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(54) **Title:** COMPOSITIONS AND METHODS FOR TREATMENT OF CANCER

(57) **Abstract:** Provided are methods of treating metastatic cancer in a patient in need thereof, comprising administering to a subject in need thereof a composition comprising one or more molecules targeting Rac1 and/or Rac2, and/or Rac3 and Cdc42. Also provided are methods of prolonging survival in a cancer patient in need thereof, comprising administering to a subject in need thereof a composition comprising one or more molecules targeting Rac and/or Rac2, and/or Rac3 and Cdc42. Also provided are methods of suppressing tumor growth in a cancer patient in need thereof, comprising administering to a subject in need thereof a composition comprising one or more molecules targeting Rac and/or Rac2, and/or Rac3 and Cdc42.

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COMPOSITIONS AND METHODS FOR TREATMENT OF CANCER

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 63/363,976, filed May 2, 2022, the disclosure of which is incorporated by reference herein in its entirety.

INCORPORATION OF SEQUENCE LISTING

[0002] The sequence listing that is contained in the file named "MBQ0005-401-PC.xml," which is 9 kilobytes as measured in Microsoft Windows operating system and was created on April 24, 2023, is filed electronically herewith and incorporated herein by reference.

[0003] Cancer is one of the leading causes of death worldwide, accounting for nearly ten million deaths in 2020, or nearly one in six deaths. The most common cancer types are breast, lung, colon, and rectal and prostate cancers. In addition, metastases resulting from primary tumors are responsible for 90% of cancer deaths. Metastases are difficult to treat and most patients who develop metastases do not respond to conventional chemotherapy. Thus, there is a critical need for targeted therapies for the prevention and treatment of metastases and removal of establishes metastases in the body.

[0004] Provided herein is a method of treating metastatic cancer in a patient in need thereof, comprising administering to a subject in need thereof a composition comprising one or more molecules targeting Rac1 and/or Rac2 and/or Rac3 (hereinafter Racs) and Cdc42.

[0005] Also provided is a method of prolonging survival in a cancer patient in need thereof, comprising administering to a subject in need thereof a composition comprising one or more molecules targeting Rac1 and/or Rac2 and/or Rac3 and Cdc42.

[0006] Also provided is a method of suppressing tumor growth in a cancer patient in need thereof, comprising administering to a subject in need thereof a composition comprising one or more molecules targeting Rac1 and/or Rac2 and/or Rac3 and Cdc42.

[0007] These and other embodiments disclosed herein are described in detail below.

BRIEF DESCRIPTION OF THE SEQUENCES

[0008] **SEQ ID NO:1** – Sequence of Cdc42 sh1-F – forward sequence of shRNA for knockdown of Cdc42.

[0009] **SEQ ID NO:2** – Sequence of Cdc42 sh1-R – reverse sequence of shRNA for knockdown of Cdc42.

[0010] **SEQ ID NO:3** – Sequence of Cdc42 sh1 – target sequence of shRNA for knockdown of Cdc42.

[0011] **SEQ ID NO:4** – Sequence of Rac1 sh1-F – forward sequence of shRNA for knockdown of Rac1.

[0012] **SEQ ID NO:5** – Sequence of Rac1 sh1-R – reverse sequence of shRNA for knockdown of Rac1/2.

[0013] **SEQ ID NO:6** – Sequence of Rac1 sh1 – target sequence of shRNA for knockdown of Rac1/2.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] **FIG. 1** – Shows that protein expression of Rac1 and Cdc42 correlate with poor survival in cancer patients.

[0015] **FIG. 2** – Shows that upregulation of Rac1 and Cdc42 is a risk factor in ER- and HER2+ breast cancers.

[0016] **FIG. 3** – Shows luminescence intensity of injected cancer cells in a rodent model. Left: saline control; middle top: lung tissues of saline control; middle bottom: lung tissues of rodent model injected with cancer cells expressing luciferase.

[0017] **FIG. 4** – Shows that silencing of Rac1/2 and Cdc42 abolishes lung metastases. Top row, left and middle columns: active Rac1/2 and Cdc42 on days 0 and 21; bottom row, left and middle columns: silenced Rac and Cdc42 on days 0 and 21. Right column shows tumors in lung tissue from active (top row) and silenced (bottom row) Rac1 and Cdc42.

[0018] **FIG. 5** – Shows reduced numbers of lung metastases after Rac1/2/Cdc42 silencing verses controls by hematoxylin and eosin (H&E) staining. Left: all lung metastases; right: size of metastases.

[0019] **FIG. 6** – Shows that silencing of Rac1/2 and Cdc42 promotes survival and blocks cachexia in animals with lung metastases. Left: body weight of silenced (top line) versus control (bottom line) animals; right: survival proportions of silenced versus controls.

DETAILED DESCRIPTION

Overview

[0020] Provided herein is a method of treating metastatic cancer in a patient in need thereof, comprising administering to the patient a composition comprising one or more molecules targeting Rac1 and/or Rac2 and/or Rac3 and Cdc42. Also provided is a method of prolonging survival in a cancer patient in need thereof, comprising administering to the

patient a composition comprising one or more molecules targeting Rac1 and/or Rac2 and/or Rac3 and Cdc42. Also provided is a method of suppressing tumor growth in a cancer patient in need thereof, comprising administering to the patient a composition comprising one or more molecules targeting Rac1 and/or Rac2 and/or Rac3and Cdc42.

Racs and Cdc42 in Cancer

[0021] The Rho GTPases Rac (Ras-related C3 botulinum toxin substrate) and Cdc42 (cell division control protein 42 homolog) regulate cell functions governing cancer malignancy, including cell polarity, migration, and cell cycle progression. The Rho family of GTPases in humans consists of 20 different members, and aberrant behavior in their regulatory activity has been implicated in cancer and other diseases. More than 70 Guanine nucleotide Exchange Factors (GEFs) are known, which specifically activate one or more of the GTPases. In turn, the activated GTPases can specifically interact with over 60 downstream effectors. Dysregulation of one or more cellular processes can lead to release of malignant cells from their original locations, which subsequently can establish themselves in pre-metastatic niches in most human tissues and organs, for example, but not limited to, brain, liver, intestine, muscle, bladder, bone, or lungs. It has been found that members of the Rho GTPase family, including Rac1, Rac2, Rac3, Cdc42, and Rho, play key signaling roles in these processes.

[0022] Rho GTPases regulate migration and invasion, cytoskeletal organization, transcriptional regulation, cell cycle progression, apoptosis, vesicle trafficking, and cell-to-cell and cell-to-extracellular matrix adhesions. The Rho GTPases Rac1, Rac2, Rac3, and Cdc42 are potent inducers of actin polymerization and extension of actin structures at the leading edge of motile cells. In addition, Cdc42 plays a critical role in cell polarity, and thus, promotes directed and persistent migration.

[0023] Studies have implicated hyperactive Rac1, Rac2, Rac3, and Cdc42 with increased cancer cell survival, proliferation, and invasion, as well in Ras and other oncogene-mediated transformation. Furthermore, oncogenic cell surface receptors, such as tyrosine kinase, cytokine, and G protein coupled receptors, activate Rac1, Rac2, Rac3, and Cdc42 via regulation of their upstream effector GEFs. Accordingly, Rac1, Rac2, Rac3, and Cdc42 proteins are generally not mutated in cancer but rather are overexpressed or hyperactivated. Even though some melanomas contain an activating Rac (P29S) mutation, and the hyperactive splice variant Raclb is overexpressed in some cancers, a majority of the Racs and Cdc42 in human cancer are activated due to upregulated GEFs.

[0024] Racs and Cdc42 promote metastasis via activation of the actin cytoskeleton. Of the direct downstream effectors of Racs and Cdc42, p21-activated kinases (PAK) are

overexpressed in a number of cancers and contribute to cancer transformation and progression by regulating key cellular functions, including cytoskeletal organization, cell migration, adhesion, growth, and development, leading to the development of a number of PAK inhibitors as anti-cancer therapeutics. Unfortunately, these therapeutics are limited by specificity, bioavailability, and toxicity, and successful clinical trials are lacking. Thus, there is a need for new therapeutic agents for the treatment of cancer and other hyperproliferative diseases.

[0025] Racs and Cdc42 GTPases are important cellular mediators that are hyperactive or overexpressed in metastatic tumors. Expression of the genes encoding Rac1, Rac2, Rac3, and Cdc42 proteins is known to be correlated with poor survival in cancer patients, with expression or activity of the different Rac proteins predominating in different cancer types. Rac1 and Cdc42 upregulation are risk factors in ER- and HER2+ breast cancers, including triple-negative breast cancer. A novel compound, MBQ-167, was previously identified as a first-in-class dual Rac1/Rac2/Rac3/Cdc42 inhibitor that induces suppression of primary and metastatic tumors, both alone and in combination with standard chemotherapy drugs such as paclitaxel (described in, e.g., WO 2017/189893). Described herein are methods for simultaneous or concurrent silencing of Racs and Cdc42, which drastically reduce the size and occurrence of metastatic tumors, exemplified by breast cancer metastases in the lungs, providing a novel treatment for lung cancer.

Molecule(s) targeting Racs and Cdc42

[0026] The present disclosure describes methods of treating metastatic cancer in a patient comprising administering to the patient a composition comprising one or more molecules targeting Racs and Cdc42. In some embodiments, a molecule targeting Rac1 and/or Rac2 and/or Rac3 and Cdc42 can be any biological or chemical molecule, compound, or moiety that results in inhibition, reduction, decrease, elimination, or ablation of expression of the Rac proteins and Cdc42 protein in the patient. A molecule targeting Racs and Cdc42 can be, for example, any molecule that is known or available in the art capable of being used to reduce or eliminate expression of a gene or its encoded protein. For example, such a molecule may include, but is not limited to, a small molecule drug, a molecule for RNA interference (RNAi), small interfering RNAs (siRNAs), small hairpin RNAs (shRNAs), microRNAs (miRNAs), endoribonuclease-prepared RNAs (esiRNAs), CRISPR/Cas9, transcription activator-like effector nucleases (TALENs), zinc finger nucleases (ZFNs), protein expression blockers (PEBL), antisense oligonucleotides, ribozymes, morpholinos, epigenetic modification, e.g., changes in DNA methylation or histone modification, or the like. In some

embodiments, these and any other methods, techniques, or technologies known and/or available in the art can be used in accordance with the present disclosure.

[0027] Each and every method, composition, or use described herein optionally includes the limitation "wherein the one or more molecule(s) targeting Rac1 and/or Rac2 and/or Rac3 and Cdc42 is not MBQ-167."

[0028] Small molecule drugs

[0029] In some embodiments, a molecule useful for silencing Rac1 and/or Rac2 and/or Rac3 and Cdc42 may be a small molecule. Small molecule drugs for treatment of cancer are known in the art. Small molecule drugs can interact with cancer cells for treatment of cancer by any mechanism. In some embodiments, a small molecule drug may refer to any traditional chemotherapy drug or any immunotherapy drug. A number of chemotherapeutic drugs and/or immunotherapy drugs are known in the art for treatment of cancer and may be used in accordance with the present disclosure for e.g., reducing the size of a tumor or the tumor load in a patient, reducing the number or size of metastases in a patient, reducing the number of cancer cells in a patient, increasing apoptosis of cancer cells in a patient, increasing DNA damage in cancer cells, or otherwise contributing to treatment of cancer in a patient. In some embodiments, a chemotherapeutic drug or immunotherapy drug known or available in the art may include, but is not limited to, Evista (Raloxifene Hydrochloride), Raloxifene Hydrochloride, Soltamox (Tamoxifen Citrate), Tamoxifen Citrate, Abemaciclib, Abraxane (Paclitaxel Albumin-stabilized Nanoparticle Formulation), Ado-Trastuzumab Emtansine, Afinitor (Everolimus), Afinitor Disperz (Everolimus), Alkeran (Melphan), Alpelisib, altretamine (Hexalen®), Anastrozole, anthracyclines, Aredia (Pamidronate Disodium), Arimidex (Anastrozole), Aromasin (Exemestane), Atezolizumab, Avastin (Bevacizumab), Bevacizumab, bleomycin, Capecitabine, Carboplatin, Cisplatin, Cyclophosphamide (Cytoxan®), Docetaxel, Doxorubicin Hydrochloride, Doxil (Doxorubicin Hydrochloride Liposome), Ellence (Epirubicin Hydrochloride), Enhertu (Fam-Trastuzumab Deruxtecan-nxki), Epirubicin Hydrochloride, Eribulin Mesylate, etoposide (VP-16), Everolimus, Exemestane, 5-FU (Fluorouracil Injection), Fam-Trastuzumab Deruxtecan-nxki, Fareston (Toremifene), Faslodex (Fulvestrant), Femara, (Letrozole), Fluorouracil Injection, Fulvestrant, Gemcitabine Hydrochloride, Gemzar (Gemcitabine Hydrochloride), Goserelin Acetate, Halaven (Eribulin Mesylate), Herceptin Hylecta (Trastuzumab and Hyaluronidase-oysk), Herceptin (Trastuzumab), Hycamtin (Topotecan Hydrochloride), Ibrance (Palbociclib), ifosfamide (Ifex®), Infugem (Gemcitabine Hydrochloride), irinotecan (CPT-11, Camptosar®), Ixabepilone, Ixempra (Ixabepilone), Kadcyla (Ado-Trastuzumab Emtansine),

Keytruda (Pembrolizumab), Kisqali (Ribociclib), Lapatinib Ditosylate, Letrozole, Lynparza (Olaparib), Margenza (Margetuximab-cmkb), Margetuximab-cmkb, Megestrol Acetate, Melphalan, Methotrexate Sodium, Neratinib Maleate, Nerlynx (Neratinib Maleate), Niraparib Tosylate Monohydrate, Olaparib, Paclitaxel, Paclitaxel Albumin-stabilized Nanoparticle Formulation, Palbociclib, Pamidronate Disodium, Pembrolizumab, pemetrexed (Alimta®), Perjeta (Pertuzumab), Pertuzumab, Pertuzumab, Rubraca (Rucaparib Camsylate), Trastuzumab, and Hyaluronidase-zzxf, Phesgo (Pertuzumab, Trastuzumab, and Hyaluronidase-zzxf), Piqray (Alpelisib), Ribociclib, Sacituzumab Govitecan-hziy, Soltamox (Tamoxifen Citrate), Talazoparib Tosylate, Talzenna (Talazoparib Tosylate),Tamoxifen Citrate, Taxol, Taxotere (Docetaxel), Tecentriq (Atezolizumab), Tepadina (Thiotepa), Thiotepa, Topotecan Hydrochloride, Toremifene, Trastuzumab, Trastuzumab and Hyaluronidase-oysk, Trexall (Methotrexate Sodium), Trodelvy (Sacituzumab Govitecan-hziy), Tucatinib, Tukysa (Tucatinib), Tykerb (Lapatinib Ditosylate), Verzenio (Abemaciclib), Vinblastine Sulfate, vinorelbine (Navelbine®), Xeloda (Capecitabine), Zejula (Niraparib Tosylate Monohydrate), Zoladex (Goserelin Acetate).

