

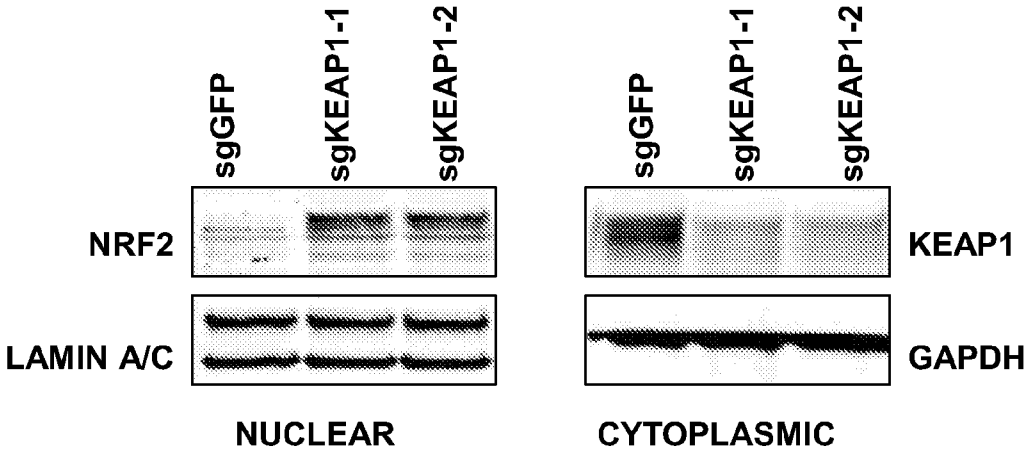

sgGFP
sgKEAP1-1
sgKEAP1-2
sgGFP
sgKEAP1-1
sgKEAP1-2
NRF2
LAMIN A/C
KEAP1
GAPDH
NUCLEAR
CYTOPLASMIC

FIG. 2C

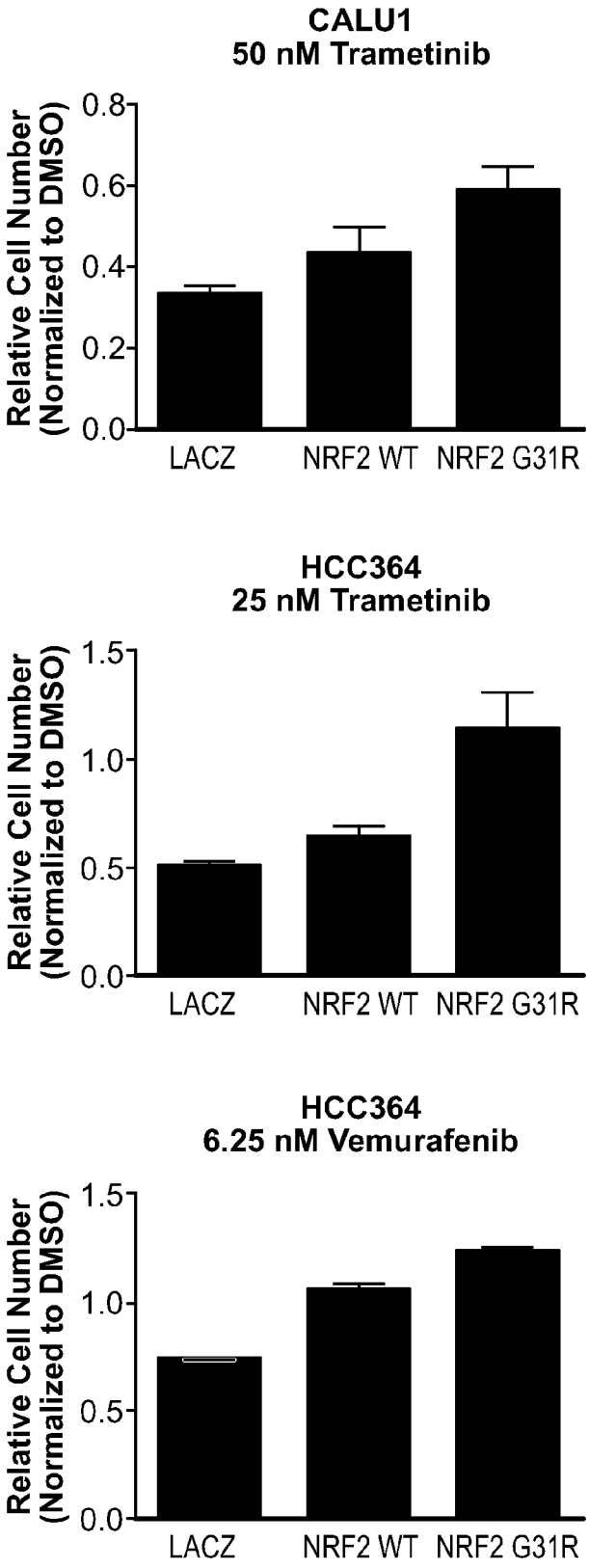

CALU1
50 nM Trametinib
Relative Cell Number
(Normalized to DMSO)
0.8
0.6
0.4
0.2
0.0
LACZ
NRF2 WT
NRF2 G31R
HCC364
25 nM Trametinib
Relative Cell Number
(Normalized to DMSO)
1.5
1.0
0.5
0.0
LACZ
NRF2 WT
NRF2 G31R
HCC364
6.25 nM Vemurafenib
Relative Cell Number
(Normalized to DMSO)
1.5
1.0
0.5
0.0
LACZ
NRF2 WT
NRF2 G31R

FIG. 3A

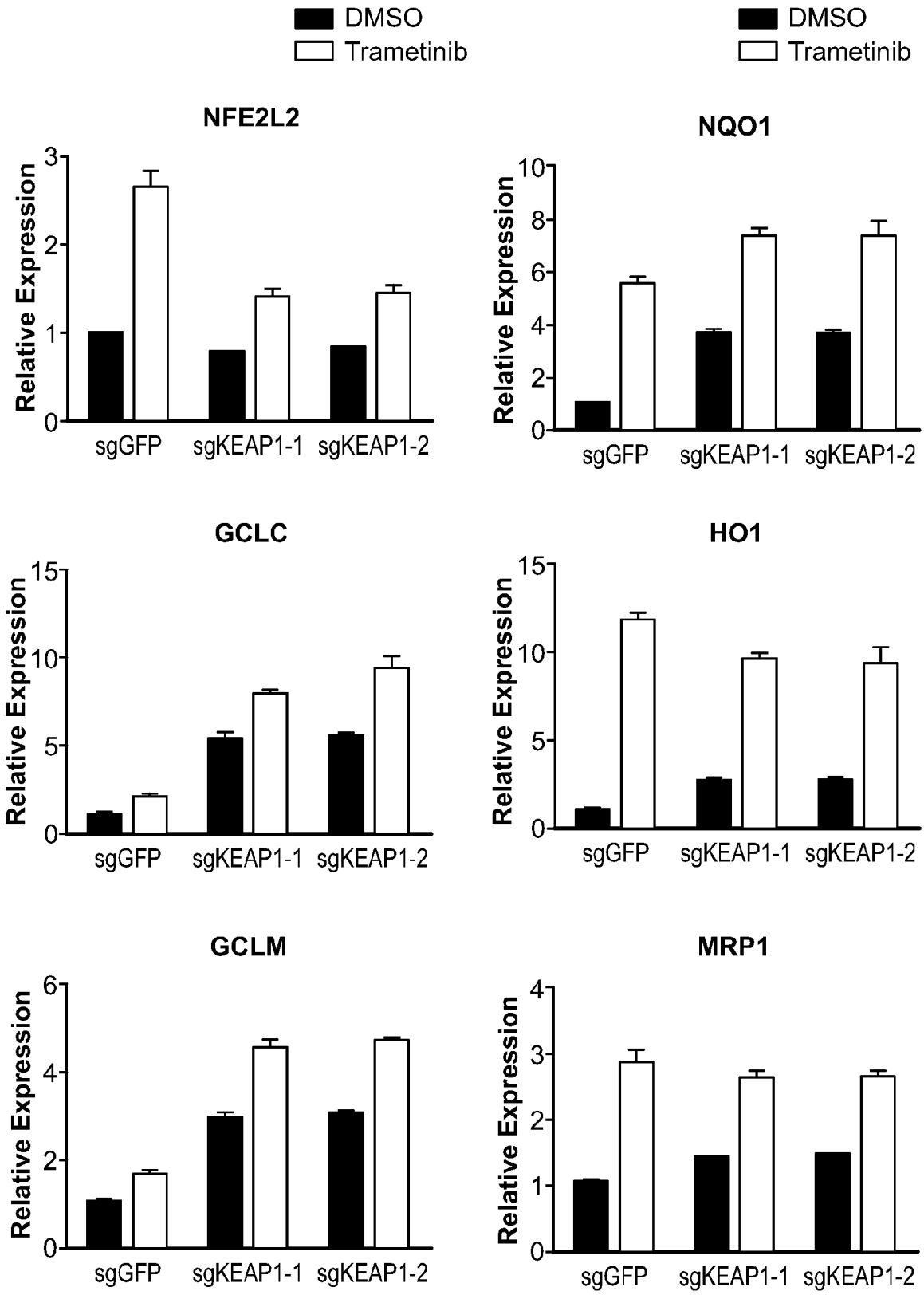

DMSO
Trametinib
DMSO
Trametinib
NFE2L2
Relative Expression
0
1
2
3
sgGFP
sgKEAP1-1
sgKEAP1-2
NQO1
Relative Expression
0
2
4
6
8
10
sgGFP
sgKEAP1-1
sgKEAP1-2
GCLC
Relative Expression
0
5
10
15
sgGFP
sgKEAP1-1
sgKEAP1-2
HO1
Relative Expression
0
5
10
15
sgGFP
sgKEAP1-1
sgKEAP1-2
GCLM
Relative Expression
0
2
4
6
sgGFP
sgKEAP1-1
sgKEAP1-2
MRP1
Relative Expression
0
1
2
3
4
sgGFP
sgKEAP1-1
sgKEAP1-2

