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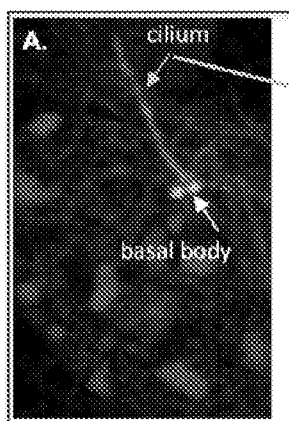
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(54) Title: BIOMARKERS FOR CANCER ASSOCIATED WITH HEDGEHOG PATHWAY ACTIVITY AND RELATED USES THEREOF

(57) Abstract: The present invention relates to biomarkers for detection and characterization of cancer cells associated with hedgehog pathway activity (e.g., non-small cell lung cancer) and related uses thereof.

FIG. 1





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BIOMARKERS FOR CANCER ASSOCIATED WITH HEDGEHOG PATHWAY ACTIVITY AND RELATED USES THEREOF

CROSS REFERENCE TO RELATED APPLICATION

5 This application claims priority to and the benefit of U.S. Provisional Application No. 62/312,262, filed March 23, 2016, which is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

10 The present invention relates to biomarkers for detection and characterization of cancer cells associated with hedgehog pathway activity (e.g., non-small cell lung cancer) and related uses thereof.

BACKGROUND OF THE INVENTION

15 Lung cancer is a highly recalcitrant cancer and remains the leading cause of cancer-related deaths worldwide with non-small cell lung cancer (NSCLC) accounting for the majority (~80%) of all cases.

 There remains an urgent need for effective therapeutic strategies to treat lung cancer.

SUMMARY OF THE INVENTION

20 The hedgehog (Hh) pathway drives tumor cell survival in many cancers, including NSCLC. Inhibitors that target the Hh pathway, such as vismodegib (2-Chloro-*N*-(4-chloro-3-pyridin-2-ylphenyl)-4-methylsulfonylbenzamide) have shown impressive disease regression in basal cell carcinoma of the skin and medulloblastoma. However, drug responsiveness varies dramatically between patients from complete remission in some patients and no response in others. Frustratingly, the use of tumor markers of increased Hh activity was not found to be predictive of patient response to Hh-targeted treatment. This failure of the biological correlate between the target and response suggests a significant and fundamental gap in understanding of the molecular mechanisms that regulate Hh-targeted drug efficacy. Addressing this gap is critical and of high potential impact in the effort to better guide the use of the Hh inhibitors for cancer treatment.

30 The present invention addresses such needs.

 In normal cells primary cilia are required for activation of the Hh pathway. However, in cancer cells new data suggests that the Hh pathway can be activated via cilia-dependent or cilia-independent mechanisms (see, e.g., Hassounah, N.B., et al., 2012 Clin

Cancer Res 18, 2429-2435). Hedgehog ligands are a family of secreted proteins that include Sonic Hh (Shh), Indian Hh (Ihh), and Desert Hh (Dhh). These Hh ligands activate the downstream Gli family of transcription factors that translocate into the nucleus to activate Hh target genes (this constitutes increased Hh signaling). Cilia-dependent activation of the Hh pathway occurs because the cilium itself is a subcellular compartment in which key Hh pathway components including Gli proteins are brought together and processed differentially depending on the absence or presence of Hh ligand (Fig. 1). In the absence of Hh, the Hh pathway stays in an 'off' state via processing of Gli transcription factors to the repressor (Gli^R) form which blocks transcriptional activation of Hh genes (Fig. 1A). This is cilia-dependent through localization of Patched (Ptch1 or Ptc1), a negative regulator of the pathway, to the ciliary membrane. In the presence of Hh, its receptor, Ptch, moves out of the cilium and Smo is phosphorylated and translocated into the cilium where it functions to promote Gli activation (Gli^A) (Fig. 1C) (see, e.g., Corbit, K.C., et al., 2005 Nature 437, 1018-1021; Milenkovic, L., et al., 2009 J Cell Biol 187, 365-374; Chen, Y., et al., 2011 PLoS Biol 9, e1001083). In contrast, cilia-independent Hh signaling occurs when cilia are lost (therefore loss of Gli^R which inhibits transcriptional activation of Hh genes). This leaves promoters of Hh genes vulnerable to activation by oncogenic transcription factors including Myc (Fig. 1B). Pharmacological inhibition of the Hh pathway in cilia-dependent cancers would therefore be mechanistically different than that of cilia-independent cancers (Fig. 2).

Experiments conducted during the course of developing embodiments for the present invention demonstrated that the primary cilia status of tumors correlates with Hh-targeted drug efficacy. Such experiments demonstrated that cancer cells activate the Hh-pathway through two distinct pathways, one that is cilia-dependent and one that is cilia-independent. Further experiments demonstrate that cilia are differentially expressed in Hh-activated NSCLC patient samples. Importantly, such experiments indicate that the relationship between the Hh- signaling pathway and cilia mechanistically influences the effectiveness of Hh-pathway drug inhibitors. Such experiments indicate that NSCLC cells that are positive for primary cilia will respond to the Hh-pathway inhibitors (e.g., Taladegib (LY2940680), Vismodegib).

Further experiments determined that individuals having cancer cells associated with 1) Hh pathway activity and 2) primary cilia expression have a unique biomarker gene expression profile or signature that can be used for predicting the presence or absence of

primary cilia expression in cancer cells associated with Hh pathway activity, and as such, can be used for predicting responsiveness (e.g., favorable or unfavorable) to a Hh inhibitor treatment regimen for treating the cancer (e.g., NSCLC).

Accordingly, in certain embodiments, the present invention provides methods for selecting an individual having cancer cells associated with Hh pathway activity for treatment with a Hh inhibitor, comprising

a) obtaining a biological sample from the individual, wherein the biological sample comprises cancer cells,

b) determining a gene expression profile for one or more biomarker genes within the obtained cancer cells; and

c) administering to the individual a therapeutically effective amount of a Hh inhibitor if the determined biomarker gene expression profile is consistent with a reference gene expression profile for cancer cells associated with Hh pathway activity and primary cilia expression, or not administering to the individual a therapeutically effective amount of a Hh inhibitor if the determined biomarker gene expression profile is inconsistent with a reference gene expression profile for cancer cells associated with Hh pathway activity and primary cilia expression.

Similarly, in certain embodiments, the present invention provides methods for treating a human subject having cancer associated with Hh pathway activity, comprising the steps of

a) performing a nucleic acid-based detection assay to determine a gene expression profile of one or more biomarker genes in a sample comprising cancer cells obtained from the human subject; b) comparing the determined gene expression profile with a reference gene expression profile for cancer cells associated with Hh pathway activity and primary cilia expression; c) determining that the human subject is responsive or unresponsive to a Hh inhibitor treatment based on such a comparison; and d) administering an effective amount of a Hh inhibitor to the human subject having a determined gene expression profile that is consistent with the reference gene expression profile for cancer cells associated with Hh pathway activity and primary cilia expression.

In certain embodiments, the present invention provides methods for determining the presence or absence of primary cilia expression in cancer cells associated with Hh pathway activity, comprising a) determining a gene expression profile of one or more biomarker genes within a sample comprising such cancer cells, b) comparing the determined gene expression profile with a reference gene expression profile for cancer cells associated with Hh pathway activity and primary cilia expression, and c) identifying such cancer cells as having primary

cilia expression if the determined gene expression profile is consistent with the reference gene expression profile for cancer cells associated with Hh pathway activity and primary cilia expression.

Such methods are not limited to specific biomarker genes for determining the gene expression profile. In some embodiments, the one or more biomarker genes include one or more genes involved in ciliogenesis. In some embodiments, the one or more biomarker genes include one or more genes involved in cilia function. In some embodiments, the one or more biomarker genes include one or more genes involved in downstream pathway activity related to ciliogenesis and/or cilia function. In some embodiments, the one or more biomarker genes include one or more genes associated with cilia disease. In some embodiments, the one or more biomarker genes include one or more genes associated with Hh pathway related activity. In some embodiments, the one or more biomarker genes include one or more genes involved in downstream pathway activity related to ciliogenesis and/or cilia function and one or more genes associated with Hh pathway related activity.

In some embodiments, the one or more biomarker genes are selected from the biomarker genes recited in Figs. 7, 8, and/or 9 (e.g., ABHD4, ACSS2, ARL14, BLOC1S2, C17orf91, CDKN1A, CLIC3, CLIP3, CYP4F11, DDIT3, DDIT4, GABARAPL1, GCNT2, GDF15, GPX3, GULP1, HKDC1, IDI1, IL1RN, INSIG1, KIAA1370, LCN2, LCP1, LPIN1, MVD, NUPR1, OSGIN1, PANX2, PCSK9, RBCK1, SC4MOL, SCD, SDCBP2, STOX2, TIMP4, TP53INP1, TRIM31, UBC, YPEL5, ARHGAP11A, ASF1B, AURKB, BIRC5, BLM, C15orf42, C16orf59, CASC5, CCNA2, CCNE2, CDC20, CDC6, CENPM, DDN, DLGAP5, DTL, E2F2, EXO1, FAM64A, GINS2, HELLS, HJURP, KIAA0101, KIF15, KIF20A, KIFC1, LRRc45, LRRC26, MCM10, MCM2, MCM4, MCM5, MCM8, MYO19, NDC80, NMU, NRGN, NUDT1, PKMYT1, POLE, POLE2, PSMC3IP, RECQL4, SLC39A10, SLC43A3, SPC24, SPC25, TK1, TOP2A, TYMS, UBE2C, XRCC3, GLI1, PTCH1, LRRC45, IFT43, WDR35, IFT122, TTC21B, IFT140, WDR19, TULP3, IFT20, RABL5, HSPB11, IFT27, IFT46, IFT52, TRAF3IP1, IFT57, TTC30B, IFT74, IFT80, IFT81, IFT88, IFT172, KIF3A, KIF3B, KIFAP3, KIF17, DYNC2H1, DYNC2LI1, WDR34, DYNLT1, BBS1, BBS2, ARL6, BBS4, BBS5, MKKS, BBS7, TTC8, BBS9, BBS10, TRIM32, BBS12, RAB3IP, RAB8A, PCM1, PKD1, PKD2, PKHD1, AHI1, ARL13B, INPP5E, TMEM216, MKS1, TMEM67, CC2D2A, SDCCAG8, SEPT7, NPHP1, INVS, NPHP3, NPHP4, IQCB1, CEP290, GLIS2, RPGRIP1L, NEK8, EVC, EVC2, VHL, OFD1, STIL, and RPGR).

In some embodiments, the one or more biomarker genes are selected from TMEM216, PCM1, IFT140, IFT122, TRIM32, MKKS, BBS1, PHKHD1, RABL5, HSPB11, PKD1, SDCCAG8, CDP290, STIL, IFT81, WDR34, TULP3, and IQCB1 (see, e.g., Example VI).

5 Such methods are not limited to incorporating a specific number of such biomarker genes in determining the gene expression profile. In some embodiments, 1 biomarker gene is utilized. In some embodiments, 5 biomarker genes are utilized in determining the gene expression profile. In some embodiments, more than 10 (e.g., 10, 11, 12, 15, 20, 25, 50, 100, 1000, etc.) are utilized in determining the gene expression profile.

10 In some embodiments, determining the gene expression profile involves quantifying mRNA expression related to the specific biomarker genes. In some embodiments, determining the gene expression profile involves quantifying protein expression related to the specific biomarker genes. In some embodiments, determining the gene expression profile involves quantifying mRNA expression and protein expression related to the specific
15 biomarker genes.

In such methods, the cancer having cells associated with Hh pathway activity is selected from NSCLC, bladder cancer, basal cell carcinoma, medulloblastoma, colon cancer, breast cancer, and pancreatic cancer.

Such methods are not limited to a particular type of Hh inhibitor.

20 In some embodiments, the Hh inhibitor is a cilia dependent Hh inhibitor.

In some embodiments, the Hh inhibitor is Taladegib (LY2940680). In some embodiments, the Hh inhibitor is vismodegib. In some embodiments, the Hh inhibitor is selected from Taladegib (LY2940680), sulforaphane, vismodegib, TAK-441, itraconazole, and erismodegib.

25 In some embodiments, the Hh inhibitor is selected from Hh ligand inhibitors (e.g., 5E1, robotnikinin), SMO antagonists (e.g., Taladegib (LY2940680), vismodegib, IPI-926, HhAntag), and Gli-processing inhibitors (e.g., HPI-2, HPI-3, HPI-4, arsenic trioxide). In some embodiments, the Hh inhibitor is described and disclosed in U.S. Patent 7,230,004, U.S. Patent Application Publication No. 2008/0293754, U.S. Patent Application Publication
30 No. 2008/0287420, U.S. Patent Application Publication No. 2008/0293755.

Additional examples of suitable Hh inhibitors include, but are not limited to, those described in U.S. Patent Application Publication Nos. US 2002/0006931, US 2007/0021493 and US 2007/0060546, and International Application Publication Nos. WO 2001/19800, WO 2001/26644, WO 2001/27135, WO 2001/49279, WO 2001/74344, WO 2003/011219, WO

2003/088970, WO 2004/020599, WO 2005/013800, WO 2005/033288, WO 2005/032343, WO 2005/042700, WO 2006/028958, WO 2006/050351, WO 2006/078283, WO 2007/054623, WO 2007/059157, WO 2007/120827, WO 2007/131201, WO 2008/070357, WO 2008/110611, WO 2008/112913, and WO 2008/131354.

5 Additional examples of suitable Hh inhibitors include, but are not limited to, BMS-833923 (also known as XL139) (see, e.g., Siu L. et al., J. Clin. Oncol. 2010; 28: 15s (suppl; abstr 2501)); LDE-225 (see, e.g., Pan S. et al., ACS Med. Chem. Lett., 2010; 1(3): 130-134); LEQ-506 (see, e.g., National Institute of Health Clinical Trial Identifier No. NCT01106508); PF-04449913 (see, e.g., National Institute of Health Clinical Trial Identifier No. NCT00953758); Hh pathway antagonists disclosed in U.S. Patent Application Publication No. 2010/0286114; SMOi2-17 (see, e.g., U.S. Patent Application Publication No. 2010/0093625); SANT-1 and SANT-2 (see, e.g., Rominger CM. et al., J. Pharmacol. Exp. Ther. 2009; 329(3):995-1005; 1-piperaziny1-4-arylphthalazines or analogues thereof (see, e.g., Lucas B.S. et al., Bioorg. Med. Chem. Lett. 2010; 20(12):3618-22).

15 In some embodiments, the reference gene expression profile for cancer cells associated with Hh pathway activity and primary cilia expression is generated from one or more subjects (e.g., 1, 5, 10, 100, 1000, 10,000, etc) diagnosed as having a cancer (e.g., NSCLC, bladder cancer, basal cell carcinoma, medulloblastoma, colon cancer, breast cancer, and pancreatic cancer) having cells (e.g., NSCLC cells, bladder cancer cells, basal cell carcinoma cells, medulloblastoma cells, colon cancer cells, breast cancer cells, and pancreatic cancer cells) associated with Hh pathway activity and primary cilia expression. In some
20 embodiments, the reference gene expression profile for cancer cells associated with Hh pathway activity and primary cilia expression is generated as described in Example VI.

In certain embodiments, such methods can be used to predict the likelihood of a
25 patient's ability to effectively respond to a Hh inhibitor based treatment for a cancer associated with Hh pathway activity.

In certain embodiments, such methods can be used for screening the effectiveness of new Hh inhibiting drugs in the treatment of a cancer associated with Hh pathway activity.

In certain embodiments, such methods can be used for identifying new Hh inhibiting
30 drugs in the treatment of a cancer associated with Hh pathway activity.

In certain embodiments, the present invention provides methods for determining the presence or absence of primary cilia expression in cancer cells associated with Hh pathway activity, comprising obtaining a biological sample comprising cancer cells, exposing the cancer cells to antibodies against a ciliary axoneme, antibodies against a cilia associated

centrosome (e.g., a centrosome attached to a ciliary axoneme), antibodies against markers for activated Hh signaling, and three different fluorescently conjugated secondary antibodies to distinguish between the antibodies against a ciliary axoneme, the antibodies against a cilia associated centrosome, and the antibodies against markers for activated Hh signaling, and
5 detecting the presence or absence of binding between the antibodies against a ciliary axoneme, detecting the presence or absence of binding between antibodies against a cilia associated centrosome, and detecting the presence or absence of binding between antibodies against markers for activated Hh signaling, wherein a detected presence of binding between the antibodies against a ciliary axoneme, the antibodies against a cilia associated centrosome,
10 and the antibodies against markers for activated Hh signaling indicates the presence of primary cilia expression in the cancer cells.

Such methods for determining the presence or absence of primary cilia expression in cancer cells associated with Hh pathway activity are not limited to utilizing specific antibodies. In some embodiments, the antibody against a ciliary axoneme is anti-acetylated-
15 tubulin. In some embodiments, the antibodies against a cilia associated centrosome is anti-gamma (γ)-tubulin. In some embodiments, the antibodies against markers for activated Hh signalling is anti-Ptch1 antibody and/or anti-Gli1 antibody and/or anti-SMO antibody.

In certain embodiments, the present invention provides methods for selecting an individual having cancer cells associated with Hh pathway activity for treatment with a Hh
20 inhibitor, comprising obtaining a biological sample from the individual, wherein the biological sample comprises cancer cells, detecting the presence or absence of primary cilia expression within the cancer cells, and administering to the individual a therapeutically effective amount of a cilia-dependent Hh inhibitor if primary cilia expression is detected in the cancer cells, or not administering to the individual a therapeutically effective amount of a
25 cilia-dependent Hh inhibitor if primary cilia expression is not detected in the cancer cells.

Similarly, in certain embodiments, the present invention provides methods for treating a human subject having cancer cells associated with Hh pathway activity, comprising the steps of obtaining a biological sample from the human subject, wherein the biological sample comprises cancer cells, detecting the presence or absence of primary cilia expression within
30 the cancer cells, and administering to the individual a therapeutically effective amount of a cilia-dependent Hh inhibitor if primary cilia expression is detected in the cancer cells, or not administering to the individual a therapeutically effective amount of a cilia-dependent Hh inhibitor if primary cilia expression is not detected in the cancer cells.

In such methods, the cancer cells associated with Hh pathway activity are selected from NSCLC cells, bladder cancer cells, basal cell carcinoma cells, medulloblastoma cells, colon cancer cells, breast cancer cells, and pancreatic cancer cells.

In such methods, the cilia-dependent Hh inhibitor is selected from Taladegib
 5 (LY2940680), sulforaphane, vismodegib, TAK-441, itraconazole, erismodegib, 5E1, robotnikinin, IPI-926, HPI-2, HPI-3, HPI-4, and arsenic trioxide.

Additional embodiments will be apparent to persons skilled in the relevant art based on the teachings contained herein.

10 DESCRIPTION OF THE DRAWINGS

FIG. 1 demonstrates that primary cilia are both positive and negative regulators of the Hh pathway. A. Immunofluorescent staining of primary cilia (grey-scaled white arrows) with antibodies recognizing Acetylated-Tubulin (grey-scaled red, ciliary axoneme or cilium) and gamma-Tubulin (grey-scaled green, basal body). B. Cilia-dependent inhibition of the Hh
 15 pathway via processing of Gli^R (in the absence of Hh ligand). Gli^R inhibits non-canonical activation of Hh target genes. C. Cilia-dependent activation of the Hh pathway via Smo localization to the cilium and processing of Gli^A (in the presence of Hh ligand).

FIG. 2A-D shows cilia-dependent activation of the Hh pathway.

FIG. 3 shows the presence of cilia on basal cell carcinoma is predictive of
 20 responsiveness to GDC-0449 treatment. A. Human tissues were stained for primary cilia (example marked with white arrow) using antibodies that recognize Acetylated-Tubulin (grey-scaled red, ciliary axoneme) and gamma-tubulin (grey-scaled green, centrosome). Staining for Epcam (grey-scaled white) was used to mark epithelia and cancer cells. B. Human BCC tissues that went on to be treated with GDC-0449 were stained for markers of
 25 primary cilian and assigned a cilia score based on percentage of ciliated cells normalized to cell cycle (Ki67 index) (n=20). Best response was evaluated on tumor size evaluated following treatment and included: Progressive Disease (PD, >20% increase in tumor size), Stable Disease (SD, 0-30% decrease in tumor size), Partial Response (PR, 30-99% decrease) and Complete Response (CR, 100% decrease). *p<0.05

30 FIG. 4 shows that primary cilia are expressed on NSCLC cells. A Human NSCLC tissues and B. human NSCLC cell A549 were stained for primary cilia (grey-scaled white arrows) using antibodies that recognize Acetylated -Tubulin (grey-scaled red, ciliary axoneme) and gamma-Tubulin (grey-scaled green, centrosome). Dashed box shows highly ciliated cancer cells.

FIG. 5 shows NSCLC patient-derived xenografts have differential expression of primary cilia. LAC PDX from patient #1 (Cilia-High) and patient #2 (Cilia-Low) were stained for markers of primary cilia and assigned a cilia score based on percentage of ciliated cells normalized to cell cycle (Ki67 index).

5 FIG. 6 shows Hh signature correlates with high ciliogenesis signature in a subset of NSCLC patients.

FIG. 7 shows a list of genes upregulated when the cancer cells are treated with GANT61 (an inhibitor of GLI1 and GLI2, both of which are transcriptional regulators of Hh signaling). Therefore, these genes would have downregulated expression when Hh is ON.

10 FIG. 8 shows a list of genes downregulated when the cancer cells are treated with GANT61 Inhibitor (an inhibitor of GLI1 and GLI2, both of which are transcriptional regulators of Hh signaling). Therefore, these genes would have upregulated expression when Hh is ON.

FIG. 9 shows a list of genes that are associated with primary cilia in one or more of the following ways: 1) required for ciliogenesis, 2) required for ciliary functions including intraflagellar transport, signal transduction and calcium regulation, 3) localized to the primary cilium or basal body, and 4) mutation in this gene is associated with a known disease referred to as a ciliopathy.