[0030] RNA interference (RNAi)

[0031] RNAi refers to a group of non-coding RNA (ncRNA) molecules that includes small interfering RNA (siRNA) and microRNA (miRNA) and that are involved in post-transcriptional gene silencing (PTGS). Specific gene expression silencing by RNAi is a mechanism of transcriptional regulation in the eukaryotic cell that is mediated by small RNAs of 21-23 nucleotides in length (siRNA). Cancer is one of the main targets for RNAi-based therapy. Several studies conducted *in vivo* and *in vitro* showed that RNAi-based therapy can be used for treating single-gene disorders and those with overexpression of proteins. There are different types of small synthetic RNA used in cancer therapy, such as siRNA, shRNA, and bifunctional shRNA (bishRNA). SiRNA degrades 99% of its target in 46 hours making it suitable for acute diseases, while shRNA can be expressed for up to 3 years, making it a good choice in chronic disease.

[0032] As a cancer therapy, RNAi has promise, due to the silencing mechanism, specificity, and lack of side effects compared to chemotherapies. Oncogenes, mutated tumor suppressor genes, and several other genes involved in tumor progression are good targets for gene silencing by RNAi-based therapy. The major advantage of RNAi in cancer therapy is targeting multiple genes of various cellular pathways involved in tumor progression. Simultaneous or concurrent inhibition of multiple genes is an effective approach to treat cancer, as well as reduction of the possibility of multiple drugs' resistance caused by

overdose of chemical drugs. Another advantage of this type of treatment is developing suitable personalized drugs for a specific patient. Personalized drugs are likely to be more effective than others in controlling tumor growth. Many studies have been done in the field of RNAi-based drugs leading to introduction of several RNAi based drugs. For example, animal studies indicate that targeting essential proteins in the cell cycle, e.g., kinesin spindle protein (KSP) and polo-like kinase 1 (PLK1), using specific siRNAs exhibit potent antitumor activity in both subcutaneous and hepatic tumor models. In addition, targeting protein kinase N3 (PKN3) with RNAi reduces lymph node metastases in orthotopic prostate cancer models, resulting in significant tumor growth and lymph node metastasis inhibition, and inhibition of metastasis after administration in animal models of metastatic lung cancer and breast cancer metastasis to the lung.

[0033] In some embodiments, RNAi-based drugs useful as molecule(s) for cancer treatment as described herein may include, but are not limited to, CALAA-01, Atu027, ALN-VSP02, siG12D LODER, EZN-2968, SNALP-PLK1, Bcr-Abl siRNA, FANG vaccine, iPsiRNA, and ATN-RNA. One of skill in the art will recognize that other or additional RNAi-based drugs may be used as described herein without deviating from the scope of the present disclosure.

[0034] In some embodiments, in order to minimize drug resistance, one or more different treatments may be administered to a patient. For example, targeting more than one gene at a time reduces the risk of cancer, such as with the use of more than one specific RNAi-based therapy, e.g., with the use of 2, 3, 4, or more siRNAs, shRNAs, or the like. In some embodiments, the use of one or more RNAi-based molecules, e.g., siRNA or shRNA, enables inhibition or suppression of more than one gene involved in cancer or metastasis thereof.

[0035] In some embodiments, RNAi-based therapies for cancer are based on individual treatments, e.g., targeting of a single gene. In some embodiments, RNAi-based therapies are based on targeting multiple genes as described herein. In some embodiments, a RNAi-based approach to cancer treatment is based on disruption of signaling pathways, or pathways leading to cell division or apoptosis.

[0036] siRNA is produced in two stages, i.e., starting and effecting stages. In the starting stage, a long double-stranded RNA (500-200 bp) is cleaved into fragments with a length of 23-21 nucleotides, and the functional siRNA is produced. Double-stranded siRNAs are separated, and the antisense strand is directed to the RNA-induced silencing complex (RISC), which is then directed to the target mRNA and degrades the target mRNA, preventing it from being translated into protein and thus effectively suppressing the gene.

[0037] Small hairpin RNA (shRNA) is similar to siRNA, but functions after transfection into the genome by vectors. shRNA targets gene expression more unstably and less transiently than siRNA. Contrary to siRNA, shRNA is synthesized in the nucleus and then transported into the cytoplasm, where it undergoes final processing and performs its suppression activities. In some embodiments, useful shRNAs for targeting Cdc42 are disclosed herein as SEQ ID NOs:1-3. In some embodiments, useful shRNAs for targeting Rac1 are disclosed herein as SEQ ID NOs:4-6. It would be known by one of skill in the art that other shRNA molecules may be used herein to suppress or reduce the expression of one or more genes involved in cancer or metastasis thereof.

[0038] Bifuncational shRNA (BishRNA) is a type of shRNA that offers increases efficacy and durability of RNAi and hastens gene expression silencing. Compared to shRNA, bishRNA is able to target mRNA degradation and inhibits translation of the mRNA individually. BishRNA shows high efficiency and effectiveness as the leading (antisense) strand in bishRNA is loaded onto at least two RISC complexes, which increases its gene silencing activity. Moreover, both cleavage-dependent and -independent pathways are activated, resulting in target mRNA degradation and inhibition of target mRNA translation.

[0039] MicroRNAs (miRNAs)

[0040] MicroRNAs (miRNAs) are one of the most well-studied non-coding RNAs that play a critical role in gene regulation, some of which are dysregulated in association with certain cancer types. MiRNAs can function as tumor suppressors to induce apoptosis or to inhibit the cell cycle. Unique miRNA transcriptomes are being studied for each cancer type, enabling better classification of specific cancer types and prognostic information.

[0041] Correlation between the dysregulation of miRNA machinery and disease progression and poor prognosis has been reported in several tumor types, such as ovarian cancer, neuroblastoma, and lung cancer. In most cases, cancer cells exhibit a distinct miRNA pattern that contributes to specific traits of malignancies. MiRNA binding through a partial complementary sequence within the target mRNA 3' untranslated region (UTR) primarily inhibits translation or promotes its degradation. A specific miRNA can regulate hundreds of genes, but only 30-40% of genes are regulated by miRNAs, and the target sites are often selectively conserved within the 3'UTR. In addition, dysregulation of proteins involved in miRNA biogenesis or processing, e.g., RNase III type enzymes, nuclear DROSHA and cytoplasmic DICER, as well as components of the RISC complex, e.g., TARBP2 and AGO2, have been reported in association with various types of cancers. The emerging role of the growth-promoting tumor microenvironment involves intra-cellular miRNA regulation.

Recent research reported that miRNAs play key roles in the crosstalk between cancer-associated fibroblasts (CAFs) and tumor cells, which were transferred by cell-derived extracellular vesicles. Extracellular miRNAs from cancer-associated fibroblasts grant favorable traits such as growth promotion, survival, and drug resistance in various tumor types, including colorectal cancer, breast cancer, and pancreatic cancer.

[0042] MiRNAs present altered expression patterns in malignant tissues and cells. Some miRNAs regulate essential genes for cellular homeostasis in which the alteration will result in abnormal biological changes, including uncontrolled cell proliferation, angiogenesis, metabolism, and apoptosis, leading to malignant formation. All of the key cancer pathways have an association with miRNA alterations; sensitivity to growth signals (e.g., let-7 family, miR-21); insensitivity to antigrowth signals (e.g., miR-17-92 cluster, miR-195); apoptosis escape (e.g., miR-34a, miR-185, miR-15/miR-16); angiogenesis (e.g., miR-210, miR-26, miR-15b, miR-155); invasion and metastases (e.g., miR-10b, miR-31, miR-200 family, miR-21, miR-15b); evading immune destruction (e.g., miR-124, miR-155, miR-17-92); tumor-promoting inflammation (e.g., miR-23b, miR-155, let-7d); and genomic instability (e.g., miR-21, miR-155, miR-15b). The malignant transformation will alter cell- and cellular state-specific miRNA expression profiles in healthy tissues. Furthermore, miRNAs are capable of driving malignant formation either by repressing tumor suppressor genes or increasing oncogene expression, thus emphasizing the importance of miRNAs in malignant transformation and as biomarkers to further subclassify cancer types.

[0043] Tumor-related miRNAs are broadly classified into two groups: Oncogenic miRNAs (OncomiRs), and Tumor Suppressor miRNAs (TS-miRNAs, TS-miRs). Most, if not all, types of cancers display an induced expression of some oncomiRs, which promote tumorigenesis by blocking the translation of tumor-suppressive mRNAs (e.g., miR-17-22, miR-125b, and miR-125), whereas the reduced expression of TS-miRNAs (e.g., miR-133a, miR-145, and miR-143) directly inhibits the translation of oncoproteins. Thus, miRNA expression versatility emphasizes their utility in cancer therapeutic strategies. Antisense-miRNA (anti-miRs) or miRNA mimics were developed for miRNA therapy and are widely used to repress oncomiRs or to restore TS-miRs, respectively.

[0044] Reducing oncomiRs, which are frequently overexpressed in human cancers, allows for the restoration of target tumor-suppressor expression that could work as a therapeutic strategy. Thus, in some embodiments, commonly used miRNA inhibitors, single-stranded antisense, anti-miR oligonucleotides (AMOs), locked nucleic acid (LNA) anti-miRs,

antagomiRs, miRNA sponges, and small molecule inhibitors of miRNAs (SMIRs), interfere with miRNA biogenesis or block miRNA-mRNA interaction.

[0045] In some embodiments, a miRNA useful in accordance with the present disclosure may be a miRNA that regulates one or more genes involved in tumor growth, progression, or the like. One of skill in the art is capable of identifying one or more miRNAs that may be useful as described herein for administration to a patient having cancer as described herein.

[0046] Endoribonuclease-prepared RNAs (esiRNAs)

[0047] Endoribonuclease-prepared RNAs (esiRNAs) technology has been widely used as an efficient tool for mediating RNA interference (RNAi) for coding transcripts in mammalian cells, ranging from single transcript targeting to genome-wide loss-of-function (LOF) screening. Furthermore, esiRNAs have been shown to be efficient in various cell types, including diverse human cancer cell lines. EsiRNAs are pools of several hundreds of individual siRNAs generated by enzymatic digestion of a typically 300- to 600-base-pairs-long double-stranded RNA (dsRNA) derived from a single target transcript. Pooling of siRNAs has been shown to increase on-target specificity by decreasing off-target effects, because the siRNAs that make up the pool exist in comparable amounts and have the same on-target competence. Therefore, the silencing capacity for the intended target is additive, while off-target effects are diluted out. Genome-scale esiRNA libraries for coding transcripts of mouse and human origins have been used intensively for LOF screening and are commercially available.

[0048] CRISPR/Cas9

[0049] Clustered regularly interspersed short palindromic repeats (CRISPR/Cas9) can be employed to engineer oncolytic viruses and immune cells for cancer therapeutic applications. CRISPR/Cas9 has the ability to precisely edit genes, not only in model organisms, but also in humans, which permits its use in therapeutic analysis. CRISPR can be used to identify genotype-specific vulnerabilities and identify 'essential' genes that can be potential drug targets, as their functional depletion leads to a reduced viability. Compared to shRNAs, CRISPR is more sensitive in detecting essential genes and thus, CRISPR/Cas9 screens have been conducted to systematically discover essential genes across many cancer cell lines, as well as genetic interactions in cancer cells, thereby revealing novel context-dependent essential genes.

[0050] Mechanistically, two distinct RNAs – the CRISPR targeting (crRNA) and the trans-activating RNA (tracrRNA) – activate and guide Cas proteins to bind viral DNA sequences which are subsequently cleaved. A subgroup of these CRISPR systems, the type II

system, relies on a single Cas protein to target a defined DNA sequence and is therefore particularly attractive to be used as a tool for genome editing. In some embodiments, crRNAs are combined with tracrRNAs into a single guide RNA (sgRNA). Before cleaving the target DNA, the Cas9 nuclease undergoes conformational changes upon sgRNA binding and is directed to its target site. Binding specificity is determined by a 20-nucleotide sequence preceding the three-nucleotide protospacer adjacent motif (PAM), which consists of a NGG or NAG sequence. After unwinding the DNA, binding to the PAM and DNA-sgRNA hybrid formation, two nuclease domains introduce a double strand break (DSB) in the target sequence. A DSB can be corrected by the host cell with non-homologues end joining (NHEJ), which is an error-prone repair mechanism that often leads to insertions or deletions (indel), which in turn can cause frameshift mutations, premature stop codons or/and non-sense mediated decay to the target gene, resulting in a loss of function. Homology-directed repair (HDR), in contrast, uses assisted recombination of DNA donor templates to reconstruct cleaved DNA. This mechanism can be exploited to introduce well defined mutations by transferring altered donor templates into targeted cells. The strength of the nuclease activity is determined largely by the binding efficiency of Cas9. Systematic modifications of the sgRNA scaffold identified an optimized scaffold structure that is associated with a higher binding efficiency of Cas9 to the target DNA.