FIG. 3B

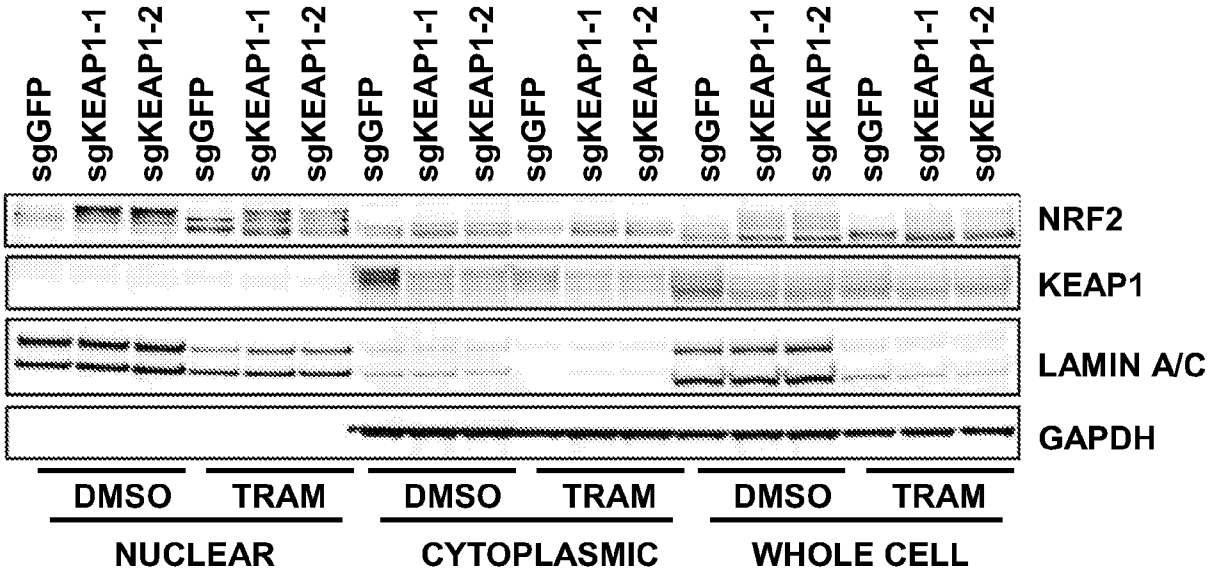

sgGFP
sgKEAP1-1
sgKEAP1-2
sgGFP
sgKEAP1-1
sgKEAP1-2
sgGFP
sgKEAP1-1
sgKEAP1-2
sgGFP
sgKEAP1-1
sgKEAP1-2
sgGFP
sgKEAP1-1
sgKEAP1-2
sgGFP
sgKEAP1-1
sgKEAP1-2
NRF2
KEAP1
LAMIN A/C
GAPDH
DMSO
TRAM
DMSO
TRAM
DMSO
TRAM
NUCLEAR
CYTOPLASMIC
WHOLE CELL

