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(57) Abstract: Provided herein are pharmaceutical products comprising therapeutically effective combinations of ALDH inhibitors (e.g., disuliram and/or derivatives thereof) and targeted therapeutics, as well as methods of using said combinations for the treatment of cancer.

(54) Title: METHODS OF TREATING CANCER AND PREVENTING DRUG RESISTANCE
METHODS OF TREATING CANCER AND PREVENTING DRUG RESISTANCE

FIELD

[0001] Provided herein are therapies for the treatment of pathological conditions, such as cancer, using ALDH inhibitors and targeted therapeutics.

BACKGROUND

[0002] The relatively rapid acquisition of resistance to cancer drugs remains a key obstacle to successful cancer therapy. Substantial efforts to elucidate the molecular basis for such drug resistance have revealed a variety of mechanisms, including drug efflux, acquisition of drug binding-deficient mutants of the target, engagement of alternative survival pathways, epigenetic alterations). Such mechanisms are generally believed to reflect the existence of rare, stochastic, resistance-conferring genetic alterations within a tumor cell population that are selected during drug treatment. Sharma et al., Cell 141(1):69-80 (2010). An increasingly observed phenomenon in cancer therapy is the so-called "re-treatment response." For example, some non-small cell lung cancer (NSCLC) patients who respond well to treatment with EGFR (epidermal growth factor receptor) tyrosine kinase inhibitors (TKIs), and who later experience therapy failure, demonstrate a second response to EGFR TKI re-treatment after a "drug holiday" Kurata et al., Ann. Oncol. 15:173-174 (2004); Yano et al., Oncol. Res. 15:107-111 (2005). Similar re-treatment responses are well established for several other anti-cancer agents. Cara and Tannock, Ann. Oncol. 12:23-27 (2001). Such findings suggest that acquired resistance to cancer drugs may involve a reversible "drug-tolerant" state, whose mechanistic basis remains to be established.

[0003] The existence of a reversibly "drug-tolerant" cell population within various human tumor cell lines has been shown to be maintained via engagement of IGF-1 receptor signaling and an altered chromatin state that requires the histone demethylase KDM5A. While some specific resistance-conferring mutations have indeed been identified in many cancer patients demonstrating acquired drug resistance, the relative contribution of mutational and non-mutational mechanisms to drug resistance, and the role of tumor cell subpopulations remain somewhat unclear. New treatment methods are needed to successfully address heterogeneity within cancer cell populations and the emergence of cancer cells resistant to drug treatments.

SUMMARY

[0004] Provided herein are combinations comprising an ALDH inhibitor (e.g., disulfiram and/or derivatives thereof) and a targeted therapeutic (e.g., TKI). Provided herein are methods of treating
cancer in an individual comprising concomitantly administering to the individual an effective amount of an ALDH inhibitor and an effective amount of a targeted therapeutic. In some embodiments, the respective amounts of the ALDH inhibitor (e.g., disulfiram and/or derivatives thereof) and the targeted therapeutic (e.g., TKI) are effective to increase efficacy of a cancer treatment comprising a targeted therapeutic (e.g., TKI). For example, in some embodiments, the respective amounts of the ALDH inhibitor (e.g., disulfiram and/or derivatives thereof) and the targeted therapeutic (e.g., TKI) are effective to increased efficacy compared to a standard treatment comprising administering an effective amount of the targeted therapeutic (e.g., TKI) without (in the absence of) the ALDH inhibitor (e.g., disulfiram and/or derivatives thereof). In some embodiments, the respective amounts of the ALDH inhibitor (e.g., disulfiram and/or derivatives thereof) and the targeted therapeutic (e.g., TKI) are effective to increased response (e.g., complete response) compared to a standard treatment comprising administering an effective amount of the targeted therapeutic (e.g., TKI) without (in the absence of) the ALDH inhibitor (e.g., disulfiram and/or derivatives thereof). In some embodiments of any of the methods, the ALDH inhibitor is disulfiram and/or derivatives thereof. In some embodiments, the ALDH inhibitor is gossypol.

[0005] Also provided herein are methods of increasing efficacy of a cancer treatment comprising a targeted therapeutic in an individual comprises concomitantly administering to the individual an effective amount of the targeted therapeutic and an effective amount of an ALDH inhibitor.

[0006] Provided herein are methods of treating cancer in an individual wherein cancer treatment comprising concomitantly administering to the individual an effective amount of a targeted therapeutic and an effective amount of an ALDH inhibitor, wherein the cancer treatment has increased efficacy compared to a standard treatment comprising administering an effective amount of the targeted therapeutic without (in the absence of) the targeted therapeutic. Further, provided herein are methods of delaying and/or preventing development of cancer resistant to a targeted therapeutic in an individual, comprising concomitantly administering to the individual an effective amount of an ALDH inhibitor and an effective amount of the targeted therapeutic.

[0007] Provided herein are methods of treating an individual with cancer who has increased likelihood of developing resistance to a targeted therapeutic comprising concomitantly administering to the individual an effective amount of an ALDH inhibitor and an effective amount of the targeted therapeutic. In addition, provided herein are methods of increasing sensitivity to a targeted therapeutic in
an individual with cancer comprising concomitantly administering to the individual an effective amount of an ALDH inhibitor and an effective amount of the targeted therapeutic.

[0008] In another aspect, provided herein are methods of extending the period of an targeted therapeutic sensitivity in an individual with cancer comprising concomitantly administering to the individual an effective amount of an ALDH inhibitor and an effective amount of targeted therapeutic. In addition, provided herein are methods of extending the duration of response to a targeted therapeutic in an individual with cancer comprising concomitantly administering to the individual an effective amount of an ALDH inhibitor and an effective amount of the targeted therapeutic.

[0010] In some embodiments of any of the methods, the ALDH inhibitor is a small molecule ALDH inhibitor. In some embodiments, the small molecule ALDH inhibitor is disulfiram or an ALDH-inhibiting derivative or metabolite thereof. In some embodiments, the ALDH inhibitor is N,N-diethyl[(diethylcarbamothioyl) disulfanyl]carbothioamide or pharmaceutically acceptable salt thereof. In some embodiments, the ALDH inhibitor is N,N-diethyl[(diethylcarbamothioyl) disulfanyl]carbothioamide. In some embodiments of any of the methods, the ALDH inhibitor is gossypol and/or an ALDH-inhibiting derivative or metabolite thereof. In some embodiments, the ALDH inhibitor is gossypol. In some embodiments, the ALDH inhibitor is 2,2′-bis-(Formyl-1,6,7-trihydroxy-5-isopropyl-3-methylnaphthalene) or pharmaceutically acceptable salt thereof. In some embodiments, the ALDH inhibitor is 2,2′-bis-(Formyl-1,6,7-trihydroxy-5-isopropyl-3-methylnaphthalene).

[0011] In some embodiments of any of the methods, the targeted therapeutic is a tyrosine kinase inhibitor (TKI). In some embodiments, the TKI is an EGFR inhibitor, HER2 inhibitor, MET inhibitor, ALK inhibitor, BRAF inhibitor, ROSI inhibitor, and/or MEK inhibitor. In some embodiments, the TKI is a receptor tyrosine kinase inhibitor (RTKI). In some embodiments, the RTKI is an EGFR inhibitor, HER2 inhibitor, MET inhibitor, and/or ALK inhibitor. In some embodiments, the inhibitor is an antibody inhibitor, a small molecule inhibitor, a binding polypeptide inhibitor, and/or a polynucleotide antagonist. In some embodiments, the TKI is N-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)quinoxalin-4-amine or a pharmaceutically acceptable salt thereof (e.g., erlotinib). In some embodiments, the TKI is N-(4-(3-fluorobenzyloxy)-3-chlorophenyl)-6-(5-((2-(methylsulfonyl)ethylamino)methyl)furan-2-yl)quinoxalin-4-amine, di4-methylbenzenesulfonate or a pharmaceutically acceptable salt thereof (e.g., lapatinib). In some embodiments, the TKI is (S)-N-(2,3-dihydroxypropyl)-3-(2-fluro-4-iodophenylamino)isonicotinamide) or a pharmaceutically acceptable salt thereof (e.g., AS703026). In some embodiments, the TKI is vemurafenib. In some embodiments, the TKI is 3-((R)-1-(2,6-dichloro-3-
fluorophenyl)ethoxy)-5-(1-(piperidin-4-yl)-1H-pyrazol-4-yl)pyridin-2-amine or a pharmaceutically acceptable salt thereof (e.g., crizotinib).

[0012] In some embodiments of any of the methods, the cancer is gastric cancer, lung cancer (e.g., non-small cell lung cancer (NSCL)), colorectal cancer (e.g., colon cancer and/or rectal cancer), or basal cell carcinoma.

[0013] In another embodiment, there is provided a pharmaceutical product comprising a) as a first component an effective amount of an ALDH inhibitor, and b) as a second component an effective amount of a targeting agent (targeted therapeutic) for the concomitant or sequential use for the treatment of cancer.

[0014] In another embodiment, there is provided the pharmaceutical product as indicated above, wherein the ALDH inhibitor is a small molecule ALDH inhibitor. In another embodiment, there is provided the pharmaceutical product as indicated above, wherein the ALDH inhibitor is disulfiram or an ALDH-inhibiting derivative or metabolite thereof. In another embodiment, there is provided the pharmaceutical product as indicated above, wherein the ALDH inhibitor is N,N-diethyl[(diethylcarbamothioyl) disulfanyl]carbothioamide or pharmaceutically acceptable salt thereof. In another embodiment, there is provided the pharmaceutical product as indicated above, wherein the ALDH inhibitor is N,N-diethyl[(diethylcarbamothioyl) disulfanyl]carbothioamide. In another embodiment, there is provided the pharmaceutical product as indicated above, wherein the ALDH inhibitor is 2,2′-bis-(Formyl-l,6,7-trihydroxy-5-isopropyl-3-methylnaphthalene) or pharmaceutically acceptable salt thereof. In another embodiment, there is provided the pharmaceutical product as indicated above, wherein the ALDH inhibitor is 2,2′-bis-(Formyl-l,6,7-trihydroxy-5-isopropyl-3-methylnaphthalene).

[0015] In another embodiment, there is provided the pharmaceutical product as indicated above, wherein the targeted therapeutic is a tyrosine kinase inhibitor (TKI). In another embodiment, there is provided the pharmaceutical product as indicated above, wherein the TKI is an EGFR inhibitor, HER2 inhibitor, MET inhibitor, ALK inhibitor, BRAF inhibitor, ROS1 inhibitor, and/or MEK inhibitor. In another embodiment, there is provided the pharmaceutical product as indicated above, wherein the TKI is a receptor tyrosine kinase inhibitor (RTKI). In another embodiment, there is provided the pharmaceutical product as indicated above, wherein the RTKI is an EGFR inhibitor, HER2 inhibitor, MET inhibitor, and/or ALK inhibitor. In another embodiment, there is provided the pharmaceutical product as indicated above, wherein the inhibitor is an antibody inhibitor, a small molecule inhibitor, a binding polypeptide inhibitor, and/or a polynucleotide antagonist. In another embodiment, there is
provided the pharmaceutical product as indicated above, wherein the TKI is N-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)quinazolin-4-amine or a pharmaceutically acceptable salt thereof, in particular erlotinib. In another embodiment, there is provided the pharmaceutical product as indicated above, wherein the TKI is N-(4-(3-fluorobenzyloxy)-3-chlorophenyl)-6-(5-((2-(methylsulfonyl)ethylamino)methyl)furan-2-yl)quinazolin-4-amine, di4-methylbenzenesulfonate or a pharmaceutically acceptable salt thereof, in particular lapatinib. In another embodiment, there is provided the pharmaceutical product as indicated above, wherein the TKI is (S)-N-(2,3-dihydroxypropyl)-3-(2-fluoro-4-iodophenylamino)isonicotinamide or a pharmaceutically acceptable salt thereof, in particular AS703026. In another embodiment, there is provided the pharmaceutical product as indicated above, wherein the TKI is vemurafenib. In another embodiment, there is provided the pharmaceutical product as indicated above, wherein the TKI is 3-((R)-1-(2,6-dichloro-3-fluorophenyl)ethoxy)-5-(1-(piperidin-4-yl)-1H-pyrazol-4-yl)pyridin-2-amine or a pharmaceutically acceptable salt thereof, in particular crizotinib.

[0016] In another embodiment, there is provided the pharmaceutical product as indicated above, wherein the cancer is gastric cancer, lung cancer, non-small cell lung cancer (NSCL), colon cancer and/or rectal cancer, or basel cell carcinoma.

[0017] In addition to providing improved treatment for cancer, administration of certain combinations described herein may improve the quality of life for a patient compared to the quality of life experienced by the same patient receiving a different treatment. For example, administration of a combination of a targeted therapeutic (e.g., TKI), and a ALDH inhibitor (e.g., disulfiram and/or derivatives thereof), as described herein to an individual may provide an improved quality of life compared to the quality of life the same patient would experience if they received only the targeted therapeutic as therapy. For example, the combined therapy with the combination described herein may lower the dose of targeted therapeutic needed, thereby lessening the side-effects associated with the therapeutic (e.g. nausea, vomiting, hair loss, rash, decreased appetite, weight loss, etc.). The combination may also cause reduced tumor burden and the associated adverse events, such as pain, organ dysfunction, weight loss, etc. Accordingly, one aspect of the invention provides ALDH inhibitor (e.g., disulfiram and/or derivatives thereof) for therapeutic use for improving the quality of life of a patient treated for a cancer with a targeted therapeutic (e.g., TKI). Accordingly, another aspect of the invention provides ALDH inhibitor (e.g., disulfiram and/or derivatives thereof) for therapeutic use for improving the quality of life of an individual treated for a cancer disorder with a targeted therapeutic, or a pharmaceutically acceptable salt thereof.
BRIEF DESCRIPTION OF THE FIGURES

[0018] Figure 1A-C. I Drug Tolerant Gastric Cancer Cells Express High level of ALDH1A1. (A) ALDH activity was measured in Kato II parental and crizotinib (luM) tolerant cells using Aldeflour assay (Stem Cell Technology). The bodipy labeled substrate emit fluorescence when oxidized by ALDH to corresponding acid. Photographs showing enrichment of ALDH^{high} cells following crizotinib treatment. Arrows marked the ALDH^{high} cells in the parental population. (B) RNA expression level of 19 ALDH family members was determined using oligonucleotide based microarrays. Kato II parental cells were incubated with Aldeflour substrate at 37°C for 30 and ALDH^{high} and ALDH^{low} cells were sorted by flowcytometry. Gene expression analysis was performed using RNAs isolated from ALDH^{high} and ALDH^{low} cells. The bar graph illustrates differential expression of only one ALDH family members, ALDH1A1 in ALDH^{high} cells. (C) Immunoblots illustrating higher expression level of ALDH1A1 protein in ALDH^{high} cells and in crizotinib tolerant Kato II and GTL-16 cells compared to ALDH^{low} cells and the parental cells respectively.

[0019] Figure 2A-C. I ALDH inhibitor Disulfiram eliminates drug tolerant cells. Parental Kato II (A) and GTL-16 (B) cells were treated with luM crizotinib for 25 days and disulfiram, 200nM, was added either on day1 (dl) or at different time intervals during crizotinib treatment. (C) Parental PC9 cells were treated with erlotinib and disulfiram, 200nM, which was added at different time points during erlotinib treatment. The bar graphs representing quantitative measurements made from triplicate wells per treatment show lethal effect of disulfiram on drug tolerant cells as measured by Syto60 viability assay (Wilson et al., 2011). The data is expressed as fractions of no treatment control, the error bar reflects SEM values.

[0020] Figure 3A-B. I Disulfiram kills drug tolerant cells of various cancer types. (A) Illustrating the effect of disulfiram and targeted cancer drug combinations on cancer cells of various tissue origins, which are addicted to different oncogenes. The cancer cells sensitive to erlotinib (HCC827 and HCC4006), lapatinib (HCC1419, SKBR3 and MDA-MB-175 v2), MEK inhibitor AS703026 (A549 and EBC-1) and BRAF inhibitor vemurafenib (Colo-205) were treated with appropriate drug, either alone or in combination with 200nM-300nM disulfiram. The duration of treatment varied from 11 to 25 days depending on the time it took for TKI-disulfiram treatment to kill almost all drug tolerant cells. (B) The bar graphs representing quantitative measurements (triplicate wells per treatment) of the effect of targeted cancer drug, disulfiram and their combination as measured by Syto60 viability assay illustrate the dependence of drug tolerant cells, in general, on ALDH for their survival. The data is expressed as fractions of no treatment control, the error bar reflects SEM values.
Figure 4A-C. Increased Mitochondrial Respiration and ROS Level in Drug Tolerant Cells. (A) ROS level was detected using fluorescein based H2DCFDA reagent (Molecular probes) and measured by flow cytometry. PC9-derived and GTL-16 derived DTPs were treated with disulfiram (200nM) and NAC (5mM) in the presence of TKI for 48h and the effect of TKI, disulfiram and NAC were measured. The bar graphs representing fold change in ROS level compared to untreated parental cells illustrates role of ALDH as ROS scavenger. (B) Oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) to measure energy production by mitochondrial respiration and glycolysis respectively of GTL-16 and PC9-derived DTPs was determined using Seahorse XF 96. The bar graphs illustrate increased use of mitochondrial respiration in the drug tolerant cell. (C) Immunoblots illustrates increased double stranded DNA breaks and activation of DNA repair mechanisms as a result of high ROS level in GTL-16 and PC9 drug tolerant cells.

Figure 5A-C. IROS scavenger N-acetyl cysteine reverses the effect of disulfiram. (A) PC9 and (B) GTL-16 parental cells were treated for 15 days with erlotinib and crizotinib respectively either alone or in combination with disulfiram, NAC and disulfiram+NAC. The bar graphs depicting the effect of these treatments on cell viability illustrate the ability of NAC to rescue the lethal effect of disulfiram on cell viability. (C1-2) GTL-16-derived DTPs were treated with disulfiram and NAC for 48h. Immunoblot data demonstrating reversal of the effect of disulfiram on γH2A, BimEL, BimS, and cleaved PARP level by NAC. Disulfiram increases ROS level and induce apoptosis in DTPs.