[0051] Somatic gene therapy traditionally refers to the introduction of novel genetic material into somatic cells to express therapeutic gene products for the treatment of diseases. New gene editing technologies may allow permanent modification of somatic cells, both *in vivo* and *ex vivo*. Clinical trials have been done to assess the safety of programmed cell death protein-1 (PD-1) knockout engineered T cells *ex vivo* in treating metastatic non-small cell lung cancer that has progressed after all standard treatments. Immune checkpoint regulator PD-1 is a T-cell receptor responsible for inhibition of T-cell activation, thereby regulating immune tolerance and decreasing autoimmune reactions, but also allowing immune escape of cancers. Antibodies that neutralize PD-1 or its ligand PD-L1 are successfully used for the treatment of lung cancers, among others. Patients enrolled in the gene-editing trial provide peripheral blood lymphocytes, and PD-1 knockout of T-cells by CRISPR/Cas9 is performed *ex vivo*. The edited lymphocytes are selected, expanded, and subsequently infused back into the patient. This same concept of PD-1 knockout is used for the treatment of other cancer types, including prostate, bladder, esophageal, and renal cell cancer.

[0052] CRISPR/Cas9 provides a way to manipulate the genome, transcriptome, and epigenome across a broad range of organisms. Pooled CRISPR screens provide a comprehensive set of essential genes across most cancer cell lines. This, combined with genetic and epigenetic characteristics of cancer cell lines, enables the extensive identification of synthetic lethal interactions and facilitate the discovery of novel drug targets.

[0053] Transcription activator-like effector nucleases (TALENs) and Zinc finger nucleases (ZFNs)

[0054] Transcription activator-like effector nucleases (TALENs) and zinc finger nucleases (ZFNs) are restriction enzymes that can be engineered to excise specific sequences of DNA, e.g., cutting out or removing genes or sections of nucleic acid. In some embodiments, a section of a gene or DNA to be removed may be, e.g., a gene that leads to tumor progression or cell division as described herein, such as Rac1 and/or Rac2 and/or Rac3 and Cdc42.

[0055] Protein expression blockers (PEBL)

[0056] Specific constructs, named protein expression blockers (PEBLs), prevent transport of targeted proteins to the cell membrane. PEBL constructs can be readily combined with other gene modifications and be incorporated into existing clinical-grade protocols for ex vivo cell processing of immune cells.

[0057] Antisense oligonucleotides (ASOs)

[0058] Antisense oligonucleotides (ASOs) are short oligonucleotides that are used to modify gene expression and mRNA splicing in the nervous system. Antisense oligonucleotides bind to specific molecules of RNA and block the ability of the RNA to make a protein or perform other functions. Antisense oligonucleotides may be used to block the production of proteins needed for cell growth or differentiation. Binding of the antisense oligonucleotide to a mRNA target of interest results in the inability of that mRNA to be translated into protein. In some embodiments, antisense oligonucleotides complementary to all or a portion of the mRNA transcribed from the Rac1 gene and the Cdc42 gene can be administered to a patient having cancer or metastases thereof in order to prevent the translation of these mRNAs into their respective proteins.

[0059] In some embodiments, ASOs that contain RNA-DNA hybrid structures may be used to target Racs and Cdc42 as described herein. Such RNA-DNA hybrid structures recruit RNaseH RNA degrading activity, resulting in degradation of transcripts of Racs and Cdc42.

[0060] Ribozymes

[0061] Ribozymes are RNA molecules that have the ability to catalyze specific biochemical reactions, including RNA splicing in gene expression, similar to the action of protein enzymes. Ribozymes are catalytically active RNA molecules that occur naturally in various sizes and shapes. Most frequently, ribozymes catalyze cleavage and ligation of specific phosphodiester bonds in *cis* or in *trans*. However, peptide bond formation during protein synthesis on the ribosome is catalyzed by ribosomal RNA. Despite the limited chemical repertoire, the biological functions of ribozymes are diverse and they play central roles during transfer RNA maturation, intron splicing, replication of RNA viruses or viroids, the regulation of messenger RNA stability, and protein synthesis.

[0062] Morpholinos

[0063] Morpholinos are synthetic molecules that are the product of a redesign of natural nucleic acid structure. Typically 25 bases in length, morpholinos bind to complementary sequences of RNA or single-stranded DNA by standard nucleic acid base-pairing to modify gene expression.

[0064] Epigenetic modifications

[0065] Epigenetic modifications refer to changes in DNA methylation or histone modification. DNA methylation is associated with gene suppression and can be used in some embodiments to inhibit, reduce, or eliminate expression of, e.g., Rac1 and/or Rac2 and/or Rac3 and Cdc42 in a patient.

[0066] The molecule types or technologies described herein represent a small portion of the possible types that can be used to target Rac1 and/or Rac2 and/or Rac3 and Cdc42 for treatment of cancer. One of skill in the art will recognize that any method of simultaneous or concurrent silencing or suppression of Rac1 and/or Rac2 and/or Rac3 and Cdc42 can be used as described herein without deviating from the scope of the present disclosure. In some embodiments, more than one method or technology described herein or known in the art can be combined together for silencing, eliminating, or suppressing gene expression of one or more genes.

[0067] In some embodiments, the Rac1 and/or Rac2 and/or Rac3 and Cdc42 genes may be optionally deleted in a tumor cell, using any known or available methods in the art, including, but not limited to, disruption by insertion of an exogenous sequence into the gene, blocking its expression; gene editing with, e.g., TALENs, ZFNs, or CRISPR; expression of an scFv with an endoplasmic reticulum (ER) binding tether to bind the cytokine in the ER and prevent secretion; and transfection of shRNAs or siRNAs. In some embodiments,

targeted deletion of Racs and Cdc42 in a tumor cell may be accomplished by, e.g., disruption of enzymes that cancer cells need to replicate, hormone therapies, signal transduction inhibitors, gene expression modulators, apoptosis inducers, angiogenesis inhibitors, immunotherapies, and toxin delivery molecules.

[0068] Hormone therapies slow or stop the growth of hormone-sensitive tumors, which require certain hormones to grow. Hormone therapies act by preventing the body from producing the hormones, or by interfering with the action of the hormones. Hormone therapies are available for both breast cancer and prostate cancer.

[0069] Signal transduction inhibitors block the activities of molecules that participate in signal transduction, the process by which a cell responds to signals from its environment. During this process, once a cell has received a specific signal, the signal is relayed within the cell through a series of biochemical reactions that ultimately produce the appropriate response(s). In some cancers, malignant cells are stimulated to divide continuously without being prompted to do so by external growth factors. Signal transduction inhibitors interfere with this inappropriate signaling.

[0070] Gene expression modulators modify the function of proteins that play a role in controlling gene expression.

[0071] Apoptosis inducers cause cancer cells to undergo a process of controlled cell death called apoptosis. Apoptosis is one method the body uses to get rid of unneeded or abnormal cells, but cancer cells have strategies to avoid apoptosis. Apoptosis inducers can get around these strategies to induce the death of cancer cells.

[0072] Angiogenesis inhibitors block the growth of new blood vessels to tumors. A blood supply is necessary for tumors to grow beyond a certain size (blood provides the oxygen and nutrients tumors need for continued growth) and thus, interfering with angiogenesis blocks tumor growth. Some targeted therapies that inhibit angiogenesis interfere with the action of vascular endothelial growth factor (VEGF), a substance that stimulates new blood vessel formation. Other angiogenesis inhibitors can target other molecules that stimulate new blood vessel growth.

[0073] Immunotherapies trigger the immune system to destroy cancer cells. Some immunotherapies are monoclonal antibodies that recognize specific molecules on the surface of cancer cells. Binding of the monoclonal antibody to the target molecule results in the immune destruction of cells that express that target molecule. Other monoclonal antibodies bind to certain immune cells to help these cells better kill cancer cells.

[0074] Monoclonal antibodies that deliver toxic molecules can cause the specific death of cancer cells. Once the antibody has bound to its target cell, the toxic molecule that is linked to the antibody (e.g., a radioactive substance or poisonous chemical) is taken up by the cell, resulting in the death of the cell. The toxin will not affect cells that lack the target for the antibody, i.e., the vast majority of cells in the body.

[0075] In some embodiments, gene silencing occurs during transcription of a gene, e.g., Rac1 and/or Cdc42. Well-known methods of transcriptional gene silencing include, but are not limited to, genomic imprinting, paramutation, transposon silencing, histone modification, transgene silencing, position effects, RNA-directed DNA methylation, among others.

[0076] In some embodiments, gene silencing occurs during translation (i.e., post-transcriptional) of a gene, e.g., Rac1 and/or Cdc42. Well-known methods of transcriptional gene silencing include, but are not limited to, RNA interference (RNAi), RNA silencing, e.g., siRNA, miRNA, shRNAs, piwi-associated RNAs (piRNAs), nonsense-mediated decay, among others. These technologies are known in the art and would be easily performed by one of skill in the art.

Chemotherapeutic Drugs for Treatment of Cancer

[0077] In some embodiments, a method for treatment of cancer as described herein by simultaneously or concurrently silencing Rac1 and/or Rac2 and/or Rac3 and Cdc42 may also be combined with additional treatments for cancer as deemed appropriate by a clinician or medical professional, such as administration of one or more standard chemotherapeutic drugs or immunotherapy drugs.

[0078] Any other drugs known or available in the art may also be used in combination with simultaneous or concurrent silencing of Rac1 and/or Rac2 and/or Rac3 and Cdc42 as described herein without deviating from the scope of the present disclosure.

[0079] In some specific embodiments, depending on the type of cancer to be treated, additional treatment for cancer may include a cancer-specific chemotherapeutic drug. In some embodiments, specific drug treatments, e.g., chemotherapeutic drugs or immunotherapy drugs, may be better suited for a particular type of cancer and may represent a preferred addition to treatment by silencing Rac1 and/or Rac2 and/or Rac3 and Cdc42 as described herein. Chemotherapeutic drugs are discussed in detail herein elsewhere.

[0080] In some embodiments, one or more chemotherapeutic drugs may be used together in combination with simultaneous or concurrent silencing of Rac1 and/or Rac2 and/or Rac3 and Cdc42 as described herein, as deemed appropriate by a clinician, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 chemotherapeutic drugs, or the like.

[0081] Administration of a chemotherapeutic drug in combination with simultaneous or concurrent silencing of Rac1 and/or Rac2 and/or Rac3 and Cdc42 as described herein may be by any route appropriate for the drug given, such as intravenous, intraperitoneal, intramuscular, oral, or the like. Treatment may be administered for a specified period of time, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 days, or the like; or for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, weeks, or the like; or for 1, 2, 3, 4, 5 6 7 8 9, 10, 11, 12, 13, 14 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 months or the like. Any length of time or any number of administrations or treatments may be used or given as deemed appropriate by a clinician.

Methods of Treatment for Cancer

[0082] Provided herein are methods of treating primary or metastatic cancer in a patient in need thereof, comprising administering to the patient therapeutically effective amounts of one or more molecules targeting Racs and Cdc42. Also provided are methods of prolonging survival of in a patient having cancer, comprising administering to the patient therapeutically effective amounts of one or more molecules targeting Racs and Cdc42. Also provided are methods of suppressing tumor growth in a patient in need thereof, comprising administering to the patient therapeutically effective amounts of one or more molecules targeting Racs and Cdc42, either alone or in combination therapy.

[0083] In some embodiments, the methods described herein may be used to treat cancer in a patient as described herein. In some embodiments, the type of cancer to be treated as described herein may be a cancer type that harbors one or more DNA repair deficiencies described herein. In some embodiments, a cancer type that can be treated as described herein is treatable with one or more molecules targeting Racs and Cdc42, either alone or in combination with one or more chemotherapeutic or immunotherapy drugs. In some embodiments, cancers that overexpress or increase activation of Racs and/or Cdc42 proteins are all particularly good targets for the therapeutic methods described herein, such as including, but not limited to, lung cancer, pancreatic cancer, colon cancer, prostate cancer, ovarian cancer, cervical cancer, melanoma, thyroid cancer, breast cancer (e.g., triple-negative breast cancer), colorectal cancer, gastric cancer, hepatocellular carcinoma, or the like. In some embodiments, the type of cancer to be treated is lung cancer. In some embodiments, the cancer is primary cancer or metastatic cancer. In some embodiments, the patient achieves remission for cancer and the cancer recurs.

[0084] In some embodiments, the type of cancer to be treated as described herein is a cancer that can be characterized by increased expression of Rac1 and/or Cdc42. For example, increased Rac1 and/or Cdc42 expression can be found at least in breast cancer (e.g., triple-

negative breast cancer), lung cancer, colorectal cancer, gastric cancer, prostate cancer, hepatocellular carcinoma, melanoma, and ovarian cancer. In some embodiments, the methods described herein are useful for treatment of cancer resulting from mutation of Rac1 and/or Cdc42 resulting in increased expression. Mutation of Rac1 is found, for example, in melanoma and sarcoma. In some embodiments, the methods described herein are useful for treatment of a primary tumor and for metastases resulting from a primary tumor. In some embodiments, simultaneous or concurrent silencing of Rac1 and/or Rac2 and/or Rac3 and Cdc42 is useful for treating lung cancer, as well as metastases from lung cancer.