FIG. 4A

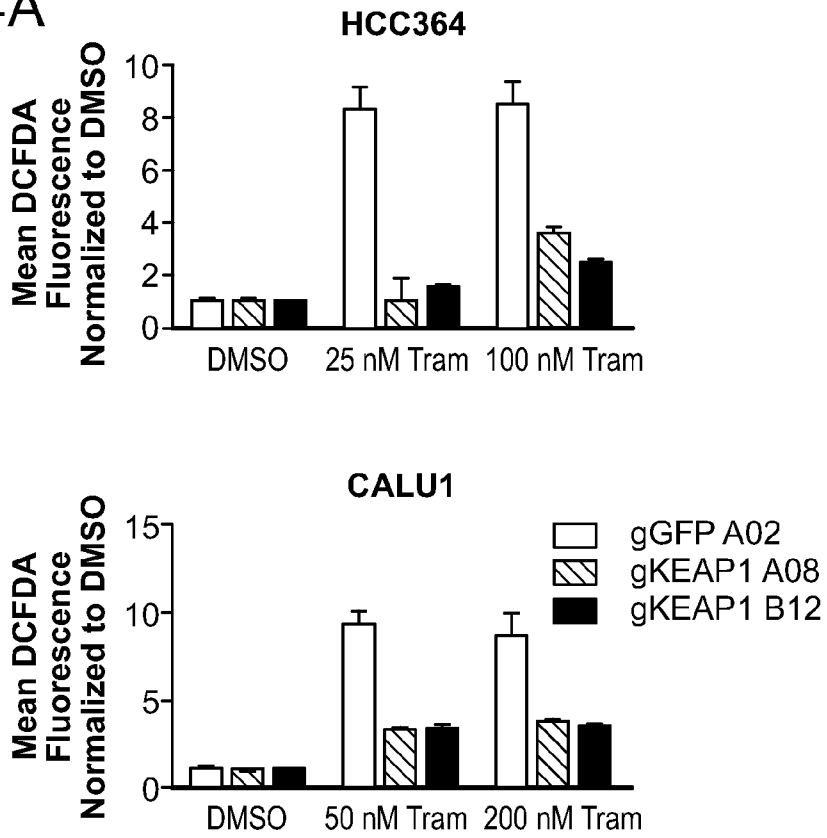


FIG. 4B

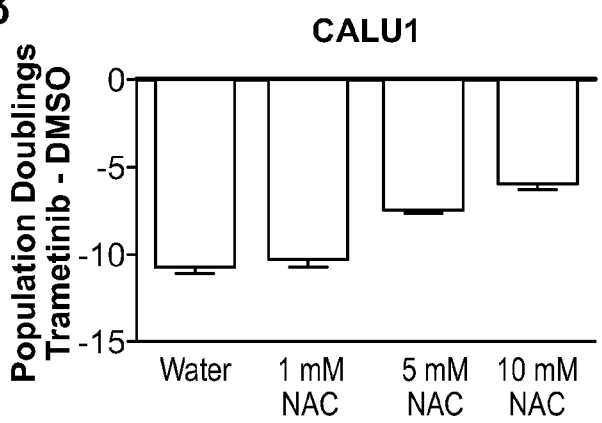


FIG. 4C

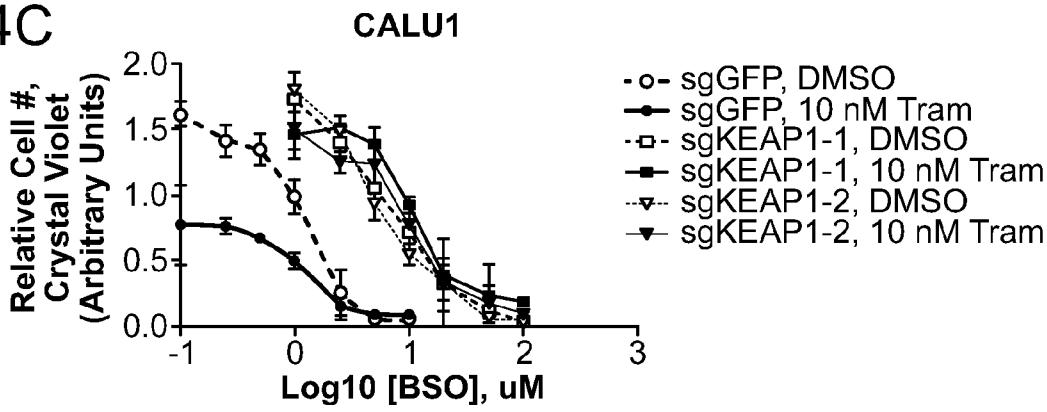


FIG. 4D

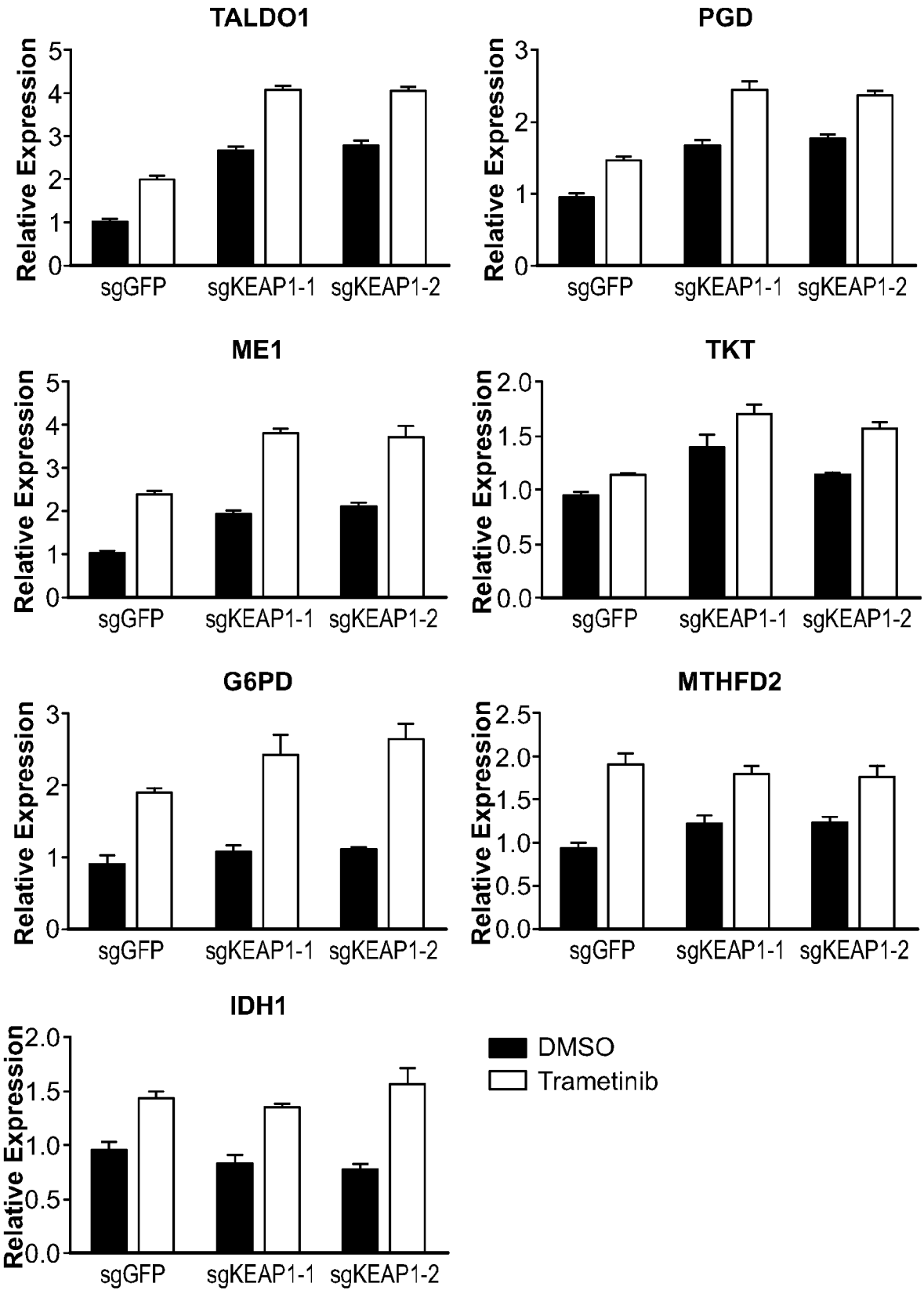

TALDO1
PGD
ME1
TKT
G6PD
MTHFD2
IDH1
Relative Expression
sgGFP
sgKEAP1-1
sgKEAP1-2
DMSO
Trametinib

FIG. 4E

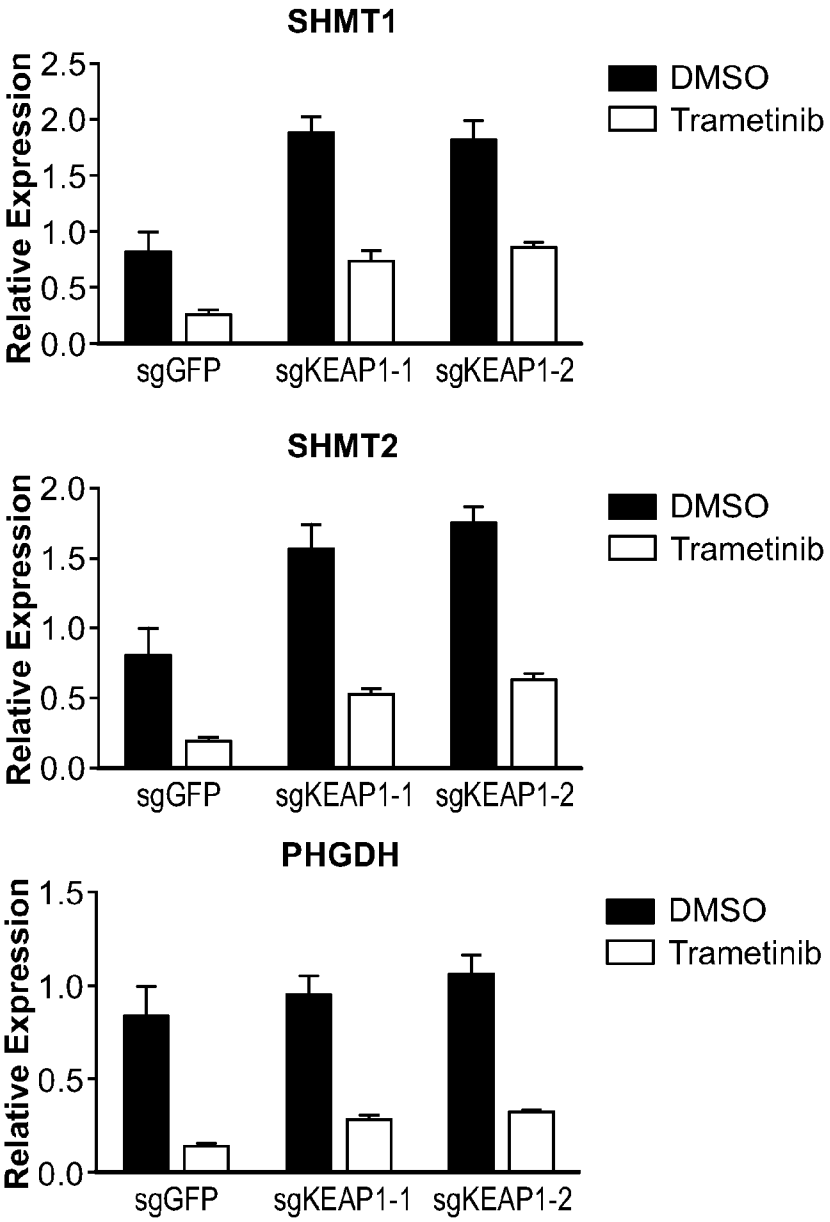

SHMT1
Relative Expression
2.5
2.0
1.5
1.0
0.5
0.0
sgGFP
sgKEAP1-1
sgKEAP1-2
DMSO
Trametinib
SHMT2
Relative Expression
2.0
1.5
1.0
0.5
0.0
sgGFP
sgKEAP1-1
sgKEAP1-2
DMSO
Trametinib
PHGDH
Relative Expression
1.5
1.0
0.5
0.0
sgGFP
sgKEAP1-1
sgKEAP1-2
DMSO
Trametinib

