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(54) Title: L-MYC PATHWAY TARGETING AS A TREATMENT FOR SMALL CELL LUNG CANCER

(57) Abstract: L-Myc pathway targeting treatments as a treatment for small cell lung cancer (SCLC) are described. The treatments can be genetic and/or pharmacologic and can particularly target L-Myc, L-Myc's partner protein MAX and/or RNA Polymerase I. Specifically, the disclosure provides a method of treating small cell lung cancer (SCLC) in a subject in need thereof comprising administering a therapeutically effective amount of an L-Myc inactivator to the subject, wherein the L-Myc inactivator comprises CX-5461, Actinomycin D, or a guide RNA.

## **L-MYC PATHWAY TARGETING AS A TREATMENT FOR SMALL CELL LUNG CANCER**

### **STATEMENT OF GOVERNMENT INTEREST**

**[0001]** This invention was made with government support under CA148867 awarded by the National Institutes of Health. The government has certain rights in the invention.

### **CROSS-REFERENCE TO RELATED APPLICATION**

**[0002]** This application claims priority to U.S. Provisional Patent Application No. 62/342,024 filed May 26, 2016 which is incorporated herein by reference in its entirety as if fully set forth herein.

### **FIELD OF THE DISCLOSURE**

**[0003]** The current disclosure provides L-Myc pathway targeting as a treatment for small cell lung cancer (SCLC). The treatments can be genetic and/or pharmacologic and can particularly target L-Myc, L-Myc's partner protein MAX and/or RNA Polymerase I.

### **BACKGROUND OF THE DISCLOSURE**

**[0004]** Cancer refers to a class of diseases in which a group of cells display uncontrolled growth (division beyond the normal limits), invasion (intrusion on and destruction of adjacent tissues), and sometimes metastasis. "Metastasis" refers to the spread of cancer cells from their original site of proliferation (e.g., a primary tumor) to another part of the body. The vast majority of cancer deaths are due to metastasis.

**[0005]** While almost all cancers share the attributes described in the preceding paragraph, the biological mechanisms initiating and supporting the growth and metastasis of different types of cancer vary widely. For example, anti-estrogen therapy is effective in subsets of patients with breast cancer (i.e., those with expression of the estrogen receptor), whereas estrogen inhibition is irrelevant to the biology of most cancer types. While cancers all exhibit uncontrolled cell proliferation, the unique biology of the cells that give rise to different types of cancers results in unique vulnerabilities towards inhibition of key molecules and signaling pathways that are differentially important depending on the cancer type.

**[0006]** Small cell lung cancer (SCLC) particularly is a very aggressive highly metastatic neuroendocrine carcinoma that represents 10-15% of lung cancer cases. Treatments for SCLC have not significantly improved over the last four decades and there are no currently approved targeted therapies. It is therefore essential that the biology of major genes that drive SCLC particularly be linked to novel therapeutic approaches for this cancer type.

## SUMMARY OF THE DISCLOSURE

**[0007]** L-Myc is a gene that has little importance in the vast majority of cell types and the vast majority of cancer types. As disclosed herein, however, L-Myc plays a critical role in a rare cell population in the lung, i.e. in the neuroendocrine cells that give rise to small cell lung cancer (SCLC). More particularly, L-Myc is permissive of and promotes the development of SCLC. Thus, the present disclosure provides targeting L-Myc and/or L-Myc's pathway as a novel therapeutic approach for the treatment of SCLC. The disclosed treatments may be used as a first line therapy and/or following the emergence of chemoresistance following initial treatment.

## BRIEF DESCRIPTION OF THE FIGURES

**[0008]** FIGs. 1A-1K. L-Myc converts preneoplastic lung neuroendocrine cells to SCLC. (1A) Pulmonary neuroendocrine cells (PNECs, arrowheads) in Chga-GFP mice positive for GFP and synaptophysin (Syn). Right: GFP immunofluorescence (top) and phase contrast (bottom) images of PNECs isolated by FACS. (1B) Schematic of isolating preSCs using FACS. The dotted line highlights GFP-positive cells. Right: Images of preSC and SCLC cells. (1C) Nude mice, 1 month after injection of PreSC or SCLC cells. Arrow points to tumor. (1D) Genotyping PCR showing deletion of Rb and p53 in preSC and SCLC tumor cells. (1E) RT-qPCR data showing Myc member expression and neuroendocrine markers and in normal lung (NL), preSC, and SCLC (n=3). Data were normalized to levels of ARBP P0 and expressed relative to expression in normal lung (1F) MTT assay results for cell viability after Cisplatin treatment (n=3). (1G) Images of mouse SCLC cells compared to preSCs infected with the retroviruses expressing GFP or L-Myc (n=3). (1H) RT-qPCR data showing expression of L-Myc normalized to ARBP P0 in SCLC cells or in preSCs infected with GFP or L-Myc (n=3). (1I) Results of soft-agar assay (n=3). (1J) Nude mice, 1 month after injection of cells infected with retro-GFP or L-Myc. Arrow points to tumor. (1K) Hematoxylin and eosin (H+E) and Uchl1 (Pgp9.5) staining of mouse SCLC or L-Myc-PreSCs. Scale bars: A, B (10  $\mu$ m), C (1 cm) G, 10 $\mu$ m; J, 1cm; K, 0.5cm (far left) and 100 $\mu$ m.

**[0009]** FIGs. 2A-2E. (2A) RT-qPCR data showing expression of several neuroendocrine-specific markers and other oncogenes in normal lung (NL), preSC, and SCLC (n=3) relative to ARBP P0 control primers. (2B) preSC infected with the retroviruses expressing N-Myc, and different levels of c-Myc (n=3). (2C) Nude mice, 1 month after injection of cells infected with retro-GFP, retro-N-Myc, and retro-c-Myc. Arrow points to a tumor. Asterisk indicates an area of injection of control preSC. (2D) RT-qPCR data showing expression of the Myc family genes in the GFP control and Myc family member infected preSCs shown in 2A. Expression is relative to ARBP P0. (2E)

CGRP immunohistochemistry showing neuroendocrine features of L-Myc-preSC allograft similar to SCLC allograft. Size bars: B, 10 $\mu$ m; C, 1cm.

**[0010]** FIGs. 3A-3I. Deletion of L-Myc suppresses SCLC (3A) H+E staining of Rb<sup>lox/lox</sup>;p53<sup>lox/lox</sup>;p130<sup>lox/lox</sup> mouse lungs either L-Myc<sup>+/+</sup>, L-Myc<sup>+/-</sup> or L-Myc<sup>lox/lox</sup> 6 months post Ad-CMV-Cre treatment. Right: tumor area quantification (3B) Phospho histone H3 staining showing reduced proliferation in Rb/p53/p130 mice that were L-Myc<sup>lox/lox</sup> compared to L-Myc<sup>+/+</sup>, quantified in (3C). (3D) H+E staining of Rb<sup>lox/lox</sup>;p53<sup>lox/lox</sup>;Pten<sup>lox/lox</sup> mouse lungs that were L-Myc<sup>lox/lox</sup> or L-Myc<sup>+/+</sup> 4 months post Ad-CGRP-Cre treatment. Data are representative of 5 mice per genotype examined. (3E) Kaplan-Meier analysis showing increased survival in Rb/p53/Pten; L-Myc<sup>lox/lox</sup> mutants, p=0.0005 (log-rank). (3F) Classification of major tumor type in mice from cohorts. SCLC: small cell lung cancer, NSCLC: non small cell lung cancer (3G)  $\beta$ -gal staining of the mouse SCLC cells transfected with control-CRISPR and LacZ-CRISPR. The expression of lacZ knock-in reporter is almost ubiquitous in the control cells but drastically reduced or absent in subsets of the cells targeted with lacZ-CRISPR. (3H) Results of soft-agar assay for the Myc family targeting CRISPR transfected SCLCs. (3I) Quantification of soft-agar assay (3H). Colonies bigger than 0.20 mm diameter were counted (n=3). Scale Bar: B, 200 $\mu$ m; G, 100 $\mu$ m.

**[0011]** FIGs. 4A and 4B. (4A) CGRP immunohistochemistry showing positive staining in Rb/p53/p130 and Rb/p53/p130/ L-Myc mutant SCLC. (4B) Sequencing traces showing CRISPR-mediated deletions of MYC members in mouse embryo fibroblasts. Scale bar = 200 microns.

**[0012]** FIGs. 5A-5E. Overexpression of L-Myc promotes ribosomal transcription programs (5A) Schematic of Affymetrix array analyses and the list of differentially regulated genes between preSC and L-Myc-preSC. Right, a partial list of top 20 up-regulated and down-regulated genes. (5B) Top 10 molecular pathways deduced from the differentially expressed genes. (5C) Real time PCR showing relative levels of pre-rRNA (internally transcribed spacer 1) of the 47S pre-rRNA relative to  $\beta$ 2 microglobulin in preSC and L-Myc-PreSC (5D) Results of MTT assay measuring viability of cells treated with CX-5461 (a RNA Pol I inhibitor) dissolved in DMSO. KP1, 3, 5 are mouse SCLC cells and H1650, A549, H2009 are human NSCLC cell lines. The rest of the cells above are human SCLC cell lines. These treatment and MTT assays have been repeated with similar results at least once. (5E) Results of soft-agar assay for the mouse Rb/p53 deleted SCLC cells (KP1, 3, and 5) treated with CX-5461 (0.2 $\mu$ M) every three days for a month.

**[0013]** FIG. 6. RT-qPCR data showing expression of MYC members relative to ARBP P0. Levels are expressed relative to those in 293T cells.

**[0014]** FIGs. 7A-7G. Inhibition of RNA Pol I suppresses SCLC in autochthonous model (7A) Representative MR images showing images from baseline scan and 2-week time point. Tumor is outlined (7B) Waterfall plot showing tumor volume changes from baseline to 2-week time point quantified from MRI in untreated (control) and CX-5461 treated mice. (7C) Proportion of control and CX-5461 treated mice with progressive disease (PD), stable disease (SD) or partial response (PR). (7D) Real time PCR showing relative levels of pre-rRNA (internally transcribed spacer 1) of the 47S pre-rRNA relative to  $\beta 2$  microglobulin in tumors from control mice or undergoing 2 weeks of CX-5461 treatment (7E) BrdU and PH3 analysis of Rb/p53 tumors in untreated and mice treated for 2 weeks with CX-5461 (7F) Quantification of BrdU and PH3 levels from (7E). (7G) Gene set enrichment analysis (GSEA) normalized enrichment plot using RNAseq from 5 controls compared to 5 CX-5461 treated SCLC tumors. The 50 gene set "Hallmarks signatures" set from MSigDB was queried.

**[0015]** FIG. 8 provides exemplary sequences supporting the disclosure.

**[0016]** FIG. 9 shows reductions in Myc-related and cell-cycle related gene sets associated with CX-5461 treatment.

#### DETAILED DESCRIPTION

**[0017]** Cancer refers to a class of diseases in which a group of cells display uncontrolled growth (division beyond the normal limits), invasion (intrusion on and destruction of adjacent tissues), and sometimes metastasis. "Metastasis" refers to the spread of cancer cells from their original site of proliferation (e.g., a primary tumor) to another part of the body. The vast majority of cancer deaths are due to metastasis.

**[0018]** While almost all cancers share the attributes described in the preceding paragraph, the biological mechanisms initiating and supporting the growth and metastasis of different types of cancer vary widely. For example, anti-estrogen therapy is effective in subsets of patients with breast cancer (i.e. those with expression of the estrogen receptor), whereas estrogen inhibition is irrelevant to the biology of most cancer types. While cancers all exhibit uncontrolled cell proliferation, the unique biology of the cells that give rise to different types of cancers results in unique vulnerabilities towards inhibition of key molecules and signaling pathways that are differentially important depending on the cancer type.

**[0019]** Small cell lung cancer (SCLC) particularly is a very aggressive highly metastatic neuroendocrine carcinoma that represents 10-15% of lung cancer cases. Treatments for SCLC have not significantly improved over the last four decades and there are no currently approved

targeted therapies. It is therefore essential that the biology of major genes that drive SCLC particularly be linked to novel therapeutic approaches for this cancer type.

**[0020]** L-Myc is a gene that has little importance in the vast majority of cell types and the vast majority of cancer types. As disclosed herein, however, L-Myc plays a critical role in a rare cell population in the lung, i.e. in the neuroendocrine cells that give rise to small cell lung cancer (SCLC). More particularly, L-Myc is permissive of and promotes the development of SCLC. Thus, the present disclosure provides targeting L-Myc and/or L-Myc's pathway in neuroendocrine cells of the lung as a novel therapeutic approach for the treatment of SCLC. The disclosed treatments may be used as a first line therapy and/or following the emergence of chemoresistance following initial treatment.

**[0021]** As described herein, the L-Myc pathway includes primary pathway members and redundant pathway members. Primary pathway members are those that are critical for the SCLC tumor promoting activity of the pathway in the neuroendocrine cells of the lung, such that their inhibition is SCLC tumor inhibitory. These primary pathway members are integral to L-Myc function in SCLC. While cells can respond to many perturbations by changing the activity or expression of other proteins to compensate, the primary pathway members are such that mechanisms to compensate are unavailable to the L-MYC-reliant SCLC cell. Examples of primary pathway members include L-Myc; L-Myc's partner protein, MAX; and RNA Polymerase I. Redundant pathway members are those that L-Myc may contribute to altered gene expression or activity, but that are not critical for the SCLC tumor promoting activity of L-Myc in the neuroendocrine cells of the lung. In some cases these may be genes or proteins for which SCLC cells can buffer the response to their perturbation by activating or inhibiting a different gene or protein. Examples of redundant pathway members include mTor, S6K, and 4EBP.

**[0022]** The distinction between primary and redundant L-myc pathway members is drawn because the pathway can be targeted at many levels, and in SCLC, only primary pathway members provide effective therapeutic targets.

**[0023]** For example, L-Myc loss results in strong suppression of SCLC tumor development (in many cases resulting in complete tumor elimination). Deletion of L-Myc using CRISPR technology leads to suppression of SCLC cell based cancer phenotypes. Moreover, using a pharmacological approach, targeting RNA Pol I suppresses SCLC tumor development. These genetic and pharmacological results demonstrate that primary L-MYC pathway members are key therapeutic targets for SCLC.

**[0024]** Redundant L-Myc pathway members do not provide therapeutic targets for SCLC. For example, mTOR inhibition using everolimus was unsuccessful in a Phase 2 clinical trial testing

patients who failed first line therapy. Tarhini, et al., 2010. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research*, 16(23): 5900-7. Similarly, in the Rb/p53 autochthonous mouse model, mTOR inhibition using the active site inhibitor AZD8055 lacked efficacy as a monotherapy, although this treatment enhanced the effects of a BCL2 inhibitor. Faber, et al., 2015. *Proc Natl Acad Sci USA*, 112(11): E1288-96.

**[0025]** Without being bound by theory, it is thought that primary L-myc pathway members provide effective therapeutic targets against SCLC because there is no mechanism for the cell to adequately compensate for the absence of L-myc primary pathway members. Redundant pathway members are ineffective as therapeutic targets against SCLC due to the ability of the cell to buffer the effect of L-Myc suppression by altering other signaling pathway components and minimizing the anti-SCLC effects of L-Myc suppression.

**[0026]** L-Myc. L-Myc is the protein encoded by the human MYCL gene (Entrez Gene ID: 4610). The canonical protein sequence for L-Myc (UniProt ID P12524) is provided as SEQ ID NO: 1 in FIG. 8. L-Myc is a transcription factor that binds to its partner protein MAX, and together they act at specific DNA sequence elements "E-Boxes" as well as other DNA sequences to modify RNA transcription.

**[0027]** MAX is a partner protein for L-Myc. The Entrez Gene ID for MAX is: 4149. The canonical protein sequence for MAX (UniProt ID P61244) is provided as SEQ ID NO: 2 in FIG. 8. MAX is a member of the basic helix-loop-helix leucine zipper family of transcription factors. MAX binds with L-MYC and other MYC members to bind to E-Box elements and other sites on DNA to regulate transcription.

**[0028]** RNA Polymerase I (Gene name POLR1B). RNA polymerases are enzymes that produce primary transcript RNA. Different RNA polymerases synthesize different types of primary transcript RNA. RNA Polymerase I (RNA Pol I) synthesizes RNA that forms significant portions of the ribosome (ribosomal RNA or rRNA). An exemplary RNA Pol I sequence includes Entrez Gene ID NO: 84172, UniProt Protein ID Q9H9Y6, provided as SEQ ID NO: 3 in FIG. 8.

**[0029]** Regarding RNA Pol I inhibition particularly, this has been shown to be effective in lymphoma models as well as in cell line xenograft studies using a single pancreatic cell line and a single melanoma cell line, when injected into immune deficient mice (see, for example, FIG. 7 of Drygin, et al., 2011. *Cancer Research* 71(4):1418-30). In lymphoma, RNA Pol I inhibition suppressed tumors by activating a p53 pathway (*Cancer Cell*. 2012. Jul 10; 22(1):51-65). These findings cannot be reasonably predicted to be extended to the treatment of SCLC, however, because the p53 pathway is irrelevant to the therapeutic action in SCLC (i.e., p53 is almost always deleted/mutated in SCLC). Also, L-Myc plays no roles in lymphoma, melanoma or

pancreatic cancers, but L-Myc dramatically activates RNA Pol I in SCLC. Thus, the described results using RNA Pol I inhibition in settings outside of SCLC are very different from the unexpected findings disclosed herein that L-Myc signaling is a critical node for targeting in SCLC.

**[0030]** The current disclosure provides both genetic and pharmacologic approaches for targeting primary members of the L-Myc pathway for down-regulation in the neuroendocrine cells of the lung.

**[0031]** Genetic Approaches. In particular embodiments, genetic approaches disclosed herein can down-regulate functional expression of primary L-Myc pathway members. Down-regulating functional expression of primary L-Myc pathway members can be through, for example, reduction of a gene's copy number, insertion of a foreign set of base pairs into a gene (e.g., into a coding region), deletion of any portion of the gene (e.g., of all or part of a promoter or coding region), substitution of base pairs within the gene (e.g., into a coding region), translation of an incomplete protein; incorrect folding of a protein; expression of an unstable protein; reduced transcription of a gene; incomplete transcription of a gene, or by any other activity resulting in reduced presence, expression or activity of a primary pathway member in the L-Myc pathway that promotes pathway activation. As indicated, these approaches generally rely on (i) disrupting expression of endogenous nucleotide sequences (e.g., endogenous genes) of a primary pathway member; (ii) up-regulating expression of an endogenous gene whose expression down-regulates a primary pathway member; and/or (iii) up-regulating expression of an exogenous nucleotide sequence that down-regulates a primary pathway member within a treated cell. Most of these approaches generally rely on altering the endogenous genome by introducing and/or removing a nucleotide sequence.

**[0032]** In particular embodiments because genetic therapies may not reach 100% of cancer cells, the genetic therapy is utilized as part of a combination therapy.

**[0033]** Numerous techniques for the introduction or deletion of one or more nucleotide sequences in a cell to alter the endogenous genome can be used alone or in various combinations. Most techniques involve a nucleotide sequence carrier or tool. In the case of naked DNA, DNA complexes and/or triplex DNA, the carrier may be a liquid. Additional carriers include liposomes (Tarahovsky and Ivanitsky, 1998, *Biochemistry* (Mosc) 63:607-618), ribozymes (Branch and Klotman, 1998, *Exp. Nephrol.* 6:78-83), vectors, cells for cell fusion, chromosomes for chromosome-mediated gene transfer, transposons/transposases, guide RNA (for example, for CRISPR applications), transcription activator-like effector nucleases (TALENs), meganucleases, meganuclease-TALEN fusions (megaTALs), zinc fingers nucleases, and/or

flanking regions of homology (e.g., homology arms). As is understood by one of ordinary skill in the art, some of these carriers or tools are used to introduce nucleotide sequences into the endogenous genome whereas others can remove nucleotide sequences from the endogenous genome (e.g., gene editing tools).

**[0034]** A "vector" is a nucleic acid molecule capable of transporting a nucleotide sequence into a cell. Vectors may be, e.g., viruses, phage, a DNA vector, a RNA vector, a viral vector, a bacterial vector, a plasmid vector, a cosmid vector, or an artificial chromosome vector. An "expression vector" is any type of vector that is capable of directing the expression of a nucleotide sequence (e.g., a therapeutic protein and/or interfering RNA (iRNA) encoded by one or more genes carried by the vector) when it is present in the appropriate environment.

**[0035]** Viral vectors are usually non-replicating or replication-impaired vectors, which means that the viral vector cannot replicate to any significant extent in normal cells (e.g., human cells), as measured by conventional means (e.g. via measuring DNA synthesis and/or viral titer). Non-replicating or replication-impaired vectors may have become so naturally (i.e., they have been isolated as such from nature) or artificially (e.g., by breeding in vitro or by genetic manipulation). There will generally be at least one cell-type in which the replication-impaired viral vector can be grown--for example, modified vaccinia Ankara (MVA) can be grown in CEF cells. Typically, viral vectors are incapable of causing a significant infection in a subject, typically in a mammalian subject.

**[0036]** "Retroviruses" are viruses having an RNA genome. In particular embodiments, a retroviral vector contains all of the cis-acting sequences necessary for the packaging and integration of the viral genome, i.e., (a) a long terminal repeat (LTR), or portions thereof, at each end of the vector; (b) primer binding sites for negative and positive strand DNA synthesis; and (c) a packaging signal, necessary for the incorporation of genomic RNA into virions. More detail regarding retroviral vectors can be found in Boesen, *et al.*, 1994, *Biotherapy* 6:291-302; Clowes, *et al.*, 1994, *J. Clin. Invest.* 93:644-651; Kiem, *et al.*, 1994, *Blood* 83:1467-1473; Salmons and Gunzberg, 1993, *Human Gene Therapy* 4:129-141; Miller, *et al.*, 1993, *Meth. Enzymol.* 217:581-599; and Grossman and Wilson, 1993, *Curr. Opin. in Genetics and Devel.* 3:110-114.

**[0037]** "Gammaretroviruses" refers to a genus of the retroviridae family. Exemplary gammaretroviruses include mouse stem cell virus, murine leukemia virus, feline leukemia virus, feline sarcoma virus, and avian reticuloendotheliosis viruses.

**[0038]** Widely used retroviral vectors include those based upon murine leukemia virus (MuLV), gibbon ape leukemia virus (GaLV), simian immunodeficiency virus (SIV), human immunodeficiency virus (HIV), and combinations thereof (see, e.g., Buchscher *et al.*, *J. Virol.*

66:2731-2739, 1992; Johann et al., J. Virol. 66:1635-1640, 1992; Sommerfelt et al., Virol. 176:58-59, 1990; Wilson et al., J. Virol. 63:2374-2378, 1989; Miller et al., J. Virol. 65:2220-2224, 1991; and PCT/US94/05700).

**[0039]** Lentiviral vectors refer to a genus of retroviruses that are capable of infecting dividing and non-dividing cells and typically produce high viral titers. Several examples of lentiviruses include HIV (human immunodeficiency virus: including HIV type 1, and HIV type 2); equine infectious anemia virus; feline immunodeficiency virus (FIV); bovine immune deficiency virus (BIV); and simian immunodeficiency virus (SIV).

**[0040]** In particular embodiments, other retroviral vectors can be used. These include, e.g., vectors based on human foamy virus (HFV) or other viruses in the Spumavirus genera.

**[0041]** Foamy viruses (FVs) are the largest retroviruses known today and are widespread among different mammals, including all non-human primate species, however are absent in humans. This complete apathogenicity qualifies FV vectors as ideal gene transfer vehicles for genetic therapies in humans and clearly distinguishes FV vectors as gene delivery system from HIV-derived and also gammaretrovirus-derived vectors.