[0085] In some embodiments, a method described herein further comprises one or more chemotherapeutic drugs, such as a chemotherapeutic drug, or a combination of chemotherapeutic drugs, described herein or known in the art.

[0086] In some embodiments, the treatment occurs outside of a clinical trial setting. In some embodiments, the methods described herein may be administered in a clinical setting or may be administered in an alternate setting as deemed appropriate by a clinician or practitioner.

[0087] In some embodiments, one or more molecules targeting Racs and Cdc42 may be combined with other cancer treatment drugs, for example, a PARP inhibitor as described herein. In some embodiments, useful PARP inhibitors can include any PARP inhibitors known or available in the art, such as including, but not limited to, olaparib (AZD 2281, Lynparza®), rucaparib (Rubraca®), niraparib (Zejula®), iniparib (BSI-201), nivolumab (Opdivo®), ipilimumab (Yervoy®), capmatinib (Tabrecta®), sotorasib (Lumakras®), veliparib (ABT-888), LY2603618 (IC-83), talazoparib (Talzenna®).

[0088] As would be understood by one of skill in the art, simultaneous or concurrent silencing of Rac1 and/or Rac2 and/or Rac3 and Cdc42 and treatment with one or more chemotherapeutic drugs as described herein are administered in any form necessary or useful to the subject for treatment of cancer, for example, a liquid (e.g., injectable and infusible solutions), a semi-solid, a solid, an aqueous solution, a suspension, an emulsion, a gel, a magma, a mixture, a tincture, a powder, a capsule, a dispersion, a tablet, a pellet, a pill, a powder, a liposome, a lozenge, a troche, a liniment, an ointment, a lotion, a paste, a suppository, a spray, an inhalant, or the like. In some embodiments, a drug as described herein for treatment of cancer may be administered in a liquid or aqueous form for injection into a patient. The form can depend on the intended mode of administration and therapeutic application. Typically, compositions for the agents described herein are in the form of injectable or infusible solutions.

[0089] In some embodiments, a drug as described herein for treatment of cancer in a patient may be administered by any route or mode of administration, such as intravenous (IV), oral (p.o.), sublingual, rectal, vaginal, ocular, otic, nasal, cutaneous, enteral, epidural, intra-arterial, intravascular, nasal, respiratory, subcutaneous (s.c.), topical, transdermal, intramuscular, intra-peritoneal (i.p.), or the like.

[0090] Unless otherwise specified herein, the methods described herein can be performed in accordance with the procedures exemplified herein or routinely practiced methods well known in the art. The following sections provide additional guidance for practicing the methods of the present disclosure.

Pharmaceutical Compositions for Targeting Rac1 and/or Rac2 and/or Rac3 and Cdc42

[0091] In some embodiments, one or more molecules targeting Racs and Cdc42 and one or more chemotherapeutic drugs may be administered together as a single composition, i.e., both or all drugs may be combined together in a solution or other drug form as described herein. In some embodiments, each drug may be administered separately (while still being administered concurrently), i.e., in separate solutions or drug forms as described herein. For example, one or more molecules targeting Racs and Cdc42 as described herein may be administered to a patient in an aqueous solution for intravenous administration, and one or more chemotherapeutic drugs may be administered in one or more separate or distinct aqueous solution(s) for intravenous administration. Pharmaceutical formulation is well established and known in the art.

[0092] In some embodiments, one or more molecules targeting Racs and Cdc42 and one or more chemotherapeutic drugs may be formulated with excipient materials, such as sodium citrate, sodium dibasic phosphate heptahydrate, sodium monobasic phosphate, Tween-80, and a stabilizer. The one or more molecules targeting Racs and Cdc42 and one or more chemotherapeutic drugs can be provided, for example, in a buffered solution at a suitable concentration and can be stored at an appropriate temperature to maintain the efficacy of the drug(s), for example a temperature of 2-8°C. In some other embodiments, the pH of the composition is between about 5.5 and about 7.5 (e.g., 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, or 7.5).

[0093] A pharmaceutical composition described herein can also include agents that reduce aggregation of the drug when formulated. Examples of aggregation reducing agents include one or more amino acids selected from the group consisting of methionine, arginine, lysine, aspartic acid, glycine, and glutamic acid. The pharmaceutical compositions can also include a sugar (e.g., sucrose, trehalose, mannitol, sorbitol, or xylitol) and/or a tonicity

modifier (e.g., sodium chloride, mannitol, or sorbitol) and/or a surfactant (e.g., polysorbate-20 or polysorbate-80).

[0094] A composition comprising one or more molecules targeting Racs and Cdc42 can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable for stable storage at high concentration. Sterile injectable solutions can be prepared by incorporating an agent described herein in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating an agent described herein into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the methods of preparation are vacuum drying and freeze drying that yield a powder of an agent described herein plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

[0095] In certain embodiments, a composition comprising one or more molecules targeting Racs and Cdc42 may be prepared with a carrier that will protect the components against rapid release, such as a controlled release formulation, including implants, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known. *See*, e.g., Sustained and Controlled Release Drug Delivery Systems, J. R. Robinson, ed., Marcel Dekker, Inc., New York (1978).

[0096] In some embodiments, a composition comprising one or more molecules targeting Racs and Cdc42 is formulated in sterile distilled water or phosphate buffered saline. The pH of the pharmaceutical formulation may be between about 5.5 and about 7.5 (e.g., 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, or 7.5).

Administration of one or more molecules targeting Racs and Cdc42

[0097] One or more molecules targeting Racs and Cdc42 as described herein can be administered to a subject, e.g., a patient in need thereof, by a variety of methods. For many applications, the route of administration or delivery is one of: intratumoral injection (e.g., in the case of miRNA mimics or inhibitors), intratumoral convection-enhanced delivery,

nanoparticle-mediated intratumoral delivery, systemic delivery, intravenous injection or infusion (IV), subcutaneous injection (SC), intraperitoneally (IP), intramuscular injection, intra-arterial, intrathecal, intracapsular, intraocular, intracardiac, intradermal, transtracheal, subcuticular, intra-articular, subcapsular, subarachnoid, intra-arterial, intrathecal, intracapsular, intraocular, intracardiac, intradermal, transtracheal, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, and epidural and intrasternal injection. The route and/or mode of administration of the one or more molecules targeting Racs and Cdc42, or compositions comprising these, can also be tailored for the individual case, e.g., by monitoring the patient.

[0098] In some embodiments, a delivery system may be used to deliver one or more molecule(s) targeting Rac1 and/or Rac2 and/or Rac3 and Cdc42. For example, in some embodiments, such molecule(s) may be delivered to a patient using any delivery system known or available in the art. Non-limiting examples include viral vectors (e.g., lentivirus, adenovirus, adeno-associated virus), non-viral vectors (e.g., nanoparticles, lipid-based vectors (e.g., cationic, neutral, or ionizable liposomes), polymer-based vectors (e.g., synthetic biodegradable polymers, copolymers, natural polymers, such as collagen, chitosan, or gelatin, polyamidoamine dendrimers, PEI-PEG, PEI, polyurethane-PEI, polymeric micelles, PACE polymers), inorganic vectors (e.g., calcium phosphate, carbonate apatite, porous silica, gold and carbon nanotubes, calcium phosphate (CaP)), exosome/extracellular vesicle-based vectors (e.g., exosomes, exosome-GE11 peptides, microvesicles, apoptotic bodies), and/or other biomaterials (e.g., atelocollagen).

[0099] The composition(s) comprising one or more molecules targeting Racs and Cdc42 can be administered as a fixed dose, or in a mg/kg dose. The dose can also be chosen to reduce or avoid production of antibodies against the one or more molecules targeting Racs and Cdc42. Dosage regimens are adjusted to provide the desired response, e.g., a therapeutic response or a combinatorial therapeutic effect. Generally, doses of the one or more molecules targeting Racs and Cdc42, and optionally additional agent(s), can be used in order to provide a subject with the agent in bioavailable quantities.

[0100] One or more molecules targeting Racs and Cdc42 can be administered, e.g., at a periodic interval over a period of time (a course of treatment) sufficient to encompass at least 1 dose, 2 doses, 3 doses, 4 doses, 5 doses, 6 doses, 7 doses, 8 doses, 9 doses, 10 doses, 11 doses, 12 doses, 13 doses, 14 doses, 15 doses, 16 doses, 17 doses, 18 doses, 19 does, 20 doses, or more, e.g., once daily, twice daily, three times daily, or about one to four times per week, or such as weekly, biweekly (every two weeks), every three weeks, monthly, e.g., for

between about 1 to 12 weeks, such as between 2 to 8 weeks, such as between about 3 to 7 weeks, such as for about 4, 5, or 6 weeks, or every 5 weeks, or every 6 weeks, or any interval deemed appropriate by a clinician. In some embodiments, one or more molecules targeting Racs and Cdc42 as described herein may be administered three times daily in certain tissue sites that wash out more readily, e.g., bladder. Factors that may influence the dosage and timing required to effectively treat a subject, include, e.g., the stage or severity of the disease or disorder, formulation, route of delivery, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of the molecule(s) targeting Rac1 and/or Rac2 and/or Rac3 and Cdc42, or compositions comprising these, can include a single treatment or can include a series of treatments.

[0101] If a subject is at risk for developing a disorder described herein, the molecule(s) targeting Rac1 and/or Rac2 and/or Rac3 and Cdc42 can be administered before the full onset of the disorder, e.g., as a preventative measure. The duration of such preventative treatment can be a single dosage of the composition, or the treatment may continue (e.g., multiple dosages). For example, a subject at risk for the disorder or who has a predisposition for the disorder may be treated with a composition as described herein for days, weeks, months, or even years, so as to prevent the disorder from occurring or fulminating.

[0102] For patients receiving treatment for cancer, resistance of the cancer cells to the one or more molecules targeting Racs and Cdc42 can reduce the efficacy of the molecule(s). For these patients, administration of a combination of one or more molecules targeting Racs and Cdc42 can increase the sensitivity of cancer cells to the one or more molecules targeting Racs and Cdc42, thus prolonging the effects of the drugs and thereby prolonging the survival of the patient having cancer.

[0103] In some embodiments, one or more molecules targeting Racs and Cdc42 may be administered to a patient in order to extend the duration of remission or to prevent a relapse or reduce the incidence of relapse of a cancer patient in remission.

[0104] A combination of one or more molecules targeting Racs and Cdc42 can be administered to a patient in need thereof (e.g., a patient that has had or is at risk of having primary or metastatic cancer) alone or in combination with (i.e., by co-administration or sequential administration) other therapeutic treatments or drugs for treating cancer (e.g., one or more chemotherapeutic or immunotherapy drugs or treatments). In one embodiment, the additional therapeutic treatments or drugs are included in a pharmaceutical composition as described herein. In other embodiments, the additional therapeutic treatments or drugs are co-

administered, administered concurrently, or administered sequentially in separate or distinct compositions.

Kits

[0105] One or more molecules targeting Racs and Cdc42 for treatment of cancer in a patient can be provided in a kit. In one embodiment, the kit includes (a) a container that contains the molecule(s) targeting Rac1 and/or Rac2 and/or Rac3 and Cdc42 as described herein, and optionally (b) informational material. The informational material can be descriptive, instructional, marketing or other material that relates to the methods described herein and/or the use of the agents for therapeutic benefit.

[0106] In one embodiment, the kit also includes additional agents (e.g., one or more chemotherapeutic or immunotherapy drugs described herein) for treating cancer described herein. For example, the kit includes a first container that contains the molecule(s) targeting Rac1 and/or Rac2 and/or Rac3 and Cdc42, and a second container that includes the chemotherapeutic or immunotherapy drug. In another embodiment, the kit includes a first container that contains the molecule(s) targeting Rac1 and/or Rac2 and/or Rac3 and Cdc42, a second container that contains the chemotherapeutic drug, and a third container that contains the additional chemotherapeutic or immunotherapy agent(s).

[0107] The informational material of the kits is not limited in its form. In one embodiment, the informational material can include information about production of the compound, molecular weight of the compound, concentration, date of expiration, batch or production site information, and so forth. In one embodiment, the informational material relates to methods of administering the molecule(s) targeting Rac1 and/or Rac2 and/or Rac3 and Cdc42, as well as the additional chemotherapeutic or immunotherapy drug, e.g., in a suitable dose, dosage form, or mode of administration (e.g., a dose, dosage form, or mode of administration described herein), to treat a subject who has had or who is at risk for cancer. The information can be provided in a variety of formats, include printed text, computer readable material, video recording, or audio recording, or information that provides a link or address to substantive material, e.g., on the internet.

[0108] In addition to the molecule(s) targeting Rac1 and/or Rac2 and/or Rac3 and Cdc42, and including any additional chemotherapeutic or immunotherapy drug(s) if applicable, the kit can include other ingredients, such as a solvent or buffer, a stabilizer, or a preservative. The molecule(s) targeting Rac1 and/or Rac2 and/or Rac3 and Cdc42, and/or one or more chemotherapeutic drugs or immunotherapy drugs, can be provided in any form described herein, e.g., liquid, dried or lyophilized form, substantially pure and/or sterile. When the

agents are provided in a liquid solution, the liquid solution is an aqueous solution. When the agents are provided as a lyophilized product, the lyophilized powder is generally reconstituted by the addition of a suitable solvent. The solvent, e.g., sterile water or buffer (e.g., PBS), can optionally be provided in the kit.