FIG. 5

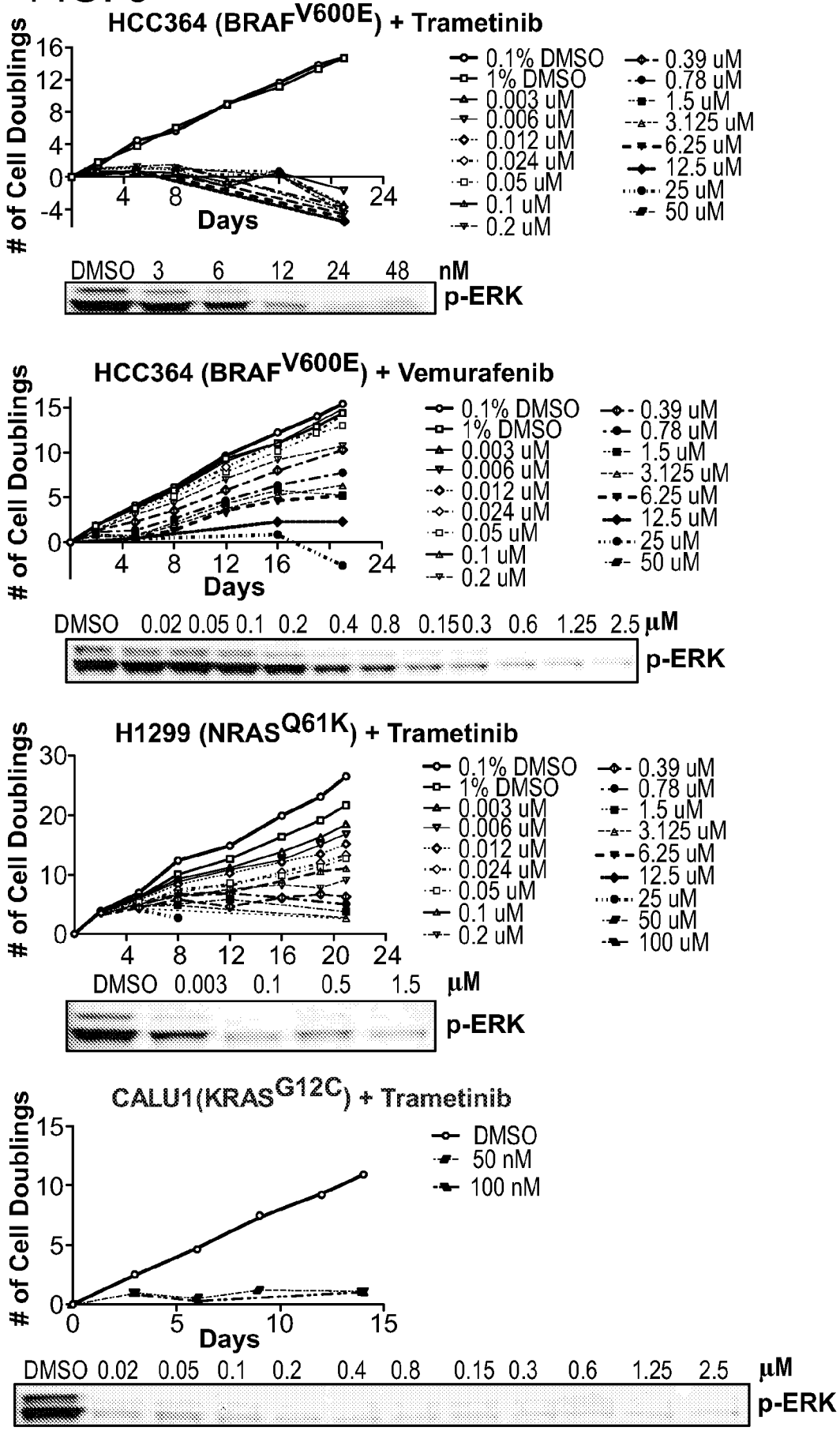
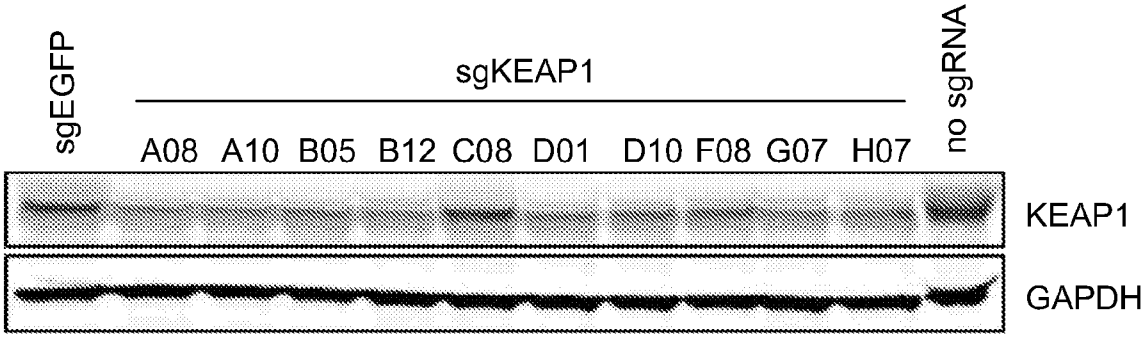

HCC364 (BRAF V600E) + Trametinib
# of Cell Doublings
Days
0.1% DMSO
1% DMSO
0.003 uM
0.006 uM
0.012 uM
0.024 uM
0.05 uM
0.1 uM
0.2 uM
0.39 uM
0.78 uM
1.5 uM
3.125 uM
6.25 uM
12.5 uM
25 uM
50 uM
DMSO 3 6 12 24 48 nM
p-ERK
HCC364 (BRAF V600E) + Vemurafenib
# of Cell Doublings
Days
DMSO 0.02 0.05 0.1 0.2 0.4 0.8 0.15 0.3 0.6 1.25 2.5 µM
p-ERK
H1299 (NRAS Q61K) + Trametinib
# of Cell Doublings
Days
100 uM
DMSO 0.003 0.1 0.5 1.5 µM
p-ERK
CALU1(KRAS G12C) + Trametinib
# of Cell Doublings
Days
DMSO
50 nM
100 nM
DMSO 0.02 0.05 0.1 0.2 0.4 0.8 0.15 0.3 0.6 1.25 2.5 µM
p-ERK