**[0042]** FV vectors are suitable for gene therapy applications because they can (1) accommodate large transgenes (> 9kb), (2) transduce slowly dividing cells efficiently (making them especially appropriate for prophylactic treatments in SCLC), and (3) integrate as a provirus into the genome of target cells, thus enabling stable long term expression of the transgene(s). FV vectors do need cell division for the pre-integration complex to enter the nucleus, however the complex is stable for at least 30 days and still infective. The intracellular half-life of the FV pre-integration complex is comparable to the one of lentiviruses and significantly higher than for gammaretroviruses, therefore FV are also - similar to LV vectors - able to transduce dividing and rarely dividing cells. FV vectors are natural self-inactivating vectors and characterized by the fact that they seem to have hardly any potential to activate neighboring genes. In addition, FV vectors can enter any cells known (although the receptor is not identified yet) and infectious vector particles can be concentrated 100-fold without loss of infectivity due to a stable envelope protein.

**[0043]** Additional examples of viral vectors include those derived from adenoviruses (e.g., adenovirus 5 (Ad5), adenovirus 35 (Ad35), adenovirus 11 (Ad11), adenovirus 26 (Ad26), adenovirus 48 (Ad48) or adenovirus 50 (Ad50)), adeno-associated virus (AAV; see, e.g., U.S. Pat. No. 5,604,090; Kay et al., Nat. Genet. 24:257 (2000); Nakai et al., Blood 91:4600 (1998)), alphaviruses, cytomegaloviruses (CMV), flaviviruses, herpes viruses (e.g., herpes simplex), influenza viruses, papilloma viruses (e.g., human and bovine papilloma virus; see, e.g., U.S.

Pat. No. 5,719,054), poxviruses, vaccinia viruses, etc. See Kozarsky and Wilson, 1993, *Current Opinion in Genetics and Development* 3:499-503, Rosenfeld, *et al.*, 1991, *Science* 252:431-434; Rosenfeld, *et al.*, 1992, *Cell* 68:143-155; Mastrangeli, *et al.*, 1993, *J. Clin. Invest.* 91:225-234; Walsh, *et al.*, 1993, *Proc. Soc. Exp. Biol. Med.* 204:289-300; and Lundstrom, 1999, *J. Recept. Signal Transduct. Res.* 19: 673-686. Examples include modified vaccinia Ankara (MVA) and NYVAC, or strains derived therefrom. Other examples include avipox vectors, such as a fowlpox vectors (e.g., FP9) or canarypox vectors (e.g., ALVAC and strains derived therefrom).

**[0044]** Exemplary chromosome carriers include artificial chromosome vectors such as mammalian artificial chromosomes (Vos, 1998, *Curr. Op. Genet. Dev.* 8:351-359) and yeast artificial chromosomes (YAC). YAC are typically used when the inserted nucleic acids are too large for more conventional vectors (e.g., greater than 12 kb).

**[0045]** In particular embodiments, the efficiency of integration, the size of the nucleotide sequence that can be integrated, and the number of copies of a nucleotide sequence that can be integrated into a genome can be improved by using transposons. Transposons or transposable elements include a short nucleic acid sequence with terminal repeat sequences upstream and downstream. Active transposons can encode enzymes that facilitate the excision and insertion of nucleic acid into a target nucleotide sequence.

**[0046]** A number of transposable elements have been described in the art that facilitate insertion of nucleic acids into the genome of vertebrates, including humans. Examples include sleeping beauty (e.g., derived from the genome of salmonid fish); piggyback (e.g., derived from lepidopteran cells and/or the *Myotis lucifugus*); mariner (e.g., derived from *Drosophila*); frog prince (e.g., derived from *Rana pipiens*); Tol2 (e.g., derived from medaka fish); TcBuster (e.g., derived from the red flour beetle *Tribolium castaneum*) and spinON.

**[0047]** Vectors and other carriers can include regulatory sequences to control the expression of nucleotide sequences (e.g., therapeutic proteins or iRNA). These regulatory sequences can be eukaryotic or prokaryotic in nature. In particular embodiments, the regulatory sequence can be a tissue specific promoter such that the expression of the one or more nucleotide sequences will be substantially greater in the target tissue type (i.e., neuroendocrine cells of the lung) compared to other types of tissue. Examples of neuroendocrine specific promoters include ASCL1 and CALCA genes. In particular embodiments, the regulatory sequence can result in the constitutive expression of the one or more nucleotide sequences upon entry of the carrier into the cell. Alternatively, the regulatory sequences can include inducible sequences. Inducible regulatory sequences are well known to those skilled in the art and are those sequences that require the presence of an additional inducing factor to result in expression of the one or more

nucleotide sequences. Examples of suitable regulatory sequences include binding sites corresponding to tissue-specific transcription factors based on endogenous nuclear proteins, sequences that direct expression in a specific cell type, the lac operator, the tetracycline operator and the steroid hormone operator. Any inducible regulatory sequence known to those of skill in the art may be used.

**[0048]** In particular embodiments, the nucleotide sequence is stably integrated into the genome of a cell. In particular embodiments, the nucleotide sequence is stably integrated into the genome of a cell so that the nucleotide sequence is expressible by the cell and preferably heritable and expressible by its cell progeny. In particular embodiments, the nucleic acid is stably maintained in a cell as a separate, episomal segment.

**[0049]** In particular embodiments, inserted nucleotide sequences include genes encoding therapeutic proteins and/or interfering RNA (iRNA). Genes may include not only coding sequences but also non-coding regulatory regions such as promoters, enhancers, and termination regions. The term further can include all introns and other DNA sequences spliced from the mRNA transcript, along with variants resulting from alternative splice sites. Nucleic acid sequences encoding proteins can be DNA or RNA that directs the expression of protein or RNA. These nucleic acid sequences may be a DNA strand sequence that is transcribed into RNA or an RNA sequence that is translated into protein. The nucleic acid sequences include both the full-length nucleic acid sequences as well as non-full-length sequences derived from the full-length protein or RNA. The sequences can also include degenerate codons of the native sequence or sequences that may be introduced to provide codon preference. Thus, a gene refers to a unit of inheritance that occupies a specific locus on a chromosome and includes transcriptional and/or translational regulatory sequences and/or a coding region and/or non-translated sequences (*i.e.*, introns, 5' and 3' untranslated sequences). The term "gene" includes various sequence polymorphisms, mutations, and/or sequence variants. In particular embodiments, the sequence polymorphisms, mutations, and/or sequence variants do not affect the function of the encoded transcript. A coding sequence is any nucleotide sequence that contributes to the code for the product of a gene. A non-coding sequence thus refers to any nucleic acid sequence that does not contribute to the code for the product of a gene.

**[0050]** Interfering RNA (iRNA) includes any type of RNA molecule capable of down-regulating expression of a target gene or protein including antisense RNA, short interfering RNA (siRNA), microRNA (miRNA), double-stranded RNA (dsRNA), hairpin RNA (hRNA, including short hRNA (shRNA)), sense RNA, ribozyme, and the like.

**[0051]** MicroRNA are genomically encoded non-coding RNAs that regulate gene expression by directing their target mRNAs for degradation or translational repression. Mature miRNAs are structurally similar to short interfering RNAs (siRNA), derived from cleavage of exogenous or foreign dsRNA. However, miRNAs differ from siRNAs in that miRNAs, especially those in animals, have incomplete base pairing to a target and inhibit translation of many different mRNAs with similar sequences, while siRNAs base-pair perfectly and induce mRNA cleavage only at a specific target.

**[0052]** In particular embodiments, the iRNA molecule has a length of at least 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 220, 240, 250, 260, 270, 280, 300, 400, 500, or 600 nucleotides.

**[0053]** Methods to assay for functional iRNA molecules are well known in the art. The methods include detecting reductions in RNA or protein levels which include RNA solution hybridization, Northern hybridization, reverse transcription (e.g. quantitative RT-PCR analysis), microarray analysis, antibody binding, enzyme-linked immunosorbent assay (ELISA) and Western blotting.

**[0054]** Guide RNA can be used, for example, with gene-editing tools such as CRISPR-Cas systems. CRISPR-Cas systems include CRISPR repeats and a set of CRISPR-associated genes (Cas).

**[0055]** The CRISPR repeats (clustered regularly interspaced short palindromic repeats) include a cluster of short direct repeats separated by spacers of short variable sequences of similar size as the repeats. The repeats range in size from 24 to 48 base pairs and have some dyad symmetry which implies the formation of a secondary structure, such as a hairpin, although the repeats are not truly palindromic. The spacers, separating the repeats, match exactly the sequences from prokaryotic viruses, plasmids, and transposons. The Cas genes encode nucleases, helicases, RNA-binding proteins, and a polymerase that unwind and cut DNA. Cas1, Cas2, and Cas9 are examples of Cas genes.

**[0056]** The source of CRISPR spacers indicates that CRISPR-Cas systems play a role in adaptive immunity in bacteria. There are at least three types of CRISPR-Cas immune system reactions, and Cas1 and Cas2 genes are involved in spacer acquisition in all three. Spacer acquisition, involving the capture and insertion of invading viral DNA into a CRISPR locus occurs in the first stage of adaptive immunity. More particularly, spacer acquisition begins with Cas1 and Cas2 recognizing invading DNA and cleaving a protospacer, which is ligated to the direct repeat adjacent to a leader sequence. Subsequently, single strand extension repairs take place and the direct repeat is duplicated.

**[0057]** The next stage of CRISPR-related adaptive immunity involves CRISPR RNA (crRNA) biogenesis, which occurs differently in each type of CRISPR-Cas system. In general, during this stage, the CRISPR transcript is cleaved by Cas nuclease to produce crRNAs. In the type I system, Cas6e/Cas6f cleaves the transcript. The type II system employs a transactivating (tracr) RNA to form a dsRNA, which is cleaved by Cas and RNase III. The type III system uses a Cas6 homolog for cleavage.

**[0058]** In the final stage of CRISPR-related adaptive immunity, processed crRNAs associate with Cas to form interference complexes. In type I and type II systems, the Cas interacts with protospacer adjacent motifs (PAMs), which are short 3-5 bp DNA sequences, for degradation of invading DNA, while the type III systems do not require interaction with a PAM for degradation. In the type III-B system, the crRNA basepairs with the mRNA, instead of the targeted DNA, for degradation.

**[0059]** CRISPR-Cas systems thus function as an RNAi-like immune system in prokaryotes. The CRISPR-Cas technology has been exploited to inactivate genes in human cell lines and cells. As an example, the CRISPR-Cas9 system, which is based on the type II system, has been used as a tool in genome editing.

**[0060]** The type II system requires three components: Cas9, crRNA, and tracrRNA. The system can be simplified by combining tracrRNA and crRNA into a single synthetic single guide RNA (sgRNA).

**[0061]** At least three different Cas9 nucleases have been developed for genome editing. The first is the wild type Cas9 which introduces double-stranded breaks (DSBs) at a specific DNA site, resulting in the activation of DSB repair machinery. DSBs can be repaired by the non-homologous end joining (NHEJ) pathway or by homology-directed repair (HDR) pathway. The second is a mutant Cas9, known as the Cas9D10A, with only nickase activity, which means that it only cleaves one DNA strand and does not activate NHEJ. Thus, the DNA repairs proceed via the HDR pathway only. The third is a nuclease-deficient Cas9 (dCas9) which does not have cleavage activity but is able to bind DNA. Therefore, dCas9 is able to target specific sequences of a genome without cleavage. By fusing dCas9 with various effector domains, dCas9 can be used either as a gene silencing or activation tool.

**[0062]** In particular embodiments, CRISPR screening can be used in cell culture to identify additional factors that target one or more primary L-Myc pathway members.

**[0063]** Transcription activator-like effector nucleases (TALENs) refer to fusion proteins including a transcription activator-like effector (TALE) DNA binding protein and a non-specific DNA

cleavage domain. TALENs have been engineered to bind a target sequence and cut DNA at a specific location.

**[0064]** TALEs are DNA binding proteins secreted by *Xanthomonas* bacteria. The DNA binding domain of TALE contains a highly conserved 33 or 34 amino acid repeat, with divergent residues at the 12<sup>th</sup> and 13<sup>th</sup> positions of each repeat. These two positions, referred to as the Repeat Variable Diresidue (RVD), show a strong correlation with specific nucleotide recognition. Accordingly, targeting specificity can be improved by changing the amino acids in the RVD and incorporating nonconventional RVD amino acids.

**[0065]** An example of a nuclease that can be included in the non-specific DNA cleavage domain is the FokI endonuclease. Both wild-type and variant FokI cleavage domains have been used with TALEN technology. The FokI domain functions as a dimer requiring two constructs with unique DNA binding domains for sites on the target sequence.

**[0066]** TALENs are used to edit genes and genomes by inducing DSBs in the DNA, which induce repair mechanisms in cells. Two TALENs must bind and flank each side of the target site for FokI to dimerize and induce a DSB. The DSB is repaired in the cell by NHEJ or by homologous recombination (HR) with an exogenous double-stranded donor DNA fragment.

**[0067]** MegaTALs have a single chain rare-cleaving nuclease structure in which a TALE is fused with the DNA cleavage domain of a meganuclease. Meganucleases, also known as homing endonucleases, are single peptide chains that have both DNA recognition and nuclease function in the same domain. In contrast to the TALEN, the megaTAL only requires the delivery of a single peptide chain for functional activity.

**[0068]** Zinc finger nucleases (ZFNs) are a class of site-specific nucleases engineered to bind and cleave DNA at specific positions. ZFNs are used to introduce DSBs at a specific site in a DNA sequence which enables the ZFNs to target unique sequences within a genome in a variety of different cells. Moreover, subsequent to double-stranded breakage, homologous recombination or non-homologous end joining takes place to repair the DSB, thus enabling genome editing.

**[0069]** ZFNs are synthesized by fusing a zinc finger DNA-binding domain to a DNA cleavage domain. The DNA-binding domain includes three to six zinc finger proteins which are transcription factors. The DNA cleavage domain includes the catalytic domain of, for example, FokI endonuclease.

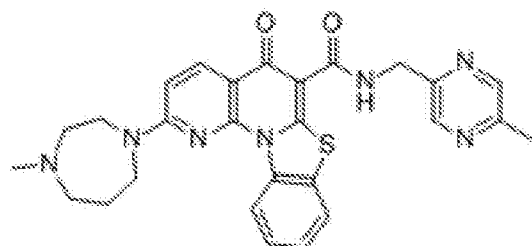
**[0070]** Because the FokI catalytic domain must dimerize to cleave DNA, the FokI catalytic domain mediates the dimerization of ZFNs at the targeted DNA site, with each of the monomer of the ZFN binding to a half-site at the correct orientation and spacing. The FokI cleavage

domain cleaves within a five or six base pair spacer sequence separating the two inverted half-sites. The requirement of dimerization by FokI enables specific DNA targeting.

**[0071]** ZFNs are useful tools for genome editing, for example gene disruption, gene editing by homologous recombination, and gene therapy to insert therapeutic genes at the appropriate chromosomal target sites with a human genome.

**[0072]** Regions of homology to aid in integration may be any suitable length such as, for example, 100 bp to 30,000 bp (e.g., at least 500 bp, at least 1,000 bp, at least 2,000 bp, at least 5,000 bp, at least 10,000 bp, or at least 20,000 bp). Any length suitable to drive integration into the genome of a target cell and resulting genetic modification may be used.

**[0073]** Pharmacologic Approaches. Pharmacologic Therapies. Pharmacologic therapies include direct administration of therapeutic proteins and/or small molecules that down-regulate primary members of the L-Myc pathway and do not rely on a genetic modification in the subject. One example includes the RNA Polymerase I inhibitor, CX-5461:



**[0074]** Previous studies have focused on the ability of CX-5461 to induce nucleolar stress and p53 activation in mediating in vivo efficacy. Devlin, et al., 2015. Combination therapy targeting ribosome biogenesis and mRNA translation synergistically extends survival in MYC-driven lymphoma. Cancer Discovery, PMID: 26490423; Bywater, et al., 2012. Cancer Cell, 22(1): 51-65. However, the current disclosure describes significant efficacy in p53-null SCLC, ruling out p53 activation as a relevant factor in the SCLC model. Further, treatment of SCLC with CX-5461 in vivo was not associated with a strong apoptotic response. In SCLC, CX-5461 drove a p53-independent anti-proliferative effect associated with reduced E2F and MYC target gene expression (FIGs. 3E-3G). In one panel of 44 cell lines from multiple tumor types, there was no correlation between p53 genetic status and sensitivity to CX-5461. Drygin, et al., 2011. Cancer Res, 71(4): 1418-30. In contrast, p53 inactivation was clearly associated with reduced effects of CX-5461 in cell lines derived from an Eμ-MYC mouse lymphoma model. Bywater, et al., 2012. Cancer Cell, 22(1): 51-65. The importance of a p53-dependent anti-proliferative vs. apoptotic

response to CX-5461 likely differs across different tumor types with effects in SCLC mediated by p53-independent anti-proliferative effects.

**[0075]** US 20150284410 describes modifications to CX-5461 and analogs thereof (described in US 20090093465), to reduce or even reverse capability to participate in hydrogen bond formation. Actinomycin D is another example of an RNA Pol I inhibitor that can be used within the pharmacologic approaches disclosed herein.

**[0076]** Particular embodiments include variants of nucleotide or protein sequences disclosed herein. Variants include sequences having one or more additions, deletions, stop positions, or substitutions, as compared to a reference sequence.

**[0077]** An amino acid substitution can be a conservative or a non-conservative substitution. A “conservative substitution” involves a substitution found in one of the following conservative substitutions groups: Group 1: Alanine (Ala; A), Glycine (Gly; G), Serine (Ser; S), Threonine (Thr; T); Group 2: Aspartic acid (Asp; D), Glutamic acid (Glu; E); Group 3: Asparagine (Asn; N), Glutamine (Gln; Q); Group 4: Arginine (Arg; R), Lysine (Lys; K), Histidine (His; H); Group 5: Isoleucine (Ile; I), Leucine (Leu; L), Methionine (Met; M), Valine (Val; V); and Group 6: Phenylalanine (Phe; F), Tyrosine (Tyr; Y), Tryptophan (Trp; W).

**[0078]** Additionally, amino acids can be grouped into conservative substitution groups by similar function, chemical structure, or composition (e.g., acidic, basic, aliphatic, aromatic, sulfur-containing). For example, an aliphatic grouping may include, for purposes of substitution, Gly, Ala, Val, Leu, and Ile. Other groups containing amino acids that are considered conservative substitutions for one another include: sulfur-containing: Met and Cys; acidic: Asp, Glu, Asn, and Gln; small aliphatic, nonpolar or slightly polar residues: Ala, Ser, Thr, Pro, and Gly; polar, negatively charged residues and their amides: Asp, Asn, Glu, and Gln; polar, positively charged residues: His, Arg, and Lys; large aliphatic, nonpolar residues: Met, Leu, Ile, Val, and Cys; and large aromatic residues: Phe, Tyr, and Trp. As indicated, in particular embodiments, conservative substitutions can include substituting Asp56 with Glu, Ser, Thr or Tyr.

**[0079]** Non-conservative substitutions include those that affect the function of a protein in a statistically-significant manner. Non-conservative substitutions include those in which (i) a hydrophilic residue (e.g. Ser or Thr) is substituted by a hydrophobic residue (e.g. Leu, Ile, Phe, Val, or Ala); (ii) a Cys or Pro is substituted by any other residue; (iii) a residue having an electropositive side chain (e.g. Lys, Arg, or His) is substituted by an electronegative residue (e.g. Gln or Asp); or (iv) a residue having a bulky side chain (e.g. Phe), is substituted by one not having a bulky side chain, (e.g. Gly). Additional information is found in Creighton (1984) *Proteins*, W.H. Freeman and Company.

**[0080]** Variants incorporating stop positions can be biologically active fragments. Biologically active fragments have 0.1, 0.5, 1, 2, 5, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99, 100, 110, 120, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000% or more of the activity of a reference sequence.

**[0081]** In particular embodiments, a nucleotide or protein sequence that has at least 85% sequence identity; 86% sequence identity; 87% sequence identity; 88% sequence identity; 89% sequence identity; 90% sequence identity; 91% sequence identity; 92% sequence identity; 93% sequence identity; 94% sequence identity; 95% sequence identity; 96% sequence identity; 97% sequence identity; 98% sequence identity; or 99% sequence identity to a nucleotide or protein disclosed herein can be used.

**[0082]** "% sequence identity" refers to a relationship between two or more sequences, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between sequences as determined by the match between strings of such sequences. "Identity" (often referred to as "similarity") can be readily calculated by known methods, including those described in: Computational Molecular Biology (Lesk, A. M., ed.) Oxford University Press, NY (1988); Biocomputing: Informatics and Genome Projects (Smith, D. W., ed.) Academic Press, NY (1994); Computer Analysis of Sequence Data, Part I (Griffin, A. M., and Griffin, H. G., eds.) Humana Press, NJ (1994); Sequence Analysis in Molecular Biology (Von Heijne, G., ed.) Academic Press (1987); and Sequence Analysis Primer (Gribskov, M. and Devereux, J., eds.) Oxford University Press, NY (1992). Preferred methods to determine sequence identity are designed to give the best match between the sequences tested. Methods to determine sequence identity and similarity are codified in publicly available computer programs. Sequence alignments and percent identity calculations may be performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR, Inc., Madison, Wisconsin). Multiple alignment of the sequences can also be performed using the Clustal method of alignment (Higgins and Sharp, *CAB/OS*, 1989; 5:151-153 with default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Relevant programs also include the GCG suite of programs (Wisconsin Package Version 9.0, Genetics Computer Group (GCG), Madison, Wisconsin); BLASTP, BLASTN, BLASTX (Altschul *et al.*, *J. Mol. Biol.*, 1990; 215:403-410; DNASTAR (DNASTAR, Inc., Madison, Wisconsin); and the FASTA program incorporating the Smith-Waterman algorithm (Pearson, *Comput. Methods Genome Res.*, [Proc. Int. Symp.] (1994), Meeting Date 1992, 111-20. Editor(s): Suhai, Sandor. Publisher: Plenum, New York, N.Y.). Within the context of this disclosure it will be understood that where sequence analysis software is used for analysis, the results of the analysis are based on the "default

values" of the program referenced. As used herein "default values" will mean any set of values or parameters which originally load with the software when first initialized.

**[0083]** Compositions. Therapeutics include any compound (e.g., protein, nucleotide sequence, small molecule) that provides a therapeutic effect as disclosed herein. Therapeutics include pharmaceutically acceptable salts, tautomers, isomers, and prodrugs of therapeutics disclosed herein. Exemplary pharmaceutically acceptable salts include acetate, acid citrate, acid phosphate, ascorbate, benzenesulfonate, benzoate, besylate, bisulfate, bitartrate, bromide, chloride, citrate, ethanesulfonate, formate, fumarate, gentisinate, gluconate, glucuronate, glutamate, lactate, methanesulfonate, nitrate, iodide, isonicotinate, maleate, oleate, oxalate, p-toluenesulfonate, pamoate (i.e., 1,1'-methylene-bis-(2-hydroxy-3-naphthoate)), pantothenate, phosphate, saccharate, salicylate, succinate, sulfate, tannate and tartrate salts.

