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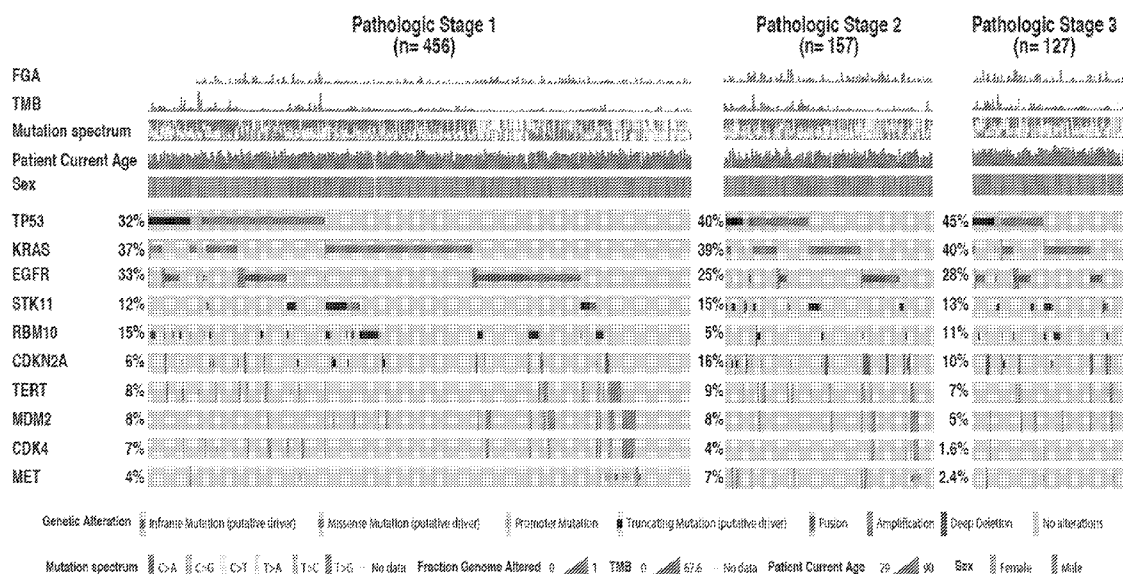


FIG. 1A

(57) Abstract: The present disclosure relates to methods for determining whether a patient diagnosed with lung or colon cancer and undergoing tumor resection surgery will benefit from treatment with intraoperative opioid analgesics or opioid-free intraoperative analgesics. The present disclosure relates to methods for determining whether a patient diagnosed with lung or colon cancer and undergoing tumor resection surgery will benefit from treatment with intraoperative opioid analgesics or opioid-free intraoperative analgesics. In some embodiments, the methods disclosed herein are based on screening a lung cancer patient for elevated FGA, mutations in CDKN2A and/o mutations in the Wnt or Hippo signaling pathways.

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## METHODS FOR TAILORING ANALGESIC REGIMEN IN LUNG OR COLON CANCER PATIENTS BASED ON TUMOR GENOMICS

### CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of and priority to U.S. Provisional Patent  
Application No. 63/162,265, filed March 17, 2021, the entire contents of which are  
incorporated herein by reference.

### TECHNICAL FIELD

[0002] The present technology relates to methods for determining whether a patient  
diagnosed with lung or colon cancer and undergoing tumor resection surgery will benefit  
from treatment with intraoperative opioid analgesics or opioid-free intraoperative analgesics  
(*e.g.*, intraoperative ester-type or amide-type local anesthetics). In some embodiments, the  
methods disclosed herein are based on screening a lung cancer patient for elevated FGA,  
mutations in CDKN2A and/or mutations in the Wnt or Hippo signaling pathways. In certain  
embodiments, the methods disclosed herein are based on screening the DNA mismatch repair  
(MMR) subtype of a colon cancer patient.

### STATEMENT OF GOVERNMENT SUPPORT

[0003] This invention was made with government support under grant number CA008748  
awarded by the National Cancer Institute. The government has certain rights in the invention.

### BACKGROUND

[0004] The following description of the background of the present technology is provided  
simply as an aid in understanding the present technology and is not admitted to describe or  
constitute prior art to the present technology.

[0005] In spite of the dependence risk, opioids are a necessary component in the perioperative  
analgesic regimen. Studies suggest that opioids augment tumor growth and metastasis,  
possibly through reduction of natural killer (NK) cell activity, T-lymphocyte proliferation,  
and cytokine secretion (Cata JP, Gottumukkala V, Sessler DI, *European Journal of Pain  
Supplements*. 5(2):345-355 (2011)). And while surgery remains the primary and most  
effective treatment option for most resectable cancers, tumor resection itself can induce  
systemic dissemination of cancer cells despite optimal surgical technique (Bar-Yosef S *et al.*,  
*Journal of the American Society of Anesthesiologists*. 94(6):1066-1073 (2001)). By  
combining the immunosuppressive effects of opioids administered intraoperatively with the

surgical stress response, the surgical environment has been hypothesized to magnify the proliferation of minimal residual disease (Looney *et al.*, *Journal of the American Society of Anesthesiologists*. 113(5):1118-1125 (2010); Retsky M *et al.*, *International Journal of Surgery*. 3(3):179-187 (2005)).

5 [0006] The relationship between opioid exposure and oncologic outcomes may vary according to cancer type or subtype. While opioids may support tumor pathogenesis in some solid cancers (Maher DP *et al.*, *Br J Anaesth*. 113 Suppl 1:i88-94 (2014); Cata JP *et al.* *Journal of Clinical Anesthesia*. 27(8):672-679 (2015); Cata JP *et al.*, *Cancer medicine*. 3(4):900-908 (2014); Silagy *et al.* *British journal of anaesthesia*. S0007-0912 (0020) 30496-10 30497 (2020)), in others they may be neutral or even protective (Du KN *et al.*, *Anesthesia & Analgesia*. 127(1):210-216 (2018)).

[0007] Accordingly, there is an urgent need for developing reliable and accurate methods for predicting whether a cancer patient undergoing tumor resection surgery for lung or colon cancer will benefit from treatment with intraoperative opioid analgesics.

## 15 SUMMARY OF THE PRESENT TECHNOLOGY

[0008] In one aspect, the present disclosure provides a method for selecting a cancer patient undergoing tumor resection surgery for lung cancer for treatment with an intraoperative opioid analgesic comprising (a) detecting the presence of at least one mutation in one or more genes that results (i) in hyperactivation of Wnt pathway and/or (ii) reduced activity of the Hippo pathway in a biological sample obtained from the cancer patient; and (b) administering to the cancer patient an effective amount of an intraoperative opioid analgesic during the tumor resection surgery. The one or more genes may be selected from the group consisting of NF2, LATS1, FAT1, RNF43, CTNNB1, AXIN2, APC and AMER1. Additionally or alternatively, in some embodiments, the at least one mutation is a frameshift mutation, a missense mutation, a deletion, an insertion, a nonsense mutation, an inversion, or a translocation. The at least one mutation may be detected using any nucleic acid detection assay known in the art such as next-generation sequencing, PCR, real-time quantitative PCR (qPCR), digital PCR (dPCR), Southern blotting, Reverse transcriptase-PCR (RT-PCR), Northern blotting, microarray, dot or slot blots, *in situ* hybridization, or fluorescent *in situ* hybridization (FISH). In some embodiments, the biological sample comprises genomic DNA, cDNA, RNA, and/or mRNA.

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**[0009]** In one aspect, the present disclosure provides a method for prolonging survival of a cancer patient undergoing tumor resection surgery for lung cancer comprising administering to the cancer patient an effective amount of an intraoperative opioid analgesic during the tumor resection surgery, wherein mRNA or polypeptide expression and/or activity levels of one or more of NF2, LATS1, FAT1, RNF43, AXIN2, APC and AMER1 in a biological sample obtained from the cancer patient are reduced compared to that observed in a control sample obtained from a healthy subject or a predetermined threshold. In another aspect, the present disclosure provides a method for prolonging survival of a cancer patient undergoing tumor resection surgery for lung cancer comprising administering to the cancer patient an effective amount of an intraoperative opioid analgesic during the tumor resection surgery, wherein mRNA or polypeptide expression and/or activity levels of CTNNB1 in a biological sample obtained from the cancer patient are elevated compared to that observed in a control sample obtained from a healthy subject or a predetermined threshold. Additionally or alternatively, in some embodiments, mRNA expression levels are detected via real-time quantitative PCR (qPCR), digital PCR (dPCR), Reverse transcriptase-PCR (RT-PCR), Northern blotting, microarray, dot or slot blots, *in situ* hybridization, or fluorescent *in situ* hybridization (FISH). Additionally or alternatively, in certain embodiments, polypeptide expression levels are detected via Western blotting, enzyme-linked immunosorbent assays (ELISA), dot blotting, immunohistochemistry, immunofluorescence, immunoprecipitation, immunoelectrophoresis, or mass-spectrometry.

**[0010]** In any of the preceding embodiments of methods disclosed herein, the intraoperative opioid analgesic is fentanyl, hydromorphone, morphine, oxycodone, hydrocodone, codeine, meperidine, remifentanyl, or sufentanyl. The effective amount of the intraoperative opioid analgesic may range from about 1 MME to about 200 MMEs. In certain embodiments, the effective amount of the intraoperative opioid analgesic is about 1 MME to about 20 MMEs, about 20 MMEs to about 45 MMEs, or about 45 MMEs to about 200 MMEs. In some embodiments, the effective amount of the intraoperative opioid analgesic is about 1 MME, about 2 MMEs, about 3 MMEs, about 4 MMEs, about 5 MMEs, about 6 MMEs, about 7 MMEs, about 8 MMEs, about 9 MMEs, about 10 MMEs, about 11 MMEs, about 12 MMEs, about 13 MMEs, about 14 MMEs, about 15 MMEs, about 16 MMEs, about 17 MMEs, about 18 MMEs, about 19 MMEs, about 20 MMEs, about 21 MMEs, about 22 MMEs, about 23 MMEs, about 24 MMEs, about 25 MMEs, about 26 MMEs, about 27 MMEs, about 28 MMEs, about 29 MMEs, about 30 MMEs, about 31 MMEs, about 32 MMEs, about 33

MMEs, about 34 MMEs, about 35 MMEs, about 36 MMEs, about 37 MMEs, about 38 MMEs, about 39 MMEs, about 40-45 MMEs, about 45-50 MMEs, about 50-55 MMEs, about 55-60 MMEs, about 60-65 MMEs, about 65-70 MMEs, about 70-75 MMEs, about 75-80 MMEs, about 80-85 MMEs, about 85-90 MMEs, about 90-95 MMEs, about 95-100 MMEs, about 100-110 MMEs, about 110-120 MMEs, about 120-130 MMEs, about 130-140 MMEs, about 140-150 MMEs, about 150-160 MMEs, about 160-170 MMEs, about 170-180 MMEs, about 180-190 MMEs, or about 190-200 MMEs. Additionally or alternatively, in some embodiments, the effective amount of the intraoperative opioid analgesic is administered as a series of bolus doses or as a continuous infusion during the tumor resection surgery. In certain embodiments, the effective amount of the intraoperative opioid analgesic is administered to the cancer patient prior to incision. Additionally or alternatively, in some embodiments, the effective amount of the intraoperative opioid analgesic is administered intravenously.

**[0011]** Additionally or alternatively, in some embodiments, the methods of the present technology further comprise administering to the cancer patient an effective amount of a local anesthetic solution via an epidural catheter before, during and/or after the tumor resection surgery. The effective amount of the local anesthetic solution may range from about 0.05%-4% local anesthetic solution in a volume of 1-10 ml per hour when administered via an epidural catheter. Additionally or alternatively, in some embodiments, the effective amount of the local anesthetic solution is administered as a single injection, a series of bolus doses or as a continuous infusion during the tumor resection surgery. In other embodiments, the effective amount of the local anesthetic solution may be administered before, during and/or after the tumor resection surgery using any regional anesthesia technique directed at nerves innervating the thorax and chest wall (*e.g.*, via serratus plane nerve block, intercostal nerve block, or paravertebral block). The effective amount of the local anesthetic solution may range from about 0.05%-4% local anesthetic solution in a volume of 10-40 ml when administered using any regional anesthesia technique directed at nerves innervating the thorax and chest wall (*e.g.*, via serratus plane nerve block, intercostal nerve block, or paravertebral block). Examples of suitable local anesthetics include, but are not limited to, lidocaine, mepivacaine, prilocaine, bupivacaine, etidocaine, ropivacaine, levobupivacaine, cocaine, procaine, tetracaine, chlorprocaine, and benzocaine. Additionally or alternatively, in certain embodiments, the local anesthetic solution may further comprise an opioid (*e.g.*, fentanyl, hydromorphone, morphine, oxycodone, hydrocodone, codeine, meperidine, remifentanyl, or

sufentanil). In some embodiments, the local anesthetic solution may comprise 0.5 mcg/ml-50 mcg/ml opioid.

**[0012]** Additionally or alternatively, in certain embodiments, the methods of the present technology further comprise administering to the cancer patient an effective amount of a post-operative opioid analgesic after the tumor resection surgery. Examples of post-operative opioid analgesics include, but are not limited to, fentanyl, hydromorphone, morphine, oxycodone, hydrocodone, codeine, meperidine, remifentanyl, or sufentanil. In some embodiments, the post-operative opioid analgesic and the intraoperative opioid analgesic are the same opioid analgesic or different opioid analgesics. In other embodiments, the effective amount of the post-operative opioid analgesic and the effective amount of the intraoperative opioid analgesic are the same or different. Additionally or alternatively, in some embodiments, the effective amount of the post-operative opioid analgesic is administered to the cancer patient as a bolus of about 0.005 mg to about 100 mg. In some embodiments, the effective amount of the post-operative opioid analgesic is administered to the cancer patient as a bolus of about 0.005 mg, about 0.006 mg, about 0.007 mg, about 0.008 mg, about 0.009 mg, about 0.01 mg, about 0.02 mg, about 0.03 mg, about 0.04 mg, about 0.05 mg, about 0.06 mg, about 0.07 mg, about 0.08 mg, about 0.09 mg, about 0.1 mg, about 0.2 mg, about 0.3 mg, about 0.4 mg, about 0.5 mg, about 0.6 mg, about 0.7 mg, about 0.8 mg, about 0.9 mg, about 1-5 mg, about 5-10 mg, about 1-5 mg, about 5-10 mg, about 1-5 mg, about 5-10 mg, about 1-5 mg, about 5-10 mg, about 10-15 mg, about 15-20 mg, about 20-25 mg, about 25-30 mg, about 30-35 mg, about 35-40 mg, about 40-45 mg, about 45-50 mg, about 50-55 mg, about 55-60 mg, about 60-65 mg, about 65-70 mg, about 70-75 mg, about 75-80 mg, about 80-85 mg, about 85-90 mg, about 90-95 mg, or about 95-100 mg. In other embodiments, the effective amount of the post-operative opioid analgesic may be continuously delivered to the cancer patient at a per hour rate of about 0.01 mg/hr to about 10 mg/hr. In certain embodiments, the effective amount of the post-operative opioid analgesic is continuously delivered to the cancer patient at a per hour rate of about 0.01 mg/hr, about 0.02 mg/hr, about 0.03 mg/hr, about 0.04 mg/hr, about 0.05 mg/hr, about 0.06 mg/hr, about 0.07 mg/hr, about 0.08 mg/hr, about 0.09 mg/hr, about 0.1 mg/hr, about 0.2 mg/hr, about 0.3 mg/hr, about 0.4 mg/hr, about 0.5 mg/hr, about 0.6 mg/hr, about 0.7 mg/hr, about 0.8 mg/hr, about 0.9 mg/hr, about 1 mg/hr, about 1.5 mg/hr, about 2 mg/hr, about 2.5 mg/hr, about 3 mg/hr, about 3.5 mg/hr, about 4 mg/hr, about 4.5 mg/hr, about 5 mg/hr, about 5.5 mg/hr, about 6 mg/hr, about 6.5 mg/hr, about 7 mg/hr, about 7.5 mg/hr, about 8 mg/hr, about 8.5 mg/hr, about 9 mg/hr,

about 9.5 mg/hr, or about 10 mg/hr. Additionally or alternatively, in some embodiments, the effective amount of the post-operative opioid analgesic is administered intravenously, orally, or transdermally.

[0013] In one aspect, the present disclosure provides a method for selecting a cancer patient undergoing tumor resection surgery for lung cancer for treatment with an opioid-free intraoperative analgesic comprising (a) (i) detecting the presence of at least one mutation in CDKN2A in a biological sample obtained from the cancer patient, wherein the at least one mutation reduces CDKN2A expression and/or activity levels, and/or (ii) detecting elevated FGA in a biological sample obtained from the cancer patient compared to a control sample obtained from a healthy subject or a predetermined threshold; and (b) administering to the cancer patient an effective amount of an opioid-free intraoperative analgesic during the tumor resection surgery. The at least one mutation in CDKN2A may be a frameshift mutation, a missense mutation, a deletion, an insertion, a nonsense mutation, an inversion, or a translocation. The at least one mutation may be detected using any nucleic acid detection assay known in the art such as next-generation sequencing, PCR, real-time quantitative PCR (qPCR), digital PCR (dPCR), Southern blotting, Reverse transcriptase-PCR (RT-PCR), Northern blotting, microarray, dot or slot blots, *in situ* hybridization, or fluorescent *in situ* hybridization (FISH). In some embodiments, the biological sample comprises genomic DNA, cDNA, RNA, and/or mRNA. Additionally or alternatively, in some embodiments, FGA is detected via next-generation sequencing.

[0014] In one aspect, the present disclosure provides a method for prolonging survival of a cancer patient undergoing tumor resection surgery for lung cancer comprising administering to the cancer patient an effective amount of an opioid-free intraoperative analgesic during the tumor resection surgery, wherein mRNA or polypeptide expression and/or activity levels of CDKN2A in a biological sample obtained from the cancer patient are reduced compared to that observed in a control sample obtained from a healthy subject or a predetermined threshold. Additionally or alternatively, in some embodiments, mRNA expression levels are detected via real-time quantitative PCR (qPCR), digital PCR (dPCR), Reverse transcriptase-PCR (RT-PCR), Northern blotting, microarray, dot or slot blots, *in situ* hybridization, or fluorescent *in situ* hybridization (FISH). Additionally or alternatively, in certain embodiments, polypeptide expression levels are detected via Western blotting, enzyme-linked immunosorbent assays (ELISA), dot blotting, immunohistochemistry, immunofluorescence, immunoprecipitation, immunoelectrophoresis, or mass-spectrometry.

In another aspect, the present disclosure provides a method for prolonging survival of a cancer patient undergoing tumor resection surgery for lung cancer comprising administering to the cancer patient an effective amount of an opioid-free intraoperative analgesic during the tumor resection surgery, wherein FGA in a biological sample obtained from the cancer patient is elevated compared to that observed in a control sample obtained from a healthy subject or a predetermined threshold. In some embodiments, FGA is detected via next-generation sequencing.

**[0015]** Additionally or alternatively, in some embodiments of the methods disclosed herein, the opioid-free intraoperative analgesic is an amide-type local anesthetic or an ester-type local anesthetic. Examples of amide-type local anesthetics include, but are not limited to, lidocaine, mepivacaine, prilocaine, bupivacaine, etidocaine, ropivacaine, or levobupivacaine. Examples of ester-type local anesthetics include, but are not limited to, cocaine, procaine, tetracaine, chloroprocaine, or benzocaine. The opioid-free intraoperative analgesic may be administered via an epidural catheter. Additionally or alternatively, in some embodiments of the methods disclosed herein, the effective amount of the opioid-free intraoperative analgesic is about 0.05%-4% amide-type or ester-type local anesthetic solution in a volume of 1-10 ml per hour when administered via an epidural catheter. In certain embodiments, the effective amount of the opioid-free intraoperative analgesic may be administered as a single injection, a series of bolus doses, or as a continuous infusion during the tumor resection surgery.

**[0016]** In certain embodiments, the opioid-free intraoperative analgesic may be administered via any regional anesthesia technique directed at nerves innervating the thorax and chest wall, such as via a serratus plane nerve block, or via an intercostal nerve block, or via a paravertebral block. In some embodiments, the effective amount of the opioid-free intraoperative analgesic is about 0.05%-4% amide-type or ester-type local anesthetic solution in a volume of 10-40 ml when administered via any regional anesthesia technique directed at nerves innervating the thorax and chest wall, such as via a serratus plane nerve block, or via an intercostal nerve block, or via a paravertebral block.

**[0017]** Additionally or alternatively, in some embodiments, the methods of the present technology further comprise administering to the cancer patient an effective amount of an opioid-free post-operative analgesic after the tumor resection surgery. Examples of suitable opioid-free post-operative analgesics include, but are not limited to, lidocaine, mepivacaine, prilocaine, bupivacaine, etidocaine, ropivacaine, levobupivacaine, cocaine, procaine,

tetracaine, chloroprocaine, and benzocaine. In some embodiments, the opioid-free post-operative analgesic and the opioid-free intraoperative analgesic are the same analgesic or different analgesics. In other embodiments, the effective amount of the opioid-free post-operative analgesic and the effective amount of the opioid-free intraoperative analgesic are the same or different. In some embodiments, the effective amount of the opioid-free post-operative analgesic is about 0.05 %, about 0.06 %, about 0.07 %, about 0.08 %, about 0.09 %, about 0.1 %, about 0.15 %, about 0.2 %, about 0.25 %, about 0.3 %, about 0.35 %, about 0.4 %, about 0.45 %, about 0.5 %, about 0.55 %, about 0.6 %, about 0.65 %, about 0.7 %, about 0.75 %, about 0.8 %, about 0.85 %, about 0.9 %, about 0.95 %, about 1.0 %, about 1.1 %, about 1.2 %, about 1.3 %, about 1.4 %, about 1.5 %, about 1.6 %, about 1.7 %, about 1.8 %, about 1.9 %, about 2.0 %, about 2.1 %, about 2.2 %, about 2.3 %, about 2.4 %, about 2.5 %, about 2.6 %, about 2.7 %, about 2.8 %, about 2.9 %, about 3.0 %, about 3.1 %, about 3.2 %, about 3.3 %, about 3.4 %, about 3.5 %, about 3.6 %, about 3.7 %, about 3.8 %, about 3.9 %, or about 4.0 % amide-type or ester-type local anesthetic solution in a volume of about 10 ml, about 12.5 ml, about 15 ml, about 17.5 ml, about 20 ml, about 22.5 ml, about 25 ml, about 27.5 ml, about 30 ml, about 32.5 ml, about 35 ml, about 37.5 ml, or about 40 ml when administered via any regional anesthesia technique directed at nerves innervating the thorax and chest wall, such as via a serratus plane nerve block, or via an intercostal nerve block, or via a paravertebral block.

**[0018]** In any and all embodiments of the methods disclosed herein, the cancer patient exhibits stage I, stage II or stage III lung cancer. Additionally or alternatively, in some embodiments, the cancer patient has been diagnosed with lung adenocarcinoma (LUAD). The histologic subtype of the lung adenocarcinoma may be lepidic, acinar, papillary, micropapillary, solid or unknown.

**[0019]** In any of the preceding embodiments of the methods disclosed herein, the cancer patient may or may not have received an adjuvant therapy. The adjuvant therapy may be chemotherapy, lobectomy, radiation therapy or chemoradiation therapy.

**[0020]** Additionally or alternatively, in some embodiments of the methods disclosed herein, the patient is human.

**[0021]** In any and all embodiments of the methods disclosed herein, the biological sample obtained from the cancer patient comprises biopsied tumor tissue, whole blood, plasma, or serum.

**[0022]** In one aspect, the present disclosure provides a method for selecting a cancer patient undergoing tumor resection surgery for colon cancer for treatment with an intraoperative opioid analgesic comprising (a) assaying gene expression levels of MLH1, MSH2, MSH6, and PMS2 in a biological sample obtained from the cancer patient and (b) administering to the cancer patient an effective amount of an intraoperative opioid analgesic during the tumor resection surgery, wherein the gene expression levels of one or more of MLH1, MSH2, MSH6, and PMS2 in the biological sample obtained from the cancer patient are reduced or undetectable compared to that observed in a control sample obtained from a healthy subject or a predetermined threshold. In certain embodiments, expression levels of MLH1, MSH2, MSH6, and/or PMS2 are assayed via next-generation sequencing, PCR, quantitative PCR (qPCR), digital PCR (dPCR), Reverse transcriptase-PCR (RT-PCR), Northern blotting, microarray, dot or slot blots, *in situ* hybridization, fluorescent *in situ* hybridization (FISH), RNA-seq, Western blotting, enzyme-linked immunosorbent assays (ELISA), dot blotting, immunohistochemistry, immunofluorescence, immunoprecipitation, immunoelectrophoresis, or mass-spectrometry. Additionally or alternatively, in some embodiments, the biological sample comprises genomic DNA, cDNA, RNA, and/or mRNA.

**[0023]** In one aspect, the present disclosure provides a method for selecting a cancer patient undergoing tumor resection surgery for colon cancer for treatment with an intraoperative opioid analgesic comprising (a) assaying gene expression levels of one or more genes selected from among CCK, NTSR2, IFNG, NTSR1, MLN, LIPN, CXCL8, FCGR3B, PTGS2, CALB2, CXCL10, IL1RN, CCL5, CCL4, IL1B, CCL4L2, FCGR3A, ITIH4, GAD1, CD8A, HTR3A, SLC1A3, LHB, ADRB1, CXCR2, HRH2, CCL3, ESR2, CSF2, ENO2, GRM5, CXCR4, PRKCG, CD68, IL10, TLR2, ICAM1, RGS2, CX3CL1 and KCNJ3 in a biological sample obtained from the cancer patient; and (b) administering to the cancer patient an effective amount of an intraoperative opioid analgesic during the tumor resection surgery, wherein the gene expression levels of one or more of CCK, NTSR2, IFNG, NTSR1, MLN, LIPN, CXCL8, FCGR3B, PTGS2, CALB2, CXCL10, IL1RN, CCL5, CCL4, IL1B, CCL4L2, FCGR3A, ITIH4, GAD1, CD8A, HTR3A, SLC1A3, LHB, ADRB1, CXCR2, HRH2, CCL3, ESR2, CSF2, ENO2, GRM5, CXCR4, PRKCG, CD68, IL10, TLR2, ICAM1, RGS2, CX3CL1 and KCNJ3 in the biological sample obtained from the cancer patient are elevated relative to a reference sample obtained from a colon cancer subject having microsatellite stable (MSS) tumors or a predetermined threshold. In certain embodiments, expression levels of CCK, NTSR2, IFNG, NTSR1, MLN, LIPN, CXCL8, FCGR3B, PTGS2, CALB2,

CXCL10, IL1RN, CCL5, CCL4, IL1B, CCL4L2, FCGR3A, ITIH4, GAD1, CD8A, HTR3A, SLC1A3, LHB, ADRB1, CXCR2, HRH2, CCL3, ESR2, CSF2, ENO2, GRM5, CXCR4, PRKCG, CD68, IL10, TLR2, ICAM1, RGS2, CX3CL1 and KCNJ3 are assayed via next-generation sequencing, PCR, quantitative PCR (qPCR), digital PCR (dPCR), Reverse transcriptase-PCR (RT-PCR), Northern blotting, microarray, dot or slot blots, *in situ* hybridization, fluorescent *in situ* hybridization (FISH), RNA-seq, Western blotting, enzyme-linked immunosorbent assays (ELISA), dot blotting, immunohistochemistry, immunofluorescence, immunoprecipitation, immunoelectrophoresis, or mass-spectrometry. Additionally or alternatively, in some embodiments, the biological sample comprises genomic DNA, cDNA, RNA, and/or mRNA.

**[0024]** In one aspect, the present disclosure provides a method for selecting a cancer patient undergoing tumor resection surgery for colon cancer for treatment with an intraoperative opioid analgesic comprising (a) assaying gene expression levels of one or more genes selected from among GHRH, RBFOX3, NOS1, KISS1, NPFFR2, GRIA2, SLC6A2, ADCY5, HCRT, OPCML, NPY1R, PPP1R14C, NTRK2, CHAT, LEP, CPB1, NR1I2, ADCY8, EGF, F2, UGT1A8, PLG, GRPR, PYY, DRD1, ABCB1, SCT, NTS, NPFFR1, TH, CYP2B6, HTR2C, TAC1, CCKBR, CALCA, ATP12A, LINC01411, P2RX3, ALB, REN, SLC6A4, KNG1 and DRD2 in a biological sample obtained from the cancer patient; and (b) administering to the cancer patient an effective amount of an intraoperative opioid analgesic during the tumor resection surgery, wherein the gene expression levels of one or more of GHRH, RBFOX3, NOS1, KISS1, NPFFR2, GRIA2, SLC6A2, ADCY5, HCRT, OPCML, NPY1R, PPP1R14C, NTRK2, CHAT, LEP, CPB1, NR1I2, ADCY8, EGF, F2, UGT1A8, PLG, GRPR, PYY, DRD1, ABCB1, SCT, NTS, NPFFR1, TH, CYP2B6, HTR2C, TAC1, CCKBR, CALCA, ATP12A, LINC01411, P2RX3, ALB, REN, SLC6A4, KNG1 and DRD2 in the biological sample obtained from the cancer patient are reduced relative to a reference sample obtained from a colon cancer subject having microsatellite stable (MSS) tumors or a predetermined threshold. In certain embodiments, expression levels of GHRH, RBFOX3, NOS1, KISS1, NPFFR2, GRIA2, SLC6A2, ADCY5, HCRT, OPCML, NPY1R, PPP1R14C, NTRK2, CHAT, LEP, CPB1, NR1I2, ADCY8, EGF, F2, UGT1A8, PLG, GRPR, PYY, DRD1, ABCB1, SCT, NTS, NPFFR1, TH, CYP2B6, HTR2C, TAC1, CCKBR, CALCA, ATP12A, LINC01411, P2RX3, ALB, REN, SLC6A4, KNG1 and DRD2 are assayed via next-generation sequencing, PCR, quantitative PCR (qPCR), digital PCR (dPCR), Reverse transcriptase-PCR (RT-PCR), Northern blotting, microarray, dot or slot blots, *in situ*

hybridization, fluorescent *in situ* hybridization (FISH), RNA-seq, Western blotting, enzyme-linked immunosorbent assays (ELISA), dot blotting, immunohistochemistry, immunofluorescence, immunoprecipitation, immunoelectrophoresis, or mass-spectrometry. Additionally or alternatively, in some embodiments, the biological sample comprises  
5 genomic DNA, cDNA, RNA, and/or mRNA.

**[0025]** In another aspect, the present disclosure provides a method for selecting a cancer patient undergoing tumor resection surgery for colon cancer for treatment with an intraoperative opioid analgesic comprising (a) detecting the expression levels or activity of one or more genes that result (i) in reduced activity of Wnt pathway and/or (ii) hyperactivation  
10 of Th1 immune response signature in a biological sample obtained from the cancer patient relative to a predetermined threshold; and (b) administering to the cancer patient an effective amount of an intraoperative opioid analgesic during the tumor resection surgery. Additionally or alternatively, in some embodiments, the cancer patient comprises at least one mutation that results in reduced activity of Wnt pathway and/or (ii) hyperactivation of Th1 immune  
15 response signature. Additionally or alternatively, in some embodiments, the at least one mutation is a frameshift mutation, a missense mutation, a deletion, an insertion, a nonsense mutation, an inversion, or a translocation. The at least one mutation may be detected using any nucleic acid detection assay known in the art such as next-generation sequencing, PCR, real-time quantitative PCR (qPCR), digital PCR (dPCR), Southern blotting, Reverse  
20 transcriptase-PCR (RT-PCR), Northern blotting, microarray, dot or slot blots, *in situ* hybridization, or fluorescent *in situ* hybridization (FISH). In some embodiments, the biological sample comprises genomic DNA, cDNA, RNA, and/or mRNA.

**[0026]** Additionally or alternatively, in some embodiments, the reduced activity of Wnt pathway is determined by detecting the expression levels or activity of one or more genes  
25 selected from among ADAM17, AXIN1, AXIN2, CCND2, CSNK1E, CTNNB1, CUL1, DKK1, DKK4, DLL1, DVL2, FRAT1, FZD1, FZD8, GNAI1, HDAC11, HDAC2, HDAC5, HEY1, HEY2, JAG1, JAG2, KAT2A, LEF1, MAML1, MYC, NCOR2, NCSTN, NKD1, NOTCH1, NOTCH4, NUMB, PPARD, PSEN2, PTCH1, RBPJ, SKP2, TCF7, TP53, WNT1, WNT5B, and WNT6. In certain embodiments, the expression levels of one or more genes  
30 selected from among ADAM17, AXIN1, AXIN2, CCND2, CSNK1E, CTNNB1, CUL1, DKK1, DKK4, DLL1, DVL2, FRAT1, FZD1, FZD8, GNAI1, HDAC11, HDAC2, HDAC5, HEY1, HEY2, JAG1, JAG2, KAT2A, LEF1, MAML1, MYC, NCOR2, NCSTN, NKD1, NOTCH1, NOTCH4, NUMB, PPARD, PSEN2, PTCH1, RBPJ, SKP2, TCF7, TP53, WNT1,

WNT5B, and WNT6 are detected via next-generation sequencing, PCR, quantitative PCR (qPCR), digital PCR (dPCR), Reverse transcriptase-PCR (RT-PCR), Northern blotting, microarray, dot or slot blots, *in situ* hybridization, fluorescent *in situ* hybridization (FISH), RNA-seq, Western blotting, enzyme-linked immunosorbent assays (ELISA), dot blotting, immunohistochemistry, immunofluorescence, immunoprecipitation, immunoelectrophoresis, or mass-spectrometry. Additionally or alternatively, in some embodiments, the biological sample comprises genomic DNA, cDNA, RNA, and/or mRNA.