[0109] The kit can include one or more containers for the drugs or compositions. In some embodiments, the kit contains separate containers, dividers or compartments for the drugs and informational material. For example, the molecule(s) targeting Rac1 and/or Rac2 and/or Rac3 and Cdc42, and any chemotherapeutic or immunotherapy drugs, if applicable, can be contained in a bottle, vial, or syringe, and the informational material can be contained in a plastic sleeve or packet. In other embodiments, the separate elements of the kit are contained within a single, undivided container. For example, the molecule(s) targeting Rac1 and/or Rac2 and/or Rac3 and Cdc42, and chemotherapeutic or immunotherapy drugs, if applicable, are contained in a bottle, vial or syringe that has attached thereto the informational material in the form of a label. In some embodiments, the kit includes a plurality (e.g., a pack) of individual containers, each containing one or more unit dosage forms (e.g., a dosage form described herein) of the agents. The containers can include a combination unit dosage, e.g., a unit that includes both the molecule(s) targeting Rac1 and/or Rac2 and/or Rac3 and Cdc42, and the chemotherapeutic or immunotherapy drugs, if applicable, e.g., in a desired ratio. For example, the kit includes a plurality of syringes, ampules, foil packets, blister packs, or medical devices, e.g., each containing a single combination unit dose. The containers of the kits can be air-tight, waterproof (e.g., impermeable to changes in moisture or evaporation), and/or light-tight.

[0110] The kit optionally includes a device suitable for administration of the molecule(s) targeting Rac1 and/or Rac2 and/or Rac3 and Cdc42, and chemotherapeutic or immunotherapy drugs, if applicable, e.g., a syringe or other suitable delivery device. The device can be provided pre-loaded with one or both of the agents or can be empty, but suitable for loading.

Definitions

[0111] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the disclosure. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the disclosure, subject to any specifically excluded limit in the stated

range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the disclosure.

[0112] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present disclosure, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited. The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present disclosure is not entitled to antedate such publication by virtue of prior disclosure. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

[0113] Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by one of ordinary skill in the art to which the disclosure pertains. Specific terminology of particular importance to the description of the present disclosure is defined below.

[0114] As used in this specification and the appended claims, the singular forms "a," "an," and "the," along with similar references used in the context of describing a particular embodiment (especially in the context of certain of the following claims), can be construed to cover both the singular and the plural, unless specifically noted otherwise. Thus, for example, "an active agent" refers not only to a single active agent, but also to a combination of two or more different active agents, "a dosage form" refers to a combination of dosage forms, as well as to a single dosage form, and the like. In some embodiments, the term "or" as used herein, including the claims, is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive.

[0115] In some embodiments, numbers expressing quantities of ingredients, properties such as molecular weight, reaction conditions, and so forth, used to describe and claim certain embodiments of the present disclosure are to be understood as being modified in some instances by the term "about." In some embodiments, the term "about" is used to indicate that a value includes the standard deviation of the mean for the device or method being employed to determine the value. In some embodiments, the numerical parameters set forth in the written description and attached claims are approximations that can vary depending upon the desired properties sought to be obtained by a particular embodiment. In some embodiments,

the numerical parameters should be construed in light of the number of reported significant digits and by applying ordinary rounding techniques. Notwithstanding that the numerical ranges and parameters setting forth the broad scope of some embodiments of the present disclosure are approximations, the numerical values set forth in the specific examples are reported as precisely as practicable. The numerical values presented in some embodiments of the present disclosure may contain certain errors necessarily resulting from the standard deviation found in their respective testing measurements. The recitation of ranges of values herein is merely intended to serve as a shorthand method of referring individually to each separate value falling within the range. Unless otherwise indicated herein, each individual value is incorporated into the specification as if it were individually recited herein. In some embodiments, "about" refers to a specified value +/- 10%.

[0116] The terms "comprise," "have," and "include" are open-ended linking verbs. Any forms or tenses of one or more of these verbs, such as "comprises," "comprising," "has," "having," "includes," and "including," are also open-ended. For example, any method that "comprises," "has," or "includes" one or more steps is not limited to possessing only those one or more steps and can also cover other unlisted steps. Similarly, any composition or device that "comprises," "has," or "includes" one or more features is not limited to possessing only those one or more features and can cover other unlisted features.

[0117] As used herein, "Cdc42" refers to Cell Division Control Protein 42, or any synonyms thereof, including, but not limited to, CDC42, CDC42Hs, G25K, Cell division cycle 42, Cell division control protein 42 homolog, GTP binding protein, 25kDa, G25K GTP-binding protein, DJ224A6.1.1 (cell division cycle 42 (GTP-binding protein, 25kD)), DJ224A6.1.2 (cell division cycle 42 (GTP-binding protein, 25kD)), Cell Division Cycle 42 (GTP Binding Protein, 25kDa), Cell Division Cycle 42 (GTP-Binding Protein, 25kD), Small GTP Binding Protein CDC42, Growth-Regulating Protein, EC 3.6.5.2, TKS, or any other synonyms known or available in the art. In some embodiments, Cdc42 refers interchangeably to the Cdc42 gene, transcripts thereof, or the Cdc42 protein.

[0118] As used herein, "clinical response" is an indicator of therapeutic efficacy in combination with other indicators. In some embodiments, a clinical response refers to a percentage of patients whose cancer (e.g., primary or metastatic tumor) reduces, shrinks, lessens, etc. after treatment. In some embodiments, clinical response varies with the type of cancer, disease state, and stage of disease. For example, in some embodiments, simultaneous or concurrent silencing of the Racs and Cdc42 genes may produce stable disease, wherein

tumor growth and progression is halted, or wherein tumor shrinkage that provides an objective clinical response in a patient is observed.

[0119] As used herein, "co-administration" refers to the simultaneous or concurrent administration of one or more drugs with another. In some embodiments, both drugs are administered at the same time. Co-administration may also refer to any particular time period of administration of either drug, or both drugs. For example, as described herein, a drug may be administered hours or days before administration of another drug and still be considered to have been co-administered. In some embodiments, co-administration may refer to any time of administration of either drug such that both drugs are present in the body of a patient at the same. In some embodiments, either drug may be administered before or after the other, so long as they are both present within the patient for a sufficient amount of time that the patient received the intended clinical or pharmacological benefits.

[0120] Conservative amino acid substitutions providing functionally similar amino acids are well known in the art. The following six groups each contain amino acids that are conservative substitutions for one another: 1) Alanine (A), Serine (S), Threonine (T); 2) Aspartic acid (D), Glutamic acid (E); 3) Asparagine (N), Glutamine (Q); Arginine (R), Lysine (K); 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W). Not all residue positions within a protein will tolerate an otherwise "conservative" substitution. For instance, if an amino acid residue is essential for a function of the protein, even an otherwise conservative substitution may disrupt that activity, for example the specific binding of an antibody to a target epitope may be disrupted by a conservative mutation in the target epitope.

[0121] In some embodiments, conservative amino acid substitutions, e.g., substituting one acidic or basic amino acid for another, can often be made without affecting the biological activity of a recombinant polypeptide as described herein. Minor variations in sequence of this nature may be made in any of the peptides disclosed herein, provided that these changes do not substantially alter (e.g., by 15% or more) the desired activity of the protein.

[0122] As used herein, a dosage unit form or "fixed dose" as used herein refers to physically discrete units suited as unitary dosages for the patients to be treated; each unit contains a predetermined quantity of the molecule(s) targeting Rac1 and/or Rac2 and/or Rac3 and Cdc42 and/or one or more chemotherapeutic or immunotherapy drugs described herein calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier and optionally in association with the other agent. Single or multiple dosages may be given. Alternatively, or in addition, the molecule(s) targeting Rac1 and/or

Rac2 and/or Rac3 and Cdc42 and/or one or more chemotherapeutic or immunotherapy drugs, or composition(s) comprising these may be administered via continuous infusion.

[0123] As used herein, the phrases "parenteral administration" and "administered parenterally" as used herein mean modes of administration other than enteral and topical administration, usually by injection, and include, without limitation, intravenous, intramuscular, intra-arterial, intrathecal, intracapsular, intraocular, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection, and infusion.

[0124] A pharmaceutical composition(s) comprising molecule(s) targeting Rac1 and/or Rac2 and/or Rac3 and Cdc42 and/or one or more chemotherapeutic drugs as described herein may include a "therapeutically effective amount" of the molecule(s) targeting Rac1 and/or Rac2 and/or Rac3 and Cdc42 and/or the one or more chemotherapeutic or immunotherapy drugs as described herein. The term "therapeutically effective amount," "pharmacologically effective dose," "pharmacologically effective amount," or simply "effective amount" may be used interchangeably and refers to that amount of an agent effective to produce the intended pharmacological, therapeutic or preventive result, e.g., a reduction of cancerous cells or lessened cancer cell burden (i.e., reduction in number of cancer cells), tumor size, tumor density, lymph node involvement, metastases, or associated symptoms in the patient. The pharmacologically effective amount results in the amelioration of one or more symptoms of a disorder (e.g., lung cancer), or prevents the advancement of a disorder, or causes the regression of the disorder, or prevents the disorder. Such effective amounts can be determined based on the effect of the administered agent, or the combinatorial effect of agents if more than one agent is used. A therapeutically effective amount of an agent may also vary according to factors such as the disease stage, state, age, sex, and weight of the individual, and the ability of the compound to elicit a desired response in the individual, e.g., amelioration of at least one disorder parameter or amelioration of at least one symptom of the disorder. A therapeutically effective amount is also one in which any toxic or detrimental effects of the composition are outweighed by the therapeutically beneficial effects. In some examples, an "effective amount" is one that treats (including prophylaxis) one or more symptoms and/or underlying causes of cancer. In one example, an effective amount is a therapeutically effective amount. In one example, an effective amount is an amount that prevents one or more signs or symptoms of a particular disease or condition from developing.

[0125] As used herein, "gene expression" or "expression" refers to the process of gene transcription, translation, and post-translational modification.

[0126] As used herein, "gene silencing" refers to the regulation of gene expression in a cell to prevent the expression of a certain gene. In some embodiments, gene silencing occurs during transcription of a gene, e.g., Rac1 and/or Cdc42. In some embodiments, gene silencing occurs during translation of a gene, e.g., Rac1 and/or Cdc42. In some embodiments, gene silencing is used to describe knockdown, i.e., reduced expression of a gene. In some embodiments, gene silencing is used to describe knockout, i.e., abolished, eliminated, or absence of expression of a gene. As described herein, any methods known in the art to reduce or eliminate expression of a gene or its encoded protein are encompassed within the present disclosure, such as including, but not limited to, RNAi, CRISPR, siRNA, or the like.

[0127] As used herein, "molecule(s) targeting Rac1 and/or Rac2 and/or Rac3 and Cdc42" refers to any biological or chemical molecule, compound, or moiety that effects gene silencing of Rac1 and/or Rac2 and/or Rac3 and Cdc42. Such molecules are described in detail herein and can include, but are not limited to, a molecule that blocks interaction of Rac and/or Cdc42 with their target molecules. A molecule targeting Rac1 and Cdc42 described herein can affect expression of the Rac1 and/or Cdc42 genes, or can affect activity of the resulting Rac1 and/or Rac2 and/or Rac3 and/or Cdc42 proteins.

[0128] By "pharmaceutically acceptable" is meant a material that is not biologically or otherwise undesirable, i.e., the material may be incorporated into a pharmaceutical composition administered to a patient without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the composition in which it is contained. When the term "pharmaceutically acceptable" is used to refer to a pharmaceutical carrier or excipient, it is implied that the carrier or excipient has met the required standards of toxicological and manufacturing testing or that it is included on the Inactive Ingredient Guide prepared by the U.S. Food and Drug administration. "Pharmacologically active" (or simply "active") as in a "pharmacologically active" (or "active") derivative or analog, refers to a derivative or analog having the same type of pharmacological activity as the parent compound and approximately equivalent in degree. The term "pharmaceutically acceptable salts" include acid addition salts which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like.

[0129] As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. The composition can include a pharmaceutically acceptable salt, e.g., an acid addition salt or a base addition salt.

[0130] As used herein, "Racs" refers to Rac1 and/or Rac2 and/or Rac3, which are the Ras-related C3 botulinum Toxin Substrate 1, 2, and 3, respectively, or any synonyms thereof. Rac1 refers interchangeably to the Rac1 gene, transcripts thereof, or the Rac1 protein, Rac2 refers interchangeably to the Rac2 gene, transcripts thereof, or the Rac2 protein, and Rac3 refers interchangeably to the Rac3 gene, transcripts thereof, or the Rac3 protein. The methods described herein may silence one or all of the Rac genes, transcripts, or proteins, together with the Cdc42 gene, transcript, or protein.

[0131] As used herein, "reducing" refers to a lowering or lessening, such as reducing cancer cell burden. In some embodiments, simultaneous or concurrent silencing of Rac and Cdc42 as described herein may result in "reduced" or lessened cancer cell burden (i.e., reduction in number of cancer cells), tumor number, tumor size, tumor density, lymph node involvement, metastases, or associated symptoms in the patient compared to a patient not been administered such drugs. "Reducing" may also refer to a reduction in disease symptoms as a result of a treatment as described herein, either alone, or co-administered with another drug.

[0132] As used herein, "reduced expression" or "silenced" refers to simultaneous or concurrent reduction of gene expression, downregulation, knockdown, suppression, or otherwise eliminating expression of Rac1 and/or Rac2 and/or Rac3 and Cdc42 in a patient having cancer. In some embodiments, a silenced Rac1 gene and a silenced Cdc42 gene result in the cancer cells lacking expression of both of these genes.