**[0084]** "Prodrugs" refer to compounds that can undergo biotransformation (e.g., either spontaneous or enzymatic) within a subject to release, or to convert (e.g., enzymatically, mechanically, electromagnetically, etc.) an active or more active form of the therapeutic after administration. Prodrugs can be used to overcome issues associated with stability, toxicity, lack of specificity, or limited bioavailability and often offer advantages related to solubility, tissue compatibility, and/or delayed release (See e.g., Bundgard, Design of Prodrugs, pp. 7-9, 21-24, Elsevier, Amsterdam (1985); and Silverman, The Organic Chemistry of Drug Design and Drug Action, pp. 352-401, Academic Press, San Diego, CA (1992)).

**[0085]** Therapeutics disclosed herein can be formulated into compositions for administration to a subject. Compositions can advantageously include any pharmaceutically acceptable carriers which include those that do not produce significantly adverse, allergic or other untoward reactions that outweigh the benefit of administration, whether for research, prophylactic and/or therapeutic treatments. Exemplary pharmaceutically acceptable carriers and formulations are disclosed in Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990. Moreover, compositions can be prepared to meet sterility, pyrogenicity, general safety and purity standards as required by United States FDA Office of Biological Standards and/or other relevant foreign regulatory agencies.

**[0086]** Exemplary generally used pharmaceutically acceptable carriers include any and all bulking agents or fillers, solvents or co-solvents, dispersion media, coatings, surfactants, antioxidants (e.g., ascorbic acid, methionine, vitamin E), preservatives, isotonic agents, absorption delaying agents, salts, stabilizers, buffering agents, chelating agents (e.g., EDTA), gels, binders, disintegration agents, and/or lubricants.

**[0087]** Exemplary buffering agents include citrate buffers, succinate buffers, tartrate buffers, fumarate buffers, gluconate buffers, oxalate buffers, lactate buffers, acetate buffers, phosphate buffers, histidine buffers and trimethylamine salts.

**[0088]** Exemplary preservatives include phenol, benzyl alcohol, meta-cresol, methyl paraben, propyl paraben, octadecyldimethylbenzyl ammonium chloride, benzalkonium halides, hexamethonium chloride, alkyl parabens such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol and 3-pentanol.

**[0089]** Exemplary isotonic agents include polyhydric sugar alcohols including trihydric or higher sugar alcohols, such as glycerin, erythritol, arabitol, xylitol, sorbitol and mannitol.

**[0090]** Exemplary stabilizers include organic sugars, polyhydric sugar alcohols, polyethylene glycol; sulfur-containing reducing agents, amino acids, low molecular weight polypeptides, proteins, immunoglobulins, hydrophilic polymers and polysaccharides.

**[0091]** For injection, compositions can be made as aqueous solutions, such as in buffers such as Hanks' solution, Ringer's solution, or physiological saline. The solutions can contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the composition can be in lyophilized and/or powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

**[0092]** For oral administration, the compositions can be made as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like. For oral solid compositions such as, for example, powders, capsules and tablets, suitable excipients include binders (gum tragacanth, acacia, cornstarch, gelatin), fillers such as sugars, e.g. lactose, sucrose, mannitol and sorbitol; dicalcium phosphate, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate; cellulose preparations such as maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxy-methylcellulose, and/or polyvinylpyrrolidone (PVP); granulating agents; and binding agents. If desired, disintegrating agents can be added, such as corn starch, potato starch, alginic acid, cross-linked polyvinylpyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate. If desired, solid dosage forms can be sugar-coated or enteric-coated using standard techniques. Flavoring agents, such as peppermint, oil of wintergreen, cherry flavoring, orange flavoring, etc. can also be used.

**[0093]** For administration by inhalation, compositions can be made as aerosol sprays from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by

providing a valve to deliver a metered amount. Capsules and cartridges of gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

**[0094]** Compositions can also be depot preparations. Such long acting compositions may be administered by, without limitation, implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, compounds can be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as sparingly soluble salts.

**[0095]** Additionally, compositions can be delivered using sustained-release systems, such as semipermeable matrices of solid polymers containing at least one compound disclosed herein. Various sustained-release materials have been established and are well known by those of ordinary skill in the art. Sustained-release capsules may, depending on their chemical nature, release the compound following administration for a few weeks up to over 100 days.

**[0096]** Methods of Use. Methods disclosed herein include treating subjects (humans, veterinary animals (dogs, cats, reptiles, birds, etc.) livestock (horses, cattle, goats, pigs, chickens, etc.) and research animals (monkeys, rats, mice, fish, etc.) with therapeutic compositions disclosed herein. Treating subjects includes delivering therapeutically effective amounts. Therapeutically effective amounts include those that provide effective amounts, prophylactic treatments and/or therapeutic treatments.

**[0097]** An "effective amount" is the amount of a compound necessary to result in a desired physiological change in the subject. Effective amounts are often administered for research purposes. Effective amounts disclosed herein can cause a statistically-significant effect in an animal model or in vitro assay relevant to the assessment of SCLC development or progression.

**[0098]** A "prophylactic treatment" includes a treatment administered to a subject who does not display signs or symptoms of SCLC or displays only early signs or symptoms of SCLC such that treatment is administered for the purpose of diminishing or decreasing the risk of developing SCLC further. Thus, a prophylactic treatment functions as a preventative treatment against SCLC. In particular embodiments, prophylactic treatments reduce, delay, or prevent metastasis from a primary SCLC tumor site from occurring.

**[0099]** A "therapeutic treatment" includes a treatment administered to a subject who displays symptoms or signs of SCLC and is administered to the subject for the purpose of diminishing or eliminating those signs or symptoms of SCLC. The therapeutic treatment can reduce, control, or eliminate the presence or activity of SCLC and/or reduce control or eliminate side effects of

SCLC. In particular embodiments, therapeutic treatments reduce, delay, or prevent further metastasis from occurring.

**[00100]** Particular embodiments include suppressing SCLC in a subject by administering an L-Myc inactivator. SCLC suppression includes one or more of decreasing the number of SCLC cells in a subject, decreasing the number of metastases in a subject, decreasing tumor volume in a subject, increasing life expectancy in a subject, inducing chemo- or radiosensitivity in SCLC cells in a subject, inhibiting angiogenesis near SCLC cells in a subject, inhibiting SCLC cell proliferation in a subject, inhibiting tumor growth in a subject, preventing, reducing, or delaying metastases in a subject, prolonging a subject's life, reducing cancer-associated pain in a subject, and/or reducing or delaying relapse or re-occurrence of SCLC following treatment in a subject. Examples of L-Myc inactivators include CX-5461, Actinomycin D, or guide RNA comprising SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14 and/or SEQ ID NO: 15. Within these embodiments, L-Myc inactivation need not be complete inactivation, but must be sufficient to produce SCLC suppression.

**[0101]** In particular embodiments, compositions are administered as a first line treatment. In particular embodiments, compositions are administered in combination with a first line chemotherapeutic treatment. Examples of chemotherapeutic treatment agents include actinomycin, all-trans retinoic acid, azacitidine, azathioprine, bleomycin, bortezomib, carboplatin, capecitabine, cisplatin, chlorambucil, cyclophosphamide, cytarabine, daunorubicin, docetaxel, doxifluridine, doxorubicin, epirubicin, epothilone, etoposide, fluorouracil, gemcitabine, hydroxyurea, idarubicin, imatinib, irinotecan, mechlorethamine, mercaptopurine, methotrexate, mitoxantrone, oxaliplatin, paclitaxel, pemetrexed, teniposide, tioguanine, topotecan, valrubicin, vinblastine, vincristine, vindesine, and vinorelbine.

**[0102]** In particular embodiments, compositions are administered following the emergence of chemoresistance. Chemoresistance refers to a clinical stage when cancer cell(s) do not respond to the cell-killing effects of chemotherapeutic drugs. Cancer cells may be chemoresistant at the beginning of treatment, or may become resistant during the course of treatment. SCLC particularly is characterized by exquisite chemosensitivity followed by chemoresistance at recurrence.

**[0103]** In the context of SCLC, therapeutically effective amounts can decrease the number of SCLC cells, decrease the number of metastases, decrease tumor volume, increase life expectancy, induce chemo- or radiosensitivity in SCLC cells, inhibit angiogenesis near SCLC cells, inhibit SCLC cell proliferation, inhibit tumor growth, prevent or reduce metastases, prolong

a subject's life, reduce cancer-associated pain, and/or reduce relapse or re-occurrence of SCLC following treatment.

**[0104]** A "tumor" is a swelling or lesion formed by an abnormal growth of cells (called neoplastic cells or tumor cells). A "tumor cell" is an abnormal cell that grows by a rapid, uncontrolled cellular proliferation and continues to grow after the stimuli that initiated the new growth cease. Tumors show partial or complete lack of structural organization and functional coordination with the normal tissue, and usually form a distinct mass of tissue, which may be benign, pre-malignant or malignant.

**[0105]** For administration, therapeutically effective amounts (also referred to herein as doses) can be initially estimated based on results from in vitro assays and/or animal model studies. The actual dose amount administered to a particular subject can be determined by a physician, veterinarian or researcher taking into account parameters such as physical and physiological factors including target, body weight, severity of SCLC, type of SCLC, stage of SCLC, previous or concurrent therapeutic interventions, idiopathy of the subject and route of administration.

**[0106]** Useful doses can range from 0.01 to 500 µg/kg or from 0.01 to 500 mg/kg. Therapeutically effective amounts can be achieved by administering single or multiple doses during the course of a treatment regimen (e.g., daily, every other day, weekly, monthly, every 6 months, or yearly).

**[0107]** The compositions described herein can be administered by, for example, injection, inhalation, infusion, perfusion, lavage or ingestion. Routes of administration can include intravenous, intradermal, intraarterial, intraparenteral, intranasal, intranodal, intralymphatic, intraperitoneal, intralesional, intraprostatic, intravaginal, intrarectal, topical, intrathecal, intratumoral, intramuscular, intravesicular, oral, subcutaneous, and/or sublingual administration and more particularly by intravenous, intradermal, intraarterial, intraparenteral, intranasal, intranodal, intralymphatic, intraperitoneal, intralesional, intraprostatic, intravaginal, intrarectal, topical, intrathecal, intratumoral, intramuscular, intravesicular, oral, subcutaneous, and/or sublingual injection.

**[0108]** Further, the current disclosure describes a method for determining whether a treatment is appropriate for a subject diagnosed with SCLC. As an example, a biological sample from the subject can be screened for the presence of L-Myc (e.g., L-Myc amplification) or evidence of L-Myc signaling. If present and/or if there is evidence of L-Myc signaling, a treatment that targets a primary L-Myc pathway member as disclosed herein would be appropriate.

**[0109]** Determining whether a treatment is appropriate for a subject includes performing a test to assess whether the subject is more or less likely to respond to a given therapeutic

intervention, such as treatment with a compound that targets a primary L-Myc pathway member. Actual response to the therapeutic intervention is not required.

**[0110]** Evidence of L-Myc amplification or signaling can be identified using methods well known to those of ordinary skill in the art. An “increase” or a “decrease” (e.g., up-regulation or down-regulation) can be measured against a relevant control condition as disclosed herein. In particular embodiments, conclusions are drawn based on whether a measure is statistically significantly different or not statistically significantly different from a reference level of a relevant control. A measure is not statistically significantly different if the difference is within a level that would be expected to occur based on chance alone. In contrast, a statistically significant difference or increase is one that is greater than what would be expected to occur by chance alone. Statistical significance or lack thereof can be determined by any of various systems and methods used in the art. An example of a commonly used measure of statistical significance is the p-value. The p-value represents the probability of obtaining a given result equivalent to a particular datapoint, where the datapoint is the result of random chance alone. A result is often considered significant (not random chance) at a p-value less than or equal to 0.05.

**[0111]** Examples of biological samples from a subject include a tissue biopsy sample, a tumor biopsy sample, or a bronchoalveolar lavage sample.

**[0112]** The current disclosure also includes selecting subjects for enrollment in clinical trials. As an example, a biological sample from the subject can be screened for the presence of L-Myc amplification or L-Myc pathway signaling. The presence of the L-Myc amplification or L-Myc pathway signaling in the sample could direct the subject for inclusion or exclusion from a clinical trial.

**[0113]** The Exemplary Embodiments and Examples below are included to demonstrate particular embodiments of the disclosure. Those of ordinary skill in the art should recognize in light of the present disclosure that many changes can be made to the specific embodiments disclosed herein and still obtain a like or similar result without departing from the spirit and scope of the disclosure.

**[0114]** Exemplary Embodiments.

1. A method of treating small cell lung cancer (SCLC) in a subject in need thereof including administering a therapeutically effective amount of a composition including a compound that down-regulates a primary pathway member of the L-Myc signaling pathway, thereby treating the SCLC in the subject.
2. A method of embodiment 1, wherein the primary pathway member is L-Myc, MAX, or RNA Polymerase I (RNA Pol I).

3. A method of embodiment 1 or 2, wherein the compound includes a nucleic acid sequence that down-regulates expression of the primary pathway member.
4. A method of any of embodiments 1-3, wherein the compound includes a nucleic acid sequence that down-regulates function of the primary pathway member.
5. A method of embodiments 3 or 4, wherein the nucleic acid includes a vector or guide RNA.
6. A method of embodiment 5, wherein the guide RNA includes SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14 and/or SEQ ID NO: 15.
7. A method of any of embodiments 1-6, wherein the compound includes a RNA Pol I inhibitor.
8. A method of embodiment 7, wherein the RNA Pol I inhibitor is CX-5461 or Actinomycin D.
9. A method for determining whether therapy that down-regulates a primary pathway member of the L-Myc signaling pathway is appropriate for a subject diagnosed with SCLC, wherein the method includes obtaining a biological sample from the subject; testing the biological sample for the presence of L-Myc amplification or L-Myc signaling; and determining that therapy with a primary L-Myc pathway inhibitor is appropriate for the subject if the L-Myc amplification or L-Myc signaling is present.
10. The method of embodiment 9, wherein the evidence of L-Myc amplification or L-Myc signaling includes increased L-Myc, increased RNA Pol I and/or increased rRNA synthesis.
11. The method of embodiment 9 or 10, wherein the biological sample is a tissue biopsy sample, a tumor biopsy sample, or a bronchoalveolar lavage sample.
12. A method for determining whether a subject should be enrolled in a clinical trial aimed at examining the efficacy of a therapeutic treatment against a cancer including obtaining a biological sample from the subject; testing the biological sample for the presence of a testing the biological sample for the presence of L-Myc amplification or L-Myc signaling; and determining that enrollment in the clinical trial is appropriate for the subject if the L-Myc amplification or L-Myc signaling is present.
13. The method of embodiment 12, wherein the evidence of L-Myc amplification or L-Myc signaling includes increased L-Myc, increased RNA Pol I, and/or increased rRNA synthesis.
14. The method of embodiment 12 or 13, wherein the biological sample is a tissue biopsy sample, a tumor biopsy sample, or a bronchoalveolar lavage sample.

**[0115]** Example 1. Introduction and Summary. MYC family genes, most frequently L-Myc (also referred to as MYCL), are amplified in a subset of human SCLC but their roles in SCLC progression are poorly understood. When preneoplastic neuroendocrine cells were isolated from a mouse model of SCLC it was found that ectopic expression of L-Myc conferred tumor-forming capacity. In this system, L-Myc promoted pre-rRNA synthesis and transcriptional

programs associated with ribosomal biogenesis. L-Myc was then deleted in two genetically engineered models of SCLC. In both models, L-Myc inactivation resulted in strong suppression of SCLC. The high degree of suppression suggested that L-Myc constitutes a therapeutic target for a broad subset of SCLC, rather than only for L-Myc-amplified SCLC. An RNA Polymerase I inhibitor was then used to inhibit rRNA synthesis in an autochthonous Rb/p53 deleted mouse SCLC model and clear tumor inhibition was observed. These data reveal L-Myc signaling as an axis of vulnerability and its targeting as a therapeutic strategy for this recalcitrant cancer.

**[0116]** Methods. Mouse strains, Adeno-Cre (Ad-Cre) infection, and Subcutaneous Allografts. Chga-GFP mice, a transgenic strain expressing green fluorescent protein under control of the Chromogranin A (Chga) promoter are described in Gong et al., 2002. *Genome Res.* 12(12): 1992-8. SCLC mouse models bearing deletions in p53, Rb, p130, or Pten have been described in Schaffer, et al., 2010. *Cancer Research*, 70(10): 3877-83; Cui, et al., 2014. PTEN is a Potent Suppressor of Small Cell Lung Cancer. *Mol Cancer Res*, PMID: 24482365; and Meuwissen, et al., 2003. *Cancer Cell*, 4(3): 181-9.

**[0117]** Compound mice were maintained on a mixed background (129/SvJ; C57BL/6). Multiple cohorts of independent litters were analyzed to control for background effects.

**[0118]** Ad-Cre was purchased from Vector Development Laboratory at Baylor College of Medicine (Houston, Texas) (Ad-CMV-Cre) or from the University of Iowa Gene Transfer Vector Core (Ad-CMV-Cre and Ad-CGRP-Cre). Intra-tracheal instillation of Ad-Cre was performed essentially as previously described. DuPage, et al., 2009. *Nat Protoc*, 4(7): 1064-72.

**[0119]** For subcutaneous allografts,  $1.0 \times 10^5$  mouse pre-cancerous cells (preSC) or mouse tumor cells were injected in the flanks of immune-deficient nude mice (Harlan). Mice were maintained according to practices prescribed by the NIH (Bethesda, MD). All animal procedures were approved by the Animal Care and Use Committee at both University of Virginia (UVA) and the Fred Hutchinson Cancer Research Center, accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC).

**[0120]** Plasmids and chemicals. Retroviral plasmids (pMXs-GW, pMXs-L-Myc, pMXs-N-Myc, and pMXs-c-Myc) were a gift from Shinya Yamanaka (Addgene plasmid # 13375). CRISPR/Cas9 plasmid (px330-U6-Chimeric BB-CBh-hSpCas9) was a gift from Feng Zhang (Addgene plasmid #42230). Guide RNA sequences for mouse genome were retrieved using the CRISPR design tool at [crispr.mit.edu](http://crispr.mit.edu). The sequence information is shown in Table 1.

Table 1.

| Target | Forward (5'-3') | Reverse (5'-3') |
|--------|-----------------|-----------------|
|--------|-----------------|-----------------|

|              |                                     |                                     |
|--------------|-------------------------------------|-------------------------------------|
| <i>L-Myc</i> | CAGACTAGGAGTGCCGTCCG (SEQ ID NO: 4) | CGGACGGCACTCCTAGTCTG (SEQ ID NO: 5) |
| <i>N-Myc</i> | TGGTCGCCGGGGCGCTAGTG (SEQ ID NO: 6) | CACTAGCGCCCCGGCGACCA (SEQ ID NO: 7) |
| <i>c-Myc</i> | GGGGTCAATGCACTCGGAGG (SEQ ID NO: 8) | CCTCCGAGTGCATTGACCCC (SEQ ID NO: 9) |

**[0121]** Guide RNA sequences for human genome were retrieved from the Genome-scale CRISPR Knock-Out (GeCKO) v2.0 libraries [Shalem and Sanjana et al. 2014 PMID: 24336571]. The sequence information is shown in Table 2.

Table 2.

| Target       | Forward (5'-3')                       | Reverse (5'-3')                      |
|--------------|---------------------------------------|--------------------------------------|
| <i>L-MYC</i> | GGGGTCTGCTCGCACCGTGA (SEQ ID NO: 10)  | TCACGGTGCGAGCAGACCCC (SEQ ID NO: 11) |
| <i>L-MYC</i> | GCGCCAAGAATCGCGAACGC (SEQ ID NO: 12)  | GCGTTCGCGATTCTTGGCGC (SEQ ID NO: 13) |
| <i>L-MYC</i> | AGGTCCTTACCCGAGTCGCT (SEQ ID NO: 14)  | AGCGACTCGGGTAAGGACCT (SEQ ID NO: 15) |
| <i>N-MYC</i> | CAGGCCGAACGCGTCCTCCT (SEQ ID NO: 16)  | AGGAGGACGCGTTCGGCCTG (SEQ ID NO: 17) |
| <i>N-MYC</i> | ATGACACTCTTGAGCGGACG (SEQ ID NO: 18)  | CGTCCGCTCAAGAGTGTCAT (SEQ ID NO: 19) |
| <i>N-MYC</i> | CACGGCGGGATCCACGCACT (SEQ ID NO: 20)  | AGTGCGTGGATCCCGCCGTG (SEQ ID NO: 21) |
| <i>c-MYC</i> | AACGTTGAGGGGCGATCGTCG (SEQ ID NO: 22) | CGACGATGCCCTCAACGTT (SEQ ID NO: 23)  |
| <i>c-MYC</i> | GCCGTATTTCTACTGCGACG (SEQ ID NO: 24)  | CGTCGCAGTAGAAATACGGC (SEQ ID NO: 25) |
| <i>c-MYC</i> | TGCGTAGTTGTGCTGATGTG (SEQ ID NO: 26)  | CACATCAGCACAACTACGCA (SEQ ID NO: 27) |

**[0122]** Cisplatin was purchased from Sigma-Aldrich, CX-5461 was purchased from EMD Millipore and Selleck. For in vivo studies, CX-5461 was suspended in 50 mM NaH<sub>2</sub>PO<sub>4</sub>, pH 4.5. Cisplatin and CX-5461 were dissolved in the appropriate solvent according to the manufacturer's instructions. The solvents were used as vehicle control.

**[0123]** FACS analysis and sorting. Whole lungs or dissected lung tumors from the transgenic mice (Chga-GFP) that express GFP in lung neuroendocrine cells were minced and digested rotating for 30 minutes in 25 unit/mL Dispase (Gibco). Single-cell suspensions were stained with

DAPI (Sigma) to visualize dead cells. GFP-positive live cells were sorted using BD Aria and FlowJo software (Tree Star Inc.).

**[0124]** Cell lines and in vitro assays. All mouse SCLC cell lines were described previously and were authenticated by genotyping for the mutant alleles and the expression of neuroendocrine markers. Human SCLC cell lines H209, H2141, and H1184 were gifts of Dr. Hisashi Harada (Virginia Commonwealth University). NHJ29 and the remaining cell lines were gifts of Dr. Julien Sage (Stanford University) [Jahchan et al., Cancer Discov. 2013 Dec;3(12):1364-77].

**[0125]** These human and mouse cells were maintained in a growth media that is RPMI1640 (Corning) supplemented with 10% bovine growth serum (BGS) (Hyclone) and 1% penicillin/streptomycin (PS) (Life Technologies) and using the 37°C/5% CO<sub>2</sub> incubator. For cell counting, the cells were treated with 0.05% Trypsin-EDTA for less than 5 minutes and counted using hemocytometer. MTT assay was performed to measure relative cell viability using (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; thiazolyl blue) tetrazolium salt and plate reader. Cells were seeded at  $2.5 \times 10^4$  per well in 96-well plates at day 0, and drugs were added 5 hours later. The MTT reagents were added on day 3 or 4 depending on the experiments. The percentage survival was determined as the ratio of treated cells versus vehicle control after background subtraction. Soft agar assay was performed following standard protocols, seeding  $3 \times 10^4$  mouse SCLC cells (per well in 6-well plates) resuspended in 1.5mL of the RPMI growth media containing 0.35% agar (Invitrogen) on bottom layer that contained 1.5mL of the growth media containing 0.5% agar. The media was regularly changed every 3 days for 4 weeks. After incubation, colonies with anchorage-independent growth were counted by using Image J software.

**[0126]** Analyses of DNA and RNA. Genomic DNA from mouse tail was purified using lysis buffer containing Proteinase K and genotyping PCR was performed using the primers described in Table 3.