FIG. 10 demonstrates the ciliogenesis signature is predictive of cilia-positive staining in NSCLC cell lines. A. Human NSCLC cell lines (NCI-H2073, NCI-H2228, NCI-H1975, and NCI-H2030) were grown *in vitro* and stained for primary cilia using antibodies that recognize acetylated-tubulin (grey-scaled green, ciliary axoneme) and γ -tubulin (grey-scaled red, centrosome). Images represents example of NCI-H2228 cells positive for markers of primary cilia (inset). Hoechst dye was used to counterstain nuclei. B. Bar graph represents quantification of percentage of cilia-positive NSCLC cells analyzed in A. C. Ciliogenesis gene signature score (y-axis) and Hh gene signature score (x-axis) plotted for each of the 47 NSCLC cell lines.

DEFINITIONS

30 To facilitate an understanding of the present invention, a number of terms and phrases are defined below:

The term “gene signature” or “gene expression signature” or “gene expression profile” refers to a group of genes in a cell whose combined expression pattern is uniquely characteristic of a biological phenotype or medical condition.

The term “treat,” “treating,” or “treatment” refers to alleviating or abrogating a disease, e.g., NSCLC, or one or more of the symptoms associated with the disease; or alleviating or eradicating the cause(s) of the disease itself.

The term “therapeutically effective amount” of a compound refers to the amount of a compound (e.g., Hh inhibitor) that, when administered, is sufficient to prevent development of, or alleviate to some extent, one or more of the symptoms of a disease, e.g., NSCLC, being treated. The term also refers to the amount of a compound that is sufficient to elicit the biological or medical response of a biological molecule (e.g., a protein, enzyme, RNA, or DNA), cell, tissue, system, animal, or human, which is being sought by a researcher, veterinarian, medical doctor, or clinician. Furthermore, a therapeutically effective amount of a compound means an amount of a therapeutic agent, alone or in combination with other therapies, which provides a therapeutic benefit in the treatment or management of a disease, e.g., NSCLC. The term encompasses an amount that improves overall therapy, reduces, or avoids symptoms or causes of a disease, e.g., NSCLC, or enhances the therapeutic efficacy of another therapeutic agent.

The term “level” refers to the amount, accumulation, or rate of a biomarker molecule. A level can be represented, for example, by the amount or the rate of synthesis of a messenger RNA (mRNA) encoded by a gene, the amount or the rate of synthesis of a polypeptide or protein encoded by a gene, or the amount or the rate of synthesis of a biological molecule accumulated in a cell or biological fluid. The term “level” refers to an absolute amount of a molecule in a sample or to a relative amount of the molecule, determined under steady-state or non-steady-state conditions.

The term “responsiveness” or “responsive” when used in reference to a treatment refer to the degree of effectiveness of the treatment in lessening or decreasing the symptoms of a disease, e.g., NSCLC, being treated. For example, the term “increased responsiveness” when used in reference to a treatment of a cell or a subject refers to an increase in the effectiveness in lessening or decreasing the symptoms of the disease when measured using any methods known in the art. In certain embodiments, the increase in the effectiveness is at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, or at least about 50%.

The term “effective patient response” refers to an increase in the therapeutic benefit to a patient in treating a disease, e.g., NSCLC. In certain embodiments, the increase is at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, or at least about 50%. An “effective patient tumor response” can be, for example, an about 5%,

about 10%, about 25%, about 50%, or about 100% decrease in one or more physical symptoms of the disease or the tumor size.

The term “likelihood” refers to an increase in the probability of an event. The term “likelihood” when used in reference to the effectiveness of a patient response to a treatment
5 of a disease, e.g., NSCLC, contemplates an increased probability that the symptoms of the disease will be lessened or decreased.

The term “predict” generally means to determine or tell in advance. When used to “predict” the effectiveness of the treatment of a disease (e.g., NSCLC), for example, the term “predict” can mean that the likelihood of the outcome of the treatment can be determined at
10 the outset, before the treatment has begun, or before the treatment period has progressed substantially.

The terms “determining”, “measuring”, “evaluating”, “assessing” and “assaying” are used interchangeably herein to refer to a form of measurement, including determining if an element is present or not. The measurement can be quantitative and/or qualitative
15 determinations. “Assessing the presence of” can include determining the amount of something present, as well as determining whether it is present or absent.

The term “biological sample” as used herein refers to a sample obtained from a biological subject, including a sample of biological tissue or fluid origin, obtained, reached, or collected in vivo or in situ. A biological sample also includes samples from a region of a
20 biological subject containing precancerous or cancer cells or tissues. Such samples can be, but are not limited to, organs, tissues, fractions and cells isolated from a mammal. Exemplary biological samples include, but are not limited to, cell lysate, a cell culture, a cell line, a tissue, oral tissue, gastrointestinal tissue, an organ, an organelle, a biological fluid, a blood sample, a urine sample, a skin sample, and the like. In certain embodiments, biological
25 samples include, but are not limited to, whole blood, partially purified blood, PBMCs, tissue biopsies, and the like.

DETAILED DESCRIPTION OF THE INVENTION

Non-small cell lung cancers (NSCLC) remain the leading cause of cancer deaths in
30 men and women worldwide (see, Ferlay, J., et al., 2008 *Int J Cancer* 127, 2893-2917). While treatment advances over the past few decades are making small gains in progression free survival, NSCLC remains a largely terminal disease. As such, there is a critical need to identify game changing drug targets.

Abnormal activation of the oncogenic Hedgehog (Hh) signaling pathway is common in solid tumors, including NSCLC. Activation of the Hh pathway increases proliferation and activates pro-survival signals. In preclinical studies, blockade of the Hh pathway has been shown to decrease tumor growth and therefore, is emerging as a promising drug target for cancer treatment (see, e.g., Chang, A.L., et al., (2014) *J Am Acad Dermatol* 70, 60-69). The effectiveness of these compounds in preclinical models was highly promising. However, when tested in clinical trials across many cancer types, the results were highly inconsistent and ranged from complete response in some patients and progression on agent in others; results that support an underlying mechanism of individual patient response heterogeneity. Classic measures of Hh-pathway activation did not, however, prove predictive in correlative biomarker studies. Thus, a clear need exists to establish the mechanism(s) that underlie Hh-targeted drug response/resistance for the purpose of developing tumor biomarkers to optimize patient selection for use of Hh-inhibitors as well as for the long-term goal of developing next generation Hh pathway inhibitors.

Experiments conducted during the course of developing embodiments for the present invention demonstrated that primary cilia expression levels on an aggressive form of basal cell carcinoma (BCC) predicts patient response to Hh inhibitors. The primary cilium is a cellular organelle shown to regulate the Hh pathway in response to extracellular cues. While cilia are required for activation of the Hh pathway in normal cells, cancer cells activate the Hh pathway via cilia-dependent or -independent mechanisms suggesting a deregulation of the Hh/cilia interaction in some but not all cancers (see, e.g., Hassounah, N.B., et al., 2012 *Clin Cancer Res* 18, 2429-2435). For aggressive BCC, it was shown that cilia (present or absent) profoundly influences tumor response to Hh pathway inhibitors. Specifically, the presence of cilia (normalized to cell cycle) was highly correlated with tumor regression and clinical remission in a subset of BCC patient receiving Vismodegib (GDC-0449). This contrasted with the failure of tumor Hh activity status to predict patient response to Hh inhibitors. Importantly, such experiments conducted to understand the mechanism of Hh-inhibitor responsiveness suggests a dependence of Vismodegib anti-tumor activity on the presence of cilia. Furthermore, this dependence on cilia is mediated through a post-translational processing of Gli into a transcriptional repressor of tumor growth.

Further experiments determined that individuals having cancer cells associated with 1) Hh pathway activity and 2) primary cilia expression have a unique biomarker gene

expression profile or signature that can be used for predicting the presence or absence of primary cilia expression in cancer cells associated with Hh pathway activity, and as such, can be used for predicting responsiveness (e.g., favorable or unfavorable) to a Hh inhibitor treatment regimen for treating the cancer (e.g., NSCLC).

5 Accordingly, methods, systems, compositions, arrays, kits, reagents, computers software, and reports are provided herein for use in analyzing one or more of the biomarkers (e.g., presence of primary cilia expression in cancer cells) or biomarker genes (e.g., gene expression profiles) disclosed herein.

In one aspect, disclosed herein are methods for stratifying an individual having a
 10 cancer associated with Hh pathway activity (e.g., NSCLC) for treatment, based on the presence or absence of a specific gene expression profile for one or more biomarker genes selected from Figs. 7, 8 and/or 9 (e.g., ABHD4, ACSS2, ARL14, BLOC1S2, C17orf91, CDKN1A, CLIC3, CLIP3, CYP4F11, DDIT3, DDIT4, GABARAPL1, GCNT2, GDF15, GPX3, GULP1, HKDC1, IDI1, IL1RN, INSIG1, KIAA1370, LCN2, LCP1, LPIN1, MVD,
 15 NUPR1, OSGIN1, PANX2, PCSK9, RBCK1, SC4MOL, SCD, SDCBP2, STOX2, TIMP4, TP53INP1, TRIM31, UBC, YPEL5, ARHGAP11A, ASF1B, AURKB, BIRC5, BLM, C15orf42, C16orf59, CASC5, CCNA2, CCNE2, CDC20, CDC6, CENPM, DDN, DLGAP5, DTL, E2F2, EXO1, FAM64A, GINS2, HELLS, HJURP, KIAA0101, KIF15, KIF20A, KIFC1, LRRc45, LRRC26, MCM10, MCM2, MCM4, MCM5, MCM8, MYO19, NDC80,
 20 NMU, NRGN, NUDT1, PKMYT1, POLE, POLE2, PSMC3IP, RECQL4, SLC39A10, SLC43A3, SPC24, SPC25, TK1, TOP2A, TYMS, UBE2C, XRCC3, GLI1, PTCH1, LRRC45, IFT43, WDR35, IFT122, TTC21B, IFT140, WDR19, TULP3, IFT20, RABL5, HSPB11, IFT27, IFT46, IFT52, TRAF3IP1, IFT57, TTC30B, IFT74, IFT80, IFT81, IFT88, IFT172, KIF3A, KIF3B, KIFAP3, KIF17, DYNC2H1, DYNC2LI1, WDR34, DYNLT1,
 25 BBS1, BBS2, ARL6, BBS4, BBS5, MKKS, BBS7, TTC8, BBS9, BBS10, TRIM32, BBS12, RAB3IP, RAB8A, PCM1, PKD1, PKD2, PKHD1, AHI1, ARL13B, INPP5E, TMEM216, MKS1, TMEM67, CC2D2A, SDCCAG8, SEPT7, NPHP1, INVS, NPHP3, NPHP4, IQCB1, CEP290, GLIS2, RPGRIP1L, NEK8, EVC, EVC2, VHL, OFD1, STIL, and RPGR).

In one aspect, disclosed herein are methods for stratifying an individual having a
 30 cancer associated with Hh pathway activity (e.g., NSCLC) for treatment, based on the presence or absence of a specific gene expression profile for one or more biomarker genes selected from TMEM216, PCM1, IFT140, IFT122, TRIM32, MKKS, BBS1, PKHD1, RABL5, HSPB11, PKD1, SDCCAG8, CDP290, STIL, IFT81, WDR34, TULP3, and IQCB1 (see, e.g., Example VI).

In some embodiments, determining the gene expression profile involves quantifying mRNA expression related to the specific biomarker genes. In some embodiments, determining the gene expression profile involves quantifying protein expression related to the specific biomarker genes. In some embodiments, determining the gene expression profile involves quantifying mRNA expression and protein expression related to the specific biomarker genes.

In some cases, the specific gene expression profile for such one or more biomarker genes is for an individual having cancer cells associated with Hh pathway activity and primary cilia expression. In some cases, the presence of such a specific gene signature indicates that the individual would be responsive to a Hh inhibitor (e.g., a SMO inhibitor (e.g., Taladegib (LY2940680), Vismodegib) as a treatment of a cancer associated with Hh pathway activity (e.g., NSCLC). In some cases, the absence of such a specific gene signature indicates that the individual would not be responsive to a Hh inhibitor (e.g., a SMO inhibitor (e.g., Taladegib (LY2940680), Vismodegib)) as a treatment of a cancer associated with Hh pathway activity (e.g., NSCLC). In other cases, an individual's therapeutic regimen is optimized, e.g. modifying, discontinuing, or continuing the treatment, based on the presence or absence of such a specific gene expression signature for the one or more biomarker genes selected from Figs. 7, 8 and/or 9. In other cases, an individual's therapeutic regimen is optimized, e.g. modifying, discontinuing, or continuing the treatment, based on the presence or absence of such a specific gene expression signature for the one or more biomarker genes selected from TMEM216, PCM1, IFT140, IFT122, TRIM32, MKKS, BBS1, PHKHD1, RABL5, HSPB11, PKD1, SDCCAG8, CDP290, STIL, IFT81, WDR34, TULP3, and IQCB1 (see, e.g., Example VI).

In one aspect, disclosed herein are methods for stratifying an individual having a cancer associated with Hh pathway activity (e.g., NSCLC) for treatment, based on the presence or absence of primary cilia expression detected in obtained cancer cells from the individual. In some embodiments, detection of the presence or absence of primary cilia expression in such obtained cancer cells is accomplished with a cilia staining technique (see, e.g., Example VII). In some cases, the presence of primary cilia expression in such obtained cancer cells indicate that the individual would be responsive to a Hh inhibitor (e.g., a SMO inhibitor (e.g., Taladegib (LY2940680), Vismodegib)) as a treatment of a cancer associated with Hh pathway activity (e.g., NSCLC). In some cases, the absence of primary cilia expression in such obtained cancer cells indicate that the individual would not be responsive to a Hh inhibitor (e.g., a SMO inhibitor (e.g., Taladegib (LY2940680), Vismodegib)) as a

treatment of a cancer associated with Hh pathway activity (e.g., NSCLC). In other cases, an individual's therapeutic regimen is optimized, e.g. modifying, discontinuing, or continuing the treatment, based on the presence or absence of primary cilia expression in such obtained cancer cells.

Also disclosed herein are systems for using biomarkers (e.g., primary cilia expression in cancer cells) or biomarker gene expression profiles as disclosed herein (e.g., the genes recited in Figs. 7, 8 and/or 9) (e.g., one or more genes selected from TMEM216, PCM1, IFT140, IFT122, TRIM32, MKKS, BBS1, PHKHD1, RABL5, HSPB11, PKD1, SDCCAG8, CDP290, STIL, IFT81, WDR34, TULP3, and IQCB1 (see, e.g., Example VI)) for assessing an individual having a cancer associated with Hh pathway activity (e.g., NSCLC) for treatment with a Hh inhibitor (e.g., a SMO inhibitor (e.g., Taladegib (LY2940680), Vismodegib)).

In some cases, the systems comprise the analysis of a biological sample (e.g., a biological sample comprising cancer cells associated with Hh pathway activity; NSCLC cancer cells) by analytical techniques to derive biomarker gene expression profile data and/or analytical measurements. In some embodiments, such biomarker gene expression profile data or analytical measurements are subsequently compiled by software into a dataset, which is then analyzed to determine one or more biomarker indications, such as the presence or absence of a gene expression profile consistent with a reference gene expression profile for cancer cells associated with Hh pathway activity and primary cilia expression. The results are used to stratify patients prior to or during therapy regimens, to monitor the progress of a therapy regimen, or to optimize a therapy regimen. In some cases, the results are compiled into a report format for sending to a user.

Further disclosed herein are kits and arrays for using biomarkers (e.g., primary cilia expression in cancer cells) or biomarker gene expression profiles disclosed herein for use with the methods and systems disclosed above.

In some embodiments, kits disclosed herein comprise one or more reagents for determining the presence or absence of primary cilia expression in obtained cancer cells from an individual (see, e.g., the technique described in Example VII).

In some embodiments, kits disclosed herein comprise one or more reagents for determining a gene expression profile for the one or more biomarker genes shown in Figs. 7, 8 and/or 9, and a reference gene expression profile for cancer cells associated with Hh pathway activity and primary cilia expression. In some embodiments, kits disclosed herein comprise one or more reagents for determining a gene expression profile for the one or more

biomarker genes shown in TMEM216, PCM1, IFT140, IFT122, TRIM32, MKKS, BBS1, PHKHD1, RABL5, HSPB11, PKD1, SDCCAG8, CDP290, STIL, IFT81, WDR34, TULP3, and IQCB1, and a reference gene expression profile for cancer cells associated with Hh pathway activity and primary cilia expression.

5 In some embodiments, instructions are provided for comparing the determined gene expression profile with the reference gene expression profile for cancer cells associated with Hh pathway activity and primary cilia expression.

 In some embodiments, the present invention provides a nucleic acid hybridization array comprising nucleic acid probes for evaluating if an individual receiving a Hh inhibitor
10 such as a SMO inhibitor (e.g., Taladegib (LY2940680), Vismodegib) for treatment of a cancer associated with Hh activity (e.g., NSCLC) has developed or is likely to develop resistance to the therapy, comprising nucleic acid probes which hybridize to biomarker genes selected from Figs. 7, 8 and/or 9.

 In some embodiments, the present invention provides a nucleic acid hybridization
15 array comprising nucleic acid probes for evaluating if an individual receiving a Hh inhibitor such as a SMO inhibitor (e.g., Taladegib (LY2940680), Vismodegib) for treatment of a cancer associated with Hh activity (e.g., NSCLC) has developed or is likely to develop resistance to the therapy, comprising nucleic acid probes which hybridize to biomarker genes selected TMEM216, PCM1, IFT140, IFT122, TRIM32, MKKS, BBS1, PHKHD1, RABL5,
20 HSPB11, PKD1, SDCCAG8, CDP290, STIL, IFT81, WDR34, TULP3, and IQCB1.

 Cancers associated with Hh pathway activity are a diverse group of cancer that include, but are not limited to, NSCLC, bladder cancer, basal cell carcinoma, medulloblastoma, colon cancer, breast cancer, and pancreatic cancer.

 Also disclosed herein are methods of using biomarkers or biomarker gene expression
25 profiles for monitoring a patient during treatment of a cancer associated with Hh pathway activity (e.g., NSCLC). Further disclosed herein are methods of using biomarkers or biomarker gene expression profiles for optimizing a treatment regimen.

 In some embodiments, the treatment comprises administration of a Hh inhibitor. Such treatment is not limited to a particular type or kind of a Hh inhibitor. In some embodiments,
30 the Hh inhibitor is Taladegib (LY2940680). In some embodiments, the Hh inhibitor is vismodegib. In some embodiments, the Hh inhibitor is selected from Taladegib (LY2940680), sulforaphane, vismodegib, TAK-441, itraconazole, and erismodegib. In some embodiments, the Hh inhibitor is selected from Hh ligand inhibitors (e.g., 5E1, robotnikinin),

SMO antagonists (e.g., Taladegib (LY2940680), vismodegib, IPI-926, HhAntag), and Gli-processing inhibitors (e.g., HPI-2, HPI-3, HPI-4, arsenic trioxide).

Methods for determining a gene expression profile are well known in the art.

Mutations or modifications and expression levels of biomarkers are measured by RT-PCR, 5
Q_t-PCR, microarray, Northern blot, or other similar technologies. Circulating levels of biomarkers in a blood sample obtained from a candidate subject are measured, for example, by ELISA, radioimmunoassay (RIA), electrochemiluminescence (ECL), Western blot, multiplexing technologies, or other similar methods. Cell surface expression of biomarkers are measured, for example, by flow cytometry, immunohistochemistry, Western Blot, 10
immunoprecipitation, magnetic bead selection, and quantification of cells expressing either of these cell surface markers.

As disclosed herein, determining the presence, modifications, or expression of the biomarker of interest at the protein or nucleotide level are accomplished using any detection method known to those of skill in the art. By “determining the modification(s)” is intended to 15
determine a mutation within the biomarker gene or a biomarker protein. As used herein, “modification” and “mutation” are used interchangeably. The term “biomarker” refers to in some cases the protein of interest. In some cases, “biomarker” refers to the gene of interest. In some cases, the terms “biomarker” and “biomarker gene” are used interchangeably. By “detecting expression” or “detecting the level of” is intended determining the expression level 20
or presence of a biomarker protein or gene in the biological sample. Thus, “detecting expression” encompasses instances where a biomarker is determined not to be expressed, not to be detectably expressed, expressed at a low level, expressed at a normal level, or overexpressed.

In certain aspects, the modifications, expression, or presence of these various 25
biomarkers and any clinically useful prognostic markers in a biological sample are detected at the protein or nucleic acid level, using, for example, immunohistochemistry techniques or nucleic acid-based techniques such as in situ hybridization and RT-PCR. In one embodiment, the modifications, expression, or presence of one or more biomarkers is carried out by a means for nucleic acid amplification, a means for nucleic acid sequencing, a means 30
utilizing a nucleic acid microarray (DNA and RNA), or a means for in situ hybridization using specifically labeled probes.

In some embodiments, the determining the modification, expression, or presence of one or more biomarkers is carried out through gel electrophoresis. In one embodiment, the

determination is carried out through transfer to a membrane and hybridization with a specific probe.

In other embodiments, the determining the modification, expression, or presence of one or more biomarkers carried out by a diagnostic imaging technique.

5 In still other embodiments, the determining the modification, expression, or presence of one or more biomarkers carried out by a detectable solid substrate. In one embodiment, the detectable solid substrate is paramagnetic nanoparticles functionalized with antibodies.

Methods for detecting the modification and expression of the biomarkers described herein, within the test and control biological samples comprise any methods that determine
10 the quantity or the presence of these markers either at the nucleic acid or protein level. Such methods are well known in the art and include but are not limited to western blots, northern blots, ELISA, immunoprecipitation, immunofluorescence, flow cytometry, immunohistochemistry, nucleic acid hybridization techniques, nucleic acid reverse transcription methods, and nucleic acid amplification methods. In some embodiments,
15 expression of a biomarker is detected on a protein level using, for example, antibodies that are directed against specific biomarker proteins. These antibodies are used in various methods such as Western blot, ELISA, multiplexing technologies, immunoprecipitation, or immunohistochemistry techniques. In some embodiments, detection of biomarkers is accomplished by ELISA. In some embodiments, detection of biomarkers is accomplished by
20 electrochemiluminescence (ECL).

In some embodiments, the modification, expression, or presence of one or more of the biomarkers described herein are determined at the nucleic acid level. Nucleic acid-based techniques for assessing expression are well known in the art and include, for example, determining the level of biomarker mRNA in a biological sample. Many expression detection
25 methods use isolated RNA. Any RNA isolation technique that does not select against the isolation of mRNA is utilized for the purification of RNA (see, e.g., Ausubel et al., ed. (1987-1999) Current Protocols in Molecular Biology (John Wiley & Sons, New York). Additionally, large numbers of tissue samples are readily processed using techniques well known to those of skill in the art, such as, for example, the single-step RNA isolation process
30 disclosed in U.S. Pat. No. 4,843,155.