FIG. 6A



HCC364
A08 = sgKEAP1-1
B12 = sgKEAP1-2

FIG. 6B

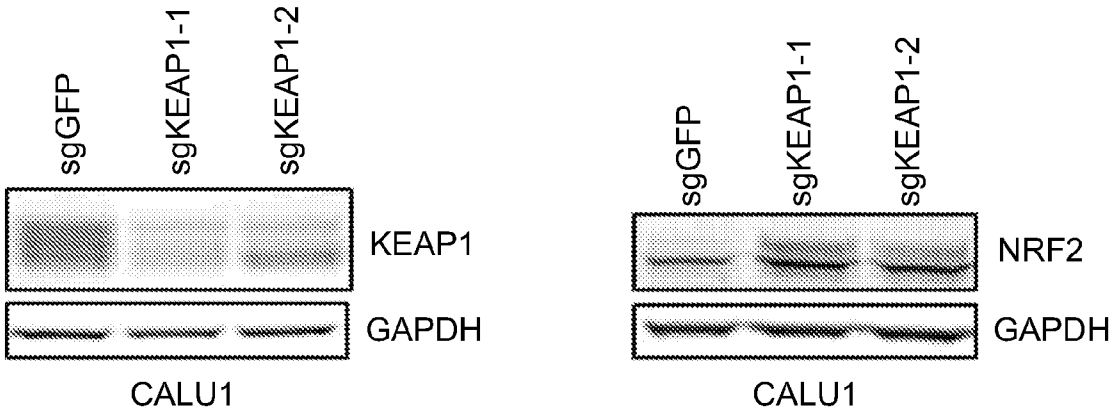


FIG. 6C

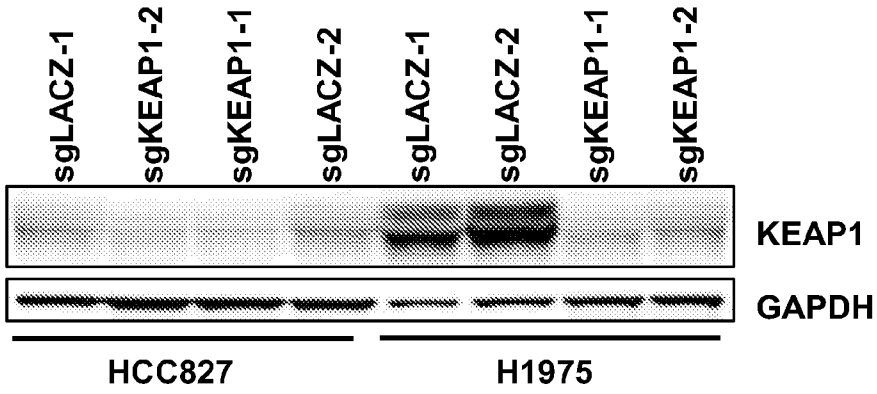


FIG. 6D

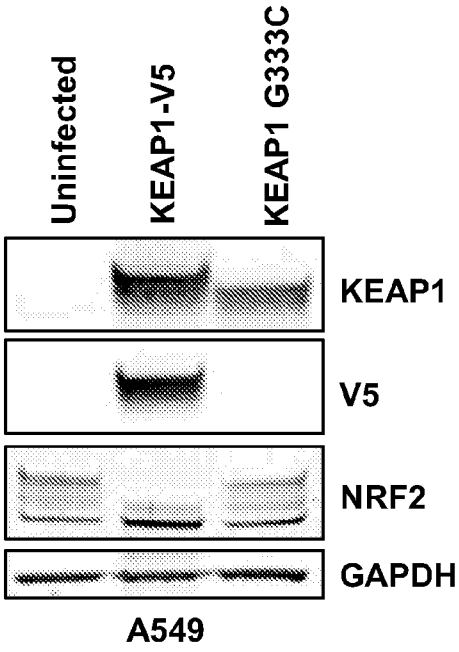


FIG. 6E

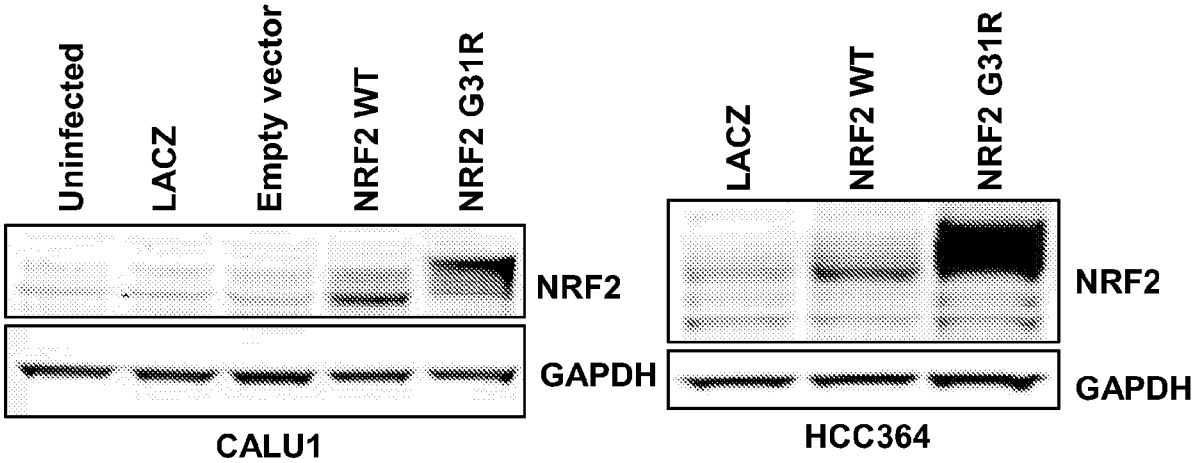


FIG. 7

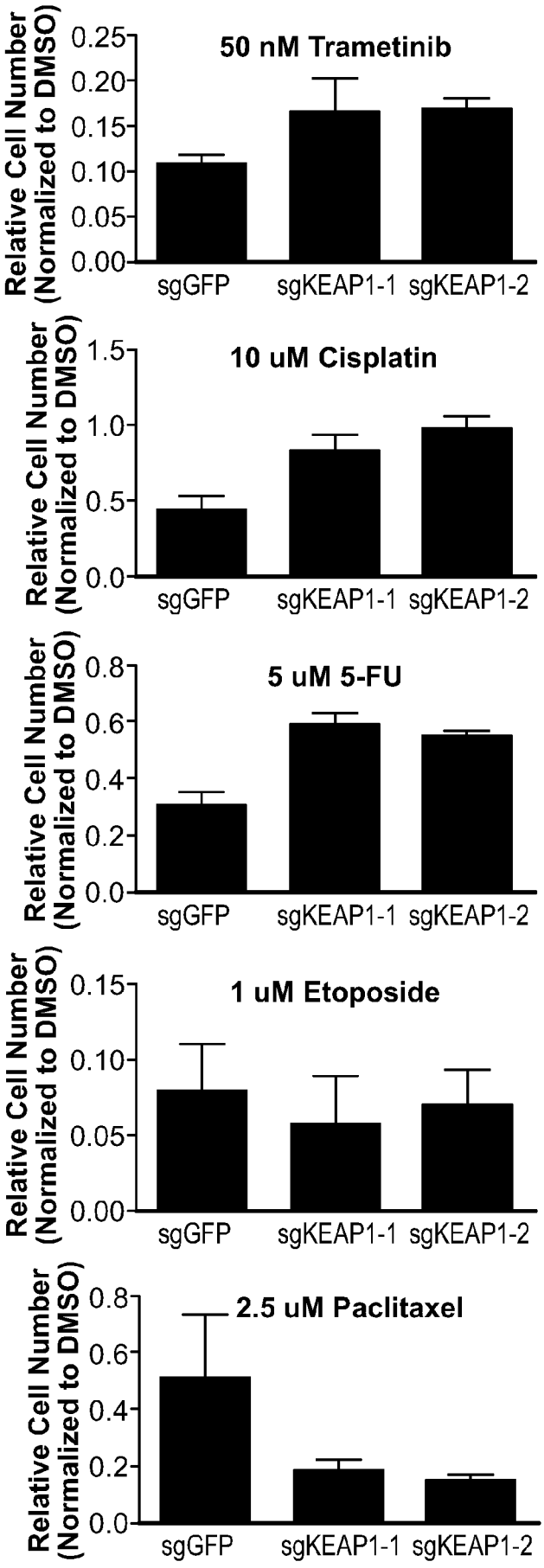

50 nM Trametinib
Relative Cell Number (Normalized to DMSO)
0.25
0.20
0.15
0.10
0.05
0.00
sgGFP
sgKEAP1-1
sgKEAP1-2
10 uM Cisplatin
Relative Cell Number (Normalized to DMSO)
1.5
1.0
0.5
0.0
sgGFP
sgKEAP1-1
sgKEAP1-2
5 uM 5-FU
Relative Cell Number (Normalized to DMSO)
0.8
0.6
0.4
0.2
0.0
sgGFP
sgKEAP1-1
sgKEAP1-2
1 uM Etoposide
Relative Cell Number (Normalized to DMSO)
0.15
0.10
0.05
0.00
sgGFP
sgKEAP1-1
sgKEAP1-2
2.5 uM Paclitaxel
Relative Cell Number (Normalized to DMSO)
0.8
0.6
0.4
0.2
0.0
sgGFP
sgKEAP1-1
sgKEAP1-2

FIG. 8A

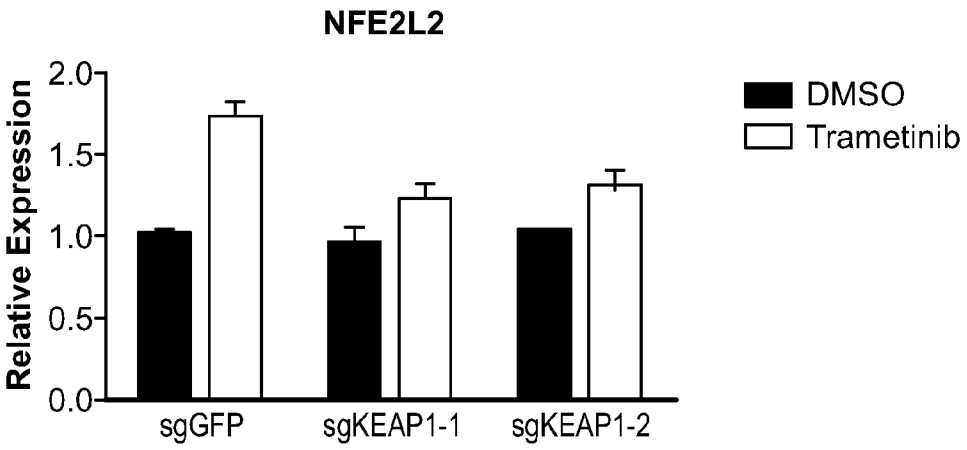
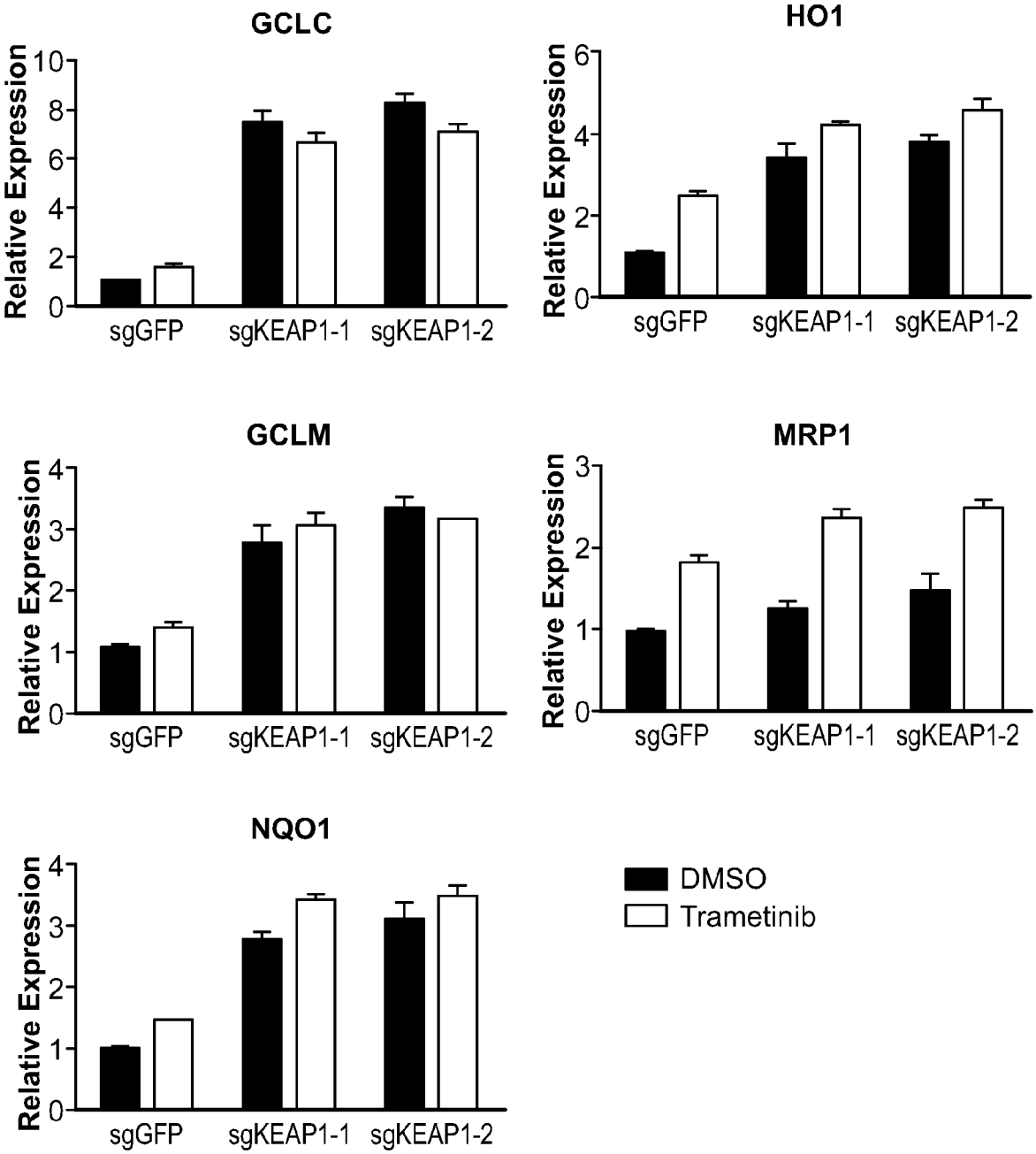