Table 3.

| Target                    | Forward (5'-3')                            | Reverse (5'-3')                             |
|---------------------------|--------------------------------------------|---------------------------------------------|
| <i>Rb</i> <sup>lox</sup>  | CTCTAGATCCTCTCATTCTTCCC<br>(SEQ ID NO: 28) | CCTTGACCATAGCCCAGCAC<br>(SEQ ID NO: 29)     |
| <i>Rb</i> <sup>Δ</sup>    | CTCTAGATCCTCTCATTCTTCCC<br>(SEQ ID NO: 30) | GCAGGAGGCAAAAATCCACATAAC<br>(SEQ ID NO: 31) |
| <i>p53</i> <sup>lox</sup> | CACAAAAACAGGTAAACCCAG<br>(SEQ ID NO: 32)   | AGCACATAGGAGGCAGAGAC<br>(SEQ ID NO: 33)     |

|                             |                                           |                                                   |
|-----------------------------|-------------------------------------------|---------------------------------------------------|
| <i>p53</i> <sup>Δ</sup>     | CACAAAAACAGGTTAAACCCAG<br>(SEQ ID NO: 34) | GAAGACAGAAAAGGGGAGGG<br>(SEQ ID NO: 35)           |
| <i>p130</i> <sup>lox</sup>  | GTGTTGTAAACATTCTCGTGGG<br>(SEQ ID NO: 36) | GACTGCTGGTATTAGAACCC<br>(SEQ ID NO: 37)           |
| <i>L-Myc</i> <sup>lox</sup> | CATTAGAAGGTTGTATTGGGGC<br>(SEQ ID NO: 38) | TGCCTGTATTCCCAATAACAATCTTC<br>TTC (SEQ ID NO: 39) |

**[0127]** RNA was isolated from cells or tumors using TRIzol (Life Technologies) according to the manufacturer's protocol. The purified RNAs were processed for Affymetrix Mouse 430 2.0 gene-chip assay at the DNA Science Core at UVA and also for real-time quantitative PCR using the Dynamo cDNA synthesis kit (New England Biolabs). Quantitative PCR (RT-qPCR) was performed using SYBR Green with Applied Biosystems 7900 or StepOnePlus™ following the manufacturer's protocol. The sequences of qPCR primers were retrieved from the online Universal Probe Library at Roche and are described in Table 4.

Table 4.

| Target                  | Forward (5'-3')                             | Reverse (5'-3')                           |
|-------------------------|---------------------------------------------|-------------------------------------------|
| <i>Chga</i>             | CGATCCAGAAAGATGATGG<br>TC (SEQ ID NO: 40)   | CGGAAGCCTCGTCTTTCC<br>(SEQ ID NO: 41)     |
| <i>Cgrp</i>             | TGCAGGACTATATGCAGAT<br>GAAA (SEQ ID NO: 42) | GGATCTCTTCTGAGCAGTGACA (SEQ<br>ID NO: 43) |
| <i>Syp</i>              | CTGGCAGACATGGACGTG<br>(SEQ ID NO: 44)       | CTTGACCACCCGGAAGT<br>(SEQ ID NO: 45)      |
| <i>Ascl</i>             | GCTCTCCTGGGAATGGACT<br>(SEQ ID NO: 46)      | CGTTGGCGAGAAACACTAAG<br>(SEQ ID NO: 47)   |
| <i>Ncam1</i>            | AGGGCAAGGCTGCTTTCT<br>(SEQ ID NO: 48)       | CCCCATCATGGTTTGGAGT<br>(SEQ ID NO: 49)    |
| <i>E-Cad</i>            | CAGAATGACAACAGGCCAGA<br>(SEQ ID NO: 50)     | TTCATCACGGAGGTTCTG<br>(SEQ ID NO: 51)     |
| <i>Nfib</i>             | GGGACTAAGCCCAAGAGACC<br>(SEQ ID NO: 52)     | GTCCAGTCACAAATCCTCAGC<br>(SEQ ID NO: 53)  |
| <i>E2f1</i>             | TGCCAAGAAGTCCAAGAAT<br>CA (SEQ ID NO: 54)   | CTTCAAGCCGCTTACCAATC<br>(SEQ ID NO: 55)   |
| <i>L-Myc</i><br>(mouse) | ACGGCACTCCTAGTCTGG<br>AA (SEQ ID NO: 56)    | CCACGTCAATCTCTTCACCTT<br>(SEQ ID NO: 57)  |
| <i>N-Myc</i><br>(mouse) | CCTCCGGAGAGGATACCTTG<br>(SEQ ID NO: 58)     | TCTCTACGGTGACCACATCG<br>(SEQ ID NO: 59)   |

|                                       |                                               |                                             |
|---------------------------------------|-----------------------------------------------|---------------------------------------------|
| <i>c-Myc</i><br>(mouse)               | CCTAGTGCTGCATGAGGAGA<br>(SEQ ID NO: 60)       | TCTTCCTCATCTTCTTGCTCTTC (SEQ<br>ID NO: 61)  |
| <i>L-MYC</i><br>(human)               | GTGGGTAGGGGGTGGTAA<br>GT (SEQ ID NO: 62)      | ATTTGGAAGTAGCAGCTGGTTT (SEQ<br>ID NO: 63)   |
| <i>N-MYC</i><br>(human)               | CCACAAGGCCCTCAGTACC<br>(SEQ ID NO: 64)        | TCTTCCTCTTCATCATCTTCATCA<br>(SEQ ID NO: 65) |
| <i>c-MYC</i><br>(human)               | CACCAGCAGCGACTCTGA<br>(SEQ ID NO: 66)         | GATCCAGACTCTGACCTTTTGC (SEQ<br>ID NO: 67)   |
| <i>Arbp P0</i><br>(mouse)             | GATGCCCAGGGAAGACAG<br>(SEQ ID NO: 68)         | ACAATGAAGCATTTTGGATAATCA<br>(SEQ ID NO: 69) |
| <i>ARB P0</i><br>(human)              | GGCACCATTGAAATCCTGAG<br>(SEQ ID NO: 70)       | GAAGGGGGAGATGTTGAGC<br>(SEQ ID NO: 71)      |
| <i>45S rRNA</i><br><i>ITS</i> (human) | TTACCCTACTGATGATGTGTTG<br>TTG (SEQ ID NO: 72) | CCTGCGGTTCTCTCGTA<br>(SEQ ID NO: 73)        |
| $\beta 2M$<br>(human)                 | TTCTGGCCTGGAGGCTATC<br>(SEQ ID NO: 74)        | TCAGGAAATTTGACTTTCCATTC<br>(SEQ ID NO: 75)  |
| $\beta 2m$<br>(mouse):                | TTCACCCCCACTGAGACTGAT<br>(SEQ ID NO: 76)      | GTCTTGGGCTCGGCCATA<br>(SEQ ID NO: 77)       |
| <i>47S rRNA</i><br><i>ITS</i> (mouse) | CCGGCTTGCCCGATTT<br>(SEQ ID NO: 78)           | GCCAGCAGGAACGAAACG<br>(SEQ ID NO: 79)       |

**[0128]** For RNAseq analysis, total RNA was isolated using Trizol and RNAseq libraries prepared using NEBNext Ultra RNA library preparation kit (New England Biolabs). 50bp single end sequencing was performed using an Illumina Hiseq2500, reads were aligned to the mm9 genome using Tophat (Trapnell et al., 2009. Bioinformatics, 1;25(9):1105-11 (PMID:19289445)) and Cuffdiff (Trapnell et al., 2010. Nat Biotechnol. 28:511-515) was used to generate FPKM expression values used for Gene Set Enrichment Analysis (<http://www.broadinstitute.org/gsea/>) (Subramanian et al., 2005. Proc Natl Acad Sci USA. 102(43):15545–15550).

**[0129]** Microarray analysis. RNA from cells or tumors was isolated using TRIzol (Invitrogen) and then purified using a RNeasy column (QIAGEN) following the manufacturer's protocol. The purified RNAs were processed and hybridized to Affymetrix Mouse Genome 430 2.0 expression array at the DNA Science Core at UVA. Array data has been uploaded to the GEO database (Submission ID: GSE77385).

**[0130]** More particularly, before discovering differentially expressed genes between preSC and L-Myc-expressed cells, all gene expression microarray data were normalized using quantile-normalization method to be comparable and to reduce any unexpected technical batch effects. Descriptive characteristics of expression of probe sets targeting L-Myc were first examined to confirm L-Myc status in preSC and L-Myc cell samples. Furthermore, array-wise correlation analysis and principal components-based cluster analysis was performed as a quality control analysis to filter microarrays with low quality out of the following differential gene expression analysis. Significant analysis of microarray (SAM) (Tusher et al., 2001. Proc Natl Acad Sci USA 98(9):5116–21 PMID:PMC33173)), linear model for microarray (LIMMA) (Ritchie et al., 2015. Nucleic Acids Research, 43(7):e47 (PMID:25605792 PMID:PMC4402510)), and local pooled error (LPE)-based analyses (Jain et al., 2003. Bioinformatics. Oct 12;19(15):1945-51 (PMID:14555628)) were performed to identify a set of genes that are consistently identified to be differentially expressed between preSC and L-Myc-expressing preSC in all analyses. The Benjamini-Hochberg multiple testing correction technique was used to control FDR at 20% and adjusted two-sided p-values < 0.2 were considered statistically significant in each analysis. Next, to capture functionally related genes giving significant effects on a pathway, the online tools at DAVID Bioinformatics Resources 6.7 were used to discover significantly enriched functional categories of the differentially expressed genes, especially focusing on KEGG-pathways Gene ontology analysis (<http://david.abcc.ncifcrf.gov/>). Also, to identify canonical pathways significantly enriched in differentially expressed genes were analyzed with Ingenuity Pathways Analysis software (QIAGEN).

**[0131]** Histology and immunostaining. Five-micron paraffin sections were used for Hematoxylin-Eosin (H&E) staining and immunostaining. For immunofluorescence, paraffin sections were dewaxed and rehydrated using Trilogy (Cell Marque) according to the manufacturer's instruction. The primary antibodies used were Synaptophysin (Neuromics, MO20000), phospho-histone H3 (Upstate, 06-570), Ki67 (BD Pharmingen, 550609), cleaved caspase 3 (Cell signaling, 9661), UCHL1 (Sigma, HPA005993), CGRP (Sigma c-8198) and anti-BRDU (BD Pharmingen). Alexa Fluor-conjugated secondary antibodies (Invitrogen) were used for antibody detection and anti-fade reagents with DAPI (Vector Lab) were used for preserving fluorescence and nuclear counter-staining. All microscopic images were acquired using Nikon ECLIPSE Ni-U microscope. Image analysis and automated quantification were performed using NIS-Elements Basic Research (Nikon). Macroscopic images of lung were acquired using an Olympus MVX10. Areas of tumors and whole

lung were quantified using ImageJ software. For quantification of the number of phospho-histone H3 (pHH3)-positive cells, tumors of similar size and area were included. For pathological analyses, entire slides were digitally scanned at high (40x) resolution using the NanoZoomer 2.0 HT Digital Pathology System (Hamamatsu Photonics, Hamamatsu City, JP) and examined using the manufacturer's software. A pathologist (AFG) examined all of the scanned images in detail and captured multiple representative images. Terminology for neuroendocrine tumors was as described previously. Gazdar, et al., 2015. International Association for the Study of Lung Cancer, 10(4): 553-64.

**[0132]** Animal imaging and tumor quantification. For therapeutic studies in the autochthonous model,  $Rb^{lox/lox};p53^{lox/lox}$  mice were infected intratracheally with Ad-CMV-Cre. Mice were screened for detectable tumor between 8 and 14 months following infection using a Bruker Icon small animal MRI. MRI covered the entire thoracic region at 1mm intervals. That tumor be present on at least three consecutive 1mm slices was required for study entry. Typically, a single measurable tumor was detected but in cases with multiple tumors, the largest tumor was quantified. Tumor volume was measured using ImageJ.

**[0133]** Statistical analysis. Except where indicated otherwise, statistical significance was assayed by a Student t test with the Prism GraphPad software (two-tailed unpaired and paired t test depending on the experiment). Unless noted otherwise, pooled data is represented as the mean  $\pm$  SEM (standard error of mean). For the survival curve analysis and comparison, the Mantel-Cox test was used.

**[0134]** Results. Isolation of preneoplastic precursors of SCLC. To characterize precursor cells of SCLC, a BAC transgenic strain (Chga-GFP) that expresses green fluorescence protein (GFP) under the control of 190 kilobase pairs of genomic sequences flanking Chga locus was employed. Lung neuroendocrine cells in this strain were specifically labeled and isolated using fluorescence-activated cell sorting (FACS) (FIG. 2A). Next, the Chga-GFP mice were crossed with  $Rb^{lox/lox};p53^{lox/lox};p130^{lox/lox}$  mice and SCLC was initiated via intratracheal instillation of Ad-CMV-Cre. Schaffer, et al., 2010. Cancer Research, 70(10): 3877-83. This Rb/p53/p130 deleted mouse model recapitulates key features of human SCLC, including neuroendocrine characteristics and metastatic spread. Schaffer, et al., 2010. Cancer Research, 70(10): 3877-83; Gazdar, et al., 2015. International Association for the Study of Lung Cancer, 10(4): 553-64.

**[0135]** One month after Cre delivery, at which time macroscopic lesions are not yet evident, a small number of GFP-positive cells were isolated from the lungs of Chga-GFP;  $Rb^{lox/lox};p53^{lox/lox};p130^{lox/lox}$  mice using FACS (FIG. 1B). The GFP cells from early stages of tumorigenesis grew attached to culture dish in monolayer, whereas mouse SCLC cells formed

spheres or aggregates and grew in suspension or loosely attached to the culture dish. The GFP-positive cells continued to proliferate in RPMI1640 media supplemented with 10% bovine growth serum, but did not form subcutaneous tumors in immune-deficient nude mice. By contrast, the mouse SCLC tumor cells when introduced in the same number, readily formed palpable tumors (FIG. 1C). These cells from an early stage of tumor development acquired unlimited replicative potential likely owing to deletion of both Rb and p53 genes, a common cause of cell immortalization. However, they were not yet tumorigenic potentially owing to the lack of other key oncogenic factors being activated. These mutant neuroendocrine cells are referred to as preneoplastic precursors of SCLC (hereafter preSC). Genotyping PCR and RT-qPCR showed deletion of targeted Rb and p53 exons and expression of various neuroendocrine cell markers including Ncam1, Chga, Syp, Cgrp, and Ascl1 in both the preSC and SCLC cells (FIG. 1D, 1E and FIG. 2A). However, unlike the tumor cells, the bulk preSC cell population did not express high levels of L-Myc. The preSC cells also maintained normal levels of E-Cadherin relative to normal lung that is decreased in the SCLC tumor cells. Interestingly, preSC cells tolerated significantly higher doses of cisplatin than tumor cells (FIG. 1F), likely due to reduced proliferation compared to that of SCLC cells.

**[0136]** L-Myc drives tumorigenic progression of preneoplastic SCLC precursors. It was hypothesized that utilizing the preSC would be a potentially powerful system to test the roles of candidate oncogenes and tumor suppressor genes in SCLC. One of the most prevalent oncogenic events in SCLC is amplification of L-Myc. However, functional roles for L-Myc in SCLC are unclear. The preSC system allowed delineation of roles for L-Myc in promoting SCLC. The effect of L-Myc overexpression in preSCs was first studied using a retroviral vector. In less than 2 weeks, the preSCs infected with retroviral-L-Myc (L-Myc-preSCs) form spheres typical of both human SCLC and mouse SCLC cells in culture (FIG. 1G). Transition from adherent cell culture to sphere formation indicates loss of contact inhibition and anchorage independence, two of the hallmarks of cancer cells. Additionally, the L-Myc-preSC cells formed colonies in soft agar (FIG. 1I) and palpable tumors with typical SCLC morphology in the flanks of athymic nude mice (FIG. 1J), whereas the control preSCs infected with retroviral-GFP were morphologically the same as uninfected cells and failed to form palpable tumors in nude mice. In human SCLC, L-Myc, MYCN or MYC can be amplified in a mutually exclusive manner, suggesting that key oncogenic activities may be shared among these family members. Similar to L-Myc, the retroviral expression of N-Myc or c-Myc also transformed preSCs, and the resulting phenotypes in culture and allograft experiments were almost identical to those caused by L-Myc (FIG. 2B, 2C). Expression of L-Myc or N-Myc in each group of transformed cells is

drastically higher (40-60 fold) than control preSCs, but c-Myc levels in the c-Myc-preSCs is increased only 3-4 fold (FIG. 1H and FIG. 2D). Notably, higher MYC expression (20 fold increase) caused morphological changes of spheroid cells into multigonal, attached cells (FIG. 2B). The allograft tumors generated from the preSC-expressing MYC family genes showed histological features of SCLC in H&E staining and also stained for neuroendocrine markers including CGRP and UCHL1 (FIG. 1K and FIG. 2E). Thus, this novel system revealed that overexpression of L-Myc, N-Myc or c-Myc is each sufficient to drive a transition from preneoplastic to neoplastic SCLC.

**[0137]** L-Myc inactivation suppresses SCLC. To determine whether L-Myc is required for SCLC development, a floxed allele for L-Myc with loxP sites upstream of the 1st exon and downstream of exon 3 was employed. L-Myc was conditionally inactivated at the time of tumor initiation in two different highly penetrant mouse models of SCLC. In the first cross, a model was employed where intratracheal AdenoCre (Ad-CMV-Cre) drives deletion of  $Rb^{lox/lox};p53^{lox/lox};p130^{lox/lox}$  alleles in the lung epithelium, in which SCLC, often with a large cell neuroendocrine component, rapidly arises from expanding neuroendocrine cells. Schaffer, et al., 2010. Cancer Research, 70(10): 3877-83; Gazdar, et al., 2015. International Association for the Study of Lung Cancer, 10(4): 553-64. Human SCLC almost always exhibits RB/P53 deletion and occasionally RBL2/130 deletion. George, et al., 2015. Nature, 524(7563): 47-53. The L-Myc floxed allele was bred into the model, allowing comparison of littermate  $Rb^{lox/lox};p53^{lox/lox};p130^{lox/lox}$  mice that were L-Myc<sup>+/+</sup>, L-Myc<sup>+/lox</sup> or L-Myc<sup>lox/lox</sup>. Six months after Ad-CMV-Cre infection, the lungs of the infected mice were analyzed. The mice with homozygous L-Myc floxed alleles exhibited drastically reduced tumor burden compared to those with wild type or heterozygous floxed alleles (FIG. 3A). Significant decreases in tumor burden were also observed between the mice with heterozygous L-Myc floxed vs. wild type alleles (FIG. 3A). Histology and immunostaining showed that the tumors and lesions with each genotype exhibit well-known SCLC features including scanty cytoplasm and positive staining for Uchl1 and CGRP (FIG. 4A and not shown). The decrease in tumor burden correlated significantly with lower rates of proliferation as measured by quantification of cells positive for phosphorylated histone H3 (pHH3) (FIG. 3B, 3C) but not with higher rates of cell death (not shown).

**[0138]** To determine whether the importance of L-Myc is broadly relevant to SCLC of differing genotypes, L-Myc was also deleted in a mouse model driven by Rb/p53 and Pten deletion. The PTEN pathway is altered through inactivating deletions/mutations in PTEN or activating mutations in PIK3CA in a subset of human SCLCs. In this mouse model,  $Rb^{lox/lox};p53^{lox/lox};Pten^{lox/lox}$  mice were infected intratracheally with Adenoviral Cre driven by a

neuroendocrine (Calcitonin/CGRP) promoter (Ad-CGRP-Cre, as in McFadden, et al., 2014. *Cell*, 156(6): 1298-311; Sutherland, et al., 2011. *Cancer Cell*, 19(6): 754-64). The Ad-CGRP-Cre approach was taken because combined deletion of Rb/p53/Pten throughout the lung using the more widely active Ad-CMV-Cre leads to substantial adenocarcinoma that impairs study of SCLC. Cui, et al., 2014. PTEN is a Potent Suppressor of Small Cell Lung Cancer. *Mol Cancer Res*, PMID: 24482365. When mice were examined 4 months following Ad-CGRP-Cre delivery, clear reductions in number and size of early tumors in the L-Myc-deleted model (FIG. 3D) were found. In a long-term survival study. Kaplan-Meier analysis revealed striking suppression of SCLC in the absence of L-Myc (FIG. 3E) ( $p=0.0005$ , log rank test). Indeed, 40% of the animals in the L-Myc<sup>lox/lox</sup> cohort were free of tumors when euthanized at the end of study at 380 days. While Ad-CGRP-Cre Rb/p53/Pten mice developed SCLC as the major phenotype, with occasional small foci of adenocarcinoma, increased heterogeneity in tumor spectrum with additional tumor types beyond SCLC was apparent upon Rb/p53/Pten and L-Myc inactivation (FIG. 3F). For example, SCLC and adenosquamous carcinoma were found in one case, in another, SCLC and adenocarcinoma were found. The possibility of leakiness in the Ad-CGRP-Cre system cannot be ruled out and one Rb/p53/Pten/L-Myc animal developed adenocarcinoma in the absence of SCLC. Increased heterogeneity in L-Myc-deficient tumors may be a consequence of the longer time that these mice had to develop tumors (FIG. 3E) and this increased heterogeneity in tumor spectrum was not apparent at the 4-month time point (FIG. 3D). These genetic data indicate that L-Myc inactivation strongly suppresses SCLC and show that inhibition of L-Myc and/or its downstream effectors provides a broad approach to treating SCLC, even in tumors without L-Myc amplification.

**[0139]** Tumor inhibition in the Rb/p53/p130 and Rb/p53/Pten mouse models precluded characterizing a continued role for L-Myc in late-stage tumor cells. Thus, to test roles of L-Myc in cells derived from SCLC, L-Myc function was acutely ablated in Rb/p53 deleted-mouse SCLC cell lines using CRISPR/Cas9-mediated gene targeting. Targeting L-Myc reduced the ability of targeted cells to form colonies in soft-agar compared to controls, indicating that L-Myc is important for the continuing growth of the tumor cells in culture (FIG. 3G-3I). Targeting Mycn also reduced the colony-forming capacity of tumor cells, whereas CRISPR inactivation of Myc did not lead to significant change in colony formation. CRISPR-mediated frame-shift mutations of the MYC family genes were validated by sequencing the genome of mouse lung fibroblast cells, which were targeted with the same vector but not affected (FIG. 4B). The inhibitory effect of targeting L-Myc and Mycn in mouse SCLC cells was congruent with their high expression in mouse SCLC cells relative to normal lung (FIG. 1E). The lack of inhibitory effect of targeting

Myc on the cell growth was associated with lower levels of Myc relative to normal lung (FIG. 1E). Thus, in contrast to L-Myc, basal levels of Myc expression may not be important for the long-term growth of SCLC cells, even though overexpression of Myc promoted SCLC (FIG. 2C).

**[0140]** Identification of effector pathways underlying L-Myc-induced SCLC progression. The results support the concept of L-Myc inhibition as a strategy for therapy and prevention of SCLC. Given the lack of a direct inhibitor of L-Myc, however, effectors of the L-Myc-driven oncogenic pathway as alternative targets were explored. To identify potential L-Myc-driven oncogenic pathways, genome-wide gene expression profiles of control preSC and L-Myc-preSC were compared using Affymetric microarrays (FIG. 5A). Using three gene expression analysis platforms that employ different statistical methods, including SAM (Significance Analysis of Microarrays), LIMMA (Linear Models for Microarray data), and LPE (Local-Pooled-Error), a set of 1017 annotated genes differentially expressed between the preSC and L-Myc-preSC were identified and defined as the L-Myc signature: 711 genes were up-regulated and 306 down-regulated in L-Myc-preSC compared to control preSC (FIG. 5A).