Figure 6A-B. IDisulfiram delays Tumor Relapse. (A) PC9 parental cells were treated with erlotinib alone or in combination with disulfiram. After 6 days of TKI treatment the DTPs were allowed to grow in erlotinib-free growth media with or without disulfiram. The cell viability data demonstrating delayed growth of PC9-derived DTPs that received disulfiram first 6 days and even longer delay in growth for those which continued to receive disulfiram for four subsequent days. The bar graph shows the effect of DS on PC9-derived DTPs measured from triplicate wells and expressed as mean +/- SD. (B) In vivo data showing delayed relapse of PC9 derived tumors in xenograft mice model treated with erlotinib and disulfiram combo compared to erlotinib alone.

Figure 7A-C. IPre-treatment with disulfiram is not sufficient to kill all DTPs. PC9 (A) and GTL-16 (B) parental cells were treated with DS first for 3 or 6 days and then with erlotinib for PC9 cells and crizotinib for GTL-16 cells in absence of DS. Syto60 cell viability staining showing brief exposure to DS reduces the number of DTPs but do not eliminate them. (C) Knock-down of ALDH1A1 alone has no significant effect on crizotinib drug sensitivity in GLT16 cells. Graph shows relative expression of
ALDHlA1 in GTL16 cells using multiple shRNAs. Table shows relative percentage of GTL16-derived DTPs upon treatment with crizotinib in ALDHlA1 knock down cells.

[0025] Figure 8A-C. IDrug treatment induces expression of multiple ALDH family members in multiple cell lines using multiple TKI inhibitors. (A) Relative change in RNA expression of ALDH family members in GTL16-derived DTPs (parental cells treated with crizotinib) and PC9-derived DTPs (parental cells treated with erlotinib). (B) RNA expression levels of ALDH family members in GTL16 parental cells and GTL16-derived DTPs (parental cells treated with crizotinib) and PC9 parental cells and PC9-derived DTPs (parental cells treated with erlotinib). (C) ALDHlA1 is upregulated GTL-16-derived DTP (parental cells treated with crizotinib) compared to GTL-16 parental cells. Conversely, ALDHlA1 expression is not significantly expressed in PC9-derived DTP (parental cells treated with erlotinib) or PC9 parental cells, and there is no significant change in expression levels of ALDHlA1 in PC9-derived DTP (parental cells treated with erlotinib) compared to PC9 parental cells.

[0026] Figure 9. IALDH inhibitor Gossypol significantly reduces drug tolerant cells. (A) Parental PC9 cells were treated with 2uM erlotinib and 1.5uM Gossypol either alone or combination for 8 days. (B) Parental GTL-16 cells were treated with 1uM crizotinib and 1.5uM Gossypol either alone or combination for 17 days. The bar graphs representing quantitative measurements of cell viability by Syto60 assay from triplicate wells per treatment show similar to DS but weaker effect of gossypol on drug tolerant cells.

DETAILED DESCRIPTION

1. Definitions

[0027] As used herein, the term "ALDH" or "aldehyde dehydrogenase" refers to an enzyme or a class of enzymes which are capable of oxidizing aldehydes. Aldehyde dehydrogenase (ALDH) (Enzyme Commission 1.2.1.3) is an enzyme responsible for oxidizing intracellular aldehydes and plays a role in metabolism of ethanol, vitamin A, cyclophosphamide and other oxazaphosphorines. Examples of ALDH enzymes in humans include ALDH1A1, ALDH1A2, ALDH1A3, ALDH1B1, ALDH1L1, ALDH1L2, ALDH2, ALDH3A1, ALDH3A2, ALDH3B1, ALDH3B2, ALDH4A1, ALDH5A1, ALDH6A1, ALDH7A1, ALDH8A1, ALDH9A1, ALDH16A1, ALDH18A1. The term "wild type ALDH" generally refers to a polypeptide comprising the amino acid sequence of a naturally occurring ALDH protein.

[0028] The terms "HER2", "ErbB2" "c-Erb-B2" are used interchangeably. Unless indicated otherwise, the terms "ErbB2" "c-Erb-B2" and "HER2" when used herein refer to the human protein, and "erbB2," "c-erb-B2," and "her2" refer to human gene. The human erbB2 gene and ErbB2 protein are, for example, described in Semba et al., PNAS (USA) 82:6497-6501 (1985) and Yamamoto et al. Nature
319:230-234 (1986) (Genebank accession number X03363). ErbB2 comprises four domains (Domains 1-4). The term "wild type HER2" generally refers to a polypeptide comprising the amino acid sequence of a naturally occurring HER2 protein.

[0029] By "EGFR" is meant the receptor tyrosine kinase polypeptide Epidermal Growth Factor Receptor which is described in Ullrich et al, Nature (1984) 309:418425, alternatively referred to as Her-1 and the c-erbB gene product, as well as variants thereof such as EGFRvIII. Variants of EGFR also include deletional, substitutional and insertional variants, for example those described in Lynch et al. (NEJM 2004, 350:2129), Paez et al. (Science 2004, 304:1497), Pao et al. (PNAS 2004, 101:13306). The term "wild type EGFR" generally refers to a polypeptide comprising the amino acid sequence of a naturally occurring EGFR protein.

[0030] The term "c-met" or "Met", as used herein, refers, unless indicated otherwise, to any native or variant (whether native or synthetic) c-met polypeptide. The term "wild type c-met" generally refers to a polypeptide comprising the amino acid sequence of a naturally occurring c-met protein.

[0031] The term "BRAF", as used herein, refers, unless indicated otherwise, to any native or variant (whether native or synthetic) BRAF polypeptide. The term "wild type BRAF" generally refers to a polypeptide comprising the amino acid sequence of a naturally occurring BRAF protein.

[0032] The term "ALK" refers to Anaplastic Lymphoma Kinase. ALK (Anaplastic Lymphoma Kinase) (GenBank accession Number: AB209477, UniProt Accession No. Q9UM73) is a receptor tyrosine kinase. This protein (which is 1620 amino acids long in humans) has a transmembrane domain in the central part and has a carboxyl-terminal tyrosine kinase region and an amino-terminal extracellular domain (Oncogene. 1997 Jan. 30; 14 (4): 439-49). See Pulford et al., J. of Cellular Physiol., 199:330-358, 2004 for a comprehensive review relating to ALK. The full-length ALK sequence is disclosed in U.S. Pat. No. 5,770,421. The term "wild type ALK" generally refers to a polypeptide comprising the amino acid sequence of a naturally occurring ALK protein.

[0033] An "antagonist" (interchangeably termed "inhibitor") of a polypeptide of interest is an agent that interferes with activation or function of the polypeptide of interest, e.g., partially or fully blocks, inhibits, or neutralizes a biological activity mediated by a polypeptide of interest. For example, an antagonist of polypeptide X may refers to any molecule that partially or fully blocks, inhibits, or neutralizes a biological activity mediated by polypeptide X. Examples of inhibitors include antibodies; ligand antibodies; small molecule antagonists; antisense and inhibitory RNA (e.g., shRNA) molecules. Preferably, the inhibitor is an antibody or small molecule which binds to the polypeptide of interest. In a particular embodiment, an inhibitor has a binding affinity (dissociation constant) to the polypeptide of
interest of about 1,000 nM or less. In another embodiment, inhibitor has a binding affinity to the polypeptide of interest of about 100 nM or less. In another embodiment, an inhibitor has a binding affinity to the polypeptide of interest of about 50 nM or less. In a particular embodiment, an inhibitor is covalently bound to the polypeptide of interest. In a particular embodiment, an inhibitor inhibits signaling of the polypeptide of interest with an IC_{50} of 1,000 nM or less. In another embodiment, an inhibitor inhibits signaling of the polypeptide of interest with an IC_{50} of 500 nM or less. In another embodiment, an inhibitor inhibits signaling of the polypeptide of interest with an IC_{50} of 50 nM or less. In certain embodiments, the antagonist reduces or inhibits, by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more, the expression level or biological activity of the polypeptide of interest.

[0034] As used herein, the term "targeted therapeutic" refers to a therapeutic agent that binds to polypeptide(s) of interest and inhibits the activity and/or activation of the specific polypeptide(s) of interest. Examples of such agents include antibodies and small molecules that bind to the polypeptide of interest. In some embodiments, the targeted therapeutic is a TKI. In some embodiments, the TKI is a RTKI.

[0035] A "tyrosine kinase inhibitor" or "TKI" refers to an agent that interferes with activation or function mediated by the tyrosine kinase activity of a tyrosine kinase, e.g., partially or fully blocks, inhibits, or neutralizes a biological activity mediated by the tyrosine kinase activity of a tyrosine kinase.

[0036] A "receptor tyrosine kinase inhibitor" or "RTKI" refers to an agent that interferes with activation or function mediated by the tyrosine kinase activity of a receptor tyrosine kinase, e.g., partially or fully blocks, inhibits, or neutralizes a biological activity mediated by the tyrosine kinase activity of a receptor tyrosine kinase.

[0037] The term "polypeptide" as used herein, refers to any native polypeptide of interest from any vertebrate source, including mammals such as primates (e.g., humans) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses "full-length," unprocessed polypeptide as well as any form of the polypeptide that results from processing in the cell. The term also encompasses naturally occurring variants of the polypeptide, e.g., splice variants or allelic variants.

[0038] "Polynucleotide," or "nucleic acid," as used interchangeably herein, refer to polymers of nucleotides of any length, and include DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a polymer by DNA or RNA polymerase, or by a synthetic reaction. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and their analogs. If present, modification to the nucleotide structure may be imparted before or after assembly of the polymer. The
sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified after synthesis, such as by conjugation with a label. Other types of modifications include, for example, "caps", substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as, for example, those with uncharged linkages (e.g., methyl phosphonates, phosphoriesters, phosphoamidates, carbamates, etc.) and with charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), those containing pendant moieties, such as, for example, proteins (e.g., nucleases, toxins, antibodies, signal peptides, ply-L-lysine, etc.), those with intercalators (e.g., acridine, psoralen, etc.), those containing chelators (e.g., metals, radioactive metals, boron, oxidative metals, etc.), those containing alkylators, those with modified linkages (e.g., alpha anomic nucleic acids, etc.), as well as unmodified forms of the polynucleotide(s). Further, any of the hydroxyl groups ordinarily present in the sugars may be replaced, for example, by phosphonate groups, phosphate groups, protected by standard protecting groups, or activated to prepare additional linkages to additional nucleotides, or may be conjugated to solid or semi-solid supports. The 5' and 3' terminal OH can be phosphorylated or substituted with amines or organic capping group moieties of from 1 to 20 carbon atoms. Other hydroxyls may also be derivatized to standard protecting groups. Polynucleotides can also contain analogous forms of ribose or deoxyribose sugars that are generally known in the art, including, for example, 2'-0-methyl-, 2'-0-allyl, 2'-fluoro- or 2'-azido-ribose, carbocyclic sugar analogs, anomic sugars, epimeric sugars such as arabinose, xyloses or lycose, pyranose sugars, furanose sugars, sedoheptuloses, acyclic analogs and abasic nucleoside analogs such as methyl riboside. One or more phosphodiester linkages may be replaced by alternative linking groups. These alternative linking groups include, but are not limited to, embodiments wherein phosphate is replaced by P(0)S("thioate"), P(S)S ("dithioate"), "(0)NR₂ ("amidate"), P(0)R, P(0)OR, CO or CH₂ ("formacetal"), in which each R or R' is independently H or substituted or unsubstituted alkyl (1-20 C) optionally containing an ether (-0-) linkage, aryl, alkenyl, cycloalkyl, cycloalkenyl or araldyl. Not all linkages in a polynucleotide need be identical. The preceding description applies to all polynucleotides referred to herein, including RNA and DNA.

[0039] The term "small molecule" refers to any molecule with a molecular weight of about 2000 daltons or less, preferably of about 500 daltons or less.

[0040] An "isolated" antibody is one which has been separated from a component of its natural environment. In some embodiments, an antibody is purified to greater than 95% or 99% purity as determined by, for example, electrophoretic (e.g., SDS-PAGE, isoelectric focusing (IEF), capillary
electrophoresis) or chromatographic (e.g., ion exchange or reverse phase HPLC). For review of methods for assessment of antibody purity, see, e.g., Flatman et al., J. Chromatogr. B 848:79-87 (2007).

The term "antibody" herein is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments so long as they exhibit the desired antigen-binding activity.

The terms anti-polypeptide of interest antibody and "an antibody that binds to" a polypeptide of interest refer to an antibody that is capable of binding a polypeptide of interest with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting a polypeptide of interest. In one embodiment, the extent of binding of an anti-polypeptide of interest antibody to an unrelated, non-polypeptide of interest protein is less than about 10% of the binding of the antibody to a polypeptide of interest as measured, e.g., by a radioimmunoassay (RIA). In certain embodiments, an antibody that binds to a polypeptide of interest has a dissociation constant (Kd) of ≤ 1μM, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, ≤ 0.1 nM, ≤ 0.01 nM, or ≤ 0.001 nM (e.g., 10^-6 M or less, e.g., from 10^-8 M to 10^-13 M, e.g., from 10^-9 M to 10^-13 M). In certain embodiments, an anti-polypeptide of interest antibody binds to an epitope of a polypeptide of interest that is conserved among polypeptides of interest from different species.

A "blocking antibody" or an "antagonist antibody" is one which inhibits or reduces biological activity of the antigen it binds. Preferred blocking antibodies or antagonist antibodies substantially or completely inhibit the biological activity of the antigen.

"Affinity" refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (e.g., an antibody) and its binding partner (e.g., an antigen). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g., antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (Kd). Affinity can be measured by common methods known in the art, including those described herein. Specific illustrative and exemplary embodiments for measuring binding affinity are described in the following.

An "antibody fragment" refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, (ab')2; diabodies; linear antibodies; single-chain antibody molecules (e.g., scFv); and multispecific antibodies formed from antibody fragments.
An "antibody that binds to the same epitope" as a reference antibody refers to an antibody that blocks binding of the reference antibody to its antigen in a competition assay by 50% or more, and conversely, the reference antibody blocks binding of the antibody to its antigen in a competition assay by 50% or more.

The term "chimeric" antibody refers to an antibody in which a portion of the heavy and/or light chain is derived from a particular source or species, while the remainder of the heavy and/or light chain is derived from a different source or species.

The terms "full length antibody," "intact antibody," and "whole antibody" are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure or having heavy chains that contain an Fc region.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variant antibodies, e.g., containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. Thus, the modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including but not limited to the hybridoma method, recombinant DNA methods, phage-display methods, and methods utilizing transgenic animals containing all or part of the human immunoglobulin loci, such methods and other exemplary methods for making monoclonal antibodies.

A "human antibody" is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human or a human cell or derived from a non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues.

A "humanized" antibody refers to a chimeric antibody comprising amino acid residues from non-human HVRs and amino acid residues from human FRs. In certain embodiments, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the HVRs (e.g., CDRs) correspond to those of a non-human antibody, and all or
substantially all of the FRs correspond to those of a human antibody. A humanized antibody optionally may comprise at least a portion of an antibody constant region derived from a human antibody. A "humanized form" of an antibody, e.g., a non-human antibody, refers to an antibody that has undergone humanization.

[0052] An "immunoconjugate" is an antibody conjugated to one or more heterologous molecule(s), including but not limited to a cytotoxic agent.

[0053] "Individual response" or "response" can be assessed using any endpoint indicating a benefit to the individual, including, without limitation, (1) inhibition, to some extent, of disease progression (e.g., cancer progression), including slowing down and complete arrest; (2) a reduction in tumor size; (3) inhibition (i.e., reduction, slowing down or complete stopping) of cancer cell infiltration into adjacent peripheral organs and/or tissues; (4) inhibition (i.e. reduction, slowing down or complete stopping) of metasisis; (5) relief, to some extent, of one or more symptoms associated with the disease or disorder (e.g., cancer); (6) increase in the length of progression free survival; and/or (9) decreased mortality at a given point of time following treatment.

[0054] The term "substantially the same," as used herein, denotes a sufficiently high degree of similarity between two numeric values, such that one of skill in the art would consider the difference between the two values to be of little or no biological and/or statistical significance within the context of the biological characteristic measured by said values (e.g., Kd values or expression). The difference between said two values is, for example, less than about 50%, less than about 40%, less than about 30%, less than about 20%, and/or less than about 10% as a function of the reference/comparator value.

[0055] The phrase "substantially different," as used herein, denotes a sufficiently high degree of difference between two numeric values such that one of skill in the art would consider the difference between the two values to be of statistical significance within the context of the biological characteristic measured by said values (e.g., Kd values). The difference between said two values is, for example, greater than about 10%, greater than about 20%, greater than about 30%, greater than about 40%, and/or greater than about 50% as a function of the value for the reference/comparator molecule.

[0056] An "effective amount" of a substance/molecule, e.g., pharmaceutical composition, refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result.

[0057] A "therapeutically effective amount" of a substance/molecule may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the substance/molecule to elicit a desired response in the individual. A therapeutically effective amount is also one in which any
toxic or detrimental effects of the substance/molecule are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically but not necessarily, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

[0058] The term "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

[0059] A "pharmaceutically acceptable carrier" refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

[0060] The phrase "pharmaceutically acceptable salt" as used herein, refers to pharmaceutically acceptable organic or inorganic salts of a compound.

[0061] As used herein, "treatment" (and grammatical variations thereof such as "treat" or "treating") refers to clinical intervention in an attempt to alter the natural course of the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, antibodies of the invention are used to delay development of a disease or to slow the progression of a disease.