**[0027]** Additionally or alternatively, in some embodiments, the hyperactivation of Th1 immune response signature is determined by detecting the expression levels or activity of one or more genes selected from among IFNG, LTA, APBB2, DOK5, IL12RB2, APOD, ZBTB32, CD38, CSF2, CTLA4, CD70, DPP4, EGFL6, BST2, DUSP5, LRP8, IL22, DGKI, CCL4, GGT1, LRRN3, SYNGR3, ATP9A, BTG3, CMAHP, HBEGF, and SGCB. In certain embodiments, the expression levels of one or more genes selected from among IFNG, LTA, APBB2, DOK5, IL12RB2, APOD, ZBTB32, CD38, CSF2, CTLA4, CD70, DPP4, EGFL6, BST2, DUSP5, LRP8, IL22, DGKI, CCL4, GGT1, LRRN3, SYNGR3, ATP9A, BTG3, CMAHP, HBEGF, and SGCB are detected via next-generation sequencing, PCR, quantitative PCR (qPCR), digital PCR (dPCR), Reverse transcriptase-PCR (RT-PCR), Northern blotting, microarray, dot or slot blots, *in situ* hybridization, fluorescent *in situ* hybridization (FISH), RNA-seq, Western blotting, enzyme-linked immunosorbent assays (ELISA), dot blotting, immunohistochemistry, immunofluorescence, immunoprecipitation, immunoelectrophoresis, or mass-spectrometry. Additionally or alternatively, in some embodiments, the biological sample comprises genomic DNA, cDNA, RNA, and/or mRNA.

**[0028]** In one aspect, the present disclosure provides a method for reducing the risk of tumor recurrence in a cancer patient undergoing tumor resection surgery for colon cancer comprising administering to the cancer patient an effective amount of an intraoperative opioid analgesic during the tumor resection surgery, wherein the cancer patient comprises at least one alteration in DNA mismatch repair (MMR) system (*e.g.*, MMR deficiency). In some embodiments, mRNA or polypeptide expression levels of one or more of MLH1, MSH2, MSH6, and PMS2 in a biological sample obtained from the cancer patient are reduced or undetectable compared to that observed in a control sample obtained from a healthy subject or a predetermined threshold. Additionally or alternatively, in some embodiments, the mRNA or polypeptide expression levels of one or more of CCK, NTSR2, IFNG, NTSR1, MLN, LIPN, CXCL8, FCGR3B, PTGS2, CALB2, CXCL10, IL1RN, CCL5, CCL4, IL1B,

CCL4L2, FCGR3A, ITIH4, GAD1, CD8A, HTR3A, SLC1A3, LHB, ADRB1, CXCR2, HRH2, CCL3, ESR2, CSF2, ENO2, GRM5, CXCR4, PRKCG, CD68, IL10, TLR2, ICAM1, RGS2, CX3CL1 and KCNJ3 in a biological sample obtained from the cancer patient are elevated relative to a reference sample obtained from a colon cancer subject having  
 5 microsatellite stable (MSS) tumors or a predetermined threshold. Additionally or alternatively, in some embodiments, the mRNA or polypeptide levels of one or more of GHRH, RBFOX3, NOS1, KISS1, NPFFR2, GRIA2, SLC6A2, ADCY5, HCRT, OPCML, NPY1R, PPP1R14C, NTRK2, CHAT, LEP, CPB1, NR1I2, ADCY8, EGF, F2, UGT1A8, PLG, GRPR, PYY, DRD1, ABCB1, SCT, NTS, NPFFR1, TH, CYP2B6, HTR2C, TAC1,  
 10 CCKBR, CALCA, ATP12A, LINC01411, P2RX3, ALB, REN, SLC6A4, KNG1 and DRD2 in the biological sample obtained from the cancer patient are reduced relative to a reference sample obtained from a colon cancer subject having microsatellite stable (MSS) tumors or a predetermined threshold. Additionally or alternatively, in some embodiments, the cancer patient comprises at least one genetic mutation or altered expression levels or activity of one  
 15 or more genes that result (i) in reduced activity of Wnt pathway and/or (ii) hyperactivation of Th1 immune response signature. In some embodiments, the reduced activity of Wnt pathway is determined by detecting the expression levels or activity of one or more genes selected from among ADAM17, AXIN1, AXIN2, CCND2, CSNK1E, CTNNB1, CUL1, DKK1, DKK4, DLL1, DVL2, FRAT1, FZD1, FZD8, GNAI1, HDAC11, HDAC2, HDAC5, HEY1,  
 20 HEY2, JAG1, JAG2, KAT2A, LEF1, MAML1, MYC, NCOR2, NCSTN, NKD1, NOTCH1, NOTCH4, NUMB, PPARD, PSEN2, PTCH1, RBPJ, SKP2, TCF7, TP53, WNT1, WNT5B, and WNT6. In other embodiments, the hyperactivation of Th1 immune response signature is determined by detecting the expression levels or activity of one or more genes selected from among IFNG, LTA, APBB2, DOK5, IL12RB2, APOD, ZBTB32, CD38, CSF2, CTLA4,  
 25 CD70, DPP4, EGFL6, BST2, DUSP5, LRP8, IL22, DGKI, CCL4, GGT1, LRRN3, SYNGR3, ATP9A, BTG3, CMAHP, HBEGF, and SGCB.

**[0029]** Additionally or alternatively, in some embodiments of the preceding embodiments, genetic mutations or mRNA expression levels are detected via next generation sequencing, RNA-seq, real-time quantitative PCR (qPCR), digital PCR (dPCR), Reverse transcriptase-  
 30 PCR (RT-PCR), Northern blotting, microarray, dot or slot blots, *in situ* hybridization, or fluorescent *in situ* hybridization (FISH). Additionally or alternatively, in certain embodiments, polypeptide expression levels are detected via Western blotting, enzyme-

linked immunosorbent assays (ELISA), dot blotting, immunohistochemistry, immunofluorescence, immunoprecipitation, immunoelectrophoresis, or mass-spectrometry.

**[0030]** In any of the preceding embodiments of methods disclosed herein, the intraoperative opioid analgesic is fentanyl, hydromorphone, morphine, oxycodone, hydrocodone, codeine, meperidine, remifentanyl, or sufentanyl. The effective amount of the intraoperative opioid analgesic may range from about 1 MME to about 200 MMEs. In certain embodiments, the effective amount of the intraoperative opioid analgesic is about 1 MME to about 20 MMEs, about 20 MMEs to about 45 MMEs, or about 45 MMEs to about 200 MMEs. In some embodiments, the effective amount of the intraoperative opioid analgesic is about 1 MME, about 2 MMEs, about 3 MMEs, about 4 MMEs, about 5 MMEs, about 6 MMEs, about 7 MMEs, about 8 MMEs, about 9 MMEs, about 10 MMEs, about 11 MMEs, about 12 MMEs, about 13 MMEs, about 14 MMEs, about 15 MMEs, about 16 MMEs, about 17 MMEs, about 18 MMEs, about 19 MMEs, about 20 MMEs, about 21 MMEs, about 22 MMEs, about 23 MMEs, about 24 MMEs, about 25 MMEs, about 26 MMEs, about 27 MMEs, about 28 MMEs, about 29 MMEs, about 30 MMEs, about 31 MMEs, about 32 MMEs, about 33 MMEs, about 34 MMEs, about 35 MMEs, about 36 MMEs, about 37 MMEs, about 38 MMEs, about 39 MMEs, about 40-45 MMEs, about 45-50 MMEs, about 50-55 MMEs, about 55-60 MMEs, about 60-65 MMEs, about 65-70 MMEs, about 70-75 MMEs, about 75-80 MMEs, about 80-85 MMEs, about 85-90 MMEs, about 90-95 MMEs, about 95-100 MMEs, about 100-110 MMEs, about 110-120 MMEs, about 120-130 MMEs, about 130-140 MMEs, about 140-150 MMEs, about 150-160 MMEs, about 160-170 MMEs, about 170-180 MMEs, about 180-190 MMEs, or about 190-200 MMEs. Additionally or alternatively, in some embodiments, the effective amount of the intraoperative opioid analgesic is administered as a series of bolus doses or as a continuous infusion during the tumor resection surgery. In certain embodiments, the effective amount of the intraoperative opioid analgesic is administered to the cancer patient prior to incision. Additionally or alternatively, in some embodiments, the effective amount of the intraoperative opioid analgesic is administered intravenously.

**[0031]** In any and all of the preceding embodiments of the methods disclosed herein, the cancer patient comprises microsatellite instability-high (MSI-H) tumors.

**[0032]** Additionally or alternatively, in some embodiments, the methods of the present technology further comprise administering to the cancer patient an effective amount of a local anesthetic solution via an epidural catheter before, during and/or after the tumor resection surgery. The effective amount of the local anesthetic solution may range from about 0.05%-

4% local anesthetic solution in a volume of 1-10 ml per hour when administered via an epidural catheter. In some embodiments, the effective amount of the local anesthetic solution is about 0.05 %, about 0.06 %, about 0.07 %, about 0.08 %, about 0.09 %, about 0.1 %, about 0.15 %, about 0.2 %, about 0.25 %, about 0.3 %, about 0.35 %, about 0.4 %, about 0.45 %, about 0.5 %, about 0.55 %, about 0.6 %, about 0.65 %, about 0.7 %, about 0.75 %, about 0.8 %, about 0.85 %, about 0.9 %, about 0.95 %, about 1.0 %, about 1.1 %, about 1.2 %, about 1.3 %, about 1.4 %, about 1.5 %, about 1.6 %, about 1.7 %, about 1.8 %, about 1.9 %, about 2.0 %, about 2.1 %, about 2.2 %, about 2.3 %, about 2.4 %, about 2.5 %, about 2.6 %, about 2.7 %, about 2.8 %, about 2.9 %, about 3.0 %, about 3.1 %, about 3.2 %, about 3.3 %, about 3.4 %, about 3.5 %, about 3.6 %, about 3.7 %, about 3.8 %, about 3.9 %, or about 4.0 % local anesthetic solution in a volume of about 1 ml per hour, about 1.5 ml per hour, about 2 ml per hour, about 2.5 ml per hour, about 3 ml per hour, about 3.5 ml per hour, about 4 ml per hour, about 4.5 ml per hour, about 5 ml per hour, about 5.5 ml per hour, about 6 ml per hour, about 6.5 ml per hour, about 7 ml per hour, about 7.5 ml per hour, about 8 ml per hour, about 8.5 ml per hour, about 9 ml per hour, about 9.5 ml per hour, or about 10 ml per hour when administered via an epidural catheter. Additionally or alternatively, in some embodiments, the effective amount of the local anesthetic solution is administered as a single injection, series of bolus doses or as a continuous infusion during the tumor resection surgery. Examples of suitable local anesthetics include, but are not limited to, lidocaine, mepivacaine, prilocaine, bupivacaine, etidocaine, ropivacaine, levobupivacaine, cocaine, procaine, tetracaine, chlorprocaine, and benzocaine.

**[0033]** In other embodiments, the effective amount of the local anesthetic solution may be administered before, during and/or after the tumor resection surgery using any suitable regional anesthesia technique for colon cancer (*e.g.*, transversus abdominis plane (TAP) blocks, Ilioinguinal (II) and iliohypogastric (IH) blocks, Rectus sheath blocks, Transversalis fascia plane blocks, quadratus lumborum blocks). The effective amount of the local anesthetic solution may range from about 0.05%-4% local anesthetic solution in a volume of 10-40 ml when administered using any suitable regional anesthesia technique for colon cancer (*e.g.*, transversus abdominis plane (TAP) blocks, Ilioinguinal (II) and iliohypogastric (IH) blocks, Rectus sheath blocks, Transversalis fascia plane blocks, quadratus lumborum blocks). In some embodiments, the effective amount of the local anesthetic solution is about 0.05 %, about 0.06 %, about 0.07 %, about 0.08 %, about 0.09 %, about 0.1 %, about 0.15 %, about 0.2 %, about 0.25 %, about 0.3 %, about 0.35 %, about 0.4 %, about 0.45 %, about 0.5 %,

about 0.55 %, about 0.6 %, about 0.65 %, about 0.7 %, about 0.75 %, about 0.8 %, about 0.85 %, about 0.9 %, about 0.95 %, about 1.0 %, about 1.1 %, about 1.2 %, about 1.3 %, about 1.4 %, about 1.5 %, about 1.6 %, about 1.7 %, about 1.8 %, about 1.9 %, about 2.0 %, about 2.1 %, about 2.2 %, about 2.3 %, about 2.4 %, about 2.5 %, about 2.6 %, about 2.7 %, about 2.8 %, about 2.9 %, about 3.0 %, about 3.1 %, about 3.2 %, about 3.3 %, about 3.4 %, about 3.5 %, about 3.6 %, about 3.7 %, about 3.8 %, about 3.9 %, or about 4.0 % local anesthetic solution in a volume of about 10 ml, about 12.5 ml, about 15 ml, about 17.5 ml, about 20 ml, about 22.5 ml, about 25 ml, about 27.5 ml, about 30 ml, about 32.5 ml, about 35 ml, about 37.5 ml, or about 40 ml when administered using any suitable regional anesthesia technique for colon cancer (*e.g.*, transversus abdominis plane (TAP) blocks, Ilioinguinal (II) and iliohypogastric (IH) blocks, Rectus sheath blocks, Transversalis fascia plane blocks, quadratus lumborum blocks). Examples of suitable local anesthetics include, but are not limited to, lidocaine, mepivacaine, prilocaine, bupivacaine, etidocaine, ropivacaine, levobupivacaine, cocaine, procaine, tetracaine, chloroprocaine, and benzocaine.

**[0034]** Additionally or alternatively, in certain embodiments, the local anesthetic solution may further comprise an opioid (*e.g.*, fentanyl, hydromorphone, morphine, oxycodone, hydrocodone, codeine, meperidine, remifentanyl, or sufentanyl). In some embodiments, the local anesthetic solution may comprise 0.5 mcg/ml-50 mcg/ml opioid. In certain embodiments, the local anesthetic solution may comprise about 0.5 mcg/ml, about 0.6 mcg/ml, about 0.7 mcg/ml, about 0.8 mcg/ml, about 0.9 mcg/ml, about 1.0 mcg/ml, about 1.5 mcg/ml, about 2.0 mcg/ml, about 2.5 mcg/ml, about 3.0 mcg/ml, about 3.5 mcg/ml, about 4.0 mcg/ml, about 4.5 mcg/ml, about 5.0 mcg/ml, about 5.5 mcg/ml, about 6.0 mcg/ml, about 6.5 mcg/ml, about 7.0 mcg/ml, about 7.5 mcg/ml, about 8.0 mcg/ml, about 8.5 mcg/ml, about 9.0 mcg/ml, about 10 mcg/ml, about 15 mcg/ml, about 20 mcg/ml, about 25 mcg/ml, about 30 mcg/ml, about 35 mcg/ml, about 40 mcg/ml, about 45 mcg/ml, or about 50 mcg/ml.

**[0035]** Additionally or alternatively, in certain embodiments, the methods of the present technology further comprise administering to the cancer patient an effective amount of a post-operative opioid analgesic after the tumor resection surgery. Examples of post-operative opioid analgesics include, but are not limited to, fentanyl, hydromorphone, morphine, oxycodone, hydrocodone, codeine, meperidine, remifentanyl, or sufentanyl. In some embodiments, the post-operative opioid analgesic and the intraoperative opioid analgesic are the same opioid analgesic or different opioid analgesics. In other embodiments, the effective amount of the post-operative opioid analgesic and the effective amount of the intraoperative

opioid analgesic are the same or different. Additionally or alternatively, in some embodiments, the effective amount of the post-operative opioid analgesic is administered to the cancer patient as a bolus of about 0.005 mg to about 100 mg. In some embodiments, the effective amount of the post-operative opioid analgesic is administered to the cancer patient as a bolus of about 0.005 mg, about 0.006 mg, about 0.007 mg, about 0.008 mg, about 0.009 mg, about 0.01 mg, about 0.02 mg, about 0.03 mg, about 0.04 mg, about 0.05 mg, about 0.06 mg, about 0.07 mg, about 0.08 mg, about 0.09 mg, about 0.1 mg, about 0.2 mg, about 0.3 mg, about 0.4 mg, about 0.5 mg, about 0.6 mg, about 0.7 mg, about 0.8 mg, about 0.9 mg, about 1-5 mg, about 5-10 mg, about 1-5 mg, about 5-10 mg, about 1-5 mg, about 5-10 mg, about 1-5 mg, about 5-10 mg, about 10-15 mg, about 15-20 mg, about 20-25 mg, about 25-30 mg, about 30-35 mg, about 35-40 mg, about 40-45 mg, about 45-50 mg, about 50-55 mg, about 55-60 mg, about 60-65 mg, about 65-70 mg, about 70-75 mg, about 75-80 mg, about 80-85 mg, about 85-90 mg, about 90-95 mg, or about 95-100 mg. In other embodiments, the effective amount of the post-operative opioid analgesic may be continuously delivered to the cancer patient at a per hour rate of about 0.01 mg/hr to about 10 mg/hr. In certain embodiments, the effective amount of the post-operative opioid analgesic is continuously delivered to the cancer patient at a per hour rate of about 0.01 mg/hr, about 0.02 mg/hr, about 0.03 mg/hr, about 0.04 mg/hr, about 0.05 mg/hr, about 0.06 mg/hr, about 0.07 mg/hr, about 0.08 mg/hr, about 0.09 mg/hr, about 0.1 mg/hr, about 0.2 mg/hr, about 0.3 mg/hr, about 0.4 mg/hr, about 0.5 mg/hr, about 0.6 mg/hr, about 0.7 mg/hr, about 0.8 mg/hr, about 0.9 mg/hr, about 1 mg/hr, about 1.5 mg/hr, about 2 mg/hr, about 2.5 mg/hr, about 3 mg/hr, about 3.5 mg/hr, about 4 mg/hr, about 4.5 mg/hr, about 5 mg/hr, about 5.5 mg/hr, about 6 mg/hr, about 6.5 mg/hr, about 7 mg/hr, about 7.5 mg/hr, about 8 mg/hr, about 8.5 mg/hr, about 9 mg/hr, about 9.5 mg/hr, or about 10 mg/hr. Additionally or alternatively, in some embodiments, the effective amount of the post-operative opioid analgesic is administered intravenously, orally, or transdermally.

**[0036]** Additionally or alternatively, in any and all embodiments of the methods disclosed herein, the methods of the present technology further comprise administering to the cancer patient an effective amount of one or more non-opioid analgesics selected from among ketamine, dexmedetomidine, a carboxylic acid derivative NSAID, or acetaminophen.

**[0037]** Additionally or alternatively, in some embodiments, the ketamine is administered intravenously. The effective amount of ketamine may be administered intraoperatively, preoperatively, and/or postoperatively.

**[0038]** Additionally or alternatively, in some embodiments, the ketamine is infused at a rate of 0.04 mg/kg/hr-2.5 mg/kg/hr. In some embodiments of the methods disclosed herein, the ketamine is infused at a rate of about 0.04 mg/kg/hr, about 0.05 mg/kg/hr, about 0.06 mg/kg/hr, about 0.07 mg/kg/hr, about 0.08 mg/kg/hr, about 0.09 mg/kg/hr, about 0.1 mg/kg/hr, about 0.2 mg/kg/hr, about 0.3 mg/kg/hr, about 0.4 mg/kg/hr, about 0.5 mg/kg/hr, about 0.6 mg/kg/hr, about 0.7 mg/kg/hr, about 0.8 mg/kg/hr, about 0.9 mg/kg/hr, about 1.0 mg/kg/hr, about 1.1 mg/kg/hr, about 1.2 mg/kg/hr, about 1.3 mg/kg/hr, about 1.4 mg/kg/hr, about 1.5 mg/kg/hr, about 1.6 mg/kg/hr, about 1.7 mg/kg/hr, about 1.8 mg/kg/hr, about 1.9 mg/kg/hr, about 2.0 mg/kg/hr, about 2.1 mg/kg/hr, about 2.2 mg/kg/hr, about 2.3 mg/kg/hr, about 2.4 mg/kg/hr, or about 2.5 mg/kg/hr.

**[0039]** Additionally or alternatively, in some embodiments, the ketamine is administered as a bolus of 0.5 mg/kg -4.5 mg/kg. In some embodiments of the methods disclosed herein, the ketamine is administered as a bolus of about 0.04 mg/kg, about 0.05 mg/kg, about 0.06 mg/kg, about 0.07 mg/kg, about 0.08 mg/kg, about 0.09 mg/kg, about 0.1 mg/kg, about 0.2 mg/kg, about 0.3 mg/kg, about 0.4 mg/kg, about 0.5 mg/kg, about 0.6 mg/kg, about 0.7 mg/kg, about 0.8 mg/kg, about 0.9 mg/kg, about 1.0 mg/kg, about 1.1 mg/kg, about 1.2 mg/kg, about 1.3 mg/kg, about 1.4 mg/kg, about 1.5 mg/kg, about 1.6 mg/kg, about 1.7 mg/kg, about 1.8 mg/kg, about 1.9 mg/kg, about 2.0 mg/kg, about 2.1 mg/kg, about 2.2 mg/kg, about 2.3 mg/kg, about 2.4 mg/kg, about 2.5 mg/kg, about 2.6 mg/kg, about 2.7 mg/kg, about 2.8 mg/kg, about 2.9 mg/kg, about 3.0 mg/kg, about 3.1 mg/kg, about 3.2 mg/kg, about 3.3 mg/kg, about 3.4 mg/kg, about 3.5 mg/kg, about 3.6 mg/kg, about 3.7 mg/kg, about 3.8 mg/kg, about 3.9 mg/kg, about 4.0 mg/kg, about 4.1 mg/kg, about 4.2 mg/kg, about 4.3 mg/kg, about 4.4 mg/kg, or about 4.5 mg/kg.

**[0040]** Additionally or alternatively, in some embodiments, the ketamine is administered intramuscularly at a dose of about 4-13 mg/kg. In certain embodiments of the methods disclosed herein, the ketamine is administered intramuscularly at a dose of 4 mg/kg, 5 mg/kg, 6 mg/kg, 7 mg/kg, 8 mg/kg, 9 mg/kg, 10 mg/kg, 11 mg/kg, 12 mg/kg, or 13 mg/kg. Additionally or alternatively, in some embodiments, the ketamine is administered orally at a dose of about 6-10 mg/kg. In certain embodiments of the methods disclosed herein, the ketamine is administered orally at a dose of 6 mg/kg, 7 mg/kg, 8 mg/kg, 9 mg/kg, or 10 mg/kg.

**[0041]** In any and all of the preceding embodiments of methods disclosed herein, the carboxylic acid derivative NSAID is an acetic acid NSAID or a propionic acid NSAID. Examples of the carboxylic acid derivative NSAID include, but are not limited to, ibuprofen,

dexibuprofen, naproxen, fenoprofen, ketoprofen, dexketoprofen, flurbiprofen, oxaprozin, loxoprofen, pelubiprofen, zaltoprofen, indomethacin, tolmetin, sulindac, etodolac, ketorolac, diclofenac, aceclofenac, bromfenac, or nabumeton. In some embodiments, the carboxylic acid derivative NSAID is administered orally, intravenously, or intramuscularly. Additionally or alternatively, in some embodiments, the effective amount of the carboxylic acid derivative NSAID is administered intraoperatively and/or postoperatively. The effective amount of the carboxylic acid derivative NSAID may be administered to the cancer patient during closing of an incision.

**[0042]** Additionally or alternatively, in some embodiments of the methods disclosed herein, the effective amount of the carboxylic acid derivative NSAID is about 10 mg-1500 mg. In some embodiments of the methods disclosed herein, the effective amount of the carboxylic acid derivative NSAID is about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 30 mg, about 35 mg, about 40 mg, about 45 mg, about 50 mg, about 55 mg, about 60 mg, about 65 mg, about 70 mg, about 75 mg, about 80 mg, about 85 mg, about 90 mg, about 95 mg, about 100 mg, about 150 mg, about 200 mg, about 250 mg, about 300 mg, about 350 mg, about 400 mg, about 450 mg, about 500 mg, about 550 mg, about 600 mg, about 650 mg, about 700 mg, about 750 mg, about 800 mg, about 850 mg, about 900 mg, about 950 mg, about 1000 mg, about 1050 mg, about 1100 mg, about 1150 mg, about 1200 mg, about 1250 mg, about 1300 mg, about 1350 mg, about 1400 mg, about 1450 mg, or about 1500 mg.

**[0043]** In certain embodiments, the effective amount of the carboxylic acid derivative NSAID is administered as a bolus dose during the tumor resection surgery. In a further embodiment, the carboxylic acid derivative NSAID is administered as a bolus of 0.25 mg/kg-10 mg/kg. In some embodiments of the methods disclosed herein, the carboxylic acid derivative NSAID is administered as a bolus of about 0.25 mg/kg, about 0.5 mg/kg, about 0.75 mg/kg, about 1 mg/kg, about 1.5 mg/kg, about 2 mg/kg, about 2.5 mg/kg, about 3 mg/kg, about 3.5 mg/kg, about 4 mg/kg, about 4.5 mg/kg, about 5 mg/kg, about 5.5 mg/kg, about 6 mg/kg, about 6.5 mg/kg, about 7 mg/kg, about 7.5 mg/kg, about 8 mg/kg, about 8.5 mg/kg, about 9 mg/kg, about 9.5 mg/kg, or about 10 mg/kg.

**[0044]** Additionally or alternatively, in some embodiments, the effective amount of the acetaminophen is administered preoperatively, intraoperatively and/or postoperatively. In certain embodiments, the effective amount of the acetaminophen may range from about 80 mg to about 1000 mg. In some embodiments, the effective amount of the acetaminophen is about 80 mg, about 90 mg, about 100 mg, about 110 mg, about 120 mg, about 130 mg, about

140 mg, about 150 mg, about 160 mg, about 170 mg, about 180 mg, about 190 mg, about 200 mg, about 210 mg, about 220 mg, about 230 mg, about 240 mg, about 250 mg, about 260 mg, about 270 mg, about 280 mg, about 290 mg, about 300 mg, about 310 mg, about 320 mg, about 330 mg, about 340 mg, about 350 mg, about 360 mg, about 370 mg, about 380 mg, about 390 mg, about 400 mg, about 410 mg, about 420 mg, about 430 mg, about 440 mg, about 450 mg, about 460 mg, about 470 mg, about 480 mg, about 490 mg, about 500 mg, about 510 mg, about 520 mg, about 530 mg, about 540 mg, about 550 mg, about 560 mg, about 570 mg, about 580 mg, about 590 mg, about 600 mg, about 610 mg, about 620 mg, about 630 mg, about 640 mg, about 650 mg, about 660 mg, about 670 mg, about 680 mg, about 690 mg, about 700 mg, about 710 mg, about 720 mg, about 730 mg, about 740 mg, about 750 mg, about 760 mg, about 770 mg, about 780 mg, about 790 mg, about 800 mg, about 810 mg, about 820 mg, about 830 mg, about 840 mg, about 850 mg, about 860 mg, about 870 mg, about 880 mg, about 890 mg, about 900 mg, about 910 mg, about 920 mg, about 930 mg, about 940 mg, about 950 mg, about 960 mg, about 970 mg, about 980 mg, about 990 mg, or about 1000 mg. Additionally or alternatively, in some embodiments, the effective amount of the acetaminophen may be administered as a series of bolus doses, or as a continuous infusion during the tumor resection surgery. In some embodiments, the acetaminophen is administered intravenously, or orally.

**[0045]** Additionally or alternatively, in some embodiments, the effective amount of dexmedetomidine may be administered intraoperatively, preoperatively, and/or postoperatively. In some embodiments, the dexmedetomidine is administered intravenously, transdermally, intramuscularly, orally, buccally, or intranasally.

**[0046]** Additionally or alternatively, in some embodiments, the dexmedetomidine is infused at a rate of 0.05 µg/kg/hr to 0.7 µg/kg/hr. In some embodiments of the methods disclosed herein, the dexmedetomidine is infused at a rate of about 0.05 µg/kg/hr, about 0.06 µg/kg/hr, about 0.07 µg/kg/hr, about 0.08 µg/kg/hr, about 0.09 µg/kg/hr, about 0.1 µg/kg/hr, 0.15 µg/kg/hr, about 0.2 µg/kg/hr, about 0.25 µg/kg/hr, about 0.3 µg/kg/hr, about 0.35 µg/kg/hr, about 0.4 µg/kg/hr, about 0.45 µg/kg/hr, about 0.5 µg/kg/hr, about 0.55 µg/kg/hr, about 0.6 µg/kg/hr, about 0.65 µg/kg/hr, or about 0.7 µg/kg/hr.

**[0047]** Additionally or alternatively, in some embodiments, the dexmedetomidine is administered as a bolus of 0.05 µg/kg – 1 µg/kg. In some embodiments of the methods disclosed herein, the dexmedetomidine is administered as a bolus of about 0.05 µg/kg, about 0.06 µg/kg, about 0.07 µg/kg, about 0.08 µg/kg, about 0.09 µg/kg, about 0.1 µg/kg, about

0.15 µg/kg, about 0.2 µg/kg, about 0.25 µg/kg, about 0.3 µg/kg, about 0.35 µg/kg, about 0.4 µg/kg, about 0.45 µg/kg, about 0.5 µg/kg, about 0.55 µg/kg, about 0.6 µg/kg, about 0.65 µg/kg, about 0.7 µg/kg, about 0.75 µg/kg, about 0.8 µg/kg, about 0.85 µg/kg, about 0.9 µg/kg, about 0.95 µg/kg, or about 1.0 µg/kg.

5 **[0048]** In one aspect, the present disclosure provides a method for selecting a cancer patient undergoing tumor resection surgery for colon cancer for treatment with an intraoperative opioid analgesic or intraoperative non-opioid analgesic comprising (a) assaying gene expression levels of MLH1, MSH2, MSH6, and PMS2 in a biological sample obtained from the cancer patient and (b) administering to the cancer patient an effective  
10 amount of an intraoperative opioid analgesic or intraoperative non-opioid analgesic during the tumor resection surgery, wherein the gene expression levels of MLH1, MSH2, MSH6, and PMS2 in the biological sample obtained from the cancer patient are comparable to that observed in a control sample obtained from a healthy subject or a predetermined threshold. In certain embodiments, expression levels of MLH1, MSH2, MSH6, and PMS2 are assayed  
15 via next-generation sequencing, PCR, quantitative PCR (qPCR), digital PCR (dPCR), Reverse transcriptase-PCR (RT-PCR), Northern blotting, microarray, dot or slot blots, *in situ* hybridization, fluorescent *in situ* hybridization (FISH), RNA-seq, Western blotting, enzyme-linked immunosorbent assays (ELISA), dot blotting, immunohistochemistry, immunofluorescence, immunoprecipitation, immunoelectrophoresis, or mass-spectrometry.  
20 Additionally or alternatively, in some embodiments, the biological sample comprises genomic DNA, cDNA, RNA, and/or mRNA. Additionally or alternatively, in some embodiments, the cancer patient comprises microsatellite instability-low (MSI-L) or MSS tumors.