**[0141]** The up-regulated genes include L-Myc and 16 genes coding for various ribosomal proteins. The L-Myc signature was analyzed using the KEGG pathway database via DAVID Bioinformatics (NIAID/NIH). Strong enrichment of pathways including ribosome and adherens junction (FIG. 5B) were identified. Also, using Ingenuity pathway analysis (IPA, Ingenuity® Systems) it was found that the top molecular pathways significantly activated in the L-Myc signature include eIF2 signaling, regulation of eIF4 and p70S6K, and mTOR signaling, all known to control ribosome biogenesis and protein synthesis. KEGG pathway analysis of the L-Myc signature via DAVID also indicated that ribosome (biogenesis) is the most significant (FIG. 5B). MYC can promote transcription of ribosomal rRNA genes (Grandori, et al., 2005. Nat Cell Biol, 7(3): 311-8; Arabi, et al., 2005. Nat Cell Biol, 7(3): 303-10), although this has not been shown for L-Myc. Real time PCR was next used to measure pre-rRNA synthesis in preSCs or in L-Myc-PreSCs. Pre-rRNA is processed rapidly to mature RNA. Pre-rRNA quantification using primers for the short-lived ITS-1 (internally transcribed spacer 1) of the 47S pre-rRNA can be used as a measure of the rate of rRNA synthesis. A striking upregulation of pre-rRNA synthesis was found upon ectopic expression of L-Myc (FIG. 5C). These results raise the possibility that L-Myc may promote tumor progression by upregulating the protein synthesis machinery to meet increasing demand for structural proteins and various enzymes essential for dividing cells.

**[0142]** Modulation of ribosome biogenesis blocks SCLC progression and continuing growth. To test whether increased ribosome biogenesis reflects a L-Myc-associated vulnerability, the

synthesis of ribosomal RNAs (rRNAs) were targeted by inhibiting RNA Pol I. CX-5461, a specific inhibitor of RNA Pol I that prevents the association of RNA Pol I specific initiation complex SL1 with DNA was employed. Drygin, et al., 2011. *Cancer Res*, 71(4): 1418-30. Four-day treatment with CX-5461 dramatically reduced the viability of 3 of 3 mouse SCLC cell lines from an Rb/p53 Ad-CMV-Cre model as well as 5 of 7 human SCLC lines in a dose-dependent manner, whereas several lung adenocarcinoma cell lines and 2 of 7 SCLC cell lines responded only mildly to the drug at the same concentrations (FIG. 5D). The drug treatment also reduced the number of colonies formed by mouse SCLC cells in a soft-agar assay (FIG. 5E). Gene expression analysis using RT-qPCR indicated that all the mouse cells and these human SCLC cell lines sensitive to CX-5461 were associated with relatively higher levels of L-Myc and MYCN while the non-responsive cells were associated with higher MYC (FIG. 1E and FIG. 6). Thus, in most SCLC cell lines, CX-5461 treatment reduced cell viability and level of proliferation.

**[0143]** CX-5461 treatment suppresses SCLC in autochthonous mouse model. The efficacy of CX-5461 was next tested in vivo, using the autochthonous Ad-CMV-Cre Rb/p53 deleted model of SCLC. Meuwissen, et al., 2003. *Cancer Cell*, 4(3): 181-9. This is an ideal preclinical model as tumors emerge from lung neuroendocrine cells and undergo frequent metastasis and genetic alterations similar to human SCLC. McFadden, et al., 2014. *Cell*, 156(6): 1298-311; Meuwissen, et al., 2003. *Cancer Cell*, 4(3): 181-9. Moreover, the Rb/p53 model is a broadly generalizable system, given the near universal deletion of RB and P53 in human SCLC. George, et al., 2015. *Nature*, 524(7563): 47-53. Notably, CX-5461 is currently being tested in Phase I clinical trials for leukemia, lymphoma and myeloma (Australia clinical trials ID: ACTRN12613001061729). Mice were monitored at by MRI for tumor emergence and then entered into CX-5461 treated or untreated groups when tumors of adequate size were detected. CX-5461 dosing was at 50 mg/kg orally, every three days, a dosing regimen previously found to be well tolerated and efficacious in mouse lymphoma models. Drygin, et al., 2011. *Cancer Res*, 71(4): 1418-30. All (9/9) untreated control mice exhibited progressive disease (PD) > 25% over two weeks (FIG. 7A-7C). In contrast, only 1/8 CX-5461 treated mice showed PD. Stable disease (SD) upon CX-5461 treatment was found in 5/8 animals and a partial response (PR) >25% reduction was seen in 3/8 cases (FIG. 7A-7C). To examine target engagement tumors were collected from 2-week treated or untreated mice, confirmed SCLC histology and extracted RNA for real time PCR analysis. A significant 2-fold reduction in pre-ribosomal 47S RNA in tumors from the treated mice (FIG. 7D) was found. Thus, SCLC suppression occurred with reduction in pre-rRNA synthesis. Proliferation and cell death was also examined in treated tumors using BrdU analysis and immunohistochemistry. At 2 weeks post CX-5461 treatment significant decreases in BrdU

positive cells and phospho histone H3 relative to untreated controls (FIG. 7E) were found. In contrast, apoptosis levels were not reduced. Thus, inhibition of RNA Pol I leads to suppressed proliferation upon treatment in vivo. Gene set enrichment analysis (GSEA) was performed using RNAseq data generated from untreated or treated mice to identify major biological pathways affected by CX-5461. Strong reductions in MYC-related and cell cycle related gene sets associated with CX-5461 treatment (FIGs. 7G and 9) were found.

**[0144]** Discussion. A major challenge in SCLC is discerning which of the many commonly mutated genes are true drivers of tumor development. This disclosure reports a novel system in which rare preneoplastic pulmonary neuroendocrine cells (PNECs) can be purified from mouse lungs in a sensitized SCLC model very early in tumor initiation. This system takes advantage of a mouse allele that uses the *Chromogranin A* (*Chga*) promoter to drive GFP expression in pulmonary neuroendocrine cells (PNECs), which previous work has identified as the major SCLC cell of origin. Sutherland, et al., 2011. Cancer Cell, 19(6): 754-64. Using this new approach PNECs can be purified in the wild type or mutant configuration. Upon *Rb/p53/p130* loss, PNECs grow in culture and retain neuroendocrine features but do not grow as colonies in soft agar or form tumors when injected into immunocompromised mice. However, overexpression of either L-Myc, N-Myc or c-Myc conferred the ability to grow in soft agar and form tumors that exhibit the histological features and express markers of human SCLC. This model can now be applied to assess oncogenic and tumor suppressive activity of many additional SCLC genes and to hone in on molecular consequences of oncogene activation.

**[0145]** The effects of inactivating L-Myc in highly penetrant mouse models of SCLC were striking. L-Myc is amplified in a subset of tumors that arise in mouse models in addition to being amplified in human tumors. The dramatic suppression of tumor development in the genetic model system suggested that inhibitory effects of L-Myc loss were not simply owing to an inability to amplify the L-Myc locus. Instead, basal levels of L-Myc are important for SCLC development. Thus, L-Myc-directed therapy is more widely relevant to SCLC beyond the subset of patients that exhibit L-Myc amplification.

**[0146]** In addition to particular sequences provided, nucleotide and protein sequences are available in publicly available databases and publications.

**[0147]** As will be understood by one of ordinary skill in the art, each embodiment disclosed herein can comprise, consist essentially of or consist of its particular stated element, step, ingredient or component. Thus, the terms “include” or “including” should be interpreted to recite: “comprise, consist of, or consist essentially of.” The transition term “comprise” or “comprises”

means includes, but is not limited to, and allows for the inclusion of unspecified elements, steps, ingredients, or components, even in major amounts. The transitional phrase “consisting of” excludes any element, step, ingredient or component not specified. The transition phrase “consisting essentially of” limits the scope of the embodiment to the specified elements, steps, ingredients or components and to those that do not materially affect the embodiment. A material effect would cause a statistically-significant reduction in suppression of SCLC in an animal model using phosphohistone H3 staining such as that described in relation to FIG. 3B.

**[0148]** Unless otherwise indicated, all numbers expressing quantities of ingredients, properties such as molecular weight, reaction conditions, and so forth used in the specification and claims are to be understood as being modified in all instances by the term “about.” Accordingly, unless indicated to the contrary, the numerical parameters set forth in the specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained by the present invention. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques. When further clarity is required, the term “about” has the meaning reasonably ascribed to it by a person skilled in the art when used in conjunction with a stated numerical value or range, i.e. denoting somewhat more or somewhat less than the stated value or range, to within a range of  $\pm 20\%$  of the stated value;  $\pm 19\%$  of the stated value;  $\pm 18\%$  of the stated value;  $\pm 17\%$  of the stated value;  $\pm 16\%$  of the stated value;  $\pm 15\%$  of the stated value;  $\pm 14\%$  of the stated value;  $\pm 13\%$  of the stated value;  $\pm 12\%$  of the stated value;  $\pm 11\%$  of the stated value;  $\pm 10\%$  of the stated value;  $\pm 9\%$  of the stated value;  $\pm 8\%$  of the stated value;  $\pm 7\%$  of the stated value;  $\pm 6\%$  of the stated value;  $\pm 5\%$  of the stated value;  $\pm 4\%$  of the stated value;  $\pm 3\%$  of the stated value;  $\pm 2\%$  of the stated value; or  $\pm 1\%$  of the stated value.

**[0149]** Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contains certain errors necessarily resulting from the standard deviation found in their respective testing measurements.

**[0150]** The terms “a,” “an,” “the” and similar referents used in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. 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. 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 herein is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention otherwise claimed. No language in the specification should be construed as indicating any non-claimed element essential to the practice of the invention.

**[0151]** Groupings of alternative elements or embodiments of the invention disclosed herein are not to be construed as limitations. Each group member may be referred to and claimed individually or in any combination with other members of the group or other elements found herein. It is anticipated that one or more members of a group may be included in, or deleted from, a group for reasons of convenience and/or patentability. When any such inclusion or deletion occurs, the specification is deemed to contain the group as modified thus fulfilling the written description of all Markush groups used in the appended claims.

**[0152]** Certain embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Of course, variations on these described embodiments will become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventor expects skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

**[0153]** Furthermore, numerous references have been made to patents, printed publications, journal articles and other written text throughout this specification (referenced materials herein). Each of the referenced materials are individually incorporated herein by reference in their entirety for their referenced teaching.

**[0154]** In closing, it is to be understood that the embodiments of the invention disclosed herein are illustrative of the principles of the present invention. Other modifications that may be employed are within the scope of the invention. Thus, by way of example, but not of limitation, alternative configurations of the present invention may be utilized in accordance with the teachings herein. Accordingly, the present invention is not limited to that precisely as shown and described.

**[0155]** The particulars shown herein are by way of example and for purposes of illustrative discussion of the preferred embodiments of the present invention only and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of various embodiments of the invention. In this regard, no attempt is made to show structural details of the invention in more detail than is necessary for the fundamental understanding of the invention, the description taken with the drawings and/or examples making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

**[0156]** Definitions and explanations used in the present disclosure are meant and intended to be controlling in any future construction unless clearly and unambiguously modified in the following examples or when application of the meaning renders any construction meaningless or essentially meaningless. In cases where the construction of the term would render it meaningless or essentially meaningless, the definition should be taken from Webster's Dictionary, 3<sup>rd</sup> Edition or a dictionary known to those of ordinary skill in the art, such as the Oxford Dictionary of Biochemistry and Molecular Biology (Ed. Anthony Smith, Oxford University Press, Oxford, 2004).

## CLAIMS

What is claimed is:

1. A method of suppressing small cell lung cancer (SCLC) in a subject in need thereof comprising administering a therapeutically effective amount of an L-Myc inactivator to the subject, thereby suppressing SCLC in the subject wherein the L-Myc inactivator comprises CX-5461, Actinomycin D, or guide RNA comprising SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14 and/or SEQ ID NO: 15.
2. A method of claim 1 wherein the L-Myc inactivator comprises CX-5461.
3. A method of claim 1 wherein the L-Myc inactivator comprises Actinomycin D.
4. A method of claim 1 wherein the L-Myc inactivator comprises guide RNA comprising SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14 and/or SEQ ID NO: 15.
5. A method of treating small cell lung cancer (SCLC) in a subject in need thereof comprising administering a therapeutically effective amount of a composition comprising a compound that down-regulates a primary pathway member of the L-Myc signaling pathway, thereby treating the SCLC in the subject.
6. A method of claim 5, wherein the primary pathway member is L-Myc, MAX, or RNA Polymerase I (RNA Pol I).
7. A method of claim 5, wherein the compound comprises a nucleic acid sequence that down-regulates expression of the primary pathway member.
8. A method of claim 5, wherein the compound comprises a nucleic acid sequence that down-regulates function of the primary pathway member.
9. A method of claim 5, wherein the compound comprises a vector or guide RNA.
10. A method of claim 9, wherein the guide RNA comprises SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14 and/or SEQ ID NO: 15.
11. A method of claim 5, wherein the compound comprises a RNA Pol I inhibitor.
12. A method of claim 11, wherein the RNA Pol I inhibitor is CX-5461 or Actinomycin D.
13. A method for determining whether therapy that down-regulates a primary pathway member of the L-Myc signaling pathway is appropriate for a subject diagnosed with SCLC, wherein the method comprises obtaining a biological sample from the subject; testing the biological sample for the presence of L-Myc amplification or L-Myc signaling; and determining that therapy with a primary L-Myc pathway inhibitor is appropriate for the subject if the L-Myc amplification or L-Myc signaling is present.

14. The method of claim 13, wherein the evidence of L-Myc amplification or L-Myc signaling comprises increased L-Myc, increased RNA Pol I, and/or increased rRNA synthesis.

15. The method of claim 13, wherein the biological sample is a tissue biopsy sample, a tumor biopsy sample, or a bronchoalveolar lavage sample.

16. A method for determining whether a subject should be enrolled in a clinical trial aimed at examining the efficacy of a therapeutic treatment against a cancer comprising obtaining a biological sample from the subject; testing the biological sample for the presence of a testing the biological sample for the presence of L-Myc amplification or L-Myc signaling; and determining that enrollment in the clinical trial is appropriate for the subject if the L-Myc amplification or L-Myc signaling is present.

17. The method of claim 16, wherein the evidence of L-Myc amplification or L-Myc signaling comprises increased L-Myc, increased RNA Pol I, and/or increased rRNA synthesis.

18. The method of claim 16, wherein the biological sample is a tissue biopsy sample, a tumor biopsy sample, or a bronchoalveolar lavage sample.

FIG. 1A

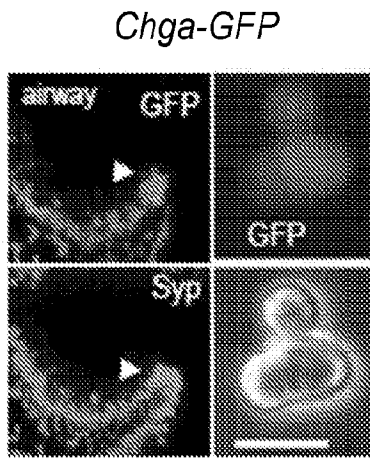


FIG. 1B

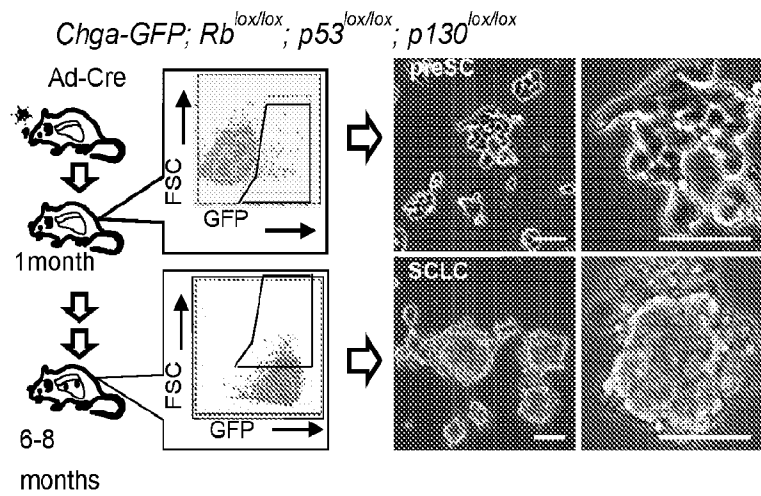


FIG. 1C

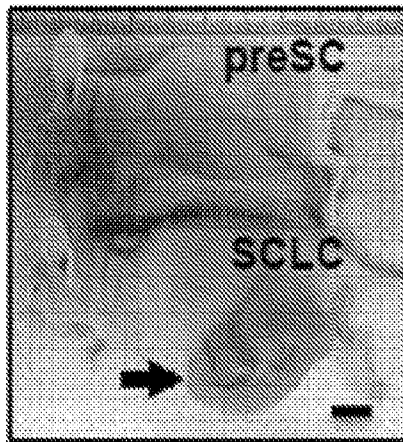
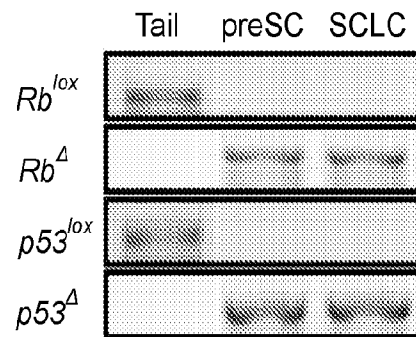


FIG. 1D



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FIG. 1E

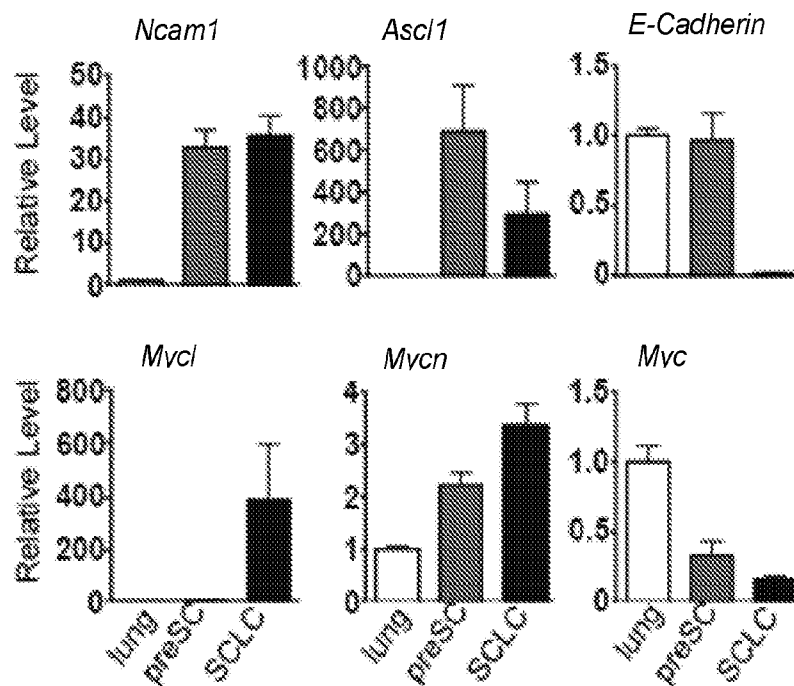
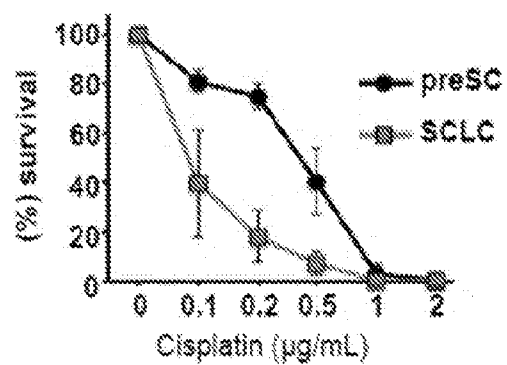


FIG. 1F



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FIG. 1G

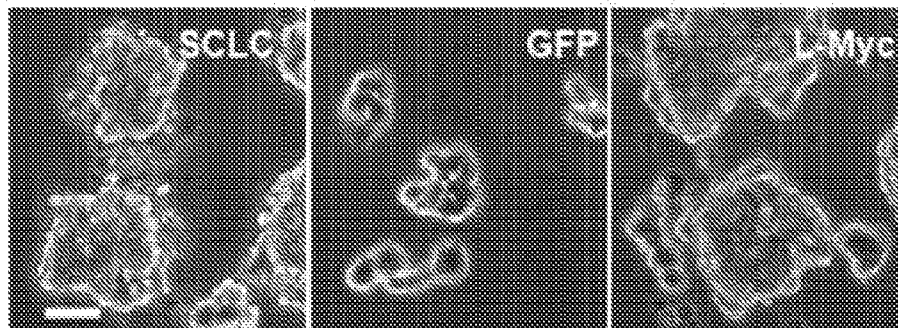


FIG. 1H

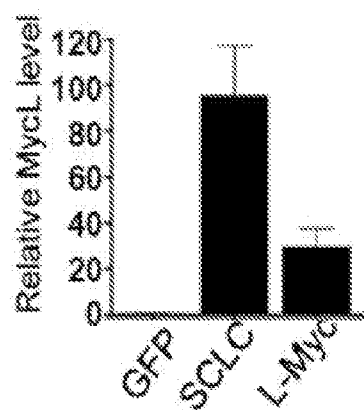
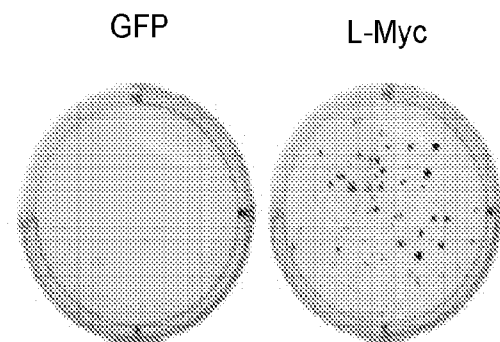


FIG. 1I



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FIG. 1J

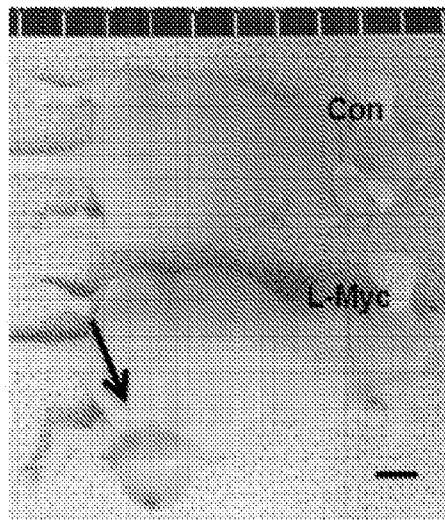
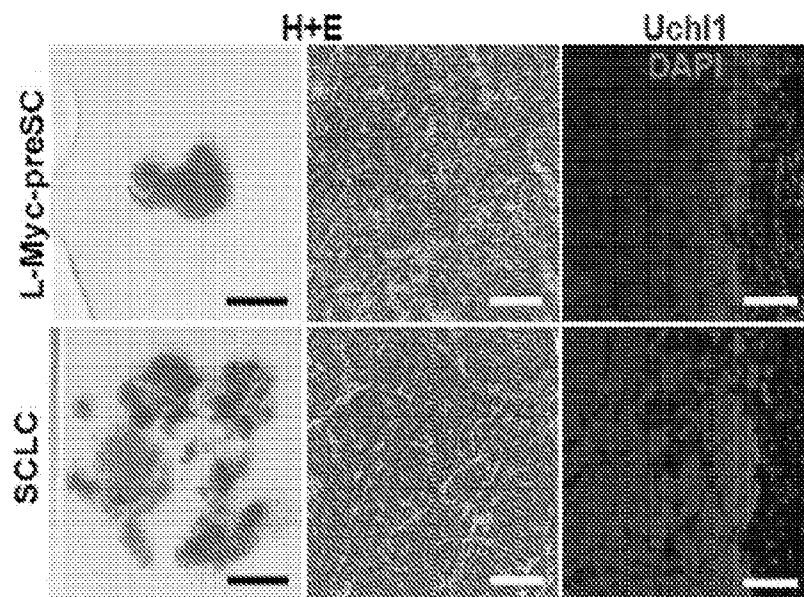