[0062] The term "anti-cancer therapy" refers to a therapy useful in treating cancer. Examples of anti-cancer therapeutic agents include, but are limited to, e.g., chemotherapeutic agents, growth inhibitory agents, cytotoxic agents, agents used in radiation therapy, anti-angiogenesis agents, apoptotic agents, anti-tubulin agents, and other agents to treat cancer, anti-CD20 antibodies, platelet derived growth factor inhibitors (e.g., Gleevec™(Imatinib Mesylate)), a COX-2 inhibitor (e.g., celecoxib), interferons, cytokines, antagonists (e.g., neutralizing antibodies) that bind to one or more of the following targets PDGFR-beta, BlyS, APRIL, BCMA receptor(s), TRAIL/Apo2, and other bioactive and organic chemical agents, etc. Combinations thereof are also included in the invention.

[0063] The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents a cellular function and/or causes cell death or destruction. The term is intended to include radioactive isotopes
(e.g., $\text{At}^{211}$, $\text{Al}^{31}$, $\text{Nd}^{125}$, $\text{Y}^{90}$, $\text{Re}^{186}$, $\text{Re}^{188}$, $\text{Sm}^{153}$, $\text{Bi}^{212}$, $\text{Pb}^{212}$, and radioactive isotopes of Lu), chemotherapeutic agents or drugs (e.g., methotrexate, adriamycin, vinca alkaloids (vincristine, vinblastine, etoposide), doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents), growth inhibitory agents, enzymes and fragments thereof such as nucleolytic enzymes, antibiotics, and toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof, and the various antitumor or anticancer agents disclosed below. Other cytotoxic agents are described below. A tumoricidal agent causes destruction of tumor cells.

A "chemotherapeutic agent" refers to a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclophosphamide (CYTOXAN®); alkyl sulfonates such as busulfan, imposulfan and pipsulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide and trimethylomelamine; acetogenins (especially bullatacin and bullatacinone); delta-9-tetrahydrocannabinol (dronabinol, MARINOL®); beta-lapachone; lapachol; colchicines; betulinic acid; a camptothecin (including the synthetic analogue topotecan (HYCAMTIN®, CPT-11 (irinotecan, CAMPTOSAR®), acetylcamptothecin, scopolectin, and 9-aminocamptothecin); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); podophyllotoxin; podophyllinic acid; teniposide; cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlorambazine, chlorophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosoureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimustine; antibiotics such as the enediyne antibiotics (e.g., calicheamicin, especially calicheamicin gammall and calicheamicin omegall (see, e.g., Nicolaou et al., Angew. Chem. Int. Ed. Engl., 33: 183-186 (1994)); CDP323, an oral alpha-4 integrin inhibitor; dynemicin, including dynemicin A; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabican, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including ADRIAMYCIN®, morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin, doxorubicin HC1 liposome injection (DOXIL®), liposomal
doxorubicin TLC D-99 (MYOCET®), peglylated liposomal doxorubicin (CAELYX®), and
deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin
C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, porfiromycin, puromycin, quelamycin,
rodorubicin, streptonigrin, streptozocin, tuberculid, ubenimex, zinostatin, zorubicin; anti-metabolites
such as methotrexate, gemcitabine (GEMZAR®), tegafur (UFTORAL®), capecitabine (XELODA®), an
epitholone, and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate,
pteroterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine,
thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine,
dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as clustosterone, dromostanolone
propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane,
trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside;
aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine;
demecolcine; diaziquone; elfomithine; elliptinium acetate; an epitholone; etogluclid; gallium nitrate;
hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone;
mitoxantrone; mopidannol; nitaerine; pentostatin; phenamet; pirurubicin; losoxantrone; 2-
ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, OR);
razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2'-
trichlorotriethylamine; trichotheccenes (especially T-2 toxin, verrucarin A, rosidin A and anguidine);
urethan; vindesine (ELDISINE®, FILDESIN®); dacarbazine; mammomustine; mitobronitol; mitolactol;
pipobroman; gacytosine; arabinoside ("Ara-C"); thiotepa; taxoid, e.g., paclitaxel (TAXOL®), albumin-
engineered nanoparticle formulation of paclitaxel (ABRAXANE™), and docetaxel (TAXOTERE®);
chlorambucil; 6-thioguanine; mercaptopurine; methotrexate; platinum agents such as cisplatin,
oxaliplatin (e.g., ELOXATIN®), and carboplatin; vincas, which prevent tubulin polymerization from
forming microtubules, including vinblastine (VELBAN®), vincristine (ONCOVIN®), vindesine
(ELDISINE®, FILDESIN®), and vinorelbine (NAVELBINE®); etoposide (VP-16); ifosfamide;
mitoxantrone; leucovorin; novantronе; edatrexate; daunomycin; aminopterin; ibandronate;
topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid,
including bexarotene (TARGETTIN®); bisphosphonates such as clodronate (for example, BONEFOS®
or OSTAT®), etidronate (DIDROCAL®, NE-58095, zoledronic acid/zoledronate (ZOMETA®),
alendronate (FOSAMAX®), pamidronate (AREDIA®), tiludronate (SKELED®, or risedronate
(ACTONEL®); trox citabine (a 1,3-dioxolane nucleoside cytosine analog); antisense oligonucleotides,
particularly those that inhibit expression of genes in signaling pathways implicated in aberrant cell
proliferation, such as, for example, PKC-alpha, Raf, H-Ras, and epidermal growth factor receptor (EGF-R); vaccines such as THERATOPE® vaccine and gene therapy vaccines, for example, ALLOVECTIN® vaccine, LEUVECTIN® vaccine, and VAXID® vaccine; topoisomerase 1 inhibitor (e.g., LURTOTECAN®); rmRH (e.g., ABARELIX®); BAY439006 (sorafenib; Bayer); SU-11248 (sunitinib, SUTENT®, Pfizer); perifosine, COX-2 inhibitor (e.g., celecoxib or etoricoxib), proteosome inhibitor (e.g., PS341); bortezomib (VELCADE®); CCI-779; tipifarnib (R11577); orafenib, ABT510; Bcl-2 inhibitor such as oblimersen sodium (GENASENSE®); pixantrone; EGFR inhibitors (see definition below); tyrosine kinase inhibitors (see definition below); serine-threonine kinase inhibitors such as rapamycin (sirolimus, RAPAMUNE®); farnesyltransferase inhibitors such as lonafarnib (SCH 6636, SARASAR™); and pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above such as CHOP, an abbreviation for a combined therapy of cyclophosphamide, doxorubicin, vincristine, and prednisolone; and FOLFOX, an abbreviation for a treatment regimen with oxaliplatin (ELOXATIN™) combined with 5-FU and leucovorin.

Chemotherapeutic agents as defined herein include "anti-hormonal agents" or "endocrine therapeutics" which act to regulate, reduce, block, or inhibit the effects of hormones that can promote the growth of cancer. They may be hormones themselves, including, but not limited to: anti-estrogens with mixed agonist/antagonist profile, including, tamoxifen (NOLVADEX®), 4-hydroxytamoxifen, toremifene (FARESTON®), idoxifene, droloxifene, raloxifene (EVISTA®), trioxifene, keoxifene, and selective estrogen receptor modulators (SERMs) such as SERM3; pure anti-estrogens without agonist properties, such as fulvestrant (FASLODEX®), and EM800 (such agents may block estrogen receptor (ER) dimerization, inhibit DNA binding, increase ER turnover, and/or suppress ER levels); aromatase inhibitors, including steroidal aromatase inhibitors such as formestane and exemestane (AROMASIN®), and nonsteroidal aromatase inhibitors such as anastrazole (ARIMIDEX®, letrozole (FEMARA®) and aminoglutethimide, and other aromatase inhibitors include vorozole (RIVISOR®), megestrol acetate (MEGASE®, fadrozole, and 4(5)-imidazoles; lutenizing hormone-releaseing hormone agonists, including leuprolide (LUPRON® and ELIGARD®), goserelin, buserelin, and tript erelin; sex steroids, including progestines such as megestrol acetate and medroxyprogesterone acetate, estrogens such as diethylstilbestrol and premarin, and androgens/retinoids such as fluoxymesterone, all transretionic acid and fenretinide; onapristone; anti-progesterones; estrogen receptor down-regulators (ERDs); anti-androgens such as flutamide, nilutamide and bicalutamide; and pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above.
The term "prodrug" as used in this application refers to a precursor or derivative form of a pharmaceutically active substance that is less cytotoxic to tumor cells compared to the parent drug and is capable of being enzymatically activated or converted into the more active parent form. See, e.g., Wilman, "Prodrugs in Cancer Chemotherapy" Biochemical Society Transactions, 14, pp. 375-382, 615th Meeting Belfast (1986) and Stella et al., "Prodrugs: A Chemical Approach to Targeted Drug Delivery," Directed Drug Delivery, Borchardt et al., (ed.), pp. 247-267, Humana Press (1985). The prodrugs of this invention include, but are not limited to, phosphate-containing prodrugs, thiophosphate-containing prodrugs, sulfate-containing prodrugs, peptide-containing prodrugs, D-amino acid-modified prodrugs, glycosylated prodrugs, β-lactam-containing prodrugs, optionally substituted phenoxycetamide-containing prodrugs or optionally substituted phenylacetamide-containing prodrugs, 5-fluorocytosine and other 5-fluorouridine prodrugs which can be converted into the more active cytotoxic free drug. Examples of cytotoxic drugs that can be derivatized into a prodrug form for use in this invention include, but are not limited to, those chemotherapeutic agents described above.

A "growth inhibitory agent" when used herein refers to a compound or composition which inhibits growth of a cell (e.g., a cell whose growth is dependent upon the activity of the polypeptide of interest either in vitro or in vivo). Examples of growth inhibitory agents include agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), taxanes, and topoisomerase II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechloretamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in THE MOLECULAR BASIS OF CANCER, Mendelsohn and Israel, eds., Chapter 1, entitled "Cell cycle regulation, oncogenes, and antineoplastic drugs" by Murakami et al. (WB Saunders: Philadelphia, 1995), especially p. 13. The taxanes (paclitaxel and docetaxel) are anticancer drugs both derived from the yew tree. Docetaxel (TAXOTERE®, Rhone-Poulenc Rorer), derived from the European yew, is a semisynthetic analogue of paclitaxel (TAXOL®, Bristol-Myers Squibb). Paclitaxel and docetaxel promote the assembly of microtubules from tubulin dimers and stabilize microtubules by preventing depolymerization, which results in the inhibition of mitosis in cells.
treatment. Typical treatments are given as a one-time administration and typical dosages range from 10 to 200 units (Grays) per day.

[0069] An "individual" or "subject" is a mammal. Mammals include, but are not limited to, domesticated animals (e.g., cows, sheep, cats, dogs, and horses), primates (e.g., humans and non-human primates such as monkeys), rabbits, and rodents (e.g., mice and rats). In certain embodiments, the individual or subject is a human.

[0070] The term "concomitantly" is used herein to refer to administration of two or more therapeutic agents, give in close enough temporal proximity where their individual therapeutic effects overlap in time. Accordingly, concurrent administration includes a dosing regimen when the administration of one or more agent(s) continues after discontinuing the administration of one or more other agent(s). In some embodiments, the concomitantly administration is concurrently, sequentially, and/or simultaneously.

[0071] By "reduce or inhibit" is meant the ability to cause an overall decrease of 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, or greater. Reduce or inhibit can refer to the symptoms of the disorder being treated, the presence or size of metastases, or the size of the primary tumor.

[0072] The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products.

[0073] An "article of manufacture" is any manufacture (e.g., a package or container) or kit comprising at least one reagent, e.g., a medicament for treatment of a disease or disorder (e.g., cancer), or a probe for specifically detecting a biomarker described herein. In certain embodiments, the manufacture or kit is promoted, distributed, or sold as a unit for performing the methods described herein.

[0074] As is understood by one skilled in the art, reference to "about" a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se. For example, description referring to "about X" includes description of "X".

[0075] It is understood that aspect and embodiments of the invention described herein include "consisting" and/or "consisting essentially of" aspects and embodiments. As used herein, the singular form "a", "an", and "the" includes plural references unless indicated otherwise.

II. Methods and Uses

[0076] Provided herein are methods utilizing an ALDH inhibitor (e.g., disulfiram and/or derivatives thereof) and a targeted therapeutic (e.g., TKI) for treating cancer.
[0077] In particular, provided herein are methods of treating cancer in an individual comprising concomitantly administering to the individual an ALDH inhibitor (e.g., disulfiram and/or derivatives thereof) and a targeted therapeutic (e.g., TKI). In some embodiments, the respective amounts of the ALDH inhibitor (e.g., disulfiram and/or derivatives thereof) and the targeted therapeutic (e.g., TKI) are effective to increase the period of cancer sensitivity and/or delay the development of cell resistance to the targeted therapeutic (e.g., TKI). In some embodiments, the respective amounts of the ALDH inhibitor (e.g., disulfiram and/or derivatives thereof) and the targeted therapeutic (e.g., TKI) are effective to increase efficacy of a cancer treatment comprising a targeted therapeutic (e.g., TKI). For example, in some embodiments, the respective amounts of the ALDH inhibitor (e.g., disulfiram and/or derivatives thereof) and the targeted therapeutic (e.g., TKI) are effective to increased efficacy compared to a standard treatment comprising administering an effective amount of the targeted therapeutic (e.g., TKI) without (in the absence of) the ALDH inhibitor (e.g., disulfiram and/or derivatives thereof). In some embodiments, the respective amounts of the ALDH inhibitor (e.g., disulfiram and/or derivatives thereof) and the targeted therapeutic (e.g., TKI) are effective to increased response (e.g., complete response) compared to a standard treatment comprising administering an effective amount of the targeted therapeutic (e.g., TKI) without (in the absence of) the ALDH inhibitor (e.g., disulfiram and/or derivatives thereof). In some embodiments, the targeted therapeutic is a tyrosine kinase inhibitor (TKI). In some embodiments, the TKI is an EGFR inhibitor, HER2 inhibitor, MET/HGF inhibitor, ALK inhibitor, BRAF inhibitor, ROS1 inhibitor, and/or MEK inhibitor. In some embodiments, the TKI is a receptor tyrosine kinase inhibitor (RTKI). In some embodiments, the RTKI is an EGFR inhibitor, HER2 inhibitor, MET inhibitor, and/or ALK inhibitor.

[0078] Further provided herein are methods of increasing efficacy of a cancer treatment comprising a targeted therapeutic (e.g., TKI) in an individual comprises concomitantly administering to the individual an effective amount of the targeted therapeutic (e.g., TKI) and an effective amount of an ALDH inhibitor (e.g., disulfiram and/or derivatives thereof). Provided herein are also methods of treating cancer in an individual wherein cancer treatment comprising concomitantly administering to the individual an effective amount of targeted therapeutic (e.g., TKI) and an effective amount of an ALDH inhibitor (e.g., disulfiram and/or derivatives thereof), wherein the cancer treatment has increased efficacy compared to a standard treatment comprising administering an effective amount of the targeted therapeutic (e.g., TKI) without (in the absence of) the ALDH inhibitor (e.g., disulfiram and/or derivatives thereof). In some embodiments, the targeted therapeutic is a TKI. In some embodiments, the TKI is an EGFR inhibitor, HER2 inhibitor, MET/HGF inhibitor, ALK inhibitor, BRAF inhibitor, ROS1
inhibitor, and/or MEK inhibitor. In some embodiments, the TKI is a RTKI. In some embodiments, the RTKI is an EGFR inhibitor, HER2 inhibitor, MET/HGF inhibitor, and/or ALK inhibitor.

[0079] In addition, provided herein are methods of delaying and/or preventing development of cancer resistance to a targeted therapeutic (e.g., TKI) in an individual, comprising concomitantly administering to the individual an effective amount of an ALDH inhibitor (e.g., disulfiram and/or derivatives thereof) and an effective amount of the targeted therapeutic (e.g., TKI). Provided herein are also methods of increasing sensitivity to a targeted therapeutic (e.g., TKI) in an individual comprising concomitantly administering to the individual an effective amount of an ALDH inhibitor (e.g., disulfiram and/or derivatives thereof) and an effective amount of the targeted therapeutic (e.g., TKI). In some embodiments, the targeted therapeutic is a TKI. In some embodiments, the TKI is an EGFR inhibitor, HER2 inhibitor, MET/HGF inhibitor, ALK inhibitor, BRAF inhibitor, ROS1 inhibitor, and/or MEK inhibitor. In some embodiments, the TKI is a RTKI. In some embodiments, the RTKI is an EGFR inhibitor, HER2 inhibitor, MET/HGF inhibitor, and/or ALK inhibitor.

[0080] Further, provided herein are methods of extending the period of a targeted therapeutic (e.g., TKI) sensitivity in an individual with cancer comprising concomitantly administering to the individual an effective amount of an ALDH inhibitor (e.g., disulfiram and/or derivatives thereof) and an effective amount of the targeted therapeutic (e.g., TKI). Provided herein are also methods of extending the duration of response to a targeted therapeutic (e.g., TKI) in an individual with cancer comprising concomitantly administering to the individual an effective amount of an ALDH inhibitor (e.g., disulfiram and/or derivatives thereof) and an effective amount of the targeted therapeutic (e.g., TKI). In some embodiments, the targeted therapeutic is a TKI. In some embodiments, the TKI is an EGFR inhibitor, HER2 inhibitor, MET/HGF inhibitor, ALK inhibitor, BRAF inhibitor, ROS1 inhibitor, and/or MEK inhibitor. In some embodiments, the TKI is a RTKI. In some embodiments, the RTKI is an EGFR inhibitor, HER2 inhibitor, MET/HGF inhibitor, and/or ALK inhibitor.

[0081] In some embodiments of any of the methods, the ALDH inhibitor and/or targeted therapeutic (e.g., TKI) is an antibody, binding polypeptide, binding small molecule, or polynucleotide such as those described herein. In some embodiments, the ALDH inhibitor is disulfiram and/or derivatives thereof. In some embodiments, the ALDH inhibitor is disulfiram. In some embodiments of any of the methods, the ALDH inhibitor is gossypol and/or an ALDH-inhibiting derivative or metabolite thereof. In some embodiments, the ALDH inhibitor is gossypol. In some embodiments, the ALDH inhibitor is 2,2'-bis-(Formyl-1,6,7-trihydroxy-5-isopropyl-3-methylnaphthalene) or pharmaceutically acceptable salt thereof.
In some embodiments, the ALDH inhibitor is 2,2'-bis-(Formyl-1,6,7-trihydroxy-5-isopropyl-3-methylnaphthalene).