**[0049]** In one aspect, the present disclosure provides a method for selecting a cancer  
25 patient undergoing tumor resection surgery for colon cancer for treatment with an intraoperative opioid analgesic or intraoperative non-opioid analgesic comprising (a) assaying gene expression levels of one or more genes selected from among CCK, NTSR2, IFNG, NTSR1, MLN, LIPN, CXCL8, FCGR3B, PTGS2, CALB2, CXCL10, IL1RN, CCL5, CCL4, IL1B, CCL4L2, FCGR3A, ITIH4, GAD1, CD8A, HTR3A, SLC1A3, LHB, ADRB1,  
30 CXCR2, HRH2, CCL3, ESR2, CSF2, ENO2, GRM5, CXCR4, PRKCG, CD68, IL10, TLR2, ICAM1, RGS2, CX3CL1 and KCNJ3 in a biological sample obtained from the cancer patient; and (b) administering to the cancer patient an effective amount of an intraoperative opioid analgesic or intraoperative non-opioid analgesic during the tumor resection surgery, wherein

the gene expression levels of one or more of CCK, NTSR2, IFNG, NTSR1, MLN, LIPN, CXCL8, FCGR3B, PTGS2, CALB2, CXCL10, IL1RN, CCL5, CCL4, IL1B, CCL4L2, FCGR3A, ITIH4, GAD1, CD8A, HTR3A, SLC1A3, LHB, ADRB1, CXCR2, HRH2, CCL3, ESR2, CSF2, ENO2, GRM5, CXCR4, PRKCG, CD68, IL10, TLR2, ICAM1, RGS2, CX3CL1 and KCNJ3 in the biological sample obtained from the cancer patient are comparable relative to a reference sample obtained from a colon cancer subject having microsatellite stable (MSS) tumors or a predetermined threshold. In certain embodiments, expression levels of CCK, NTSR2, IFNG, NTSR1, MLN, LIPN, CXCL8, FCGR3B, PTGS2, CALB2, CXCL10, IL1RN, CCL5, CCL4, IL1B, CCL4L2, FCGR3A, ITIH4, GAD1, CD8A, HTR3A, SLC1A3, LHB, ADRB1, CXCR2, HRH2, CCL3, ESR2, CSF2, ENO2, GRM5, CXCR4, PRKCG, CD68, IL10, TLR2, ICAM1, RGS2, CX3CL1 and KCNJ3 are assayed via next-generation sequencing, PCR, quantitative PCR (qPCR), digital PCR (dPCR), Reverse transcriptase-PCR (RT-PCR), Northern blotting, microarray, dot or slot blots, *in situ* hybridization, fluorescent *in situ* hybridization (FISH), RNA-seq, Western blotting, enzyme-linked immunosorbent assays (ELISA), dot blotting, immunohistochemistry, immunofluorescence, immunoprecipitation, immunoelectrophoresis, or mass-spectrometry. Additionally or alternatively, in some embodiments, the biological sample comprises genomic DNA, cDNA, RNA, and/or mRNA. Additionally or alternatively, in some embodiments, the cancer patient comprises microsatellite instability-low (MSI-L) or MSS tumors.

**[0050]** In one aspect, the present disclosure provides a method for selecting a cancer patient undergoing tumor resection surgery for colon cancer for treatment with an intraoperative opioid analgesic or intraoperative non-opioid analgesic comprising (a) assaying gene expression levels of one or more genes selected from among GHRH, RBFOX3, NOS1, KISS1, NPFFR2, GRIA2, SLC6A2, ADCY5, HCRT, OPCML, NPY1R, PPP1R14C, NTRK2, CHAT, LEP, CPB1, NR1I2, ADCY8, EGF, F2, UGT1A8, PLG, GRPR, PYY, DRD1, ABCB1, SCT, NTS, NPFFR1, TH, CYP2B6, HTR2C, TAC1, CCKBR, CALCA, ATP12A, LINC01411, P2RX3, ALB, REN, SLC6A4, KNG1 and DRD2 in a biological sample obtained from the cancer patient; and (b) administering to the cancer patient an effective amount of an intraoperative opioid analgesic or intraoperative non-opioid analgesic during the tumor resection surgery, wherein the gene expression levels of one or more of GHRH, RBFOX3, NOS1, KISS1, NPFFR2, GRIA2, SLC6A2, ADCY5, HCRT, OPCML, NPY1R, PPP1R14C, NTRK2, CHAT, LEP, CPB1, NR1I2, ADCY8, EGF, F2, UGT1A8,

PLG, GRPR, PYY, DRD1, ABCB1, SCT, NTS, NPFFR1, TH, CYP2B6, HTR2C, TAC1, CCKBR, CALCA, ATP12A, LINC01411, P2RX3, ALB, REN, SLC6A4, KNG1 and DRD2 in the biological sample obtained from the cancer patient are comparable relative to a reference sample obtained from a colon cancer subject having microsatellite stable (MSS) tumors or a predetermined threshold. In certain embodiments, expression levels of GHRH, RBFOX3, NOS1, KISS1, NPFFR2, GRIA2, SLC6A2, ADCY5, HCRT, OPCML, NPY1R, PPP1R14C, NTRK2, CHAT, LEP, CPB1, NR1I2, ADCY8, EGF, F2, UGT1A8, PLG, GRPR, PYY, DRD1, ABCB1, SCT, NTS, NPFFR1, TH, CYP2B6, HTR2C, TAC1, CCKBR, CALCA, ATP12A, LINC01411, P2RX3, ALB, REN, SLC6A4, KNG1 and DRD2 are assayed via next-generation sequencing, PCR, quantitative PCR (qPCR), digital PCR (dPCR), Reverse transcriptase-PCR (RT-PCR), Northern blotting, microarray, dot or slot blots, *in situ* hybridization, fluorescent *in situ* hybridization (FISH), RNA-seq, Western blotting, enzyme-linked immunosorbent assays (ELISA), dot blotting, immunohistochemistry, immunofluorescence, immunoprecipitation, immunoelectrophoresis, or mass-spectrometry. Additionally or alternatively, in some embodiments, the biological sample comprises genomic DNA, cDNA, RNA, and/or mRNA. Additionally or alternatively, in some embodiments, the cancer patient comprises microsatellite instability-low (MSI-L) or MSS tumors.

**[0051]** In another aspect, the present disclosure provides a method for selecting a cancer patient undergoing tumor resection surgery for colon cancer for treatment with an intraoperative opioid analgesic or intraoperative non-opioid analgesic comprising (a) detecting the expression levels or activity of one or more genes that result (i) in hyperactivation of Wnt pathway and/or (ii) reduced activity of Th1 immune response signature in a biological sample obtained from the cancer patient relative to a predetermined threshold; and (b) administering to the cancer patient an effective amount of an intraoperative opioid analgesic or intraoperative non-opioid analgesic during the tumor resection surgery. Additionally or alternatively, in some embodiments, the cancer patient comprises at least one mutation that results in hyperactivation of Wnt pathway and/or (ii) reduced activity of Th1 immune response signature. Additionally or alternatively, in some embodiments, the at least one mutation is a frameshift mutation, a missense mutation, a deletion, an insertion, a nonsense mutation, an inversion, or a translocation. The at least one mutation may be detected using any nucleic acid detection assay known in the art such as next-generation sequencing, PCR, real-time quantitative PCR (qPCR), digital PCR (dPCR), Southern blotting, Reverse

transcriptase-PCR (RT-PCR), Northern blotting, microarray, dot or slot blots, *in situ* hybridization, or fluorescent *in situ* hybridization (FISH). In some embodiments, the biological sample comprises genomic DNA, cDNA, RNA, and/or mRNA. Additionally or alternatively, in some embodiments, the cancer patient comprises microsatellite instability-low (MSI-L) or MSS tumors.

**[0052]** Additionally or alternatively, in some embodiments, the hyperactivation of Wnt pathway is determined by detecting the expression levels or activity of one or more genes selected from among ADAM17, AXIN1, AXIN2, CCND2, CSNK1E, CTNNB1, CUL1, DKK1, DKK4, DLL1, DVL2, FRAT1, FZD1, FZD8, GNAI1, HDAC11, HDAC2, HDAC5, HEY1, HEY2, JAG1, JAG2, KAT2A, LEF1, MAML1, MYC, NCOR2, NCSTN, NKD1, NOTCH1, NOTCH4, NUMB, PPARD, PSEN2, PTCH1, RBPJ, SKP2, TCF7, TP53, WNT1, WNT5B, and WNT6. In certain embodiments, the expression levels of one or more genes selected from among ADAM17, AXIN1, AXIN2, CCND2, CSNK1E, CTNNB1, CUL1, DKK1, DKK4, DLL1, DVL2, FRAT1, FZD1, FZD8, GNAI1, HDAC11, HDAC2, HDAC5, HEY1, HEY2, JAG1, JAG2, KAT2A, LEF1, MAML1, MYC, NCOR2, NCSTN, NKD1, NOTCH1, NOTCH4, NUMB, PPARD, PSEN2, PTCH1, RBPJ, SKP2, TCF7, TP53, WNT1, WNT5B, and WNT6 are detected via next-generation sequencing, PCR, quantitative PCR (qPCR), digital PCR (dPCR), Reverse transcriptase-PCR (RT-PCR), Northern blotting, microarray, dot or slot blots, *in situ* hybridization, fluorescent *in situ* hybridization (FISH), RNA-seq, Western blotting, enzyme-linked immunosorbent assays (ELISA), dot blotting, immunohistochemistry, immunofluorescence, immunoprecipitation, immunoelectrophoresis, or mass-spectrometry. Additionally or alternatively, in some embodiments, the biological sample comprises genomic DNA, cDNA, RNA, and/or mRNA.

**[0053]** Additionally or alternatively, in some embodiments, the reduced activity of Th1 immune response signature is determined by detecting the expression levels or activity of one or more genes selected from among IFNG, LTA, APBB2, DOK5, IL12RB2, APOD, ZBTB32, CD38, CSF2, CTLA4, CD70, DPP4, EGFL6, BST2, DUSP5, LRP8, IL22, DGKI, CCL4, GGT1, LRRN3, SYNGR3, ATP9A, BTG3, CMAHP, HBEGF, and SGCB. In certain embodiments, the expression levels of one or more genes selected from among IFNG, LTA, APBB2, DOK5, IL12RB2, APOD, ZBTB32, CD38, CSF2, CTLA4, CD70, DPP4, EGFL6, BST2, DUSP5, LRP8, IL22, DGKI, CCL4, GGT1, LRRN3, SYNGR3, ATP9A, BTG3, CMAHP, HBEGF, and SGCB are detected via next-generation sequencing, PCR, quantitative PCR (qPCR), digital PCR (dPCR), Reverse transcriptase-PCR (RT-PCR), Northern blotting,

microarray, dot or slot blots, *in situ* hybridization, fluorescent *in situ* hybridization (FISH), RNA-seq, Western blotting, enzyme-linked immunosorbent assays (ELISA), dot blotting, immunohistochemistry, immunofluorescence, immunoprecipitation, immunoelectrophoresis, or mass-spectrometry. Additionally or alternatively, in some embodiments, the biological sample comprises genomic DNA, cDNA, RNA, and/or mRNA.

**[0054]** In one aspect, the present disclosure provides a method for reducing the risk of tumor recurrence in a cancer patient undergoing tumor resection surgery for colon cancer comprising administering to the cancer patient an effective amount of an intraoperative opioid analgesic or intraoperative non-opioid analgesic during the tumor resection surgery, wherein the cancer patient does not comprise any alterations in DNA mismatch repair (MMR) system. In some embodiments, mRNA or polypeptide expression levels of MLH1, MSH2, MSH6, and PMS2 in a biological sample obtained from the cancer patient are comparable relative to that observed in a control sample obtained from a healthy subject or a predetermined threshold. Additionally or alternatively, in some embodiments, the mRNA or polypeptide expression levels of one or more of CCK, NTSR2, IFNG, NTSR1, MLN, LIPN, CXCL8, FCGR3B, PTGS2, CALB2, CXCL10, IL1RN, CCL5, CCL4, IL1B, CCL4L2, FCGR3A, ITIH4, GAD1, CD8A, HTR3A, SLC1A3, LHB, ADRB1, CXCR2, HRH2, CCL3, ESR2, CSF2, ENO2, GRM5, CXCR4, PRKCG, CD68, IL10, TLR2, ICAM1, RGS2, CX3CL1 and KCNJ3 in a biological sample obtained from the cancer patient are comparable relative to a reference sample obtained from a colon cancer subject having microsatellite stable (MSS) tumors or a predetermined threshold. Additionally or alternatively, in some embodiments, the mRNA or polypeptide levels of one or more of GHRH, RBFOX3, NOS1, KISS1, NPFFR2, GRIA2, SLC6A2, ADCY5, HCRT, OPCML, NPY1R, PPP1R14C, NTRK2, CHAT, LEP, CPB1, NR1I2, ADCY8, EGF, F2, UGT1A8, PLG, GRPR, PYY, DRD1, ABCB1, SCT, NTS, NPFFR1, TH, CYP2B6, HTR2C, TAC1, CCKBR, CALCA, ATP12A, LINC01411, P2RX3, ALB, REN, SLC6A4, KNG1 and DRD2 in the biological sample obtained from the cancer patient are comparable relative to a reference sample obtained from a colon cancer subject having microsatellite stable (MSS) tumors or a predetermined threshold. Additionally or alternatively, in some embodiments, the cancer patient comprises at least one genetic mutation or altered expression levels or activity of one or more genes that result (i) in hyperactivation of Wnt pathway and/or (ii) reduced activity of Th1 immune response signature. In some embodiments, the hyperactivation of Wnt pathway is determined by detecting the expression levels or activity of one or more genes selected from among

ADAM17, AXIN1, AXIN2, CCND2, CSNK1E, CTNNB1, CUL1, DKK1, DKK4, DLL1, DVL2, FRAT1, FZD1, FZD8, GNAI1, HDAC11, HDAC2, HDAC5, HEY1, HEY2, JAG1, JAG2, KAT2A, LEF1, MAML1, MYC, NCOR2, NCSTN, NKD1, NOTCH1, NOTCH4, NUMB, PPARD, PSEN2, PTCH1, RBPJ, SKP2, TCF7, TP53, WNT1, WNT5B, and WNT6.

5 In other embodiments, the reduced activity of Th1 immune response signature is determined by detecting the expression levels or activity of one or more genes selected from among IFNG, LTA, APBB2, DOK5, IL12RB2, APOD, ZBTB32, CD38, CSF2, CTLA4, CD70, DPP4, EGFL6, BST2, DUSP5, LRP8, IL22, DGKI, CCL4, GGT1, LRRN3, SYNGR3, ATP9A, BTG3, CMAHP, HBEGF, and SGCB. Additionally or alternatively, in some embodiments,  
10 the cancer patient comprises microsatellite instability-low (MSI-L) or MSS tumors.

**[0055]** Additionally or alternatively, in some embodiments of the preceding embodiments, genetic mutations or mRNA expression levels are detected via next generation sequencing, RNA-seq, real-time quantitative PCR (qPCR), digital PCR (dPCR), Reverse transcriptase-PCR (RT-PCR), Northern blotting, microarray, dot or slot blots, *in situ* hybridization, or  
15 fluorescent *in situ* hybridization (FISH). Additionally or alternatively, in certain embodiments, polypeptide expression levels are detected via Western blotting, enzyme-linked immunosorbent assays (ELISA), dot blotting, immunohistochemistry, immunofluorescence, immunoprecipitation, immunoelectrophoresis, or mass-spectrometry.

**[0056]** The intraoperative opioid analgesic may be fentanyl, hydromorphone, morphine,  
20 oxycodone, hydrocodone, codeine, meperidine, remifentanyl, or sufentanyl. The effective amount of the intraoperative opioid analgesic may range from about 1 MME to about 200 MMEs. In certain embodiments, the effective amount of the intraoperative opioid analgesic is about 1 MME to about 20 MMEs, about 20 MMEs to about 45 MMEs, or about 45 MMEs to about 200 MMEs. In some embodiments, the effective amount of the intraoperative opioid  
25 analgesic is about 1 MME, about 2 MMEs, about 3 MMEs, about 4 MMEs, about 5 MMEs, about 6 MMEs, about 7 MMEs, about 8 MMEs, about 9 MMEs, about 10 MMEs, about 11 MMEs, about 12 MMEs, about 13 MMEs, about 14 MMEs, about 15 MMEs, about 16 MMEs, about 17 MMEs, about 18 MMEs, about 19 MMEs, about 20 MMEs, about 21 MMEs, about 22 MMEs, about 23 MMEs, about 24 MMEs, about 25 MMEs, about 26 MMEs, about 27  
30 MMEs, about 28 MMEs, about 29 MMEs, about 30 MMEs, about 31 MMEs, about 32 MMEs, about 33 MMEs, about 34 MMEs, about 35 MMEs, about 36 MMEs, about 37 MMEs, about 38 MMEs, about 39 MMEs, about 40-45 MMEs, about 45-50 MMEs, about 50-55 MMEs, about 55-60 MMEs, about 60-65 MMEs, about 65-70 MMEs, about 70-75 MMEs, about 75-

80 MMEs, about 80-85 MMEs, about 85-90 MMEs, about 90-95 MMEs, about 95-100 MMEs, about 100-110 MMEs, about 110-120 MMEs, about 120-130 MMEs, about 130-140 MMEs, about 140-150 MMEs, about 150-160 MMEs, about 160-170 MMEs, about 170-180 MMEs, about 180-190 MMEs, or about 190-200 MMEs. Additionally or alternatively, in  
5 some embodiments, the effective amount of the intraoperative opioid analgesic is administered as a series of bolus doses or as a continuous infusion during the tumor resection surgery. In certain embodiments, the effective amount of the intraoperative opioid analgesic is administered to the cancer patient prior to incision. Additionally or alternatively, in some  
10 embodiments, the effective amount of the intraoperative opioid analgesic is administered intravenously.

**[0057]** Examples of non-opioid analgesics include regional anesthetics, ketamine, dexmedetomidine, a carboxylic acid derivative NSAID, or acetaminophen.

**[0058]** Additionally or alternatively, in some embodiments, the methods of the present technology further comprise administering to the cancer patient an effective amount of a  
15 regional anesthetic solution via an epidural catheter before, during and/or after the tumor resection surgery. The effective amount of the regional anesthetic solution may range from about 0.05%-4% regional anesthetic solution in a volume of 1-10 ml per hour when administered via an epidural catheter. In some embodiments, the effective amount of the regional anesthetic solution is about 0.05 %, about 0.06 %, about 0.07 %, about 0.08 %, about  
20 0.09 %, about 0.1 %, about 0.15 %, about 0.2 %, about 0.25 %, about 0.3 %, about 0.35 %, about 0.4 %, about 0.45 %, about 0.5 %, about 0.55 %, about 0.6 %, about 0.65 %, about 0.7 %, about 0.75 %, about 0.8 %, about 0.85 %, about 0.9 %, about 0.95 %, about 1.0 %, about 1.1 %, about 1.2 %, about 1.3 %, about 1.4 %, about 1.5 %, about 1.6 %, about 1.7 %, about 1.8 %, about 1.9 %, about 2.0 %, about 2.1 %, about 2.2 %, about 2.3 %, about 2.4 %, about  
25 2.5 %, about 2.6 %, about 2.7 %, about 2.8 %, about 2.9 %, about 3.0 %, about 3.1 %, about 3.2 %, about 3.3 %, about 3.4 %, about 3.5 %, about 3.6 %, about 3.7 %, about 3.8 %, about 3.9 %, or about 4.0 % regional anesthetic solution in a volume of about 1 ml per hour, about 1.5 ml per hour, about 2 ml per hour, about 2.5 ml per hour, about 3 ml per hour, about 3.5 ml per hour, about 4 ml per hour, about 4.5 ml per hour, about 5 ml per hour, about 5.5 ml  
30 per hour, about 6 ml per hour, about 6.5 ml per hour, about 7 ml per hour, about 7.5 ml per hour, about 8 ml per hour, about 8.5 ml per hour, about 9 ml per hour, about 9.5 ml per hour, or about 10 ml per hour when administered via an epidural catheter. Additionally or alternatively, in some embodiments, the effective amount of the regional anesthetic solution

is administered as a single injection, series of bolus doses or as a continuous infusion during the tumor resection surgery. Examples of suitable regional anesthetics include, but are not limited to, lidocaine, mepivacaine, prilocaine, bupivacaine, etidocaine, ropivacaine, levobupivacaine, cocaine, procaine, tetracaine, chloroprocaine, and benzocaine.

5 **[0059]** In other embodiments, the effective amount of the regional anesthetic solution may be administered before, during and/or after the tumor resection surgery using any suitable regional anesthesia technique for colon cancer (*e.g.*, transversus abdominis plane (TAP) blocks, Ilioinguinal (II) and iliohypogastric (IH) blocks, Rectus sheath blocks, Transversalis fascia plane blocks, quadratus lumborum blocks). The effective amount of the  
 10 regional anesthetic solution may range from about 0.05%-4% regional anesthetic solution in a volume of 10-40 ml when administered using any suitable regional anesthesia technique for colon cancer (*e.g.*, transversus abdominis plane (TAP) blocks, Ilioinguinal (II) and iliohypogastric (IH) blocks, Rectus sheath blocks, Transversalis fascia plane blocks, quadratus lumborum blocks). In some embodiments, the effective amount of the regional  
 15 anesthetic solution is about 0.05 %, about 0.06 %, about 0.07 %, about 0.08 %, about 0.09 %, about 0.1 %, about 0.15 %, about 0.2 %, about 0.25 %, about 0.3 %, about 0.35 %, about 0.4 %, about 0.45 %, about 0.5 %, about 0.55 %, about 0.6 %, about 0.65 %, about 0.7 %, about 0.75 %, about 0.8 %, about 0.85 %, about 0.9 %, about 0.95 %, about 1.0 %, about 1.1 %, about 1.2 %, about 1.3 %, about 1.4 %, about 1.5 %, about 1.6 %, about 1.7 %, about  
 20 1.8 %, about 1.9 %, about 2.0 %, about 2.1 %, about 2.2 %, about 2.3 %, about 2.4 %, about 2.5 %, about 2.6 %, about 2.7 %, about 2.8 %, about 2.9 %, about 3.0 %, about 3.1 %, about 3.2 %, about 3.3 %, about 3.4 %, about 3.5 %, about 3.6 %, about 3.7 %, about 3.8 %, about 3.9 %, or about 4.0 % regional anesthetic solution in a volume of about 10 ml, about 12.5 ml, about 15 ml, about 17.5 ml, about 20 ml, about 22.5 ml, about 25 ml, about 27.5 ml, about 30  
 25 ml, about 32.5 ml, about 35 ml, about 37.5 ml, or about 40 ml when administered using any suitable regional anesthesia technique for colon cancer (*e.g.*, transversus abdominis plane (TAP) blocks, Ilioinguinal (II) and iliohypogastric (IH) blocks, Rectus sheath blocks, Transversalis fascia plane blocks, quadratus lumborum blocks). Examples of suitable regional anesthetics include, but are not limited to, lidocaine, mepivacaine, prilocaine,  
 30 bupivacaine, etidocaine, ropivacaine, levobupivacaine, cocaine, procaine, tetracaine, chloroprocaine, and benzocaine.

**[0060]** Additionally or alternatively, in certain embodiments, the regional anesthetic solution may further comprise an opioid (*e.g.*, fentanyl, hydromorphone, morphine,

oxycodone, hydrocodone, codeine, meperidine, remifentanyl, or sufentanyl). In some embodiments, the regional anesthetic solution may comprise 0.5 mcg/ml-50 mcg/ml opioid. In certain embodiments, the regional anesthetic solution may comprise about 0.5 mcg/ml, about 0.6 mcg/ml, about 0.7 mcg/ml, about 0.8 mcg/ml, about 0.9 mcg/ml, about 1.0 mcg/ml, about 1.5 mcg/ml, about 2.0 mcg/ml, about 2.5 mcg/ml, about 3.0 mcg/ml, about 3.5 mcg/ml, about 4.0 mcg/ml, about 4.5 mcg/ml, about 5.0 mcg/ml, about 5.5 mcg/ml, about 6.0 mcg/ml, about 6.5 mcg/ml, about 7.0 mcg/ml, about 7.5 mcg/ml, about 8.0 mcg/ml, about 8.5 mcg/ml, about 9.0 mcg/ml, about 10 mcg/ml, about 15 mcg/ml, about 20 mcg/ml, about 25 mcg/ml, about 30 mcg/ml, about 35 mcg/ml, about 40 mcg/ml, about 45 mcg/ml, or about 50 mcg/ml.

**[0061]** Additionally or alternatively, in certain embodiments, the methods of the present technology further comprise administering to the cancer patient an effective amount of a post-operative opioid analgesic after the tumor resection surgery. Examples of post-operative opioid analgesics include, but are not limited to, fentanyl, hydromorphone, morphine, oxycodone, hydrocodone, codeine, meperidine, remifentanyl, or sufentanyl. In some embodiments, the post-operative opioid analgesic and the intraoperative opioid analgesic are the same opioid analgesic or different opioid analgesics. In other embodiments, the effective amount of the post-operative opioid analgesic and the effective amount of the intraoperative opioid analgesic are the same or different. Additionally or alternatively, in some embodiments, the effective amount of the post-operative opioid analgesic is administered to the cancer patient as a bolus of about 0.005 mg to about 100 mg. In some embodiments, the effective amount of the post-operative opioid analgesic is administered to the cancer patient as a bolus of about 0.005 mg, about 0.006 mg, about 0.007 mg, about 0.008 mg, about 0.009 mg, about 0.01 mg, about 0.02 mg, about 0.03 mg, about 0.04 mg, about 0.05 mg, about 0.06 mg, about 0.07 mg, about 0.08 mg, about 0.09 mg, about 0.1 mg, about 0.2 mg, about 0.3 mg, about 0.4 mg, about 0.5 mg, about 0.6 mg, about 0.7 mg, about 0.8 mg, about 0.9 mg, about 1-5 mg, about 5-10 mg, about 1-5 mg, about 5-10 mg, about 1-5 mg, about 5-10 mg, about 1-5 mg, about 5-10 mg, about 10-15 mg, about 15-20 mg, about 20-25 mg, about 25-30 mg, about 30-35 mg, about 35-40 mg, about 40-45 mg, about 45-50 mg, about 50-55 mg, about 55-60 mg, about 60-65 mg, about 65-70 mg, about 70-75 mg, about 75-80 mg, about 80-85 mg, about 85-90 mg, about 90-95 mg, or about 95-100 mg. In other embodiments, the effective amount of the post-operative opioid analgesic may be continuously delivered to the cancer patient at a per hour rate of about 0.01 mg/hr to about 10 mg/hr. In certain embodiments, the effective amount of the post-operative opioid analgesic is continuously

delivered to the cancer patient at a per hour rate of about 0.01 mg/hr, about 0.02 mg/hr, about 0.03 mg/hr, about 0.04 mg/hr, about 0.05 mg/hr, about 0.06 mg/hr, about 0.07 mg/hr, about 0.08 mg/hr, about 0.09 mg/hr, about 0.1 mg/hr, about 0.2 mg/hr, about 0.3 mg/hr, about 0.4 mg/hr, about 0.5 mg/hr, about 0.6 mg/hr, about 0.7 mg/hr, about 0.8 mg/hr, about 0.9 mg/hr, about 1 mg/hr, about 1.5 mg/hr, about 2 mg/hr, about 2.5 mg/hr, about 3 mg/hr, about 3.5 mg/hr, about 4 mg/hr, about 4.5 mg/hr, about 5 mg/hr, about 5.5 mg/hr, about 6 mg/hr, about 6.5 mg/hr, about 7 mg/hr, about 7.5 mg/hr, about 8 mg/hr, about 8.5 mg/hr, about 9 mg/hr, about 9.5 mg/hr, or about 10 mg/hr. Additionally or alternatively, in some embodiments, the effective amount of the post-operative opioid analgesic is administered intravenously, orally, or transdermally.

**[0062]** Additionally or alternatively, in some embodiments, the ketamine is administered intravenously. The effective amount of ketamine may be administered intraoperatively, preoperatively, and/or postoperatively.

**[0063]** Additionally or alternatively, in some embodiments, the ketamine is infused at a rate of 0.04 mg/kg/hr-2.5 mg/kg/hr. In some embodiments of the methods disclosed herein, the ketamine is infused at a rate of about 0.04 mg/kg/hr, about 0.05 mg/kg/hr, about 0.06 mg/kg/hr, about 0.07 mg/kg/hr, about 0.08 mg/kg/hr, about 0.09 mg/kg/hr, about 0.1 mg/kg/hr, about 0.2 mg/kg/hr, about 0.3 mg/kg/hr, about 0.4 mg/kg/hr, about 0.5 mg/kg/hr, about 0.6 mg/kg/hr, about 0.7 mg/kg/hr, about 0.8 mg/kg/hr, about 0.9 mg/kg/hr, about 1.0 mg/kg/hr, about 1.1 mg/kg/hr, about 1.2 mg/kg/hr, about 1.3 mg/kg/hr, about 1.4 mg/kg/hr, about 1.5 mg/kg/hr, about 1.6 mg/kg/hr, about 1.7 mg/kg/hr, about 1.8 mg/kg/hr, about 1.9 mg/kg/hr, about 2.0 mg/kg/hr, about 2.1 mg/kg/hr, about 2.2 mg/kg/hr, about 2.3 mg/kg/hr, about 2.4 mg/kg/hr, or about 2.5 mg/kg/hr.

**[0064]** Additionally or alternatively, in some embodiments, the ketamine is administered as a bolus of 0.5 mg/kg -4.5 mg/kg. In some embodiments of the methods disclosed herein, the ketamine is administered as a bolus of about 0.04 mg/kg, about 0.05 mg/kg, about 0.06 mg/kg, about 0.07 mg/kg, about 0.08 mg/kg, about 0.09 mg/kg, about 0.1 mg/kg, about 0.2 mg/kg, about 0.3 mg/kg, about 0.4 mg/kg, about 0.5 mg/kg, about 0.6 mg/kg, about 0.7 mg/kg, about 0.8 mg/kg, about 0.9 mg/kg, about 1.0 mg/kg, about 1.1 mg/kg, about 1.2 mg/kg, about 1.3 mg/kg, about 1.4 mg/kg, about 1.5 mg/kg, about 1.6 mg/kg, about 1.7 mg/kg, about 1.8 mg/kg, about 1.9 mg/kg, about 2.0 mg/kg, about 2.1 mg/kg, about 2.2 mg/kg, about 2.3 mg/kg, about 2.4 mg/kg, about 2.5 mg/kg, about 2.6 mg/kg, about 2.7 mg/kg, about 2.8 mg/kg, about 2.9 mg/kg, about 3.0 mg/kg, about 3.1 mg/kg, about 3.2 mg/kg, about 3.3 mg/kg, about 3.4 mg/kg,

about 3.5 mg/kg, about 3.6 mg/kg, about 3.7 mg/kg, about 3.8 mg/kg, about 3.9 mg/kg, about 4.0 mg/kg, about 4.1 mg/kg, about 4.2 mg/kg, about 4.3 mg/kg, about 4.4 mg/kg, or about 4.5 mg/kg.

**[0065]** Additionally or alternatively, in some embodiments, the ketamine is administered intramuscularly at a dose of about 4-13 mg/kg. In certain embodiments of the methods disclosed herein, the ketamine is administered intramuscularly at a dose of 4 mg/kg, 5 mg/kg, 6 mg/kg, 7 mg/kg, 8 mg/kg, 9 mg/kg, 10 mg/kg, 11 mg/kg, 12 mg/kg, or 13 mg/kg. Additionally or alternatively, in some embodiments, the ketamine is administered orally at a dose of about 6-10 mg/kg. In certain embodiments of the methods disclosed herein, the ketamine is administered orally at a dose of 6 mg/kg, 7 mg/kg, 8 mg/kg, 9 mg/kg, or 10 mg/kg.

**[0066]** In any and all of the preceding embodiments of methods disclosed herein, the carboxylic acid derivative NSAID is an acetic acid NSAID or a propionic acid NSAID. Examples of the carboxylic acid derivative NSAID include, but are not limited to, ibuprofen, dexibuprofen, naproxen, fenoprofen, ketoprofen, dexketoprofen, flurbiprofen, oxaprozin, loxoprofen, pelubiprofen, zaltoprofen, indomethacin, tolmetin, sulindac, etodolac, ketorolac, diclofenac, aceclofenac, bromfenac, or nabumeton. In some embodiments, the carboxylic acid derivative NSAID is administered orally, intravenously, or intramuscularly. Additionally or alternatively, in some embodiments, the effective amount of the carboxylic acid derivative NSAID is administered intraoperatively and/or postoperatively. The effective amount of the carboxylic acid derivative NSAID may be administered to the cancer patient during closing of an incision.

**[0067]** Additionally or alternatively, in some embodiments of the methods disclosed herein, the effective amount of the carboxylic acid derivative NSAID is about 10 mg-1500 mg. In some embodiments of the methods disclosed herein, the effective amount of the carboxylic acid derivative NSAID is about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 30 mg, about 35 mg, about 40 mg, about 45 mg, about 50 mg, about 55 mg, about 60 mg, about 65 mg, about 70 mg, about 75 mg, about 80 mg, about 85 mg, about 90 mg, about 95 mg, about 100 mg, about 150 mg, about 200 mg, about 250 mg, about 300 mg, about 350 mg, about 400 mg, about 450 mg, about 500 mg, about 550 mg, about 600 mg, about 650 mg, about 700 mg, about 750 mg, about 800 mg, about 850 mg, about 900 mg, about 950 mg, about 1000 mg, about 1050 mg, about 1100 mg, about 1150 mg, about 1200 mg, about 1250 mg, about 1300 mg, about 1350 mg, about 1400 mg, about 1450 mg, or about 1500 mg.