NFE2L2
Relative Expression
2.0
1.5
1.0
0.5
0.0
sgGFP
sgKEAP1-1
sgKEAP1-2
DMSO
Trametinib

FIG. 8B


GCLC
HO1
GCLM
MRP1
NQO1
Relative Expression
sgGFP
sgKEAP1-1
sgKEAP1-2
DMSO
Trametinib

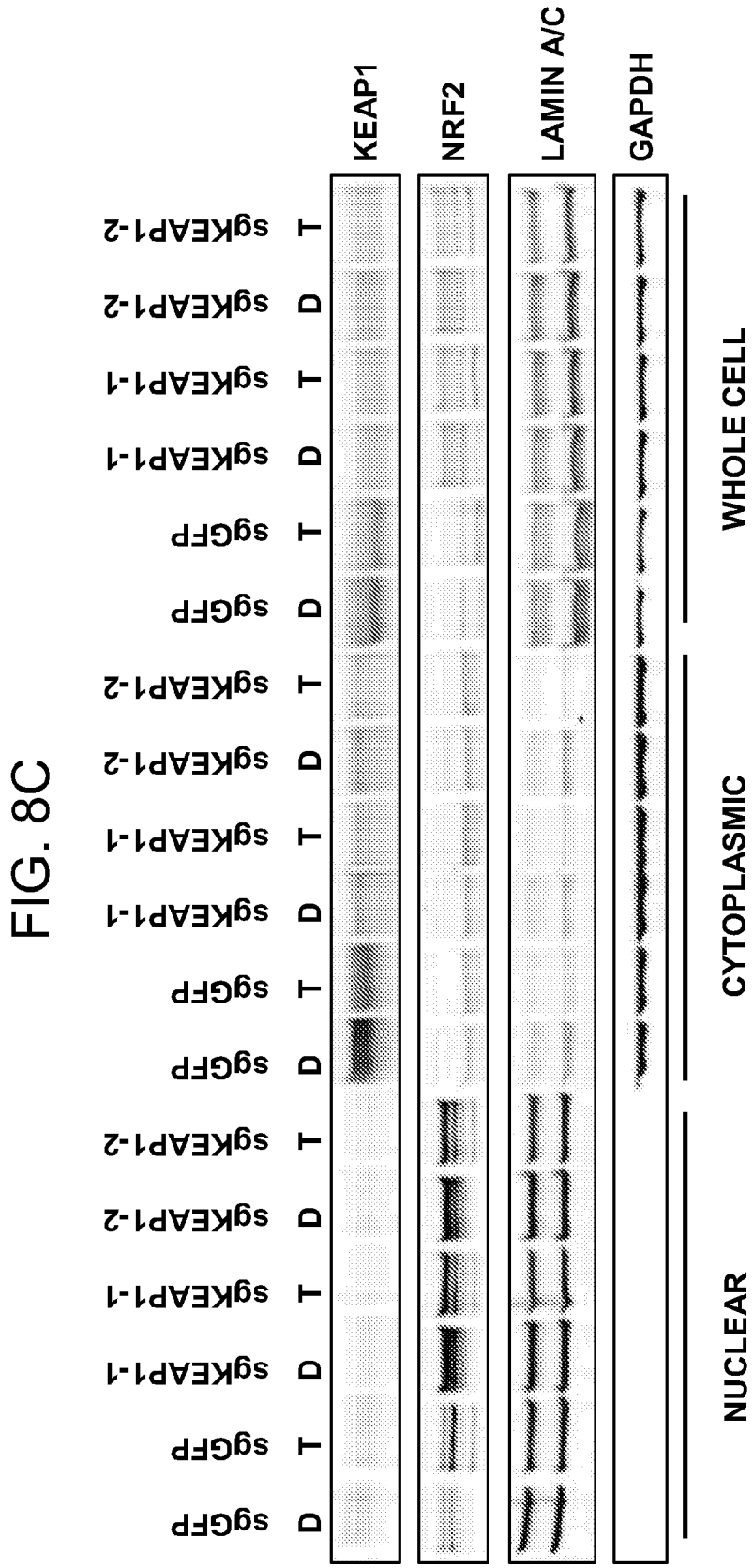

KEAP1
NRF2
LAMIN A/C
GAPDH
sgKEAP1-2 T
sgKEAP1-2 D
sgKEAP1-1 T
sgKEAP1-1 D
sgGFP T
sgGFP D
sgKEAP1-2 T
sgKEAP1-2 D
sgKEAP1-1 T
sgKEAP1-1 D
sgGFP T
sgGFP D
sgKEAP1-2 T
sgKEAP1-2 D
sgKEAP1-1 T
sgKEAP1-1 D
sgGFP T
sgGFP D
WHOLE CELL
CYTOPLASMIC
NUCLEAR