[0133] As used herein, "simultaneous silencing" or "concurrent silencing" refers to the silencing of more than one gene or its expression in an individual at the same time. Simultaneous or concurrent silencing of the Rac1 and/or Rac2 and/or Rac3 and Cdc42 genes is used to treat or prevent metastases of lung cancer or other cancers.

[0134] As used herein, "subject" or "individual" or "patient" refers to any patient for whom or which therapy is desired, and generally refers to the recipient of the therapy. A "subject" or "patient" refers to any animal classified as a mammal, e.g., human and non-human mammals. Examples of non-human animals include dogs, cats, cattle, horses, sheep, pigs, goats, rabbits, etc. Unless otherwise noted, the terms "patient" or "subject" are used

herein interchangeably. In some embodiments, a subject amenable for therapeutic applications may be a primate, e.g., human and non-human primates.

[0135] As used herein, "targeting Rac1 and/or Rac2 and/or Rac3 and Cdc42" or "targeting Racs and Cdc42" refers to silencing, knockdown, suppression, or the like, at the gene level, e.g., transcriptional silencing, of one or more of the Rac1, Rac2, and Rac3 genes, and the Cdc42 gene. Targeting Racs and Cdc42 may also refer to elimination of the gene product, i.e., following transcription. Targeting Rac1 and/or Rac2 and/or Rac3 and Cdc42 may also refer to events at the protein level, e.g., translation or post-translation. Any method of targeting of Racs and Cdc42 is encompassed within the scope of the present disclosure.

[0136] In some embodiments, "transcriptional silencing" refers to any method of silencing, knockdown, or suppression at the gene expression or transcription level. Transcriptional silencing can be effected by any method known or available in the art, such as including, but not limited to, RNA interference (RNAi), RNA-induced transcriptional silencing (RITS), RNA-directed DNA methylation (RdDM), genomic imprinting, paramutation, transposon silencing, transgene silencing, or position effects. As used herein, "post-transcriptional silencing" refers to any method of silencing, knockdown, or suppression after transcription, e.g., regulation, degradation, modification of RNA. Post-transcriptional silencing can be effected by any method known or available in the art, such as including, but not limited to, RNA interference (RNAi), RNA silencing, antisense oligonucleotides, ribozymes, or nonsense-mediated decay. As used herein, "translational silencing" refers to any method of silencing, knockdown, or suppression at the translation level, e.g., protein level. Translational silencing can be effected by any method known or available in the art, such as including, but not limited to, RNA interference (RNAi), RNA silencing, antisense oligonucleotides, ribozymes, or nonsense-mediated decay. As used herein, "post-translational silencing" refers to any method of silencing, knockdown, or suppression after translation, e.g., protein level. Translational silencing can be effected by any method known or available in the art, such as including, but not limited to, RNA interference (RNAi), RNA silencing, antisense oligonucleotides, ribozymes, or nonsense-mediated decay. These techniques are well represented in the art and would be understood by one of skill in the art.

[0137] The terms "treating" and "treatment" or "alleviating" as used herein refer to reduction or lessening in severity and/or frequency of symptoms, elimination of symptoms and/or underlying cause, and improvement or remediation of damage. In certain aspects, the term "treating" and "treatment" as used herein refer to the prevention of the occurrence of symptoms. In other aspects, the term "treating" and "treatment" as used herein refer to the

prevention of the underlying cause of symptoms associated with a disease or condition, such as breast or ovarian cancer. The phrase "administering to a patient" refers to the process of introducing a composition or drug into the patient via an art-recognized means of introduction. "Treating" or "alleviating" also includes the administration of compounds or agents to a subject to prevent or delay the onset of the symptoms, complications, or biochemical indicia of a disease (e.g., lung cancer), alleviating the symptoms or arresting or inhibiting further development of the disease, condition, or disorder. Subjects in need of treatment include those already suffering from the disease or condition, as well as those being at risk of developing the disease or condition. Treatment may be prophylactic (to prevent or delay the onset of the disease or condition, or to prevent the manifestation of clinical or subclinical symptoms thereof) or therapeutic suppression, or alleviation of symptoms after the manifestation of the disease or condition.

[0138] All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided with respect to certain embodiments herein is intended merely to better illuminate the present disclosure and does not pose a limitation on the scope of the present disclosure otherwise claimed. No language in the specification should be construed as indicating any non-claimed element essential to the practice of the present disclosure.

[0139] Groupings of alternative elements or embodiments of the present disclosure disclosed herein are not to be construed as limitations. Each group member can be referred to and claimed individually or in any combination with other members of the group or other elements found herein. One or more members of a group can be included in, or deleted from, a group for reasons of convenience or patentability.

[0140] Having described the present disclosure in detail, it will be apparent that modifications, variations, and equivalent embodiments are possible without departing the scope of the present disclosure defined in the appended claims. Furthermore, it should be appreciated that all examples in the present disclosure are provided as non-limiting examples.

EXAMPLES

[0141] Examples of embodiments of the present disclosure are provided in the following examples. The following examples are presented only by way of illustration and to assist one of ordinary skill in using the disclosure. The examples are not intended in any way to otherwise limit the scope of the disclosure.

Example 1 – Rac1 and Cdc42 are correlated with poor survival

[0142] Rac1 and Cdc42 correlate with poor survival in cancer patients. FIG. 1 shows the probability of survival for patients having liver, pancreatic, renal, and lung cancer that exhibit increased expression of either Rac1 or Cdc42. In addition, Rac1 and Cdc42 were found to be upregulated in ER- breast cancer (including triple-negative) and HER2+ breast cancer (FIG. 2).

Example 2 - Metastatic tumor cell bioluminescence tracking in mice

[0143] Cancer cells expressing luciferase were injected into the circulation in the ophthalmic venous sinus, which is anatomically found in the retro-orbital eye socket. These injected cells develop into lung metastases.

[0144] Lung metastases were detected using bioluminescence 10 days after injection of the cancer cells. As shown in FIG. 3, the intensity of luciferase activity is measured from the hydrolysis of injected luciferin. Metastatic foci are quantified by bioluminescence intensity, where red indicates high luminescence, green indicates medium luminescence, and blue indicates low intensity.

Example 3 – Silencing Rac and Cdc42 genes after establishment of metastases abolishes metastatic tumors

[0145] Lung metastases were established using doxycycline-inducible silencing of Rac1 and Cdc42 in 4T1 mouse mammary carcinoma cells. Seven days after establishment of lung metastases, Rac1/Cdc42 silencing was induced to knock down expression of Rac1 and CDC42. Two weeks after induction of silencing, the lungs of non-silenced to silenced animals were compared. Results demonstrating reduced tumors are shown in FIG. 4.

[0146] As shown in FIG. 5, silencing of Rac1 and Cdc42 resulted in reduced numbers of lung metastases verses controls. Hematoxylin and eosin (H&E) staining showed that after silencing of Rac1 and Cdc42, lung tissue from treated animals showed only 1 to 2 small metastases over a period of about 2 weeks, while controls showed an average of 12 metastases. In addition, the size of metastases was assessed using pixel numbers per area with ImageJ software, and results showed that the size of metastases in animals after silencing of Rac1 and Cdc42 was considerably smaller than controls. Thus, a dramatic reduction was seen in both number and size of the established metastases with 2 weeks of Rac1/Cdc42 silencing.

Example 4 – Silencing Rac1 and Cdc42 promotes survival and blocks cachexia in animals with lung metastases

Weight loss in animals in which Rac1 and Cdc42 were silenced was compared with control animals. Increased body weight is a surrogate for well-being and low or no metastatic burden.

As shown in FIG. 6, the Rac1/Cdc42 silenced animals (Dox +) showed increased body weight (P<0.05, Two-way ANOVA test) compared to control animals. In contrast, non-silenced control animals demonstrated significant cachexia, including loss of body weight. Non-silenced control animals all died by 30 days post-establishment of lung tumors, whereas most animals silenced for Rac1/Cdc42 survived by termination of the study at 30 days (P=0.0178, Mantel-Cox log-rank test). Rac1/Cdc42 silenced animals were healthy, showed no signs of distress. and would likely have survived.

Example 5 – shRNA for knockdown of Racs and Cdc42

[0147] For additional quantification of Rac/Cdc42 silencing, shRNA was used to known down expression of the Racs and Cdc42 genes. The shRNA used to knock down expression of Cdc42 (clone ID TRCN0000071686, NM_009861.1-277s1c1) is provided as SEQ ID NO:1 (Cdc42 sh1-F) and SEQ ID NO:2 (Cdc42 sh1-R). The target sequence is provided as SEQ ID NO:3. The knockdown level for this shRNA was 0.94 in Hepa 1-6 cells.

[0148] Cdc42 sh1-F – forward sequence of shRNA for knockdown of Cdc42: CCGGCCGCTAAGTTATCCACAGACACTCGAGTGTCTGTGGATAACTTAG CGGTTTTTG (SEQ ID NO:1)

[0149] Cdc42 sh1-R – reverse sequence of shRNA for knockdown of Cdc42: AATTCAAAAACCGCTAAGTTATCCACAGACACTCGAGTGTCTGTGGATAACTTAG CGG (SEQ ID NO:2)

[0150] Cdc42 sh1 – target sequence of shRNA for knockdown of Cdc42:

[0151] CCGCTAAGTTATCCACAGACA (SEQ ID NO:3)

[0152] The shRNA used to knock down expression of Rac1 (clone ID TRCN0000310901, NM_009007.2-833s21c1) is provided as SEQ ID NO:4 (Rac1 sh1-F) and SEQ ID NO:5 (Cdc42 sh1-R). The target sequence is provided as SEQ ID NO:6. The knockdown level for this shRNA was 0.97 in Hepa 1-6 cells.

[0153] Rac1 sh1-F – forward sequence of shRNA for knockdown of Rac1:

[0154] CCGGATCAGCGAGCCTTCGCATTTGCTCGAGCAAATGCGAAGGCTCGC TGATTTTTG (SEQ ID NO:4)

[0155] Rac1 sh1-R – reverse sequence of shRNA for knockdown of Rac1:

[0156] AATTCAAAAAATCAGCGAGCCTTCGCATTTGCTCGAGCAAATGCGAAG GCTCGCTGA (SEQ ID NO:5)

[0157] Rac1 sh1 – target sequence of shRNA for knockdown of Rac1

[0158] ATCAGCGAGCCTTCGCATTTG (SEQ ID NO:6)

Example 6 – Direct administration of molecules targeting Racs and Cdc42

[0159] A molecule targeting Racs and Cdc42 is administered directly to a patient having cancer, e.g., primary or metastatic cancer. Possible molecules targeting Racs and Cdc42 to be administered include, but are not limited to, shRNA, siRNA, miRNA, esiRNAs, morpholinos, TALENs, ZFNs RNAi, PEBL, antisense oligonucleotides (ASOs), CRISPR/Cas9, knockdown, ribozymes, and epigenetic modifications.

[0160] After administration of one or more molecules targeting Racs and Cdc42, knockdown of Racs and Cdc42 is evaluated directly by obtaining a tumor biopsy sample. Alternatively, knockdown is assessed indirectly based on tumor response following administration of the molecule targeting shRNA.

Example 7 – Administration of molecules targeting Rac1 and/or Rac2 and/or Rac3 and Cdc42 by vector

[0161] Alternatively, one or more vectors as described herein, e.g., a lentiviral vector, is used to deliver one or more siRNAs or shRNAs to the patient for the treatment of cancer. After administration of one or more vectors comprising one or more siRNAs or shRNAs targeting Racs and Cdc42, knockdown of Racs and Cdc42 is evaluated directly by obtaining a tumor biopsy sample. Alternatively, knockdown is assessed indirectly based on tumor response following administration of the molecule targeting shRNA.

CLAIMS

What is claimed is:

1. A method of treating metastatic cancer in a patient in need thereof, comprising administering to the patient a composition comprising one or more molecules targeting Rac1 and/or Rac2 and/or Rac3 and Cdc42.
2. A method of prolonging survival in a cancer patient in need thereof, comprising administering to the patient a composition comprising one or more molecules targeting Rac1 and/or Rac2 and/or Rac3 and Cdc42.
3. A method of suppressing tumor growth in a cancer patient in need thereof, comprising administering to the patient a composition comprising one or more molecules targeting Rac1 and/or Rac2 and/or Rac3 and Cdc42.
4. The method of any of claims 1-3, wherein the targeting of Rac1 and/or Rac2 and/or Rac3 and Cdc42 comprises reduced expression or silencing of Rac1 and/or Rac2 and/or Rac3 and Cdc42.
5. The method of claim 4, wherein the reduced expression or silencing comprises simultaneous or concurrent? reduction of expression, downregulation, suppression, or silencing of Rac1 and/or Rac2 and/or Rac3 and Cdc42.
6. The method of any of claims 1-5, wherein the reduced expression or silencing of Rac1 and/or Rac2 and/or Rac3 and Cdc42 is effected by targeting of the Rac1 and/or Rac2 and/or Rac3 gene and the Cdc42 gene.
7. The method of any of claims 1-6, wherein the reduced expression or silencing of Rac1 and/or Rac2 and/or Rac3 and Cdc42 is effected by targeting of the Rac1 and/or Rac2 and/or Rac3 protein and the Cdc42 protein.
8. The method of any of claims 1-7, wherein the one or more molecules targeting Rac1 and/or Rac2 and/or Rac3 and Cdc42 is selected from the group consisting of shRNA, siRNA, miRNA, esiRNAs, morpholinos, TALENs, ZFNs RNAi, PEBL, antisense oligonucleotides (ASOs), CRISPR/Cas9, knockdown, ribozymes, and epigenetic modifications.
9. The method of any of claims 1-8, wherein the one or more molecules targeting Rac1 and/or Rac2 and/or Rac3 and Cdc42 are involved in transcriptional silencing of Rac1 and/or Rac2 and/or Rac3 and Cdc42.
10. The method of claim 9, wherein the transcriptional silencing is effected by RNA interference (RNAi), RNA-induced transcriptional silencing (RITS), RNA-directed

DNA methylation (RdDM), genomic imprinting, paramutation, transposon silencing, transgene silencing, or position effects.