**[0068]** In certain embodiments, the effective amount of the carboxylic acid derivative NSAID is administered as a bolus dose during the tumor resection surgery. In a further embodiment, the carboxylic acid derivative NSAID is administered as a bolus of 0.25 mg/kg-10 mg/kg. In some embodiments of the methods disclosed herein, the carboxylic acid derivative NSAID is administered as a bolus of about 0.25 mg/kg, about 0.5 mg/kg, about 0.75 mg/kg, about 1 mg/kg, about 1.5 mg/kg, about 2 mg/kg, about 2.5 mg/kg, about 3 mg/kg, about 3.5 mg/kg, about 4 mg/kg, about 4.5 mg/kg, about 5 mg/kg, about 5.5 mg/kg, about 6 mg/kg, about 6.5 mg/kg, about 7 mg/kg, about 7.5 mg/kg, about 8 mg/kg, about 8.5 mg/kg, about 9 mg/kg, about 9.5 mg/kg, or about 10 mg/kg.

**[0069]** Additionally or alternatively, in some embodiments, the effective amount of the acetaminophen is administered preoperatively, intraoperatively and/or postoperatively. In certain embodiments, the effective amount of the acetaminophen may range from about 80 mg to about 1000 mg. In some embodiments, the effective amount of the acetaminophen is about 80 mg, about 90 mg, about 100 mg, about 110 mg, about 120 mg, about 130 mg, about 140 mg, about 150 mg, about 160 mg, about 170 mg, about 180 mg, about 190 mg, about 200 mg, about 210 mg, about 220 mg, about 230 mg, about 240 mg, about 250 mg, about 260 mg, about 270 mg, about 280 mg, about 290 mg, about 300 mg, about 310 mg, about 320 mg, about 330 mg, about 340 mg, about 350 mg, about 360 mg, about 370 mg, about 380 mg, about 390 mg, about 400 mg, about 410 mg, about 420 mg, about 430 mg, about 440 mg, about 450 mg, about 460 mg, about 470 mg, about 480 mg, about 490 mg, about 500 mg, about 510 mg, about 520 mg, about 530 mg, about 540 mg, about 550 mg, about 560 mg, about 570 mg, about 580 mg, about 590 mg, about 600 mg, about 610 mg, about 620 mg, about 630 mg, about 640 mg, about 650 mg, about 660 mg, about 670 mg, about 680 mg, about 690 mg, about 700 mg, about 710 mg, about 720 mg, about 730 mg, about 740 mg, about 750 mg, about 760 mg, about 770 mg, about 780 mg, about 790 mg, about 800 mg, about 810 mg, about 820 mg, about 830 mg, about 840 mg, about 850 mg, about 860 mg, about 870 mg, about 880 mg, about 890 mg, about 900 mg, about 910 mg, about 920 mg, about 930 mg, about 940 mg, about 950 mg, about 960 mg, about 970 mg, about 980 mg, about 990 mg, or about 1000 mg. Additionally or alternatively, in some embodiments, the effective amount of the acetaminophen may be administered as a series of bolus doses, or as a continuous infusion during the tumor resection surgery. In some embodiments, the acetaminophen is administered intravenously, or orally.

**[0070]** Additionally or alternatively, in some embodiments, the effective amount of dexmedetomidine may be administered intraoperatively, preoperatively, and/or postoperatively. In some embodiments, the dexmedetomidine is administered intravenously, transdermally, intramuscularly, orally, buccally, or intranasally.

5 **[0071]** Additionally or alternatively, in some embodiments, the dexmedetomidine is infused at a rate of 0.05 µg/kg/hr to 0.7 µg/kg/hr. In some embodiments of the methods disclosed herein, the dexmedetomidine is infused at a rate of about 0.05 µg/kg/hr, about 0.06 µg/kg/hr, about 0.07 µg/kg/hr, about 0.08 µg/kg/hr, about 0.09 µg/kg/hr, about 0.1 µg/kg/hr, 0.15 µg/kg/hr, about 0.2 µg/kg/hr, about 0.25 µg/kg/hr, about 0.3 µg/kg/hr, about 0.35  
10 µg/kg/hr, about 0.4 µg/kg/hr, about 0.45 µg/kg/hr, about 0.5 µg/kg/hr, about 0.55 µg/kg/hr, about 0.6 µg/kg/hr, about 0.65 µg/kg/hr, or about 0.7 µg/kg/hr.

**[0072]** Additionally or alternatively, in some embodiments, the dexmedetomidine is administered as a bolus of 0.05 µg/kg – 1 µg/kg. In some embodiments of the methods disclosed herein, the dexmedetomidine is administered as a bolus of about 0.05 µg/kg, about  
15 0.06 µg/kg, about 0.07 µg/kg, about 0.08 µg/kg, about 0.09 µg/kg, about 0.1 µg/kg, about 0.15 µg/kg, about 0.2 µg/kg, about 0.25 µg/kg, about 0.3 µg/kg, about 0.35 µg/kg, about 0.4 µg/kg, about 0.45 µg/kg, about 0.5 µg/kg, about 0.55 µg/kg, about 0.6 µg/kg, about 0.65 µg/kg, about 0.7 µg/kg, about 0.75 µg/kg, about 0.8 µg/kg, about 0.85 µg/kg, about 0.9 µg/kg, about 0.95 µg/kg, or about 1.0 µg/kg.

20 **[0073]** In any and all embodiments of the methods disclosed herein, the cancer patient exhibits stage I, stage II or stage III colon cancer. Additionally or alternatively, in some embodiments, the cancer patient has been diagnosed with colon adenocarcinoma (COAD). The colon adenocarcinoma may be synchronous or non-synchronous. The tumor surgery may be laparoscopic, robotic or open. In certain embodiments, the tumor surgery is segmental or  
25 extended.

**[0074]** In any of the preceding embodiments of the methods disclosed herein, the cancer patient has received/is receiving an adjuvant therapy. The adjuvant therapy may be chemotherapy, radiation therapy or chemoradiation therapy. In any of the preceding embodiments of the methods disclosed herein, the cancer patient has not received an adjuvant  
30 therapy. Additionally or alternatively, in some embodiments of the methods disclosed herein, the patient is human.

[0075] In any and all embodiments of the methods disclosed herein, the biological sample obtained from the cancer patient comprises biopsied tumor tissue, whole blood, plasma, or serum.

### BRIEF DESCRIPTION OF THE DRAWINGS

[0076] **FIGs. 1A-1D** show association of intraoperative opioid dose and genetic alterations in lung adenocarcinoma (LUAD) patients. **FIG. 1A** shows oncoprint of alteration frequencies of all genes altered  $\geq 5\%$  for the overall study cohort. Patients are subdivided by pathologic tumor stage. **FIG. 1B** shows five-year predicted overall survival for patients with high and low FGA and altered and wild-type CDKN2A with increasing intraoperative MME. **FIG. 1C** shows comparative bar graphs representing alteration rate for each genomic factor by stage. **FIG. 1D** shows co-occurrence and mutual exclusivity between genes across all tumors. FGA: fraction genome altered; WT: wildtype; MME: oral morphine milligram equivalents.

[0077] **FIGs. 2A-2D** show association of intraoperative MMEs and oncogenic pathway alterations in lung adenocarcinoma patients. **FIG. 2A** shows oncoprint of alteration frequencies of ten canonical oncogenic pathways for the overall study cohort. Patients are subdivided by pathologic tumor stage. **FIG. 2B** shows five-year predicted recurrence-specific survival for patients with altered and unaltered Wnt and Hippo pathways. **FIG. 2C** shows comparative bar graphs representing alteration rate for each oncologic signaling pathway by stage. **FIG. 2D** shows co-occurrence and mutual exclusivity between oncogenic pathways across all tumors. OS: overall survival; RSS: recurrence-specific survival; MME: oral morphine milligram equivalents.

[0078] **FIG. 3** shows Mu-opioid receptor concentrations in patient-matched lung adenocarcinoma and adjacent non-tumor lung tissue. Concentrations are based on optical density measurements derived from enzyme linked immunosorbent assay analysis. Lines signify individual patient matched samples with blue lines indicating those patients with elevated tumor concentrations of the mu-opioid receptor (MOR) compared with adjacent lung tissue and yellow lines indicating patients with elevated normal lung tissue MOR concentrations compared to tumor.

[0079] **FIG. 4** shows CONSORT diagram describing the exclusion criteria for the patient cohort. MSK-IMPACT™: Memorial Sloan Kettering–Integrated Mutation Profiling of Actionable Cancer Targets.

[0080] **FIGs. 5A-5B** show analysis of total intraoperative MME in terms of specific opioids received. **FIG. 5A** shows breakdown by patient of specific opioid type (fentanyl, hydromorphone, morphine) as a contribution to total intraoperative MME. **FIG. 5B** shows cumulative percentage of fentanyl fraction of total intraoperative MME (fentanyl dose divided by total dose).

[0081] **FIG. 6** shows estimates of interaction effects between genomic factors and intraoperative morphine milligram equivalents in the multivariable Cox models for overall survival and recurrence-specific survival\*. Genomic factors include tumor mutation burden (TMB), fraction genome altered (FGA), all genes altered in  $\geq 5\%$  of the study cohort, canonical oncogenic pathways (N=10), and number of pathways altered.

[0082] **FIG. 7** shows genes altered in oncogenic pathways stratified by pathologic stage.

[0083] **FIG. 8** shows a distribution of the FGA within the cohort.

[0084] **FIG. 9** shows a loss of function/gain of function plot of the altered oncogenic pathways.

[0085] **FIG. 10** shows the frequency of the different loss of function/gain of function gene mutations identified in the LUAD patient cohort.

[0086] **FIG. 11** shows the frequency of the different loss of function/gain of function oncogenic pathway mutations identified in the LUAD patient cohort.

[0087] **FIGs. 12A-12C** show associations between intraoperative opioid dose, DNA mismatch repair subtype and recurrence. Cumulative incidence functions of recurrence for (**FIG. 12A**) all patients, (inset) stratified by MMR subtype, and (**FIG. 12B**) stratified by intraoperative opioid dose ( $<$  median: below vs  $\geq$  median: above median) for (l) dMMR and (r) pMMR patients separately. (**FIG. 12C**) Model-estimated probability of recurrence at 5 years following surgery over a range of intraoperative MME values based on MMR subtype (for a hypothetical patient with these MVA factor values: T stage=3, N stage=0, BMI=27.5, no adjuvant chemotherapy, no adjunct, and no ketorolac). Shaded area represents 95% confidence intervals for all panels. Abbreviations: MVA, multivariable analysis; MMR, mismatch repair; dMMR, MMR deficient; pMMR, MMR proficient; MME, intraoperative oral morphine milligram equivalents.

[0088] **FIGs. 13A-13C** show associations between intraoperative opioid dose, tumour-infiltrating lymphocytes (TILs), and recurrence. (**FIG. 13A**) Distribution of absent versus

present increased TILs by DNA mismatch repair (MMR) subtype. Cumulative incidence function of recurrence (**FIG. 13B**) stratified by absent versus present increased TILs. Shaded area represents 95% confidence intervals. (**FIG. 13C**) Cumulative incidence function of recurrence stratified by increased TILs (A: absent vs P: present) and intraoperative opioid dose (< med: below vs >= med: above median) for (l) dMMR and (r) pMMR patients separately. Abbreviations: MMR, mismatch repair; dMMR, MMR deficient; pMMR, MMR proficient; TIL, tumour-infiltrating lymphocytes; MME, intraoperative oral morphine milligram equivalents; A, absent; P, present.

[0089] **FIGs. 14A-14D** show differential expression of opioid-related genes in TCGA-COAD. (**FIG. 14A**) Volcano plot showing differential expression of opioid genes for MSI (dMMR) versus MSS (pMMR) tumours.  $-\log_{10} P$ -value (adjusted for multiple testing) is plotted against  $\log_2$  (fold change). The horizontal dotted line represents  $P=0.05$ . Vertical lines dotted at an absolute fold change of 2. Genes with absolute fold change higher than 2 and  $P$ -value < 0.05 are coloured in red. (**FIG. 14B**) SSGSEA analysis correlation plot highlighting major pathways and cell types correlated with MSI upregulated and downregulated opioid genes. For each pairwise correlation the colour and the size of the circle denote the Spearman correlation. (**FIG. 14C**) SSGSEA correlation of MSI upregulated opioid genes and Th1 immune signature and (**FIG. 14D**) MSI downregulated opioid genes and Wnt beta-catenin pathway; each patient sample is labelled as MSI (blue) or MSS (yellow) and is plotted by normalized SSGSEA scores for the relevant gene sets on the x- and y-axes. Shaded area represents the 95% confidence interval around the best fit line. Abbreviations: TCGA-COAD, The Cancer Genome Atlas Colon Adenocarcinoma; MSI, microsatellite instability; MSS, microsatellite stability; SSGSEA, single-sample gene set enrichment analysis.

[0090] **FIG. 15** shows CONSORT diagram. Abbreviations: COAD, colon adenocarcinoma; MMR, mismatch repair.

[0091] **FIGs. 16A-16B** show cumulative incidence of recurrence stratified by MME (below/above median) for patients (**FIG. 16A**) without increased TILs and (**FIG. 16B**) with increased TILs for the subset of patients with TILs data. Shaded area represents 95% confidence intervals. Abbreviations: TILs, tumour-infiltrating lymphocytes; MME, oral morphine milligram equivalents.

[0092] **FIGs. 17A-17B** show Kaplan-Meier estimate of overall survival stratified by MME (below vs above median) for patients (**FIG. 17A**) without increased TILs and (**FIG. 17B**)

with increased TILs for the subset of patients with TILs data. Shaded area represents 95% confidence intervals. Abbreviations: TILs, tumour-infiltrating lymphocytes; MME, oral morphine milligram equivalents.

[0093] FIGs. 18A-18B show differential expression of opioid receptor genes in TCGA-COAD. Volcano plots showing differential expression of opioid receptor genes for (FIG. 18A) tumour versus normal and (FIG. 18B) MSI (dMMR) versus MSS (pMMR). -Log<sub>10</sub> P-value (adjusted for multiple testing) is plotted against log<sub>2</sub> (fold change). The horizontal dotted line represents  $P=0.05$ . Vertical lines dotted at an absolute fold change of 2. Genes with absolute fold change higher than 2 and  $P$ -value $<0.05$  are coloured in red. Abbreviations: TCGA-COAD, The Cancer Genome Atlas Colon Adenocarcinoma; MSS, microsatellite stability; MSI, microsatellite instability; pMMR, mismatch repair proficient; dMMR, mismatch repair deficient.

[0094] FIG. 19 shows SSGSEA analysis correlation plot showing pairwise correlations between MSI upregulated opioid gene list, MSI downregulated opioid gene list (both in purple), 50 Hallmark pathways (in red), and 25 immune cell type signatures (in blue). For each pairwise correlation, the colour and the size of the circle denote the Spearman correlation. All comparisons shown are adjusted  $P<0.05$ . Abbreviations: MSS, microsatellite stability; MSI, microsatellite instability; SSGSEA, single-sample gene set enrichment analysis.

[0095] FIG. 20 shows clinicopathologic characteristics for the entire cohort and for MMR subtypes.

[0096] FIG. 21 shows multivariable Competing Risk Regression Analysis for Recurrence. Estimates are pooled from 10 imputed datasets. Model includes clinical factors of interest (intraoperative MMEs, MMR subtype, adjunct, and ketorolac) and statistically significant factors from the univariable analysis for adjusting baseline factors, followed by backwards selection on the adjusting factors.

[0097] FIG. 22 shows univariable competing risk regression analysis for recurrence. Estimates are pooled from 10 imputed datasets.

[0098] FIG. 23 shows univariable Cox regression analysis for overall survival (OS). Estimates are pooled from 10 imputed datasets.

[0099] FIG. 24 shows multivariable Cox regression analysis for overall survival (OS). Estimates are pooled from 10 imputed datasets. Model includes clinical factors of interest (MMR subtype, intraoperative MME, adjunct, and ketorolac) and statistically significant

factors from the univariable analysis for adjusting baseline factors, followed by backwards selection on the adjusting factors.

**[00100]** FIG. 25 shows opioid\*MSI and Opioid\*MSS gene lists. Log2 (fold change) is for expression in MSI versus MSS.

5 **[00101]** FIG. 26 shows an analysis of total intraoperative morphine milligram equivalents (MMEs) in terms of specific opioids received. **FIG. 26A:** Breakdown by patient of specific opioid type (fentanyl, hydromorphone, morphine) as a contribution to total intraoperative MMEs, with patients ordered from highest to lowest MMEs (inset features the top 100 patients). **FIG. 26B:** Cumulative percentage of patients with each fentanyl fraction of total  
10 intraoperative MMEs (fentanyl dose divided by total dose); fentanyl constituted at least 90% of total MMEs for more than 50% of patients and at least 80% of total MMEs for 75% of patients.

**[00102]** FIG. 27 shows a summary of regional anaesthesia and opioid dose.

#### DETAILED DESCRIPTION

15 **[00103]** It is to be appreciated that certain aspects, modes, embodiments, variations and features of the present methods are described below in various levels of detail in order to provide a substantial understanding of the present technology. It is to be understood that the present disclosure is not limited to particular uses, methods, reagents, compounds, compositions or biological systems, which can, of course, vary. It is also to be understood  
20 that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

**[00104]** In practicing the present methods, many conventional techniques in molecular biology, protein biochemistry, cell biology, microbiology and recombinant DNA are used. See, e.g., Sambrook and Russell eds. (2001) *Molecular Cloning: A Laboratory Manual*, 3rd  
25 edition; the series Ausubel *et al.*, eds. (2007) *Current Protocols in Molecular Biology*; the series *Methods in Enzymology* (Academic Press, Inc., N.Y.); MacPherson *et al.*, (1991) *PCR 1: A Practical Approach* (IRL Press at Oxford University Press); MacPherson *et al.*, (1995) *PCR 2: A Practical Approach*; Harlow and Lane eds. (1999) *Antibodies, A Laboratory Manual*; Freshney (2005) *Culture of Animal Cells: A Manual of Basic Technique*, 5th edition;  
30 Gait ed. (1984) *Oligonucleotide Synthesis*; U.S. Patent No. 4,683,195; Hames and Higgins eds. (1984) *Nucleic Acid Hybridization*; Anderson (1999) *Nucleic Acid Hybridization*; Hames and Higgins eds. (1984) *Transcription and Translation; Immobilized Cells and*

*Enzymes* (IRL Press (1986)); Perbal (1984) *A Practical Guide to Molecular Cloning*; Miller and Calos eds. (1987) *Gene Transfer Vectors for Mammalian Cells* (Cold Spring Harbor Laboratory); Makrides ed. (2003) *Gene Transfer and Expression in Mammalian Cells*; Mayer and Walker eds. (1987) *Immunochemical Methods in Cell and Molecular Biology* (Academic Press, London); and Herzenberg *et al.*, eds (1996) *Weir's Handbook of Experimental Immunology*.

[00105] The use of intraoperative opioids have been linked to worse oncologic outcomes in cancer patients. The present disclosure demonstrates that lung cancer patients with alterations that disrupt the Hippo signaling pathway as well as alterations that hyperactivate Wnt signaling pathway were associated with improved recurrence-specific survival (RSS) at higher morphine milligram equivalents (MMEs) compared to unaltered pathways. Accordingly, such tumorigenic mutations may be useful as biomarkers to predict whether a cancer patient undergoing tumor resection surgery for lung cancer would benefit from treatment with intraoperative opioid analgesics. The present disclosure also demonstrates that higher FGA and CDKN2A loss of function mutations in lung cancer patients were correlated with worse overall survival (OS) with increasing intraoperative MMEs. The present disclosure also demonstrates that colon cancer patients harboring deficiencies in the DNA mismatch repair (MMR) system (*i.e.*, dMMR) show reduced risk of tumor recurrence when treated with intraoperative opioid analgesics, whereas colon cancer patients with wild-type/proficient DNA mismatch repair system (pMMR) benefit from either opioid or non-opioid intraoperative analgesics.

### **Definitions**

[00106] Unless defined otherwise, all technical and scientific terms used herein generally have the same meaning as commonly understood by one of ordinary skill in the art to which this technology belongs. As used in this specification and the appended claims, the singular forms “a”, “an” and “the” include plural referents unless the content clearly dictates otherwise. For example, reference to “a cell” includes a combination of two or more cells, and the like. Generally, the nomenclature used herein and the laboratory procedures in cell culture, molecular genetics, organic chemistry, analytical chemistry and nucleic acid chemistry and hybridization described below are those well-known and commonly employed in the art.

[00107] As used herein, the term “about” in reference to a number is generally taken to include numbers that fall within a range of 1%, 5%, or 10% in either direction (greater than

or less than) of the number unless otherwise stated or otherwise evident from the context (except where such number would be less than 0% or exceed 100% of a possible value).

**[00108]** The term “adapter” refers to a short, chemically synthesized, nucleic acid sequence which can be used to ligate to the end of a nucleic acid sequence in order to facilitate attachment to another molecule. The adapter can be single-stranded or double-stranded. An adapter can incorporate a short (typically less than 50 base pairs) sequence useful for PCR amplification or sequencing.

**[00109]** As used herein, the “administration” of an agent or drug to a subject includes any route of introducing or delivering to a subject a compound to perform its intended function. Administration can be carried out by any suitable route, including but not limited to, orally, intranasally, parenterally (intravenously, intramuscularly, intraperitoneally, or subcutaneously), rectally, intrathecally, intratumorally or topically. Administration includes self-administration and the administration by another.

**[00110]** As used herein, an “alteration” of a gene or gene product (*e.g.*, a marker gene or gene product) refers to the presence of a mutation or mutations within the gene or gene product, *e.g.*, a mutation, which affects the quantity or activity of the gene or gene product, as compared to the normal or wild-type gene. The genetic alteration can result in changes in the quantity, structure, and/or activity of the gene or gene product in a cancer tissue or cancer cell, as compared to its quantity, structure, and/or activity, in a normal or healthy tissue or cell (*e.g.*, a control). For example, an alteration which is associated with cancer, or predictive of responsiveness to intraoperative analgesics, can have an altered nucleotide sequence (*e.g.*, a mutation), amino acid sequence, chromosomal translocation, intra-chromosomal inversion, copy number, expression level, protein level, protein activity, in a cancer tissue or cancer cell, as compared to a normal, healthy tissue or cell. Exemplary mutations include, but are not limited to, point mutations (*e.g.*, silent, missense, or nonsense), deletions, insertions, inversions, linking mutations, duplications, translocations, inter- and intra-chromosomal rearrangements. Mutations can be present in the coding or non-coding region of the gene.

**[00111]** As used herein, the terms “amplify” or “amplification” with respect to nucleic acid sequences, refer to methods that increase the representation of a population of nucleic acid sequences in a sample. Nucleic acid amplification methods are well known to the skilled artisan and include ligase chain reaction (LCR), ligase detection reaction (LDR), ligation followed by Q-replicase amplification, PCR, primer extension, strand displacement

amplification (SDA), hyperbranched strand displacement amplification, multiple displacement amplification (MDA), nucleic acid strand-based amplification (NASBA), two-step multiplexed amplifications, rolling circle amplification (RCA), recombinase-polymerase amplification (RPA)(TwistDx, Cambridge, UK), transcription mediated  
5 amplification, signal mediated amplification of RNA technology, loop-mediated isothermal amplification of DNA, helicase-dependent amplification, single primer isothermal amplification, and self-sustained sequence replication (3SR), including multiplex versions or combinations thereof. Copies of a particular nucleic acid sequence generated *in vitro* in an amplification reaction are called “amplicons” or “amplification products.”

10 **[00112]** The terms “cancer” or “tumor” are used interchangeably and refer to the presence of cells possessing characteristics typical of cancer-causing cells, such as uncontrolled proliferation, immortality, metastatic potential, rapid growth and proliferation rate, and certain characteristic morphological features. Cancer cells are often in the form of a tumor, but such cells can exist alone within an animal, or can be a non-tumorigenic cancer cell. As  
15 used herein, the term “cancer” includes premalignant, as well as malignant cancers.

**[00113]** The terms “complementary” or “complementarity” as used herein with reference to polynucleotides (*i.e.*, a sequence of nucleotides such as an oligonucleotide or a target nucleic acid) refer to the base-pairing rules. The complement of a nucleic acid sequence as used herein refers to an oligonucleotide which, when aligned with the nucleic acid sequence  
20 such that the 5' end of one sequence is paired with the 3' end of the other, is in “antiparallel association.” For example, the sequence “5'-A-G-T-3'” is complementary to the sequence “3'-T-C-A-5'.” Certain bases not commonly found in naturally-occurring nucleic acids may be included in the nucleic acids described herein. These include, for example, inosine, 7-deazaguanine, Locked Nucleic Acids (LNA), and Peptide Nucleic Acids (PNA).  
25 Complementarity need not be perfect; stable duplexes may contain mismatched base pairs, degenerative, or unmatched bases. Those skilled in the art of nucleic acid technology can determine duplex stability empirically considering a number of variables including, for example, the length of the oligonucleotide, base composition and sequence of the oligonucleotide, ionic strength and incidence of mismatched base pairs. A complement  
30 sequence can also be an RNA sequence complementary to the DNA sequence or its complement sequence, and can also be a cDNA.

**[00114]** As used herein, a “control” is an alternative sample used in an experiment for comparison purpose. A control can be “positive” or “negative.” For example, where the

purpose of the experiment is to determine a correlation of the efficacy of a therapeutic agent for the treatment for a particular type of disease, a positive control (a compound or composition known to exhibit the desired therapeutic effect) and a negative control (a subject or a sample that does not receive the therapy or receives a placebo) are typically employed.

5 [00115] A “control nucleic acid sample” or “reference nucleic acid sample” as used herein, refers to nucleic acid molecules from a control or reference sample. In certain embodiments, the reference or control nucleic acid sample is a wild type or a non-mutated DNA or RNA sequence. In certain embodiments, the reference nucleic acid sample is purified or isolated (*e.g.*, it is removed from its natural state). In other embodiments, the reference nucleic acid  
10 sample is from a non-tumor sample, *e.g.*, a blood control, a normal adjacent tumor (NAT), or any other non-cancerous sample from the same or a different subject.

[00116] “Detecting” as used herein refers to determining the presence of a mutation or alteration in a nucleic acid of interest in a sample. Detection does not require the method to provide 100% sensitivity. Analysis of nucleic acid markers can be performed using  
15 techniques known in the art including, but not limited to, sequence analysis, and electrophoretic analysis. Non-limiting examples of sequence analysis include Maxam-Gilbert sequencing, Sanger sequencing, capillary array DNA sequencing, thermal cycle sequencing (Sears *et al.*, *Biotechniques*, 13:626-633 (1992)), solid-phase sequencing (Zimmerman *et al.*, *Methods Mol. Cell Biol*, 3:39-42 (1992)), sequencing with mass  
20 spectrometry such as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS; Fu *et al.*, *Nat. Biotechnol*, 16:381-384 (1998)), and sequencing by hybridization. Chee *et al.*, *Science*, 274:610-614 (1996); Drmanac *et al.*, *Science*, 260:1649-1652 (1993); Drmanac *et al.*, *Nat. Biotechnol*, 16:54-58 (1998). Non-limiting examples of electrophoretic analysis include slab gel electrophoresis such as agarose  
25 or polyacrylamide gel electrophoresis, capillary electrophoresis, and denaturing gradient gel electrophoresis. Additionally, next generation sequencing methods can be performed using commercially available kits and instruments from companies such as the Life Technologies/Ion Torrent PGM or Proton, the Illumina HiSEQ or MiSEQ, and the Roche/454 next generation sequencing system.

30 [00117] “Detectable label” as used herein refers to a molecule or a compound or a group of molecules or a group of compounds used to identify a nucleic acid or protein of interest. In some embodiments, the detectable label may be detected directly. In other embodiments, the detectable label may be a part of a binding pair, which can then be subsequently detected.

Signals from the detectable label may be detected by various means and will depend on the nature of the detectable label. Detectable labels may be isotopes, fluorescent moieties, colored substances, and the like. Examples of means to detect detectable labels include but are not limited to spectroscopic, photochemical, biochemical, immunochemical, electromagnetic, radiochemical, or chemical means, such as fluorescence, chemifluorescence, or chemiluminescence, or any other appropriate means.

[00118] As used herein, “DNA mismatch repair” or “MMR” system refers to a gene pathway that is integral in the maintenance of genomic stability. MMR functions in postreplicative repair by correcting DNA polymerase errors including base-base or insertion/deletion mismatches that form during DNA replication.

[00119] As used herein, the term “effective amount” refers to a quantity sufficient to achieve a desired therapeutic and/or prophylactic effect, *e.g.*, an amount which results in the prevention of, or a decrease in a disease or condition described herein or one or more signs or symptoms associated with a disease or condition described herein. In the context of therapeutic or prophylactic applications, the amount of a composition administered to the subject will vary depending on the composition, the degree, type, and severity of the disease and on the characteristics of the individual, such as general health, age, sex, body weight and tolerance to drugs. The skilled artisan will be able to determine appropriate dosages depending on these and other factors. The compositions can also be administered in combination with one or more additional therapeutic compounds. In the methods described herein, the therapeutic compositions may be administered to a subject having one or more signs or symptoms of a disease or condition described herein. As used herein, a “therapeutically effective amount” of a composition refers to composition levels in which the physiological effects of a disease or condition are ameliorated or eliminated. A therapeutically effective amount can be given in one or more administrations.

[00120] As used herein, “fraction of the genome altered” or “FGA” refers to the percentage of copy number altered regions out of all sequenced regions. In some embodiments, FGA may be defined as the number of bases in sequenced genomic segments with log2 copy number fold change  $>0.2$  or  $<-0.2$  over the total number of bases in all sequenced segments.

[00121] “Gene” as used herein refers to a DNA sequence that comprises regulatory and coding sequences necessary for the production of an RNA, which may have a non-coding function (*e.g.*, a ribosomal or transfer RNA) or which may include a polypeptide or a

polypeptide precursor. The RNA or polypeptide may be encoded by a full length coding sequence or by any portion of the coding sequence so long as the desired activity or function is retained. Although a sequence of the nucleic acids may be shown in the form of DNA, a person of ordinary skill in the art recognizes that the corresponding RNA sequence will have a similar sequence with the thymine being replaced by uracil, i.e., "T" is replaced with "U."

**[00122]** The term "hybridize" as used herein refers to a process where two substantially complementary nucleic acid strands (at least about 65% complementary over a stretch of at least 14 to 25 nucleotides, at least about 75%, or at least about 90% complementary) anneal to each other under appropriately stringent conditions to form a duplex or heteroduplex through formation of hydrogen bonds between complementary base pairs. Hybridizations are typically and preferably conducted with probe-length nucleic acid molecules, preferably 15-100 nucleotides in length, more preferably 18-50 nucleotides in length. Nucleic acid hybridization techniques are well known in the art. *See, e.g.,* Sambrook, *et al.*, 1989, *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Press, Plainview, N.Y. Hybridization and the strength of hybridization (*i.e.*, the strength of the association between the nucleic acids) is influenced by such factors as the degree of complementarity between the nucleic acids, stringency of the conditions involved, and the thermal melting point ( $T_m$ ) of the formed hybrid. Those skilled in the art understand how to estimate and adjust the stringency of hybridization conditions such that sequences having at least a desired level of complementarity will stably hybridize, while those having lower complementarity will not. For examples of hybridization conditions and parameters, *see, e.g.,* Sambrook, *et al.*, 1989, *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Press, Plainview, N.Y.; Ausubel, F. M. *et al.* 1994, *Current Protocols in Molecular Biology*, John Wiley & Sons, Secaucus, N.J. In some embodiments, specific hybridization occurs under stringent hybridization conditions. An oligonucleotide or polynucleotide (*e.g.*, a probe or a primer) that is specific for a target nucleic acid will "hybridize" to the target nucleic acid under suitable conditions.

**[00123]** As used herein, the term "library" refers to a collection of nucleic acid sequences, *e.g.*, a collection of nucleic acids derived from whole genomic, subgenomic fragments, cDNA, cDNA fragments, RNA, RNA fragments, or a combination thereof. In one embodiment, a portion or all of the library nucleic acid sequences comprises an adapter sequence. The adapter sequence can be located at one or both ends. The adapter sequence can be useful,

*e.g.*, for a sequencing method (*e.g.*, an NGS method), for amplification, for reverse transcription, or for cloning into a vector.

**[00124]** The library can comprise a collection of nucleic acid sequences, *e.g.*, a target nucleic acid sequence (*e.g.*, a tumor nucleic acid sequence), a reference nucleic acid sequence, or a combination thereof. In some embodiments, the nucleic acid sequences of the library can be derived from a single subject. In other embodiments, a library can comprise nucleic acid sequences from more than one subject (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30 or more subjects). In some embodiments, two or more libraries from different subjects can be combined to form a library having nucleic acid sequences from more than one subject.