FIG. 8C

FIG. 9A

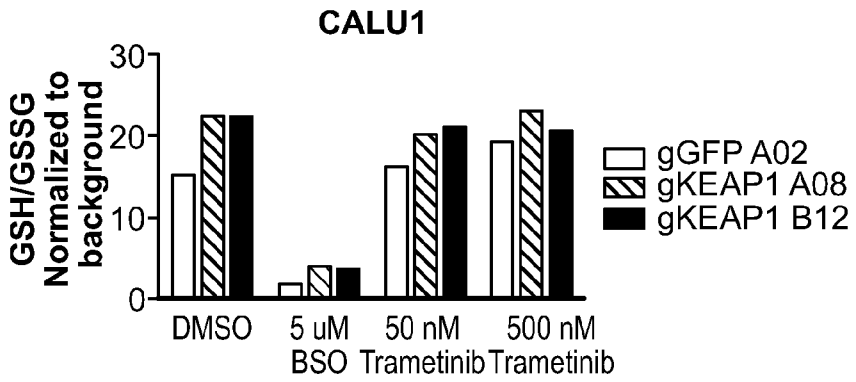


FIG. 9B

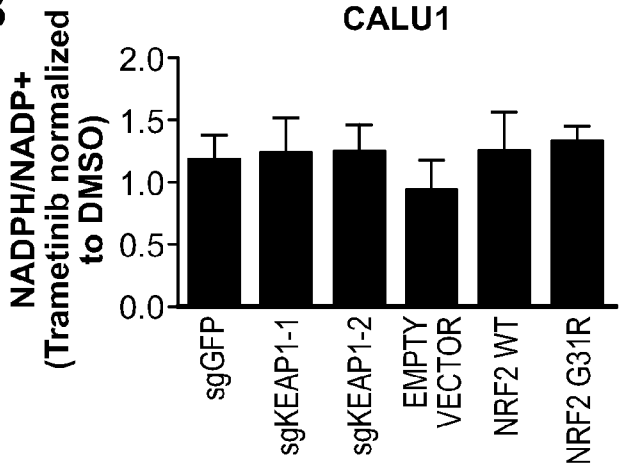


FIG. 9C

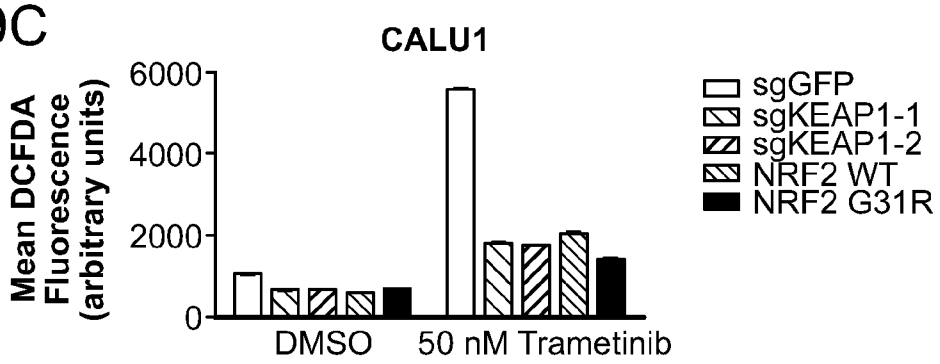


FIG. 9D

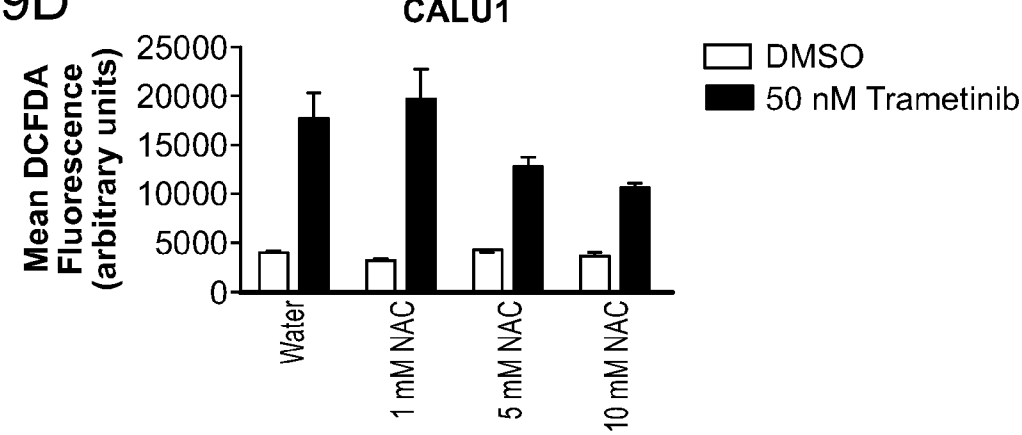


FIG. 9E

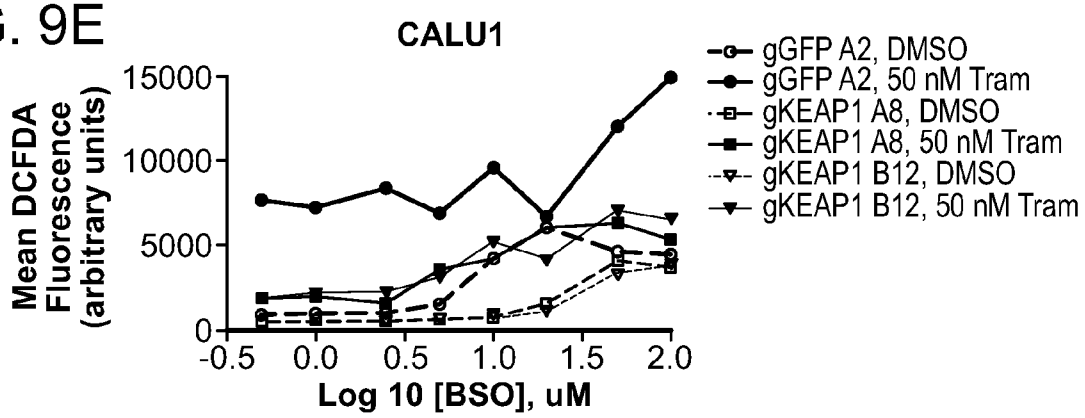


FIG. 9F

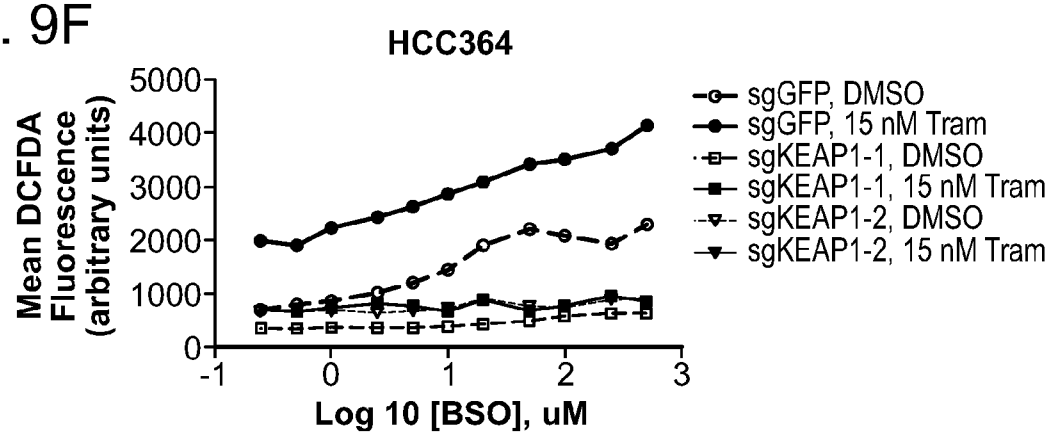


FIG. 9G

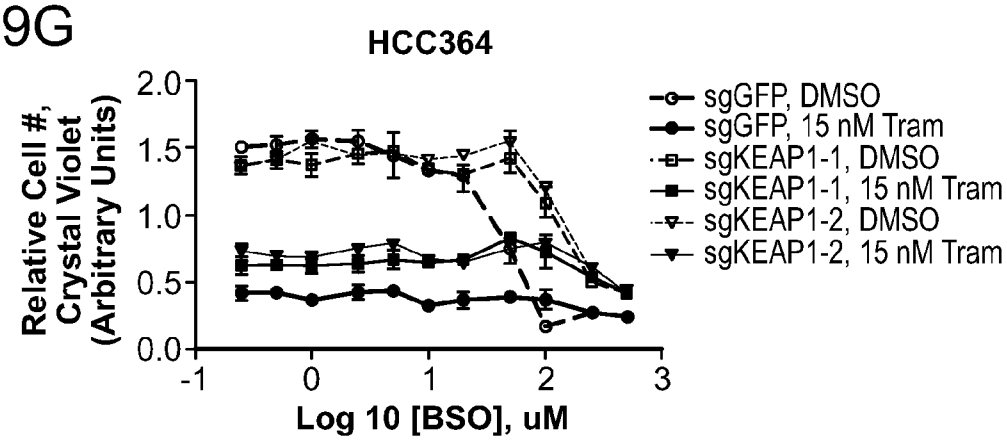


FIG. 9H

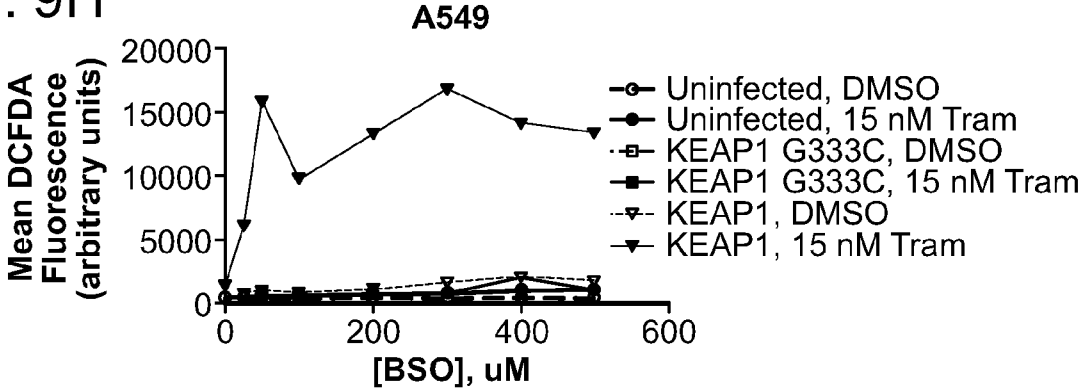


FIG. 10A

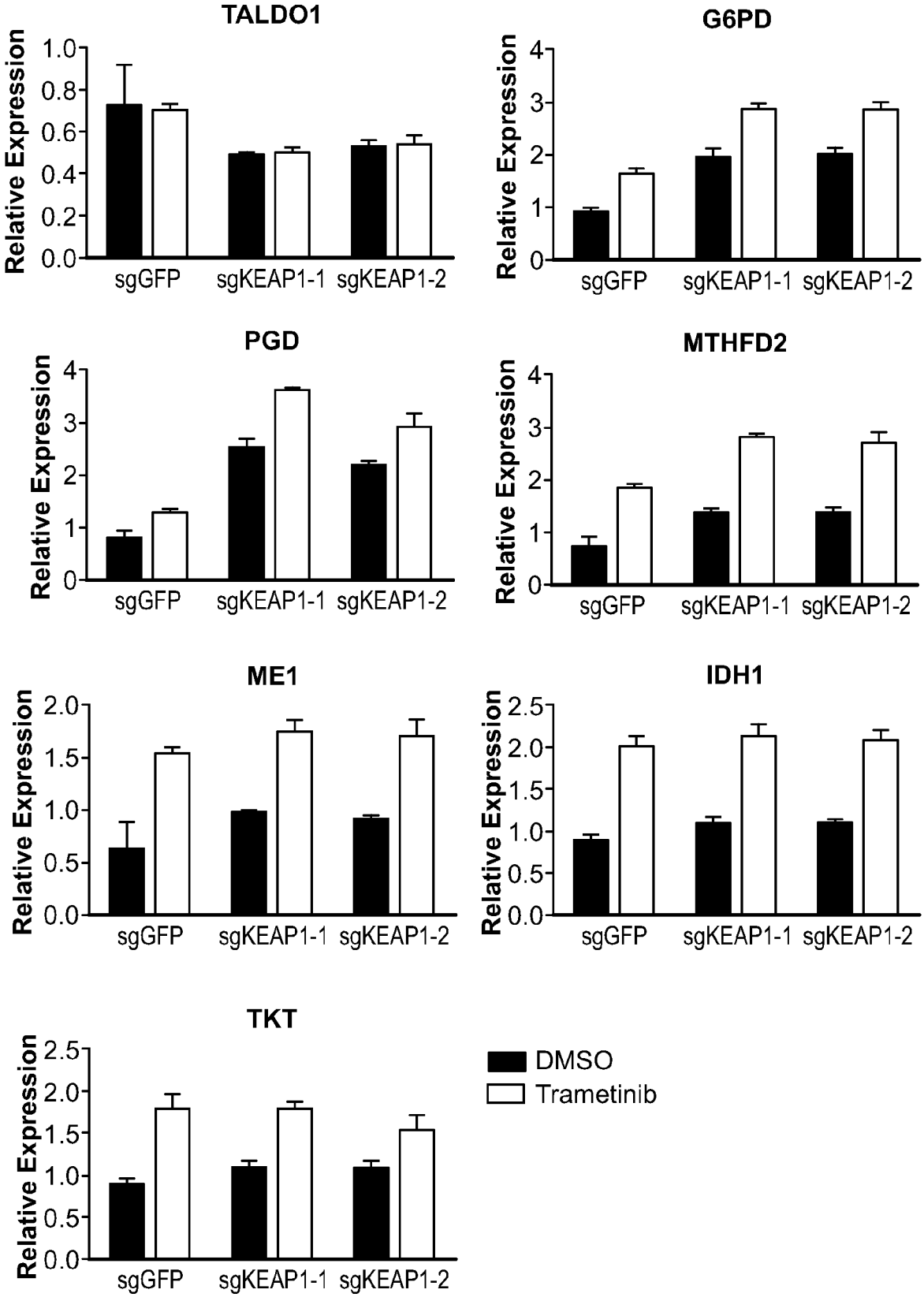

TALDO1
G6PD
PGD
MTHFD2