FIG. 1K



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FIG. 2A

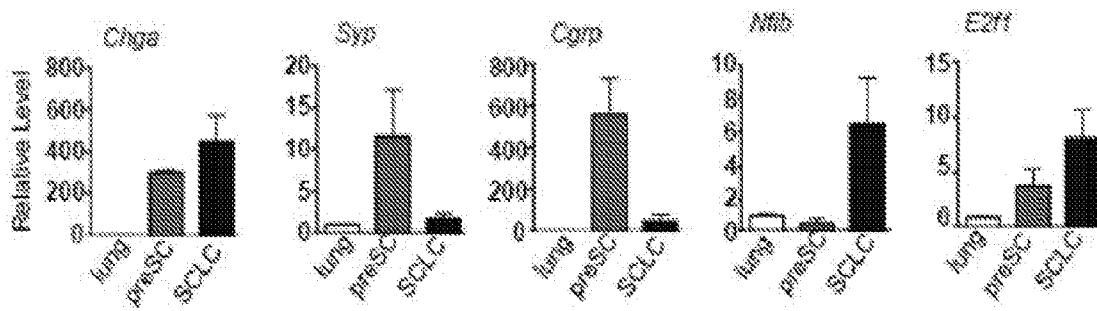


FIG. 2B

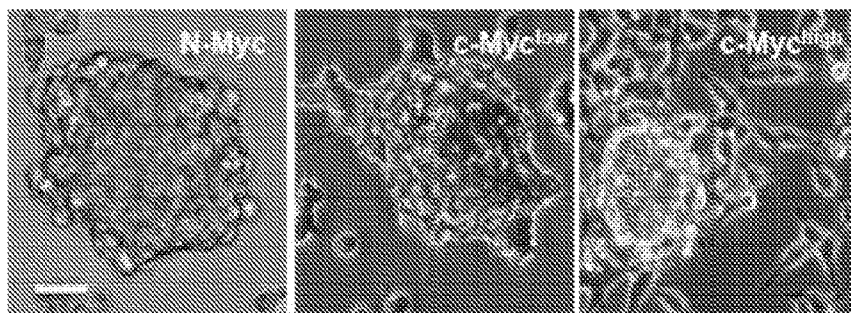
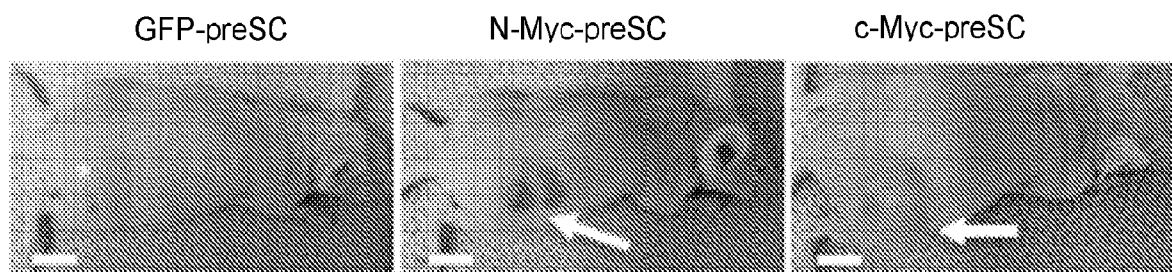


FIG. 2C



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FIG. 2D

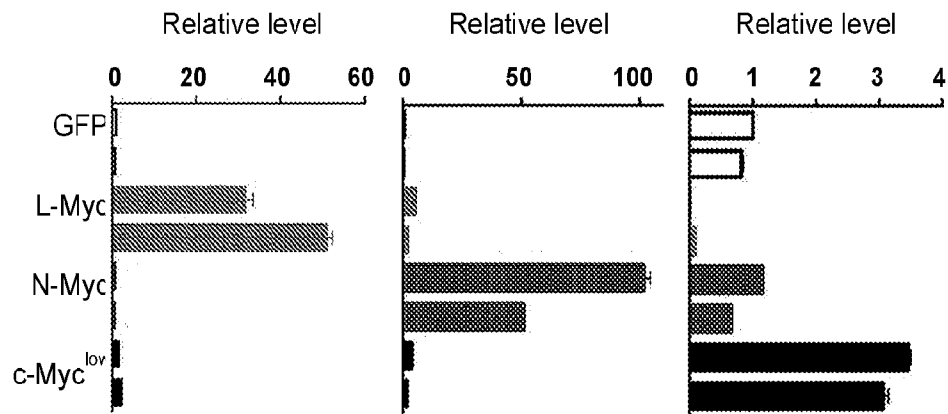
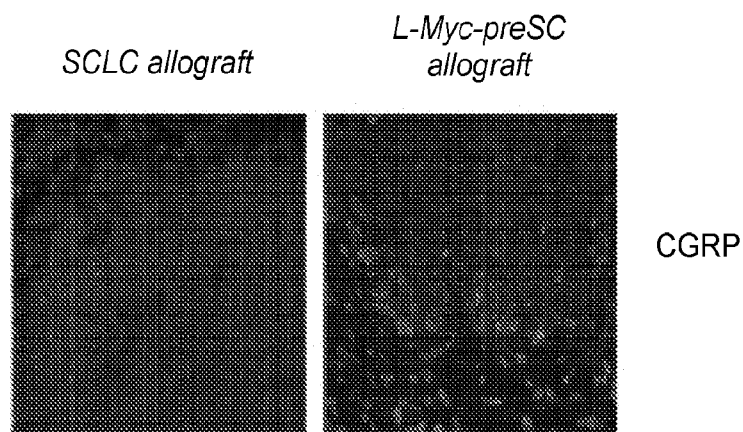


FIG. 2E



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FIG. 3A

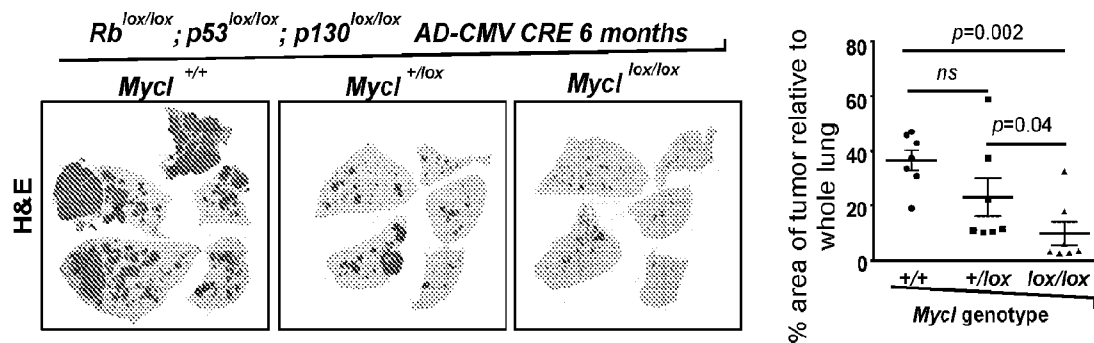


FIG. 3B

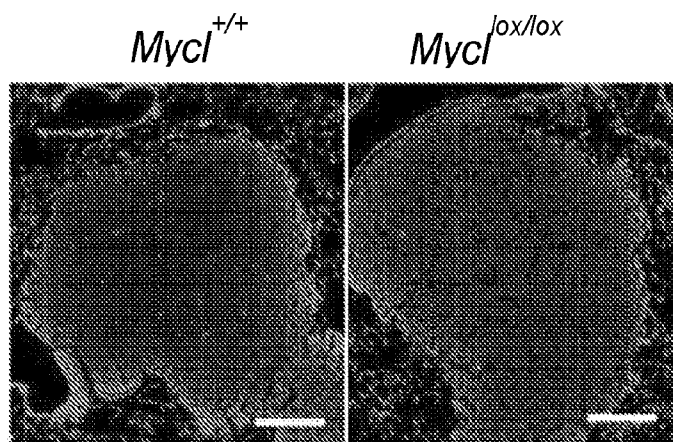
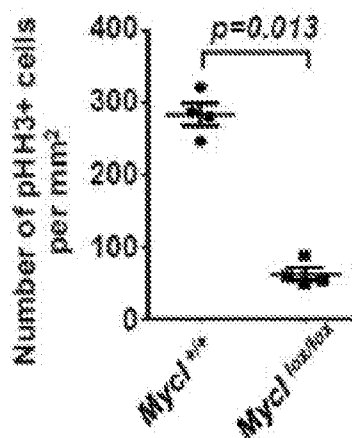


FIG. 3C



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FIG. 3D

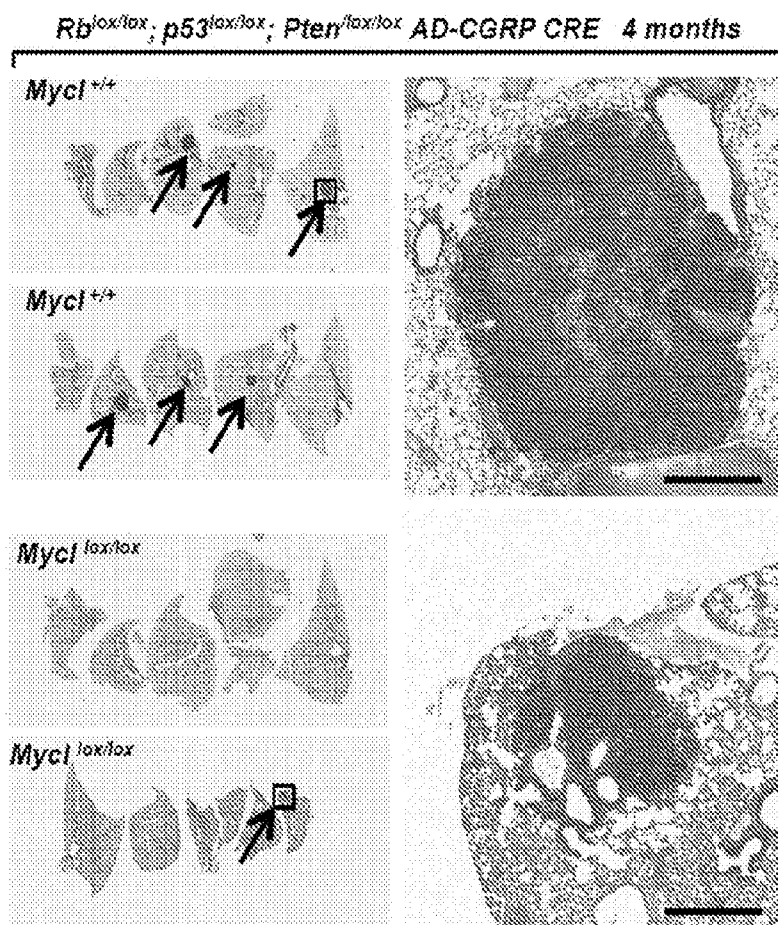
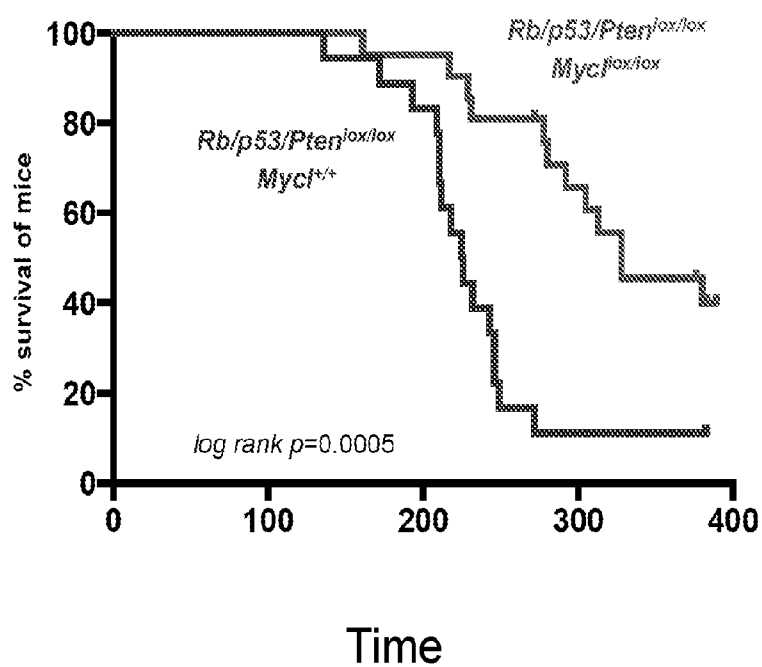


FIG. 3E



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FIG. 3F

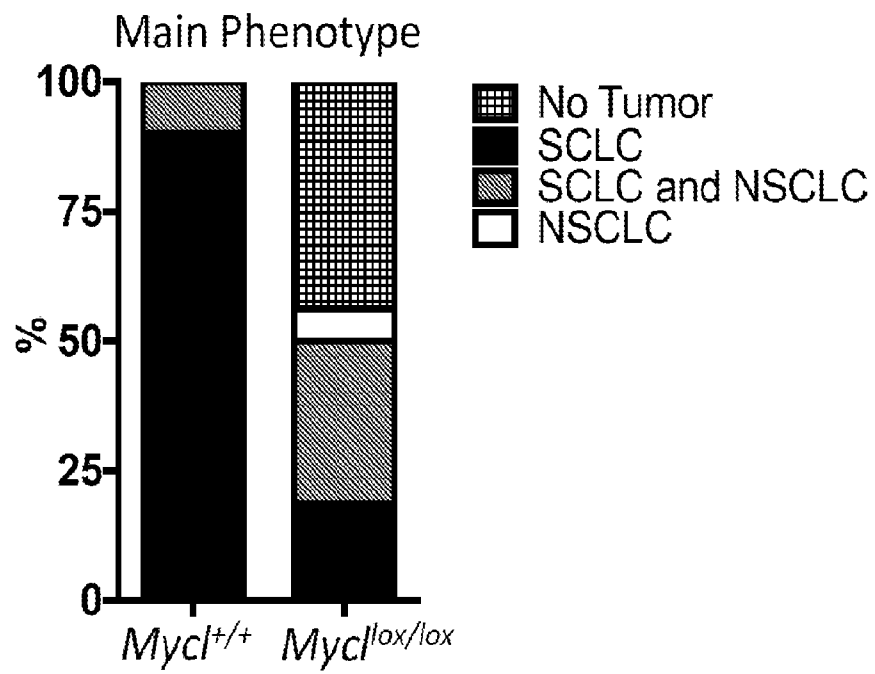
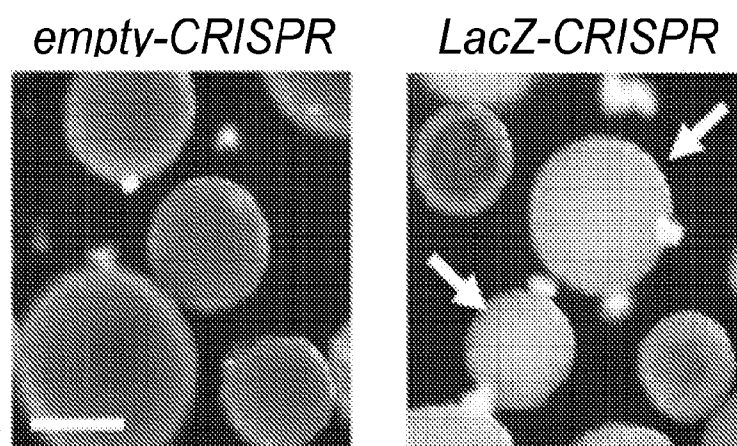


FIG. 3G



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FIG. 3H

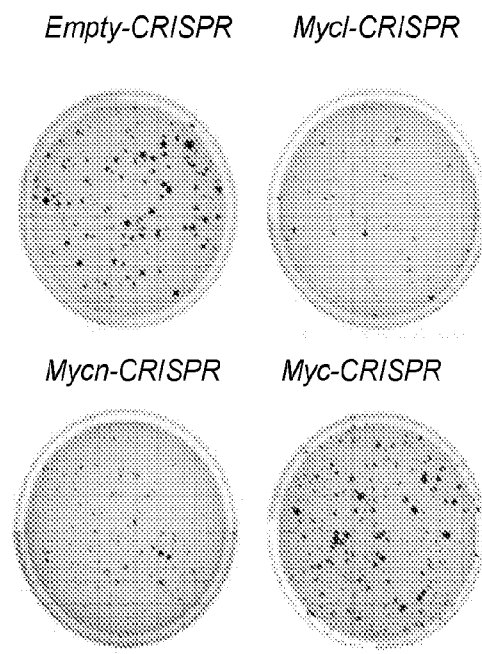
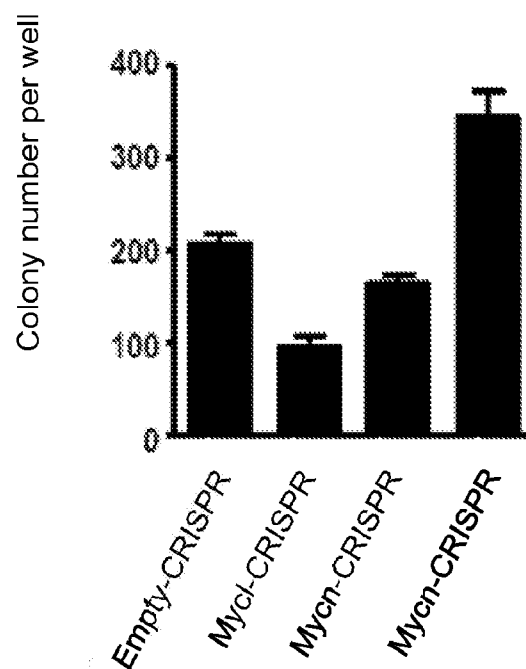
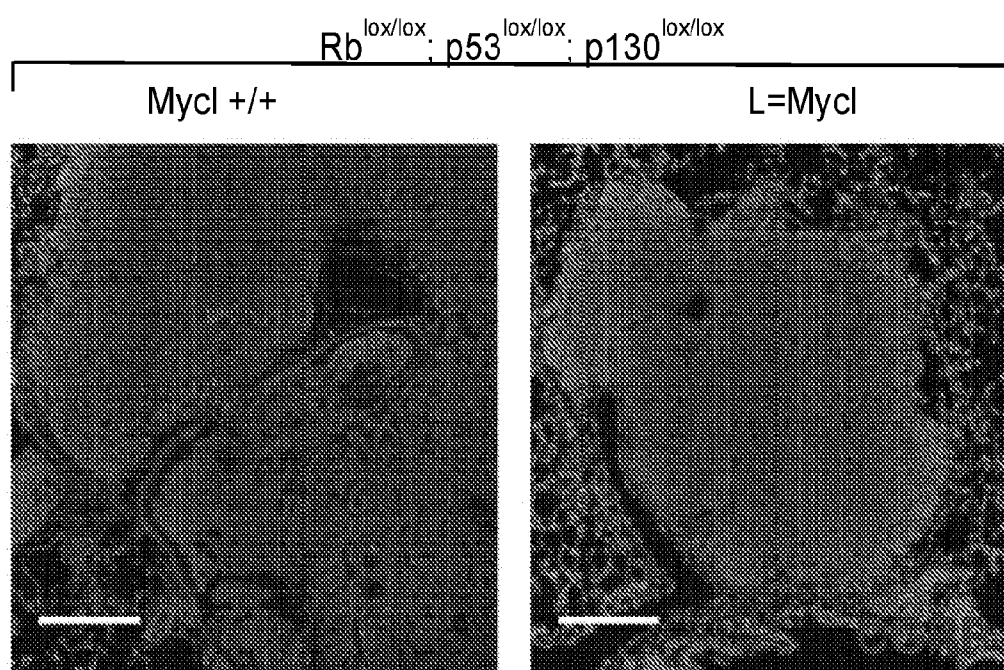


FIG. 3I



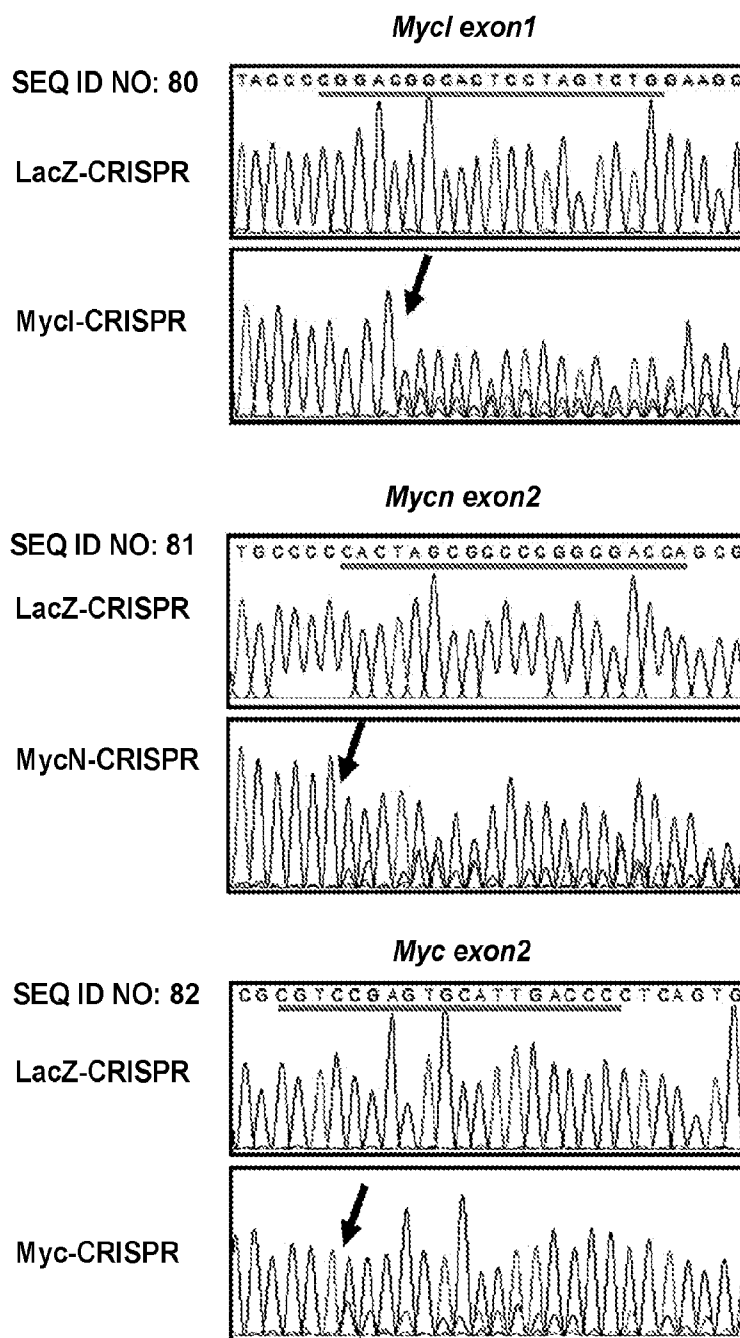
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FIG. 4A