[0082] Cancer having resistance to a therapy as used herein includes a cancer which is not responsive and/or reduced ability of producing a significant response (e.g., partial response and/or complete response) to the therapy. Resistance may be acquired resistance which arises in the course of a treatment method. In some embodiments, the acquired drug resistance is transient and/or reversible drug tolerance. Transient and/or reversible drug resistance to a therapy includes wherein the drug resistance is capable of regaining sensitivity to the therapy after a break in the treatment method. In some embodiments, the acquired resistance is permanent resistance. Permanent resistance to a therapy includes a genetic change conferring drug resistance. Permanent resistance can occur as a result of treatment with general chemotherapies-cyclophosphomide, platinum agent, and/or taxol.

[0083] Cancer having sensitivity to a therapy as used herein includes cancer which is responsive and/or capable of producing a significant response (e.g., partial response and/or complete response).

[0084] Methods of determining of assessing acquisition of resistance and/or maintenance of sensitivity to a therapy are known in the art and described in the Examples. Changes in acquisition of resistance and/or maintenance of sensitivity such as drug tolerance may be assessed by assaying the growth of drug tolerant persisters as described in the Examples and Sharma et al. Changes in acquisition of resistance and/or maintenance of sensitivity such as permanent resistance and/or expanded resisters may be assessed by assaying the growth of drug tolerant expanded persisters as described in the Examples and Sharma et al. In some embodiments, resistance may be indicated by a change in IC50, EC50 or decrease in tumor growth in drug tolerant persisters and/or drug tolerant expanded persisters. In some embodiments, the change is greater than about any of 50%, 100%, and/or 200%. In addition, changes in acquisition of resistance and/or maintenance of sensitivity may be assessed in vivo for examples by assessing response, duration of response, and/or time to progression to a therapy, e.g., partial response and complete response. Changes in acquisition of resistance and/or maintenance of sensitivity may be based on changes in response, duration of response, and/or time to progression to a therapy in a population of individuals, e.g., number of partial responses and complete responses.

[0085] In some embodiments of any of the methods, the cancer is a solid tumor cancer. In some embodiments, the cancer is gastric cancer. In some embodiments, the cancer is lung cancer (e.g., non-small cell lung cancer (NSCL)). In some embodiments, the cancer is breast cancer. In some embodiments, the cancer is colorectal cancer (e.g., colon cancer and/or rectal cancer). In some embodiments, the cancer is basel cell carcinoma. In some embodiments of any of the cancers, the cancer
is adenocarcinoma. The cancer in any of the combination therapies methods described herein when starting the method of treatment comprising the ALDH inhibitor (e.g., disulfiram and/or derivatives thereof) and the targeted therapeutic (e.g., TKI) may be sensitive (examples of sensitive include, but are not limited to, responsive and/or capable of producing a significant response (e.g., partial response and/or complete response)) to a method of treatment comprising the targeted therapeutic alone. The cancer in any of the combination therapies methods described herein when starting the method of treatment comprising the ALDH inhibitor (e.g., disulfiram and/or derivatives thereof) and the targeted therapeutic (e.g., TKI) may not be resistant (examples of resistance include, but are not limited to, not responsive and/or reduced ability and/or incapable of producing a significant response (e.g., partial response and/or complete response)) to a method of treatment comprising the targeted therapeutic alone. 

[0086] In some embodiments of any of the methods, the individual according to any of the above embodiments may be a human. 

[0087] In some embodiments of any of the methods, the combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate formulations), and separate administration, in which case, administration of the antagonist of the invention can occur prior to, simultaneously, sequentially, concurrently and/or following, administration of the additional therapeutic agent and/or adjuvant. In some embodiments, the combination therapy further comprises radiation therapy and/or additional therapeutic agents. 

[0088] An ALDH inhibitor (e.g., disulfiram and/or derivatives thereof) and/or targeted therapeutic (e.g., TKI) described herein can be administered by any suitable means, including oral, parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. Dosing can be by any suitable route, e.g., by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein. 

[0089] An ALDH inhibitor (e.g., disulfiram and/or derivatives thereof) and/or targeted therapeutic (e.g., TKI) described herein may be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The ALDH inhibitor (e.g., disulfiram and/or
derivatives thereof) and/or targeted therapeutic (e.g., TKI) need not be, but is optionally formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of the ALDH inhibitor (e.g., disulfiram and/or derivatives thereof) and/or targeted therapeutic (e.g., TKI) present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

[0090] For the prevention or treatment of disease, the appropriate dosage of an ALDH inhibitor (e.g., disulfiram and/or derivatives thereof) and/or targeted therapeutic (e.g., TKI) described herein (when used alone or in combination with one or more other additional therapeutic agents) will depend on the type of disease to be treated, the severity and course of the disease, whether the ALDH inhibitor (e.g., disulfiram and/or derivatives thereof) and/or targeted therapeutic (e.g., TKI) is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the ALDH inhibitor (e.g., disulfiram and/or derivatives thereof), and the discretion of the attending physician. The ALDH inhibitor (e.g., disulfiram and/or derivatives thereof) is suitably administered to the patient at one time or over a series of treatments. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. Such doses may be administered intermittently, e.g., every week or every three weeks (e.g., such that the patient receives from about two to about twenty, or e.g., about six doses of the ALDH inhibitor (e.g., disulfiram and/or derivatives thereof)) and/or targeted therapeutic (e.g., TKI). An initial higher loading dose, followed by one or more lower doses may be administered. An exemplary dosing regimen comprises administering. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

[0091] It is understood that any of the above formulations or therapeutic methods may be carried out using an immunoconjugate as the ALDH inhibitor and/or targeted therapeutic (e.g., TKI).

III. Therapeutic Compositions

[0092] Provided herein are combinations comprising an ALDH inhibitor (e.g., disulfiram and/or derivatives thereof) and a targeted therapeutic (e.g., TKI). In one aspect, there is provided a pharmaceutical product comprising a) as a first component an effective amount of an ALDH inhibitor, and b) as a second component an effective amount of a targeting agent (targeted therapeutic) for the concomitant or sequential use for the treatment of cancer. In certain embodiments, the combination increases the efficacy of the targeted therapeutic administered alone. In certain embodiments, the
combination delays and/or prevents development of cancer resistance to the targeted therapeutic. In certain embodiments, the combination extends the period of the targeted therapeutic sensitivity in an individual with cancer.

[0093] Also provided herein are ALDH inhibitors and/or targeted therapeutics useful in the combination therapy methods described herein. In some embodiments, the ALDH inhibitors and/or targeted therapeutics are an antibody, binding polypeptide, binding small molecule, and/or polynucleotide.

[0094] In some embodiments of any of the combination therapy methods described herein, the ALDH inhibitor inhibits one or more of ALDH1A1, ALDH1A2, ALDH1A3, ALDH1B1, ALDH1L1, ALDH1L2, ALDH2, ALDH3A1, ALDH3A2, ALDH3B1, ALDH3B2, ALDH4A1, ALDH5A1, ALDH6A1, ALDH7A1, ALDH8A1, ALDH9A1, ALDH16A1, and/or ALDH18A1. In some embodiments, ALDH inhibitors according to the invention are compounds that are capable of inhibiting the activity of one or more of the several isozymes of ALDH. In some embodiments, the ALDH inhibitor is a pan-ALDH inhibitor. ALDH inhibitors include, but are not limited to, disulfiram, coprine, cyanamide, 1-aminocyclopropanol (ACP), daidzin (i.e., the 7-glucoside of 4',7-dihydroxyisoflavone), cephalosporins, antidiabetic sulfonyl ureas, metronidazole, diethyldithiocarbamate, phenethyl isothiocyanate (PEITC), prunetin (4',5-dihydroxy-7-methoxyisoflavone), 5-hydroxydaidzin (genistin), and any of their metabolites or analogs exhibiting ALDH-inhibiting activity. In another embodiment, the ALDH inhibitor is disulfiram or an ALDH-inhibiting metabolite thereof. Such metabolites include, e.g., S-methyl N,N-diethyldithiocarbamates, S-methyl N,N-diethyldithiocarbamate sulfoxide, and S-methyl N,N-diethylthiocarbamates sulfoxide. In some embodiments, the ALDH inhibitor is disulfiram. In some embodiments of any of the methods, the ALDH inhibitor is gossypol and/or an ALDH-inhibiting derivative or metabolite thereof. In some embodiments, the ALDH inhibitor is gossypol. In some embodiments, the ALDH inhibitor is 2,2'-bis-(Formyl-1,6,7-trihydroxy-5-isopropyl-3-methylnaphthalene) or pharmaceutically acceptable salt thereof. In some embodiments, the ALDH inhibitor is 2,2'-bis-(Formyl-1,6,7-trihydroxy-5-isopropyl-3-methylnaphthalene).

[0095] ALDH inhibitors also include compounds of the formula:
wherein:

$R_1$ is selected from the group consisting of hydrogen, carboxy, halo, branched or unbranched (C$_1$-$C_6$)haloalkyl, (C$_3$-$C_6$)cycloalkoxy, (Ci-$C_6$)haloalkoxy, (C$_3$-$C_6$)cyclohaloalkoxy, (C$_3$-$C_6$)cycloalkoxyalkyl, (Cr-$C_6$)alkoxy(C$_3$-$C_6$)cycloalkyl, (C$_3$-$C_6$)cycloalkylcarbonyl, substituted or unsubstituted phenyl, phenyl (Ci-$C_6$)alkyl, heterocyclyl, and heterocyclyloxy, heterocyclylcarbonyl, wherein substituents are from one to four and are selected from the group consisting of halo, aminocarbonyl, aminothiocarbonyl, carboxy, formyl, hydroxy, amino, carbamoyl, (Ci-$C_3$)alkyl, (Cj-$C_3$)haloalkyl, (Cr-$C_3$)alkoxy, (Cr-$C_3$)haloalkoxy, (C$_1$-$C_3$)alkylammonio, di(Ci-$C_3$)alkylammonio, (Ci-$C_2$)alkoxy(Ci-$C_2$)alkyl, (Cj-$C_2$)alkylamino(Ci-$C_2$)alkyl, di(C$_1$-$C_2$)alkylamino(C$_1$-$C_2$)alkyl, (Ci-$C_3$)alkylcarbonyl, (C$_i$-$C_3$)alkoxycarbonyl, (Ci-$C_3$)alkylammonocarbonyl, and di (Ci-$C_3$)alkylaminocarbonyl;

$R_2$ is selected from the group consisting of hydrogen and alkoxy;

$R_3$ is selected from the group consisting of hydrogen Ci-$C_6$ alkoxy carbonyl, carboxy and sugar;
R₄ is selected from the group consisting of hydrogen and hydroxide;

R₅ is selected from the group consisting of hydrogen, carboxy, hydroxy, halo, branched or unbranched (C₁-C₆)alkyl, (C₁-C₆)haloalkyl, (C₂-C₆)alkenyl, (C₃-C₆)alkadienyl, (C₁-C₆)alkoxy, (C₁-C₆)cycloalkoxy, (C₁-C₆)haloalkoxy, (C₂-C₆)cycloalkoxyalkyl, (C₁-C₆)alkoxy(C₁-C₆)cycloalkyl, (C₁-C₆)alkylcarbonyl, (C₁-C₆)alkoxycarbonyl, (C₄-C₆)alkoxyalkyl, substituted or unsubstituted phenyl, phenyl(C₁-C₆)alkyl, heterocycl, heterocyclyloxy, heterocyclylcarbonyl, wherein substituents are from one to four and are selected from the group consisting of halo, aminocarbonyl, aminothiocarbonyl, carboxy, formyl, hydroxy, amino, carbamoyl, (C₁-C₃)alkyl, (C₁-C₃)haloalkyl, (C₁-C₃)alkoxy, (C₁-C₃)haloalkoxy, (C₁-C₃)alkylamino, di(C₁-C₃)alkylamino, (C₁-C₃)alkoxy(C₁-C₃)alkyl, (C₁-C₃)alkylamino(C₁-C₃)alkyl, di(C₁-C₃)alkylamino(C₁-C₃)alkyl, (C₁-C₃)alkylcarbonyl, (C₁-C₃)alkoxycarbonyl, (C₁-C₃)alkylaminocarbonyl, and di(C₁-C₃)alkylaminocarbonyl;

Rₑ is selected from the group consisting of hydrogen and hydroxide; and

R₇ is selected from the group consisting of hydrogen, halogen, and C₁-C₆ alkoxy.


[0097] ALDH inhibitors also include compounds of the formula:
wherein R1, R2 and R3, independently represent a saturated or unsaturated linear or branched Ci-C6 alkyl radical, or a salt thereof.

[0098] ALDH inhibitors also include 4-amino-4-methyl-2-pentynethioic acid (S)-methyl ester, and salts thereof.

[0099] In some embodiments of any of the combination therapy methods described herein, the targeted therapeutic is a TKI. In some embodiments, the TKI is an EGFR inhibitor, HER2 inhibitor, MET/HGF inhibitor, ALK inhibitor, BRAF inhibitor, ROSI inhibitor, and/or MEK inhibitor. In some embodiments, the TKI is a RTKI. In some embodiments, the RTKI is an EGFR inhibitor, HER2 inhibitor, MET/HGF inhibitor, and/or ALK inhibitor.

[0100] In some embodiments of any of the combination therapy methods described herein, the targeted therapeutic is an EGFR inhibitor. Exemplary EGFR inhibitors (anti-EGFR antibodies) include antibodies such as humanized monoclonal antibody known as nimotuzumab (YM Biosciences), fully human ABX-EGF (panitumumab, Abgenix Inc.) as well as fully human antibodies known as El.l, E2.4, E2.5, E6.2, E6.4, E2.11, E6.3 and E7.6. 3 and described in US 6,235,883; MDX-447 (Medarex Inc). Pertuzumab (2C4) is a humanized antibody that binds directly to HER2 but interferes with HER2-EGFR dimerization thereby inhibiting EGFR signaling. Other examples of antibodies which bind to EGFR include GA201 (RG7160; Roche Glycart AG), MAb 579 (ATCC CRL HB 8506), MAb 455 (ATCC CRL HB8507), MAb 225 (ATCC CRL 8508), MAb 528 (ATCC CRL 8509) (see, US Patent No. 4,943, 533, Mendelsohn et al.) and variants thereof, such as chimerized 225 (C225 or Cetuximab; ERBUTIX®) and reshaped human 225 (H225) (see, WO 96/40210, Imclone Systems Inc.); IMC-1 IF8, a fully human, EGFR-targeted antibody (Imclone); antibodies that bind type II mutant EGFR (US Patent No. 5,212,290); humanized and chimeric antibodies that bind EGFR as described in US Patent No. 5,891,996; and human antibodies that bind EGFR, such as ABX-EGF (see WO98/50433, Abgenix); EMD 55900 (Stragliotto et al. Eur. J. Cancer 32A:636-640 (1996)); EMD7200 (matuzumab) a humanized EGFR antibody directed against EGFR that competes with both EGF and TGF-alpha for EGFR binding; and mAb 806 or humanized mAb 806 (Johns et al., J. Biol. Chem. 279(29):30375-30384 (2004)). The anti-EGFR antibody may be conjugated with a cytotoxic agent, thus generating an immunoconjugate (see, e.g., EP659,439A2, Merck Patent GmbH). In some embodiments, the anti-EGFR antibody is cetuximab. In some embodiments, the anti-EGFR antibody is panitumumab. In some embodiments, the anti-EGFR antibody is zalutumumab, nimotuzumab, and/or matuzumab.

[0101] Anti-EGFR antibodies that are useful in the methods include any antibody that binds with sufficient affinity and specificity to EGFR and can reduce or inhibit EGFR activity. The antibody selected will normally have a sufficiently strong binding affinity for EGFR, for example, the antibody may bind human c-met with a Kd value of between 100 nM-1 pM. Antibody affinities may be determined by a surface plasmon...
resonance based assay (such as the BIAcore assay as described in PCT Application Publication No. WO2005/012359); enzyme-linked immunoabsorbent assay (ELISA); and competition assays (e.g., RIA's), for example. Preferably, the anti-EGFR antibody of the invention can be used as a therapeutic agent in targeting and interfering with diseases or conditions wherein EGFR/EGFR ligand activity is involved. Also, the antibody may be subjected to other biological activity assays, e.g., in order to evaluate its effectiveness as a therapeutic. Such assays are known in the art and depend on the target antigen and intended use for the antibody. In some embodiments, an EGFR arm may be combined with an arm which binds to a triggering molecule on a leukocyte such as a T-cell receptor molecule (e.g. CD2 or CD3), or Fc receptors for IgG (FcyR), such as FcyRI (CD64), FcyRII (CD32) and FcyRIII (CD16) so as to focus cellular defense mechanisms to the EGFR-expressing cell. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express EGFR. These antibodies possess an EGFR-binding arm and an arm which binds the cytotoxic agent (e.g. saporin, anti-interferon-a, vinca alkaloid, ricin A chain, methotrexate or radioactive isotope hapten). Bispecific antibodies can be prepared as full length antibodies or antibody fragments (e.g., F(ab')2 bispecific antibodies).