11. The method of any of claims 1-10, wherein the one or more molecules targeting Rac1 and/or Rac2 and/or Rac3 and Cdc42 are involved in post-transcriptional silencing of Rac1 and/or Rac2 and/or Rac3 and Cdc42.
12. The method of claim 11, wherein the post-transcriptional silencing is effected by RNA interference (RNAi), RNA silencing, antisense oligonucleotides, ribozymes, or nonsense-mediated decay.
13. The method of any of claims 1-12, wherein the one or more molecules targeting Rac1 and/or Rac2 and/or Rac3 and Cdc42 are involved in translational silencing of Rac1 and/or Rac2 and/or Rac3 and Cdc42.
14. The method of claim 13, wherein the translational silencing is effected by RNA interference (RNAi), RNA silencing, antisense oligonucleotides, ribozymes, or nonsense-mediated decay.
15. The method of any of claims 1-14, wherein the one or more molecules targeting Rac1 and/or Rac2 and/or Rac3 and Cdc42 are involved in post-translational silencing of Rac1 and/or Rac2 and/or Rac3 and Cdc42.
16. The method of claim 15, wherein the post-translational silencing is effected by RNA interference (RNAi), RNA silencing, antisense oligonucleotides, ribozymes, or nonsense-mediated decay.
17. The method of any of claims 1-16, wherein the RNAi comprises use of one or more of siRNA, shRNA, and miRNA.
18. The method of any of claims 1-17, wherein the shRNA comprises a sequence set forth in SEQ ID NOs:1-6.
19. The method of any one of claims 1-18, wherein the metastatic cancer is breast, lung, pancreatic, colorectal, or liver cancer.
20. The method of claim 19, wherein the breast cancer is triple-negative breast cancer.
21. The method of any one of claims 1-20, wherein the simultaneous or concurrent silencing of Rac1 and/or Rac2 and/or Rac3 and Cdc42 results in reduction, shrinkage, or lessening of cancer after treatment.
22. The method of any one of claims 1-21, wherein the simultaneous or concurrent silencing of Rac1 and/or Rac2 and/or Rac3 and Cdc42 inhibits growth of cancer cells.

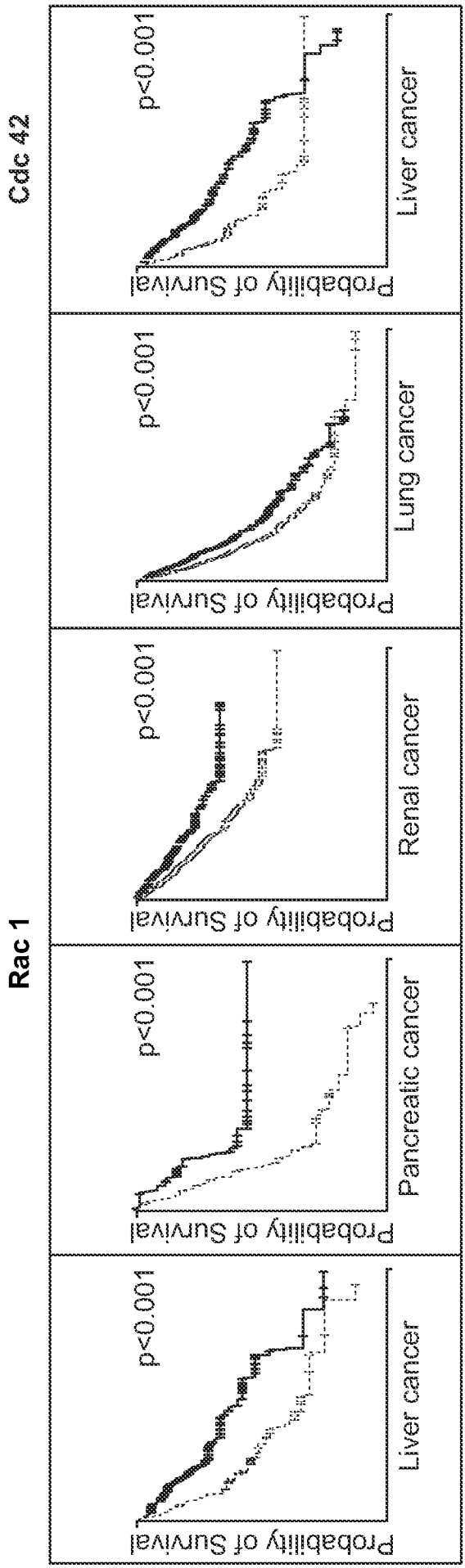

Rac 1
Cdc 42
Probability of Survival
p<0.001
Liver cancer
Pancreatic cancer
Renal cancer
Lung cancer
Liver cancer

FIG. 1

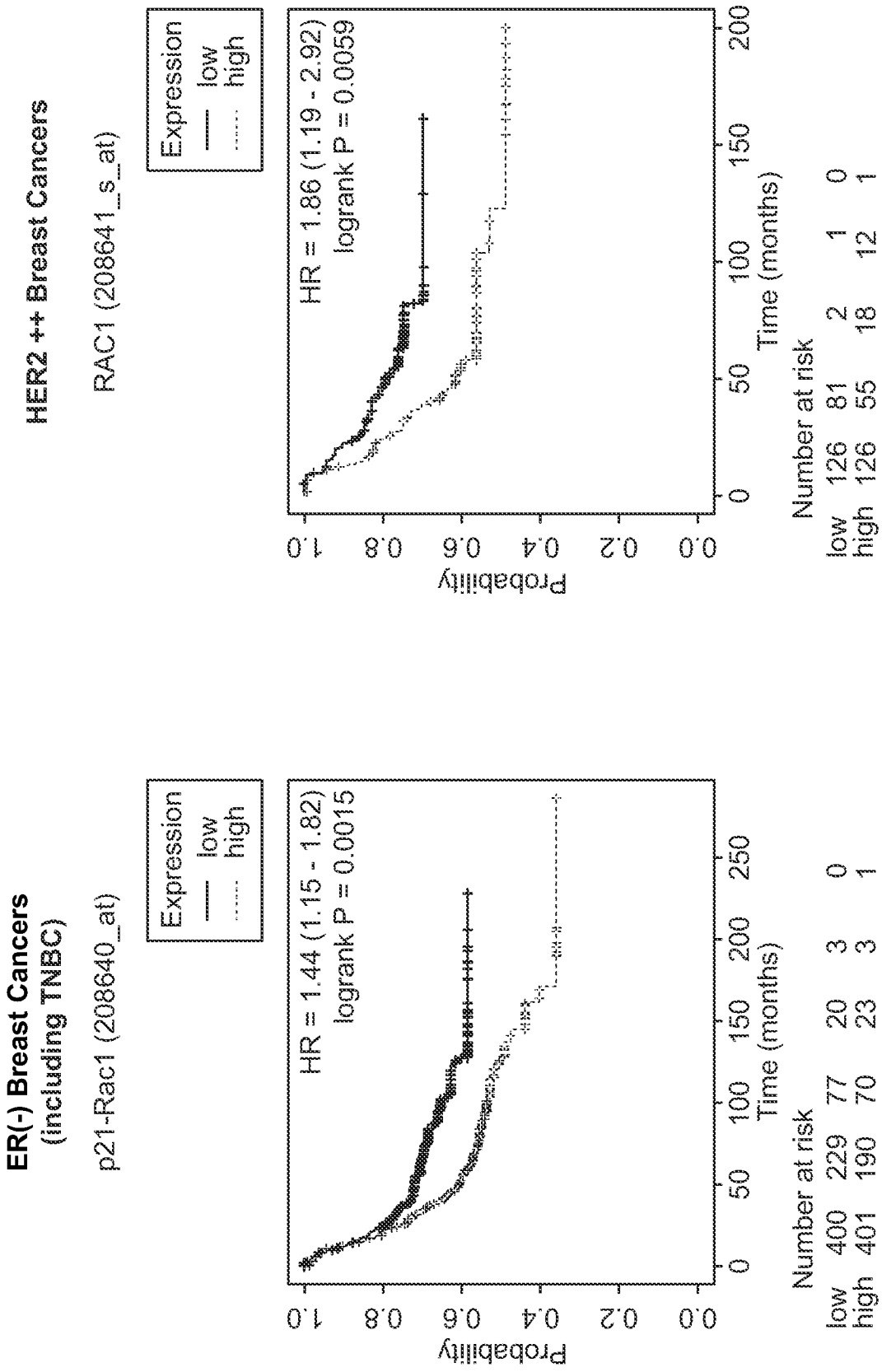

ER(-) Breast Cancers
(including TNBC)
p21-Rac1 (208640_at)
Expression
low
high
HR = 1.44 (1.15 - 1.82)
logrank P = 0.0015
Probability
Time (months)
Number at risk
low 400 229 77 20 3 0
high 401 190 70 23 3 1
HER2 ++ Breast Cancers
RAC1 (208641_s_at)
Expression
low
high
HR = 1.86 (1.19 - 2.92)
logrank P = 0.0059
Probability
Time (months)
Number at risk
low 126 81 2 1 0
high 126 55 18 12 1