Thus, in some embodiments, the detection of a biomarker or other protein of interest is assayed at the nucleic acid level using nucleic acid probes. The term “nucleic acid probe” refers to any molecule that is capable of selectively binding to a specifically intended target nucleic acid molecule, for example, a nucleotide transcript. Probes are synthesized by one of

skill in the art, or derived from appropriate biological preparations. Probes are specifically designed to be labeled, for example, with a radioactive label, a fluorescent label, an enzyme, a chemiluminescent tag, a colorimetric tag, or other labels or tags that are discussed above or that are known in the art. Examples of molecules that are utilized as probes include, but are not limited to, RNA and DNA.

For example, isolated mRNA are used in hybridization or amplification assays that include, but are not limited to, Southern or Northern analyses, polymerase chain reaction analyses and probe arrays. One method for the detection of mRNA levels involves contacting the isolated mRNA with a nucleic acid molecule (probe) that hybridize to the mRNA encoded by the gene being detected. The nucleic acid probe comprises of, for example, a full-length cDNA, or a portion thereof, such as an oligonucleotide of at least 7, 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to an mRNA or genomic DNA encoding a biomarker, biomarker described herein above. Hybridization of an mRNA with the probe indicates that the biomarker or other target protein of interest is being expressed.

In one embodiment, the mRNA is immobilized on a solid surface and contacted with a probe, for example by running the isolated mRNA on an agarose gel and transferring the mRNA from the gel to a membrane, such as nitrocellulose. In an alternative embodiment, the probe(s) are immobilized on a solid surface and the mRNA is contacted with the probe(s), for example, in a gene chip array. A skilled artisan readily adapts known mRNA detection methods for use in detecting the level of mRNA encoding the biomarkers or other proteins of interest.

An alternative method for determining the level of an mRNA of interest in a sample involves the process of nucleic acid amplification, e.g., by RT-PCR (see, for example, U.S. Pat. No. 4,683,202), ligase chain reaction (Barany (1991) Proc. Natl. Acad. Sci. USA 88:189193), self-sustained sequence replication (Guatelli et al. (1990) Proc. Natl. Acad. Sci. USA 87:1874-1878), transcriptional amplification system (Kwoh et al. (1989) Proc. Natl. Acad. Sci. USA 86:1173-1177), Q-Beta Replicase (Lizardi et al. (1988) Bio/Technology 6:1197), rolling circle replication (U.S. Pat. No. 5,854,033) or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers. In particular aspects of the invention, biomarker expression is assessed by quantitative fluorogenic RT-PCR (i.e., the TaqMan[®] System).

Modifications or expression levels of an RNA of interest are monitored using a membrane blot (such as used in hybridization analysis such as Northern, dot, and the like), or microwells, sample tubes, gels, beads or fibers (or any solid support comprising bound nucleic acids). See U.S. Pat. Nos. 5,770,722, 5,874,219, 5,744,305, 5,677,195 and 5,445,934, which are incorporated herein by reference. The detection of expression also comprises using nucleic acid probes in solution.

In some embodiments, microarrays are used to determine expression or presence of one or more biomarkers. Microarrays are particularly well suited for this purpose because of the reproducibility between different experiments. DNA microarrays provide one method for the simultaneous measurement of the expression levels of large numbers of genes. Each array consists of a reproducible pattern of capture probes attached to a solid support. Labeled RNA or DNA is hybridized to complementary probes on the array and then detected by laser scanning. Hybridization intensities for each probe on the array are determined and converted to a quantitative value representing relative gene expression levels. See, U.S. Pat. Nos. 6,040,138, 5,800,992, 6,020,135, 6,033,860, 6,344,316, and U.S. Pat. Application 20120208706. High-density oligonucleotide arrays are particularly useful for determining the gene expression profile for a large number of RNA's in a sample. Exemplary microarray chips include FoundationOne and FoundationOne Heme from Foundation Medicine, Inc; GeneChip® Human Genome U133 Plus 2.0 array from Affymetrix; and Human DiscoveryMAP® 250+ v. 2.0 from Myraid RBM.

Techniques for the synthesis of these arrays using mechanical synthesis methods are described in, e.g., U.S. Pat. No. 5,384,261. In some embodiments, an array is fabricated on a surface of virtually any shape or even a multiplicity of surfaces. In some embodiments, an array is a planar array surface. In some embodiments, arrays include peptides or nucleic acids on beads, gels, polymeric surfaces, fibers such as fiber optics, glass or any other appropriate substrate, see U.S. Pat. Nos. 5,770,358, 5,789,162, 5,708,153, 6,040,193 and 5,800,992, each of which is hereby incorporated in its entirety for all purposes. In some embodiments, arrays are packaged in such a manner as to allow for diagnostics or other manipulation of an all-inclusive device.

Any means for specifically identifying and quantifying a biomarker in the biological sample of a candidate subject is contemplated. Thus, in some embodiments, expression level of a biomarker protein of interest in a biological sample is detected by means of a binding protein capable of interacting specifically with that biomarker protein or a biologically active variant thereof. In some embodiments, labeled antibodies, binding portions thereof, or other

binding partners are used. The word “label” when used herein refers to a detectable compound or composition that is conjugated directly or indirectly to the antibody so as to generate a “labeled” antibody. In some embodiments, the label is detectable by itself (e.g., radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, catalyzes chemical alteration of a substrate compound or composition that is detectable.

The antibodies for detection of a biomarker protein are either monoclonal or polyclonal in origin, or are synthetically or recombinantly produced. The amount of complexed protein, for example, the amount of biomarker protein associated with the binding protein, for example, an antibody that specifically binds to the biomarker protein, is determined using standard protein detection methodologies known to those of skill in the art. A detailed review of immunological assay design, theory and protocols are found in numerous texts in the art (see, for example, Ausubel et al., eds. (1995) *Current Protocols in Molecular Biology*) (Greene Publishing and Wiley-Interscience, NY)); Coligan et al., eds. (1994) *Current Protocols in Immunology* (John Wiley & Sons, Inc., New York, N.Y.).

The choice of marker used to label the antibodies will vary depending upon the application. However, the choice of the marker is readily determinable to one skilled in the art. These labeled antibodies are used in immunoassays as well as in histological applications to detect the presence of any biomarker or protein of interest. The labeled antibodies are either polyclonal or monoclonal. Further, the antibodies for use in detecting a protein of interest are labeled with a radioactive atom, an enzyme, a chromophoric or fluorescent moiety, or a colorimetric tag as described elsewhere herein. The choice of tagging label also will depend on the detection limitations desired. Enzyme assays (ELISAs) typically allow detection of a colored product formed by interaction of the enzyme-tagged complex with an enzyme substrate. Radionuclides that serve as detectable labels include, for example, ¹³¹I, ¹²³I, ¹²⁵I, ⁹⁰Y, ¹⁸⁸Re, ¹⁸⁶Re, ²¹¹At, ⁶⁷Cu, ²¹²Bi, and ¹⁰⁹Pd. Examples of enzymes that serve as detectable labels include, but are not limited to, horseradish peroxidase, alkaline phosphatase, beta-galactosidase, and glucose-6-phosphate dehydrogenase. Chromophoric moieties include, but are not limited to, fluorescein and rhodamine. The antibodies are conjugated to these labels by methods known in the art. For example, enzymes and chromophoric molecules are conjugated to the antibodies by means of coupling agents, such as dialdehydes, carbodiimides, dimaleimides, and the like. Alternatively, conjugation occurs through a ligand-receptor pair. Examples of suitable ligand-receptor pairs are biotin-avidin or biotin-streptavidin, and antibody-antigen.

In certain embodiments, expression or presence of one or more biomarkers or other proteins of interest within a biological sample, for example, a sample of bodily fluid, is determined by radioimmunoassays or enzyme-linked immunoassays (ELISAs), competitive binding enzyme-linked immunoassays, dot blot (see, for example, Promega Protocols and Applications Guide, Promega Corporation (1991), Western blot (see, for example, Sambrook et al. (1989) Molecular Cloning, A Laboratory Manual, Vol. 3, Chapter 18 (Cold Spring Harbor Laboratory Press, Plainview, N.Y.), chromatography such as high performance liquid chromatography (HPLC), or other assays known in the art. Thus, the detection assays involve steps such as, but not limited to, immunoblotting, immunodiffusion, immunoelectrophoresis, or immunoprecipitation.

In some embodiments, the sample for use in the methods is obtained from cells of a cancer cell line (e.g., a cancer associated with Hh pathway activity). In some embodiments, the sample is obtained from cells of NSCLC, basal cell carcinoma, pancreatic cancer, medulloblastoma, colon cancer, breast cancer, and/or bladder cancer.

In some embodiments, the sample for use in the methods is from any tissue or fluid from a patient. Samples include, but are not limited, to whole blood, dissociated bone marrow, bone marrow aspirate, pleural fluid, peritoneal fluid, central spinal fluid, abdominal fluid, pancreatic fluid, cerebrospinal fluid, brain fluid, ascites, pericardial fluid, urine, saliva, bronchial lavage, sweat, tears, ear flow, sputum, hydrocele fluid, semen, vaginal flow, milk, amniotic fluid, and secretions of respiratory, intestinal or genitourinary tract. In particular embodiments, the sample is a blood serum sample. In particular embodiments, the sample is from a fluid or tissue that is part of, or associated with, the lymphatic system or circulatory system. In some embodiments, the sample is a blood sample that is a venous, arterial, peripheral, tissue, cord blood sample. In particular embodiments, the sample is a blood cell sample containing one or more peripheral blood mononuclear cells (PBMCs). In some embodiments, the sample contains one or more circulating tumor cells (CTCs). In some embodiments, the sample contains one or more disseminated tumor cells (DTC, e.g., in a bone marrow aspirate sample).

In some embodiments, the samples are obtained from the individual by any suitable means of obtaining the sample using well-known and routine clinical methods. Procedures for obtaining fluid samples from an individual are well known. For example, procedures for drawing and processing whole blood and lymph are well-known and can be employed to obtain a sample for use in the methods provided. Typically, for collection of a blood sample, an anti-coagulation agent (e.g., EDTA, or citrate and heparin or CPD (citrate, phosphate,

dextrose) or comparable substances) is added to the sample to prevent coagulation of the blood. In some examples, the blood sample is collected in a collection tube that contains an amount of EDTA to prevent coagulation of the blood sample.

In some embodiments, the collection of a sample from the individual is performed at
5 regular intervals, such as, for example, one day, two days, three days, four days, five days, six days, one week, two weeks, weeks, four weeks, one month, two months, three months, four months, five months, six months, one year, daily, weekly, bimonthly, quarterly, biyearly or yearly.

In some embodiments, the collection of a sample is performed at a predetermined
10 time or at regular intervals relative to treatment with a Hh inhibitor (e.g., SMO inhibitor). For example, a sample is collected from a patient at a predetermined time or at regular intervals prior to, during, or following treatment or between successive treatments with a Hh inhibitor. In particular examples, a sample is obtained from a patient prior to administration of a Hh inhibitor, and then again at regular intervals after treatment with the Hh inhibitor has been
15 effected. In some embodiments, the patient is administered a Hh inhibitor and one or more additional therapeutic agents.

In some embodiments, a Hh inhibitor is administered in combination with an additional therapeutic agent for the treatment of a cancer associated with Hh pathway activity. In some embodiments, the Hh inhibitor includes Hh ligand inhibitors (e.g., 5E1,
20 robotnikinin), SMO antagonists (e.g., vismodegib, IPI-926, HhAntag), and Gli-processing inhibitors (e.g., HPI-2, HPI-3, HPI-4, arsenic trioxide).

In certain embodiments, an Hh inhibitor is administered in combination with an additional therapeutic agent for the treatment of a cancer associated with Hh pathway activity (e.g., NSCLC). In some embodiments, the additional therapeutic agent including, but not
25 limited to, chemotherapeutic antineoplastics, apoptosis-modulating agents, antimicrobials, antivirals, antifungals, and anti-inflammatory agents) and/or therapeutic technique (e.g., surgical intervention, and/or radiotherapies). In a particular embodiment, the additional therapeutic agent(s) is an anticancer agent.

A number of suitable anticancer agents are contemplated for use in the methods of the
30 present invention. Indeed, the present invention contemplates, but is not limited to, administration of numerous anticancer agents such as: agents that induce apoptosis; polynucleotides (e.g., anti-sense, ribozymes, siRNA); polypeptides (e.g., enzymes and antibodies); biological mimetics; alkaloids; alkylating agents; antitumor antibiotics; antimetabolites; hormones; platinum compounds; monoclonal or polyclonal antibodies (e.g.,

antibodies conjugated with anticancer drugs, toxins, defensins), toxins; radionuclides; biological response modifiers (*e.g.*, interferons (*e.g.*, IFN- α) and interleukins (*e.g.*, IL-2)); adoptive immunotherapy agents; hematopoietic growth factors; agents that induce tumor cell differentiation (*e.g.*, all-trans-retinoic acid); gene therapy reagents (*e.g.*, antisense therapy reagents and nucleotides); tumor vaccines; angiogenesis inhibitors; proteasome inhibitors; NF-KB modulators; anti-CDK compounds; HDAC inhibitors; and the like. Numerous other examples of chemotherapeutic compounds and anticancer therapies suitable for co-administration with Hh inhibitors are known to those skilled in the art.

In certain embodiments, anticancer agents comprise agents that induce or stimulate apoptosis. Agents that induce apoptosis include, but are not limited to, radiation (*e.g.*, X-rays, gamma rays, UV); tumor necrosis factor (TNF)-related factors (*e.g.*, TNF family receptor proteins, TNF family ligands, TRAIL, antibodies to TRAIL-R1 or TRAIL-R2); kinase inhibitors (*e.g.*, epidermal growth factor receptor (EGFR) kinase inhibitor, vascular growth factor receptor (VGFR) kinase inhibitor, fibroblast growth factor receptor (FGFR) kinase inhibitor, platelet-derived growth factor receptor (PDGFR) kinase inhibitor, and Bcr-Abl kinase inhibitors (such as GLEEVEC)); antisense molecules; antibodies (*e.g.*, HERCEPTIN, RITUXAN, ZEVALIN, and AVASTIN); anti-estrogens (*e.g.*, raloxifene and tamoxifen); anti-androgens (*e.g.*, flutamide, bicalutamide, finasteride, aminoglutethamide, ketoconazole, and corticosteroids); cyclooxygenase 2 (COX-2) inhibitors (*e.g.*, celecoxib, meloxicam, NS-398, and non-steroidal anti-inflammatory drugs (NSAIDs)); anti-inflammatory drugs (*e.g.*, butazolidin, DECADRON, DELTASONE, dexamethasone, dexamethasone intensol, DEXONE, HEXADROL, hydroxychloroquine, METICORTEN, ORADEXON, ORASONE, oxyphenbutazone, PEDIAPRED, phenylbutazone, PLAQUENIL, prednisolone, prednisone, PRELONE, and TANDEARIL); and cancer chemotherapeutic drugs (*e.g.*, irinotecan (CAMPTOSAR), CPT-11, fludarabine (FLUDARA), dacarbazine (DTIC), dexamethasone, mitoxantrone, MYLOTARG, VP-16, cisplatin, carboplatin, oxaliplatin, 5-FU, doxorubicin, gemcitabine, bortezomib, gefitinib, bevacizumab, TAXOTERE or TAXOL); cellular signaling molecules; ceramides and cytokines; staurosporine, and the like.

In still other embodiments, the compositions and methods of the present invention provide a Hh inhibitor and at least one anti-hyperproliferative or antineoplastic agent selected from alkylating agents, antimetabolites, and natural products (*e.g.*, herbs and other plant and/or animal derived compounds).

Alkylating agents suitable for use in the present compositions and methods include, but are not limited to: 1) nitrogen mustards (*e.g.*, mechlorethamine, cyclophosphamide,

ifosfamide, melphalan (L-sarcolysin); and chlorambucil); 2) ethylenimines and methylmelamines (*e.g.*, hexamethylmelamine and thiotepa); 3) alkyl sulfonates (*e.g.*, busulfan); 4) nitrosoureas (*e.g.*, carmustine (BCNU); lomustine (CCNU); semustine (methyl-CCNU); and streptozocin (streptozotocin)); and 5) triazenes (*e.g.*, dacarbazine (DTIC);
 5 dimethyltriazenoimid-azolecarboxamide).

In some embodiments, antimetabolites suitable for use in the present compositions and methods include, but are not limited to: 1) folic acid analogs (*e.g.*, methotrexate (amethopterin)); 2) pyrimidine analogs (*e.g.*, fluorouracil (5-fluorouracil; 5-FU), floxuridine (fluorode-oxyuridine; FudR), and cytarabine (cytosine arabinoside)); and 3) purine analogs
 10 (*e.g.*, mercaptopurine (6-mercaptopurine; 6-MP), thioguanine (6-thioguanine; TG), and pentostatin (2'-deoxycorformycin)).

In still further embodiments, chemotherapeutic agents suitable for use in the compositions and methods of the present invention include, but are not limited to: 1) vinca alkaloids (*e.g.*, vinblastine (VLB), vincristine); 2) epipodophyllotoxins (*e.g.*, etoposide and
 15 teniposide); 3) antibiotics (*e.g.*, dactinomycin (actinomycin D), daunorubicin (daunomycin; rubidomycin), doxorubicin, bleomycin, plicamycin (mithramycin), and mitomycin (mitomycin C)); 4) enzymes (*e.g.*, L-asparaginase); 5) biological response modifiers (*e.g.*, interferon-alfa); 6) platinum coordinating complexes (*e.g.*, cisplatin (cis-DDP) and carboplatin); 7) anthracenediones (*e.g.*, mitoxantrone); 8) substituted ureas (*e.g.*,
 20 hydroxyurea); 9) methylhydrazine derivatives (*e.g.*, procarbazine (N-methylhydrazine; MIH)); 10) adrenocortical suppressants (*e.g.*, mitotane (o,p'-DDD) and aminoglutethimide); 11) adrenocorticosteroids (*e.g.*, prednisone); 12) progestins (*e.g.*, hydroxyprogesterone caproate, medroxyprogesterone acetate, and megestrol acetate); 13) estrogens (*e.g.*, diethylstilbestrol and ethinyl estradiol); 14) antiestrogens (*e.g.*, tamoxifen); 15) androgens
 25 (*e.g.*, testosterone propionate and fluoxymesterone); 16) antiandrogens (*e.g.*, flutamide); and 17) gonadotropin-releasing hormone analogs (*e.g.*, leuprolide).

Any oncolytic agent that is routinely used in a cancer therapy context finds use in the compositions and methods of the present invention. For example, the U.S. Food and Drug Administration maintains a formulary of oncolytic agents approved for use in the United
 30 States. International counterpart agencies to the U.S.F.D.A. maintain similar formularies. Table 1 provides a list of exemplary antineoplastic agents approved for use in the U.S. Those skilled in the art will appreciate that the "product labels" required on all U.S. approved chemotherapeutics describe approved indications, dosing information, toxicity data, and the like, for the exemplary agents.