[0102] Exemplary EGFR inhibitors also include small molecules such as compounds described in US5616582, US5457105, US5475001, US5654307, US5679683, US6084095, US6265410, US6455534, US6521620, US6596726, US6713484, US5770599, US6140332, US5866572, US6399602, US6344459, US6602863, US6391874, WO9814451, WO9850038, WO9909016, WO9924037, WO9935146, WO0132651, US6344455, US5760041, US6002008, and/or US5747498. Particular small molecule EGFR antagonists include OSI-774 (CP-358774, erlotinib, OSI Pharmaceuticals); PD 198305 (CI 1033, 2-propenamide, N-[4-[(3-chloro-4-fluorophenyl)amino]-7-[3-(4-morpholinyl)propoxy]-6-quinazolynyl]-, dihydrochloride, Pfizer Inc.); Iressa® (ZD1839, gefitinib, AstraZeneca); ZM 105180 ((6-amino-4-(3-methylphenylamino)-quinazoline, Zeneca); BIBX-1382 (N8-(3-chloro-4-fluoro-phenyl)-N2-(1-methylpiperidin-4-yl)-pyrirriido[5,4-d]pyrimidine-2,8-diamine, Boehringer Ingelheim); PKI-166 ((R)-4-[[1-phenylethyl]amino]-1H-pyrrolo[2,3-d]pyrimidin-6-yl]-phenol); (R)-6-(4-hydroxyphenyl)-4-[[1-phenylethyl]amino]-7H-pyrrolo[2,3-d]pyrimidine; CL-387785 (N-[4-[(3-bromophenyl)amino]-6-quinazolynyl]-2-butyramidie); EKB-569 (N-[4-[(3-chloro-4-fluorophenyl)amino]-3-cyano-7-ethoxy-6-quinolynyl]-4-(dimethylamino)-2-butenamide); lapatinib (Tykerb, GlaxoSmithKline); ZD6474 (Zactima, AstraZeneca); CUDC-101 (Curis); canertinib (CI-1033); AEE788 (6-[4-[(4-ethyl-1-piperazinyl)methyl]phenyl]-N-[[(I)-1-phenylethyl]-7H-pyrrolo[2,3-d]pyrimidin-4-amine, WO20030 13541, Novartis) and PKI166 4-[[1-phenylethyl]amino]-7H-pyrrolo[2,3-d]pyrimidin-6-yl]-phenol, WO9702266 Novartis). In some embodiments, the EGFR antagonist is N-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)-4-quinazolinamine and/or a pharmaceutical acceptable salt thereof (e.g., N-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)-4-quinazolinamine-HCl). In some embodiments, the EGFR
antagonist is gefitinib and/or a pharmaceutical acceptable salt thereof. In some embodiments, the EGFR antagonist is lapatinib and/or a pharmaceutical acceptable salt thereof. In some embodiments, the EGFR antagonist is gefitinib and/or erlotinib.

[0103] In some embodiments of any of the combination therapy methods described herein, the targeted therapeutic is an HGF/MET inhibitor. Exemplary HGF/MET inhibitors (anti-HGF and/or anti-MET antibodies) include antibodies such as anti-MET antibodies disclosed in WO05/016382 (including but not limited to antibodies 13.3.2, 9.1.2, 8.70.2, 8.90.3); an anti-MET antibodies produced by the hybridoma cell line deposited with ICLC number PD 03001 at the CBA in Genoa, or that recognizes an epitope on the extracellular domain of the β chain of the HGF receptor, and said epitope is the same as that recognized by the monoclonal antibody; anti-met antibodies disclosed in WO2007/126799 (including but not limited to 04536, 05087, 05088, 05091, 05092, 04687, 05097, 05098, 05100, 05101, 04541, 05093, 05094, 04537, 05102, 05105, 04696, 04682); anti met antibodies disclosed in WO2009/007427 (including but not limited to an antibody deposited at CNCM, Institut Pasteur, Paris, France, on March 14, 2007 under the number 1-3731, on March 14, 2007 under the number 1-3732, on July 6, 2007 under the number 1-3786, on March 14, 2007 under the number 1-3724; an anti-met antibody disclosed in 20110129481; an anti-met antibody disclosed in US201 10104176; an anti-met antibody disclosed in WO2009/134776; an anti-met antibody disclosed in WO2010/059654; an anti-met antibody disclosed in WO201 1020925 (including but not limited to an antibody secreted from a hybridoma deposited at the CNCM, Institut Pasteur, Paris, France, on Mar. 12, 2008 under the number 1-3949 and the hybridoma deposited on January 14, 2010 under the number 1-4273); and/or MetMaB (onartuzumab) or a biosimilar version thereof (WO2006/015371; Jin et al, Cancer Res (2008) 68:4360). In some embodiments, the MET/HGF inhibitor is onartuzumab.

[0104] In some embodiments of any of the combination therapy methods described herein, the MET/HGF inhibitor is an anti-hepatocyte growth factor (HGF) antibody, for example, humanized anti-HGF antibody TAK701, rilotumumab, Ficlatuzumab, and/or humanized antibody 2B8 described in WO2007/143090. In some embodiments, the anti-HGF antibody is the anti-HGF antibody described in US7718174B2.

[0105] In certain embodiments of any of the combination therapy methods described herein, the MET/HGF inhibitor is any one of: SGX-523, Crizotinib (PF-02341066; 3-[1(IR)-l-(2,6-dichloro-3-fluorophenyl)ethoxy]-5-(1-piperidin-4-ylpyrazol-4-yl)pyridin-2-amine; CAS no. 877399-52-5); JNJ-38877605 (CAS no. 943540-75-8), BMS-698769, PHA-665752 (Pfizer), SU5416, INC-280 (Incyte; SU1 1274 (Sugen; [(3Z)-N-(3-chlorophenyl)-3-((3,5-dimethyl-4-[(4-methylpiperazin-1-yl)carbonyl]-1H-pyrrol-2-yl)methylene)-N-methyl-2-oxoindoline-5-sulfonamide; CAS no. 658084-23-2)), Foretinib (GSK1363089), XL880 (CAS no. 849217-64-7; XL880 is a inhibitor of MET/HFand VEGFR2 and KDR); MGCD-265 (MethylGene; MGCD-265 targets the met, VEGFR1, VEGFR2, VEGFR3, Ron and Tie-2
receptors; CAS no. 875337-44-3), Tivantinib (ARQ 197; (-)(3R,4R)-3-(5,6-dihydro-4H-pyrrolo[3,2,1-ij]quinolin-1-yl)-4-[(IH-indol-3-yl)pyrroldine-2,5-dione; see Munchi et al, Mol Cancer Ther June 2010 9; 1544; CAS no. 905854-02-6), LY-2801653 (Lilly), LY2875358 (Lilly), MP-470, Rilotumumab (AMG 102, anti-HGF monoclonal antibody), antibody 223C4 or humanized antibody 223C4 (WO2009/007427),

humanized L2G7 (humanized TAK701; humanized anti-HGF monoclonal antibody); EMD 1214063 (Merck Serono), EMD 1204831 (Merck Serono), NK4, Cabozantinib (XL-184, CAS no. 849217-68-1; carbozantinib is a dual inhibitor of MET/HGF and VEGFR2), MP-470 (SuperGen; is a novel inhibitor of c-KIT, MET, PDGFR, Flt3, and AXL), Comp-1, Ficlatuzumab (AV-299; anti-HGF monoclonal antibody), E7050 (Cas no. 1196681-49-8; E7050 is a dual MET/HGF and VEGFR2 inhibitor (Esai)). MK-2461 (Merck; N-((2R)-1,4-Dioxan-2-ylmethyl)-N-methyl-N’-[3-(1-methyl-1H-pyrazol-4-yl)-5-oxo-5H-benzo[4,5]cyclohepta[1,2-b]pyridin-7-yl]sulfamide; CAS no. 917879-39-1); MK8066 (Merck), PF4217903 (Pfizer), AMG208 (Amgen), SGX-126, RP1040, LY2801653, AMG458, EMD637830, BAY-853474, DP-3590. In certain embodiments, the met inhibitor is any one or more of crizotinib, tivantinib, carbozantinib, MGCD-265, ficlatuzumab, humanized TAK-701, rilotumumab, foretinib, h224Gill, DN-30, GDC-0712, MK-2461, E7050, MK-8033, PF-4217903, AMG208, JNJ-38877605, EMD1204831, INC-280, LY-2801653, SGX-126, RP1040, LY2801653, BAY-853474, and/or LA480. In certain embodiments, the met inhibitor is any one or more of crizotinib, tivantinib, carbozantinib, MGCD-265, ficlatuzumab, humanized TAK-701, rilotumumab, and/or foretinib. In some embodiments, the met inhibitor is crizotinib.

In some embodiments of any of the combination therapy methods described herein, the targeted therapeutic is a BRAF inhibitor. Exemplary BRAF inhibitors are known in the art and include, for example, sorafenib, PLX4720, PLX-3603, dabrafenib (GSK21 18436), GDC-0879, RAF265 (Novartis), XL281, ARQ736, BAY73-4506, vemurafenib and those described in WO2007/002325, WO2007/002433, WO20091 11278, WO20091 11279, WO20091 11277, WO20091 11280 and U.S. Pat. No. 7,491,829. In some embodiments, the BRAF inhibitor is a selective BRAF inhibitor. In some embodiments, the BRAF inhibitor is a selective inhibitor of BRAF V600. In some embodiments, BRAF V600 is BRAF V600E, BRAF V600K, and/or V600D. In some embodiments, BRAF V600 is BRAF V600R. In some embodiments, the BRAF inhibitor is vemurafenib. In some embodiments, the BRAF inhibitor is vemurafenib.

Vemurafenib (RG7204, PLX-4032, CAS Reg. No. 1029872-55-5) has been shown to cause programmed cell death in various cancer cell lines, for example melanoma cell lines. Vemurafenib interrupts the BRAF/MEK step on the BRAF/MEK/ERK pathway - if the BRAF has the common V600E mutation. Vemurafenib works in patients, for example in melanoma patients as approved by the FDA, whose cancer has a V600E BRAF mutation (that is, at amino acid position number 600 on the
BRAF protein, the normal valine is replaced by glutamic acid). About 60% of melanomas have the V600E BRAF mutation. The V600E mutation is present in a variety of other cancers, including lymphoma, colon cancer, melanoma, thyroid cancer and lung cancer. Vemurafenib has the following structure:

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CH3
O

[0108] ZELBORAF® (vemurafenib) (Genentech, Inc.) is a drug product approved in the U.S. and indicated for treatment of patients with unresectable or metastatic melanoma with BRAF V600E mutation as detected by an FDA-approved test. ZELBORAF® (vemurafenib) is not recommended for use in melanoma patients who lack the BRAF V600E mutation (wild-type BRAF melanoma).

In some embodiments of any of the combination therapy methods described herein, the targeted therapeutic is a MEK inhibitor. In some embodiments, the MEK inhibitor is a MEK1 inhibitor, MEK2 inhibitor, and/or MEK1/2 inhibitor. Exemplary MEK inhibitors include, but are not limited to, trametinib (GSK 1120212), MEK162, selumetinib (AZD 6244, ARRY-142886), pimasertib (MSC1936369B, AS-703026, AS703026), GDC-0973, GDC-0623, PD-325901, GDC-0973, CI-1040, PD035901. In some embodiments, the Mek inhibitor is selumetinib, pimasertib, GDC-0973, GDC-0623 or trametinib. In certain embodiments, the Mek inhibitor is GDC-0973.

GDC-0973 (XL518) is a selective inhibitor of MEK, also known as mitogen activated protein kinase kinase (MAPKK), which is a key component of the RAS/RAF/MEK/ERK pathway that is frequently activated in human tumors. Inappropriate activation of the MEK/ERK pathway promotes cell growth in the absence of exogenous growth factors. Clinical trials evaluating GDC-0973 for solid tumors is ongoing. GDC-0973 can be prepared as described in International Patent Application Publication Number WO2007044515(Al). GDC-0973 has the name: (S)-(3,4-difluoro-2-(2-fluoro-4-iodophenylamino)phenyl)(3-hydroxy-3-(piperidin-2-yl)azetidin-1-yl)methanone, and the following structure:

![Structure of GDC-0973](image)

Trametinib (GSK 1120212, CAS Registry No. 871700-17-3) has the name N-(3-{3-Cyclopropyl-5-[2-fluoro-4-iodophenylamino]-6,8-dimethyl-2,4,7-trioxo-3,4,6,7-tetrahydropyrido[4,3-d]pyrimidin-1(2H)-yl}phenyl)acetamide, and the following structure:

![Structure of Trametinib](image)
In some embodiments of any of the TKI and/or RTKI, the inhibitor may be a specific inhibitor for the polypeptide of interest, e.g., an inhibitor specific for EGFR, HER2, MET/HGF, ALK, BRAF, ROS1, and/or MEK. In some embodiments of any of the TKI and/or RTKI, the inhibitor may be a dual inhibitor or pan inhibitor wherein the TKI and/or RTKI inhibits one or more polypeptides of interest, e.g., an inhibitor specific for EGFR, HER2, MET/HGF, ALK, BRAF, ROS1, and/or MEK, and one or more other target polypeptides.

A. Antibodies

Provided herein isolated antibodies that bind to a polypeptide of interest, such as ALDH and/or tyrosine kinase (e.g., receptor tyrosine kinase), for use in the methods described herein. In any of the above embodiments, an antibody is humanized. Further, the antibody according to any of the above embodiments is a monoclonal antibody, including a chimeric, humanized or human antibody. In one embodiment, the antibody is an antibody fragment, e.g., a Fv, Fab, Fab', scFv, diabody, or F(ab')2 fragment. In another embodiment, the antibody is a full length antibody, e.g., an "intact IgGl" antibody or other antibody class or isotype as defined herein.

In a further aspect, an antibody according to any of the above embodiments may incorporate any of the features, singly or in combination, as described in Sections below:

1. Antibody Affinity

In certain embodiments, an antibody provided herein has a dissociation constant (Kd) of ≤ 1μM, ≤ 10 nM, ≤ 1 nM, ≤ 0.1 nM, ≤ 0.01 nM, or ≤ 0.001 nM (e.g., 10^-8 M or less, e.g., from 10^-8 M to 10^-13 M, e.g., from 10^-9 M to 10^-11 M). In one embodiment, Kd is measured by a radiolabeled antigen binding assay (RIA). In one embodiment, the RIA is performed with the Fab version of an antibody of interest and its antigen. For example, solution binding affinity of Fabs for antigen is measured by equilibrating Fab with a minimal concentration of (125I)-labeled antigen in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate (see, e.g., Chen et al., J. Mol. Biol. 293:865-881(1999)). To establish conditions for the assay, MICROTRITETER® multi-well plates (Thermo Scientific) are coated overnight with 5 μg/ml of a capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate (pH 9.6), and subsequently blocked with 2% (w/v) bovine serum albumin in PBS for two to five hours at room temperature (approximately 23°C). In a non-adsorbent plate (Nunc #269620), 100 pM or 26 pM 125I-antigen are mixed with serial dilutions of a Fab of interest (e.g., consistent with assessment of the anti-VEGF antibody, Fab-12, in Presta et al., Cancer Res. 57:4593-4599 (1997)). The Fab of interest is then incubated overnight; however, the incubation may continue for a longer period (e.g., about 65 hours) to ensure that
equilibrium is reached. Thereafter, the mixtures are transferred to the capture plate for incubation at room temperature (e.g., for one hour). The solution is then removed and the plate washed eight times with 0.1% polysorbate 20 (TWEEN-20®) in PBS. When the plates have dried, 150 µl/well of scintillant (MICROSCINT-20™; Packard) is added, and the plates are counted on a TOPCOUNT™ gamma counter (Packard) for ten minutes. Concentrations of each Fab that give less than or equal to 20% of maximal binding are chosen for use in competitive binding assays.

[0117] According to another embodiment, Kd is measured using a BIACORE® surface plasmon resonance assay. For example, an assay using a BIACORE®-2000 or a BIACORE®-3000 (BIAcore, Inc., Piscataway, NJ) is performed at 25°C with immobilized antigen CM5 chips at -10 response units (RU).

In one embodiment, carboxymethylated dextran biosensor chips (CM5, BIAcore, Inc.) are activated with N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) according to the supplier's instructions. Antigen is diluted with 10 mM sodium acetate, pH 4.8, to 5 µg/ml (-0.2 µM) before injection at a flow rate of 5 µl/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% polysorbate 20 (TWEEN-20™) surfactant (PBST) at 25°C at a flow rate of approximately 25 µl/min. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a simple one-to-one Langmuir binding model (BIACORE® Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (Kd) is calculated as the ratio k_{off}/k_{on}. See, e.g., Chen et al., J. Mol. Biol. 293:865-881 (1999). If the on-rate exceeds 10^6 M^-1 s^-1 by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation = 295 nm; emission = 340 nm, 16 nm band-pass) at 25°C of a 20 nM anti-antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophotometer (Aviv Instruments) or a 8000-series SLM-AMINCO™ spectrophotometer (ThermoSpectronic) with a stirred cuvette.

2. Antibody Fragments

[0118] In certain embodiments, an antibody provided herein is an antibody fragment. Antibody fragments include, but are not limited to, Fab, Fab', Fab'-SH, F(ab')_2, Fv, and scFv fragments, and other fragments described below. For a review of certain antibody fragments, see Hudson et al. Nat. Med. 9:129-134 (2003). For a review of scFv fragments, see, e.g., Pluckthiin, in The Pharmacology of

Diabodies are antibody fragments with two antigen-binding sites that may be bivalent or bispecific. See, for example, EP 404,097; WO 1993/01161; Hudson et al., Nat. Med. 9:129-134 (2003); and Hollinger et al., Proc. Natl. Acad. Sci. USA 90: 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al., Nat. Med. 9:129-134 (2003).

Single-domain antibodies are antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, MA; see, e.g., U.S. Patent No. 6,248,516 Bl).

Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells (e.g., E. coli or phage), as described herein.

3. Chimeric and Humanized Antibodies

In certain embodiments, an antibody provided herein is a chimeric antibody. Certain chimeric antibodies are described, e.g., in U.S. Patent No. 4,816,567; and Morrison et al., Proc. Natl. Acad. Sci. USA, 81:6851-6855 (1984). In one example, a chimeric antibody comprises a non-human variable region (e.g., a variable region derived from a mouse, rat, hamster, rabbit, or non-human primate, such as a monkey) and a human constant region. In a further example, a chimeric antibody is a "class switched" antibody in which the class or subclass has been changed from that of the parent antibody. Chimeric antibodies include antigen-binding fragments thereof.