FIG. 2

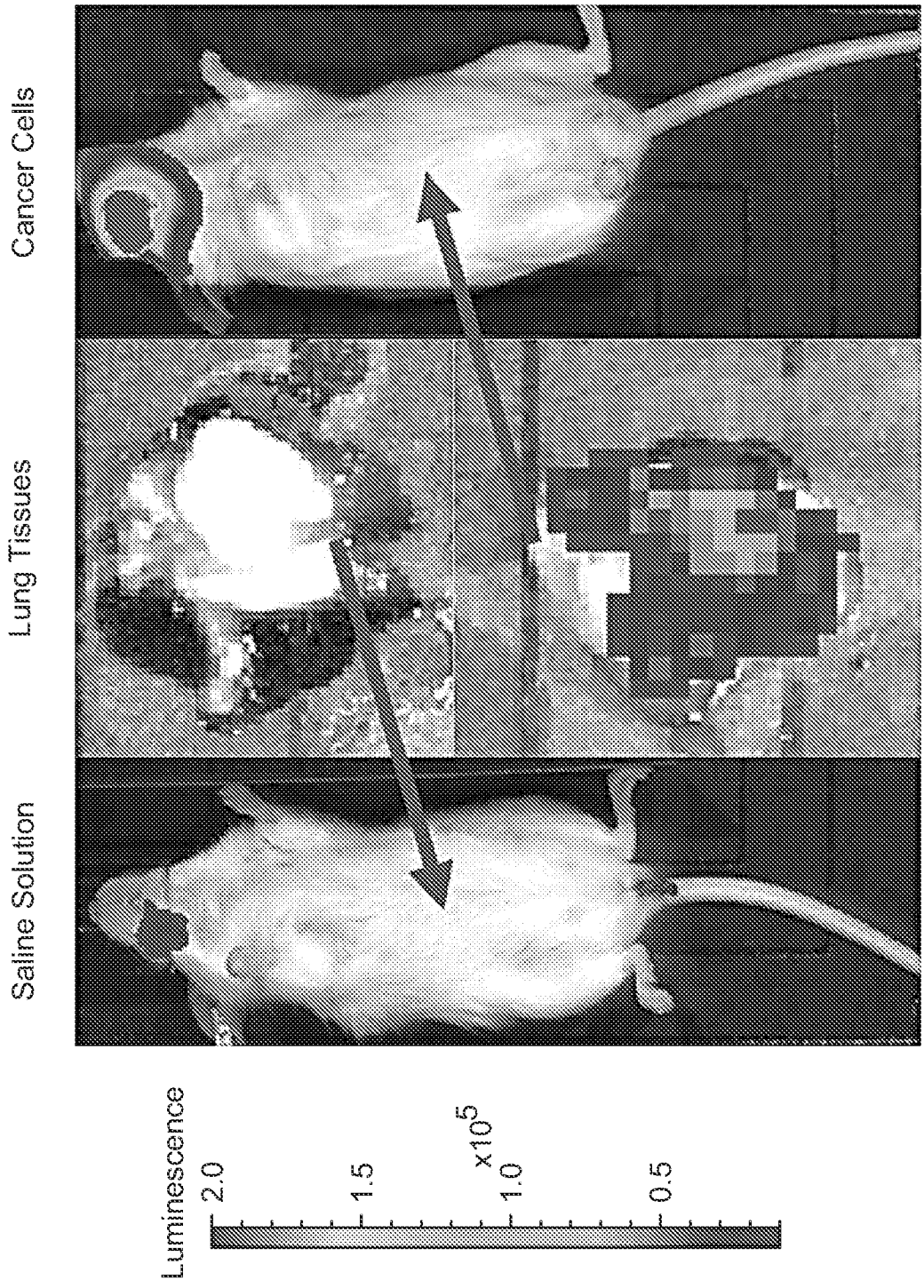

Saline Solution
Lung Tissues
Cancer Cells
Luminescence
2.0
1.5
1.0
0.5
x10^5

FIG. 3

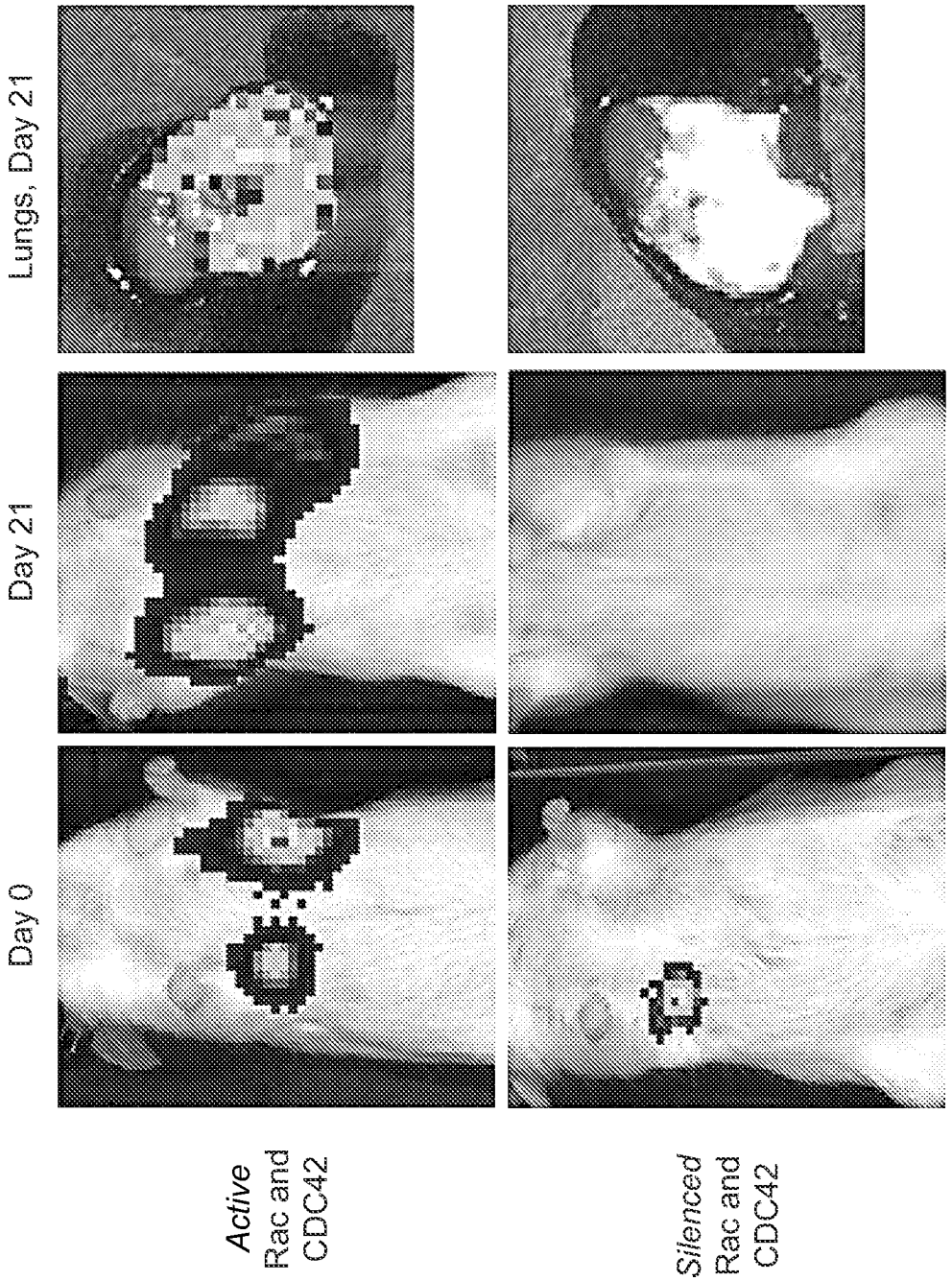

Lungs, Day 21
Day 21
Day 0
Active Rac and CDC42
Silenced Rac and CDC42

FIG. 4

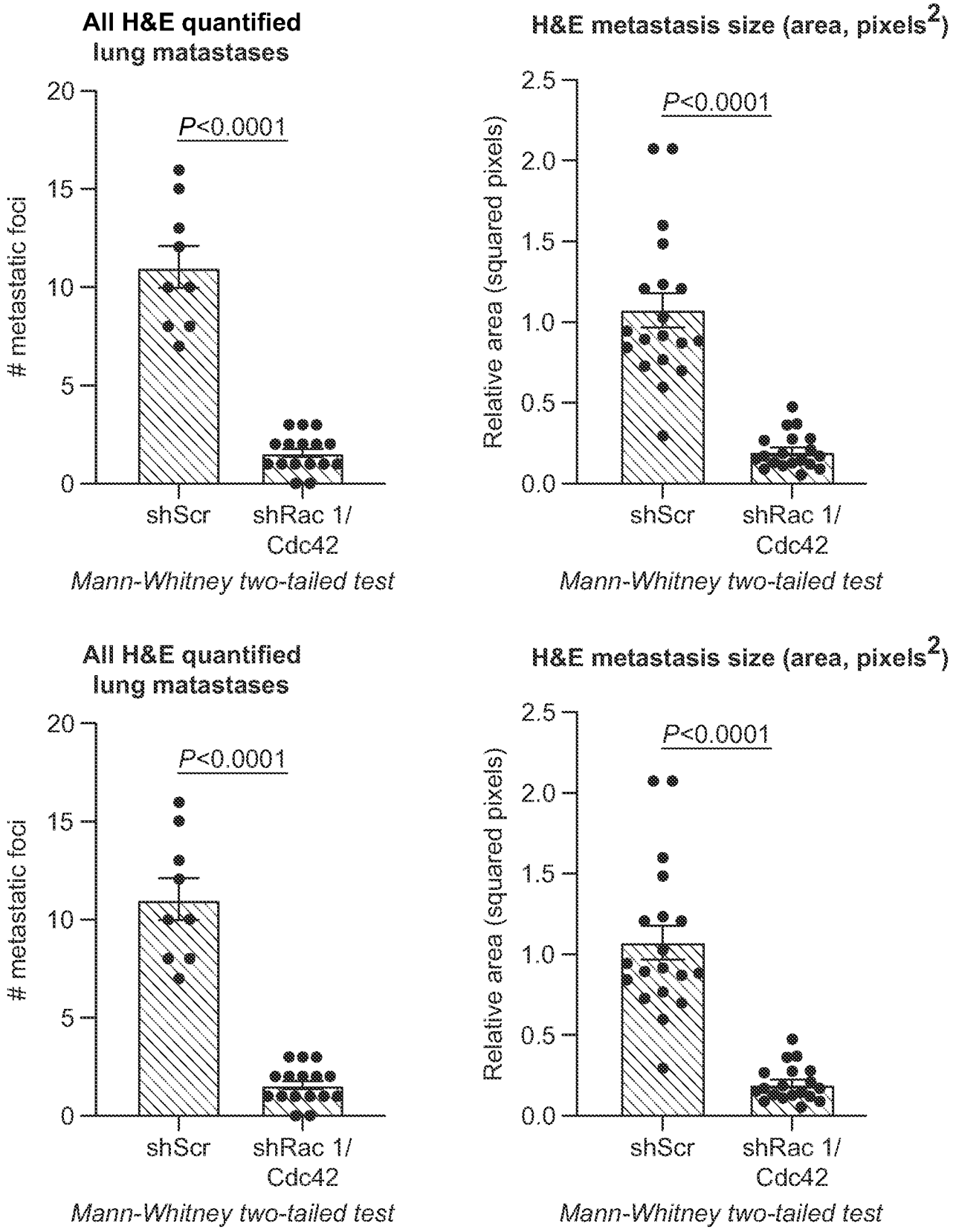
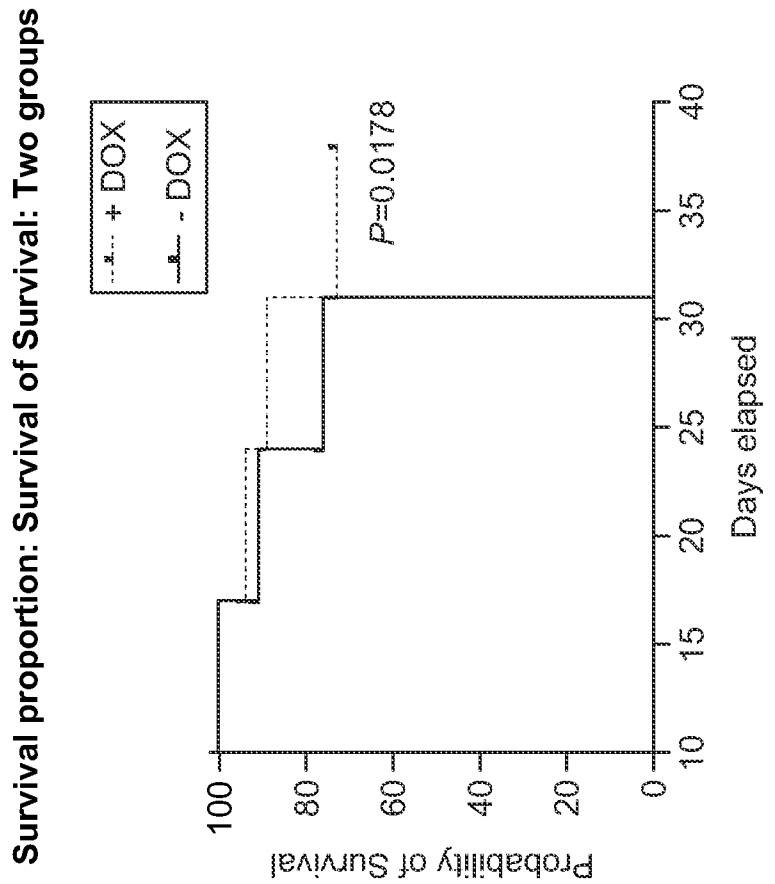

All H&E quantified lung matastases
metastatic foci
20
15
10
5
0
P<0.0001
shScr
shRac 1/ Cdc42
Mann-Whitney two-tailed test
H&E metastasis size (area, pixels2)
Relative area (squared pixels)
2.5
2.0
1.5
1.0
0.5
0.0
P<0.0001
shScr
shRac 1/ Cdc42
Mann-Whitney two-tailed test
All H&E quantified lung matastases
metastatic foci
20
15
10
5
0
P<0.0001
shScr
shRac 1/ Cdc42
Mann-Whitney two-tailed test
H&E metastasis size (area, pixels2)
Relative area (squared pixels)
2.5
2.0
1.5
1.0
0.5
0.0
P<0.0001
shScr
shRac 1/ Cdc42
Mann-Whitney two-tailed test

FIG. 5


Survival proportion: Survival of Survival: Two groups
+ DOX
- DOX
P=0.0178
Probability of Survival
100
80
60
40
20
0
10
15
20
25
30
35
40
Days elapsed

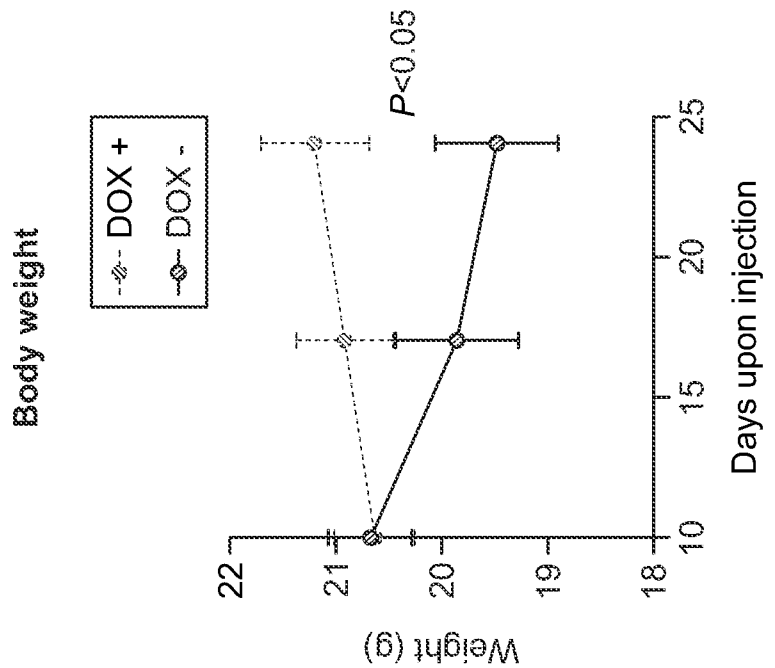

Body weight
DOX +
DOX -
P<0.05
Weight (g)
22
21
20
19
18
10
15
20
25
Days upon injection

FIG. 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 23/66444

A. CLASSIFICATION OF SUBJECT MATTER

IPC - INV. A61K 31/713, A61K 48/00, A61P 35/04, C12N 15/113 (2023.01)
ADD.

CPC - INV. A61K 31/713, A61K 48/005, A61P 35/04, C12N 15/1135

ADD. C12N 2320/31

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 2017/182002 A1 (STC.UNM) 29 June 2017 (29.06.2017) para [0024], [0076], [0094]-[0095], [0097], [0102], [0109], [0119]	1-3 -- 4-5
Y	WO 2020/223118 A1 (DHARMACON, INC.) 05 November 2020 (05.11.2020) para [0030], [0033], [0085], [00103], [00105], [00107], [00147]-[00148], [00156]-[00157]	4-5
A	US 2003/0199467 A1 (ROBERTS et al.) 23 October 2003 (23.10.2003); entire document	1-5

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

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
31 July 2023	SEP 12 2023
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Kari Rodriquez Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 23/66444

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as fi led.

   b. ☐ furnished subsequent to the international fi ling date for the purposes of international search (Rule 13ter.1(a)),

      ☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 23/66444

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

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

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

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

3. ☒ Claims Nos.: 6-22
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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

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

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

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

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.