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FIG. 4B

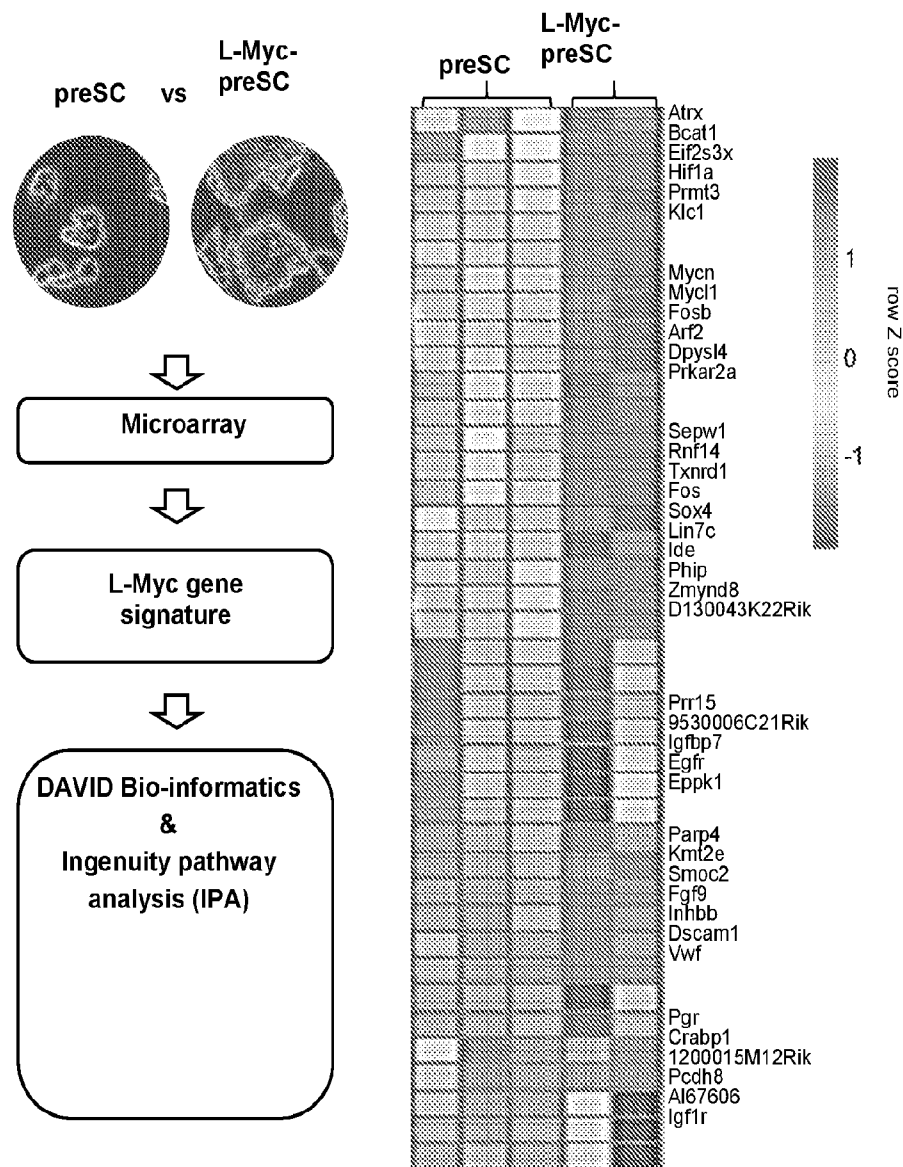


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Genomic regions targeted by guide RNA

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FIG. 5A



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FIG. 5B

**DAVID: KEGG pathways**

1. Ribosome ( $p=1.2 \times 10^{-9}$ )
2. Oocyte meiosis ( $p=4.6 \times 10^{-5}$ )
3. Regulation of actin cytoskeleton ( $p=8.8 \times 10^{-5}$ )
4. Adherens junction ( $p=1.4 \times 10^{-4}$ )

**IPA: Top molecular pathways**

1. eIF2 signaling ( $p=2.8 \times 10^{-5}$ )
2. Huntington's Disease ( $p=2.5 \times 10^{-4}$ )
3. Integrin signaling ( $p=2.5 \times 10^{-4}$ )
4. ERK5 signaling ( $p=4.2 \times 10^{-4}$ )
5. Hypoxia signaling in the cardiovascular system ( $p=5.2 \times 10^{-4}$ )
6. mTOR signaling ( $p=5.5 \times 10^{-4}$ )
7. PI3K/AKT signaling ( $p=6.2 \times 10^{-4}$ )
8. Regulation of eIF4 and p70S6K signaling ( $p=6.6 \times 10^{-4}$ )
9. Molecular mechanism of cancer ( $p=1.5 \times 10^{-4}$ )
10. Remodeling of adherent junctions ( $p=1.6 \times 10^{-4}$ )

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FIG. 5C

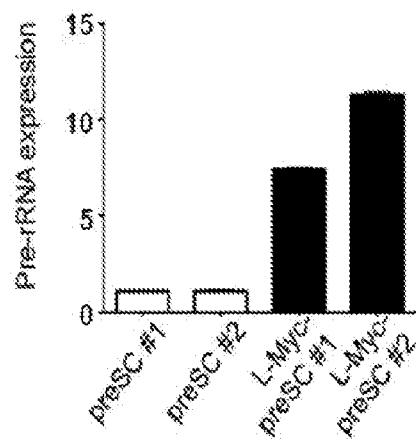
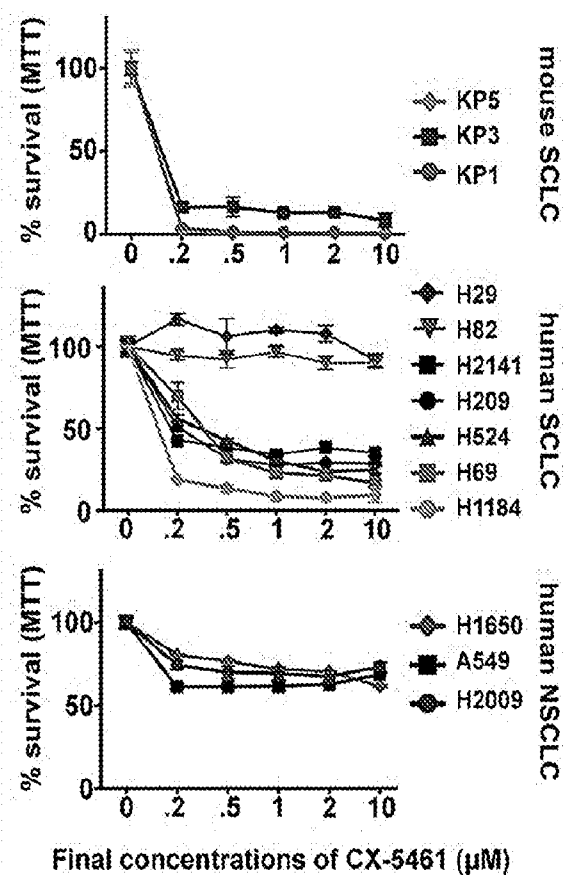


FIG. 5D



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FIG. 5E

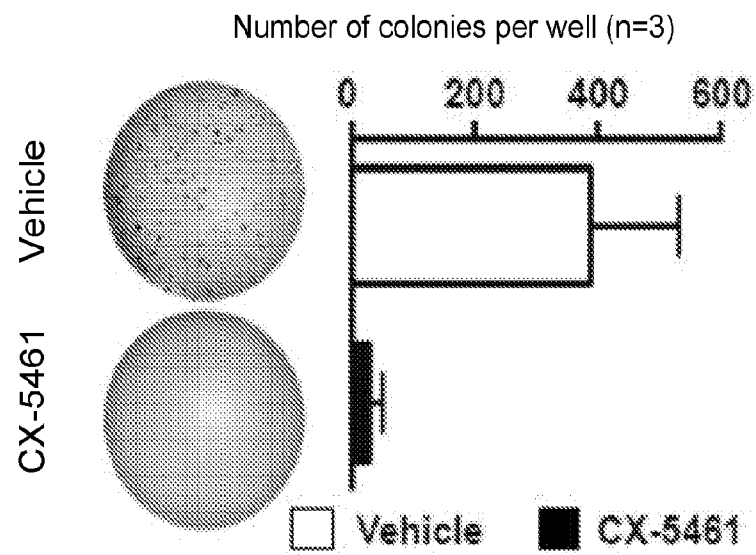
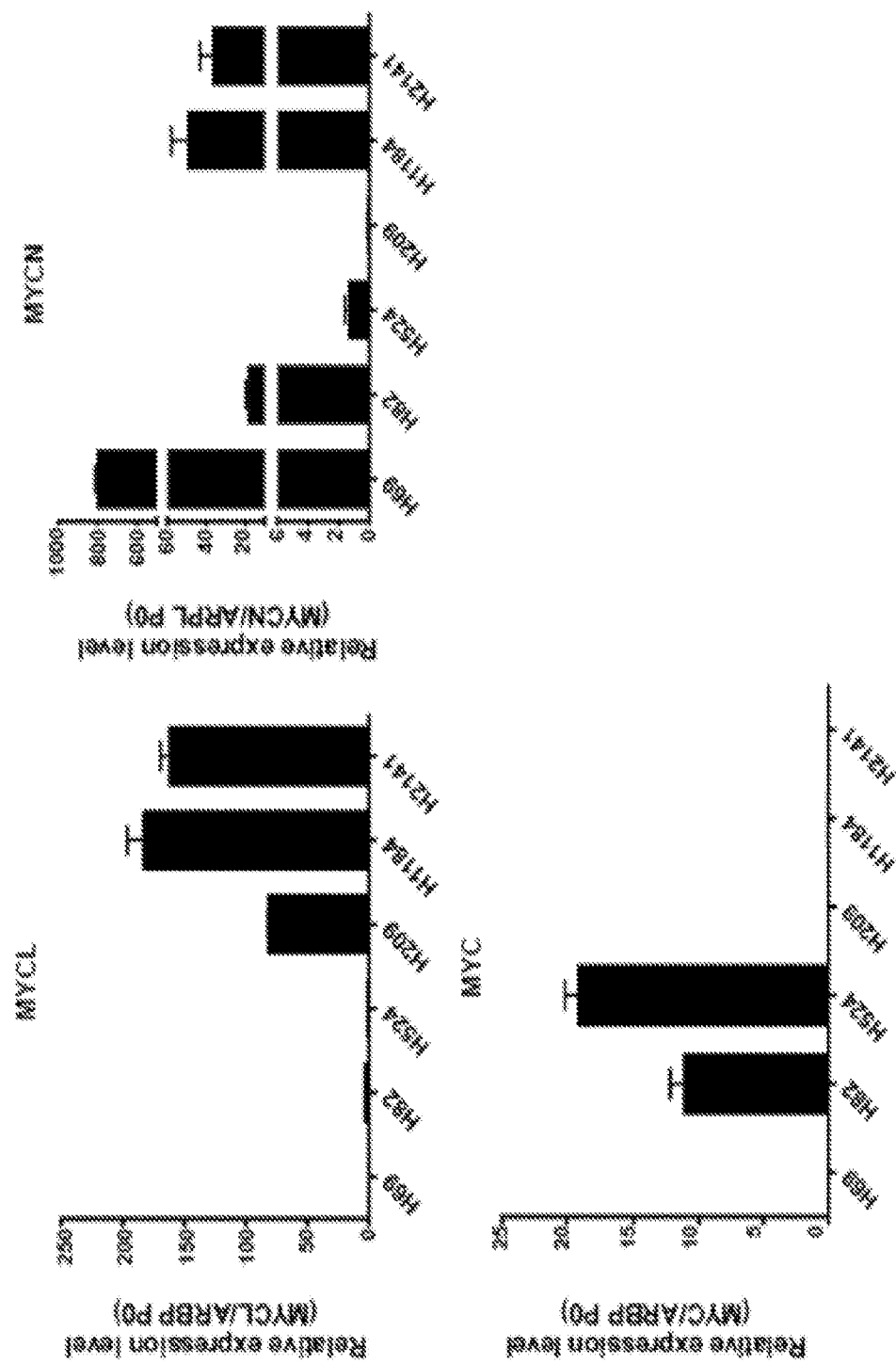


FIG. 6



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FIG. 7A

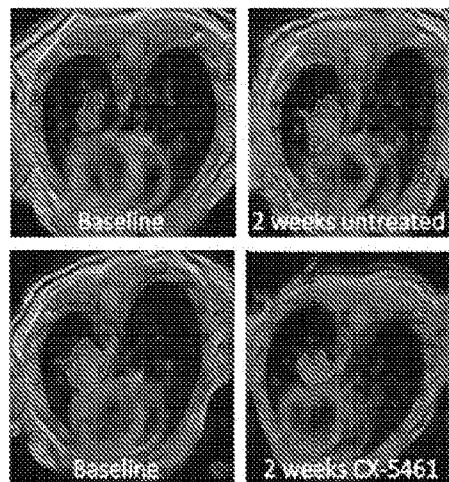
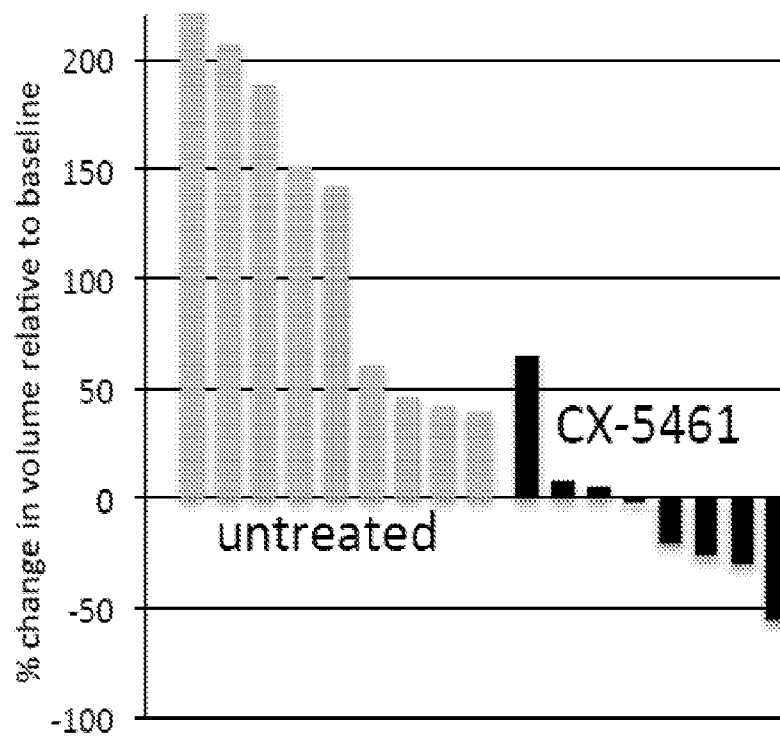


FIG. 7B



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FIG. 7C

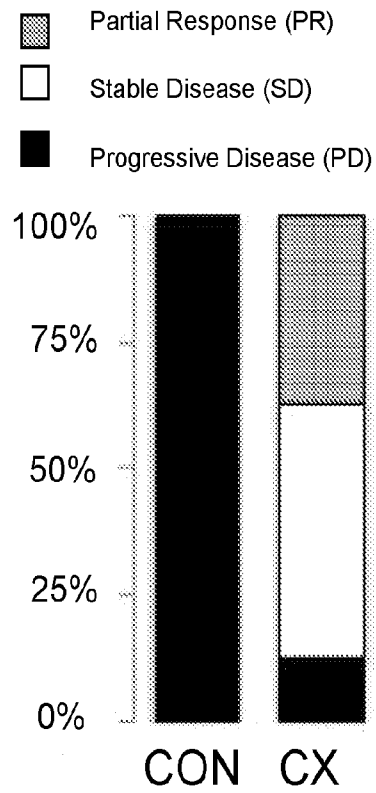
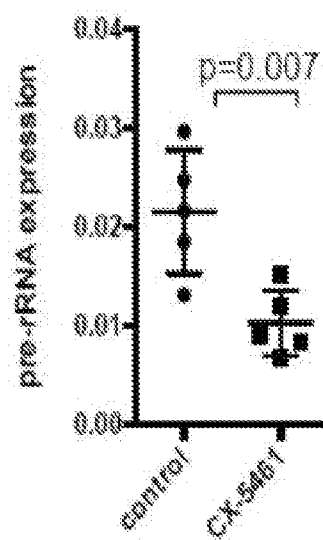


FIG. 7D



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FIG. 7E

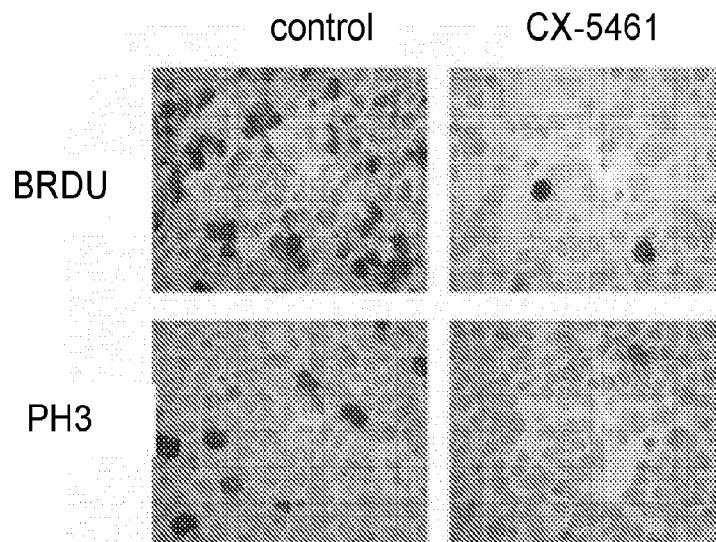
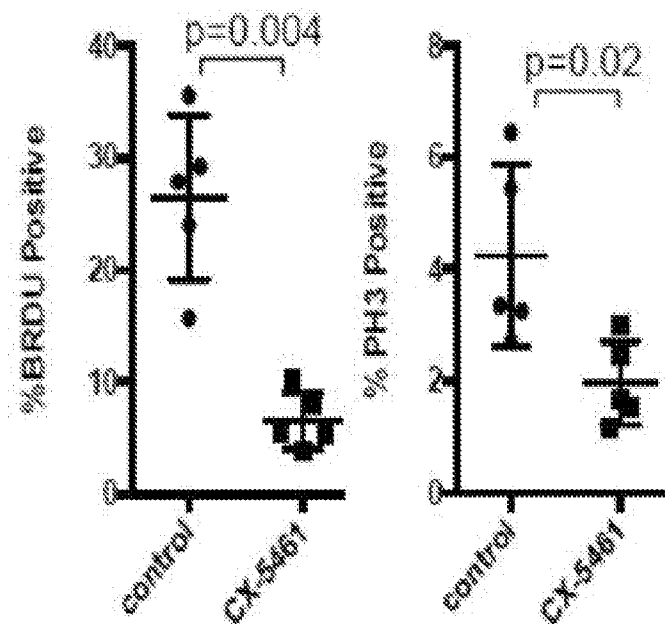


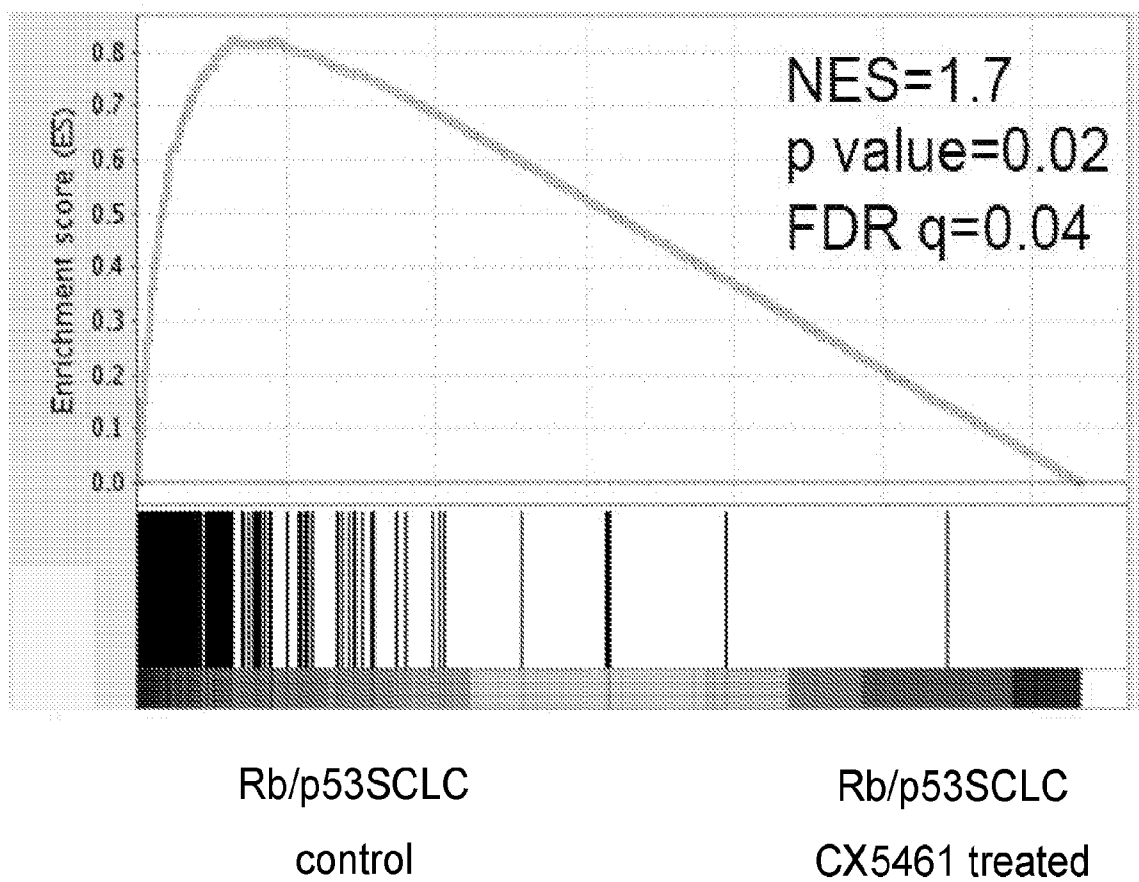
FIG. 7F



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FIG. 7G

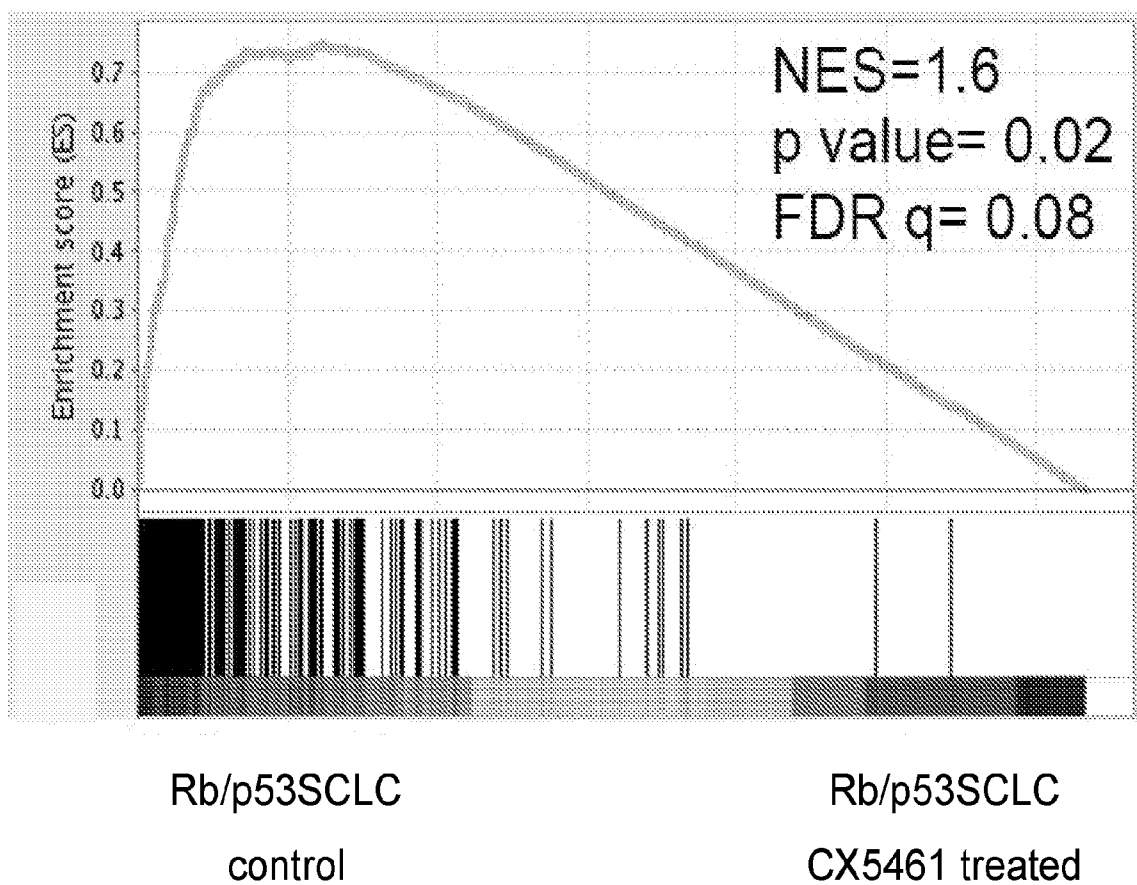
## Hallmark E2F targets



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FIG. 7G cont'd

## Hallmark MYC targets



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## FIG. 8

UniProt ID P12524 – Canonical L-Myc Protein Sequence (SEQ ID NO: 1)