In certain embodiments, a chimeric antibody is a humanized antibody. Typically, a non-human antibody is humanized to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. Generally, a humanized antibody comprises one or more variable domains in which HVRs, e.g., CDRs, (or portions thereof) are derived from a non-human antibody, and FRs (or portions thereof) are derived from human antibody sequences. A humanized antibody optionally will also comprise at least a portion of a human constant region. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (e.g., the antibody from which the HVR residues are derived), e.g., to restore or improve antibody specificity or affinity.


4. Human Antibodies

[0126] In certain embodiments, an antibody provided herein is a human antibody. Human antibodies can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk and van de Winkel, Curr. Opin. Pharmacol. 5:368-74 (2001) and Lonberg, Curr. Opin. Immunol. 20:450-459 (2008).

[0127] Human antibodies may be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic mice, the endogenous immunoglobulin loci have generally been inactivated. For review of methods for obtaining human antibodies from transgenic animals, see Lonberg, Nat. Biotech. 23:1117-1125 (2005). See also, e.g., U.S. Patent Nos. 6,075,181 and 6,150,584 describing XENOMOUSE™ technology; U.S. Patent No. 5,770,429 describing HuMab® technology; U.S. Patent No. 7,041,870 describing K-M MOUSE® technology, and U.S. Patent Application Publication No. US 2007/0061900, describing
VelociMouse® technology). Human variable regions from intact antibodies generated by such animals may be further modified, e.g., by combining with a different human constant region.


Human antibodies may also be generated by isolating Fv clone variable domain sequences selected from human-derived phage display libraries. Such variable domain sequences may then be combined with a desired human constant domain. Techniques for selecting human antibodies from antibody libraries are described below.

5. Library-Derived Antibodies


In certain phage display methods, repertoires of VH and VL genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter et al., Ann. Rev. Immunol., 12: 433-455 (1994). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab...
fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (e.g., from human) to provide a single source of antibodies to a wide range of non-self and also self antigens without any immunization as described by Griffiths et al., EMBO J., 12: 725-734 (1993).


[0132] Antibodies or antibody fragments isolated from human antibody libraries are considered human antibodies or human antibody fragments herein.

6. Multispecific Antibodies

[0133] In certain embodiments, an antibody provided herein is a multispecific antibody, e.g., a bispecific antibody. Multispecific antibodies are monoclonal antibodies that have binding specificities for at least two different sites. In certain embodiments, one of the binding specificities is a polypeptide of interest, such as ALDH and/or tyrosine kinase (e.g., receptor tyrosine kinase), and the other is for any other antigen. In certain embodiments, bispecific antibodies may bind to two different epitopes of a polypeptide of interest, such as ALDH and/or tyrosine kinase (e.g., receptor tyrosine kinase). Bispecific antibodies may also be used to localize cytotoxic agents to cells which express a polypeptide of interest, such as ALDH and/or tyrosine kinase (e.g., receptor tyrosine kinase). Bispecific antibodies can be prepared as full length antibodies or antibody fragments.

[0134] Techniques for making multispecific antibodies include, but are not limited to, recombinant co-expression of two immunoglobulin heavy chain-light chain pairs having different specificities (see Milstein and Cuello, Nature 305: 537 (1983)), WO 93/08829, and Traunecker et al., EMBO J. 10: 3655 (1991)), and "knob-in-hole" engineering (see, e.g., U.S. Patent No. 5,731,168). Multi-specific antibodies may also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (WO 2009/089004A1); cross-linking two or more antibodies or fragments (see, e.g., US Patent No. 4,676,980, and Brennan et al., Science, 229: 81 (1985)); using leucine zippers to produce bispecific antibodies (see, e.g., Kostelny et al., J. Immunol., 148(5): 1547-1553 (1992)); using "diabody" technology for making bispecific antibody fragments (see, e.g., Hollinger et al., Proc. Natl. Acad. Sci. USA 90:6444-6448 (1993)); and using single-chain Fv (sFv) dimers (see,e.g., Gruber et al., J. Immunol.,

[0135] Engineered antibodies with three or more functional antigen binding sites, including "Octopus antibodies," are also included herein (see, e.g., US 2006/0025576A1).

[0136] The antibody or fragment herein also includes a "Dual Acting FAb" or "DAF" comprising an antigen binding site that binds to a polypeptide of interest, such as ALDH and/or tyrosine kinase (e.g., receptor tyrosine kinase), as well as another, different antigen (see, US 2008/0069820, for example).

7. Antibody Variants
   a) Glycosylation variants

[0137] In certain embodiments, an antibody provided herein is altered to increase or decrease the extent to which the antibody is glycosylated. Addition or deletion of glycosylation sites to an antibody may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

[0138] Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the Fc region. See, e.g., Wright et al. TIBTECH 15:26-32 (1997). The oligosaccharide may include various carbohydrates, e.g., mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the "stem" of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in an antibody of the invention may be made in order to create antibody variants with certain improved properties.

[0139] In one embodiment, antibody variants are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. For example, the amount of fucose in such antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (e.g. complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (Eu numbering of Fc region residues); however, Asn297 may also be located about ± 3 amino acids upstream or downstream of position 297, i.e., between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function. See, e.g., US Patent Publication Nos. US 2003/0157108 (Presta, L.); US 2004/0093621 (Kyowa Hakko Kogyo Co.,

[0140] Antibodies variants are further provided with bisected oligosaccharides, e.g., in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by GlcNAc. Such antibody variants may have reduced fucosylation and/or improved ADCC function. Examples of such antibody variants are described, e.g., in WO 2003/01 1878 (Jean-Maires et al.); US Patent No. 6,602,684 (Umana et al.); and US 2005/0123546 (Umana et al.). Antibody variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, e.g., in WO 1997/30087 (Patel et al.); WO 1998/58964 (Raju, S.); and WO 1999/22764 (Raju, S.).

b) Fc region variants

[0141] In certain embodiments, one or more amino acid modifications may be introduced into the Fc region of an antibody provided herein, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (e.g., a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (e.g., a substitution) at one or more amino acid positions.

[0142] In certain embodiments, the invention contemplates an antibody variant that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half life of the antibody in vivo is important yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. In vitro and/or in vivo cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express FcRIII only, whereas monocytes express FcRI, FcRII and FcRIII. FcR expression on hematopoietic cells is
summarized in Table 3 on page 464 of Ravetch and Kinet, Annu. Rev. Immunol. 9:457-492 (1991). Non-limiting examples of in vitro assays to assess ADCC activity of a molecule of interest is described in U.S. Patent No. 5,500,362 (see, e.g., Hellstrom, I. et al. Proc. Nat'l Acad. Sci. USA 83:7059-7063 (1986)) and Hellstrom, I et al., Proc. Natl Acad. Sci. USA 82: 1499-1502 (1985); 5,821,337 (see Bruggemann, M. et al., J. Exp. Med. 166:1351-1361 (1987)). Alternatively, non-radioactive assays methods may be employed (see, for example, ACTITM non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA; and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, WI). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed in vivo, e.g., in an animal model such as that disclosed in Clynes et al. Proc. Nat'l Acad. Sci. USA 95:652-656 (1998). Clq binding assays may also be carried out to confirm that the antibody is unable to bind Clq and hence lacks CDC activity. See, e.g., Clq and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro et al., J. Immunol. Methods 202:163 (1996); Cragg, M.S. et al., Blood 101:1045-1052 (2003); and Cragg, M.S. and MJ. Glennie, Blood 103:2738-2743 (2004)). FcRn binding and in vivo clearance/half life determinations can also be performed using methods known in the art (see, e.g., Petkova, S.B. et al., Int'l. Immunol. 18(12): 1759-1769 (2006)).

[0143] Antibodies with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Patent No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (US Patent No. 7,332,581).

[0144] Certain antibody variants with improved or diminished binding to FcRs are described. (See, e.g., U.S. Patent No. 6,737,056; WO 2004/056312, and Shields et al., J. Biol. Chem. 9(2): 6591-6604 (2001).) In certain embodiments, an antibody variant comprises an Fc region with one or more amino acid substitutions which improve ADCC, e.g., substitutions at positions 298, 333, and/or 334 of the Fc region (EU numbering of residues). In some embodiments, alterations are made in the Fc region that result in altered (i.e., either improved or diminished) Clq binding and/or Complement Dependent Cytotoxicity (CDC), e.g., as described in US Patent No. 6,194,551, WO 99/51642, and Idusogie et al. J. Immunol. 164: 4178-4184 (2000).
[0145] Antibodies with increased half-lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., J. Immunol. 117:587 (1976) and Kim et al., J. Immunol. 24:249 (1994)), are described in US2005/0014934A1 (Hinton et al.). Those antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Such Fc variants include those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434, e.g., substitution of Fc region residue 434 (US Patent No. 7,371,826). See also Duncan & Winter, Nature 322:738-40 (1988); U.S. Patent No. 5,648,260; U.S. Patent No. 5,624,821; and WO 94/29351 concerning other examples of Fc region variants.

[0146] In certain embodiments, it may be desirable to create cysteine engineered antibodies, e.g., "thioMAbs," in which one or more residues of an antibody are substituted with cysteine residues. In particular embodiments, the substituted residues occur at accessible sites of the antibody. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the antibody and may be used to conjugate the antibody to other moieties, such as drug moieties or linker-drug moieties, to create an immunonoconjugate, as described further herein. In certain embodiments, any one or more of the following residues may be substituted with cysteine: V205 (Kabat numbering) of the light chain; A18 (EU numbering) of the heavy chain; and S400 (EU numbering) of the heavy chain Fc region. Cysteine engineered antibodies may be generated as described, e.g., in U.S. Patent No. 7,521,541.

B. Immunonoconjugates

[0147] Further provided herein are immunonoconjugates comprising antibody which binds a polypeptide of interest, such as ALDH and/or tyrosine kinase (e.g., receptor tyrosine kinase), or immunonoconjugates comprising an antibody which binds a polypeptide of interest, such as ALDH and/or tyrosine kinase (e.g., receptor tyrosine kinase), conjugated to one or more cytotoxic agents, such as chemotherapeutic agents or drugs, growth inhibitory agents, toxins (e.g., protein toxins, enzymatically active toxins of bacterial, fungal, plant, or animal origin, or fragments thereof), or radioactive isotopes for use in the methods described herein.

[0148] In one embodiment, an immunonoconjugate is an antibody-drug conjugate (ADC) in which an antibody is conjugated to one or more drugs, including but not limited to a maytansinoid (see U.S. Patent Nos. 5,208,020, 5,416,064 and European Patent EP 0 425 235 B1); an auristatin such as

[0149] In another embodiment, an immunoconjugate comprises an antibody as described herein conjugated to an enzymatically active toxin or fragment thereof, including but not limited to diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from Pseudomonas aeruginosa), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthrin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, saponaaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes.

[0150] In another embodiment, an immunoconjugate comprises an antibody as described herein conjugated to a radioactive atom to form a radioconjugate. A variety of radioactive isotopes are available for the production of radioconjugates. Examples include At<sup>211</sup>, I<sup>131</sup>, I<sup>125</sup>, Y<sup>90</sup>, Re<sup>186</sup>, Re<sup>188</sup>, Sm<sup>153</sup>, Bi<sup>212</sup>, Pd<sup>32</sup>, Pb<sup>212</sup> and radioactive isotopes of Lu. When the radioconjugate is used for detection, it may comprise a radioactive atom for scintigraphic studies, for example Te<sup>99m</sup> or I<sup>123</sup>, or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, mri), such as iodine-123 again, iodine-131, indium-III, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron.

[0151] Conjugates of an antibody and cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-l-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidade HC1), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene).
For example, a ricin immunotoxin can be prepared as described in Vitetta et al., Science 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyl diethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See W094/1 1026. The linker may be a "cleavable linker" facilitating release of a cytotoxic drug in the cell.

For example, an acid-labile linker, peptidase-sensitive linker, photolabile linker, dimethyl linker or disulfide-containing linker (Chad et al., Cancer Res. 52:127-131 (1992); U.S. Patent No. 5,208,020) may be used.

[0152] The immunoconjugates or ADCs herein expressly contemplate, but are not limited to such conjugates prepared with cross-linker reagents including, but not limited to, BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and SVSB (succinimidyl-(4-vinylsulfone)benzoate) which are commercially available (e.g., from Pierce Biotechnology, Inc., Rockford, IL., U.S.A).

C. Binding Polypeptides

[0153] Binding polypeptides are polypeptides that bind, preferably specifically, to a polypeptide of interest, such as ALDH and/or tyrosine kinase (e.g., receptor tyrosine kinase), are also provided for use in the methods described herein as described herein. In some embodiments, the binding polypeptides are antagonists of a polypeptide of interest, such as ALDH and/or tyrosine kinase (e.g., receptor tyrosine kinase).

[0154] Binding polypeptides may be chemically synthesized using known polypeptide synthesis methodology or may be prepared and purified using recombinant technology. Binding polypeptides are usually at least about 5 amino acids in length, alternatively at least about 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, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 amino acids in length or more, wherein such binding polypeptides that are capable of binding, preferably specifically, to a polypeptide of interest, such as ALDH and/or tyrosine kinase (e.g., receptor tyrosine kinase).

[0155] Binding polypeptides may be identified without undue experimentation using well known techniques. In this regard, it is noted that techniques for screening polypeptide libraries for binding polypeptides that are capable of specifically binding to a polypeptide target are well known in the art (see, e.g., U.S. Patent Nos. 5,556,762, 5,750,373, 4,708,871, 4,833,092, 5,223,409, 5,403,484,

Methods of generating peptide libraries and screening these libraries are also disclosed in U.S. Patent Nos. 5,723,286, 5,432,018, 5,580,717, 5,427,908, 5,498,530, 5,770,434, 5,734,018, 5,698,426, 5,763,192, and 5,723,323.

D. Binding Small Molecules

[0156] Provided herein are binding small molecules for use as a small molecule ALDH inhibitor (e.g., disulfiram and/or derivatives thereof) and/or small molecule targeted therapeutic (e.g., small molecule TKI (e.g., small molecule RTKI) for use in the methods described above.

[0157] Binding small molecules are preferably organic molecules other than binding polypeptides or antibodies as defined herein that bind, preferably specifically, to a polypeptide of interest, such as ALDH and/or tyrosine kinase (e.g., receptor tyrosine kinase). Binding organic small molecules may be identified and chemically synthesized using known methodology (see, e.g., PCT Publication Nos. WO00/00823 and WO00/39585). Binding organic small molecules are usually less than about 2000 daltons in size, alternatively less than about 1500, 750, 500, 250 or 200 daltons in size, wherein such organic small molecules that are capable of binding, preferably specifically, to a polypeptide as described herein may be identified without undue experimentation using well known techniques. In this regard, it is noted that techniques for screening organic small molecule libraries for molecules that are capable of binding to a polypeptide of interest are well known in the art (see, e.g., PCT Publication Nos. WO00/00823 and WO00/39585). Binding organic small molecules may be, for example, aldehydes, ketones, oximes, hydrazones, semicarbazones, carbazides, primary amines, secondary amines, tertiary amines, N-substituted hydrazines, hydrazides, alcohols, ethers, thiols, thioethers, disulfides, carboxylic acids, esters, amides, ureas, carbamates, carbonates, ketals, thioketals, acetics, thioacetals, aryl halides, aryl sulfonates, alkyl halides, alkyl sulfonates, aromatic compounds, heterocyclic compounds, anilines, alkenes, alkynes, diols, amino alcohols, oxazolidines, oxazolines, thiazolidines, thiazolines, enamines, sulfonamides, epoxides, aziridines, isocyanates, sulfonyl chlorides, diazo compounds, acid chlorides, or the like.
E. Antagonist Polynucleotides

[0158] Provided herein are also polynucleotide antagonists for use in the methods described herein. The polynucleotide may be an antisense nucleic acid and/or a ribozyme. The antisense nucleic acids comprise a sequence complementary to at least a portion of an RNA transcript of a gene of interest, such as ALDH and/or tyrosine kinase (e.g., receptor tyrosine kinase). However, absolute complementarity, although preferred, is not required.

[0159] A sequence "complementary to at least a portion of an RNA," referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double stranded antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the larger the hybridizing nucleic acid, the more base mismatches with a RNA it may contain and still form a stable duplex (or triplex as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

[0160] Polynucleotides that are complementary to the 5' end of the message, e.g., the 5' untranslated sequence up to and including the AUG initiation codon, should work most efficiently at inhibiting translation. However, sequences complementary to the 3' untranslated sequences of mRNAs have been shown to be effective at inhibiting translation of mRNAs as well. See generally, Wagner, R., 1994, Nature 372:333-335. Thus, oligonucleotides complementary to either the 5'- or 3'-non-translated, non-coding regions of the gene, could be used in an antisense approach to inhibit translation of endogenous mRNA. Polynucleotides complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon. Antisense polynucleotides complementary to mRNA coding regions are less efficient inhibitors of translation but could be used in accordance with the invention. Whether designed to hybridize to the 5'-, 3'- or coding region of an mRNA, antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides or at least 50 nucleotides.

F. Antibody and Binding Polypeptide Variants

[0161] In certain embodiments, amino acid sequence variants of the antibodies and/or the binding polypeptides provided herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody and/or binding polypeptide. Amino acid sequence variants of an antibody and/or binding polypeptides may be prepared by introducing
appropriate modifications into the nucleotide sequence encoding the antibody and/or binding polypeptide, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody and/or binding polypeptide. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, e.g., antigen-binding.