**[00125]** A “library nucleic acid sequence” refers to a nucleic acid molecule, *e.g.*, a DNA, RNA, or a combination thereof, that is a member of a library. Typically, a library nucleic acid sequence is a DNA molecule, *e.g.*, genomic DNA or cDNA. In some embodiments, a library nucleic acid sequence is fragmented, *e.g.*, sheared or enzymatically prepared, genomic DNA. In certain embodiments, the library nucleic acid sequences comprise sequence from a subject and sequence not derived from the subject, *e.g.*, adapter sequence, a primer sequence, or other sequences that allow for identification, *e.g.*, “barcode” sequences.

**[00126]** “Microsatellites” are repeated DNA sequences that occur approximately every 50–100 Kb base pairs throughout the human genome. “MSI” or “microsatellite instability” refers to a hypermutable phenotype caused by the loss of DNA mismatch repair activity and is implicated in the development of CRC and endometrial cancer. MSI is detected in about 15% of all CRCs; 3% are of which are associated with Lynch syndrome and the other 12% are caused by sporadic, acquired hypermethylation of the promoter of the *MLH1* gene, which occurs in tumors with the CpG island methylator phenotype. Colorectal tumors with MSI have distinctive features, including a tendency to arise in the proximal colon, lymphocytic infiltrate, and a poorly differentiated, mucinous or signet ring appearance.

**[00127]** “MMR-deficient” or “dMMR” refers to a defect in DNA mismatch repair or to cells exhibiting epigenetic inactivation of a gene in the MMR pathway, or loss of protein function of a MMR gene. Examples of genes in the MMR pathway are MSH2, MLH1, MSH6, PMS1 or PMS2. Mutations in MMR genes are often associated with an increase in the frequency of spontaneous mutation and carcinogenesis. Defects in MMR are often characterized by microsatellite instability (MSI) caused by expansion or contraction of short

nucleotide repeats in the absence of efficient MMR and, as such, MSI is detectable in the majority of colorectal cancers arising in carriers of germ-line MMR mutations.

**[00128]** “MMR-proficient” or “pMMR” refers to cells comprising wild-type MMR genes. MMR-proficient cancers are characterized by microsatellite stability (MSS).

5 **[00129]** The term “multiplex PCR” as used herein refers to amplification of two or more PCR products or amplicons which are each primed using a distinct primer pair.

**[00130]** “Next-generation sequencing or NGS” as used herein, refers to any sequencing method that determines the nucleotide sequence of either individual nucleic acid molecules (*e.g.*, in single molecule sequencing) or clonally expanded proxies for individual nucleic acid molecules in a high throughput parallel fashion (*e.g.*, greater than  $10^3$ ,  $10^4$ ,  $10^5$  or more molecules are sequenced simultaneously). In one embodiment, the relative abundance of the nucleic acid species in the library can be estimated by counting the relative number of occurrences of their cognate sequences in the data generated by the sequencing experiment. Next generation sequencing methods are known in the art, and are described, *e.g.*, in Metzker, M. *Nature Biotechnology Reviews* 11:31-46 (2010).

15 **[00131]** As used herein, “oligonucleotide” refers to a molecule that has a sequence of nucleic acid bases on a backbone comprised mainly of identical monomer units at defined intervals. The bases are arranged on the backbone in such a way that they can bind with a nucleic acid having a sequence of bases that are complementary to the bases of the oligonucleotide. The most common oligonucleotides have a backbone of sugar phosphate units. A distinction may be made between oligodeoxyribonucleotides that do not have a hydroxyl group at the 2' position and oligoribonucleotides that have a hydroxyl group at the 2' position. Oligonucleotides may also include derivatives, in which the hydrogen of the hydroxyl group is replaced with organic groups, *e.g.*, an allyl group. Oligonucleotides of the method which function as primers or probes are generally at least about 10-15 nucleotides long and more preferably at least about 15 to 25 nucleotides long, although shorter or longer oligonucleotides may be used in the method. The exact size will depend on many factors, which in turn depend on the ultimate function or use of the oligonucleotide. The oligonucleotide may be generated in any manner, including, for example, chemical synthesis, DNA replication, restriction endonuclease digestion of plasmids or phage DNA, reverse transcription, PCR, or a combination thereof. The oligonucleotide may be modified *e.g.*, by

addition of a methyl group, a biotin or digoxigenin moiety, a fluorescent tag or by using radioactive nucleotides.

**[00132]** As used herein, the term “overall survival” or “OS” means the observed length of life from the start of treatment to death or the date of last contact.

5 **[00133]** As used herein, the term “perioperative” refers to the time period of a patient's surgical procedure. It commonly includes ward admission, anesthesia, surgery, and recovery. The perioperative period is characterized by a sequence including the time preceding an operation when a patient is being prepared for surgery (“the preoperative period”), followed by the time spent in surgery (“the intraoperative period”), and by the time  
10 following an operation when the patient is closely monitored for complications while recovering from the effects of anesthesia (“the postoperative period”).

**[00134]** As used herein, the term “primer” refers to an oligonucleotide, which is capable of acting as a point of initiation of nucleic acid sequence synthesis when placed under conditions in which synthesis of a primer extension product which is complementary to a  
15 target nucleic acid strand is induced, *i.e.*, in the presence of different nucleotide triphosphates and a polymerase in an appropriate buffer (“buffer” includes pH, ionic strength, cofactors *etc.*) and at a suitable temperature. One or more of the nucleotides of the primer can be modified for instance by addition of a methyl group, a biotin or digoxigenin moiety, a fluorescent tag or by using radioactive nucleotides. A primer sequence need not reflect the exact sequence  
20 of the template. For example, a non-complementary nucleotide fragment may be attached to the 5' end of the primer, with the remainder of the primer sequence being substantially complementary to the strand. The term primer as used herein includes all forms of primers that may be synthesized including peptide nucleic acid primers, locked nucleic acid primers, phosphorothioate modified primers, labeled primers, and the like. The term “forward primer”  
25 as used herein means a primer that anneals to the anti-sense strand of dsDNA. A “reverse primer” anneals to the sense-strand of dsDNA.

**[00135]** As used herein, “primer pair” refers to a forward and reverse primer pair (*i.e.*, a left and right primer pair) that can be used together to amplify a given region of a nucleic acid of interest.

30 **[00136]** “Probe” as used herein refers to nucleic acid that interacts with a target nucleic acid *via* hybridization. A probe may be fully complementary to a target nucleic acid sequence or partially complementary. The level of complementarity will depend on many factors based,

in general, on the function of the probe. A probe or probes can be used, for example to detect the presence or absence of a mutation in a nucleic acid sequence by virtue of the sequence characteristics of the target. Probes can be labeled or unlabeled, or modified in any of a number of ways well known in the art. A probe may specifically hybridize to a target nucleic acid. Probes may be DNA, RNA or a RNA/DNA hybrid. Probes may be oligonucleotides, artificial chromosomes, fragmented artificial chromosome, genomic nucleic acid, fragmented genomic nucleic acid, RNA, recombinant nucleic acid, fragmented recombinant nucleic acid, peptide nucleic acid (PNA), locked nucleic acid, oligomer of cyclic heterocycles, or conjugates of nucleic acid. Probes may comprise modified nucleobases, modified sugar moieties, and modified internucleotide linkages. A probe may be used to detect the presence or absence of a target nucleic acid. Probes are typically at least about 10, 15, 20, 25, 30, 35, 40, 50, 60, 75, 100 nucleotides or more in length.

**[00137]** As used herein, “recurrence-specific survival” or “RSS” means the observed length of life from the time of surgical resection to the time of first recurrence of the cancer, otherwise censored at the time of last follow-up. In RSS, deaths not involving recurrence of cancer are excluded.

**[00138]** As used herein, a “sample” refers to a substance that is being assayed for the presence of a mutation in a nucleic acid of interest. Processing methods to release or otherwise make available a nucleic acid for detection are well known in the art and may include steps of nucleic acid manipulation. A biological sample may be a body fluid or a tissue sample. In some cases, a biological sample may consist of or comprise blood, plasma, sera, urine, feces, epidermal sample, vaginal sample, skin sample, cheek swab, sperm, amniotic fluid, cultured cells, bone marrow sample, tumor biopsies, aspirate and/or chorionic villi, cultured cells, and the like. Fresh, fixed or frozen tissues may also be used. In one embodiment, the sample is preserved as a frozen sample or as formaldehyde- or paraformaldehyde-fixed paraffin-embedded (FFPE) tissue preparation. For example, the sample can be embedded in a matrix, e.g., an FFPE block or a frozen sample. Whole blood samples of about 0.5 to 5 ml collected with EDTA, ACD or heparin as anti-coagulant are suitable.

**[00139]** The term “sensitivity,” as used herein in reference to the methods of the present technology, is a measure of the ability of a method to detect a preselected sequence variant in a heterogeneous population of sequences. A method has a sensitivity of S % for variants of F % if, given a sample in which the preselected sequence variant is present as at least F % of

the sequences in the sample, the method can detect the preselected sequence at a preselected confidence of C %, S % of the time. By way of example, a method has a sensitivity of 90% for variants of 5% if, given a sample in which the preselected variant sequence is present as at least 5% of the sequences in the sample, the method can detect the preselected sequence at a preselected confidence of 99%, 9 out of 10 times (F=5%; C=99%; S=90%).

**[00140]** As used herein, the term “separate” therapeutic use refers to an administration of at least two active ingredients at the same time or at substantially the same time by different routes.

**[00141]** As used herein, the term “sequential” therapeutic use refers to administration of at least two active ingredients at different times, the administration route being identical or different. More particularly, sequential use refers to the whole administration of one of the active ingredients before administration of the other or others commences. It is thus possible to administer one of the active ingredients over several minutes, hours, or days before administering the other active ingredient or ingredients. There is no simultaneous treatment in this case.

**[00142]** As used herein, the term “simultaneous” therapeutic use refers to the administration of at least two active ingredients by the same route and at the same time or at substantially the same time.

**[00143]** The term “specific” as used herein in reference to an oligonucleotide primer means that the nucleotide sequence of the primer has at least 12 bases of sequence identity with a portion of the nucleic acid to be amplified when the oligonucleotide and the nucleic acid are aligned. An oligonucleotide primer that is specific for a nucleic acid is one that, under the stringent hybridization or washing conditions, is capable of hybridizing to the target of interest and not substantially hybridizing to nucleic acids which are not of interest. Higher levels of sequence identity are preferred and include at least 75%, at least 80%, at least 85%, at least 90%, at least 95% and more preferably at least 98% sequence identity.

**[00144]** “Specificity,” as used herein, is a measure of the ability of a method to distinguish a truly occurring preselected sequence variant from sequencing artifacts or other closely related sequences. It is the ability to avoid false positive detections. False positive detections can arise from errors introduced into the sequence of interest during sample preparation, sequencing error, or inadvertent sequencing of closely related sequences like pseudo-genes or members of a gene family. A method has a specificity of X % if, when applied to a sample

set of  $N_{\text{Total}}$  sequences, in which  $X_{\text{True}}$  sequences are truly variant and  $X_{\text{Not true}}$  are not truly variant, the method selects at least  $X\%$  of the not truly variant as not variant. *E.g.*, a method has a specificity of 90% if, when applied to a sample set of 1,000 sequences, in which 500 sequences are truly variant and 500 are not truly variant, the method selects 90% of the 500 not truly variant sequences as not variant. Exemplary specificities include 90, 95, 98, and 99%.

[00145] The term “stringent hybridization conditions” as used herein refers to hybridization conditions at least as stringent as the following: hybridization in 50% formamide, 5xSSC, 50 mM  $\text{NaH}_2\text{PO}_4$ , pH 6.8, 0.5% SDS, 0.1 mg/mL sonicated salmon sperm DNA, and 5x Denhart's solution at 42° C. overnight; washing with 2x SSC, 0.1% SDS at 45° C; and washing with 0.2x SSC, 0.1% SDS at 45° C. In another example, stringent hybridization conditions should not allow for hybridization of two nucleic acids which differ over a stretch of 20 contiguous nucleotides by more than two bases.

[00146] As used herein, the terms “subject”, “patient”, or “individual” can be an individual organism, a vertebrate, a mammal, or a human. In some embodiments, the subject, patient or individual is a human.

[00147] As used herein, the terms “target sequence” and “target nucleic acid sequence” refer to a specific nucleic acid sequence to be detected and/or quantified in the sample to be analyzed.

[00148] “Treating” or “treatment” as used herein covers the treatment of a disease or disorder described herein, in a subject, such as a human, and includes: (i) inhibiting a disease or disorder, *i.e.*, arresting its development; (ii) relieving a disease or disorder, *i.e.*, causing regression of the disorder; (iii) slowing progression of the disorder; and/or (iv) inhibiting, relieving, or slowing progression of one or more symptoms of the disease or disorder. In some embodiments, treatment means that the symptoms associated with the disease are, *e.g.*, alleviated, reduced, cured, or placed in a state of remission.

[00149] It is also to be appreciated that the various modes of treatment of disorders as described herein are intended to mean “substantial,” which includes total but also less than total treatment, and wherein some biologically or medically relevant result is achieved. The treatment may be a continuous prolonged treatment for a chronic disease or a single, or few time administrations for the treatment of an acute condition.

**Methods for Detecting Polynucleotides Associated with Positive or Negative Responsiveness to Intraoperative Opioid Analgesics in Lung Cancer**

[00150] Polynucleotides associated with responsiveness to intraoperative opioid analgesics may be detected by a variety of methods known in the art. Non-limiting examples of detection methods are described below. The detection assays in the methods of the present technology may include purified or isolated DNA (genomic or cDNA), RNA or protein or the detection step may be performed directly from a biological sample without the need for further DNA, RNA or protein purification/isolation.

**Nucleic Acid Amplification and/or Detection**

[00151] Polynucleotides associated with responsiveness to intraoperative opioid analgesics can be detected by the use of nucleic acid amplification techniques that are well known in the art. The starting material may be genomic DNA, cDNA, RNA or mRNA. Nucleic acid amplification can be linear or exponential. Specific variants or mutations may be detected by the use of amplification methods with the aid of oligonucleotide primers or probes designed to interact with or hybridize to a particular target sequence in a specific manner, thus amplifying only the target variant.

[00152] Non-limiting examples of nucleic acid amplification techniques include polymerase chain reaction (PCR), real-time quantitative PCR (qPCR), digital PCR (dPCR), reverse transcriptase polymerase chain reaction (RT-PCR), nested PCR, ligase chain reaction (see Abravaya, K. *et al.*, *Nucleic Acids Res.* (1995), 23:675-682), branched DNA signal amplification (see Urdea, M. S. *et al.*, *AIDS* (1993), 7(suppl 2):S11- S14), amplifiable RNA reporters, Q-beta replication, transcription-based amplification, boomerang DNA amplification, strand displacement activation, cycling probe technology, isothermal nucleic acid sequence based amplification (NASBA) (see Kievits, T. *et al.*, *J Virological Methods* (1991), 35:273-286), Invader Technology, next-generation sequencing technology or other sequence replication assays or signal amplification assays.

[00153] *Primers*: Oligonucleotide primers for use in amplification methods can be designed according to general guidance well known in the art as described herein, as well as with specific requirements as described herein for each step of the particular methods described. In some embodiments, oligonucleotide primers for cDNA synthesis and PCR are 10 to 100 nucleotides in length, preferably between about 15 and about 60 nucleotides in length, more preferably 25 and about 50 nucleotides in length, and most preferably between about 25 and about 40 nucleotides in length.

[00154]  $T_m$  of a polynucleotide affects its hybridization to another polynucleotide (*e.g.*, the annealing of an oligonucleotide primer to a template polynucleotide). In certain embodiments of the disclosed methods, the oligonucleotide primer used in various steps selectively hybridizes to a target template or polynucleotides derived from the target template (*i.e.*, first and second strand cDNAs and amplified products). Typically, selective hybridization occurs when two polynucleotide sequences are substantially complementary (at least about 65% complementary over a stretch of at least 14 to 25 nucleotides, preferably at least about 75%, more preferably at least about 90% complementary). See Kanehisa, M., *Polynucleotides Res.* (1984), 12:203, incorporated herein by reference. As a result, it is expected that a certain degree of mismatch at the priming site is tolerated. Such mismatch may be small, such as a mono-, di- or tri-nucleotide. In certain embodiments, 100% complementarity exists.

[00155] *Probes*: Probes are capable of hybridizing to at least a portion of the nucleic acid of interest or a reference nucleic acid (*i.e.*, wild-type sequence). Probes may be an oligonucleotide, artificial chromosome, fragmented artificial chromosome, genomic nucleic acid, fragmented genomic nucleic acid, RNA, recombinant nucleic acid, fragmented recombinant nucleic acid, peptide nucleic acid (PNA), locked nucleic acid, oligomer of cyclic heterocycles, or conjugates of nucleic acid. Probes may be used for detecting and/or capturing/purifying a nucleic acid of interest.

[00156] Typically, probes can be about 10 nucleotides, about 20 nucleotides, about 25 nucleotides, about 30 nucleotides, about 35 nucleotides, about 40 nucleotides, about 50 nucleotides, about 60 nucleotides, about 75 nucleotides, or about 100 nucleotides long. However, longer probes are possible. Longer probes can be about 200 nucleotides, about 300 nucleotides, about 400 nucleotides, about 500 nucleotides, about 750 nucleotides, about 1,000 nucleotides, about 1,500 nucleotides, about 2,000 nucleotides, about 2,500 nucleotides, about 3,000 nucleotides, about 3,500 nucleotides, about 4,000 nucleotides, about 5,000 nucleotides, about 7,500 nucleotides, or about 10,000 nucleotides long.

[00157] Probes may also include a detectable label or a plurality of detectable labels. The detectable label associated with the probe can generate a detectable signal directly. Additionally, the detectable label associated with the probe can be detected indirectly using a reagent, wherein the reagent includes a detectable label, and binds to the label associated with the probe.

[00158] In some embodiments, detectably labeled probes can be used in hybridization assays including, but not limited to Northern blots, Southern blots, microarray, dot or slot blots, and *in situ* hybridization assays such as fluorescent *in situ* hybridization (FISH) to detect a target nucleic acid sequence within a biological sample. Certain embodiments may employ hybridization methods for measuring expression of a polynucleotide gene product, such as mRNA. Methods for conducting polynucleotide hybridization assays have been well developed in the art. Hybridization assay procedures and conditions will vary depending on the application and are selected in accordance with the general binding methods known including those referred to in: Maniatis *et al.* *Molecular Cloning: A Laboratory Manual* (2nd Ed. Cold Spring Harbor, N.Y., 1989); Berger and Kimmel *Methods in Enzymology*, Vol. 152, Guide to Molecular Cloning Techniques (Academic Press, Inc., San Diego, Calif, 1987); Young and Davis, *PNAS*. 80: 1194 (1983).

[00159] Detectably labeled probes can also be used to monitor the amplification of a target nucleic acid sequence. In some embodiments, detectably labeled probes present in an amplification reaction are suitable for monitoring the amount of amplicon(s) produced as a function of time. Examples of such probes include, but are not limited to, the 5'-exonuclease assay (TAQMAN® probes described herein (see also U.S. Pat. No. 5,538,848) various stem-loop molecular beacons (see for example, U.S. Pat. Nos. 6,103,476 and 5,925,517 and Tyagi and Kramer, 1996, *Nature Biotechnology* 14:303- 308), stemless or linear beacons (see, *e.g.*, WO 99/21881), PNA Molecular Beacons™ (see, *e.g.*, U.S. Pat. Nos. 6,355,421 and 6,593,091), linear PNA beacons (see, for example, Kubista *et al.*, 2001, *SPIE* 4264:53-58), non-FRET probes (see, for example, U.S. Pat. No. 6,150,097), Sunrise®/Amplifluor™ probes (U.S. Pat. No. 6,548,250), stem-loop and duplex Scorpion probes (Solinas *et al.*, 2001, *Nucleic Acids Research* 29:E96 and U.S. Pat. No. 6,589,743), bulge loop probes (U.S. Pat. No. 6,590,091), pseudo knot probes (U.S. Pat. No. 6,589,250), cyclicons (U.S. Pat. No. 6,383,752), MGB Eclipse™ probe (Epoch Biosciences), hairpin probes (U.S. Pat. No. 6,596,490), peptide nucleic acid (PNA) light-up probes, self-assembled nanoparticle probes, and ferrocene-modified probes described, for example, in U.S. Pat. No. 6,485,901 ; Mhlanga *et al.*, 2001, *Methods* 25:463-471 ; Whitcombe *et al.*, 1999, *Nature Biotechnology*. 17:804-807; Isacson *et al.*, 2000, *Molecular Cell Probes*. 14:321-328; Svanvik *et al.*, 2000, *Anal Biochem*. 281 :26-35; Wolffs *et al.*, 2001, *Biotechniques* 766:769-771 ; Tsourkas *et al.*, 2002, *Nucleic Acids Research*. 30:4208-4215; Riccelli *et al.*, 2002, *Nucleic Acids Research* 30:4088-4093; Zhang *et al.*, 2002 *Shanghai*. 34:329-332; Maxwell *et al.*, 2002, *J. Am. Chem.*

*Soc.* 124:9606-9612; Broude *et al.*, 2002, *Trends Biotechnol.* 20:249-56; Huang *et al.*, 2002, *Chem. Res. Toxicol.* 15:118- 126; and Yu *et al.*, 2001, *J. Am. Chem. Soc.* 14:11155-11161.

**[00160]** In some embodiments, the detectable label is a fluorophore. Suitable fluorescent moieties include but are not limited to the following fluorophores working individually or in combination: 4-acetamido-4'-isothiocyanatostilbene- 2,2'-disulfonic acid; acridine and derivatives: acridine, acridine isothiocyanate; Alexa Fluors: Alexa Fluor® 350, Alexa Fluor® 488, Alexa Fluor® 546, Alexa Fluor® 555, Alexa Fluor® 568, Alexa Fluor® 594, Alexa Fluor® 647 (Molecular Probes); 5-(2- aminoethyl)aminonaphthalene-1 -sulfonic acid (EDANS); 4-amino-N-[3- vinylsulfonyl]phenyl]naphthalimide-3,5 disulfonate (Lucifer Yellow VS); N-(4-anilino-1- naphthyl)maleimide; anthranilamide; Black Hole Quencher™ (BHQ™) dyes (biosearch Technologies); BODIPY dyes: BODIPY® R-6G, BOPIPY® 530/550, BODIPY® FL; Brilliant Yellow; coumarin and derivatives: coumarin, 7-amino-4-methylcoumarin (AMC, Coumarin 120), 7-amino-4-trifluoromethylcoumarin (Coumarin 151); Cy2®, Cy3®, Cy3.5®, Cy5®, Cy5.5®; cyanosine; 4',6-diaminidino-2-phenylindole (DAPI); 5', 5''-dibromopyrogallol- sulfonephthalein (Bromopyrogallol Red); 7-diethylamino-3-(4'- isothiocyanatophenyl)-4- methylcoumarin; diethylenetriamine pentaacetate; 4,4'- diisothiocyanatodihydro-stilbene-2,2'- disulfonic acid; 4,4'-diisothiocyanatostilbene-2,2'- disulfonic acid; 5- [dimethylamino]naphthalene-1 -sulfonyl chloride (DNS, dansyl chloride); 4-(4'- dimethylaminophenylazo)benzoic acid (DABCYL); 4- dimethylaminophenylazophenyl-4'- isothiocyanate (DABITC); Eclipse™ (Epoch Biosciences Inc.); eosin and derivatives: eosin, eosin isothiocyanate; erythrosin and derivatives: erythrosin B, erythrosin isothiocyanate; ethidium; fluorescein and derivatives: 5-carboxyfluorescein (FAM), 5-(4,6-dichlorotriazin-2- yl)amino fluorescein (DTAF), 2',7'- dimethoxy-4'5'-dichloro-6-carboxyfluorescein (JOE), fluorescein, fluorescein isothiocyanate (FITC), hexachloro-6-carboxyfluorescein (HEX), QFITC (XRITC), tetrachlorofluorescein (TET); fluorescamine; IR144; IR1446; lanthamide phosphors; Malachite Green isothiocyanate; 4-methylumbelliferone; ortho cresolphthalein; nitrotyrosine; pararosaniline; Phenol Red; B-phycoerythrin, R-phycoerythrin; allophycocyanin; o-phthalaldehyde; Oregon Green®; propidium iodide; pyrene and derivatives: pyrene, pyrene butyrate, succinimidyl 1 -pyrene butyrate; QSY® 7; QSY® 9; QSY® 21; QSY® 35 (Molecular Probes); Reactive Red 4 (Cibacron®Brilliant Red 3B-A); rhodamine and derivatives: 6-carboxy-X-rhodamine (ROX), 6-carboxyrhodamine (R6G), lissamine rhodamine B sulfonyl chloride, rhodamine (Rhod), rhodamine B, rhodamine 123, rhodamine green, rhodamine X

isothiocyanate, riboflavin, rosolic acid, sulforhodamine B, sulforhodamine 101, sulfonyl chloride derivative of sulforhodamine 101 (Texas Red); terbium chelate derivatives; N,N,N',N'-tetramethyl-6-carboxyrhodamine (TAMRA); tetramethyl rhodamine; tetramethyl rhodamine isothiocyanate (TRITC); and VIC®. Detector probes can also comprise sulfonate derivatives of fluorescein dyes with SO<sub>3</sub> instead of the carboxylate group, phosphoramidite forms of fluorescein, phosphoramidite forms of CY 5 (commercially available for example from Amersham).

[00161] Detectably labeled probes can also include quenchers, including without limitation black hole quenchers (Biosearch), Iowa Black (IDT), QSY quencher (Molecular Probes), and Dabsyl and Dabcel sulfonate/carboxylate Quenchers (Epoch).

[00162] Detectably labeled probes can also include two probes, wherein for example a fluorophore is on one probe, and a quencher is on the other probe, wherein hybridization of the two probes together on a target quenches the signal, or wherein hybridization on the target alters the signal signature *via* a change in fluorescence.

[00163] In some embodiments, interchelating labels such as ethidium bromide, SYBR® Green I (Molecular Probes), and PicoGreen® (Molecular Probes) are used, thereby allowing visualization in real-time, or at the end point, of an amplification product in the absence of a detector probe. In some embodiments, real-time visualization may involve the use of both an intercalating detector probe and a sequence-based detector probe. In some embodiments, the detector probe is at least partially quenched when not hybridized to a complementary sequence in the amplification reaction, and is at least partially unquenched when hybridized to a complementary sequence in the amplification reaction.

[00164] In some embodiments, the amount of probe that gives a fluorescent signal in response to an excited light typically relates to the amount of nucleic acid produced in the amplification reaction. Thus, in some embodiments, the amount of fluorescent signal is related to the amount of product created in the amplification reaction. In such embodiments, one can therefore measure the amount of amplification product by measuring the intensity of the fluorescent signal from the fluorescent indicator.

[00165] Primers or probes may be designed to selectively hybridize to any portion of a nucleic acid sequence encoding a polypeptide selected from among CDKN2A, NF2, LATS1, FAT1, RNF43, CTNNB1, AXIN2, APC and AMER1. Exemplary nucleic acid sequences of the human orthologs of these genes are provided below:

[00166] *Homo sapiens* cyclin dependent kinase inhibitor 2A (CDKN2A), transcript variant 1, mRNA (NCBI Reference Sequence: NM\_000077.5) (SEQ ID NO: 1)

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1 gagagcagggc agcggggcggc ggggagcagc atggagccgg cggcggggag cagcatggag
61 ccttcggctg actggctggc cagggccggc gcccggggtc gggtagagga ggtgcggggc
5 121 ctgctggagg cggggggcgt gcccaacgca ccgaatagtt acggtcggag gccgatccag
181 gtcatgatga tgggcagcgc ccgagtggcg gagctgctgc tgctccacgg cgcggagccc
241 aactgcgccc accccgccac tctcaccoga cccgtgcaag acgctgcccg ggagggcttc
301 ctggacacgc tgggtgggtgct gcaccggggc ggggcggcggc tggacgtgcg cgatgcctgg
361 ggccgtctgc ccgtggacct ggctgaggag ctggggccatc gcgatgtcgc acggtacctg
10 421 cgcgcggctg cggggggcac cagaggcagt aacctatgcc gcatagatgc cgcggaaggt
481 ccctcagaca tccccgattg aaagaaccag agaggctctg agaaacctcg ggaaacttag
541 atcatcagtc accgaaggtc ctacagggcc acaactgcc cgcgcacaac ccaccccgct
601 ttcgtagttt tcatttagaa aatagagctt ttaaaaatgt cctgcctttt aacgtagata
661 tatgccttcc cccactaccg taaatgtcca tttatatcat tttttatata ttcttataaa
15 721 aatgtaaaaa agaaaaacac cgcttctgcc ttttcaactgt gttggagtgt tctggagtga
781 gcactcacgc cctaagcgca cattcatgtg ggcatctctt gcgagcctcg cagcctccgg
841 aagctgtcga cttcatgaca agcattttgt gaactaggga agctcagggg ggttactggc
901 ttctcttgag tcacactgct agcaaatggc agaaccaaag ctcaaataaa aataaaataa
961 ttttcattca ttcactca
20

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[00167] *Homo sapiens* cyclin dependent kinase inhibitor 2A (CDKN2A), transcript variant 4, mRNA (NCBI Reference Sequence: NM\_058195.4) (SEQ ID NO: 2)

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1 acctctggtg ccaaagggcg gcgcagcggc tgccgagctc ggccctggag gcggcgagaa
61 catggtgcgc aggttcttgg tgaccctccg gattcggcgc gcgtgcggcc cgcgcgagat
25 121 gagggttttc gtggttcaca tcccgcggtc caggggggag tgggcagcgc caggggcgcc
181 cgcgcgtgtg gccctcgtgc tgatgtact gaggagccag cgtctagggc agcagccgct
241 tcctagaaga ccaggtcatg atgatgggca gcgccgagat ggccgagctg ctgctgctcc
301 acgggcgcgga gcccaactgc gcgcaccccg ccactctcac ccgaccgtg cacgacgctg
361 cccgggaggg cttcctggac acgctggtgg tgctgcaccg ggccggggcg cggctggacg
30 421 tgccgcgatg ctggggcgct ctgcccggtg acctggctga ggagctgggc catcgcgatg
481 tcgcacggta cctgcgcgcg gctgcggggg gcaccagagg cagtaacctat gccgcgatg
541 atgcgcgga aggtccctca gacatccccg attgaaagaa ccagagaggc tctgagaaac
601 ctgcggaaac ttagatcatc agtcaccgaa ggtcctacag ggccacaact gccccgcca
661 caaccacccc cgctttcgta gttttcattt agaaaataga gcttttaaaa atgtcctgcc
35 721 ttttaacgta gatatatgcc ttccccact accgtaaatg tccatttata tcatttttta
781 tatattctta taaaaatgta aaaaagaaaa acaccgcttc tgccttttca ctgtgttga
841 gttttctgga gtgagcactc acgcctaag cgcacattca tgtgggcatt tcttgcgagc
901 ctgcgagcct ccggaagctg tcgacttcat gacaagcatt ttgtgaacta ggaagctca
961 ggggggttac tggcttctct tgagtcacac tgctagcaaa tggcagaacc aaagctcaaa
40 1021 taaaaataaa ataattttca ttcattcact ca

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[00168] *Homo sapiens* FAT atypical cadherin 1 (FAT1), mRNA (NCBI Reference Sequence: NM\_005245.4) (SEQ ID NO: 3)

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1 gagcgtgagg cgcgggcgcg gagctggggc gccgggcgcg gggagagggc gcgggagcgg
45 61 ctgctgcggc aggtaccatg cggacgcgcg agcccggcga gggccccggc aggcccggtc
121 cctgctcggg ggcgcgctga gacggcgggt gagctccacg agagcgccgt cgccacttcg
181 ggccaacttt gcgattcccg acagttaagc aatggggaga catttggtt tgctcctgct
241 tctgctcctt ctcttccaac attttggaga cagtgatggc agccaacgac ttgaacagac
301 tcctctgcag tttacacacc tcgagtacaa cgtcacccgt caggagaact ctgcagctaa
50 361 cagttatgtg gggcatcctg tcaagatggg tgtttacatt acacatccag cgtgggaagt
421 aaggtacaaa attgtttccg gagacagtga aaacctgttc aaagctgaag agtacattct
481 cggagacttt tgcttttctaa gaataaggac caaaggagga aatacagcta ttcttaatag