ME1
IDH1
TKT
Relative Expression
sgGFP
sgKEAP1-1
sgKEAP1-2
DMSO
Trametinib

FIG. 10B

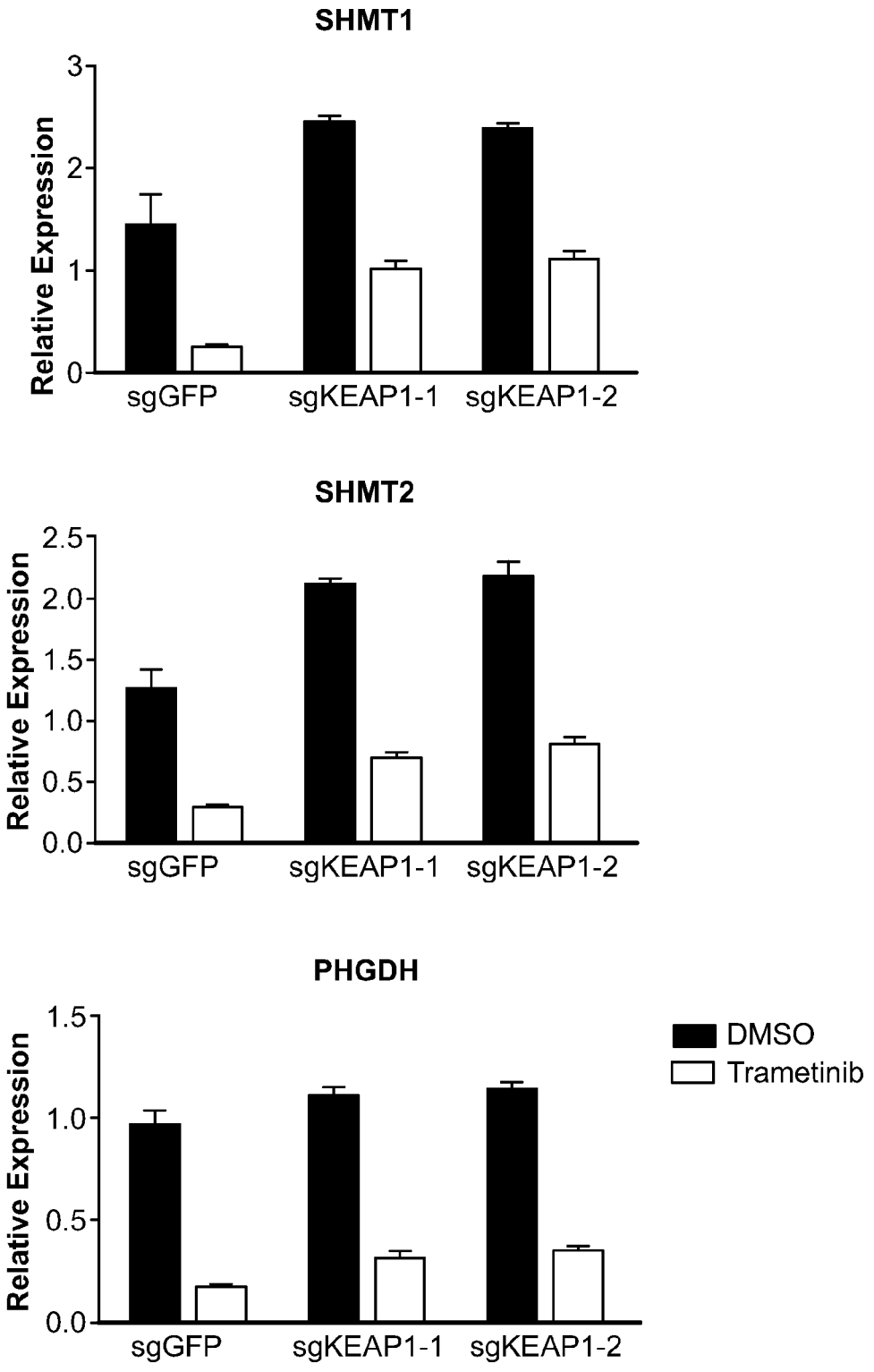
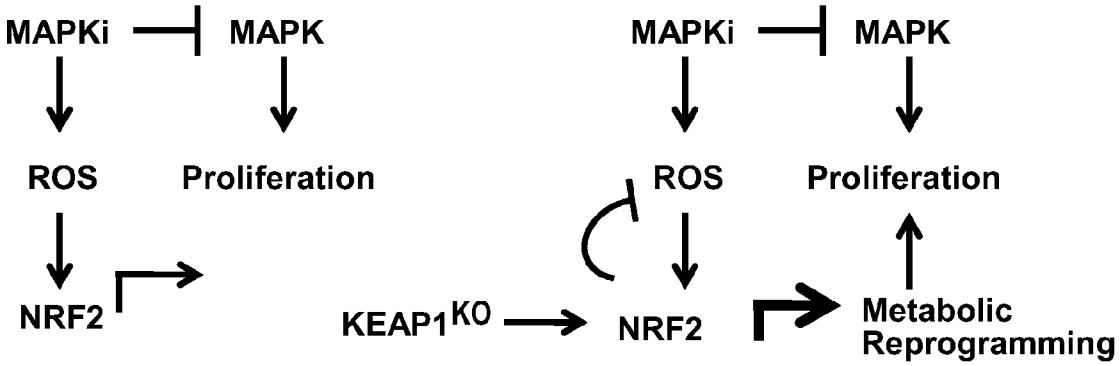

SHMT1
Relative Expression
3
2
1
0
sgGFP
sgKEAP1-1
sgKEAP1-2
SHMT2
Relative Expression
2.5
2.0
1.5
1.0
0.5
0.0
sgGFP
sgKEAP1-1
sgKEAP1-2
PHGDH
Relative Expression
1.5
1.0
0.5
0.0
sgGFP
sgKEAP1-1
sgKEAP1-2
DMSO
Trametinib

FIG. 11


MAPKi
MAPK
ROS
Proliferation
NRF2
MAPKi
MAPK
ROS
Proliferation
KEAP1KO
NRF2
Metabolic
Reprogramming