[0162] In certain embodiments, antibody variants and/or binding polypeptide variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the HVRs and FRs. Conservative substitutions are shown in Table 1 under the heading of "preferred substitutions." More substantial changes are provided in Table 1 under the heading of "exemplary substitutions," and as further described below in reference to amino acid side chain classes. Amino acid substitutions may be introduced into an antibody and/or binding polypeptide of interest and the products screened for a desired activity, e.g., retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

**TABLE 1**

<table>
<thead>
<tr>
<th>Original Residue</th>
<th>Exemplary Substitutions</th>
<th>Preferred Substitutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala (A)</td>
<td>Val; Leu; Ile</td>
<td>Val</td>
</tr>
<tr>
<td>Arg (R)</td>
<td>Lys; Gln; Asn</td>
<td>Lys</td>
</tr>
<tr>
<td>Asn (N)</td>
<td>Gln; His; Asp, Lys; Arg</td>
<td>Gln</td>
</tr>
<tr>
<td>Asp (D)</td>
<td>Glu; Asn</td>
<td>Glu</td>
</tr>
<tr>
<td>Cys (C)</td>
<td>Ser; Ala</td>
<td>Ser</td>
</tr>
<tr>
<td>Gln (Q)</td>
<td>Asn; Glu</td>
<td>Asn</td>
</tr>
<tr>
<td>Glu (E)</td>
<td>Asp; Gln</td>
<td>Asp</td>
</tr>
<tr>
<td>Gly (G)</td>
<td>Ala</td>
<td>Ala</td>
</tr>
<tr>
<td>His (H)</td>
<td>Asn; Gln; Lys; Arg</td>
<td>Arg</td>
</tr>
<tr>
<td>Ile (I)</td>
<td>Leu; Val; Met; Ala; Phe; Norleucine</td>
<td>Leu</td>
</tr>
<tr>
<td>Leu (L)</td>
<td>Norleucine; Ile; Val; Met; Ala; Phe</td>
<td>Ile</td>
</tr>
<tr>
<td>Lys (K)</td>
<td>Arg; Gln; Asn</td>
<td>Arg</td>
</tr>
<tr>
<td>Met (M)</td>
<td>Leu; Phe; Ile</td>
<td>Leu</td>
</tr>
<tr>
<td>Phe (F)</td>
<td>Trp; Leu; Val; Ile; Ala; Tyr</td>
<td>Tyr</td>
</tr>
</tbody>
</table>
Amino acids may be grouped according to common side-chain properties:

1. Hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;
2. Neutral hydrophilic: Cys, Ser, Thr, Asn, Gin;
3. Acidic: Asp, Glu;
4. Basic: His, Lys, Arg;
5. Residues that influence chain orientation: Gly, Pro;
6. Aromatic: Trp, Tyr, Phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

G. Antibody and Binding Polypeptide Derivatives

In certain embodiments, an antibody and/or binding polypeptide provided herein may be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the antibody and/or binding polypeptide include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody and/or binding polypeptide may vary, and if more than one polymer are attached, they can be the same or different.
molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody and/or binding polypeptide to be improved, whether the antibody derivative and/or binding polypeptide derivative will be used in a therapy under defined conditions, etc.

[0166] In another embodiment, conjugates of an antibody and/or binding polypeptide to nonproteinaceous moiety that may be selectively heated by exposure to radiation are provided. In one embodiment, the nonproteinaceous moiety is a carbon nanotube (Kam et al., Proc. Natl. Acad. Sci. USA 102: 11600-11605 (2005)). The radiation may be of any wavelength, and includes, but is not limited to, wavelengths that do not harm ordinary cells, but which heat the nonproteinaceous moiety to a temperature at which cells proximal to the antibody and/or binding polypeptide-nonproteinaceous moiety are killed.

IV. Methods of Screening and/or Identifying ALDH inhibitor and/or Targeted Therapeutics With Desired Function

[0167] Additional antagonists of a polypeptide of interest, such as ALDH and/or tyrosine kinase (e.g., receptor tyrosine kinase) for use in the methods described herein, including antibodies, binding polypeptides, and/or binding small molecules provided herein may be identified, screened for, or characterized for their physical/chemical properties and/or biological activities by various assays known in the art.

[0168] Amino acid sequences of various human ALDH family members (e.g., "isozymes") are known in the art and are publicly available. See, e.g., GenBank Accession No. NP.sub.—000680 (ALDH 1, member Al); GenBank Accession No. NP_000684 (ALDH 1, member A3); GenBank Accession Nos. AAH02967 and NP.sub.—000681 (ALDH 2); GenBank Accession No. NP.sub.—001026976 (ALDH 3, member A2, isoform 1); GenBank Accession No. CA139494 (ALDH 4, member Al); GenBank Accession No. CAA20248 (ALDH 5, member Al); GenBank Accession No. EAW81160 (ALDH 6, member Al, isoform CRA_b); GenBank Accession No. AAH02515 (ALDH 7, member Al); GenBank Accession No. NP.sub.—072090 (ALDH 8, member Al, isoform 1); GenBank Accession No. NP.sub.—000687 (ALDH 9, member Al); GenBank Accession No. AAG42417 (ALDH 12); GenBank Accession No. AAG42417 (ALDH 12); GenBank Accession No. NP.sub.—699160 (ALDH 16); and GenBank Accession No. CA116766 (ALDH 18, member Al).

[0169] The crystal structures of wild-type ALDH2 and a C302S mutant of ALDH2 are known in the art (U.S. Pat. No. 8,124,389), and can be used in the design and preparation of ALDH inhibitors for use in the methods and compositions described herein.
In certain embodiments, a computer system comprising a memory comprising atomic coordinates of an ALDH polypeptide are useful as models for rationally identifying compounds that a ligand binding site of an ALDH polypeptide. Such compounds may be designed either de novo, or by modification of a known compound, for example. In other cases, binding compounds may be identified by testing known compounds to determine if the "dock" with a molecular model of an ALDH polypeptide. Such docking methods are generally well known in the art.

ALDH crystal structure data can be used in conjunction with computer-modeling techniques to develop models of binding of various ALDH-binding compounds by analysis of the crystal structure data. The site models characterize the three-dimensional topography of site surface, as well as factors including van der Waals contacts, electrostatic interactions, and hydrogen-bonding opportunities. Computer simulation techniques are then used to map interaction positions for functional groups including but not limited to protons, hydroxyl groups, amine groups, divalent cations, aromatic and aliphatic functional groups, amide groups, alcohol groups, etc. that are designed to interact with the model site. These groups may be designed into a pharmacophore or candidate compound with the expectation that the candidate compound will specifically bind to the site. Pharmacophore design thus involves a consideration of the ability of the candidate compounds falling within the pharmacophore to interact with a site through any or all of the available types of chemical interactions, including hydrogen bonding, van der Waals, electrostatic, and covalent interactions, although in general, pharmacophores interact with a site through non-covalent mechanisms.

The ability of a pharmacophore or candidate compound to bind to an ALDH polypeptide can be analyzed in addition to actual synthesis using computer modeling techniques. Only those candidates that are indicated by computer modeling to bind the target (e.g., an ALDH polypeptide binding site) with sufficient binding energy (in one example, binding energy corresponding to a dissociation constant with the target on the order of $10^{-2}$ M or tighter) may be synthesized and tested for their ability to bind to an ALDH polypeptide and to inhibit ALDH enzymatic function using enzyme assays known to those of skill in the art and/or as described herein. The computational evaluation step thus avoids the unnecessary synthesis of compounds that are unlikely to bind an ALDH polypeptide with adequate affinity.

An ALDH pharmacophore or candidate compound may be computationally evaluated and designed by means of a series of steps in which chemical entities or fragments are screened and selected for their ability to associate with individual binding target sites on an ALDH polypeptide. One skilled in the art may use one of several methods to screen chemical entities or fragments for their ability to associate with an ALDH polypeptide, and more particularly with target sites on an ALDH polypeptide.
The process may begin by visual inspection of, for example a target site on a computer screen, based on
the ALDH polypeptide coordinates, or a subset of those coordinates known in the art.

[0174] To select for an ALDH inhibitor which enhances induction cancer cell death, loss of membrane
integrity as indicated by, e.g., propidium iodide (PI), trypan blue or 7AAD uptake in combination with a	
5 targeted therapeutic (e.g., TKI) may be assessed relative to a reference. A PI uptake assay can be
performed in the absence of complement and immune effector cells. Tumor cells are incubated with
medium alone or medium containing the appropriate combination of an ALDH and/or targeted
therapeutic (TKI). The cells are incubated for a 3-day time period. Following each treatment, cells are
washed and aliquoted into 35 mm strainer-capped 12 x 75 tubes (1 ml per tube, 3 tubes per treatment
group) for removal of cell clumps. Tubes then receive PI (10 µg/ml). Samples may be analyzed using a
FACSCAN® flow cytometer and FACSCONVERT® CellQuest software (Becton Dickinson). Those
ALDH inhibitor in combination with a targeted therapeutic (e.g., TKI) that induce statistically
significant levels of cell death compared to media alone and/or targeted therapeutic (e.g., TKI) alone as
determined by PI uptake may be selected as cell death-inducing antibodies, binding polypeptides or
binding small molecules.

[0175] In some embodiments of any of the methods of screening and/or identifying, the candidate
ALDH inhibitor is an antibody, binding polypeptide, binding small molecule, or polynucleotide. In some
embodiments, the ALDH inhibitor (e.g., disulfiram and/or derivatives thereof) is a small molecule.

V. Pharmaceutical Formulations

[0176] Pharmaceutical formulations of an antagonist of an ALDH inhibitor (e.g., disulfiram and/or
derivatives thereof) and/or a targeted therapeutic (e.g., TKI) as described herein are prepared by mixing
such antibody having the desired degree of purity with one or more optional pharmaceutically
acceptable carriers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the
form of lyophilized formulations or aqueous solutions. In some embodiments, the antagonist of an
ALDH inhibitor (e.g., disulfiram and/or derivatives thereof) and/or a targeted therapeutic (e.g., TKI) is a
binding small molecule, an antibody, binding polypeptide, and/or polynucleotide. Pharmaceutically
acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and
include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants
including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium
chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or
benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-
pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such
as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g., Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG). Exemplary pharmaceutically acceptable carriers herein further include interstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX®, Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including rHuPH20, are described in US Patent Publication Nos. 2005/0260186 and 2006/0104968. In one aspect, a sHASEGP is combined with one or more additional glycosaminoglycanases such as chondroitinases. [0177] Exemplary lyophilized formulations are described in US Patent No. 6,267,958. Aqueous antibody formulations include those described in US Patent No. 6,171,586 and WO2006/044908, the latter formulations including a histidine-acetate buffer. [0178] The formulation herein may also contain more than one active ingredients as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Such active ingredients are suitably present in combination in amounts that are effective for the purpose intended. [0179] Active ingredients may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980). [0180] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antagonist of an ALDH inhibitor (e.g., disulfiram and/or derivatives thereof) and/or a targeted therapeutic (e.g., TKI), which matrices are in the form of shaped articles, e.g., films, or microcapsules. [0181] The formulations to be used for in vivo administration are generally sterile. Sterility may be readily accomplished, e.g., by filtration through sterile filtration membranes.
VI. Articles of Manufacture

[0182] In another aspect of the invention, an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an antagonist of an ALDH inhibitor (e.g., disulfiram and/or derivatives thereof) and/or a targeted therapeutic (e.g., TKI) described herein. The label or package insert indicates that the composition is used for treating the condition of choice. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises an antagonist of an ALDH inhibitor (e.g., disulfiram and/or derivatives thereof) and/or a targeted therapeutic (e.g., TKI); and (b) a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent.

[0183] In some embodiments, the article of manufacture comprises a container, a label on said container, and a composition contained within said container; wherein the composition includes one or more reagents (e.g., primary antibodies that bind to one or more biomarkers or probes and/or primers to one or more of the biomarkers described herein), the label on the container indicating that the composition can be used to evaluate the presence of one or more biomarkers in a sample, and instructions for using the reagents for evaluating the presence of one or more biomarkers in a sample. The article of manufacture can further comprise a set of instructions and materials for preparing the sample and utilizing the reagents. In some embodiments, the article of manufacture may include reagents such as both a primary and secondary antibody, wherein the secondary antibody is conjugated to a label, e.g., an enzymatic label. In some embodiments, the article of manufacture one or more probes and/or primers to one or more of the biomarkers described herein.

[0184] In some embodiments of any of the article of manufacture, the antagonist of an ALDH inhibitor (e.g., disulfiram and/or derivatives thereof) and/or a targeted therapeutic (e.g., TKI) is an antibody, binding polypeptide, binding small molecule, or polynucleotide. In some embodiments, the antagonist of an ALDH inhibitor (e.g., disulfiram and/or derivatives thereof) and/or a targeted therapeutic (e.g., TKI) is a small molecule. In some embodiments, the antagonist of an ALDH inhibitor (e.g., disulfiram and/or
derivatives thereof) and/or a targeted therapeutic (e.g., TKI) is an antibody. In some embodiments, the antibody is a monoclonal antibody. In some embodiments, the antibody is a human, humanized, or chimeric antibody. In some embodiments, the antibody is an antibody fragment and the antibody fragment binds an ALDH inhibitor (e.g., disulfiram and/or derivatives thereof) and/or a targeted therapeutic (e.g., TKI).

[0185] The article of manufacture in this embodiment of the invention may further comprise a package insert indicating that the compositions can be used to treat a particular condition. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

[0186] Other optional components in the article of manufacture include one or more buffers (e.g., block buffer, wash buffer, substrate buffer, etc), other reagents such as substrate (e.g., chromogen) which is chemically altered by an enzymatic label, epitope retrieval solution, control samples (positive and/or negative controls), control slide(s) etc.

[0187] It is understood that any of the above articles of manufacture may include an immunoconjugate described herein in place of or in addition to an antagonist of an ALDH inhibitor (e.g., disulfiram and/or derivatives thereof) and/or a targeted therapeutic (e.g., TKI).
EXAMPLES

[0188] The following are examples of methods and compositions of the invention. It is understood that various other embodiments may be practiced, given the general description provided above.

5 Example 1
Materials and Methods

Human Cancer Cell Lines and Reagents
[0189] Human cancer cell lines were grown in RPMI media supplemented with sodium pyruvate, 10% Fetal bovine serum and antibiotics penicillin and streptomycin at 37°C in the presence of 5% CO₂.

10 ALDH Activity Assay
[0190] A bodipy labeled ALDH substrate (Aldefluor Kit, Stem Cell Technology), reconstituted according to the vendor's protocol, was used to detect ALDH activity. The substrate was diluted in RPMI media (5ul substrate/ml media) and added to the adherent cells. After 30 minutes of incubation at 37°C in the CO₂ incubator cells were washed twice with RPMI media and the images were taken using IncuCyte HD System (Essen Bioscience) and a 10x objective.

Flow Cytometry and RNA Extraction
[0191] Aldefluor assay was used to detect ALDH activity in Kato II parental cells. ALDH<sup>high</sup> and ALDH<sup>low</sup> cells representing ~5% of parental cells with highest and lowest ALDH activity, respectively, were sorted using flow cytometry. Kato II cells incubated with the bodipy labeled substrate in the presence of DEAB, a cold competitive substrate, were used as negative control. Total RNA extracted using RNeasy column (Qiagen) was used for microarray based gene expression analysis.

Cell Viability Assay
[0192] The cells were fixed with 4% paraformaldehyde at the end of the assay period and the viability was determined using nucleic acid stain Syto60 (Life Technologies) diluted 1:5000 in water. The fluorescence intensity was measured using SpectraMax M5 (excitation 635nm and emission 695nm; Molecular Device). The viability was expressed as % of no treatment control.

Generation of Drug Tolerant Cells
[0193] Kato II and GTL-16 DTPs were generated by treating parental cells with 1μM crizotinib for 30 days. PC9 parental cells were treated with 2μM erlotinib for 9 days for DTP generation. In all cases media was changed every three days.

Immunobloting
[0194] Proteins were extracted from cell pellet using NP-40 lysis buffer containing protease and phosphatase inhibitors. Proteins were separated using SDS-PAGE gels (BioRad) and immunodetection was performed using standard protocols. The antibodies to ALDH1A1 was purchased from R&D Systems, GAPDH, cleaved PARP and phospho-ATM/ATR substrate antibodies were purchased from Cell Signaling Technology and phospho-γH2A.x antibody was purchased from Millipore.

ROS Assay

[0195] ROS assay was performed using carboxy derivative of fluorescein, CM-H$_2$DCFDA (Molecular Probes). Reconstituted ROS indicator was added to the growth media in the plates containing DTPs and incubated for 30 minutes. DTPs were detached from the plate using trypsin-EDTA and ROS level was detected using flow-cytometry using untreated parental cells as controls.

Xenograft Tumor Studies

[0196] PC-9, PC-9-GFP, EBC-1, and GTL-16 cells were cultured in growth media (RPMI 1640, 10% heat-inactivated fetal calf serum, 2 mM L-glutamine) to 80% confluency and then trypsinized, washed once with PBS, and resuspended in either Hank's Balanced Salt Solution (HBSS) or a 1:1 mixture of HBSS with matrigel [growth factor reduced; catalog #356231 (BD Biosciences, West Grove, PA)] to a final concentration of 5 x 10$^7$ cells/ml. Each xenograft tumor model was established using 5 x10$^6$ cells (100 μL) inoculated subcutaneously (s.c.) in the rear right flank of immunocompromised mice. GTL-16 cells were implanted in HBSS without matrigel in nude (nu/nu) mice (Charles River Laboratories, Hollister, CA). PC-9 and PC-9-GFP cells were implanted in HBSS with matrigel in nude (nu/nu) mice (Charles River Laboratories, Hollister, CA). EBC-1 cells were implanted in HBSS without matrigel in nude (nu/nu) mice (Charles River Laboratories, Hollister, CA). When tumor volumes reach approximately 100-200 mm$^3$, mice were separated into groups of 10-15 animals with similarly sized tumors, and treatment was initiated the day after grouping. Mice were dosed via daily (QD) oral gavage (PO) with GDC-0712 (Genentech, Inc. - a MET small molecule inhibitor, at 100 mg/kg formulated in water), erlotinib (50 mg/kg in 7.5% Captisol) and/or disulfiram (Sigma - Tetraethylthiuram, Catalog # 86720, dosed at 200 mg/kg formulated in safflower oil 95%, benzyl alcohol 5%), or with corresponding vehicle only. Tumor volumes were determined using digital calipers (Fred V. Fowler Company, Inc.) using the formula (L x W x W)/2. Tumor growth inhibition (%TGI) was calculated as the percentage of the area under the fitted curve (AUC) for the respective dose group per day in relation to the vehicle, such that %TGI = 100 x 1-(AUC treatment/day)/(AUC vehicle/day). Curve fitting was applied to Log2 transformed individual tumor volume data using a linear mixed-effects (LME) model using the R package nlme, version 3.1-97 in R v2.12.0.
Seahorse Assay

Approximately 5,000 parental cells and 15,000 DTPs cells were plated per well in XF 96-well cell culture microplates (SeahorseBioscience) and incubated for 24 h at 37°C in a5% CO_2 incubator. Disulfiram and NAC treatment was performed for 48h in the presence of TKI. The oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) measurements were performed in bicarbonate-free, serum-free, 37°C pre-warmed media. After completion of analysis the cells were fixed with 4% paraformaldehyde, stained with Hoechst and 4 quadrants/well was imaged using a Molecular Devices ImageXpress HCS and average nuclei number per quadrant was counted. The bar graphs represented the mean +/- SEM of normalized (cell number) OCR and ECAR measurements from six wells.