Table 1

Aldesleukin (des-alanyl-1, serine-125 human interleukin-2)	Proleukin	Chiron Corp., Emeryville, CA
Alemtuzumab (IgG1κ anti CD52 antibody)	Campath	Millennium and ILEX Partners, LP, Cambridge, MA
Alitretinoin (9-cis-retinoic acid)	Panretin	Ligand Pharmaceuticals, Inc., San Diego CA
Allopurinol (1,5-dihydro-4 H -pyrazolo[3,4-d]pyrimidin-4-one monosodium salt)	Zyloprim	GlaxoSmithKline, Research Triangle Park, NC
Altretamine (N,N,N',N',N'',N''-, hexamethyl-1,3,5-triazine-2, 4, 6- triamine)	Hexalen	US Bioscience, West Conshohocken, PA
Amifostine (ethanethiol, 2-[(3-aminopropyl)amino]-, dihydrogen phosphate (ester))	Ethyol	US Bioscience
Anastrozole (1,3-Benzenediacetonitrile, a, a, a', a'-tetramethyl-5-(1H- 1,2,4-triazol-1-ylmethyl))	Arimidex	AstraZeneca Pharmaceuticals, LP, Wilmington, DE
Arsenic trioxide	Trisenox	Cell Therapeutic, Inc., Seattle, WA
Asparaginase (L-asparagine amidohydrolase, type EC-2)	Elspar	Merck & Co., Inc., Whitehouse Station, NJ
BCG Live (lyophilized preparation of an attenuated strain of <i>Mycobacterium bovis</i> (<i>Bacillus Calmette-Guérin</i> [BCG], substrain Montreal)	TICE BCG	Organon Teknika, Corp., Durham, NC
bexarotene capsules (4-[1-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2- naphthalenyl) ethenyl] benzoic acid)	Targretin	Ligand Pharmaceuticals
bexarotene gel	Targretin	Ligand Pharmaceuticals
Bleomycin (cytotoxic glycopeptide antibiotics produced by <i>Streptomyces verticillus</i> ; bleomycin A ₂ and bleomycin B ₂)	Blenoxane	Bristol-Myers Squibb Co., NY, NY
Capecitabine	Xeloda	Roche

(5'-deoxy-5-fluoro-N-[(pentyloxy)carbonyl]-cytidine)		
Carboplatin (platinum, diammine [1,1-cyclobutanedicarboxylato(2-)-0,0']-, (SP-4-2))	Paraplatin	Bristol-Myers Squibb
Carmustine (1,3-bis(2-chloroethyl)-1-nitrosourea)	BCNU, BiCNU	Bristol-Myers Squibb
Carmustine with Polifeprosan 20 Implant	Gliadel Wafer	Guilford Pharmaceuticals, Inc., Baltimore, MD
Celecoxib (as 4-[5-(4-methylphenyl)-3- (trifluoromethyl)-1H-pyrazol-1-yl] benzenesulfonamide)	Celebrex	Searle Pharmaceuticals, England
Chlorambucil (4-[bis(2chloroethyl)amino]benzenebutanoic acid)	Leukeran	GlaxoSmithKline
Cisplatin (PtCl ₂ H ₆ N ₂)	Platinol	Bristol-Myers Squibb
Cladribine (2-chloro-2'-deoxy-b-D-adenosine)	Leustatin, 2-CdA	R.W. Johnson Pharmaceutical Research Institute, Raritan, NJ
Cyclophosphamide (2-[bis(2-chloroethyl)amino] tetrahydro-2H-13,2-oxazaphosphorine 2-oxide monohydrate)	Cytosan, Neosar	Bristol-Myers Squibb
Cytarabine (1-b-D-Arabinofuranosylcytosine, C ₉ H ₁₃ N ₃ O ₅)	Cytosar-U	Pharmacia & Upjohn Company
cytarabine liposomal	DepoCyt	Skye Pharmaceuticals, Inc., San Diego, CA
Dacarbazine (5-(3,3-dimethyl-1-triazeno)-imidazole-4-carboxamide (DTIC))	DTIC-Dome	Bayer AG, Leverkusen, Germany
Dactinomycin, actinomycin D (actinomycin produced by <i>Streptomyces parvullus</i> , C ₆₂ H ₈₆ N ₁₂ O ₁₆)	Cosmegen	Merck
Darbepoetin alfa (recombinant peptide)	Aranesp	Amgen, Inc., Thousand Oaks, CA
daunorubicin liposomal	DanuoXome	Nexstar

((8S-cis)-8-acetyl-10-[(3-amino-2,3,6-trideoxy- α -L-lyxo-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacenedione hydrochloride)		Pharmaceuticals, Inc., Boulder, CO
Daunorubicin HCl, daunomycin ((1 S ,3 S)-3-Acetyl-1,2,3,4,6,11-hexahydro-3,5,12-trihydroxy-10-methoxy-6,11-dioxo-1-naphthacenyl 3-amino-2,3,6-trideoxy-(α)-L- /yxo -hexopyranoside hydrochloride)	Cerubidine	Wyeth Ayerst, Madison, NJ
Denileukin diftitox (recombinant peptide)	Ontak	Seragen, Inc., Hopkinton, MA
Dexrazoxane ((S)-4,4'-(1-methyl-1,2-ethanediyl)bis-2,6-piperazinedione)	Zinecard	Pharmacia & Upjohn Company
Docetaxel ((2R,3S)-N-carboxy-3-phenylisoserine, N-tert-butyl ester, 13-ester with 5b-20-epoxy-12a,4,7b,10b,13a-hexahydroxytax- 11-en-9-one 4-acetate 2-benzoate, trihydrate)	Taxotere	Aventis Pharmaceuticals, Inc., Bridgewater, NJ
Doxorubicin HCl (8S,10S)-10-[(3-amino-2,3,6-trideoxy- α -L-lyxo-hexopyranosyl)oxy] -8-glycolyl-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacenedione hydrochloride)	Adriamycin, Rubex	Pharmacia & Upjohn Company
doxorubicin	Adriamycin PFS Intravenous injection	Pharmacia & Upjohn Company
doxorubicin liposomal	Doxil	Sequus Pharmaceuticals, Inc., Menlo park, CA
dromostanolone propionate (17b-Hydroxy-2a-methyl-5a-androstan-3-one propionate)	Dromostanolone	Eli Lilly & Company, Indianapolis, IN
dromostanolone propionate	Masterone injection	Syntex, Corp., Palo Alto, CA
Elliott's B Solution	Elliott's B Solution	Orphan Medical, Inc
Epirubicin ((8S-cis)-10-[(3-amino-2,3,6-trideoxy- α -L-arabino-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-8- (hydroxyacetyl)-1-methoxy-5,12-naphthacenedione hydrochloride)	Ellence	Pharmacia & Upjohn Company
Epoetin alfa	Epogen	Amgen, Inc

(recombinant peptide)		
Estramustine (estra-1,3,5(10)-triene-3,17-diol(17(beta))- 3-[bis(2-chloroethyl)carbamate] 17-(dihydrogen phosphate), disodium salt, monohydrate, or estradiol 3-[bis(2-chloroethyl)carbamate] 17-(dihydrogen phosphate), disodium salt, monohydrate)	Emcyt	Pharmacia & Upjohn Company
Etoposide phosphate (4'-Demethylepipodophyllotoxin 9-[4,6-O-(R)-ethylidene-(beta)-D-glucopyranoside], 4'-(dihydrogen phosphate))	Etopophos	Bristol-Myers Squibb
etoposide, VP-16 (4'-demethylepipodophyllotoxin 9-[4,6-O-(R)-ethylidene-(beta)-D-glucopyranoside])	Vepesid	Bristol-Myers Squibb
Exemestane (6-methylenandrosta-1,4-diene-3, 17-dione)	Aromasin	Pharmacia & Upjohn Company
Filgrastim (r-metHuG-CSF)	Neupogen	Amgen, Inc
floxuridine (intraarterial) (2'-deoxy-5-fluorouridine)	FUDR	Roche
Fludarabine (fluorinated nucleotide analog of the antiviral agent vidarabine, 9-b -D-arabinofuranosyladenine (ara-A))	Fludara	Berlex Laboratories, Inc., Cedar Knolls, NJ
Fluorouracil, 5-FU (5-fluoro-2,4(1H,3H)-pyrimidinedione)	Adrucil	ICN Pharmaceuticals, Inc., Humacao, Puerto Rico
Fulvestrant (7-alpha-[9-(4,4,5,5,5-penta fluoropentylsulphonyl) nonyl]estra-1,3,5-(10)- triene-3,17-beta-diol)	Faslodex	IPR Pharmaceuticals, Guayama, Puerto Rico
Gemcitabine (2'-deoxy-2', 2'-difluorocytidine monohydrochloride (b-isomer))	Gemzar	Eli Lilly
Gemtuzumab Ozogamicin (anti-CD33 hP67.6)	Mylotarg	Wyeth Ayerst
Goserelin acetate	Zoladex Implant	AstraZeneca Pharmaceuticals
Hydroxyurea	Hydrea	Bristol-Myers Squibb
Ibritumomab Tiuxetan (immunoconjugate resulting from a thiourea covalent bond between the monoclonal antibody Ibritumomab and the	Zevalin	Biogen IDEC, Inc., Cambridge MA

linker-chelator tiuxetan [N-[2-bis(carboxymethyl)amino]-3-(p-isothiocyanatophenyl)-propyl]-[N-[2-bis(carboxymethyl)amino]-2-(methyl)-ethyl]glycine)		
Idarubicin (5, 12-Naphthacenedione, 9-acetyl-7-[(3-amino-2,3,6-trideoxy-(alpha)-L- lyxo -hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,9,11-trihydroxyhydrochloride, (7S- cis))	Idamycin	Pharmacia & Upjohn Company
Ifosfamide (3-(2-chloroethyl)-2-[(2-chloroethyl)amino]tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide)	IFEX	Bristol-Myers Squibb
Imatinib Mesilate (4-[(4-Methyl-1-piperazinyl)methyl]-N-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-phenyl]benzamide methanesulfonate)	Gleevec	Novartis AG, Basel, Switzerland
Interferon alfa-2a (recombinant peptide)	Roferon-A	Hoffmann-La Roche, Inc., Nutley, NJ
Interferon alfa-2b (recombinant peptide)	Intron A (Lyophilized Betaseron)	Schering AG, Berlin, Germany
Irinotecan HCl ((4S)-4,11-diethyl-4-hydroxy-9-[(4- piperidinopiperidino)carbonyloxy]-1H-pyrano[3', 4': 6,7] indolizino[1,2-b] quinoline-3,14(4H, 12H) dione hydrochloride trihydrate)	Camptosar	Pharmacia & Upjohn Company
Letrozole (4,4'-(1H-1,2,4 -Triazol-1-ylmethylene) dibenzonitrile)	Femara	Novartis
Leucovorin (L-Glutamic acid, N[4[[[(2-amino-5-formyl-1,4,5,6,7,8 -hexahydro-4-oxo-6-pteridiny]methyl]amino]benzoyl], calcium salt (1:1))	Wellcovorin, Leucovorin	Immunex, Corp., Seattle, WA
Levamisole HCl ((-)-(S)-2,3,5, 6-tetrahydro-6-phenylimidazo [2,1-b] thiazole monohydrochloride C ₁₁ H ₁₂ N ₂ S·HCl)	Ergamisol	Janssen Research Foundation, Titusville, NJ
Lomustine (1-(2-chloro-ethyl)-3-cyclohexyl-1-nitrosourea)	CeeNU	Bristol-Myers Squibb
Mecllorethamine, nitrogen mustard (2-chloro-N-(2-chloroethyl)-N-methylethanamine hydrochloride)	Mustargen	Merck

Megestrol acetate 17 α (acetyloxy)- 6- methylpregna- 4,6- diene- 3,20- dione	Megace	Bristol-Myers Squibb
Melphalan, L-PAM (4-[bis(2-chloroethyl) amino]-L-phenylalanine)	Alkeran	GlaxoSmithKline
Mercaptopurine, 6-MP (1,7-dihydro-6 H -purine-6-thione monohydrate)	Purinethol	GlaxoSmithKline
Mesna (sodium 2-mercaptoethane sulfonate)	Mesnex	Asta Medica
Methotrexate (N-[4-[(2,4-diamino-6-pteridiny)methyl]methylamino]benzoyl]-L-glutamic acid)	Methotrexate	Lederle Laboratories
Methoxsalen (9-methoxy-7H-furo[3,2-g][1]-benzopyran-7-one)	Uvadex	Therakos, Inc., Way Exton, Pa
Mitomycin C	Mutamycin	Bristol-Myers Squibb
mitomycin C	Mitozytrex	SuperGen, Inc., Dublin, CA
Mitotane (1,1-dichloro-2-(o-chlorophenyl)-2-(p-chlorophenyl) ethane)	Lysodren	Bristol-Myers Squibb
Mitoxantrone (1,4-dihydroxy-5,8-bis[[2- [(2-hydroxyethyl)amino]ethyl]amino]-9,10-anthracenedione dihydrochloride)	Novantrone	Immunex Corporation
Nandrolone phenpropionate	Durabolin-50	Organon, Inc., West Orange, NJ
Nofetumomab	Verluma	Boehringer Ingelheim Pharma KG, Germany
Oprelvekin (IL-11)	Neumega	Genetics Institute, Inc., Alexandria, VA
Oxaliplatin (cis-[(1R,2R)-1,2-cyclohexanediamine-N,N'] [oxalato(2-)-O,O'] platinum)	Eloxatin	Sanofi Synthelabo, Inc., NY, NY
Paclitaxel (5 β , 20-Epoxy-1,2a, 4,7 β , 10 β , 13a-hexahydroxytax-11-en-9-one 4,10-diacetate 2- benzoate 13-ester with (2R, 3 S)-N-benzoyl-3-phenylisoserine)	TAXOL	Bristol-Myers Squibb
Pamidronate (phosphonic acid (3-amino-1-hydroxypropylidene) bis-, disodium salt, pentahydrate, (APD))	Aredia	Novartis

Pegademase (monomethoxypolyethylene glycol succinimidyl 11 - 17 - adenosine deaminase)	Adagen (Pegademase Bovine)	Enzon Pharmaceuticals, Inc., Bridgewater, NJ
Pegaspargase (monomethoxypolyethylene glycol succinimidyl L- asparaginase)	Oncaspar	Enzon
Pegfilgrastim (covalent conjugate of recombinant methionyl human G-CSF (Filgrastim) and monomethoxypolyethylene glycol)	Neulasta	Amgen, Inc
Pentostatin	Nipent	Parke-Davis Pharmaceutical Co., Rockville, MD
Pipobroman	Vercyte	Abbott Laboratories, Abbott Park, IL
Plicamycin, Mithramycin (antibiotic produced by <i>Streptomyces plicatus</i>)	Mithracin	Pfizer, Inc., NY, NY
Porfimer sodium	Photofrin	QLT Phototherapeutics, Inc., Vancouver, Canada
Procarbazine (N-isopropyl-μ-(2-methylhydrazino)-p-toluamide monohydrochloride)	Matulane	Sigma Tau Pharmaceuticals, Inc., Gaithersburg, MD
Quinacrine (6-chloro-9-(1 –methyl-4-diethyl-amine) butylamino-2-methoxyacridine)	Atabrine	Abbott Labs
Rasburicase (recombinant peptide)	Elitek	Sanofi-Synthelabo, Inc.,
Rituximab (recombinant anti-CD20 antibody)	Rituxan	Genentech, Inc., South San Francisco, CA
Sargramostim (recombinant peptide)	Prokine	Immunex Corp
Streptozocin (streptozocin 2 –deoxy - 2 - [[[(methylnitrosoamino)carbonyl]amino] - a(and b) - D - glucopyranose and 220 mg citric acid anhydrous)	Zanosar	Pharmacia & Upjohn Company
Talc (Mg ₃ Si ₄ O ₁₀ (OH) ₂)	Sclerosol	Bryan, Corp., Woburn, MA

Tamoxifen ((Z)-2-[4-(1,2-diphenyl-1-butenyl) phenoxy]-N, N-dimethylethanamine 2-hydroxy-1,2,3-propanetricarboxylate (1:1))	Nolvadex	AstraZeneca Pharmaceuticals
Temozolomide (3,4-dihydro-3-methyl-4-oxoimidazo[5,1-d]-as-tetrazine-8-carboxamide)	Temodar	Schering
teniposide, VM-26 (4'-demethylepipodophyllotoxin 9-[4,6-O-(R)-2- thenylidene-(beta)-D-glucopyranoside])	Vumon	Bristol-Myers Squibb
Testolactone (13-hydroxy-3-oxo-13,17-secoandrosta-1,4-dien-17-oic acid [dgr]-lactone)	Teslac	Bristol-Myers Squibb
Thioguanine, 6-TG (2-amino-1,7-dihydro-6 H - purine-6-thione)	Thioguanine	GlaxoSmithKline
Thiotepa (Aziridine, 1,1',1"-phosphinothiolyldynetris-, or Tris (1-aziridinyl) phosphine sulfide)	Thioplex	Immunex Corporation
Topotecan HCl ((S)-10-[(dimethylamino) methyl]-4-ethyl-4,9-dihydroxy-1H-pyrano[3', 4': 6,7] indolizino [1,2-b] quinoline-3,14-(4H,12H)-dione monohydrochloride)	Hycamtin	GlaxoSmithKline
Toremifene (2-(p-[(Z)-4-chloro-1,2-diphenyl-1-butenyl]-phenoxy)-N,N-dimethylethylamine citrate (1:1))	Fareston	Roberts Pharmaceutical Corp., Eatontown, NJ
Tositumomab, I 131 Tositumomab (recombinant murine immunotherapeutic monoclonal IgG _{2a} lambda anti-CD20 antibody (I 131 is a radioimmunotherapeutic antibody))	Bexxar	Corixa Corp., Seattle, WA
Trastuzumab (recombinant monoclonal IgG ₁ kappa anti-HER2 antibody)	Herceptin	Genentech, Inc
Tretinoin, ATRA (all-trans retinoic acid)	Vesanoid	Roche
Uracil Mustard	Uracil Mustard Capsules	Roberts Labs
Valrubicin, N-trifluoroacetyladiamycin-14-valerate ((2S-cis)-2- [1,2,3,4,6,11-hexahydro-2,5,12-trihydroxy-7-methoxy-6,11-dioxo-[[4 2,3,6-trideoxy-3- [(trifluoroacetyl)-	Valstar	Anthra --> Medeva

amino- α -L-lyxo-hexopyranosyl]oxyl]-2-naphthaceny]-2-oxoethyl pentanoate)		
Vinblastine, Leurocristine (C ₄₆ H ₅₆ N ₄ O ₁₀ •H ₂ SO ₄)	Velban	Eli Lilly
Vincristine (C ₄₆ H ₅₆ N ₄ O ₁₀ •H ₂ SO ₄)	Oncovin	Eli Lilly
Vinorelbine (3',4'-didehydro-4'-deoxy-C'-norvincal leukoblastine [R-(R*,R*)-2,3-dihydroxybutanedioate (1:2)(salt)])	Navelbine	GlaxoSmithKline
Zoledronate, Zoledronic acid ((1-Hydroxy-2-imidazol-1-yl-phosphonoethyl) phosphonic acid monohydrate)	Zometa	Novartis

Anticancer agents further include compounds which have been identified to have anticancer activity. Examples include, but are not limited to, 3-AP, 12-O-tetradecanoylphorbol-13-acetate, 17AAG, 852A, ABI-007, ABR-217620, ABT-751, ADI-PEG 20, AE-941, AG-013736, AGRO100, alanosine, AMG 706, antibody G250, antineoplastons, AP23573, apaziquone, APC8015, atiprimod, ATN-161, atrasenten, azacitidine, BB-10901, BCX-1777, bevacizumab, BG00001, bicalutamide, BMS 247550, bortezomib, bryostatin-1, buserelin, calcitriol, CCI-779, CDB-2914, cefixime, cetuximab, CG0070, cilengitide, clofarabine, combretastatin A4 phosphate, CP-675,206, CP-724,714, CpG 7909, curcumin, decitabine, DENSPM, doxercalciferol, E7070, E7389, ecteinascidin 743, efaproxiral, eflornithine, EKB-569, enzastaurin, erlotinib, exisulind, fenretinide, flavopiridol, fludarabine, flutamide, fotemustine, FR901228, G17DT, galiximab, gefitinib, genistein, glufosfamide, GTI-2040, histrelin, HKI-272, homoharringtonine, HSPPC-96, hu14.18-interleukin-2 fusion protein, HuMax-CD4, iloprost, imiquimod, infliximab, interleukin-12, IPI-504, irofulven, ixabepilone, lapatinib, lenalidomide, lestaurtinib, leuprolide, LMB-9 immunotoxin, lonafarnib, luniliximab, mafosfamide, MB07133, MDX-010, MLN2704, monoclonal antibody 3F8, monoclonal antibody J591, motexafin, MS-275, MVA-MUC1-IL2, nilutamide, nitrocamptothecin, nolatrexed dihydrochloride, nolvadex, NS-9, O6-benzylguanine, oblimersen sodium, ONYX-015, oregovomab, OSI-774, panitumumab, paraplantin, PD-0325901, pemetrexed, PHY906, pioglitazone, pirfenidone, pixantrone, PS-341, PSC 833, PXD101, pyrazoloacridine, R115777, RAD001, ranpirnase, rebeccamycin analogue, rhuAngiostatin protein, rhuMab 2C4, rosiglitazone, rubitecan, S-1, S-8184, satraplatin, SB-, 15992, SGN-0010, SGN-40, sorafenib, SR31747A, ST1571, SU011248,

suberoylanilide hydroxamic acid, suramin, talabostat, talampanel, tariquidar, temsirolimus, TGF α -PE38 immunotoxin, thalidomide, thymalfasin, tipifarnib, tirapazamine, TLK286, trabectedin, trimetrexate glucuronate, TroVax, UCN-1, valproic acid, vinflunine, VNP40101M, volociximab, vorinostat, VX-680, ZD1839, ZD6474, zileuton, and zosuquidar trihydrochloride.

For a more detailed description of anticancer agents and other therapeutic agents, those skilled in the art are referred to any number of instructive manuals including, but not limited to, the Physician's Desk Reference and to Goodman and Gilman's "Pharmaceutical Basis of Therapeutics" tenth edition, Eds. Hardman *et al.*, 2002.

The present invention provides methods for administering a Hh inhibitor with radiation therapy. The invention is not limited by the types, amounts, or delivery and administration systems used to deliver the therapeutic dose of radiation to an animal. For example, the animal may receive photon radiotherapy, particle beam radiation therapy, other types of radiotherapies, and combinations thereof. In some embodiments, the radiation is delivered to the animal using a linear accelerator. In still other embodiments, the radiation is delivered using a gamma knife.

The source of radiation can be external or internal to the animal. External radiation therapy is most common and involves directing a beam of high-energy radiation to a tumor site through the skin using, for instance, a linear accelerator. While the beam of radiation is localized to the tumor site, it is nearly impossible to avoid exposure of normal, healthy tissue. However, external radiation is usually well tolerated by animals. Internal radiation therapy involves implanting a radiation-emitting source, such as beads, wires, pellets, capsules, particles, and the like, inside the body at or near the tumor site including the use of delivery systems that specifically target cancer cells (*e.g.*, using particles attached to cancer cell binding ligands). Such implants can be removed following treatment, or left in the body inactive. Types of internal radiation therapy include, but are not limited to, brachytherapy, interstitial irradiation, intracavity irradiation, radioimmunotherapy, and the like.

The animal may optionally receive radiosensitizers (*e.g.*, metronidazole, misonidazole, intra-arterial Budr, intravenous iododeoxyuridine (IudR), nitroimidazole, 5-substituted-4-nitroimidazoles, 2H-isoindolediones, [[(2-bromoethyl)-amino]methyl]-nitro-1H-imidazole-1-ethanol, nitroaniline derivatives, DNA-affinic hypoxia selective cytotoxins, halogenated DNA ligand, 1,2,4 benzotriazine oxides, 2-nitroimidazole derivatives, fluorine-containing nitroazole derivatives, benzamide, nicotinamide, acridine-intercalator, 5-thiotetrazole derivative, 3-nitro-1,2,4-triazole, 4,5-dinitroimidazole derivative, hydroxylated

texaphrins, cisplatin, mitomycin, tiripazamine, nitrosourea, mercaptopurine, methotrexate, fluorouracil, bleomycin, vincristine, carboplatin, epirubicin, doxorubicin, cyclophosphamide, vindesine, etoposide, paclitaxel, heat (hyperthermia), and the like), radioprotectors (*e.g.*, cysteamine, aminoalkyl dihydrogen phosphorothioates, amifostine (WR 2721), IL-1, IL-6, and the like). Radiosensitizers enhance the killing of tumor cells. Radioprotectors protect healthy tissue from the harmful effects of radiation.

Any type of radiation can be administered to an animal, so long as the dose of radiation is tolerated by the animal without unacceptable negative side-effects. Suitable types of radiotherapy include, for example, ionizing (electromagnetic) radiotherapy (*e.g.*, X-rays or gamma rays) or particle beam radiation therapy (*e.g.*, high linear energy radiation). Ionizing radiation is defined as radiation comprising particles or photons that have sufficient energy to produce ionization, *i.e.*, gain or loss of electrons (as described in, for example, U.S. 5,770,581 incorporated herein by reference in its entirety). The effects of radiation can be at least partially controlled by the clinician. In one embodiment, the dose of radiation is fractionated for maximal target cell exposure and reduced toxicity.