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541 agaagtgaag gatcactaca cattgatagt gaaagcactt gaaaaaata ctaatgtgga  
 601 ggcgcgaaca aaggtcaggg tgcaggtgct ggatacaaat gacttgagac cgttattctc  
 661 acccacctca tacagcgttt ctttacctga aaacacagct ataaggacca gtatcgcaag  
 721 agtcagcgcc acggatgcag acataggaac caacggggaa ttttactaca gttttaaaga  
 5 781 tcgaacagat atgtttgcta ttaccccaac cagtggtgtg atagtgttaa ctggtagact  
 841 tgattaccta gagaccaagc tctatgagat ggaaatcctc gctgcgacc gtggcatgaa  
 901 gttgtatggg agcagtgga tcagcagcat ggccaagcta acggtgcaca tcgaacaggc  
 961 caatgaatgt gctccggtga taacagcagt gacattgtca ccatcagaac tggacagggg  
 1021 cccagcatat gcaattgtga cagtggatga ctgcatcag ggtgccaatg gtgacatagc  
 10 1081 atctttaagc atcgtggcag gtgaccttct ccagcagttt agaacagtga ggtcctttcc  
 1141 agggagtaag gagtataaag tcaaagccat cgggtggcatt gattgggaca gtcattcctt  
 1201 cggctacaat ctacactac aggtctaaaga taaaggaact ccgccccagt tctcttctgt  
 1261 taaagtcatt cacgtgactt ctccacagtt caaagccggg ccagtcagt ttgaaaaggg  
 1321 tgtttacaga gcagaaataa gtgaatttgc tcctcccaac acacctgtgg tcatggtaaa  
 15 1381 ggccattcct gcttattccc atttgaggta tgttttttaa agtacacctg gaaaagctaa  
 1441 attcagttta aattacaaca ctggtctcat ttctatttta gaaccagtta aaagacagca  
 1501 ggcagcccat tttgaacttg aagtaacaac aagtgcaga aaagcgtcca ccaaggtctt  
 1561 ggtgaaagtc ttaggtgcaa atagcaatcc ccctgaattt acccagacag cgtacaaagc  
 1621 tgcttttgat gagaacgtgc ccattggtac tactgtcatg agcctgagtg ccgtagaccc  
 20 1681 tgatgagggg gagaacgggt acgtgacata cagtatcgca aatttaaata atgtgccgtt  
 1741 tgcgattgac ctttctactg gtgccgtgag tacgtcagaa aacctggact acgaactgat  
 1801 gcctcgggtt tatactctga ggattcgtgc atcagactgg ggcttgccgt accgccggga  
 1861 agtcgaagtc cttgctacaa ttactctcaa taacttgaat gacaacacac ctttgtttga  
 1921 gaaaataaat tgtgaaggga caattccag agatctaggc gtgggagagc aaataaccac  
 25 1981 tgtttctgct attgatgcag atgaacttca gttggtacag tatcagattg aagctgaaa  
 2041 tgaactggat ttcttttagt taaaccccaa ctccgggggt tgtcattaa agcgatcgct  
 2101 aatggatggc ttaggtgcaa aggtgtcttt ccacagtctg agaatcacag ctacagatgg  
 2161 agaaaatttt gccacaccat tatatatcaa cataacagtg gctgccagtc acaagctggg  
 2221 aaacttgcag tgtgaagaga ctggtgttgc caaatgctg gcagagaagc tctcgaggc  
 30 2281 aaataaatta cacaaccagg gagaggtgga ggatatttct ttcgattctc actctgtcaa  
 2341 tgctcacata ccgcagttta gaagcactct tccgactggg attcaggtaa aggaaaacca  
 2401 gcctgtgggt tccagtgtaa ttttcatgaa ctccactgac cttgacactg gcttcaatgg  
 2461 aaaactggtc tatgctgttt ctggaggaaa tgaggatagt tgcttcatga ttgatattga  
 2521 aacaggaatg ctgaaaattt tatctctctt tgaccgtgaa acaacagaca aatacacctt  
 35 2581 gaatattacc gtctatgacc ttgggatacc ccagaaggct gcgtggcgct ttctacatgt  
 2641 cgtggttgct gatgccaatg ataatccacc cgagttttta caggagagct attttgtgga  
 2701 agtgagtga gacaaggagg tacatagtga aatcatccag gttgaagcca cagataaaga  
 2761 cctggggccc aacggacacg tgacgtactc aattgttaca gacacagaca cttttcaat  
 2821 tgacagcgtg acgggtgttg ttaacatcgc acgccctctg gatcgagagc tgcagcatga  
 40 2881 gcactcctta aagattgagg ccagggaacca agccagagaa gagcctcagc tgttctccac  
 2941 tgtcgttgtg aaagtatcac tagaagatgt taatgacaac ccacctacat ttattccacc  
 3001 taattatcgt gtgaaagtcc gagaggatct tccagaagga accgtcatca tgtggttaga  
 3061 agcccacgat cctgatttag gtcagtctgg tcaggtgaga tacagccttc tggaccagg  
 3121 agaaggaaac ttcgatgtgg ataaactcag tggagcagtt aggatcgtcc agcagttgga  
 45 3181 ctttgagaag aagcaagtgt ataactctac tgtgagggcc aaagacaagg gaaagccagt  
 3241 ttctctgtct tctacttgct atgttgaagt tgaggtggtt gatgtgaatg agaacctgca  
 3301 cccaccogtg ttttccagct ttgtggaaaa ggggacagtg aaagaagatg cacctgttgg  
 3361 ttcatgtgta atgacggtgt cggctcatga tgaggacgcc agaagagatg gggagatccg  
 3421 atactccatt agagatggct ctggcggttg tgttttcaaa ataggtgaag agacaggtgt  
 50 3481 catagagacg tcagatcgac tggaccgtga atcgacctc cattattggc taacagtctt  
 3541 tgcaaccgat cagggtgtcg tgccctcttc atcgttcata gagatctaca tagaggttga  
 3601 ggatgtcaat gacaatgcac cacagacatc agagcctgtt tattaccag aaatcatgga  
 3661 aaattctcct aaagatgtat ctgtggtcca gatcgaggca tttgatccag attcgagctc  
 3721 taatgacaag ctcatgtaca aaattacaag tggaaatcca caaggattct tttcaatata  
 55 3781 tcctaaaaca ggtctcatca caactacgtc aaggaagcta gaccgagAAC agcaagatga  
 3841 acacatatta gaggttactg tgacagacaa tggtagtccc cccaaatcaa ccattgcaag  
 3901 agtcattgtg aaaatccttg atgaaaatga caacaaacct cagtttctgc aaaagttcta  
 3961 caaaatcaga ctccctgagc gggaaaagcc agaccgagaa agaaatgcc gacgggagcc  
 4021 gctctatcac gtcatagcca ccgacaagga tgagggcccc aatgcagaaa tctcctacag  
 60 4081 catcgaagac gggaaatgagc atggcaaat tttcatcgaa ccgaaaactg gagtggtttc  
 4141 gtccaagagg ttttcagcag ctggagaata tgatattctt tcaattaagg cagttgacaa

|    |      |             |            |             |             |            |             |
|----|------|-------------|------------|-------------|-------------|------------|-------------|
|    | 4201 | tggtcgccct  | caaaagtcac | caaccaccag  | actccatatt  | gaatggatct | ccaagcccaa  |
|    | 4261 | accgtccctg  | gagcccattt | catttgaaga  | atcatttttt  | acctttactg | tgatggaaag  |
|    | 4321 | tgaccccgtt  | gctcacatga | ttggagtaat  | atctgtggag  | cctcctggca | tacccttttg  |
|    | 4381 | gtttgacatc  | actggtggca | actacgacag  | tcacttcgat  | gtggacaagg | gaactggaac  |
| 5  | 4441 | catcattggt  | gccaacacct | ttgatgcaga  | acagaagtca  | aactacaacc | tcacagtcga  |
|    | 4501 | ggctacagat  | ggaaccacca | ctatcctcac  | tcagggtattc | atcaaagtaa | tagacacaaa  |
|    | 4561 | tgaccatcgt  | cctcagtttt | ctacatcaaa  | gtatgaagtt  | gttattcctg | aagatacagc  |
|    | 4621 | gccagaaaca  | gaaattttgc | aaatcagtg   | tgtggatcag  | gatgagaaaa | acaaactaat  |
|    | 4681 | ctacactctg  | cagagcagta | gagatccact  | gagtctcaag  | aaatttcgtc | ttgatcctgc  |
| 10 | 4741 | aaccggctct  | ctctatactt | ctgagaaact  | ggatcatgaa  | gctgttcacc | agcacaccct  |
|    | 4801 | cacggtcatg  | gtacgagatc | aagatgtgcc  | tgtaaaacgc  | aactttgcaa | ggattgtggt  |
|    | 4861 | caatgtcagc  | gacacgaatg | accacgcccc  | gtgggttcacc | gcttcctcct | acaaagggcg  |
|    | 4921 | ggtttatgaa  | tcggcagccg | ttggctcagt  | tgtgttgag   | gtgacggctc | tggaacaagg  |
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[00169] *Homo sapiens* neurofibromin 2 (NF2), transcript variant 1, mRNA (NCBI Reference Sequence: NM\_000268.4) (SEQ ID NO: 4)

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**[00170]** *Homo sapiens* large tumor suppressor kinase 1 (LATS1), transcript variant 1,  
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[00171] *Homo sapiens* ring finger protein 43 (RNF43), transcript variant 1, mRNA (NCBI

50 Reference Sequence: NM\_017763.6) (SEQ ID NO: 6)

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 25 1921 cacctcattc gccagcatcc cggccatgcc cactaccacc tcctgctgc ctacctgttg  
 1981 ggcccttccc ggagtgcagt ggctcggccc ccacgacctg gtcccttctt gccatcccag  
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 2161 cccctccaat ccacctcaca gcacctgtct gcttgcccag tgcccctacg ccgggcccag  
 30 2221 cccctgaca gcagtggatc tggagaaagc tattgcacag aacgcagtgg gtacctggca  
 2281 gatgggcccag ccagtgcact cagctcaggg ccctgtcatg gctcttccag tgactctgtg  
 2341 gtcaactgca cggacatcag cctacagggg gtccatggca gcagtctctac tttctgcagc  
 2401 tccctaagca gtgactttga cccctagtgt tactgcagcc ctaaagggga tccccagcga  
 2461 gtggacatgc agcctagtgt gacctctcgg cctcgttctt tggactcggg ggtgcccaca  
 35 2521 ggggaaaccc aggtttccag ccatgtccac taccaccgcc accggcacca ccactacaaa  
 2581 aagcggttcc agtggcatgg cagggaagcct ggcccagaaa ccggagtccc ccagtccagg  
 2641 cctcctattc ctcgacaca gcccagcca gagccacctt ctctgatca gcaagtacc  
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 2761 cctgagccag cccctggccc agttgacgcc tccagcatct gcccagtag cagcagtctg  
 40 2821 ttcaacttgc aaaaatccag cctctctgcc cgacacccac agaggaaaag gcgggggggg  
 2881 ccctccgagc ccacccctgg ctctcggccc caggatgcaa ctgtgcaccc agcttgccag  
 2941 atttttcccc attacacccc cagtgtggca tatecttggg ccccagagge acaccccttg  
 3001 atctgtggac ctccaggcct ggacaagagg ctgctaccag aaaccccagg cccctgttac  
 3061 tcaaattcac agccagtgtg gttgtgcctg actcctcggc agcccctgga accacatcca  
 45 3121 cctggggagg ggccttctga atggagttct gacaccgcag agggcaggcc atgccttat  
 3181 ccgactgcc aggtgctgtc ggcccagcct ggctcagagg aggaactcga ggagtgtgt  
 3241 gaacaggctg tgtgagatgt tcaggcctag ctccaaccaa gagtgtgctc cagatgtgtt  
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 3361 ttctttttgc cgtacttctt agaagcactg gaagaggact ggtgatgggt gaggggtgaga  
 50 3421 ggggtgccgtt tcctgctcca gctccagacc ttgtctgcag aaacatctg cagtgcagca  
 3481 aatccatgtc cagccaggca accagctgct gcctgtggcg tgtgtgggct ggatcccttg  
 3541 aaggctgagt ttttgagggc agaaagctag ctatgggtag ccagggtgta caaagggtgct  
 3601 gctccttctc caacccctac ttggtttccc tcaccccaag cctcatgttc ataccagcca  
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 55 3721 cagctttggc cctccacag taaggctgct acatcagggg caaccctggc tctatcattt  
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 60 4021 aggaaggatg gaatgaaggc agaaaaggca gaattacagc tgagcgggga caacaaagag  
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4141 aagcaaagtg tgacccttgg gtttggacag cccagaggcc cagctcccca gtataagcca  
 4201 tacaggccag ggacccacag gagagtggat tagagcaca gtctggcctc actgagtggga  
 4261 caagagctga tgggcctcat cagggtgaca ttcaccccag ggcagcctga ccactcttgg  
 4321 cccctcaggc attatcccat ttggaatgtg aatgtggtgg caaagtgggc agaggacccc  
 5 4381 acctgggaac ctttttccct cagttagtgg ggagactagc acctaggtac ccacatgggt  
 4441 atttatatct gaaccagaca gacgcttgaa tcaggcacta tgtaagaaa tatatttatt  
 4501 tgctaataa tttatccaca aa

[00172] *Homo sapiens* catenin beta 1 (CTNNB1), transcript variant 1, mRNA (NCBI

10 Reference Sequence: NM\_001904.4) (SEQ ID NO: 7)

1 aagcctctcg gtctgtggca gcagcgttgg cccggccccg ggagcggaga gcgaggggag  
 61 gcggagacgg aggaaggctc gaggagcagc ttcagtcccc gccgagccgc caccgcaggt  
 121 cgaggacggt cggactcccc cggcggggagg agcctgttcc cctgagggta tttgaagtat  
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 15 241 gttggacatg gccatggaac cagacagaaa agcggctgtt agtcaactggc agcaacagtc  
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 361 aggcaatcct gaggaagagg atgtggatag ctcccaagtc ctgtatgagt ggggaacaggg  
 421 attttctcag tccttccactc aagaacaagt agctgatatt gatggacagt atgcaatgac  
 481 tcgagctcag agggtagcag ctgctatggt ccctgagaca ttagatgagg gcatgcagat  
 20 541 cccatctaca cagtttgatg ctgctcatcc cactaatgtc cagcgttttg ctgaaccatc  
 601 acagatgctg aaacatgcag ttgtaaaact gattaactat caagatgatg cagaacttgc  
 661 cacacgtgca atccctgaac tgacaaaact gctaaatgac gaggaccagg tgggtggttaa  
 721 taaggctgca gttatggtcc atcagcttcc taaaaaggaa gcttccagac acgctatcat  
 781 gcgttctcct cagatggtgt ctgctattgt acgtaccatg cagaatacaa atgatgtaga  
 25 841 aacagctcgt tgtaccgctg ggacotttga taacctttcc catcatcgtg agggcttact  
 901 ggccatcttt aagtctggag gcattctcgc cctggtgaaa atgcttggtt cccagtgga  
 961 ttctgtgttg ttttatgcca ttacaactct ccacaacctt ttattacatc aagaaggagc  
 1021 taaaatggca gtgcgttttag ctggtgggct gcagaaaatg gttgccttgc tcaacaaaac  
 1081 aaatgttaaa ttcttggtta ttacgacaga ctgccttcaa attttagctt atggcaacca  
 30 1141 agaaagcaag ctcatcatalc tggctagtgg tggaccccaa gctttagtaa atataatgag  
 1201 gacctatact tacgaaaaac tactgtggac cacaagcaga gtgctgaagg tgctatctgt  
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 1321 cctgacagat ccaagtcaac gtcttgttca gaactgtctt tggactctca ggaatcttcc  
 1381 agatgctgca actaaacagg aagggatgga aggtctcctt gggactcttg ttcagcttct  
 35 1441 gggttcagat gatataaatg tggtcacctg tgcagctgga attctttcta acctcacttg  
 1501 caataattat aagaacaaga tgatgggtctg ccaagtgggt ggtatagagg ctcttggtgcg  
 1561 tactgtcctt cgggctggtg acaggggaaga catcactgag cctgccatct gtgctcttcg  
 1621 tcatctgacc agccgacacc aagaagcaga gatggcccag aatgcagttc gccttacta  
 1681 tggactacca gttgtggtta agctcttaca cccaccatcc cactggcctc tgataaaggc  
 40 1741 tactgttgga ttgattcgaa atcttgccct ttgtcccgca aatcatgcac ctttgctgta  
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 45 2041 catccaaaga gtagctgcag gggctcctctg tgaacttgct caggacaagg aagctgcaga  
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 55 2641 tggttggtag ggtgggagtg gtttaggcta tttgtaaatc tgccacaaaa acaggtatat  
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 5 3121 cctagccttg cttgttaaat tttttttttt ttttttttaa gaatatctgt aatggtagctg  
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 3361 aataaagtgt aacaattgtg tagccttttt gtataaaata gacaaataga aaatggtcca  
 10 3421 attagtttcc tttttaatat gcttaaaata agcaggtgga tctatttcat gtttttgatc  
 3481 aaaaactatt tgggatatgt atgggtaggg taaatcagta agaggtgtta tttggaacct  
 3541 tgttttggac agtttaccag ttgcctttta tcccaaagtt gttgtaacct gctgtgatac  
 3601 gatgcttcaa gagaaaatgc ggttataaaa aatgggtcag aattaaactt ttaattcatt  
 3661 c

15

**[00173]** *Homo sapiens* axin 2 (AXIN2), mRNA (NCBI Reference Sequence:  
 NM\_004655.4) (SEQ ID NO: 8)

1 gcggctgtga ttggcgcggc gggatcactg gctccgcgag cctggcccgg gggagtcggc  
 61 tggagccggc tgcgctttga taaggtoctg gcaactcagt aacagcccga gagccgggaa  
 20 121 ataaaaataa cccctcagag cgatggattt cggggccgcc cggcgccga ggcgcccgc  
 181 gaaggccctg ctgtaaaaga gaggagggtc agatgagccc ctgctgactt gagagagaca  
 241 gagagaccac gccgattgct gagaggaact ggaagaagaa aaattcccag actcagtggg  
 301 aagagctccc tcacatgag tagcgtatg ttggtgactt gcctcccga cccagcagc  
 361 agcttccgtg aggatgcccc ggggccccca gtgccagggg aagaagggga gaccccaccg  
 25 421 tgtcagccag ggggtggcaa gggccaggtc accaaaccca tgctgtctc ttcccaaccc  
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 541 acccggtgga ccaagtcctt acactcctta ttgggcgatc aagacggtgc ttacctgttc  
 601 cgaactttcc tggagaggga gaaatgcgtg gataccttag acttctgggt tgctgcaat  
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 35 1021 tgtgccgact tcaagtgcaa actttcgcca accgtgggtt gcttgtccag caaaactctg  
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 50 1921 acgcagcggg tgcactgctt ctgccctggg ggcagcagat attactgcta ctcgaaatgc  
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 3241 catccccagt ttatggatat gctgctttta acttggaagg gggagacagg aagttttaat  
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 3361 ggaattatgc tttgcactac gtccctccaa gtgaagacag actgttttag acagactttt  
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 25 3901 ccattgttac ttgtactata catctgtata tagtgtacgg caaaagagta ttaatccact  
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 4021 tgtgtcgccc tatattgagg gctcaagctt tcccttgttt tttgaaaggg gtttatgtat  
 4081 aaatatattt tatgcctttt tattacaagt cttgtactca atgacttttg tcatgacatt  
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 30 4201 gtacaataat tattgttaatt aagagatgta gcctttatta aaattttata tttttcaaaa

**[00174]** *Homo sapiens* APC regulator of WNT signaling pathway (APC), transcript variant 3, mRNA (NCBI Reference Sequence: NM\_000038.6) (SEQ ID NO: 9)

1 actggagaca gaatggaggt gctgccggac tcggaaatgg ggtccaaggg tagccaagga  
 35 61 tggctgcagc ttcatatgat cagttgttaa agcaagttga ggcactgaag atggagaact  
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 241 cttcttctgg acagattgat ttattagagc gtcttaaaaga gcttaactta gatagcagta  
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 481 ggtcattgct tcttgctgat cttgacaaag aagaaaagga aaaagactgg tattacgctc  
 541 aacttcagaa tctcactaaa agaatagata gtcttccttt aactgaaaat ttttccttac  
 601 aaacagatat gaccagaagg caattggaat atgaagcaag gcaaatcaga gttgcatgg  
 45 661 aagaacaact aggtacctgc caggatatgg aaaaacgagc acagcgaaga atagccagaa  
 721 ttcagcaaat cgaaaaggac atacttcgta tacgacagct tttacagtcc caagcaacag  
 781 aagcagagag gtcatctcag aacaagcatg aaaccggctc acatgatgct gagcggcaga  
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 1381 ctgttgaaaca tcagatctgt cctgctgtgt gtgttctaata gaaactttca tttgatgaag  
 1441 agcatagaca tgcaatgaat gaactagggg gactacaggc cattgcagaa ttattgcaag

|    |      |             |             |             |             |             |             |
|----|------|-------------|-------------|-------------|-------------|-------------|-------------|
|    | 1501 | tggactgtga  | aatgtatggg  | cttactaatg  | accactacag  | tattacacta  | agacgatatg  |
|    | 1561 | ctggaatggc  | tttgacaaac  | ttgacttttg  | gagatgtagc  | caacaaggct  | acgctatgct  |
|    | 1621 | ctatgaaagg  | ctgcatgaga  | gcacttgtgg  | cccaactaaa  | atctgaaagt  | gaagacttac  |
|    | 1681 | agcaggttat  | tgcgagtgtt  | ttgaggaatt  | tgtcttggcg  | agcagatgta  | aatagtaaaa  |
| 5  | 1741 | agacgttgcg  | agaagttgga  | agtgtgaaag  | cattgatgga  | atgtgcttta  | gaagttaaaa  |
|    | 1801 | aggaatcaac  | cctcaaaagc  | gtattgagtg  | ccttatggaa  | tttgtcagca  | cattgcactg  |
|    | 1861 | agaataaagc  | tgatatatgt  | gctgtagatg  | gtgcacttgc  | atTTTTggtt  | ggcactctta  |
|    | 1921 | cttaccggag  | ccagacaaac  | acttttagcca | ttattgaaag  | tggaggtggg  | atattacgga  |
|    | 1981 | atgtgtccag  | cttgatagct  | acaaatgagg  | accacaggca  | aatcctaaga  | gagaacaact  |
| 10 | 2041 | gtctacaaac  | tttattacaa  | cacttaaaat  | ctcatagttt  | gacaatagtc  | agtaatgcat  |
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|    | 2161 | tgggggcagt  | tagcatgctc  | aagaacctca  | ttcattcaaa  | gcacaaaatg  | attgctatgg  |
|    | 2221 | gaagtgtctg  | agctttaagg  | aatctcatgg  | caaataggcc  | tgcgaagtac  | aaggatgcca  |
|    | 2281 | atattatgtc  | tcctggctca  | agcttgccat  | ctcttcatgt  | taggaaacaa  | aaagccctag  |
| 15 | 2341 | aagcagaatt  | agatgtctcag | cacttatcag  | aaacttttga  | caatatagac  | aatttaagtc  |
|    | 2401 | ccaaggcatc  | tcctgtagt   | aagcagagac  | acaagcaaag  | tctctatggt  | gattatgttt  |
|    | 2461 | ttgacaccaa  | tcgacatgat  | gataataggt  | cagacaattt  | taatactggc  | aacatgactg  |
|    | 2521 | tcctttcacc  | atatttgaat  | actacagtgt  | taccagctc   | ctcttcatca  | agaggaagct  |
|    | 2581 | tagatagtct  | tcgttctgaa  | aaagatagaa  | gtttggagag  | agaacgcgga  | attggtctag  |
| 20 | 2641 | gcaactacca  | tccagcaaca  | gaaaatccag  | gaacttcttc  | aaagcgaggt  | ttgcagatct  |
|    | 2701 | ccaccactgc  | agcccagatt  | gccaaagtca  | tggagaaggt  | gtcagccatt  | catacctctc  |
|    | 2761 | aggaagacag  | aagttctggg  | tctaccactg  | aattacattg  | tgtgacagat  | gagagaaatg  |
|    | 2821 | cacttagaag  | aagctctgct  | gcccatcac   | attcaaacac  | ttacaatttc  | actaagtcgg  |
|    | 2881 | aaaattcaaa  | taggacatgt  | tctatgcctt  | atgccaaatt  | agaatacaag  | agatcttcaa  |
| 25 | 2941 | atgatagtgt  | aaatagtgtc  | agtagtagtg  | atggttatgg  | taaaagaggt  | caaatgaaac  |
|    | 3001 | cctcgattga  | atcctattct  | gaagatgatg  | aaagtaagtt  | ttgcagttat  | ggtcaatacc  |
|    | 3061 | cagccgacct  | agcccataaa  | atacatagtg  | caaatacatat | ggatgataat  | gatggagaac  |
|    | 3121 | tagatacacc  | aataaattat  | agtctttaa   | attcagatga  | gcagttgaac  | tctggaaggc  |
|    | 3181 | aaagtccttc  | acagaatgaa  | agatgggcaa  | gacccaaaca  | cataatgaa   | taggaaataa  |
| 30 | 3241 | aacaaagtga  | gcaaagacaa  | tcaaggaatc  | aaagtacaac  | ttatcctggt  | tatactgaga  |
|    | 3301 | gcactgtatga | taaacacctc  | aagtccaac   | cacatttttg  | acagcaggaa  | tgtgtttctc  |
|    | 3361 | catacaggtc  | acggggagcc  | aatggttcag  | aaacaaatcg  | agtgggttct  | aatcatggaa  |
|    | 3421 | ttaatcaaaa  | tgtaagccag  | tctttgtgtc  | aagaagatga  | ctatgaagat  | gataagccta  |
|    | 3481 | ccaattatag  | tgaacgttac  | tctgaagaag  | aacagcatga  | agaagaagag  | agaccaacaa  |
| 35 | 3541 | attatagcat  | aaaatataat  | gaagagaaac  | gtcatgtgga  | tcagcctatt  | gattatagtt  |
|    | 3601 | taaaatatgc  | cacagatatt  | ccttcatcac  | agaaacagtc  | atTTTctattc | tcaaagagtt  |
|    | 3661 | catctggaca  | aagcagtaaa  | accgaacata  | tgtcttcaag  | cagtgagaat  | acgtccacac  |
|    | 3721 | cttcatctaa  | tgccaagagg  | cagaatcagc  | tccatccaag  | ttctgcacag  | agtagaagtg  |
|    | 3781 | gtcagcctca  | aaaggctgcc  | acttgcaaag  | tttcttctat  | taaccaagaa  | acaatacaga  |
| 40 | 3841 | cttatttgtgt | agaagatact  | ccaatatggt  | tttcaagatg  | tagttcatta  | tcatctttgt  |
|    | 3901 | catcagctga  | agatgaaata  | ggatgtaatc  | agacgacaca  | ggaagcagat  | tctgctaata  |
|    | 3961 | ccctgcaaat  | agcagaaata  | aaagaaaaga  | ttggaactag  | gtcagctgaa  | gatcctgtga  |
|    | 4021 | gcgaagttcc  | agcagtgtca  | cagcacccta  | gaaccaaatac | cagcagactg  | cagggttcta  |
|    | 4081 | gtttatcttc  | agaatcagcc  | aggcacaag   | ctgttgaaat  | ttcttcagga  | gcgaaatctc  |
| 45 | 4141 | cctccaaaag  | tgggtgtcag  | acacccaaaa  | gtccacctga  | acactatggt  | caggagaccc  |
|    | 4201 | cactcatggt  | tagcagatgt  | acttctgtca  | gttcaattga  | tagtttttag  | agtcgttcga  |
|    | 4261 | ttgccagctc  | cgttcagagt  | gaacctgtca  | gtggaatggt  | aagtggcatt  | ataagcccca  |
|    | 4321 | gtgatcttcc  | agatagccct  | ggacaaacca  | tgccaccaag  | cagaagttaa  | acacctccac  |
|    | 4381 | cacctcctca  | aacagctcaa  | accaagcgag  | aagtaccta   | aaataaagca  | cctactgctg  |
| 50 | 4441 | aaaagagaga  | gagtggaact  | aagcaagctg  | cagtaaatgc  | tgcaagttcag | aggggtccagg |
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|    | 4561 | cttggttcac  | cagcctgagt  | gctctgagcc  | tcgatgagcc  | atTTTatacag | aaagatgtgg  |
|    | 4621 | aattaagaat  | aatgcctcca  | gttcaggaaa  | atgacaatgg  | gaatgaaaca  | gaatcagagc  |
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|    | 4801 | ctgccatgcc  | aacaaagtca  | tcacgtaaag  | caaaaaagcc  | agcccagact  | gcttcaaaat  |
|    | 4861 | tacctccacc  | tgtggcaagg  | aaaccaagtc  | agctgcctgt  | gtacaaactt  | ctaccatcac  |
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 10681 ccataaaciaa tggagataca tgca