MDYDSYQHYFYDYDCGEDFYRSTAPSEDIWKKFELVPSPTSPPWGLGPGAGDPAPGIGPPE  
 PWPGGCTGDEAESRGHSGWGRNYASIIRDCMWSGFSARERLERAVSDRLAPGAPRGNP  
 PKASAAPDCTPSLEAGNPAPAAPCPLGEPKTQACSGSESPSDSENEEIDVVTVEKRQSLGIRK  
 PVTITVRADPLDPCMKEFHISIHQQQHNYAARFPPESCSQEEASERGPQEEVLERDAAGEKED  
 EEDDEIVSPPPVESEAAQSCHPKPVSSDTEVDTKRKNHNFLEKRRNDLRSRFLALRDQVPTL  
 ASCSKAPKVVILSKALEYLQALVGAEKRMATEKRQLRCRQQQLQKRIAYLTGY

UniProt ID P61244 – Canonical Max Protein Sequence (SEQ ID NO: 2)

MSDNDIEVESDEEQPRFQSAADKRAHHNALERKRRDHIKDSFHSLRDSVPSLQGEKASRAQI  
 LDKATEYIQYMRRKNHHTHQDIDDLKRQNALLEQQVRALEKARSSAQLQTNYPSSDNSLYTNA  
 KGSTISAFDGGSDSSSESEPEEPQSRKKLRMEAS

UniProt ID P61244 – Exemplary RNA Pol I (SEQ ID NO: 3)

VISPPTVPKGITCKEANVYPAECRGRSTYRGKLTADINWAVNGISKGIKQLGYVPIMVSKSL  
 CNLRNLPPQALIEHHEEAEEMGGYFIINGIEKVIRMLIMPRNFPIAMIRPKWKTRGPGYTQYGV  
 SMHCVREEHSVNMNLHYLENGTVMLNFIYRKELFFLPLGFALKALVSFSDYQIFQELIKGED  
 DSFLRNSVSQMLRIVMEEGCSTQKQVLNVLGECFRVKLNVPDWYPNEQAAEFLNQICICHLK  
 SNTEKFYMLCLMTRKLFALAKGECMEDNPDLSVNQEVLTGQQLFLMFLKEKLEGWLVSIKIAFD  
 KKAQKTSVSMNTDNLMRIFTMGIDLTKEFEYLFATGNLRSKTGLGLLQDSGLCVVADKLNFIY  
 LSHFRCVHRGADFAKMRTTTRRLLPESWGFLCPVHTPDGEPGLMNHILTAVCEVVTQFVYT  
 ASIPALLCNLGVTPIDGAPHSYSECYPVLLDGVMVGWVDKDLAPGIADSLRHFKVLREKRIPP  
 WMEVVLIPMTGKPSLYPGLFLFTTPCRLVRPVQNLALGKEELIGTMEQIFMNVAIFEDEVFAGV  
 TTHQELFPHSLLSVIANFIPFSDHNQSPRMYQCQMGKQTMGFPLTYQDRSDNKLYRLQTP  
 QSPLVRPSMYDYDMDNYPITGNAIVAVISYTYGDMEDAMIVNKASWERGFAHGSVYKSEFID  
 LSEKIKQGDSSLVFGIKPGDPRVLQKLDDGLPFIGAKLQYGDYPYSYLNLTGESFVMYYKSK  
 ENCVVDNIKVCSNDTGSGKFKVCITMRVPRNPTIGDKFASRHGQKGILSRLWPAEDMPFTES  
 GMVPDILFNPHGFPSRMTIGMLIESMAGKSAALHGLCHDATPFIFSEENSALEYFGEMLKAAGY  
 NFYGTERLYSGISGLELEADIFIGVYYYQRLRMVSDKFQVRTTGARDRVTNQPIGGRNVQGGI  
 RFGEMERDALLAHGTSFLLHDRLFNCSDRSVAHVVCVKGCSLLSPLEKPPPSWSAMRNRKYN  
 CTLCRSRSDTIDTVSVPYVFRYFVAELAAMNIKVKLDVV

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FIG. 9

| NAME                                       | SIZE | ES         | NES        | NOM p-val  | FDR q-val  |
|--------------------------------------------|------|------------|------------|------------|------------|
| HALLMARK_G2M_CHECKPOINT                    | 191  | 0.75295436 | 1.7345501  | 0.02028398 | 0.06458388 |
| HALLMARK_E2F_TARGETS                       | 196  | 0.8239788  | 1.7120906  | 0.01814516 | 0.04498171 |
| HALLMARK_MITOTIC_SPINDLE                   | 191  | 0.51371175 | 1.6788952  | 0.03680982 | 0.04809924 |
| HALLMARK_MYC_TARGETS_V1                    | 192  | 0.7470766  | 1.6084868  | 0.01844262 | 0.07719691 |
| HALLMARK_DNA_REPAIR                        | 137  | 0.43410477 | 1.5908623  | 0.03212851 | 0.07474566 |
| HALLMARK_MYC_TARGETS_V2                    | 56   | 0.62244654 | 1.4189998  | 0.16701461 | 0.24148485 |
| HALLMARK_MTORC1_SIGNALING                  | 199  | 0.469441   | 1.3991096  | 0.09504132 | 0.2294148  |
| HALLMARK_SPERMATOGENESIS                   | 130  | 0.31924763 | 1.1809721  | 0.26824033 | 0.59627056 |
| HALLMARK_UNFOLDED_PROTEIN_RESPONSE         | 112  | 0.40292907 | 1.1562845  | 0.30638298 | 0.58249223 |
| HALLMARK_PI3K_AKT_MTOR_SIGNALING           | 103  | 0.27323437 | 1.1484215  | 0.25585938 | 0.53376454 |
| HALLMARK_GLYCOLYSIS                        | 198  | 0.28366604 | 1.0550164  | 0.37065637 | 0.68828887 |
| HALLMARK_UV_RESPONSE_UP                    | 151  | 0.24186955 | 0.93960637 | 0.5693277  | 0.89656764 |
| HALLMARK_HYPOXIA                           | 196  | 0.2556874  | 0.9174258  | 0.5426195  | 0.8664225  |
| HALLMARK_TGF_BETA_SIGNALING                | 53   | 0.24196015 | 0.90788513 | 0.61770624 | 0.8241478  |
| HALLMARK_WNT_BETA_CATENIN_SIGNALING        | 42   | 0.22318794 | 0.86914635 | 0.7672065  | 0.83752465 |
| HALLMARK_OXIDATIVE_PHOSPHORYLATION         | 191  | 0.26772067 | 0.83272827 | 0.5920792  | 0.8520848  |
| HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION | 187  | 0.25880918 | 0.7812851  | 0.7346939  | 0.8874281  |
| HALLMARK_PROTEIN_SECRETION                 | 92   | 0.17669989 | 0.6643951  | 0.86346155 | 0.99124885 |
| HALLMARK_ANGIOGENESIS                      | 36   | 0.22917311 | 0.62576365 | 0.9087049  | 0.9678163  |
| HALLMARK_APICAL_JUNCTION                   | 196  | 0.13309638 | 0.5565184  | 0.9979798  | 0.952394   |

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/34838

**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 filed:

☒ in the form of an Annex C/ST.25 text file.

☐ on paper or in the form of an image file.

b. ☐ furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

☐ in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).

☐ on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/34838

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

Groups I+, Claims 1-12, directed to a method of suppressing small cell lung cancer (SCLC) in a subject in need thereof, comprising administering a therapeutically effective amount of an L-Myc inactivator to the subject; or a method of treating small cell lung cancer (SCLC) in a subject in need thereof comprising administering a therapeutically effective amount of a composition comprising a compound that down-regulates a primary pathway member of the L-Myc signaling pathway. The L-Myc inactivator or the compound will be searched to the extent that the L-Myc inactivator or the compound encompasses the first named CX-5461. It is believed that claims 1(in part), 2, 5-6, 11, 12(in part) encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass wherein the L-Myc inactivator or the compound encompasses the first named CX-5461. Additional L-Myc inactivator or compound will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected L-Myc inactivator or compound. \*\*\*\*\*Continued in the extra sheet\*\*\*\*\*

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  
1(in part), 2, 5-6, 11, 12(in part), limited to CX-5461

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/34838

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 48/00; A61K 31/55; A61K 31/44; A61K 31/335; C12Q 1/68; G01N 33/574 (2017.01)  
 CPC - A61K 48/00; C12N 15/113; C07D 401/14; A61K 31/55; C07D 471/14; C07D 513/14; A61K 31/335; C12Q 1/68; C12Q 1/686; C12Q 1/6886

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<br>---<br>Y | US 2015/0272960 A1 (HEALTH RESEARCH, INC. et al.) 01 October 2015 (01.10.2015), Abstract, para [0002], [0024], [0025], [0159], and [0160]                                                                                                                                                                  | 5-6<br>-----<br>1-2, 11-12 |
| Y             | POORTINGA et al., Targeting RNA polymerase I to treat MYC-driven cancer. Oncogene. 2015, Vol. 34(4), p. 403-12. Title; Abstract; pg 403, col 1, para 1 and last para, and col 2, top para; pg 406, col 2, lower para, and Fig 2; pg 407, col 2, para 3, para 4, and last para; and pg 408, col 1, top para | 1-2, 11-12                 |
| A             | IKEGAKI et al., The human L-myc gene is expressed as two forms of protein in small cell lung-carcinoma cell lines: detection by monoclonal antibodies specific to two myc homology box sequences. EMBO J. 1989, Vol. 8(6), p. 1793-9. Abstract                                                             | 1-2, 5-6, 11-12            |
| A             | US 2011/0166200 A1 (ZHANG et al.) 07 July 2011 (07.07.2011), para [0003], [0008], [0028], [0086], [0102], [0103], [0104], [0114], [0121], [0123], [0204], and [0206]                                                                                                                                       | 1-2, 5-6, 11-12            |
| A             | Tansey, Mammalian MYC Proteins and Cancer. New J Sci. 2014, Vol.2014 (2014), Article ID 757534. PDF File: pg 1-28. Entire documentation, especially pg 3, Fig 2                                                                                                                                            | 1-2, 5-6, 11-12            |
| A             | US 2015/0353905 A1 (EMORY UNIVERSITY) 10 December 2015 (10.12.2015), para [0170]                                                                                                                                                                                                                           | 1-2, 5-6, 11-12            |
| A             | DEPINHO et al., The human myc gene family: structure and activity of L-myc and an L-myc pseudogene. Genes Dev. 1987, Vol. 1(10), p. 1311-26. Entire documentation, especially Abstract; and pg 1313-1315, Fig 1                                                                                            | 1-2, 5-6, 11-12            |
| A             | HOOKE et al., Of Myc and Mnt. J Cell Sci. 2006, Vol. 119(Pt 2), p. 208-16. Entire documentation, especially Abstract; pg 209, Table 1, and Fig 1; and pg 211, col 1, top para                                                                                                                              | 1-2, 5-6, 11-12            |

☒ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

\* Special categories of cited documents:

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

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

"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

"&amp;" document member of the same patent family

Date of the actual completion of the international search

24 July 2017

Date of mailing of the international search report

04 OCT 2017

Name and mailing address of the ISA/US

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Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300  
 PCT OSP: 571-272-7774

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/34838

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                       | Relevant to claim No. |
|-----------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| A         | US 2014/0086839 A1 (ACHIRON et al.) 27 March 2014 (27.03.2014), Abstract, para [0012], [0013], [0103], and [0130]                                                                                                                                                                                                        | 1-2, 5-6, 11-12       |
| P,X       | KIM et al., Genetic requirement for Mycl and efficacy of RNA Pol I inhibition in mouse models of small cell lung cancer. Genes Dev. 2016 Jun 1, Vol. 30(11), p. 1289-99. Entire documentation, especially Abstract; pg 1291, col 2, last para; pg 1292, col 2, last para; and pg 1295, col 1, middle para, and last para | 1-2, 5-6, 11-12       |

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/34838

Continuation of:  
Box No III (unity of invention is lacking)

(Continuation of Groups I+) Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be: 1) wherein the L-Myc inactivator or the compound is Actinomycin D [claims: 1(in part), 3, (5-6), (11), and 12(in part)]; or 2) wherein the L-Myc inactivator or the compound is a guide RNA comprising SEQ ID NO: 10 [claims 1(in part), 4(in part), (5-6), 7-9, 10(in part)].

Group II, claims 13-18, directed to a method for determining whether therapy that down-regulates a primary pathway member of the L-Myc signaling pathway is appropriate for a subject diagnosed with SCLC; or a method for determining whether a subject should be enrolled in a clinical trial aimed at examining the efficacy of a therapeutic treatment against a cancer.

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

## Special Technical Feature

Groups I+ include the special technical feature of suppressing or treating small cell lung cancer (SCLC) comprising administering an L-Myc inactivator or a compound that down-regulates a primary pathway member of the L-Myc signaling pathway to the subject, not required by Group II.

Group II includes the special technical feature of obtaining a biological sample from the subject; testing the biological sample for the presence of L-Myc amplification or L-Myc signaling, not required by Groups I+.

Among Groups I+, each of the L-Myc inactivator (listed in claim 1), or each of the compound that down-regulates a primary pathway member of the L-Myc signaling pathway (listed in claims 9-12), is structurally different from all others.

## Common Technical Features

The inventions of Groups I+ and II share the technical feature of association of an L-Myc pathway inhibitor or a compound that down-regulates a primary pathway member of the L-Myc signaling pathway with small cell lung cancer (SCLC) in a subject;  
--the inventions of Groups I+ further share the technical feature of a method of treating/suppressing small cell lung cancer (SCLC) in a subject in need thereof comprising administering a composition comprising a compound that down-regulates a primary pathway member of the L-Myc signaling pathway or a therapeutically effective amount of an L-Myc inactivator to the subject, thereby treating/suppressing SCLC in the subject (claim 5; or part of claim 1); and  
--the inventions of Groups I+ further share the technical feature of wherein the L-Myc inactivator or the compound comprises guide RNA (part of claim 1, or claim 9); or  
-- wherein the compound comprises a RNA Pol I inhibitor (claim 11; also inexpressively in claim 1).

However, these shared technical features do not represent a contribution over prior art as being obvious over US 2015/0272960 A1 to HEALTH RESEARCH, INC. et al. (hereinafter 'HEALTH\_RES'); or being obvious over HEALTH\_RES, in view of US 2015/0353905 A1 to EMORY UNIVERSITY (hereinafter 'EMORY'); or being obvious over HEALTH\_RES, in view of an article entitled 'Targeting RNA polymerase I to treat MYC-driven cancer' by POORTINGA et al. (hereinafter 'Poortinga'; Oncogene. 2015, Vol. 34(4), p. 403-12) as follows:

HEALTH\_RES discloses a method of treating/suppressing small cell lung cancer (SCLC) in a subject in need thereof (Abstract - 'methods for treating cancer including administration of compounds that inhibit oncoprotein Myc'; para [0024] - 'the patient has a cancer selected from ... small cell lung cancer') comprising  
--administering a therapeutically effective amount of an Myc inactivator to the subject (para [0002] - 'treating cancer by administering to an individual in thereof a therapeutically effective amount a compound capable of inhibiting the MYC oncoprotein'),  
-- thereby treating/suppressing SCLC in the subject (Abstract - 'methods for treating cancer including administration of compounds that inhibit oncoprotein Myc'; para [0159] - 'cancer is selected from... small cell lung cancer').

HEALTH\_RES further discloses wherein the Myc inactivator inhibits Myc selected from the group consisting of L-Myc (para [0002] - 'treating cancer by administering to an individual in thereof a therapeutically effective amount a compound capable of inhibiting the MYC oncoprotein'; para [0025] - 'administered compound inhibits the function of a Myc protein selected from the group consisting of N-Myc, c-Myc, and L-Myc'; para [0160] - 'compounds of the method of treating cancer inhibit the function of a Myc protein selected from the group consisting of ... L-Myc').

Although HEALTH\_RES does not expressly teach using L-Myc inactivator for treating/suppressing small cell lung cancer (SCLC), one of the ordinary skill in the art at the time the invention was made would have known to do so, based on the teaching of HEALTH\_RES and the State of the Art, because it was well known in the art long before the invention was made that the elevated L-Myc expression is associated with small cell lung cancer (SCLC), as evident by an article entitled 'The human L-myc gene is expressed as two forms of protein in small cell lung carcinoma cell lines: detection by monoclonal antibodies specific to two myc homology box sequences' by IKEGAKI et al. (EMBO J. 1989, Vol. 8(6), p. 1793-9; Abstract - 'L-myc gene is the third member of the myc family of proto-oncogenes. Amplification and elevated expression of the L-myc gene has been detected in a subset of small cell lung carcinoma (SCLC) cell lines'; Please see also see US 2011/0166200 A1 to ZHANG et al.; para [0104] - 'L-Myc is amplified in small cell lung cancer and lung carcinoma cell lines (Nau et al., Nature 318:69-73, 1985)').

\*\*\*\*\*Continued in the next extra sheet\*\*\*\*\*

Continuation of:

The previous extra sheet - Box No III (unity of invention is lacking)

Furthermore, although HEALTH\_RES does not expressly teach administering 'a composition comprising a compound that down-regulates a primary pathway member of the L-Myc signaling pathway', this limitation is encompassed by the teaching of HEALTH\_RES, because HEALTH\_RES discloses administering an L-Myc inhibitor (para [0160] - 'compounds of the method of treating cancer inhibit the function of a Myc protein selected from the group consisting of ... L-Myc'), which is 'a composition comprising a compound that down-regulates a primary pathway member of the L-Myc signaling pathway' (See Specification: para [0021] - 'Examples of primary pathway members include L-Myc').

Therefore, one of the ordinary skill in the art at the time the invention was made would have known to obtain a method of treating/suppressing small cell lung cancer (SCLC) in a subject in need thereof comprising administering a composition comprising a compound that down-regulates a primary pathway member of the L-Myc signaling pathway or a therapeutically effective amount of an L-Myc inactivator to the subject, thereby treating/suppressing SCLC in the subject, based on the teaching of HEALTH\_RES and the state of the art, in order to use the L-Myc inactivators available in the art for facilitating treating cancer with elevated L-Myc expression with a desired result and without undue experimentation.

Furthermore, EMORY discloses a method for treating cancer comprising administering a guide RNA that target to members associated with Myc in a subject (para [0170] - 'treating cancer comprising administering an effective amount of cloning vector disclosed herein that is configured to express Cas9 and a guided nucleic acid complex that targets mRNA or microRNA associated with transcription factors, e.g., myc, to a subject in need thereof'). Although EMORY does not specifically teach targeting to L-Myc, one of the ordinary skill in the art at the time the invention was made would have known to do so based on the combination of EMORY and HEALTH\_RES, in order to combine the methods, therapeutic agents, and knowledge available in the art for facilitating treating cancers with elevated L-Myc expression with a desired result and without undue experimentation, especially L-Myc sequence was cloned from small cell lung cancer (SCLC) long before the invention was made, as evident by an article entitled 'The human myc gene family: structure and activity of L-myc and an L-myc pseudogene' by DePinho et al. (Genes Dev. 1987, Vol. 1(10), p. 1311-26: Abstract - 'an L-rnyc cDNA clone derived from a human small cell lung carcinoma'; pg 1313-1315, Fig 1, the sequence).

Moreover, Poortinga discloses a method for using RNA polymerase I (Pol I) inhibitor for treating MYC-driven cancer (Title; Abstract; pg 403, col 1, last para to col 2, top para - 'small molecule inhibition of RNA Pol I, the enzyme responsible for transcribing the ribosomal RNA (rRNA) genes (rDNA) in the nucleolus and a major MYC target, selectively kills MYC-driven lymphoma in vivo'; pg 403, col 1, para 1 - 'RNA polymerase II (Pol II)'; pg 406, Fig 2), wherein the RNA polymerase I mediated transcription is regulated by Myc (pg 406, col 2, lower para - 'MYC can also interact with the rDNA repeats to regulate Pol I transcription'), and further wherein the Pol I inhibitor CX-5461 inhibits Pol I transcription across a broad range of cancer cell lines (pg 408, col 1, top para - 'CX-5461 potently inhibited Pol I transcription across a broad range of cancer cell lines'), and which has been tested in phase I clinical trial for treating lymphoma and leukemia (pg 407, col 2, para 4 - 'selectively inhibit Pol I transcription has ... initiation of phase I clinical trials of one such compound, CX-5461, in patients with lymphoma and leukemia'), and therefore can be a therapeutic choice for treating Myc-driven cancer (pg 407, col 2, para 3 - 'POL I TRANSCRIPTION-BASED THERAPIES TO TREAT MYC-DRIVEN MALIGNANCY'; pg 407, col 2, last para - 'the leading Pol I transcription inhibitor with potential for cancer therapeutics is CX-5461'). Although Poortinga does not specifically teach targeting L-Myc, one of the ordinary skill in the art at the time the invention was made would have been motivated to do so, based on the combination of Poortinga and HEALTH\_RES, in order to combine the methods, therapeutic agents, and knowledge available in the art for facilitating treating different types of cancer with elevated L-Myc expression with a desired result and without undue experimentation, especially it was known in the art long before the invention was made that all Mycs share the same transcriptional activation domain, as evident by an article entitled 'Mammalian MYC Proteins and Cancer' by Tansey (New J Sci. 2014, Vol.2014 (2014), Article ID 757534. PDF File: pg 1-28: pg 3, Fig 2).

Without a shared special technical feature, the inventions lack unity with one another.

Groups I+ and II therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Note:

I) There is no full chemical name of CX-5461 disclosed in the Specification.