In one embodiment, the total dose of radiation administered to an animal is about .01 Gray (Gy) to about 100 Gy. In another embodiment, about 10 Gy to about 65 Gy (*e.g.*, about 15 Gy, 20 Gy, 25 Gy, 30 Gy, 35 Gy, 40 Gy, 45 Gy, 50 Gy, 55 Gy, or 60 Gy) are administered over the course of treatment. While in some embodiments a complete dose of radiation can be administered over the course of one day, the total dose is ideally fractionated and administered over several days. Desirably, radiotherapy is administered over the course of at least about 3 days, *e.g.*, at least 5, 7, 10, 14, 17, 21, 25, 28, 32, 35, 38, 42, 46, 52, or 56 days (about 1-8 weeks). Accordingly, a daily dose of radiation will comprise approximately 1-5 Gy (*e.g.*, about 1 Gy, 1.5 Gy, 1.8 Gy, 2 Gy, 2.5 Gy, 2.8 Gy, 3 Gy, 3.2 Gy, 3.5 Gy, 3.8 Gy, 4 Gy, 4.2 Gy, or 4.5 Gy), or 1-2 Gy (*e.g.*, 1.5-2 Gy). The daily dose of radiation should be sufficient to induce destruction of the targeted cells. If stretched over a period, in one embodiment, radiation is not administered every day, thereby allowing the animal to rest and the effects of the therapy to be realized. For example, radiation desirably is administered on 5 consecutive days, and not administered on 2 days, for each week of treatment, thereby allowing 2 days of rest per week. However, radiation can be administered 1 day/week, 2 days/week, 3 days/week, 4 days/week, 5 days/week, 6 days/week, or all 7 days/week, depending on the animal's responsiveness and any potential side effects. Radiation therapy can be initiated at any time in the therapeutic period. In one embodiment, radiation is initiated in week 1 or week 2, and is administered for the remaining duration of the therapeutic period. For

example, radiation is administered in weeks 1-6 or in weeks 2-6 of a therapeutic period comprising 6 weeks for treating, for instance, a solid tumor. Alternatively, radiation is administered in weeks 1-5 or weeks 2-5 of a therapeutic period comprising 5 weeks. These exemplary radiotherapy administration schedules are not intended, however, to limit the present invention.

Antimicrobial therapeutic agents may also be used as therapeutic agents in the present invention. Any agent that can kill, inhibit, or otherwise attenuate the function of microbial organisms may be used, as well as any agent contemplated to have such activities.

Antimicrobial agents include, but are not limited to, natural and synthetic antibiotics, antibodies, inhibitory proteins (*e.g.*, defensins), antisense nucleic acids, membrane disruptive agents and the like, used alone or in combination. Indeed, any type of antibiotic may be used including, but not limited to, antibacterial agents, antiviral agents, antifungal agents, and the like.

Disclosed herein, in certain embodiments, are systems of assessing an individual having a cancer associated with Hh pathway activity (*e.g.*, NSCLC) for treatment comprising: (a) a digital processing device comprising an operating system configured to perform executable instructions, and an electronic memory; (b) a dataset stored in the electronic memory, wherein the dataset comprises data for one or more biomarker gene expression profiles in a sample; and (c) a computer program including instructions executable by the digital processing device to create an application comprising: (i) a first software module configured to analyze the dataset for similarity with a reference gene expression profile for cancer cells associated with Hh pathway activity and primary cilia expression; and (ii) a second software module to assign the individual as a candidate for treatment with a Hh inhibitor if there is a similarity between the determined gene expression profile and a reference gene expression profile for cancer cells associated with Hh pathway activity and primary cilia expression.

In some embodiments, the systems and methods described herein include a digital processing device, or use of the same. In further embodiments, the digital processing device includes one or more hardware central processing units (CPU) that carry out the device's functions. In still further embodiments, the digital processing device further comprises an operating system configured to perform executable instructions. In some embodiments, the digital processing device is optionally connected to a computer network. In further embodiments, the digital processing device is optionally connected to the Internet such that it accesses the World Wide Web. In still further embodiments, the digital processing device is

optionally connected to a cloud computing infrastructure. In other embodiments, the digital processing device is optionally connected to an intranet. In other embodiments, the digital processing device is optionally connected to a data storage device.

In accordance with the description herein, suitable digital processing devices include, by way of non-limiting examples, server computers, desktop computers, laptop computers, notebook computers, sub-notebook computers, netbook computers, netpad computers, set-top computers, media streaming devices, handheld computers, Internet appliances, mobile smartphones, tablet computers, personal digital assistants, video game consoles, and vehicles. Those of skill in the art will recognize that many smartphones are suitable for use in the system described herein. Those of skill in the art will also recognize that select televisions, video players, and digital music players with optional computer network connectivity are suitable for use in the system described herein. Suitable tablet computers include those with booklet, slate, and convertible configurations, known to those of skill in the art.

EXPERIMENTAL

The following examples are provided in order to demonstrate and further illustrate certain preferred embodiments and aspects of the present invention and are not to be construed as limiting the scope thereof.

Example I.

Hh-targeted inhibitors predicted to be cilia-dependent include, but are not limited to, Hh-ligand antagonists (e.g. 5E1 or robotnikinin), Smo antagonists (e.g. Vismodegib, IPI-926, HhAntag), and Gli-processing inhibitors (e.g. HPI-2,3) (Fig. 2). These inhibitors all involve inhibiting components of the Hh pathway that require cilia (see, e.g., Hassounah, N.B., et al., 2012 Clin Cancer Res 18, 2429-2435). Therefore, it is predicted that these inhibitors will be effective only if the tumor cells activate the Hh pathway via a cilia-dependent mechanism. All of the Smo inhibitors that are currently being evaluated in clinical trials (Vismodegib (Genentech), NVP-LDE225 (Novartis), IPI-926 (Infinity), and XL-139 (BMS/Exelixis)) are in this cilia-dependent class. Clinical trials utilizing Hh inhibitors are not considering cilia status of cancer cells as a criterion for determining the most effective Hh inhibitor.

Vismodegib is a Smo inhibitor and was recently shown to cause rapid tumor regression in patients with advanced basal cell carcinoma (BCC) (see, e.g., Von Hoff, D.D., et al., 2009 N Engl J Med 361, 1164-1172). Vismodegib was the first Hh inhibitor to be

approved by the FDA for treatment of BCC. However, Vismodegib is not efficacious in all BCC patients and some patients that initially responded to treatment have been documented to relapse (see, e.g., Chang, A.L., et al., (2014) *J Am Acad Dermatol* 70, 60-69; Von Hoff, D.D., et al., 2009 *N Engl J Med* 361, 1164-1172). Since being approved for treatment of BCC, Vismodegib has gone on to be investigated in 45 clinical trials involving over 18 different types of cancer. Note that NSCLC was not among the cancer types included in these clinical trials. However, several of these trials have been stopped due to poor clinical outcome. Long-term exposure to Vismodegib has been shown to result in resistant cancer cells that acquired novel mutations, which block the binding of Smo to Vismodegib (see, e.g., Rudin, C.M., et al., 2009 *N Engl J Med* 361, 1173-1178). These results explain how some patients acquire resistance to Vismodegib over time; however, these types of Smo mutations have not been observed in primary cancers and hence do not explain why some Hh-active patients do not respond to initial treatment.

Experiments conducted during the course of developing embodiments for the present invention demonstrate that the reason Vismodegib is not effective in some cancers is because such cancers activate the Hh pathway in a cilia-independent manner and do not have a significant population of ciliated cancer cells.

The percentage of ciliated cancer cells associated with response to the Smo inhibitor Vismodegib was analyzed. Primary BCC tumor samples from patients on a clinical trial with Vismodegib (Von Hoff, D.D., et al., 2009 *N Engl J Med* 361, 1164-1172) were stained. The patient samples were taken prior to treatment with Vismodegib and were stained for markers of primary cilia and then normalized to cell cycle (%Ki67 positive cancer cells) for each patient to obtain a cilia score. It was found that BCC patients with best response (complete response) to Vismodegib have the highest cilia score (median: 86%) compared to patients with partial response (median: 66%), stable disease (median: 58%) and progressive disease (median: 37%) (n=20; Fig. 3). This indicates that Smo inhibitors are cilia- dependent and suggests that BCC cancers with the highest percentage of ciliated cells are those that will benefit most from Vismodegib treatment. The significance of this observation is two-fold. First, this demonstrates that cilia status can serve as a predictive biomarker for Hh inhibitor efficacy. Second, these findings indicate that cilia status modulates tumor response to Hh inhibitors.

High Hh activity has been associated with poor overall survival and decreased relapse- free survival in many cancers including BCC, bladder cancer, pancreatic cancer, medulloblastoma and NSCLC (see, e.g., Raz G, et al., 76 *Lung Cancer* 2012, pages 191-196;

Chen, M., et al., 2010 *Cancer Prev Res (Phila)* 3, 1235-1245; Dai, J., et al., 2011 *Pancreas* 40, 233-236; Al-Halabi, H., 2011 *Acta Neuropathol* 121, 229-239). Therefore, such results indicate a significant impact by making important advances in the therapy of the many cancer types with upregulated Hh signaling. Such results directly address efficacy of Hh inhibitors in NSCLC; which, account for the greatest number of cancer-associated deaths worldwide each year. Based on this data, ~36% of all NSCLC patients have primary cilia and would benefit from Hh-targeted therapy.

Example II.

It has recently been demonstrated that the Hh signaling pathway is active in ~50% of early-stage NSCLC patient samples (see, e.g., Raz G, et al., 76 *Lung Cancer* 2012, pages 191-196). In this NSCLC cohort, *Ptch* (a Hh responsive gene indicating increased Hh signaling) correlated with decreased overall survival and decreased relapse-free survival. Such data suggest that these patients would benefit from treatment with a Hh inhibitor. The data on primary cilia highlight the importance of determining if NSCLC activate the Hh pathway via a cilia-dependent mechanism. Determining the general prevalence of NSCLC patients whose tumors are positive or negative for cilia will aid in the design and selection of patients for Hh-targeted therapies. Experiments conducted during the course of developing embodiments for the present invention strongly suggests that this strategy will expand the field of use of the Hh inhibitor Vismodegib.

Hh activity is characterized by increased transcription and expression of *Gli1* and *Ptch1*. Experiments conducted during the course of developing embodiments for the present invention demonstrates that of NSCLC cases with >25% staining intensity (based on general intensity scoring) of *Gli1* and/or *Ptch1* there was a significant correlation with *Smo* ($p=0.007$) and *Shh* ($p=0.001$) expression (see, e.g., Raz G, et al., 76 *Lung Cancer* 2012, pages 191-196). This correlation suggests a dependence on *Smo*, and therefore cilia, for activation of the Hh pathway in NSCLC (Fig. 1C). Based on this data, it was hypothesized that a subset of NSCLC activate the Hh pathway via a cilia-dependent mechanism and that these patients are positive for primary cilia.

Gli1, *Ptch1*, *Smo* and *Shh* markers alone are not sufficient to predict efficacy with the Hh-specific *Smo* inhibitor Vismodegib in clinical trials (Von Hoff, D.D., et al., 2009 *N Engl J Med* 361, 1164-1172). Demonstrating presence of primary cilia in these Hh-positive cases would provide further evidence of *Smo* activity in these patients and provide justification for clinical trials with the FDA approved *Smo* inhibitor Vismodegib.

NSCLC patient samples were stained for markers of primary cilia and it was found that 8 out of 25 NSCLC samples express primary cilia (Fig. 4A). A NSCLC cell line (A549 cells) was identified that is responsive to the cilia- dependent Smo inhibitor Vismodegib (see, e.g., Shi, S., et al., 2012 J Biol Chem 287, 7845-7858). A549 cells were obtained and utilized to demonstrate that these cells express primary cilia (Fig. 5B). Such data supports the hypothesis that Hh signaling, in a subset of Hh-active NSCLC patients, is activated via a cilia-dependent mechanism.

Example III.

This example describes the experimental design for additional experiments related to Example II.

Tissue: Clinically annotated NSCLC samples for expression of primary cilia will be examined. Such experiments will also correlate this with expression of Gli1, Ptch1, Smo and Shh. Tissue microarrays (TMA) with NSCLC samples used in a previously described Hh marker study (see, e.g., Raz G, et al., 76 Lung Cancer 2012, pages 191-196) have been obtained. The TMAs have duplicate 1mm cores representing a total of 245 NSCLC cases. The TMA includes 64.5% (n=158) adenocarcinoma, 26.9% (n=66) squamous cell carcinoma (SCC), and 21 of 'other' types of NSCLC. The majority (77.6%) had stage I disease and the rest are stage II disease. 93.6% of the NSCLCs are from current or former smokers. These patients were diagnosed between 2001 and 2007 and they are fully annotated for age at diagnosis, gender, histologic subtype, stage, smoking history, relapse-free survival and overall survival.

R&D, Staining and Imaging: Serial sections of each TMA have been obtained. The first slide will be stained for H&E in order to annotate the cancer pathology. The H&E stained slide will be used to find the areas of interest to focus on in the next serial section, which will be stained for markers of primary cilia. Each tissue core of the digitally scanned TMA will be annotated and detailed histologic information provided. The second serial sections will be stained with antibodies to detect cilia (anti-acetylated-tubulin antibody to recognize the ciliary axoneme and anti-gamma (γ)-tubulin antibody to recognize the centrosome) and to co-stain for a marker of activated Hh signaling (anti-Ptch1 antibody or anti-Gli1 antibody). Ptch1 was chosen as the third marker because 48.5% of the 245 NSCLC cases were shown to be Ptch1 positive and these had a significant correlation with Gli1, Smo and Shh. These three primary antibodies are all anti-mouse antibodies but all have different isotypes, which allows us to utilize three different fluorescently conjugated

secondary antibodies (Alexa-488, Alexa-564 and Alexa-633) to distinguish between primary antibodies. All tissue cores will be imaged using a Leica Confocal Microscope located at the University of Arizona Cancer Center. Such experiments will image with a 60X objective and obtain three fields/core for each patient to obtain maximum coverage.

5 See Fig. 1 for example of fluorescent cilia staining in paraffin tissue sections from the NSCLC cohort. The third serial section will be stained with an antibody to recognize Ki67 as a marker of proliferation. Such experiments will use standard immunohistological staining of each TMA for Ki67 as done previously (see, e.g., Hassounah, N.B., et al., 2013 PLoS ONE 8, e68521).

10 **Staining Analysis:** The Ptch1-positive and Ptch1-negative cancer cells with cilia will be scored by manually counting cilia per nuclei and calculating the percentage of cancer cells that are both cilia-positive and Ptch- positive. Note that a cell will only be considered cilia positive if both acetylated and γ -tubulin signal are detected together. The serial adjacent slides stained for Ki67 will be digitally scanned and analyzed using
15 Definiens Software. This software allows for unbiased, automated scoring of the fraction of Ki67 positive nuclei in regions of interest. Cilia are found on cells in G0 or early G1 of the cell cycle. Therefore, low percent cilia score could be due to a high percentage of proliferative cells. In order to interpret the percentage of cilia such experiments will add the percent cilia of cancer cells to the percent Ki67 for each imaged region to obtain a
20 normalized ‘cilia score’. Thus, a low ‘cilia score’ will indicate loss of cilia expression for reasons unrelated to increased cell proliferation.

Cilia scores will be grouped into four categories (>25%, 25-50%, 51-75% and 76-100%). Such experiments will correlate the four cilia score categories directly with Ptch1 positivity. Such experiments will also utilize previous data generated on these TMAs to
25 correlate cilia score categories with Gli1, Smo and Shh staining intensity (see, e.g., Raz G, et al., 76 Lung Cancer 2012, pages 191-196). The 4 cilia score categories will also be correlated with overall survival and relapse-free survival to determine if cilia status influences the aggressiveness of the disease and is useful as a novel prognostic marker.

Based on preliminary data, it is expected that roughly 50% of the NSCLC samples
30 will express markers of activated Hh signaling, resulting in 119 patient samples for assessment of percent cilia in Hh-active cases. Since the Spearman correlation coefficient is the Pearson correlation coefficient of the ranks, this sample size will allow us to detect a Spearman correlation of 0.25 or greater with 80% statistical power (assuming a two- sided alpha level of 0.05).

Based on preliminary data demonstrating Smo and Shh correlates with downstream activity in the Hh pathway, such experiments are expected to find that patient samples that are positive for Smo, Shh, Gli1 and/or Ptch1 activate the Hh pathway via primary cilia and therefore these patients will have a high cilia score. There is also preliminary data

5 demonstrating that Hh-positive NSCLC has a high percentage of ciliated cancer cells further supporting the hypothesis. As described, such experiments will correlate cilia score with pathologic subtype (adenocarcinoma, squamous cell carcinoma and other), age at diagnosis, gender, smoking history, disease stage, relapse-free survival and overall survival. High Hh activity has been associated with poor overall survival and decreased relapse-free

10 survival in bladder cancer, pancreatic adenocarcinoma and medulloblastoma (see, e.g., Chen, M., et al., 2010 Cancer Prev Res (Phila) 3, 1235-1245; Dai, J., et al., 2011 Pancreas 40, 233-236; Al-Halabi, H., et al., 2011 Acta Neuropathol 121, 229-239). In the NSCLC cohort, Ptch1 correlated with decreased overall survival and decreased relapse-free survival (see, e.g., Yuan, Z., et al., 2007 Oncogene 26, 1046-1055). Therefore, if such experiments

15 see that the Hh pathway is activated in these patients via a cilia-dependent mechanism it is also predict that the highest cilia scores will correlate with poor overall survival and decreased relapse-free survival. The opposite correlation is predicted if it is found that the Hh pathway is activated in patients via a cilia-independent mechanism. Results demonstrating that Hh-active NSCLC samples have a high percentage of ciliated cancer

20 cells will provide justification to move forward with a clinical trial using the FDA approved Hh-targeted drug Vismodegib (cilia-dependent) and preliminary data supports this expectation. Indeed, preliminary data suggests that ~36% of NLCLC cases are cilia-positive. Because the preliminary data indicates that not all NLCLC cases are cilia-positive it is predicted that the results will demonstrate absence of cilia in a subset of NSCLC cases.

Example IV.

In NSCLC, Hh signaling promotes proliferation and tumorigenesis *in vitro* and *in vivo* (see, e.g., Schidlow, D.V. (1994) Ann Allergy 73, 457-468; quiz 468-470). In addition, downregulation of Hh signaling using small molecule inhibitors (Vismodegib,

30 HhAntag, GANT61) or shRNAs targeting Gli1 or Smo were able to decrease cell viability and cell migration of NSCLC *in vitro* and reduce tumor growth of NSCLC models *in vivo*. Experiments conducted for the present invention demonstrated that, within a NSCLC cohort, high Hh activity correlated with decreased overall survival and decreased relapse-free survival. These data are consistent with the hypothesis that some NSCLC require Hh

signaling for tumorigenesis. Such results further suggests that patients with high Hh signaling would benefit from treatment with an Hh inhibitor. However, clinical trials using Vismodegib to treat BCC patients based on markers of high Hh pathway activity (Gli1 or Smo activating mutations) had mixed results indicating that these markers are not
5 sufficient to predict which patients will respond to drug. Therefore, a better understanding of the mechanisms of Vismodegib response/resistance are urgently needed to better target this drug (and other Hh inhibitors) to the correct population of patients. Hh signaling can be activated in cancer cells in a cilia-dependent and cilia-independent manner. It was hypothesized that the Hh activating protein Smo is cilia-dependent and therefore Smo
10 inhibitors such as Vismodegib will only be effective at treating cilia-positive cancer cells. Experiments conducted herein demonstrate that a subset of NSCLC are cilia-positive.

This experiment investigated if Hh-active NSCLC require cilia for targeted therapy with Vismodegib.

In vitro NSCLC cell lines. A NSCLC cell line (A549) that has high Hh signaling has
15 been identified. Previously published studies demonstrate that A549 cells have reduced cell migration *in vitro* following treatment with the Hh inhibitor Vismodegib (see, e.g., Maitah, M.Y., et al., 2011 PLoS ONE 6, e16068). Consistent with this hypothesis, such experiments determined that A549 cells are cilia-positive (Fig. 5B). Experiments will use shRNAs that knock down genes required for ciliogenesis to determine if Hh activation is dependent on
20 cilia in A549 cells and if Hh inhibitors require cilia for drug sensitivity *in vitro*. shRNAs have been designed that when individually knocked down result in loss of ciliogenesis: CCDC41, IFT88 and CEP164. Use of shRNAs to more than one ciliogenesis gene will allow determine if that drug efficacy is related to cilia in general and not to a non-ciliary function of these specific genes. Scramble shRNA will also be used as a control. Control
25 (cilia-positive) and experimental (cilia-negative) A549 cells will be analyzed for Hh activity and cell viability following treatment with Vismodegib. Such experiments will also determine the IC50/LD50 of Vismodegib in the control and experimental groups to determine if loss of cilia affects sensitivity. Briefly, qPCR will be performed to determine if control and experimental A549 cells treated with drug have expression of Hh
30 target genes including Gli1, Gli2, and Ptch1. Cell viability will be assessed using a standard MTT assay. Such experiments also propose to screen a panel of NSCLC cell lines for expression of primary cilia that are known to have high Hh signaling (including: NCI-H520, NCI-H226, SK-MES-1) (see, e.g., Huang, L., et al., 2014 Clin Cancer Res 20, 1566-1575).

Such experiments will then use the same shRNAs in the cilia-positive cell lines to assess the role of cilia in mediating Hh inhibitor efficacy more globally in NSCLC.