Results

Cancer stem cell marker gene ALDH1A1 is differentially expressed in drug tolerant cells

Microarray based gene expression analysis was performed to identify genes differentially expressed in crizotinib tolerant Kato II gastric carcinoma cells. Among many up-regulated genes ALDH1A1, a cancer stem cell marker gene, was identified in drug tolerant persister (DTP) population. ALDH activity in live Kato II cells using Aldefluor assay (Stem Cell Technology) was measured and high ALDH activity was detected in a small (~5%) population of Kato II parental cells (Fig. 1A) and in almost all Kato II DTPs after a month of crizotinib treatment. A microarray based gene expression analysis performed on RNAs isolated ALDH^{high} cells identified ALDH1A1 as the only member of the ALDH family of genes up-regulated ~8 fold compared to ALDH^{low} cells (Fig. 1b). Consistent with the RNA level ALDH1A1 protein level in Kato II was detected in ALDH^{high} cells and in DTPs derived from Kato II and GTL-16 cells (gastric carcinoma) (Fig.1c). Crizotinib treatment increased ALDH1A1 protein level in Kato II parental cells within 24h (Fig.1c), before any drug induced apoptosis was detected as measured by the appearance of cleaved PARP protein product (data not shown). Knockdown of ALDH1A1 expression in Kato II and GTL-16 cells had no significant effect on drug sensitivity or DTP formation.

Disulfiram, an ALDH inhibitor, Kills Drug Tolerant Cells

To understand the role of ALDH in drug tolerance, Kato II and GTL-16 parental cells were treated with an irreversible ALDH inhibitor called Disulfiram (DS). DS and its metabolites inhibit enzymatic activity of multiple ALDH family members (Koppaka et al., 2012). Disulfiram alone had no significant effect on the growth of these cancer cells, but in combination with crizotinib, DS eliminated drug tolerant Kato II and GTL-16 cells (Fig.2A,B). Similar effect of DS was observed on non-small cell lung carcinoma PC9 cells that do not express ALDH1A1 but express other ALDH family members (Fig.
2C). Approximately 20% PC9 DTPs starts dividing like parental PC9 cells while maintaining their drug tolerance property when maintained over 10 days in erlotinib. This growing population of erlotinib tolerant PC9 DTPs are called drug tolerant expanded persister or DTEPs (Sharma et al., 2010), which unlike DTPs are much less sensitive to DS. The bar graphs in Fig.2 represent data from triplicate wells and illustrate the combined effect of DS and TKI on the viability of DTPs from all three cell lines mentioned above. Pre-treatment of PC9 and GTL-16 cells with DS alone for 3-6 days prior to TKI exposure did not eliminate DTPs, indicating continuous suppression of ALDH activity is critical for the killing DTPs.

[0200] The effectiveness of various TKI-DS combinations were tested on eight other cancer cell lines of breast, colon and lung cancer origins and addicted to various oncogenes. While DS alone had no significant effect on the viability, all TKI-DS combinations were highly effective in either eliminating or significantly reducing the number of corresponding DTPs (Fig.3). These results further emphasize the dependence of drug tolerant cells, in general, on ALDH activity for their survival and implicate potential beneficial effect of the use of DS in combination of TKIs in eliminating/delaying relapse of various type of cancer.

Drug Tolerant Cells Have High ROS Level

[0201] Cancer cells, compared to their normal counterpart, have higher ROS level, which is believed to promote cell proliferation (Szatrowski et al., 1991; Boonstra et al., 2004). Exposure to chemotherapy and radiation therapies increase ROS level even higher in cancer cells, which can cause generation of various aldehyde products through peroxidation of membrane lipid. Some of these aldehyde products like malonaldehyde and 4-hydroxy-nonenal (4-HNE) have longer half-life and can cause DNA damage and subsequent cell death (Chiu et al., 2012; Casares et al., 2012, Li et al., 2009). A prompt activation of DNA repair pathway in CD133+ glioblastoma stem cells in response to increase in ROS level provided the basis for their resistance to radiation (Bao et al. 2006). To determine whether similar mechanisms involving ROS play role in drug tolerance, the bioenergetics, ROS level, extent of DNA damage and activity of DNA repair pathway was measured in DTPs. An over six-fold increase in ROS level was observed in both PC9 and GTL-16-derived DTPs compared to their parental cells (Fig.4A). DS treatment for 48h caused further increase in ROS level in DTPs, which can be reversed by adding NAC to the media. Increased ROS level led to increased oxygen consumption rate (OCR) (Fig.4B) and increased double-stranded DNA breaks in drug tolerant cells and activated DNA repair mechanism (Fig.4C). These results suggest that ALDH family members play a ROS scavenger role, which is critical for the survival of DTPs.
N-acetyl cysteine rescue the lethal effect of disulfiram on DTPs

Next we asked whether NAC treatment is sufficient to prevent the killing of DTPs by TKI+DS treatment. PC9 and GTL-16 cells were treated with erlotinib and crizotinib respectively either alone or in combination with DS and NAC. As expected DS and TKI combination killed all PC9 DTPs within 14 days, which was almost completely rescued by NAC when added along with DS and TKI (Fig.5A). Similar results were obtained with GTL-16 DTPs where inclusion of NAC during the treatment with TKI+DS (Fig.5B) rescued ~80% of GTL-16 DTPs from DS induced death.

To understand the mechanism of DS action, GTL-16-derived DTPs were treated with DS and NAC for 48h and performed immunoblot experiments with the extracted proteins. DS treatment caused decrease in ALDH1A1 and NFKB levels and resulted in several fold increase in γH2Aχ, which suggested extensive DNA damage in DS treated DTPs and subsequent activation of apoptotic pathway as revealed by significant increase in cleaved PARP level. Presence of NAC, a ROS scavenger, restored ALDH1A1 and NFKB levels and prevented increase in γH2Aχ and apoptosis of DTPs.

Disulfiram Delays Tumor Relapse in Xenograft Mouse Models

Xenograft mouse models were used to investigate the efficacy of DS in eliminating/delaying tumor relapse in vivo. The treatment regimen for PC9 in vivo study was first tested in an in vitro experiment where PC9 cells were treated with either erlotinib alone or in combination with DS for six days. PC9 DTPs in the erlotinib, DS and one of the erlotinib+DS group were allowed to grow without any drug while the other erlotinib+DS group continued to receive DS. As shown in Fig.6A, a significant delay in the growth of PC9 DTPs was observed from erlotinib+DS group where both drugs were withdrawn as compared to erlotinib group. As expected, no PC9 DTPs survived from erlotinib+DS sub-group which continued to receive DS. The bar graph represents data from triplicate wells illustrating the effect of DS.

For the PC9 xenograft study the mice were inoculated with PC9 cells and the tumors were allowed to grow to 100-200 mm³ in size, which were then divided into four treatment groups, namely, vehicle control, DS control, TKI alone and TKI+DS groups. The treatments were stopped after eleven days except for the animals in the TKI+DS group, which continued to receive DS until the end of the study. As shown in Fig. 6B, near complete regression of PC9 tumors was observed upon erlotinib treatment. The time of tumor progression (TTP) measured as 5xTTP was 60 days for the erlotinib treatment group with 10 PR and ICR whereas for the erlotinib+DS group the average tumor size with 9PR and 6CR was below the initial volume of the tumor (P=0.0007). In agreement with the cell line data xenograft data showed that TKI and DS combination can significantly delay tumor relapse.
References

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention. The disclosures of all patent and scientific literature cited herein are expressly incorporated in their entirety by reference.
CLAIMS

1) A method of treating cancer in an individual comprising concomitantly administering to the individual an effective amount of an ALDH inhibitor and an effective amount of a targeted therapeutic.

2) The method of claim 1, wherein the respective amounts the ALDH inhibitor and the targeted therapeutic are effective to increase the period of cancer sensitivity and/or delay the development of cell resistance to the targeted therapeutic.

3) A method of increasing efficacy of a cancer treatment comprising a targeted therapeutic in an individual comprises concomitantly administering to the individual an effective amount of the targeted therapeutic and an effective amount of an ALDH inhibitor.

4) A method of treating cancer in an individual wherein cancer treatment comprising concomitantly administering to the individual an effective amount of a targeted therapeutic and an effective amount of an ALDH inhibitor, wherein the cancer treatment has increased efficacy compared to a standard treatment comprising administering an effective amount of the targeted therapeutic without (in the absence of) the targeted therapeutic.

5) A method of delaying and/or preventing development of cancer resistant to a targeted therapeutic in an individual, comprises concomitantly administering to the individual an effective amount of an ALDH inhibitor and an effective amount of the targeted therapeutic.

6) A method of treating an individual with cancer who has increased likelihood of developing resistance to a targeted therapeutic comprising concomitantly administering to the individual an effective amount of an ALDH inhibitor and an effective amount of the targeted therapeutic.

7) A method of increasing sensitivity to a targeted therapeutic in an individual with cancer comprising concomitantly administering to the individual an effective amount of an ALDH inhibitor and an effective amount of the targeted therapeutic.

8) A method of extending the period of an targeted therapeutic sensitivity in an individual with cancer comprising concomitantly administering to the individual an effective amount of an ALDH inhibitor and an effective amount of targeted therapeutic.

9) A method of extending the duration of response to a targeted therapeutic in an individual with cancer comprising concomitantly administering to the individual an effective amount of an ALDH inhibitor and an effective amount of the targeted therapeutic.

10) The method of any one of claims 1-9, wherein the ALDH inhibitor is a small molecule ALDH inhibitor.
11) The method of claim 10, wherein the small molecule ALDH inhibitor is disulfiram or an ALDH-inhibiting derivative or metabolite thereof.

12) The method of claim 10, wherein the ALDH inhibitor is N,N-diethyl[(diethylcarbamothioyl)disulfanyl]carbothioamide or pharmaceutically acceptable salt thereof.

13) The method of claim 10, wherein the ALDH inhibitor is N,N-diethyl[(diethylcarbamothioyl)disulfanyl]carbothioamide.

14) The method of claim 10, wherein the ALDH inhibitor is 2,2'-bis-(Formyl-l,6,7-trihydroxy-5-isopropyl-3-methylnaphthalene) or pharmaceutically acceptable salt thereof.

15) The method of claim 10, wherein the ALDH inhibitor is 2,2'-bis-(Formyl-l,6,7-trihydroxy-5-isopropyl-3-methylnaphthalene).

16) The method of any one of claims 1-15, wherein the targeted therapeutic is a tyrosine kinase inhibitor (TKI).

17) The method of claim 16, wherein the TKI is an EGFR inhibitor, HER2 inhibitor, MET inhibitor, ALK inhibitor, BRAF inhibitor, ROS1 inhibitor, and/or MEK inhibitor.

18) The method of claim 16, the TKI is a receptor tyrosine kinase inhibitor (RTKI).

19) The method of claim 18, the RTKI is an EGFR inhibitor, HER2 inhibitor, MET inhibitor, and/or ALK inhibitor.

20) The method of any one of claims 16-19, wherein the inhibitor is an antibody inhibitor, a small molecule inhibitor, a binding polypeptide inhibitor, and/or a polynucleotide antagonist.

21) The method of claim 16, wherein the TKI is N-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)quinazolin-4-amine or a pharmaceutically acceptable salt thereof (e.g., erlotinib).

22) The method of claim 16, wherein the TKI is N-(4-(3-fluorobenzyloxy)-3-chlorophenyl)-6-(5-((2-methylsulfonyl)ethylamino)methyl)furan-2-yl)quinazolin-4-amine, di4-methylbenzenesulfonate or a pharmaceutically acceptable salt thereof (e.g., lapatinib).

23) The method of claim 16, wherein the TKI is (S)-N-(2,3-dihydroxypropyl)-3-(2-fluoro-4-iodophenylamino)isonicotinamide or a pharmaceutically acceptable salt thereof (e.g., AS703026).

24) The method of claim 16, wherein the TKI is vemurafenib.

25) The method of claim 16, wherein the TKI is 3-((R)-l-(2,6-dichloro-3-fluorophenyl)ethoxy)-5-(l(piperidin-4-yl)-IH-pyrazol-4-yl)pyridin-2-amine or a pharmaceutically acceptable salt thereof (e.g., crizotinib).
26) The method of any one of claims 1-25, wherein the cancer is gastric cancer, lung cancer (e.g., non-small cell lung cancer (NSCL)), colorectal cancer (e.g., colon cancer and/or rectal cancer), or basal cell carcinoma.

27) A pharmaceutical product comprising a) as a first component an effective amount of an ALDH inhibitor, and b) as a second component an effective amount of a targeting agent for the concomitant or sequential use for the treatment of cancer.

28) The pharmaceutical product of claim 27, wherein the ALDH inhibitor is a small molecule ALDH inhibitor.

29) The pharmaceutical product of claim 27 or 28, wherein the ALDH inhibitor is disulfiram or an ALDH-inhibiting derivative or metabolite thereof.

30) The pharmaceutical product of claim 27 or 28, wherein the ALDH inhibitor is N,N-diethyl[(diethylcarbamothioyl) disulfanyl]carbothioamide or pharmaceutically acceptable salt thereof.

31) The pharmaceutical product of claim 27 or 28, wherein the ALDH inhibitor is N,N-diethyl[(diethylcarbamothioyl) disulfanyl]carbothioamide.

32) The pharmaceutical product of claim 27 or 28, wherein the ALDH inhibitor is 2,2'-bis-(Formyl-1,6,7-trihydroxy-5-isopropyl-3-methylnaphthalene) or pharmaceutically acceptable salt thereof.

33) The pharmaceutical product of claim 27 or 28, wherein the ALDH inhibitor is 2,2'-bis-(Formyl-1,6,7-trihydroxy-5-isopropyl-3-methylnaphthalene).

34) The pharmaceutical product of any one of claim 27 to 33, wherein the targeted therapeutic is a tyrosine kinase inhibitor (TKI).

35) The pharmaceutical product of claim 34, wherein the TKI is an EGFR inhibitor, HER2 inhibitor, MET inhibitor, ALK inhibitor, BRAF inhibitor, ROS1 inhibitor, and/or MEK inhibitor.

36) The pharmaceutical product of claim 34, wherein the TKI is a receptor tyrosine kinase inhibitor (RTKI).

37) The pharmaceutical product of claim 36, wherein the RTKI is an EGFR inhibitor, HER2 inhibitor, MET inhibitor, and/or ALK inhibitor.

38) The pharmaceutical product of claim 34, wherein the inhibitor is an antibody inhibitor, a small molecule inhibitor, a binding polypeptide inhibitor, and/or a polynucleotide antagonist.

39) The pharmaceutical product of claim 34, wherein the TKI is N-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)quinazolin-4-amine or a pharmaceutically acceptable salt thereof, in particular erlotinib.
40) The pharmaceutical product of claim 34, wherein the TKI is N-(4-(3-fluorobenzyloxy)-3-chlorophenyl)-6-(5-((2-(methylsulfonyl)ethy lamino)methyl)furan-2-yl)quinazolin-4-amine, di-4-m ethylbenzenesulfonate or a pharmaceutically acceptable salt thereof, in particular lapatinib.

41) The pharmaceutical product of claim 34, wherein the TKI is (S)-N-(2,3-dihydroxypropyl)-3-(2-fluoro-4-iodophenylamino)isonicotinamide) or a pharmaceutically acceptable salt thereof, in particular AS703026.

42) The pharmaceutical product of claim 34, wherein the TKI is vemurafenib.

43) The pharmaceutical product of claim 34, wherein the TKI is 3-((R)-l-(2,6-dichloro-3-fluorophenyl)ethoxy)-5-(l-(piperidin-4-yl)-lH-pyrazol-4-yl)pyridin-2-amine or a pharmaceutically acceptable salt thereof, in particular crizotinib.

44) The pharmaceutical product according to any one of claims 27 to 43, wherein the cancer is gastric cancer, lung cancer, non-small cell lung cancer (NSCL), colon cancer and/or rectal cancer, or basel cell carcinoma.
Fig. 1
Fig. 2
Fig. 3
Fig. 4
Fig. 5
Fig. 6
A

PC9

Untreated  Erlot  erlot+DSF d1-15  DSF d1-3  erlot d4-15  DSF d1-6  erlot d7-15  DSF d1-15

B

GTL-16


C

GTL16 - ALDH1A1 KO

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Fig. 7
Fig. 8
Fig. 9
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K45/06

ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K

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

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

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

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*A* document member of the same patent family

Date of the actual completion of the international search

24 March 2014

Date of mailing of the international search report

08/04/2014

Name and mailing address of the ISA

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Authorized officer

Al bayrak, Timur
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<td>LIAN JIQIN ET AL: &quot;Sorafenib Sensitizes (-)-Gossypol-Induced Growth Suppression in Androgen-Independent Prostate Cancer Cells via Mcl-1 Inhibition and Bak Activation&quot;, MOLECULAR CANCER THERAPEUTICS, vol. 11, no. 2, February 2012 (2012-02), pages 416-426, XP002722170, ISSN: 1535-7163</td>
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