**[00175]** *Homo sapiens* APC membrane recruitment protein 1 (AMER1), mRNA (NCBI

35 Reference Sequence: NM\_152424.4) (SEQ ID NO: 10)

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|    | 1561 | gttaggtctt  | atcctggcct  | agcccctggg  | gaacttttga | ctcctcagag  | tgaccagcaa  |
|    | 1621 | gaatccgccc  | ccaatagtga  | tgaaggttat  | tatgactcca | ccacacctgg  | atttgaggat  |
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|    | 1741 | ggagatgccc  | tatatgagtt  | ctatgagcca  | gatgacagcc | ttgagaactc  | tccacctgga  |
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|    | 2461 | aggagggcct  | accctactta  | ttcacccct   | gaagatccag | aggaagagga  | ggttgagaag  |
|    | 2521 | gaagggaatg  | ccactgtgag  | tttctcacag  | gccctggtag | agttcaccag  | caatgggaac  |
| 20 | 2581 | ctcttttcca  | gcatgtcctg  | cagctctgac  | tctgactcat | ctttcactca  | aaacctccct  |
|    | 2641 | gagctgcctc  | ccatggtgac  | ctttgacatc  | gctgatgtgg | aacgggatgg  | ggaaggcaag  |
|    | 2701 | tgtgaagaga  | atcctgagtt  | ccacaatgat  | gaagatcttg | cagcctcctt  | ggaagccttt  |
|    | 2761 | gagctgggct  | actatcacaa  | acatgccttc  | aacaactacc | atagtcgatt  | ctaccaaggc  |
|    | 2821 | ctgccctggg  | gtgtgagcag  | cctccctcga  | tacttgggac | tgectggcct  | gcaccctcga  |
| 25 | 2881 | cctccacctg  | ctgctatggc  | cctcaacagg  | agaagccgct | ctctcgacac  | tgagagagcc  |
|    | 2941 | ctggagatgg  | agctctccaa  | ttcccacttg  | gtccagggct | acctcgagtc  | tgatgagctg  |
|    | 3001 | caggcccagc  | aggaagattc  | agatgaggag  | gacgaagagg | aggaagaagg  | agaatggagc  |
|    | 3061 | cgagacagtc  | ccctttccct  | ctatactgaa  | cccccagggg | cctatgattg  | gcctgcttgg  |
|    | 3121 | gctccctgtc  | ctcttccagt  | ggggccagg   | cctgcctgga | taagcccca   | ccagttggac  |
| 30 | 3181 | aggccttcca  | gccagtctcc  | atataggcag  | gcaacctggt | gcatacctcc  | tatgacctg   |
|    | 3241 | tcaatatcac  | tatcagtgcc  | agagtccaag  | gcacctgggg | aatctgggcc  | tcaactagct  |
|    | 3301 | cgtccctcac  | acctacacct  | gcccattggg  | ccttgctata | acctccagcc  | acaggcctcc  |
|    | 3361 | cagagcatga  | gggccaggcc  | tcgagatgtg  | ctgctgcctg | ttgatgagcc  | cagttgctct  |
|    | 3421 | tccagttctg  | gaggcttcag  | ccccagccct  | ctaccacagg | ccaagcctgt  | gggcatcacc  |
| 35 | 3481 | catggcattc  | ctcagctgcc  | cagggtccgg  | cctgagcacc | cccagcctca  | gcccactcac  |
|    | 3541 | tatgggcctt  | ccagccttga  | cctgtcaaag  | gagagggctg | agcaagggtg  | ctctcttgcc  |
|    | 3601 | accagctact  | cctccactgc  | catgaatgga  | aacctagcca | agtagttatt  | atcaattctg  |
|    | 3661 | gagtagggac  | acggggcctg  | agcatgtgaa  | tggggatcag | gggttgggca  | gaagggtggg  |
|    | 3721 | gggggtgggg  | ttgttgttta  | acttgaaaac  | cctttggggc | aaggaaaact  | cctgtgagtt  |
| 40 | 3781 | ttcaccttgg  | ttttcccaact | tgccctcttct | gccatctatg | gccacgttca  | actcaagtgc  |
|    | 3841 | atctgcccag  | ggaggctgct  | gctgctactg  | cactccagtt | tttgccttctg | aggtttcttc  |
|    | 3901 | ttgtgaccca  | cgctgggttt  | gggatgctgt  | aattttgtgc | ctattccagt  | tgccaagtta  |
|    | 3961 | gtgattatat  | atatatatat  | atatatatat  | atatatatat | atatatatat  | acacacacac  |
|    | 4021 | acacacacac  | acacacacat  | acatacacac  | acacacacaa | acacacatgc  | acacacatat  |
| 45 | 4081 | atagcatgca  | tccttgacac  | aggctaaacc  | tttagtgccc | cctgcccctt  | tcccccaatg  |
|    | 4141 | aatgatgaac  | cactgattag  | cagccaagtc  | tgacattgct | cccgccttgg  | gaagtggggc  |
|    | 4201 | ttatttgcta  | ttagcaatgt  | gaaattatgg  | tacagatcct | ccccaccctc  | attccttttg  |
|    | 4261 | gcctggcata  | gctgccatca  | ttatatcaga  | tgactataag | ctattcatcc  | cagctagctg  |
|    | 4321 | tccttagaga  | ggatcaagta  | gcgtgtgtct  | cacagcctat | cgctgtggac  | tttggcatca  |
| 50 | 4381 | tgaccagatg  | ggggcctttt  | aactgtcctg  | ccatagcttg | ggttaaagac  | aaaaatgcta  |
|    | 4441 | atccccttga  | tggagtgaata | aaattcatcc  | atttgcaaac | agaagacaaa  | acaaggttct  |
|    | 4501 | gttgtggaca  | ctgccataga  | gtgttgagct  | ccggaatgat | aggatttttt  | ttccttcttt  |
|    | 4561 | taagcttgtg  | actacctgag  | ttgcagatgc  | ttttgaattt | ttgttctttt  | ttcctctaaa  |
|    | 4621 | ggaaattttc  | ataatctcat  | cacaaggaac  | acgggtcttt | aattgagata  | agggaagaga  |
| 55 | 4681 | agcaacctaa  | attaggcttg  | actatagggg  | ctgggggttt | tgtggagtgt  | aggggaaaga  |
|    | 4741 | tagaaaatga  | gggtccattt  | atcacagcca  | gcaagaaagg | ggatttcaga  | ccatgcagag  |
|    | 4801 | cttaagggtat | ctcagtttct  | taaaagggaa  | aactcccttc | tcaagcctcc  | caacacaaaa  |
|    | 4861 | tgttcccttaa | gccttgccca  | tcccatcatt  | tggactctgt | aataaggccc  | caggtttgca  |
|    | 4921 | ctcccaagac  | aggataccaa  | aggcatctca  | gctgctgttc | tgggcccttt  | aaagagcttt  |
| 60 | 4981 | tatcttccaa  | ttagccgagt  | ccttagcatc  | atcaaatgac | agggtctctt  | ggcagttgca  |
|    | 5041 | gcccttttatg | gaatgaatgg  | cacctccct   | tctcctgcat | ccatgcagtt  | ttcatgttaa  |

|    |      |             |             |             |             |             |            |
|----|------|-------------|-------------|-------------|-------------|-------------|------------|
|    | 5101 | actaggttgg  | aaatactggt  | ggggaagagc  | ttgctgtcag  | gaagacattg  | gaacaggcta |
|    | 5161 | atcttgccaa  | gctcaacctc  | tcattaatca  | cctgtaggag  | aggggttcctt | ttacatgtct |
|    | 5221 | ttattggaaa  | tggcatatgg  | aacatttttt  | gcttggctgt  | gttcaactcaa | attatgatta |
|    | 5281 | ttgcaccctc  | gccctcacat  | cccaccatct  | ccatgcctgt  | tgagagaaga  | agtcaacagg |
| 5  | 5341 | gttcttgcca  | ttcctgagat  | atggcaatth  | agcttgggaa  | cagatgctgt  | ttgcccccta |
|    | 5401 | agtcaagggtg | actttgtggg  | caaactgtth  | cttggccagg  | ggatgtgtcc  | atgctggtct |
|    | 5461 | ttgtcaccat  | ttctcagatt  | ggggttggcc  | cagtgcctt   | actcacaat   | agcagacctg |
|    | 5521 | gctggtagaa  | gctgcacccc  | tgggaagcacc | acatgaatth  | gcatggcact  | aacaccacca |
|    | 5581 | ctcaagggtg  | ccagaccagg  | tcttcaggct  | gactttggct  | acagatacat  | tgtgaagaaa |
| 10 | 5641 | tgtatctgtt  | gtggagctgg  | agaatgaaag  | aggggtgagga | agggcctcct  | taccccaagc |
|    | 5701 | acagcattcc  | aagagagaag  | tgcccaaatt  | ccaactttct  | ggcctcttac  | caaagctgct |
|    | 5761 | ctgggatggt  | ggggctgaaa  | ttggcctggc  | tatttggcag  | gaagtaaaac  | ctcaccaat  |
|    | 5821 | gcctttacaa  | caggggaagaa | cccacctctt  | gcctcttctt  | tcagggcagc  | ttgagctcct |
|    | 5881 | gtctacatgt  | ggactgtgcc  | agccacagca  | ggcggtagag  | ccatcaccag  | cctccagacc |
| 15 | 5941 | ctgctggaga  | agggccacca  | gcctatctgg  | gaagagaaaa  | aggaggtggc  | caacctttgg |
|    | 6001 | ctaagggtctg | agagctgccc  | tcaggatcag  | gaaggcagca  | tgaggcattg  | tctctgaggg |
|    | 6061 | gcaactggcat | agccctgaca  | cactttgttg  | ggctttggaa  | ggtcacacac  | cacagagtag |
|    | 6121 | ggcctggggc  | tgagtggag   | ttcatggth   | ggacagcctt  | gctgcctgca  | agtcccttca |
|    | 6181 | ctgccctagc  | cagcccagga  | ataggatcct  | taccacctga  | agtggggcag  | ccaggatatg |
| 20 | 6241 | tggctgcaga  | agcaggttcc  | tttgaatth   | tagagctaag  | cagtttgtgg  | ctgaaacca  |
|    | 6301 | atggcagcct  | aggggaggaa  | ggaagacctg  | tctggggacc  | catggtgttg  | acagcacagg |
|    | 6361 | gtgacattgg  | gagagccagc  | caagcttcag  | gcctgttctc  | aaacacagac  | tagtgctggc |
|    | 6421 | ttgggtggca  | gcaaatacac  | cccagaccac  | taaaactgtt  | gatatcatgt  | ccacacctcc |
|    | 6481 | aagagtacta  | ctagatgaac  | aacatgtctc  | cattgcaagg  | tcttggaaata | atgcttttgg |
| 25 | 6541 | atgtgattth  | aagggaata   | gggacttagt  | ggaagtagct  | gccttcatct  | tattacccaa |
|    | 6601 | taggcctagt  | gccagacagc  | tgtgatcctg  | agaattccca  | gatgagactg  | gtccttggag |
|    | 6661 | gaccagtga   | tgtggataga  | gaaggccacc  | tagtaggcct  | ctcccagggc  | caggatgcca |
|    | 6721 | gtttcttgg   | gcttttagag  | ctccaaggaa  | gcactgcctt  | ggggcccagt  | tcaagccagc |
|    | 6781 | cctacctatc  | tggcctcagc  | ttgggtacca  | ggccacaaga  | ctgcccggat  | aatgggttca |
| 30 | 6841 | gtaaacacca  | gtctctctcc  | ctccttagtc  | ctatgccaa   | aagggaagta  | gcttcacaca |
|    | 6901 | ctcaggaggc  | tgggtgtgta  | cccttcatcc  | tgctgccagc  | agctttcctt  | acctgtgcca |
|    | 6961 | gtgccaccac  | cccacctgcc  | ctcagccccc  | agggccagtt  | gctagaggga  | ggcatgggg  |
|    | 7021 | ttagcaggga  | gacttcaagt  | ttacccatgt  | gtatgttgca  | ttcaagttgt  | ttttaatata |
|    | 7081 | tatatatata  | tatatcaaaa  | gtcaattatc  | tatttttata  | ttgtttgcat  | tttatattca |
| 35 | 7141 | tggaaaacaa  | tgtttatcaa  | ttgttctth   | ctgatttatt  | tttaagcgg   | gctgtttaca |
|    | 7201 | gttttaaa    | aaacaaagat  | gacacaaaa   | ttgctgctgc  | ttgtgcagag  | ggctggcatc |
|    | 7261 | tcaactgcct  | agggccctcc  | ctctttcccc  | tctccctacc  | ttagctgtac  | cacactgtcc |
|    | 7321 | gttccctccc  | cccatgatag  | ggacagcaga  | agatgccag   | gtactgaaga  | caaacgccaa |
|    | 7381 | gtgactcagg  | cgtgtgggac  | ctggtagaca  | gcttgtcatt  | ttctggctth  | atthttatgt |
| 40 | 7441 | tttctttctt  | ttgaacctt   | cttccccatg  | tctagacct   | ctgagagtac  | tcatgtgtct |
|    | 7501 | tattttttth  | cctcgttatg  | ttttgtgctt  | tagttttgaa  | atcagagtgc  | ccatgttgtg |
|    | 7561 | ctatttgtct  | tgttgagth   | ctccaaagga  | gcattttggg  | aacattctcc  | acccaatggg |
|    | 7621 | gaaggaagg   | ggtttcaacc  | atgtccctt   | tccccaagaa  | gctgtgggag  | acagatgaca |
|    | 7681 | atgatgcttg  | cactatcagg  | aatttaccat  | ctcgttgagg  | aaacccttgc  | ataggacttc |
| 45 | 7741 | ctaggagctc  | agttgattgt  | gacattttcc  | cagaagagag  | gctttgggga  | actccttcac |
|    | 7801 | ccctcttgct  | gctttggggg  | aatctctctc  | ctaccacca   | ttccccctcc  | cctgccttgc |
|    | 7861 | tccagctcac  | acgtggatgg  | tactttgggg  | aacagtgaact | ttcagggtga  | tggcttgctg |
|    | 7921 | gcctgatccc  | tgactttcca  | atcctatagt  | cactccaaag  | aagaaaatcc  | acatggtatg |
|    | 7981 | tatatgtgtg  | agggttgggt  | ctattgagag  | gtgggactth  | aattattgtt  | attattattg |
| 50 | 8041 | gtgcttattt  | ttcctgctgt  | atatgggggt  | gggggtgggg  | gggaataaaa  | caggaatgta |
|    | 8101 | tatttaggtc  | atgttaata   | aaatggctgg  | aggaaggagg  | gaagaaatta  | ggaactgcag |
|    | 8161 | tacatcatca  | ccccagggtg  | aagagttatt  | tctctcccag  | tgcaaaaagg  | ggagtgtggc |
|    | 8221 | ccctggctgt  | acctgagctt  | cctcttctcc  | atccctgaga  | catcacagaa  | tcacccgggt |
|    | 8281 | tctgacctct  | ccacttctag  | ctctcactga  | catctggata  | tcttgatgca  | ctgctgtth  |
| 55 | 8341 | gtaacaacat  | atacttctth  | gctgaatgct  | aataaacttc  | tgggttcaag  | aggaacccc  |
|    | 8401 | tcagtca     |             |             |             |             |            |

[00176] Primers or probes can be designed so that they hybridize under stringent conditions to mutant nucleotide sequences of at least one of CDKN2A, NF2, LATS1, FAT1,

RNF43, CTNNB1, AXIN2, APC and AMER1, but not to the respective wild-type nucleotide sequences. Primers or probes can also be prepared that are complementary and specific for the wild-type nucleotide sequence of at least one of CDKN2A, NF2, LATS1, FAT1, RNF43, CTNNB1, AXIN2, APC and AMER1, but not to any one of the corresponding mutant nucleotide sequences. In some embodiments, the mutant nucleotide sequences of at least one of CDKN2A, NF2, LATS1, FAT1, RNF43, AXIN2, APC and AMER1 may be a frameshift mutation, a missense mutation, a deletion, an insertion, a nonsense mutation, an inversion, or a translocation, that results in the loss of expression and/or activity of at least one of CDKN2A, NF2, LATS1, FAT1, RNF43, AXIN2, APC and AMER1 (*i.e.*, loss of function mutations). Alternatively, primers or probes can be designed so that they selectively hybridize to CTNNB1. In some embodiments, the mutant nucleotide sequences of CTNNB1 include mutations in the N-terminal  $\beta$ -TrCP binding motif of CTNNB1 that results in constitutive translocation of  $\beta$ -catenin to the nucleus (*i.e.*, gain of function mutations).

**[00177]** In some embodiments, detection can occur through any of a variety of mobility dependent analytical techniques based on the differential rates of migration between different nucleic acid sequences. Exemplary mobility-dependent analysis techniques include electrophoresis, chromatography, mass spectroscopy, sedimentation, gradient centrifugation, field-flow fractionation, multi-stage extraction techniques, and the like. In some embodiments, mobility probes can be hybridized to amplification products, and the identity of the target nucleic acid sequence determined *via* a mobility dependent analysis technique of the eluted mobility probes, as described in Published PCT Applications WO04/46344 and WO01/92579. In some embodiments, detection can be achieved by various microarrays and related software such as the Applied Biosystems Array System with the Applied Biosystems 1700 Chemiluminescent Microarray Analyzer and other commercially available array systems available from Affymetrix, Agilent, Illumina, and Amersham Biosciences, among others (see also Gerry *et al.*, *J. Mol. Biol.* 292:251-62, 1999; De Bellis *et al.*, *Minerva Biotec* 14:247-52, 2002; and Stears *et al.*, *Nat. Med.* 9:14045, including supplements, 2003).

**[00178]** It is also understood that detection can comprise reporter groups that are incorporated into the reaction products, either as part of labeled primers or due to the incorporation of labeled dNTPs during an amplification, or attached to reaction products, for example but not limited to, *via* hybridization tag complements comprising reporter groups or *via* linker arms that are integral or attached to reaction products. In some embodiments, unlabeled reaction products may be detected using mass spectrometry.

**NGS Platforms**

[00179] In some embodiments, high throughput, massively parallel sequencing employs sequencing-by-synthesis with reversible dye terminators. In other embodiments, sequencing is performed *via* sequencing-by-ligation. In yet other embodiments, sequencing is single molecule sequencing. Examples of Next Generation Sequencing techniques include, but are not limited to pyrosequencing, Reversible dye-terminator sequencing, SOLiD sequencing, Ion semiconductor sequencing, Helioscope single molecule sequencing *etc.*

[00180] The Ion Torrent<sup>TM</sup> (Life Technologies, Carlsbad, CA) amplicon sequencing system employs a flow-based approach that detects pH changes caused by the release of hydrogen ions during incorporation of unmodified nucleotides in DNA replication. For use with this system, a sequencing library is initially produced by generating DNA fragments flanked by sequencing adapters. In some embodiments, these fragments can be clonally amplified on particles by emulsion PCR. The particles with the amplified template are then placed in a silicon semiconductor sequencing chip. During replication, the chip is flooded with one nucleotide after another, and if a nucleotide complements the DNA molecule in a particular microwell of the chip, then it will be incorporated. A proton is naturally released when a nucleotide is incorporated by the polymerase in the DNA molecule, resulting in a detectable local change of pH. The pH of the solution then changes in that well and is detected by the ion sensor. If homopolymer repeats are present in the template sequence, multiple nucleotides will be incorporated in a single cycle. This leads to a corresponding number of released hydrogens and a proportionally higher electronic signal.

[00181] The 454<sup>TM</sup> GS FLX<sup>TM</sup> sequencing system (Roche, Germany), employs a light-based detection methodology in a large-scale parallel pyrosequencing system. Pyrosequencing uses DNA polymerization, adding one nucleotide species at a time and detecting and quantifying the number of nucleotides added to a given location through the light emitted by the release of attached pyrophosphates. For use with the 454<sup>TM</sup> system, adapter-ligated DNA fragments are fixed to small DNA-capture beads in a water-in-oil emulsion and amplified by PCR (emulsion PCR). Each DNA-bound bead is placed into a well on a picotiter plate and sequencing reagents are delivered across the wells of the plate. The four DNA nucleotides are added sequentially in a fixed order across the picotiter plate device during a sequencing run. During the nucleotide flow, millions of copies of DNA bound to each of the beads are sequenced in parallel. When a nucleotide complementary to

the template strand is added to a well, the nucleotide is incorporated onto the existing DNA strand, generating a light signal that is recorded by a CCD camera in the instrument.

**[00182]** Sequencing technology based on reversible dye-terminators: DNA molecules are first attached to primers on a slide and amplified so that local clonal colonies are formed. Four types of reversible terminator bases (RT-bases) are added, and non-incorporated nucleotides are washed away. Unlike pyrosequencing, the DNA can only be extended one nucleotide at a time. A camera takes images of the fluorescently labeled nucleotides, then the dye along with the terminal 3' blocker is chemically removed from the DNA, allowing the next cycle.

**[00183]** Helicos's single-molecule sequencing uses DNA fragments with added polyA tail adapters, which are attached to the flow cell surface. At each cycle, DNA polymerase and a single species of fluorescently labeled nucleotide are added, resulting in template-dependent extension of the surface-immobilized primer-template duplexes. The reads are performed by the Helioscope sequencer. After acquisition of images tiling the full array, chemical cleavage and release of the fluorescent label permits the subsequent cycle of extension and imaging.

**[00184]** Sequencing by synthesis (SBS), like the "old style" dye-termination electrophoretic sequencing, relies on incorporation of nucleotides by a DNA polymerase to determine the base sequence. A DNA library with affixed adapters is denatured into single strands and grafted to a flow cell, followed by bridge amplification to form a high-density array of spots onto a glass chip. Reversible terminator methods use reversible versions of dye-terminators, adding one nucleotide at a time, detecting fluorescence at each position by repeated removal of the blocking group to allow polymerization of another nucleotide. The signal of nucleotide incorporation can vary with fluorescently labeled nucleotides, phosphate-driven light reactions and hydrogen ion sensing having all been used. Examples of SBS platforms include Illumina GA and HiSeq 2000. The MiSeq® personal sequencing system (Illumina, Inc.) also employs sequencing by synthesis with reversible terminator chemistry.

**[00185]** In contrast to the sequencing by synthesis method, the sequencing by ligation method uses a DNA ligase to determine the target sequence. This sequencing method relies on enzymatic ligation of oligonucleotides that are adjacent through local complementarity on a template DNA strand. This technology employs a partition of all possible oligonucleotides of a fixed length, labeled according to the sequenced position. Oligonucleotides are annealed and ligated and the preferential ligation by DNA ligase for matching sequences results in a

dinucleotide encoded color space signal at that position (through the release of a fluorescently labeled probe that corresponds to a known nucleotide at a known position along the oligo). This method is primarily used by Life Technologies' SOLiD™ sequencers. Before sequencing, the DNA is amplified by emulsion PCR. The resulting beads, each containing  
5 only copies of the same DNA molecule, are deposited on a solid planar substrate.

[00186] SMRT™ sequencing is based on the sequencing by synthesis approach. The DNA is synthesized in zero-mode wave-guides (ZMWs)-small well-like containers with the capturing tools located at the bottom of the well. The sequencing is performed with use of unmodified polymerase (attached to the ZMW bottom) and fluorescently labeled nucleotides  
10 flowing freely in the solution. The wells are constructed in a way that only the fluorescence occurring at the bottom of the well is detected. The fluorescent label is detached from the nucleotide at its incorporation into the DNA strand, leaving an unmodified DNA strand.

#### **Methods for Selecting Intraoperative Analgesic Regimen in Lung Cancer Patients Undergoing Tumor Resection Surgery**

[00187] In one aspect, the present disclosure provides a method for selecting a cancer patient undergoing tumor resection surgery for lung cancer for treatment with an intraoperative opioid analgesic comprising (a) detecting the presence of at least one mutation in one or more genes that results (i) in hyperactivation of Wnt pathway and/or (ii) reduced activity of the Hippo pathway in a biological sample obtained from the cancer patient; and  
20 (b) administering to the cancer patient an effective amount of an intraoperative opioid analgesic during the tumor resection surgery. The one or more genes may be selected from the group consisting of NF2, LATS1, FAT1, RNF43, CTNNB1, AXIN2, APC and AMER1. Additionally or alternatively, in some embodiments, the at least one mutation is a frameshift mutation, a missense mutation, a deletion, an insertion, a nonsense mutation, an inversion, or  
25 a translocation. The at least one mutation may be detected using any nucleic acid detection assay known in the art such as next-generation sequencing, PCR, real-time quantitative PCR (qPCR), digital PCR (dPCR), Southern blotting, Reverse transcriptase-PCR (RT-PCR), Northern blotting, microarray, dot or slot blots, *in situ* hybridization, or fluorescent *in situ* hybridization (FISH). In some embodiments, the biological sample comprises genomic  
30 DNA, cDNA, RNA, and/or mRNA.

[00188] In one aspect, the present disclosure provides a method for prolonging survival of a cancer patient undergoing tumor resection surgery for lung cancer comprising administering

to the cancer patient an effective amount of an intraoperative opioid analgesic during the tumor resection surgery, wherein mRNA or polypeptide expression and/or activity levels of one or more of NF2, LATS1, FAT1, RNF43, AXIN2, APC and AMER1 in a biological sample obtained from the cancer patient are reduced compared to that observed in a control sample obtained from a healthy subject or a predetermined threshold. In another aspect, the present disclosure provides a method for prolonging survival of a cancer patient undergoing tumor resection surgery for lung cancer comprising administering to the cancer patient an effective amount of an intraoperative opioid analgesic during the tumor resection surgery, wherein mRNA or polypeptide expression and/or activity levels of CTNNB1 in a biological sample obtained from the cancer patient are elevated compared to that observed in a control sample obtained from a healthy subject or a predetermined threshold. Additionally or alternatively, in some embodiments, mRNA expression levels are detected via real-time quantitative PCR (qPCR), digital PCR (dPCR), Reverse transcriptase-PCR (RT-PCR), Northern blotting, microarray, dot or slot blots, *in situ* hybridization, or fluorescent *in situ* hybridization (FISH). Additionally or alternatively, in certain embodiments, polypeptide expression levels are detected via Western blotting, enzyme-linked immunosorbent assays (ELISA), dot blotting, immunohistochemistry, immunofluorescence, immunoprecipitation, immunoelectrophoresis, or mass-spectrometry.

**[00189]** In any of the preceding embodiments of methods disclosed herein, the intraoperative opioid analgesic is fentanyl, hydromorphone, morphine, oxycodone, hydrocodone, codeine, meperidine, remifentanyl, or sufentanyl. The effective amount of the intraoperative opioid analgesic may range from about 1 MME to about 200 MMEs. In certain embodiments, the effective amount of the intraoperative opioid analgesic is about 1 MME to about 20 MMEs, about 20 MMEs to about 45 MMEs, or about 45 MMEs to about 200 MMEs. In some embodiments, the effective amount of the intraoperative opioid analgesic is about 1 MME, about 2 MMEs, about 3 MMEs, about 4 MMEs, about 5 MMEs, about 6 MMEs, about 7 MMEs, about 8 MMEs, about 9 MMEs, about 10 MMEs, about 11 MMEs, about 12 MMEs, about 13 MMEs, about 14 MMEs, about 15 MMEs, about 16 MMEs, about 17 MMEs, about 18 MMEs, about 19 MMEs, about 20 MMEs, about 21 MMEs, about 22 MMEs, about 23 MMEs, about 24 MMEs, about 25 MMEs, about 26 MMEs, about 27 MMEs, about 28 MMEs, about 29 MMEs, about 30 MMEs, about 31 MMEs, about 32 MMEs, about 33 MMEs, about 34 MMEs, about 35 MMEs, about 36 MMEs, about 37 MMEs, about 38 MMEs, about 39 MMEs, about 40-45 MMEs, about 45-50 MMEs, about 50-55 MMEs, about

55-60 MMEs, about 60-65 MMEs, about 65-70 MMEs, about 70-75 MMEs, about 75-80 MMEs, about 80-85 MMEs, about 85-90 MMEs, about 90-95 MMEs, about 95-100 MMEs, about 100-110 MMEs, about 110-120 MMEs, about 120-130 MMEs, about 130-140 MMEs, about 140-150 MMEs, about 150-160 MMEs, about 160-170 MMEs, about 170-180 MMEs, about 180-190 MMEs, or about 190-200 MMEs. Additionally or alternatively, in some embodiments, the effective amount of the intraoperative opioid analgesic is administered as a series of bolus doses or as a continuous infusion during the tumor resection surgery. In certain embodiments, the effective amount of the intraoperative opioid analgesic is administered to the cancer patient prior to incision. Additionally or alternatively, in some  
10 embodiments, the effective amount of the intraoperative opioid analgesic is administered intravenously.

**[00190]** Additionally or alternatively, in some embodiments, the methods of the present technology further comprise administering to the cancer patient an effective amount of a local anesthetic solution via an epidural catheter before, during and/or after the tumor resection  
15 surgery. The effective amount of the local anesthetic solution may range from about 0.05%-4% local anesthetic solution in a volume of 1-10 ml per hour when administered via an epidural catheter. In some embodiments, the effective amount of the local anesthetic solution is about 0.05 %, about 0.06 %, about 0.07 %, about 0.08 %, about 0.09 %, about 0.1 %, about 0.15 %, about 0.2 %, about 0.25 %, about 0.3 %, about 0.35 %, about 0.4 %, about 0.45 %, about 0.5 %, about 0.55 %, about 0.6 %, about 0.65 %, about 0.7 %, about 0.75 %, about 0.8  
20 %, about 0.85 %, about 0.9 %, about 0.95 %, about 1.0 %, about 1.1 %, about 1.2 %, about 1.3 %, about 1.4 %, about 1.5 %, about 1.6 %, about 1.7 %, about 1.8 %, about 1.9 %, about 2.0 %, about 2.1 %, about 2.2 %, about 2.3 %, about 2.4 %, about 2.5 %, about 2.6 %, about 2.7 %, about 2.8 %, about 2.9 %, about 3.0 %, about 3.1 %, about 3.2 %, about 3.3 %, about 3.4 %, about 3.5 %, about 3.6 %, about 3.7 %, about 3.8 %, about 3.9 %, or about 4.0 % local  
25 anesthetic solution in a volume of about 1 ml per hour, about 1.5 ml per hour, about 2 ml per hour, about 2.5 ml per hour, about 3 ml per hour, about 3.5 ml per hour, about 4 ml per hour, about 4.5 ml per hour, about 5 ml per hour, about 5.5 ml per hour, about 6 ml per hour, about 6.5 ml per hour, about 7 ml per hour, about 7.5 ml per hour, about 8 ml per hour, about 8.5 ml per hour, about 9 ml per hour, about 9.5 ml per hour, or about 10 ml per hour when  
30 administered via an epidural catheter. Additionally or alternatively, in some embodiments, the effective amount of the local anesthetic solution is administered as a single injection, a series of bolus doses or as a continuous infusion during the tumor resection surgery.

Examples of suitable local anesthetics include, but are not limited to, lidocaine, mepivacaine, prilocaine, bupivacaine, etidocaine, ropivacaine, levobupivacaine, cocaine, procaine, tetracaine, chlorprocaine, and benzocaine.

**[00191]** In other embodiments, the effective amount of the local anesthetic solution may be administered before, during and/or after the tumor resection surgery using any regional anesthesia technique directed at nerves innervating the thorax and chest wall (*e.g.*, via serratus plane nerve block, intercostal nerve block, or paravertebral block). The effective amount of the local anesthetic solution may range from about 0.05%-4% local anesthetic solution in a volume of 10-40 ml when administered using any regional anesthesia technique directed at nerves innervating the thorax and chest wall (*e.g.*, via serratus plane nerve block, intercostal nerve block, or paravertebral block). In some embodiments, the effective amount of the local anesthetic solution is about 0.05 %, about 0.06 %, about 0.07 %, about 0.08 %, about 0.09 %, about 0.1 %, about 0.15 %, about 0.2 %, about 0.25 %, about 0.3 %, about 0.35 %, about 0.4 %, about 0.45 %, about 0.5 %, about 0.55 %, about 0.6 %, about 0.65 %, about 0.7 %, about 0.75 %, about 0.8 %, about 0.85 %, about 0.9 %, about 0.95 %, about 1.0 %, about 1.1 %, about 1.2 %, about 1.3 %, about 1.4 %, about 1.5 %, about 1.6 %, about 1.7 %, about 1.8 %, about 1.9 %, about 2.0 %, about 2.1 %, about 2.2 %, about 2.3 %, about 2.4 %, about 2.5 %, about 2.6 %, about 2.7 %, about 2.8 %, about 2.9 %, about 3.0 %, about 3.1 %, about 3.2 %, about 3.3 %, about 3.4 %, about 3.5 %, about 3.6 %, about 3.7 %, about 3.8 %, about 3.9 %, or about 4.0 % local anesthetic solution in a volume of about 10 ml, about 12.5 ml, about 15 ml, about 17.5 ml, about 20 ml, about 22.5 ml, about 25 ml, about 27.5 ml, about 30 ml, about 32.5 ml, about 35 ml, about 37.5 ml, or about 40 ml when administered using any regional anesthesia technique directed at nerves innervating the thorax and chest wall (*e.g.*, via serratus plane nerve block, intercostal nerve block, or paravertebral block). Examples of suitable local anesthetics include, but are not limited to, lidocaine, mepivacaine, prilocaine, bupivacaine, etidocaine, ropivacaine, levobupivacaine, cocaine, procaine, tetracaine, chlorprocaine, and benzocaine.

**[00192]** Additionally or alternatively, in certain embodiments, the local anesthetic solution may further comprise an opioid (*e.g.*, fentanyl, hydromorphone, morphine, oxycodone, hydrocodone, codeine, meperidine, remifentanyl, or sufentanyl). In some embodiments, the local anesthetic solution may comprise 0.5 mcg/ml-50 mcg/ml opioid. In certain embodiments, the local anesthetic solution may comprise about 0.5 mcg/ml, about 0.6 mcg/ml, about 0.7 mcg/ml, about 0.8 mcg/ml, about 0.9 mcg/ml, about 1.0 mcg/ml, about 1.5

mcg/ml, about 2.0 mcg/ml, about 2.5 mcg/ml, about 3.0 mcg/ml, about 3.5 mcg/ml, about 4.0 mcg/ml, about 4.5 mcg/ml, about 5.0 mcg/ml, about 5.5 mcg/ml, about 6.0 mcg/ml, about 6.5 mcg/ml, about 7.0 mcg/ml, about 7.5 mcg/ml, about 8.0 mcg/ml, about 8.5 mcg/ml, about 9.0 mcg/ml, about 10 mcg/ml, about 15 mcg/ml, about 20 mcg/ml, about 25 mcg/ml, about 30 mcg/ml, about 35 mcg/ml, about 40 mcg/ml, about 45 mcg/ml, or about 50 mcg/ml.

**[00193]** Additionally or alternatively, in certain embodiments, the methods of the present technology further comprise administering to the cancer patient an effective amount of a post-operative opioid analgesic after the tumor resection surgery. Examples of post-operative opioid analgesics include, but are not limited to, fentanyl, hydromorphone, morphine, oxycodone, hydrocodone, codeine, meperidine, remifentanyl, or sufentanyl. In some embodiments, the post-operative opioid analgesic and the intraoperative opioid analgesic are the same opioid analgesic or different opioid analgesics. In other embodiments, the effective amount of the post-operative opioid analgesic and the effective amount of the intraoperative opioid analgesic are the same or different. Additionally or alternatively, in some embodiments, the effective amount of the post-operative opioid analgesic is administered to the cancer patient as a bolus of about 0.005 mg to about 100 mg. In some embodiments, the effective amount of the post-operative opioid analgesic is administered to the cancer patient as a bolus of about 0.005 mg, about 0.006 mg, about 0.007 mg, about 0.008 mg, about 0.009 mg, about 0.01 mg, about 0.02 mg, about 0.03 mg, about 0.04 mg, about 0.05 mg, about 0.06 mg, about 0.07 mg, about 0.08 mg, about 0.09 mg, about 0.1 mg, about 0.2 mg, about 0.3 mg, about 0.4 mg, about 0.5 mg, about 0.6 mg, about 0.7 mg, about 0.8 mg, about 0.9 mg, about 1-5 mg, about 5-10 mg, about 1-5 mg, about 5-10 mg, about 1-5 mg, about 5-10 mg, about 1-5 mg, about 5-10 mg, about 10-15 mg, about 15-20 mg, about 20-25 mg, about 25-30 mg, about 30-35 mg, about 35-40 mg, about 40-45 mg, about 45-50 mg, about 50-55 mg, about 55-60 mg, about 60-65 mg, about 65-70 mg, about 70-75 mg, about 75-80 mg, about 80-85 mg, about 85-90 mg, about 90-95 mg, or about 95-100 mg. In other embodiments, the effective amount of the post-operative opioid analgesic may be continuously delivered to the cancer patient at a per hour rate of about 0.01 mg/hr to about 10 mg/hr. In certain embodiments, the effective amount of the post-operative opioid analgesic is continuously delivered to the cancer patient at a per hour rate of about 0.01 mg/hr, about 0.02 mg/hr, about 0.03 mg/hr, about 0.04 mg/hr, about 0.05 mg/hr, about 0.06 mg/hr, about 0.07 mg/hr, about 0.08 mg/hr, about 0.09 mg/hr, about 0.1 mg/hr, about 0.2 mg/hr, about 0.3 mg/hr, about 0.4 mg/hr, about 0.5 mg/hr, about 0.6 mg/hr, about 0.7 mg/hr, about 0.8 mg/hr, about 0.9 mg/hr,

about 1 mg/hr, about 1.5 mg/hr, about 2 mg/hr, about 2.5 mg/hr, about 3 mg/hr, about 3.5 mg/hr, about 4 mg/hr, about 4.5 mg/hr, about 5 mg/hr, about 5.5 mg/hr, about 6 mg/hr, about 6.5 mg/hr, about 7 mg/hr, about 7.5 mg/hr, about 8 mg/hr, about 8.5 mg/hr, about 9 mg/hr, about 9.5 mg/hr, or about 10 mg/hr. Additionally or alternatively, in some embodiments, the effective amount of the post-operative opioid analgesic is administered intravenously, orally, or transdermally.