***In vivo* patient derived xenograft model.** To evaluate the role of primary cilia and hedgehog inhibition in therapeutic treatment of NSCLC, it is proposed to perform a pre-clinical efficacy trial of the hedgehog inhibitor, Vismodegib alone or in combination with a standard of care treatment (cisplatin). For these studies, experiments will utilize two NSCLC patient derived tumor explants (PDX) derived from early stage lung adenocarcinoma (LAC), chemo-radiation naïve human patient LAC tumors that have been found to harbor either high or low scores for primary cilia expression (Fig. 5). Maintained via *in vivo* serial propagation, these LAC explants maintain both the genetic and histological characteristics of the original patient tumor. Such experiments will use LAC explants in the conventional heterotopic (flank) position, which allows assessment of therapeutic response via measurement with calipers. In addition, mice will undergo *in vivo* image analysis using a Pearl Impluse imager and a fluorescent dye, which preferentially marks tumor cells, to qualitatively assess tumor burden. Experiments have treated these LAC PDX models with cisplatin and it was found that A) the model is responsive to standard of care and B) this model is consistent across mice for tumor response and therefore useful for pre-clinical efficacy trials. While the LAC PDX model is responsive to standard of care, tumor growth is not completely inhibited. This response is consistent with what is observed in the clinic. For studies proposed in this application, LAC PDX tissue samples will be diced into slurry and mixed with growth factor depleted Matrigel. 6-8 week old SCID mice (male to female ratio = 1:1) will be injected in the flank with 200µl of the LAC PDX- Matrigel slurry. Upon development of palpable tumor, mice will undergo a baseline scan using the Pearl Impluse and then randomized into 4 therapy cohorts (n=10 mice each): a) untreated, b) Vismodegib (10mg/kg) c) Vismodegib (25mg/kg) d) Vismodegib (50mg/kg). Tumor volumes will be measured using calipers twice weekly. After four weeks of treatment, mice will undergo a final scan with the Pearl Impluse and sacrificed. Tumors will be collected for histological and biochemical analysis. Initial statistical analysis will focus upon evaluating the effects of Hh inhibition upon tumor growth via both comparison of the change in volume for individual tumors, as well as the change in tumor luminescence values (Relative light units-RLU) generated from the Pearl Impulse. Additional analysis of tumor tissue will focus upon comparisons between the ciliated and non-ciliated LAC PDXs, and will include analysis of pathology (H&E staining),

Hh activity (Gli1, Ptch1 staining), proliferation (Ki-67 staining) and apoptosis (cleaved caspase 3 staining).

Cellular response *in vitro*: It is expected that Vismodegib will inhibit Hh signaling in cilia-positive cancers and not in cilia-negative cancers. Cilia-dependent inhibition of Hh signaling will result in reduced Gli1, Gli2 and Ptch gene transcription following treatment with Vismodegib. It is also expected that treatment of the cilia-positive cells with Vismodegib will reduce cell viability. Results demonstrating that cilia-negative cancer cells are sensitive to a Smo inhibitor would suggest that Smo can activate the Hh pathway in a cilia-independent manner. This would be a novel finding and suggest that cancer cells can activate Smo without cilia. These findings would be unexpected but provide an exciting new avenue to study novel mechanisms of Hh activation in tumors.

Tumor response *in vivo*: It is expected that Vismodegib will reduce tumor growth more effectively in the LAC PDX with a high cilia score as compared to the LAC PDX with low cilia score. The LAC PDX with a high cilia score (93%) is a similar score to that resulted in complete response in BCC. The LAC PDX with a low cilia score (54%) is similar to a BCC score that resulted in minimal response (progressive or stable disease). In a colon tumor PDX model, response to Vismodegib was minimal at 10mg/Kg and response was saturated at 50mg/Kg (see, e.g., Wong, H., et al., 2011 Clin Cancer Res 17, 4682-4692). A similar dose response to Vismodegib in the LAC PDX model with a high cilia score is expected.

Example V.

Fig. 7 shows a list of genes upregulated when the cancer cells are treated with GANT61 (an inhibitor of GLI1 and GLI2, both of which are transcriptional regulators of Hh signaling). Therefore, these genes would have downregulated expression when Hh is ON.

Fig. 8 shows a list of genes downregulated when the cancer cells are treated with GANT61 Inhibitor (an inhibitor of GLI1 and GLI2, both of which are transcriptional regulators of Hh signaling). Therefore, these genes would have upregulated expression when Hh is ON.

For the Gant61 Up and Gant61 Down list of genes used for the Hh Signature, the genes listed as provided in Figure 4 of cDNA Microarray Gene Expression Profiling of Hedgehog Signaling Pathway Inhibition in Human Colon Cancer Cells. Shi, T. et. al. PLoS One, Oct 10, Vol 5, Issue 10, e13054.

Fig. 9 shows a list of genes that are associated with primary cilia in one or more of the following ways: 1) required for ciliogenesis, 2) required for ciliary functions including intraflagellar transport, signal transduction and calcium regulation, 3) localized to the primary cilium or basal body, and 4) mutation in this gene is associated with a known disease referred to as a ciliopathy.

Example VI.

This example demonstrates gene expression signatures representing Hh pathway activity and ciliogenesis.

Evidence exists that primary cilia can be used as a companion diagnostic to predict Hh-targeted drug efficacy in NSCLC. A bioinformatics approach was utilized to identify cancer patients that have expression of genes associated with the Hh pathway and with ciliogenesis.

First, patients with high levels of expression of Hh-target genes were identified. To accomplish this a Hh-target gene expression signature score was developed that when “high” represents activation of signaling by the Hh pathway. The gene signature is based on the 40 gene expression values that are known to go up (Fig.8) or the 52 gene expression values that are known to go down (Fig. 7) when cancer cells were treated with the Hh inhibitor GANT61 (Shi T, et al., PLoS ONE. 2010;5(10)). Note that 3 additional genes (GLI1, PTCH1, LRRC45) that are known to go down when the Hh pathway is inhibited based on published literature were included. The level of expression of the GANT UP and GANT DOWN genes in a published NSCLC cancer microarray dataset (Hou J, Aerts J, et al., PLoS ONE. 2010;5(4):e10312) was analyzed. A Hh-target gene expression score was calculated for each patient using the following formula:

- Hh pathway activity score (for each patient) = (median of the x GANT DOWN gene expression values) – (median of the x GANT UP gene expression values).

Next, a ciliogenesis gene expression signature was derived that can be used to predict frequency of primary cilia in cancers (see list of ciliogenesis genes). A published NSCLC microarray dataset was analyzed for all of the ciliogenesis genes listed in Fig. 9 (see, e.g., Hou J, Aerts J, et al., PLoS ONE. 2010;5(4):e10312). The top 18 ciliogenesis genes expressed in NSCLC as compared to normal control lung samples were identified. This subset of ciliogenesis genes includes TMEM216, PCM1, IFT140, IFT122, TRIM32, MKKS,

BBS1, PHKHD1, RABL5, HSPB11, PKD1, SDCCAG8, CDP290, STIL, IFT81, WDR34, TULP3, and IQCB1.

These 18 ciliogenesis genes were then utilized as the basis for the ciliogenesis gene expression signature. The level of expression of the 18 ciliogenesis genes in the same

5 NSCLC cancer microarray dataset described above was analyzed (Hou J, Aerts J, et al., PLoS ONE. 2010;5(4):e10312). A ciliogenesis gene expression score was calculated for each patient using the following formula:

- Ciliogenesis score (for each patient) = (median of the 18 ciliogenesis gene expression values).

10

For healthy patients (normal) as well as patients with NSCLC (NSCLC types: adenocarcinoma, squamous, and other) a scatter-plot was utilized to determine if there is a correlation between ciliogenesis and Hh pathway activity. Hh pathway activity score was plotted on the x axis and the ciliogenesis score was plotted on the y axis to examine the

15 relationship between the two expression scores. As expected, this scatter-plot analysis demonstrated a positive correlation between ciliogenesis score and the Hh pathway activity score amongst patients with NSCLC (Pearson correlation coefficient = 0.80) (Fig. 6).

Furthermore, the scatter-plot analysis demonstrated that samples from healthy patients (normal) had lower ciliogenesis and Hh pathway activity scores than samples from patients

20 with NSCLC.

This data strongly supports the use of the Hh pathway activity and ciliogenesis gene signatures to predict which NSCLC activate the Hh pathway via cilia-dependent mechanisms. It can further be utilized to identify patients that activate the Hh pathway via cilia-independent mechanisms.

25

Example VII.

This example shows an experimental method for cilia staining and scoring (see, e.g., Raz G, et al., 76 Lung Cancer 2012, pages 191-196).

Human tissue specimens. All tissue sections used were formalin-fixed paraffin-embedded human tissue samples. Serial tissue sections were cut for each patient sample. The

30 first serial section was stained for H&E and the entire tissue section was scanned with a 20x objective using the automated DMetrix slide scanner (DMetrix, Inc.). Digital images were annotated using the Eyepiece software (DMetrix, Inc.) by a certified pathologist for each of the areas of interest per tissue slide.

Immunofluorescence. The tissue slide serial to the H&E-stained slide was used for co-staining of ciliary axoneme, and the centrosomes. Paraffin-embedded tissue slides were deparaffinized in a dry incubator at 65°C for 15 minutes and hydrated by washing with Xylene (2 x 10 min), 100% Isopropanol (2 x 10 min), 70% Isopropanol (2 x 10 min), 50% Isopropanol (2 x 10 min), and ultrapure water (2 x 10 min). All washes were at room temperature. Antigen retrieval with a 1mM EDTA unmasking solution was performed using a 2100 Retriever (Electron Microscopy Sciences) according to manufacturer's instructions. Tissue slides were placed in Shandon Coverplates (Thermo Scientific, Cat# 72-110-017,) and then into Sequenza Slide Racks (Thermo Scientific, Cat# NC0263065). Tissue slides were blocked with ChemMate Antibody Dilution Buffer (Ventana Medical Systems, Inc., Cat# ADB250) with goat serum (5%) (Invitrogen Corporation, Cat# 16210-064) for 45 minutes at room temperature. Primary and secondary antibodies were diluted in the ChemMate Antibody Dilution Buffer at 1:1000. Primary antibodies were used against acetylated tubulin (mouse monoclonal IgG_{2B}, Sigma, Cat# T7451, clone 6-11B-1), γ -tubulin (mouse monoclonal IgG₁, Sigma, Cat# T5326, clone GTU-88), Arl13b (1:300, mouse monoclonal IgG_{2a}, UC, Davis/NIH NeuroMab Facility, clone N295B/66), and incubated on the tissue overnight at 4°C. The slides were then washed with PBS for 10 minutes (3 x 10 min). The secondary antibodies used were tetramethylrhodamine isothiocyanate (TRITC)-labeled goat anti-mouse-IgG_{2B} (Southern Biotech, Cat# 1090-03), Alexa 633-labeled goat anti-mouse-IgG₁ (Invitrogen, Cat# A21126), and Alexa 546-labeled goat anti-mouse-IgG_{2a} (Invitrogen, Cat# A21133). Secondary antibodies were incubated on the tissue for 45 minutes at room temperature. Slides were washed with PBS for 10 minutes (3 x 10 min). Hoechst 33342 (Cat# H3570, Invitrogen) was used as a counterstain at 1:1000 and incubated on slides for 10 minutes, followed by washing with PBS for 5 minutes (2 x 10 min). Slides were mounted with 1.5 coverslips (0.16-0.19 mm thickness) (Fisher Scientific, Cat# 12-544B) using Prolong Gold Antifade mounting media (Cat# P36934, Invitrogen).

Confocal imaging. The tissue slide that was serially adjacent to the digitally scanned H&E slide was fluorescently stained for markers of ciliary axoneme, centrosomes, and Hoechst. The Leica TCS SP5 II laser scanning confocal microscope (Leica Microsystems) was used to image the fluorescently-stained slides. Areas of interest were found using a low-power magnification objective (10x, 0.4 PI Apo) to visualize Hoechst counterstain on the fluorescently-stained slide and by referencing the annotated serially adjacent H&E that was digitally scanned. This allowed us to find the exact same area of interest that had been annotated by the pathologist on the H&E. Once the area of interest was found, Z stacks were

then acquired with the violet-laser diode at 405 nm to detect Hoechst staining at a total thickness of $2 \pm 0.5 \mu\text{m}$, with a Z-step taken every $1 \mu\text{m}$. Cilia were then imaged within these areas of interest using a 63x objective (1.4 NA PL Apo) with the helium neon lasers (543 nm and 633 nm), the argon laser (488 nm), and the violet-laser diode (405 nm) was used to detect
5 Hoechst staining. Z-stacks were acquired at a total thickness of $5.0 \pm 0.5 \mu\text{m}$, with a Z-step taken every $0.34 \mu\text{m}$ (image resolution 2048x2048 pixels). Such experiments acquired a range of 3-6 images per location using the 63x objective per tissue type per patient and this varied depending on the size of the location. Z images were processed post-acquisition to maximum projections using the Leica LAS AF software for image analysis.

10 **Confocal image analysis.** Cilia frequency and cilia lengths were obtained for each cell type using the Leica LAS AF software. Cilia were only scored when both ciliary axoneme and centrosome were visible together. For each cell nuclei were counted using the count tool, and cilia lengths were measured using the scale bar tool. The number of cilia per cell type was divided by total nuclei per cell type to obtain a percentage of ciliated cells.

15 **Immunohistochemistry.** Formalin-fixed paraffin-embedded tissue slides were stained with a similar protocol used for immunofluorescently stained slides described above. Antigen retrieval was performed as described but with the Vector Antigen Unmasking Solution (1:106) (Vector Laboratories, Cat# H-3300), followed by quenching of endogenous peroxidase activity at room temperature for 20 minutes using hydrogen peroxide in methanol
20 (0.3%). Slides were then washed with PBS (4 x 10 min), placed into Shandon Coverplates (Thermo Scientific, Cat# 72-110-017) and then into Sequenza Slide Racks (Thermo Scientific, Cat# NC0263065). Slides were then blocked with 2.5% normal horse serum blocking buffer (Vector Laboratories, Cat# S-2012) for 20 minutes, and then with ChemMate Antibody Dilution Buffer (Ventana Medical Systems, Inc., Cat# ADB250) with goat serum
25 (5%) (Invitrogen Corporation, Cat# 16210-064) for 45 minutes. All washes and blocking steps were at room temperature. Primary antibodies were diluted in the ChemMate Antibody Dilution Buffer. The following primary antibody was used: Ki67 (1:100, mouse monoclonal IgG₁, Dako, Cat#M7240, clone MIB-1). Tissue slides with secondary antibody only were used as a negative control. Primary antibodies were incubated on tissues overnight at 4°C.
30 Slides were then washed in PBS (3 x 10 min). A universal anti-rabbit, anti-mouse secondary antibody conjugated to peroxidase (ImmPress Universal Reagent, Vector Laboratories, Cat# MP-7500) was incubated on the tissue for 30 minutes, followed by a wash in PBS for 5 minutes. 3-amino-9-ethylcarbazole (AEC) with high sensitivity substrate chromagen (Dako, Cat# K3461) was used as the peroxidase substrate for Ki67 staining. AEC was incubated on

slides for 7 minutes for Ki67 staining. Tissue slides were rinsed with distilled water for 5 minutes. Hematoxylin 1 (Thermo Scientific, Cat# 7221) was used to counterstain the tissue slides. Hematoxylin 1 was diluted 1:3 and incubated on tissue slides for 15 seconds and then rinsed in tap water until water ran clear. Faramount Aqueous Mounting Media (Dako, Cat# S3025) was used for mounting slides using 1.5 coverslips (0.16-0.19 mm thickness) (Fisher Scientific, Cat# 12-544B).

Immunohistochemistry analysis. Whole slides stained for Ki67 were scanned using a 20x objective with the BioImagene scanner (Ventana Medical Systems). The same locations used for primary cilia analysis were found by referencing the annotated H&E slide. These areas of interest were exported as TIFF files from the Dmetrix scan files, and as JPEG files from the BioImagene scan files, and uploaded into Definiens Tissue Studio 3.0 Software (<http://www.tissuestudio.com>). Tissue Studio 3.0 Software was tested for absolute agreement with manual hand counts performed by two separate investigators. For each image, Tissue Studio 3.0 was used in conjunction to quantify the number of positive and negative cells/nuclei. A statistical test that is used to measure the consistency and absolute agreement of measurements made by different observers (intraclass correlation) was applied to the data obtained. The intraclass correlation coefficient was determined as 0.7, using SPSS 19 (Statistical Package for the Social Sciences; IBM Corporation), which is considered strong agreement.

For the Ki67 analysis with Definiens Tissue Studio, a modified Nuclei (Positive/Negative) solution was used. The epithelial/cancer and stromal compartments of cancer were separately analyzed using the Manual ROI Selection (Select Segments) segmentation tool, with a segmentation of 8. The hematoxylin and immunohistological (IHC) threshold were set at 0.12 arbitrary units (a.u.) and 0.03 a.u., respectively. The IHC threshold was determined by identifying the lightest positively-stained nucleus in the sample set and using this value as the cutoff for positivity. A Nucleus Morphology and Filter step was used to exclude objects mistakenly identified as nuclei. From the exported results, positive indices were computed per cell type per patient.

Cilia Score (Cilia + Ki67): Scoring percent ciliated cancer cells may under-represent the cilia-positive fraction of cancer cells because cilia are only expressed on cells in G0 or early-G1 of the cell cycle. To correct for this Such experiments will add the percent cilia-positive cancer cells to the percent Ki67-positive cancer cells for each imaged region to obtain a cilia + Ki67 score. Interpretation of cilia + Ki67 score will follow these examples: 1.) cilia (~30%) + Ki67 (~70%) =100%, therefore all of the cancer cells have the ability to

make cilia; 2.) cilia (~30%) + Ki67 (~30%) =60%, heterogeneity amongst the cancer cells that have the potential to make cilia; 3.) cilia (0%) + Ki67 (~50%) =50%, interpreted as a cilia-negative sample.

5 Example VIII.

To determine if the ciliogenesis gene signature could be utilized to predict expression of primary cilia, experiments were conducted that utilized a publically available gene expression dataset of 47 NSCLC cell lines to analyze the ciliogenesis gene signature. This data set was provided by Genentech (Dataset: RNA-seq of 675 commonly used human cancer
10 cell lines) is publicly available at the Expression Atlas.

Four of the NSCLC cell lines (NCI-H2073, NCI-H2228, NCI-H1975, and NCI-H2030) in this gene expression dataset were stained for markers of primary cilia. Based on immunocytochemistry for markers of primary cilia, two of the four NSCLC cell lines tested (NCI-H2228 and NCI-H2073) were positive for primary cilia expression (Figure 10A and
15 10B). Experiments analyzed the ciliogenesis gene signature in each of the 47 NSCLC cell lines and found that the two cilia-positive cell lines, NCI-H2228, and NCI-H2073, had a ciliogenesis gene signature >12 (Figure 10C). The two NSCLC cell lines that staining determined were cilia-negative (NCI-H1975 and NCI-H2030) both had a ciliogenesis gene signature score of < 11 (Figure 10B and 10C respectively). Therefore, “12” was set as a
20 minimum cilia prediction cutoff, and this predicted that 38% of NSCLC cell lines are cilia-positive (Figure 10C, horizontal dashed line).

Experiments also analyzed the Hh gene signature in each of the 47 NSCLC cell lines. Using >2 as a minimum cutoff for Hh-positive, the ciliogenesis and Hh signature scores suggest that 29% of NSCLC cell lines are both Hh-positive and Cilia-positive (Figure 10C,
25 upper right quadrant). This is consistent with previous studies staining NSCLC patient tissue samples, which demonstrated that ~30% are Hh-positive/Cilia-positive. These results strongly support the conclusion that the ciliogenesis gene signature is predictive of expression of primary cilia in NSCLC.

30 All publications and patents mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed

should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention that are obvious to those skilled in the relevant fields are intended to be within the scope of the present invention.

5 **INCORPORATION BY REFERENCE**

The entire disclosure of each of the patent documents and scientific articles referred to herein is incorporated by reference for all purposes.

EQUIVALENTS

10 The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting the invention described herein. Scope of the invention is thus indicated by the appended claims rather than by the foregoing description, and all changes that come within the meaning and range of equivalency of the
15 claims are intended to be embraced therein.

CLAIMS

1. A method for selecting an individual having cancer cells associated with Hh pathway activity for treatment with a Hh inhibitor, comprising:
 - 5 a) obtaining a biological sample from the individual, wherein the biological sample comprises cancer cells,
 - b) determining a gene expression profile for one or more biomarker genes within the obtained cancer cells; and
 - c) administering to the individual a therapeutically effective amount of a Hh
 - 10 inhibitor if the determined biomarker gene expression profile is consistent with a reference gene expression profile for cancer cells associated with Hh pathway activity and primary cilia expression, or not administering to the individual a therapeutically effective amount of a Hh inhibitor if the determined biomarker gene expression profile is inconsistent with a reference gene expression profile for cancer cells associated with Hh pathway activity and primary cilia
 - 15 expression.
2. The method of claim 1, wherein the one or more biomarker genes are selected from
 - ABHD4, ACSS2, ARL14, BLOC1S2, C17orf91, CDKN1A, CLIC3, CLIP3, CYP4F11, DDIT3, DDIT4, GABARAPL1, GCNT2, GDF15, GPX3, GULP1, HKDC1, IDI1, IL1RN,
 - 20 INSIG1, KIAA1370, LCN2, LCP1, LPIN1, MVD, NUPR1, OSGIN1, PANX2, PCSK9, RBCK1, SC4MOL, SCD, SDCBP2, STOX2, TIMP4, TP53INP1, TRIM31, UBC, YPEL5, ARHGAP11A, ASF1B, AURKB, BIRC5, BLM, C15orf42, C16orf59, CASC5, CCNA2, CCNE2, CDC20, CDC6, CENPM, DDN, DLGAP5, DTL, E2F2, EXO1, FAM64A, GINS2, HELLS, HJURP, KIAA0101, KIF15, KIF20A, KIFC1, LRRc45, LRRC26, MCM10, MCM2,
 - 25 MCM4, MCM5, MCM8, MYO19, NDC80, NMU, NRG1, NUDT1, PKMYT1, POLE, POLE2, PSMC3IP, RECQL4, SLC39A10, SLC43A3, SPC24, SPC25, TK1, TOP2A, TYMS, UBE2C, XRCC3, GLI1, PTCH1, LRRC45, IFT43, WDR35, IFT122, TTC21B, IFT140, WDR19, TULP3, IFT20, RABL5, HSPB11, IFT27, IFT46, IFT52, TRAF3IP1, IFT57, TTC30B, IFT74, IFT80, IFT81, IFT88, IFT172, KIF3A, KIF3B, KIFAP3, KIF17,
 - 30 DYNC2H1, DYNC2LI1, WDR34, DYNLT1, BBS1, BBS2, ARL6, BBS4, BBS5, MKKS, BBS7, TTC8, BBS9, BBS10, TRIM32, BBS12, RAB3IP, RAB8A, PCM1, PKD1, PKD2, PKHD1, AHI1, ARL13B, INPP5E, TMEM216, MKS1, TMEM67, CC2D2A, SDCCAG8, SEPT7, NPHP1, INVS, NPHP3, NPHP4, IQCB1, CEP290, GLIS2, RPGRIP1L, NEK8, EVC, EVC2, VHL, OFD1, STIL, and RPGR.

3. The method of claim 1, wherein the one or more biomarker genes are selected from TMEM216, PCM1, IFT140, IFT122, TRIM32, MKKS, BBS1, PHKHD1, RABL5, HSPB11, PKD1, SDCCAG8, CDP290, STIL, IFT81, WDR34, TULP3, and IQCB1.

5

4. The method of claim 1, wherein the determining the gene expression profile involves quantifying mRNA expression and protein expression related to the specific biomarker genes within the obtained cancer cells.

10 5. The method of claim 1, wherein the cancer cells associated with Hh pathway activity are selected from NSCLC cells, bladder cancer cells, basal cell carcinoma cells, medulloblastoma cells, colon cancer cells, breast cancer cells, and pancreatic cancer cells.

15 6. The method of claim 1, wherein the Hh inhibitor is selected from Taladegib (LY2940680), sulforaphane, vismodegib, TAK-441, itraconazole, erismodegib, 5E1, robotnikinin, IPI-926, HPI-2, HPI-3, HPI-4, and arsenic trioxide.

7. A method for treating a human subject having cancer cells associated with Hh pathway activity, comprising the steps of:

20 a) performing a nucleic acid-based detection assay to determine a gene expression profile of one or more biomarker genes in a sample comprising cancer cells obtained from the human subject;

b) comparing the determined gene expression profile with a reference gene expression profile for cancer cells associated with Hh pathway activity and primary cilia
25 expression;

c) determining that the human subject is responsive or unresponsive to a Hh inhibitor treatment based on such a comparison; and

d) administering an effective amount of a Hh inhibitor to the human subject having a determined gene expression profile that is consistent with the reference gene
30 expression profile for cancer cells associated with Hh pathway activity and primary cilia expression.

8. The method of Claim 7, wherein the one or more biomarker genes are selected from ABHD4, ACSS2, ARL14, BLOC1S2, C17orf91, CDKN1A, CLIC3, CLIP3, CYP4F11,

DDIT3, DDIT4, GABARAPL1, GCNT2, GDF15, GPX3, GULP1, HKDC1, IDI1, IL1RN, INSIG1, KIAA1370, LCN2, LCP1, LPIN1, MVD, NUPR1, OSGIN1, PANX2, PCSK9, RBCK1, SC4MOL, SCD, SDCBP2, STOX2, TIMP4, TP53INP1, TRIM31, UBC, YPEL5, ARHGAP11A, ASF1B, AURKB, BIRC5, BLM, C15orf42, C16orf59, CASC5, CCNA2, CCNE2, CDC20, CDC6, CENPM, DDN, DLGAP5, DTL, E2F2, EXO1, FAM64A, GINS2, HELLS, HJURP, KIAA0101, KIF15, KIF20A, KIFC1, LRRc45, LRRC26, MCM10, MCM2, MCM4, MCM5, MCM8, MYO19, NDC80, NMU, NRG1, NUDT1, PKMYT1, POLE, POLE2, PSMC3IP, RECQL4, SLC39A10, SLC43A3, SPC24, SPC25, TK1, TOP2A, TYMS, UBE2C, XRCC3, GLI1, PTCH1, LRRC45, IFT43, WDR35, IFT122, TTC21B, IFT140, WDR19, TULP3, IFT20, RABL5, HSPB11, IFT27, IFT46, IFT52, TRAF3IP1, IFT57, TTC30B, IFT74, IFT80, IFT81, IFT88, IFT172, KIF3A, KIF3B, KIFAP3, KIF17, DYNC2H1, DYNC2LI1, WDR34, DYNLT1, BBS1, BBS2, ARL6, BBS4, BBS5, MKKS, BBS7, TTC8, BBS9, BBS10, TRIM32, BBS12, RAB3IP, RAB8A, PCM1, PKD1, PKD2, PKHD1, AHI1, ARL13B, INPP5E, TMEM216, MKS1, TMEM67, CC2D2A, SDCCAG8, SEPT7, NPHP1, INVS, NPHP3, NPHP4, IQCB1, CEP290, GLIS2, RPGRIP1L, NEK8, EVC, EVC2, VHL, OFD1, STIL, and RPGR.

9. The method of claim 7, wherein the one or more biomarker genes are selected from TMEM216, PCM1, IFT140, IFT122, TRIM32, MKKS, BBS1, PHKHD1, RABL5, HSPB11, PKD1, SDCCAG8, CDP290, STIL, IFT81, WDR34, TULP3, and IQCB1.

10. The method of claim 7, wherein the cancer cells associated with Hh pathway activity is selected from NSCLC cells, bladder cancer cells, basal cell carcinoma cells, medulloblastoma cells, colon cancer cells, breast cancer cells, and pancreatic cancer cells.

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11. The method of claim 7, wherein the Hh inhibitor is selected from Taladegib (LY2940680), sulforaphane, vismodegib, TAK-441, itraconazole, erismodegib, 5E1, robotnikinin, IPI-926, HPI-2, HPI-3, HPI-4, and arsenic trioxide.

12. A method for determining the presence or absence of primary cilia expression in cancer cells associated with Hh pathway activity, comprising

a) determining a gene expression profile of one or more biomarker genes within a sample comprising such cancer cells,

b) comparing the determined gene expression profile with a reference gene expression profile for cancer cells associated with Hh pathway activity and primary cilia expression, and

c) identifying such cancer cells as having primary cilia expression if the
 5 determined gene expression profile is consistent with the reference gene expression profile for cancer cells associated with Hh pathway activity and primary cilia expression.

13. The method of claim 12, wherein the one or more biomarker genes are selected from ABHD4, ACSS2, ARL14, BLOC1S2, C17orf91, CDKN1A, CLIC3, CLIP3, CYP4F11,
 10 DDIT3, DDIT4, GABARAPL1, GCNT2, GDF15, GPX3, GULP1, HKDC1, IDI1, IL1RN, INSIG1, KIAA1370, LCN2, LCP1, LPIN1, MVD, NUPR1, OSGIN1, PANX2, PCSK9, RBCK1, SC4MOL, SCD, SDCBP2, STOX2, TIMP4, TP53INP1, TRIM31, UBC, YPEL5, ARHGAP11A, ASF1B, AURKB, BIRC5, BLM, C15orf42, C16orf59, CASC5, CCNA2, CCNE2, CDC20, CDC6, CENPM, DDN, DLGAP5, DTL, E2F2, EXO1, FAM64A, GINS2,
 15 HELLS, HJURP, KIAA0101, KIF15, KIF20A, KIFC1, LRRc45, LRRC26, MCM10, MCM2, MCM4, MCM5, MCM8, MYO19, NDC80, NMU, NRG1, NUDT1, PKMYT1, POLE, POLE2, PSMC3IP, RECQL4, SLC39A10, SLC43A3, SPC24, SPC25, TK1, TOP2A, TYMS, UBE2C, XRCC3, GLI1, PTCH1, LRRC45, IFT43, WDR35, IFT122, TTC21B, IFT140, WDR19, TULP3, IFT20, RABL5, HSPB11, IFT27, IFT46, IFT52, TRAF3IP1, IFT57,
 20 TTC30B, IFT74, IFT80, IFT81, IFT88, IFT172, KIF3A, KIF3B, KIFAP3, KIF17, DYNC2H1, DYNC2LI1, WDR34, DYNLT1, BBS1, BBS2, ARL6, BBS4, BBS5, MKKS, BBS7, TTC8, BBS9, BBS10, TRIM32, BBS12, RAB3IP, RAB8A, PCM1, PKD1, PKD2, PKHD1, AHI1, ARL13B, INPP5E, TMEM216, MKS1, TMEM67, CC2D2A, SDCCAG8, SEPT7, NPHP1, INVS, NPHP3, NPHP4, IQCB1, CEP290, GLIS2, RPGRIP1L, NEK8,
 25 EVC, EVC2, VHL, OFD1, STIL, and RPGR.

14. The method of claim 12, wherein the one or more biomarker genes are selected from TMEM216, PCM1, IFT140, IFT122, TRIM32, MKKS, BBS1, PKHD1, RABL5, HSPB11, PKD1, SDCCAG8, CEP290, STIL, IFT81, WDR34, TULP3, and IQCB1.

30

15. The method of claim 12, wherein the determining the gene expression profile involves quantifying mRNA expression and protein expression related to the specific biomarker genes within the obtained cancer cells.

16. A method for selecting an individual having cancer cells associated with Hh pathway activity for treatment with a Hh inhibitor, comprising:

- a) obtaining a biological sample from the individual, wherein the biological sample comprises cancer cells,
- 5 b) detecting the presence or absence of primary cilia expression within the cancer cells, and
- c) administering to the individual a therapeutically effective amount of a cilia-dependent Hh inhibitor if primary cilia expression is detected in the cancer cells, or not administering to the individual a therapeutically effective amount of a cilia-dependent Hh
10 inhibitor if primary cilia expression is not detected in the cancer cells.

17. The method of Claim 16, wherein detecting the presence or absence of primary cilia expression within the cancer cells comprises:

- 1) exposing the cancer cells to
15 antibodies against a ciliary axoneme,
antibodies against a cilia associated centrosome,
antibodies against markers for activated Hh signaling,
three different fluorescently conjugated secondary antibodies
to distinguish between the antibodies against a ciliary axoneme, the antibodies against a cilia
20 associated centrosome, and the antibodies against markers for activated Hh signaling, and
- 2) detecting the presence or absence of binding between the antibodies
against a ciliary axoneme, detecting the presence or absence of binding between antibodies
against a cilia associated centrosome, and detecting the presence or absence of binding
between antibodies against markers for activated Hh signaling,
25 wherein a detected presence of binding between the antibodies against
a ciliary axoneme, the antibodies against a cilia associated centrosome, and the antibodies
against markers for activated Hh signaling indicates the presence of primary cilia expression
in the cancer cells.

30 18. The method of Claim 17, wherein the antibody against a ciliary axoneme is anti-acetylated-tubulin.

19. The method of Claim 17, wherein the antibodies against a cilia associated centrosome is anti-gamma (γ)-tubulin.

20. The method of Claim 17, wherein the antibodies against markers for activated Hh signalling is anti-Ptch1 antibody and/or anti-Gli1 antibody and/or anti-SMO antibody.

5 21. The method of claim 16, wherein the cancer cells associated with Hh pathway activity are selected from NSCLC cells, bladder cancer cells, basal cell carcinoma cells, medulloblastoma cells, colon cancer cells, breast cancer cells, and pancreatic cancer cells.

22. The method of claim 16, wherein the Hh inhibitor is selected from Taladegib
10 (LY2940680), sulforaphane, vismodegib, TAK-441, itraconazole, erismodegib, 5E1, robotnikinin, IPI-926, HPI-2, HPI-3, HPI-4, and arsenic trioxide.

23. A method for treating a human subject having cancer cells associated with Hh pathway activity, comprising the steps of:

- 15 a) obtaining a biological sample from the human subject, wherein the biological sample comprises cancer cells,
- b) detecting the presence or absence of primary cilia expression within the cancer cells, and
- c) administering to the individual a therapeutically effective amount of a cilia-
20 dependent Hh inhibitor if primary cilia expression is detected in the cancer cells, or not administering to the individual a therapeutically effective amount of a cilia-dependent Hh inhibitor if primary cilia expression is not detected in the cancer cells.

24. The method of Claim 23, wherein detecting the presence or absence of primary cilia
25 expression within the cancer cells comprises:

- 1) exposing the cancer cells to
- antibodies against a ciliary axoneme,
- antibodies against a cilia associated centrosome,
- antibodies against markers for activated Hh signaling,
- 30 three different fluorescently conjugated secondary antibodies to distinguish between the antibodies against a ciliary axoneme, the antibodies against a cilia associated centrosome, and the antibodies against markers for activated Hh signaling, and
- 2) detecting the presence or absence of binding between the antibodies against a ciliary axoneme, detecting the presence or absence of binding between antibodies

against a cilia associated centrosome, and detecting the presence or absence of binding between antibodies against markers for activated Hh signaling,

wherein a detected presence of binding between the antibodies against a ciliary axoneme, the antibodies against a cilia associated centrosome, and the antibodies
5 against markers for activated Hh signaling indicates the presence of primary cilia expression in the cancer cells.

25. The method of Claim 23, wherein the antibody against a ciliary axoneme is anti-acetylated-tubulin.

10

26. The method of Claim 23, wherein the antibodies against a cilia associated centrosome is anti-gamma (γ)-tubulin.

10

27. The method of claim 23, wherein the cancer cells associated with Hh pathway activity
15 is selected from NSCLC cells, bladder cancer cells, basal cell carcinoma cells, medulloblastoma cells, colon cancer cells, breast cancer cells, and pancreatic cancer cells.

15

28. The method of claim 23, wherein the Hh inhibitor is selected from Taladegib (LY2940680), sulforaphane, vismodegib, TAK-441, itraconazole, erismodegib, 5E1,
20 robotnikinin, IPI-926, HPI-2, HPI-3, HPI-4, and arsenic trioxide.

20

29. A method for determining the presence or absence of primary cilia expression in cancer cells associated with Hh pathway activity, comprising

a) obtaining a biological sample comprising cancer cells,
25 b) exposing the cancer cells to

25

antibodies against a ciliary axoneme,
antibodies against a cilia associated centrosome,
antibodies against markers for activated Hh signaling,
three different fluorescently conjugated secondary antibodies

30 to distinguish between the antibodies against a ciliary axoneme, the antibodies against a cilia associated centrosome, and the antibodies against markers for activated Hh signaling, and

c) detecting the presence or absence of binding between the antibodies against a ciliary axoneme, detecting the presence or absence of binding between antibodies against a

cilia associated centrosome, and detecting the presence or absence of binding between antibodies against markers for activated Hh signaling,

wherein a detected presence of binding between the antibodies against a ciliary axoneme, the antibodies against a cilia associated centrosome, and the antibodies
5 against markers for activated Hh signaling indicates the presence of primary cilia expression in the cancer cells.

30. The method of Claim 29, wherein the antibody against a ciliary axoneme is anti-acetylated-tubulin.

10

31. The method of Claim 29, wherein the antibodies against a cilia associated centrosome is anti-gamma (γ)-tubulin.

32. The method of Claim 29, wherein the antibodies against markers for activated Hh
15 signalling is anti-Ptch1 antibody and/or anti-Gli1 antibody and/or anti-SMO antibody.

FIG. 1

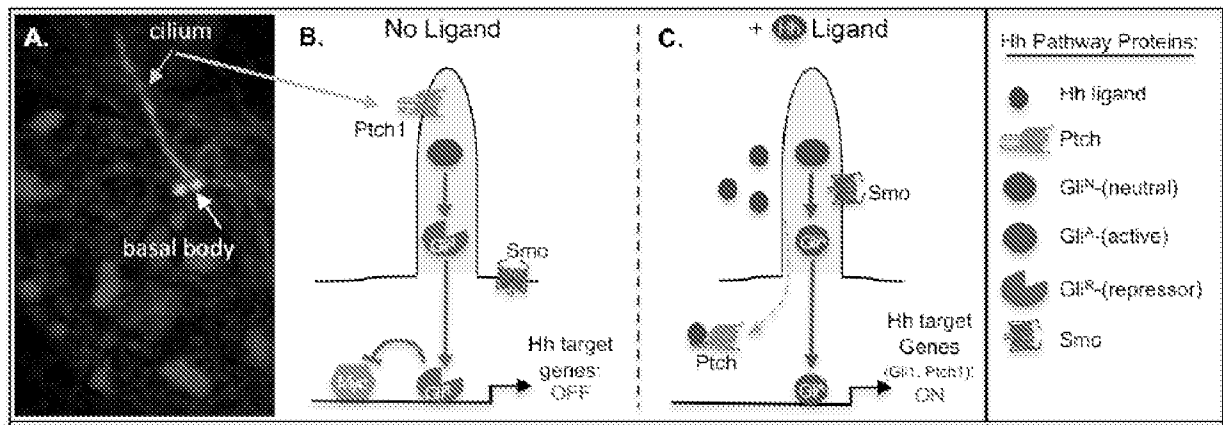


FIG. 2

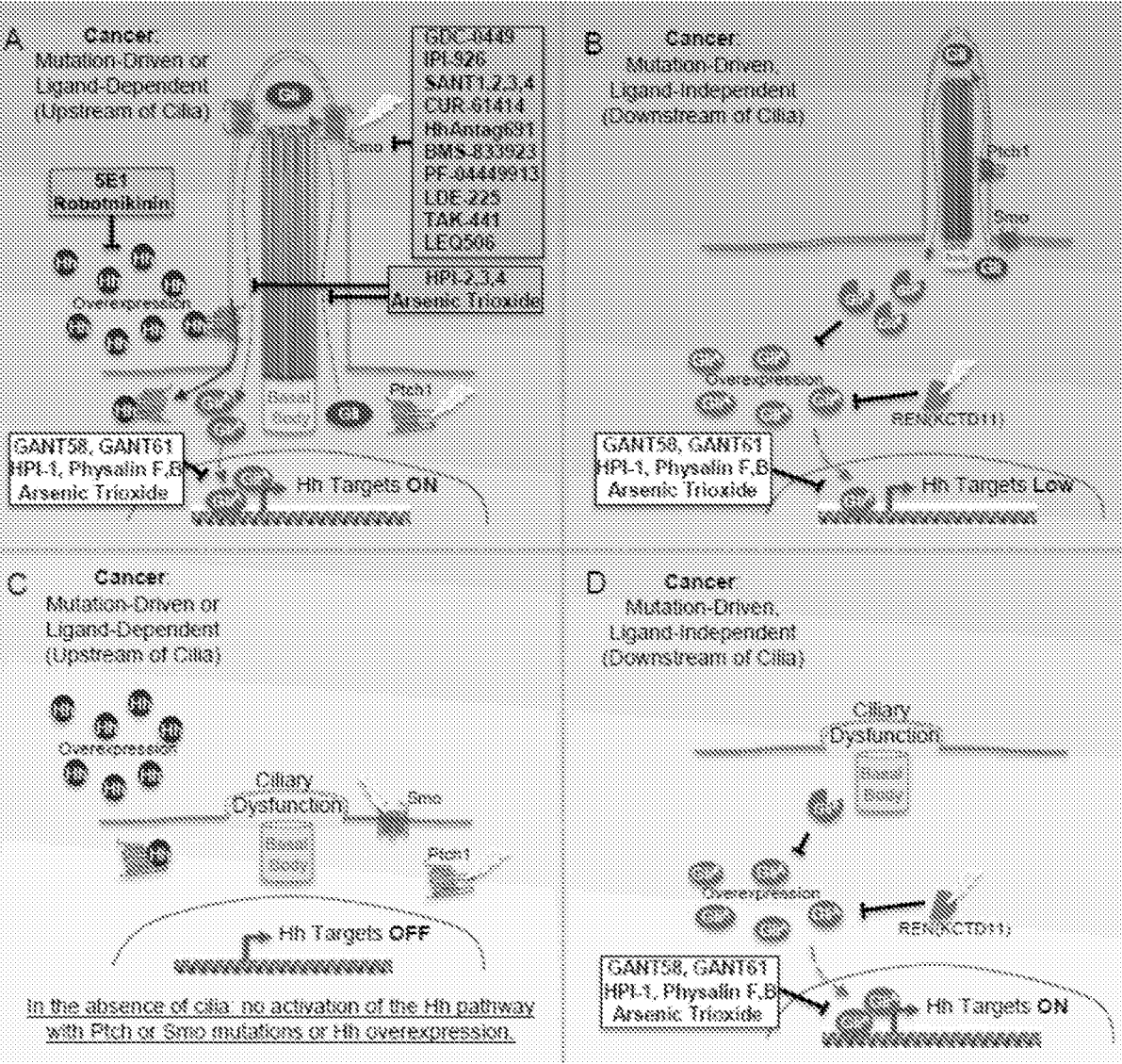


FIG. 3

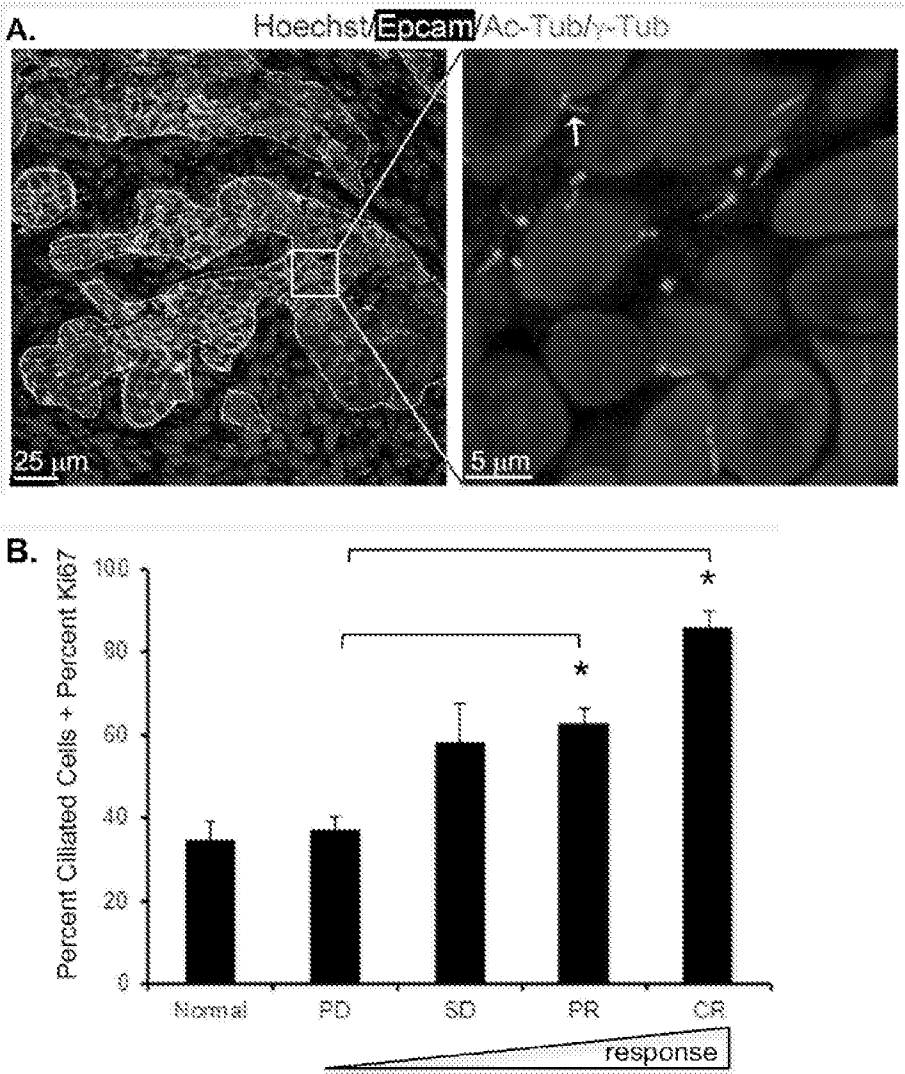


FIG. 4

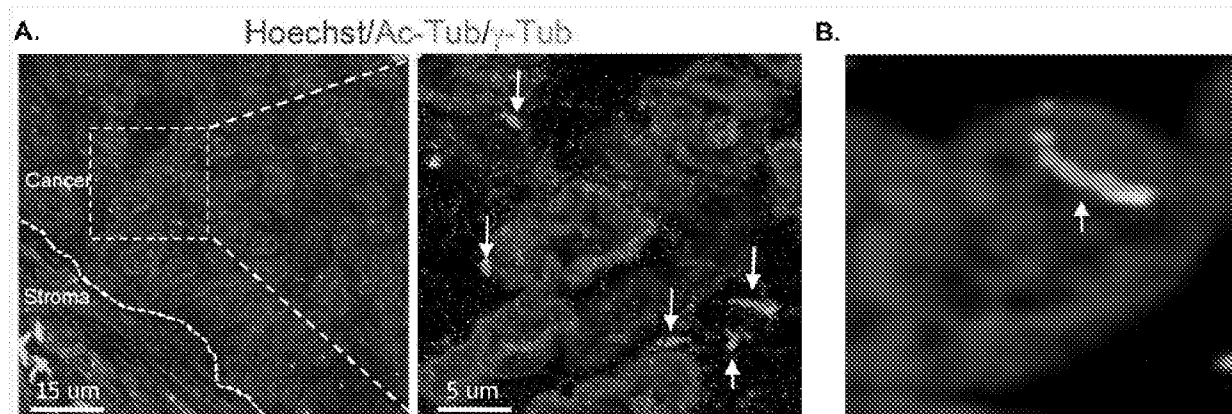
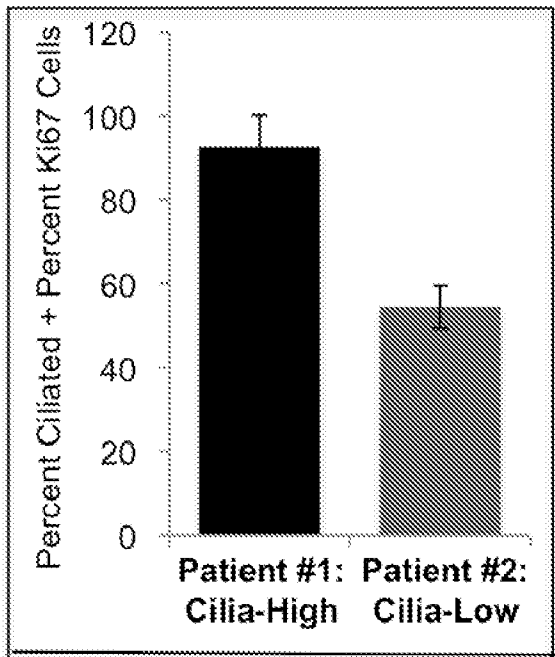


FIG. 5



6/13

FIG. 6

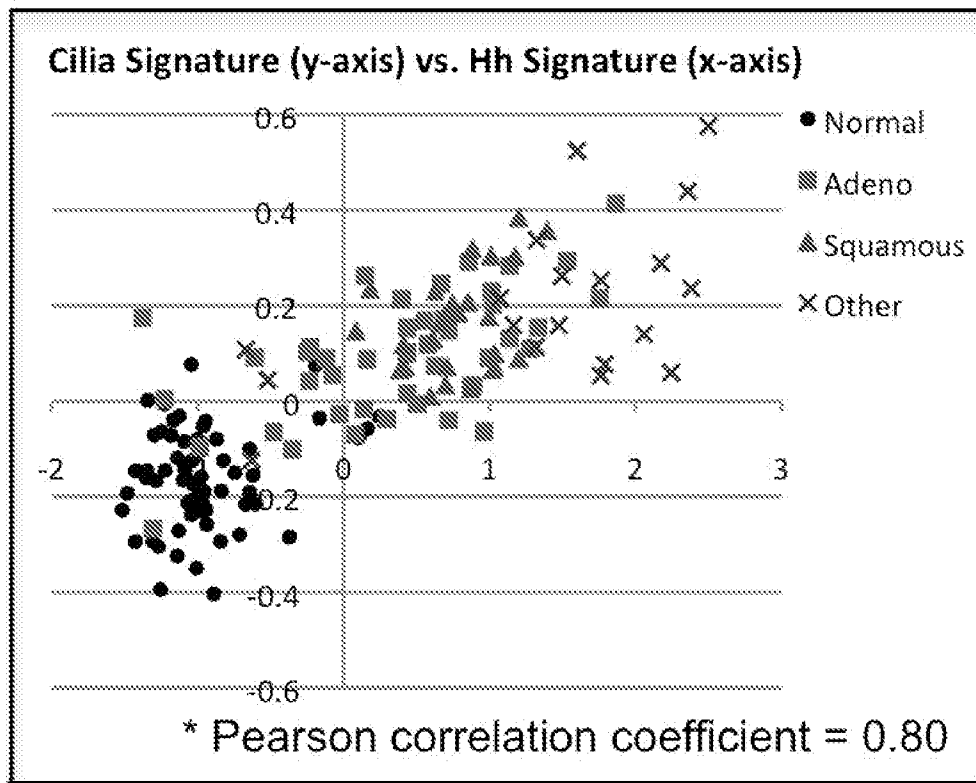


FIG. 7

ABHD4	INSIG1
ACSS2	KIAA1370
ARL14	LCN2
BLOC1S2	LCP1
C17orf91	LPIN1
CDKN1A	MVD
CLIC3	NUPR1
CLIP3	OSGIN1
CYP4F11	PANX2
DDIT3	PCSK9
DDIT4	RBCK1
GABARAPL1	SC4MOL
GCNT2	SCD
GDF15	SDCBP2
GPX3	STOX2
GULP1	TIMP4
HKDC1	TP53INP1
HMGCS1	TRIM31
IDI1	UBC
IL1RN	YPEL5

FIG. 8

ARHGAP11A	MCM10
ASF1B	MCM2
AURKB	MCM4
BIRC5	MCM5
BLM	MCM8
C15orf42	MYO19
C16orf59	NDC80
CASC5	NMU
CCNA2	NRGN
CCNE2	NUDT1
CDC20	PKMYT1
CDC6	POLE
CENPM	POLE2
DDN	PSMC3IP
DLGAP5	RECQL4
DTL	SLC39A10
E2F2	SLC43A3
EXO1	SPC24
FAM64A	SPC25
GINS2	TK1
HELLS	TOP2A
HJURP	TYMS
KIAA0101	UBE2C
KIF15	XRCC3
KIF20A	GLI1
KIFC1	PTCH1
LRRc45	LRRc45
LRRc26	

FIG. 9

Official Symbol	Human Gene ID	Chromosome Annotation Number	Human Chromosome Location	Alternative Names
IFT43	112752	NC_000014.8	14q24.3	CED3, FLJ32173, MGC16028, C14orf179
WDR35	57539	NC_000002.11	2p24.1	IFTA-1, WDR35, OSEG4, CED2, KIAA1336, MGC33196
IFT122	55764	NC_000003.11	3q21	DAF-10, OSEG, CED, SPG, WDR10, WDR10p, WDR140
TTC21B	79809	NC_000002.11	2q24.3	ZK328.7, Nbla10696, ATD4, FLJ11457, JBTS11, NPHP12, THM1
IFT140	9742	NC_000016.9	16p13.3	CHE-11, OSEG3, LA16c-313F4.1, DKFZp564L232, FLJ10306, FLJ30571, KIAA0590, WDTC2, c305C8.4, c380F5.1, gs114
WDR19	57728	NC_000004.11	4p14	DYF-2, WDR19, OSEG6, FLJ23127, IFT144, KIAA1638, ORF26, Oseg6, PWDMP
TULP3	7289	NC_000012.11	12p13.3	MGC45295, TUBL3
IFT20	90410	NC_000017.10	17q11.2	Y110A7A, CG30441, hIFT20; intraflagellar transport protein 20 homolog
RABL5	64792	NC_000007.13	7q22.1	IFTA-2, RABL5, DKFZp761N0823, FLJ13225, FLJ14117
HSPB11	51668	NC_000001.10	1p32	HSPB11, HSPC034, C1orf41, HSPC034, IFT25, PP25
IFT27	11020	NC_000022.10	22q13.1	LL22NC01-132D12.2, RABL4, RAYL
IFT46	56912	NC_000011.9	11q23.3	DYF-6, CG15161, C11orf2, C11orf60, FLJ21827
IFT52	51098	NC_000020.10	20	BLD1, OSM-6, RP5-1028D15.4, C20orf9, CGI-53, NGD2, NGD5, dJ1028D15.1
TRAF3IP1	26146	NC_000002.11	2q37.3	DKFZp434F124, MIP-T3, MIPT3

10/13

FIG. 9 (cont'd)

IFT57	55081	NC_000003.11	3q13.13	CHE-13, HIPPI, ESRRBL1, FLJ10147, MHS4R2
TTC30B	150737	NC_000002.11	2q31.2	DYF-1, TTC30A/B, FLJ30990, IFT70, fleer
IFT74	80173	NC_000009.11	9p21.2	IFT72, CCDC2, CMG-1, CMG1, FLJ22621, MGC111562
IFT80	57560	NC_000003.11	3q25.33	CHE-2, WDR56, OSEG5, ATD2, KIAA1374, MGC126543
IFT81	28981	NC_000012.11	12q24.13	CDV-1, CDV-1R, CDV1, CDV1R, MGC102777, MGC4027
IFT88	8100	NC_000013.10	13q12.1	OSM-5, Polaris, NOMPb, RP11-172H24.2, D13S1056E, DAF19, MGC26259, TG737, TTC10, hTg737
IFT172	26160	NC_000002.11	2p23.3	DKFZp434A163, KIAA1179, SLB, osm-1, wim
KIF3A	11127	NC_000005.9	5q31	
KIF3B	9371	NC_000020.10	20q11.21	HH0048, KIAA0359
KIFAP3	22920	NC_000001.10	1q24.2	RP1-190I16.1, FLJ22818, KAP-3, KAP3, SMAP, Smg-GDS, dJ190I16.1
KIF17	57576	NC_000001.10	1p36.12	RP11-401M16.8, KIAA1405, KIF17B, KIF3X
DYNC2H1	79659	NC_000011.9	11q21-q22.1	ATD3, DHC1b, DHC2, DNCH2, DYH1B, FLJ11756, hdhc11
DYNC2LI1	51626	NC_000002.11	2p25.1-p24.1	CGI-60, D2LIC, DKFZp564A033, LIC3
WDR34	89891	NC_000009.11	9q34.11	RP11-216B9.5, MGC20486, bA216B9.3
DYNLT1				
BBS1	582	NC_000011.9	11q13	BBS2L2, FLJ23590, MGC126183, MGC126184, MGC51114
BBS2	583	NC_000016.9	16q21	BBS, MGC20703
ARL6	84100	NC_000003.11	3q11.2	BBS3, RP55, MGC32934
BBS4	585	NC_000015.9	15q22.3-q23	
BBS5	129880	NC_000002.11	2q31.1	
MKKS	8195	NC_000020.10	20p12	BBS6, HMCS, KMS, MKS
BBS7	55212	NC_000004.11	4q27	BBS2L1, FLJ10715
TTC8	123016	NC_000014.8	14q31.3	BBS8, RP51
BBS9	27241	NC_000007.13	7p14	B1, C18, D1, MGC118917, PTHB1

11/13

FIG. 9 (cont'd)

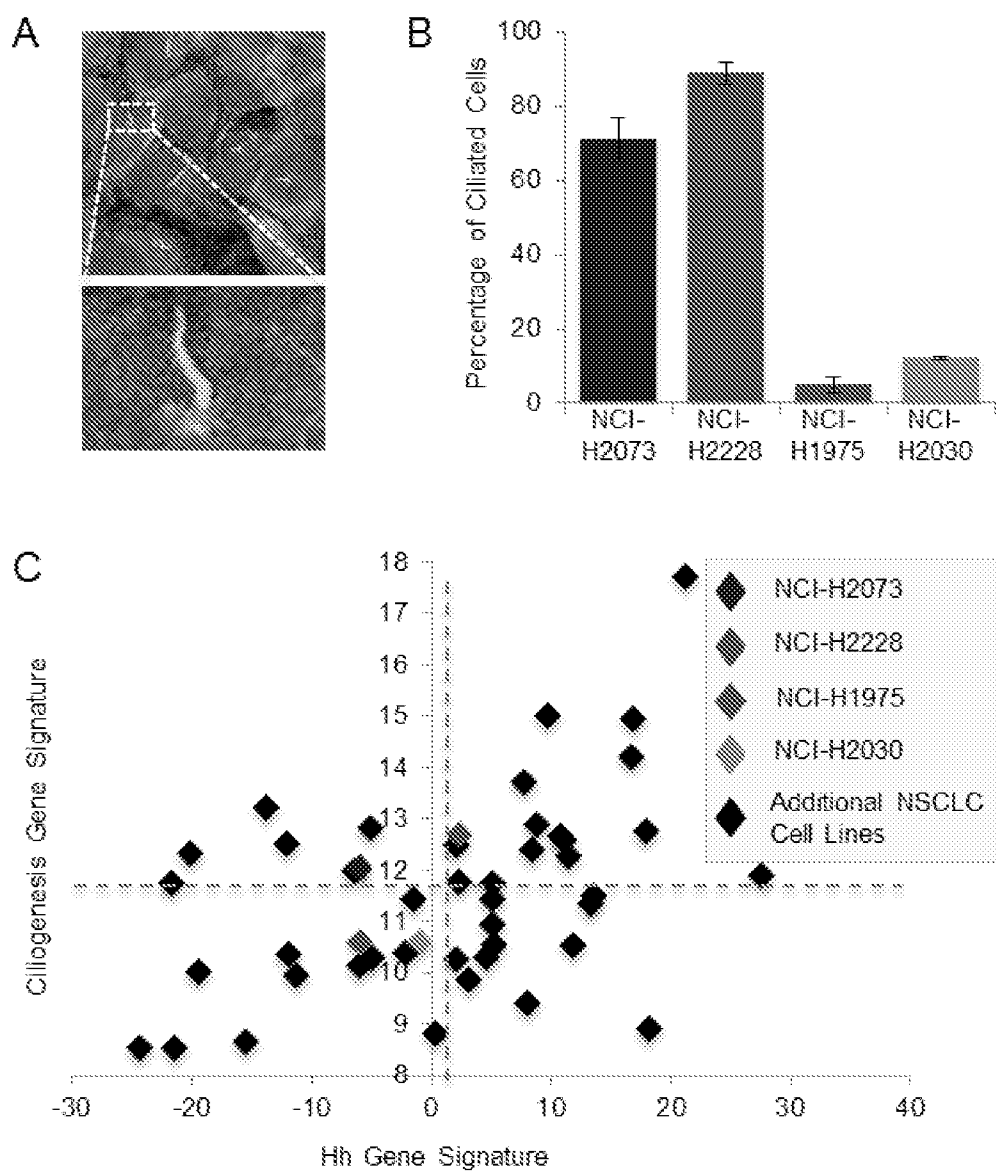
BBS10	79738	NC_000012.11	12q21.2	C12orf58, FLJ23560
TRIM32	22954	NC_000009.11	9q33.1	RP11-67K19.1, BBS11, HT2A, LGMD2H, TATIP
BBS12	166379	NC_000004.11	4q27	C4orf24, FLJ35630, FLJ41559
RAB3IP	117177	NC_000012.11	12q14.3	FLJ14660, FLJ22548, MGC71495, RABIN3
RAB8A	4218	NC_000019.9	19p13.1	MEL, RAB8
PCM1	5108	NC_000008.10	8p22-p21.3	PTC4
PKD1	5310	NC_000016.9	16p13.3	polycystin-1, PBP, Pc-1, TRPP1
PKD2	5311	NC_000004.11	4q22.1	polycystin-2, APKD2, MGC138466, MGC138468, PC2, PKD4, Pc-2, TRPP2
PKHD1	5314	NC_000006.11	6p12.2	fibrocystin, polyductin, ARPKD, DKFZp686C01112, FCYT, FLJ46150, TIGM1
AHI1	54806	NC_000006.11	6q23.3	RP1-32B1.2, AHI-1, DKFZp686J1653, FLJ14023, FLJ20069, JBTS3, ORF1, dJ71N10.1
ARL13B	200894	NC_000003.11	3q11.1	ARL2L1, DKFZp686E2075, DKFZp686L2472, DKFZp686M2074, DKFZp761H079, JBTS8, MGC1
INPP5E	56623	NC_000009.11	9q34.3	CORS1, CPD4, JBTS1, MGC117201, MORMS, PPI5PIV
TMEM216	51259	NC_000011.9	11q13.1	HSPC244, MGC13379
MKS1	54903	NC_000017.10	17q22	BBS13, FLJ20345, MES, MKS, POC12
TMEM67	91147	NC_000008.10	8q22.1	MKS3, JBTS6, NPHP11, MECKELIN, MGC26979
CC2D2A	57545	NC_000004.11	4p15.32	JBTS9, KIAA1345, MKS6
SDCCAG8	10806	NC_000001.10	1q43	HSPC085, BBS16, CCCAP, NPHP10, NY-CO-8, SLSN7, hCCCAP
SEPT7	989	NC_000007.13	7p14.2	septin 7, CDC10, CDC3, DKFZp686M10191, NBLA02942, SEPT7A
NPHP1	4867	NC_000002.11	2q13	nephrocystin-1, FLJ97602, JBTS4, NPH1, SLSN1
INVS	27130	NC_000009.11	9q31	INV, NPH2, NPHP2, KIAA0573, MGC133080, MGC133081

12/13

FIG. 9 (cont'd)

NPHP3	27031	NC_000003.11	3q22.1	DKFZp667K242, DKFZp781K1312, FLJ30691, FLJ36696, KIAA2000, MGC78666, MKS7, NPH3
NPHP4	261734	NC_000001.10	1p36	RP11-33M12.1, FLJ46306, KIAA0673, POC10, SLSN4
IQCB1	9657	NC_000003.11	3q21.1	OK/SW-cl.85, NPHP5, PIQ, SLSN5
CEP290	80184	NC_000012.11	12q21.32	3H11Ag, BBS14, CT87, FLJ13615, FLJ21979, JBTS5, JBTS6, KIAA0373, LCA10, MKS4, NPHP6, SLSN6, 3H11Ag, FLJ13615, FLJ21979, KIAA0373
GLIS2	84662	NC_000016.9	16p13.3	FLJ38247, NKL, NPHP7
RPGRIP1L	23322	NC_000016.9	16q12.2	CORS3, DKFZp686C0668, FTM, JBTS7, KIAA1005, MKS5, NPHP8
NEK8	284086	NC_000017.10	17q11.1	JCK, MGC138445, NEK12A, NPHP9
EVC	2121	NC_000004.11	4p16	DWF-1, EVC1, EVCL, MGC105107
EVC2	132884	NC_000004.11	4p16.2-p16.1	LBN
VHL	7428	NC_000003.11	3p25.3	HRCA1, RCA1, VHL1
OFD1	8481	NC_000023.10	Xp22	71-7A, CXorf5, JBTS10, MGC117039, MGC117040, SGBS2
STIL	6491	NC_000001.10	1p32	RP1-18D14.8, DKFZp686O09161, MCPH7, SIL
RPGR	6103	NC_000023.10	Xp21.1	COD1, CORDX1, CRD, PCDX, RP15, RP3, XLRP3, orf15

FIG. 10



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2017/023839

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61P 35/00; C12Q 1/68; G01N 33/574; G01N 33/68 (2017.01)

CPC - C12Q 1/6886; C12Q 2600/106; C12Q 2600/158; G01N 33/574; G01N 2800/52 (2017.02)

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

USPC - 435/6; 435/375; 435/7.23; 435/7.1 (keyword delimited)

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	US 2015/0025012 A1 (INFINITY PHARMACEUTICALS, INC) 22 January 2015 (22.01.2015) entire document	1, 2, 4-8, 10-13, 15, 16, 21-23, 27, 28
--		
Y		3, 9, 14, 17-20, 24-26, 29-32
Y	QIN et al. "Intraflagellar transport protein 122 antagonizes Sonic Hedgehog signaling and controls ciliary localization of pathway components," Proc Natl Acad Sci USA, 05 January 2011 (05.01.2011), Vol. 108, Pgs. 1456-1461. entire document	3, 9, 14
Y	BROUNS et al. "Triple immunofluorescence staining with antibodies raised in the same species to study the complex innervation pattern of intrapulmonary chemoreceptors," J Histochem Cytochem, 01 April 2002 (01.04.2002), Vol. 50, Pgs. 575-582. entire document	17-20, 24-26, 29-32
A	US 2014/0094461 A1 (BANDARU et al) 03 April 2014 (03.04.2014) entire document	1-32
A	US 2013/0274233 A1 (PRESIDENT AND FELLOW OF HARVARD COLLEGE) 17 October 2013 (17.10.2013) entire document	1-32
A	HASSOUNAH et al. "Molecular pathways: the role of primary cilia in cancer progression and therapeutics with a focus on Hedgehog signaling," Clin Cancer Res, 13 March 2012 (13.03.2012), Vol. 18, Pgs. 2429-2435. entire document	1-32
A	US 8,273,701 B2 (ROBBINS et al) 25 September 2012 (25.09.2012) entire document	1-32

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"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

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"&" document member of the same patent family

Date of the actual completion of the international search

08 May 2017

Date of mailing of the international search report

22 JUN 2017

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