**[00194]** In one aspect, the present disclosure provides a method for selecting a cancer patient undergoing tumor resection surgery for lung cancer for treatment with an opioid-free intraoperative analgesic comprising (a) (i) detecting the presence of at least one mutation in CDKN2A in a biological sample obtained from the cancer patient, wherein the at least one mutation reduces CDKN2A expression and/or activity levels, and/or (ii) detecting elevated FGA in a biological sample obtained from the cancer patient compared to a control sample obtained from a healthy subject or a predetermined threshold; and (b) administering to the cancer patient an effective amount of an opioid-free intraoperative analgesic during the tumor resection surgery. The at least one mutation in CDKN2A may be a frameshift mutation, a missense mutation, a deletion, an insertion, a nonsense mutation, an inversion, or a translocation. The at least one mutation may be detected using any nucleic acid detection assay known in the art such as next-generation sequencing, PCR, real-time quantitative PCR (qPCR), digital PCR (dPCR), Southern blotting, Reverse transcriptase-PCR (RT-PCR), Northern blotting, microarray, dot or slot blots, *in situ* hybridization, or fluorescent *in situ* hybridization (FISH). In some embodiments, the biological sample comprises genomic DNA, cDNA, RNA, and/or mRNA. Additionally or alternatively, in some embodiments, FGA is detected via next-generation sequencing.

**[00195]** In one aspect, the present disclosure provides a method for prolonging survival of a cancer patient undergoing tumor resection surgery for lung cancer comprising administering to the cancer patient an effective amount of an opioid-free intraoperative analgesic during the tumor resection surgery, wherein mRNA or polypeptide expression and/or activity levels of CDKN2A in a biological sample obtained from the cancer patient are reduced compared to that observed in a control sample obtained from a healthy subject or a predetermined threshold. Additionally or alternatively, in some embodiments, mRNA expression levels are detected via real-time quantitative PCR (qPCR), digital PCR (dPCR), Reverse transcriptase-PCR (RT-PCR), Northern blotting, microarray, dot or slot blots, *in situ* hybridization, or fluorescent *in situ* hybridization (FISH). Additionally or alternatively, in certain

embodiments, polypeptide expression levels are detected via Western blotting, enzyme-linked immunosorbent assays (ELISA), dot blotting, immunohistochemistry, immunofluorescence, immunoprecipitation, immunoelectrophoresis, or mass-spectrometry. In another aspect, the present disclosure provides a method for prolonging survival of a cancer patient undergoing tumor resection surgery for lung cancer comprising administering to the cancer patient an effective amount of an opioid-free intraoperative analgesic during the tumor resection surgery, wherein FGA in a biological sample obtained from the cancer patient is elevated compared to that observed in a control sample obtained from a healthy subject or a predetermined threshold. In some embodiments, FGA is detected via next-generation sequencing.

**[00196]** Additionally or alternatively, in some embodiments of the methods disclosed herein, the opioid-free intraoperative analgesic is an amide-type local anesthetic or an ester-type local anesthetic. Examples of amide-type local anesthetics include, but are not limited to, lidocaine, mepivacaine, prilocaine, bupivacaine, etidocaine, ropivacaine, or levobupivacaine. Examples of ester-type local anesthetics include, but are not limited to, cocaine, procaine, tetracaine, chlorprocaine, or benzocaine. The opioid-free intraoperative analgesic may be administered via an epidural catheter. Additionally or alternatively, in some embodiments of the methods disclosed herein, the effective amount of the opioid-free intraoperative analgesic is about 0.05%-4% amide-type or ester-type local anesthetic solution in a volume of 1-10 ml per hour when administered via an epidural catheter. In certain embodiments, the effective amount of the opioid-free intraoperative analgesic is about 0.05 %, about 0.06 %, about 0.07 %, about 0.08 %, about 0.09 %, about 0.1 %, about 0.15 %, about 0.2 %, about 0.25 %, about 0.3 %, about 0.35 %, about 0.4 %, about 0.45 %, about 0.5 %, about 0.55 %, about 0.6 %, about 0.65 %, about 0.7 %, about 0.75 %, about 0.8 %, about 0.85 %, about 0.9 %, about 0.95 %, about 1.0 %, about 1.1 %, about 1.2 %, about 1.3 %, about 1.4 %, about 1.5 %, about 1.6 %, about 1.7 %, about 1.8 %, about 1.9 %, about 2.0 %, about 2.1 %, about 2.2 %, about 2.3 %, about 2.4 %, about 2.5 %, about 2.6 %, about 2.7 %, about 2.8 %, about 2.9 %, about 3.0 %, about 3.1 %, about 3.2 %, about 3.3 %, about 3.4 %, about 3.5 %, about 3.6 %, about 3.7 %, about 3.8 %, about 3.9 %, or about 4.0 % amide-type or ester-type local anesthetic solution in a volume of about 1 ml per hour, about 1.5 ml per hour, about 2 ml per hour, about 2.5 ml per hour, about 3 ml per hour, about 3.5 ml per hour, about 4 ml per hour, about 4.5 ml per hour, about 5 ml per hour, about 5.5 ml per hour, about 6 ml per hour, about 6.5 ml per hour, about 7 ml per hour, about 7.5 ml per hour, about 8 ml

per hour, about 8.5 ml per hour, about 9 ml per hour, about 9.5 ml per hour, or about 10 ml per hour when administered via an epidural catheter. In certain embodiments, the effective amount of the opioid-free intraoperative analgesic may be administered as a single injection, a series of bolus doses, or as a continuous infusion during the tumor resection surgery.

**[00197]** In certain embodiments, the opioid-free intraoperative analgesic may be administered via any regional anesthesia technique directed at nerves innervating the thorax and chest wall, such as via a serratus plane nerve block, or via an intercostal nerve block, or via a paravertebral block. In some embodiments, the effective amount of the opioid-free intraoperative analgesic is about 0.05%-4% amide-type or ester-type local anesthetic solution in a volume of 10-40 ml when administered via any regional anesthesia technique directed at nerves innervating the thorax and chest wall, such as via a serratus plane nerve block, or via an intercostal nerve block, or via a paravertebral block. In some embodiments, the effective amount of the opioid-free intraoperative analgesic is about 0.05 %, about 0.06 %, about 0.07 %, about 0.08 %, about 0.09 %, about 0.1 %, about 0.15 %, about 0.2 %, about 0.25 %, about 0.3 %, about 0.35 %, about 0.4 %, about 0.45 %, about 0.5 %, about 0.55 %, about 0.6 %, about 0.65 %, about 0.7 %, about 0.75 %, about 0.8 %, about 0.85 %, about 0.9 %, about 0.95 %, about 1.0 %, about 1.1 %, about 1.2 %, about 1.3 %, about 1.4 %, about 1.5 %, about 1.6 %, about 1.7 %, about 1.8 %, about 1.9 %, about 2.0 %, about 2.1 %, about 2.2 %, about 2.3 %, about 2.4 %, about 2.5 %, about 2.6 %, about 2.7 %, about 2.8 %, about 2.9 %, about 3.0 %, about 3.1 %, about 3.2 %, about 3.3 %, about 3.4 %, about 3.5 %, about 3.6 %, about 3.7 %, about 3.8 %, about 3.9 %, or about 4.0 % amide-type or ester-type local anesthetic solution in a volume of about 10 ml, about 12.5 ml, about 15 ml, about 17.5 ml, about 20 ml, about 22.5 ml, about 25 ml, about 27.5 ml, about 30 ml, about 32.5 ml, about 35 ml, about 37.5 ml, or about 40 ml when administered via any regional anesthesia technique directed at nerves innervating the thorax and chest wall, such as via a serratus plane nerve block, or via an intercostal nerve block, or via a paravertebral block.

**[00198]** Additionally or alternatively, in some embodiments, the methods of the present technology further comprise administering to the cancer patient an effective amount of an opioid-free post-operative analgesic after the tumor resection surgery. Examples of suitable opioid-free post-operative analgesics include, but are not limited to, lidocaine, mepivacaine, prilocaine, bupivacaine, etidocaine, ropivacaine, levobupivacaine, cocaine, procaine, tetracaine, chlorprocaine, and benzocaine. In some embodiments, the opioid-free post-operative analgesic and the opioid-free intraoperative analgesic are the same analgesic or

different analgesics. In other embodiments, the effective amount of the opioid-free post-operative analgesic and the effective amount of the opioid-free intraoperative analgesic are the same or different. In some embodiments, the effective amount of the opioid-free post-operative analgesic is about 0.05 %, about 0.06 %, about 0.07 %, about 0.08 %, about 0.09  
5 %, about 0.1 %, about 0.15 %, about 0.2 %, about 0.25 %, about 0.3 %, about 0.35 %, about 0.4 %, about 0.45 %, about 0.5 %, about 0.55 %, about 0.6 %, about 0.65 %, about 0.7 %, about 0.75 %, about 0.8 %, about 0.85 %, about 0.9 %, about 0.95 %, about 1.0 %, about 1.1 %, about 1.2 %, about 1.3 %, about 1.4 %, about 1.5 %, about 1.6 %, about 1.7 %, about 1.8 %, about 1.9 %, about 2.0 %, about 2.1 %, about 2.2 %, about 2.3 %, about 2.4 %, about 2.5  
10 %, about 2.6 %, about 2.7 %, about 2.8 %, about 2.9 %, about 3.0 %, about 3.1 %, about 3.2 %, about 3.3 %, about 3.4 %, about 3.5 %, about 3.6 %, about 3.7 %, about 3.8 %, about 3.9 %, or about 4.0 % amide-type or ester-type local anesthetic solution in a volume of about 10 ml, about 12.5 ml, about 15 ml, about 17.5 ml, about 20 ml, about 22.5 ml, about 25 ml, about 27.5 ml, about 30 ml, about 32.5 ml, about 35 ml, about 37.5 ml, or about 40 ml when  
15 administered via any regional anesthesia technique directed at nerves innervating the thorax and chest wall, such as via a serratus plane nerve block, or via an intercostal nerve block, or via a paravertebral block.

**[00199]** In any and all embodiments of the methods disclosed herein, the cancer patient exhibits stage I, stage II or stage III lung cancer. Additionally or alternatively, in some  
20 embodiments, the cancer patient has been diagnosed with lung adenocarcinoma (LUAD). The histologic subtype of the lung adenocarcinoma may be lepidic, acinar, papillary, micropapillary, solid or unknown.

**[00200]** In any of the preceding embodiments of the methods disclosed herein, the cancer patient has received an adjuvant therapy. The adjuvant therapy may be chemotherapy, lobectomy, radiation therapy or chemoradiation therapy. Additionally or alternatively, in  
25 some embodiments of the methods disclosed herein, the patient is human.

**[00201]** In any and all embodiments of the methods disclosed herein, the biological sample obtained from the cancer patient comprises biopsied tumor tissue, whole blood, plasma, or serum.

**[00202]** Administration of any of the intraoperative or postoperative analgesics disclosed  
30 herein can be carried out by any suitable route, including but not limited to, orally, intranasally,

parenterally (intravenously, intramuscularly, intraperitoneally, or subcutaneously), rectally, intrathecally, intratumorally or topically.

**Methods for Selecting Intraoperative Analgesic Regimen in Colon Cancer Patients Undergoing Tumor Resection Surgery**

5 [00203] Administration of any of the intraoperative or postoperative analgesics disclosed herein can be carried out by any suitable route, including but not limited to, orally, intranasally, parenterally (intravenously, intramuscularly, intraperitoneally, or subcutaneously), rectally, intrathecally, intratumorally or topically.

[00204] In one aspect, the present disclosure provides a method for selecting a cancer  
10 patient undergoing tumor resection surgery for colon cancer for treatment with an intraoperative opioid analgesic comprising (a) assaying gene expression levels of MLH1, MSH2, MSH6, and PMS2 in a biological sample obtained from the cancer patient and (b) administering to the cancer patient an effective amount of an intraoperative opioid analgesic during the tumor resection surgery, wherein the gene expression levels of one or more of  
15 MLH1, MSH2, MSH6, and PMS2 in the biological sample obtained from the cancer patient are reduced or undetectable compared to that observed in a control sample obtained from a healthy subject or a predetermined threshold. In certain embodiments, expression levels of MLH1, MSH2, MSH6, and/or PMS2 are assayed via next-generation sequencing, PCR, quantitative PCR (qPCR), digital PCR (dPCR), Reverse transcriptase-PCR (RT-PCR),  
20 Northern blotting, microarray, dot or slot blots, *in situ* hybridization, fluorescent *in situ* hybridization (FISH), RNA-seq, Western blotting, enzyme-linked immunosorbent assays (ELISA), dot blotting, immunohistochemistry, immunofluorescence, immunoprecipitation, immunoelectrophoresis, or mass-spectrometry. Additionally or alternatively, in some embodiments, the biological sample comprises genomic DNA, cDNA, RNA, and/or mRNA.

25 [00205] In one aspect, the present disclosure provides a method for selecting a cancer patient undergoing tumor resection surgery for colon cancer for treatment with an intraoperative opioid analgesic comprising (a) assaying gene expression levels of one or more genes selected from among CCK, NTSR2, IFNG, NTSR1, MLN, LIPN, CXCL8, FCGR3B, PTGS2, CALB2, CXCL10, IL1RN, CCL5, CCL4, IL1B, CCL4L2, FCGR3A, ITIH4, GAD1,  
30 CD8A, HTR3A, SLC1A3, LHB, ADRB1, CXCR2, HRH2, CCL3, ESR2, CSF2, ENO2, GRM5, CXCR4, PRKCG, CD68, IL10, TLR2, ICAM1, RGS2, CX3CL1 and KCNJ3 in a biological sample obtained from the cancer patient; and (b) administering to the cancer patient

an effective amount of an intraoperative opioid analgesic during the tumor resection surgery, wherein the gene expression levels of one or more of CCK, NTSR2, IFNG, NTSR1, MLN, LIPN, CXCL8, FCGR3B, PTGS2, CALB2, CXCL10, IL1RN, CCL5, CCL4, IL1B, CCL4L2, FCGR3A, ITIH4, GAD1, CD8A, HTR3A, SLC1A3, LHB, ADRB1, CXCR2, HRH2, CCL3, ESR2, CSF2, ENO2, GRM5, CXCR4, PRKCG, CD68, IL10, TLR2, ICAM1, RGS2, CX3CL1 and KCNJ3 in the biological sample obtained from the cancer patient are elevated relative to a reference sample obtained from a colon cancer subject having microsatellite stable (MSS) tumors or a predetermined threshold. In some embodiments, the gene expression levels of at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 31, at least 32, at least 33, at least 34, at least 35, at least 36, at least 37, at least 38, at least 39, or 40 genes selected from among CCK, NTSR2, IFNG, NTSR1, MLN, LIPN, CXCL8, FCGR3B, PTGS2, CALB2, CXCL10, IL1RN, CCL5, CCL4, IL1B, CCL4L2, FCGR3A, ITIH4, GAD1, CD8A, HTR3A, SLC1A3, LHB, ADRB1, CXCR2, HRH2, CCL3, ESR2, CSF2, ENO2, GRM5, CXCR4, PRKCG, CD68, IL10, TLR2, ICAM1, RGS2, CX3CL1 and KCNJ3 in the biological sample obtained from the cancer patient are elevated relative to a reference sample obtained from a colon cancer subject having microsatellite stable (MSS) tumors or a predetermined threshold. In certain embodiments, expression levels of CCK, NTSR2, IFNG, NTSR1, MLN, LIPN, CXCL8, FCGR3B, PTGS2, CALB2, CXCL10, IL1RN, CCL5, CCL4, IL1B, CCL4L2, FCGR3A, ITIH4, GAD1, CD8A, HTR3A, SLC1A3, LHB, ADRB1, CXCR2, HRH2, CCL3, ESR2, CSF2, ENO2, GRM5, CXCR4, PRKCG, CD68, IL10, TLR2, ICAM1, RGS2, CX3CL1 and KCNJ3 are assayed via next-generation sequencing, PCR, quantitative PCR (qPCR), digital PCR (dPCR), Reverse transcriptase-PCR (RT-PCR), Northern blotting, microarray, dot or slot blots, *in situ* hybridization, fluorescent *in situ* hybridization (FISH), RNA-seq, Western blotting, enzyme-linked immunosorbent assays (ELISA), dot blotting, immunohistochemistry, immunofluorescence, immunoprecipitation, immunoelectrophoresis, or mass-spectrometry. Additionally or alternatively, in some embodiments, the biological sample comprises genomic DNA, cDNA, RNA, and/or mRNA.

**[00206]** In one aspect, the present disclosure provides a method for selecting a cancer patient undergoing tumor resection surgery for colon cancer for treatment with an intraoperative opioid analgesic comprising (a) assaying gene expression levels of one or more

genes selected from among GHRH, RBFOX3, NOS1, KISS1, NPFFR2, GRIA2, SLC6A2, ADCY5, HCRT, OPCML, NPY1R, PPP1R14C, NTRK2, CHAT, LEP, CPB1, NR1I2, ADCY8, EGF, F2, UGT1A8, PLG, GRPR, PYY, DRD1, ABCB1, SCT, NTS, NPFFR1, TH, CYP2B6, HTR2C, TAC1, CCKBR, CALCA, ATP12A, LINC01411, P2RX3, ALB, REN, SLC6A4, KNG1 and DRD2 in a biological sample obtained from the cancer patient; and (b) administering to the cancer patient an effective amount of an intraoperative opioid analgesic during the tumor resection surgery, wherein the gene expression levels of one or more of GHRH, RBFOX3, NOS1, KISS1, NPFFR2, GRIA2, SLC6A2, ADCY5, HCRT, OPCML, NPY1R, PPP1R14C, NTRK2, CHAT, LEP, CPB1, NR1I2, ADCY8, EGF, F2, UGT1A8, PLG, GRPR, PYY, DRD1, ABCB1, SCT, NTS, NPFFR1, TH, CYP2B6, HTR2C, TAC1, CCKBR, CALCA, ATP12A, LINC01411, P2RX3, ALB, REN, SLC6A4, KNG1 and DRD2 in the biological sample obtained from the cancer patient are reduced relative to a reference sample obtained from a colon cancer subject having microsatellite stable (MSS) tumors or a predetermined threshold. In some embodiments, the gene expression levels of at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 31, at least 32, at least 33, at least 34, at least 35, at least 36, at least 37, at least 38, at least 39, at least 40, at least 41, at least 42, or 43 genes selected from among GHRH, RBFOX3, NOS1, KISS1, NPFFR2, GRIA2, SLC6A2, ADCY5, HCRT, OPCML, NPY1R, PPP1R14C, NTRK2, CHAT, LEP, CPB1, NR1I2, ADCY8, EGF, F2, UGT1A8, PLG, GRPR, PYY, DRD1, ABCB1, SCT, NTS, NPFFR1, TH, CYP2B6, HTR2C, TAC1, CCKBR, CALCA, ATP12A, LINC01411, P2RX3, ALB, REN, SLC6A4, KNG1 and DRD2 in the biological sample obtained from the cancer patient are reduced relative to a reference sample obtained from a colon cancer subject having microsatellite stable (MSS) tumors or a predetermined threshold. In certain embodiments, expression levels of GHRH, RBFOX3, NOS1, KISS1, NPFFR2, GRIA2, SLC6A2, ADCY5, HCRT, OPCML, NPY1R, PPP1R14C, NTRK2, CHAT, LEP, CPB1, NR1I2, ADCY8, EGF, F2, UGT1A8, PLG, GRPR, PYY, DRD1, ABCB1, SCT, NTS, NPFFR1, TH, CYP2B6, HTR2C, TAC1, CCKBR, CALCA, ATP12A, LINC01411, P2RX3, ALB, REN, SLC6A4, KNG1 and DRD2 are assayed via next-generation sequencing, PCR, quantitative PCR (qPCR), digital PCR (dPCR), Reverse transcriptase-PCR (RT-PCR), Northern blotting, microarray, dot or slot blots, *in situ* hybridization, fluorescent *in situ* hybridization (FISH), RNA-seq, Western blotting, enzyme-linked immunosorbent assays (ELISA), dot blotting,

immunohistochemistry, immunofluorescence, immunoprecipitation, immunoelectrophoresis, or mass-spectrometry. Additionally or alternatively, in some embodiments, the biological sample comprises genomic DNA, cDNA, RNA, and/or mRNA.

**[00207]** In another aspect, the present disclosure provides a method for selecting a cancer patient undergoing tumor resection surgery for colon cancer for treatment with an intraoperative opioid analgesic comprising (a) detecting the expression levels or activity of one or more genes that result (i) in reduced activity of Wnt pathway and/or (ii) hyperactivation of Th1 immune response signature in a biological sample obtained from the cancer patient relative to a predetermined threshold; and (b) administering to the cancer patient an effective amount of an intraoperative opioid analgesic during the tumor resection surgery. Additionally or alternatively, in some embodiments, the cancer patient comprises at least one mutation that results in reduced activity of Wnt pathway and/or (ii) hyperactivation of Th1 immune response signature. Additionally or alternatively, in some embodiments, the at least one mutation is a frameshift mutation, a missense mutation, a deletion, an insertion, a nonsense mutation, an inversion, or a translocation. The at least one mutation may be detected using any nucleic acid detection assay known in the art such as next-generation sequencing, PCR, real-time quantitative PCR (qPCR), digital PCR (dPCR), Southern blotting, Reverse transcriptase-PCR (RT-PCR), Northern blotting, microarray, dot or slot blots, *in situ* hybridization, or fluorescent *in situ* hybridization (FISH). In some embodiments, the biological sample comprises genomic DNA, cDNA, RNA, and/or mRNA.

**[00208]** Additionally or alternatively, in some embodiments, the reduced activity of Wnt pathway is determined by detecting the expression levels or activity of one or more genes selected from among ADAM17, AXIN1, AXIN2, CCND2, CSNK1E, CTNNB1, CUL1, DKK1, DKK4, DLL1, DVL2, FRAT1, FZD1, FZD8, GNAI1, HDAC11, HDAC2, HDAC5, HEY1, HEY2, JAG1, JAG2, KAT2A, LEF1, MAML1, MYC, NCOR2, NCSTN, NKD1, NOTCH1, NOTCH4, NUMB, PPARD, PSEN2, PTCH1, RBPJ, SKP2, TCF7, TP53, WNT1, WNT5B, and WNT6. In certain embodiments, the reduced activity of Wnt pathway is determined by detecting the expression levels or activity of at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 31, at least 32, at least 33, at least 34, at least 35, at least 36, at least 37, at least 38, at least 39, at least 40, at least 41, or 42 genes selected from among

ADAM17, AXIN1, AXIN2, CCND2, CSNK1E, CTNNB1, CUL1, DKK1, DKK4, DLL1, DVL2, FRAT1, FZD1, FZD8, GNAI1, HDAC11, HDAC2, HDAC5, HEY1, HEY2, JAG1, JAG2, KAT2A, LEF1, MAML1, MYC, NCOR2, NCSTN, NKD1, NOTCH1, NOTCH4, NUMB, PPARD, PSEN2, PTCH1, RBPJ, SKP2, TCF7, TP53, WNT1, WNT5B, and WNT6.

5 In certain embodiments, the expression levels of one or more genes selected from among ADAM17, AXIN1, AXIN2, CCND2, CSNK1E, CTNNB1, CUL1, DKK1, DKK4, DLL1, DVL2, FRAT1, FZD1, FZD8, GNAI1, HDAC11, HDAC2, HDAC5, HEY1, HEY2, JAG1, JAG2, KAT2A, LEF1, MAML1, MYC, NCOR2, NCSTN, NKD1, NOTCH1, NOTCH4, NUMB, PPARD, PSEN2, PTCH1, RBPJ, SKP2, TCF7, TP53, WNT1, WNT5B, and WNT6  
10 are detected via next-generation sequencing, PCR, quantitative PCR (qPCR), digital PCR (dPCR), Reverse transcriptase-PCR (RT-PCR), Northern blotting, microarray, dot or slot blots, *in situ* hybridization, fluorescent *in situ* hybridization (FISH), RNA-seq, Western blotting, enzyme-linked immunosorbent assays (ELISA), dot blotting, immunohistochemistry, immunofluorescence, immunoprecipitation, immunoelectrophoresis,  
15 or mass-spectrometry. Additionally or alternatively, in some embodiments, the biological sample comprises genomic DNA, cDNA, RNA, and/or mRNA.

**[00209]** Additionally or alternatively, in some embodiments, the hyperactivation of Th1 immune response signature is determined by detecting the expression levels or activity of one or more genes selected from among IFNG, LTA, APBB2, DOK5, IL12RB2, APOD, ZBTB32,  
20 CD38, CSF2, CTLA4, CD70, DPP4, EGFL6, BST2, DUSP5, LRP8, IL22, DGKI, CCL4, GGT1, LRRN3, SYNGR3, ATP9A, BTG3, CMAHP, HBEGF, and SGCB. In certain embodiments, the hyperactivation of Th1 immune response signature is determined by detecting the expression levels or activity of at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at  
25 least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, or 27 genes selected from among IFNG, LTA, APBB2, DOK5, IL12RB2, APOD, ZBTB32, CD38, CSF2, CTLA4, CD70, DPP4, EGFL6, BST2, DUSP5, LRP8, IL22, DGKI, CCL4, GGT1, LRRN3, SYNGR3, ATP9A, BTG3, CMAHP, HBEGF, and SGCB. In certain embodiments, the expression levels of one  
30 or more genes selected from among IFNG, LTA, APBB2, DOK5, IL12RB2, APOD, ZBTB32, CD38, CSF2, CTLA4, CD70, DPP4, EGFL6, BST2, DUSP5, LRP8, IL22, DGKI, CCL4, GGT1, LRRN3, SYNGR3, ATP9A, BTG3, CMAHP, HBEGF, and SGCB are detected via next-generation sequencing, PCR, quantitative PCR (qPCR), digital PCR (dPCR), Reverse

transcriptase-PCR (RT-PCR), Northern blotting, microarray, dot or slot blots, *in situ* hybridization, fluorescent *in situ* hybridization (FISH), RNA-seq, Western blotting, enzyme-linked immunosorbent assays (ELISA), dot blotting, immunohistochemistry, immunofluorescence, immunoprecipitation, immunoelectrophoresis, or mass-spectrometry.

5 Additionally or alternatively, in some embodiments, the biological sample comprises genomic DNA, cDNA, RNA, and/or mRNA.

**[00210]** In one aspect, the present disclosure provides a method for reducing the risk of tumor recurrence in a cancer patient undergoing tumor resection surgery for colon cancer comprising administering to the cancer patient an effective amount of an intraoperative opioid analgesic during the tumor resection surgery, wherein the cancer patient comprises at least one alteration in DNA mismatch repair (MMR) system (*e.g.*, MMR deficiency). In some embodiments, mRNA or polypeptide expression levels of one or more of MLH1, MSH2, MSH6, and PMS2 in a biological sample obtained from the cancer patient are reduced or undetectable compared to that observed in a control sample obtained from a healthy subject or a predetermined threshold. Additionally or alternatively, in some embodiments, the mRNA or polypeptide expression levels of one or more of CCK, NTSR2, IFNG, NTSR1, MLN, LIPN, CXCL8, FCGR3B, PTGS2, CALB2, CXCL10, IL1RN, CCL5, CCL4, IL1B, CCL4L2, FCGR3A, ITIH4, GAD1, CD8A, HTR3A, SLC1A3, LHB, ADRB1, CXCR2, HRH2, CCL3, ESR2, CSF2, ENO2, GRM5, CXCR4, PRKCG, CD68, IL10, TLR2, ICAM1, RGS2, CX3CL1 and KCNJ3 in a biological sample obtained from the cancer patient are elevated relative to a reference sample obtained from a colon cancer subject having microsatellite stable (MSS) tumors or a predetermined threshold. Additionally or alternatively, in some embodiments, the mRNA or polypeptide levels of one or more of GHRH, RBFOX3, NOS1, KISS1, NPFFR2, GRIA2, SLC6A2, ADCY5, HCRT, OPCML, NPY1R, PPP1R14C, NTRK2, CHAT, LEP, CPB1, NR1I2, ADCY8, EGF, F2, UGT1A8, PLG, GRPR, PYY, DRD1, ABCB1, SCT, NTS, NPFFR1, TH, CYP2B6, HTR2C, TAC1, CCKBR, CALCA, ATP12A, LINC01411, P2RX3, ALB, REN, SLC6A4, KNG1 and DRD2 in the biological sample obtained from the cancer patient are reduced relative to a reference sample obtained from a colon cancer subject having microsatellite stable (MSS) tumors or a predetermined threshold. Additionally or alternatively, in some embodiments, the cancer patient comprises at least one genetic mutation or altered expression levels or activity of one or more genes that result (i) in reduced activity of Wnt pathway and/or (ii) hyperactivation of Th1 immune response signature. In some embodiments, the reduced activity of Wnt pathway

is determined by detecting the expression levels or activity of one or more genes selected from among ADAM17, AXIN1, AXIN2, CCND2, CSNK1E, CTNNB1, CUL1, DKK1, DKK4, DLL1, DVL2, FRAT1, FZD1, FZD8, GNAI1, HDAC11, HDAC2, HDAC5, HEY1, HEY2, JAG1, JAG2, KAT2A, LEF1, MAML1, MYC, NCOR2, NCSTN, NKD1, NOTCH1, NOTCH4, NUMB, PPARD, PSEN2, PTCH1, RBPJ, SKP2, TCF7, TP53, WNT1, WNT5B, and WNT6. In other embodiments, the hyperactivation of Th1 immune response signature is determined by detecting the expression levels or activity of one or more genes selected from among IFNG, LTA, APBB2, DOK5, IL12RB2, APOD, ZBTB32, CD38, CSF2, CTLA4, CD70, DPP4, EGFL6, BST2, DUSP5, LRP8, IL22, DGKI, CCL4, GGT1, LRRN3, SYNGR3, ATP9A, BTG3, CMAHP, HBEGF, and SGCB.

**[00211]** Additionally or alternatively, in some embodiments of the preceding embodiments, genetic mutations or mRNA expression levels are detected via next generation sequencing, RNA-seq, real-time quantitative PCR (qPCR), digital PCR (dPCR), Reverse transcriptase-PCR (RT-PCR), Northern blotting, microarray, dot or slot blots, *in situ* hybridization, or fluorescent *in situ* hybridization (FISH). Additionally or alternatively, in certain embodiments, polypeptide expression levels are detected via Western blotting, enzyme-linked immunosorbent assays (ELISA), dot blotting, immunohistochemistry, immunofluorescence, immunoprecipitation, immunoelectrophoresis, or mass-spectrometry.

**[00212]** In any of the preceding embodiments of methods disclosed herein, the intraoperative opioid analgesic is fentanyl, hydromorphone, morphine, oxycodone, hydrocodone, codeine, meperidine, remifentanyl, or sufentanyl. The effective amount of the intraoperative opioid analgesic may range from about 1 MME to about 200 MMEs. In certain embodiments, the effective amount of the intraoperative opioid analgesic is about 1 MME to about 20 MMEs, about 20 MMEs to about 45 MMEs, or about 45 MMEs to about 200 MMEs. In some embodiments, the effective amount of the intraoperative opioid analgesic is about 1 MME, about 2 MMEs, about 3 MMEs, about 4 MMEs, about 5 MMEs, about 6 MMEs, about 7 MMEs, about 8 MMEs, about 9 MMEs, about 10 MMEs, about 11 MMEs, about 12 MMEs, about 13 MMEs, about 14 MMEs, about 15 MMEs, about 16 MMEs, about 17 MMEs, about 18 MMEs, about 19 MMEs, about 20 MMEs, about 21 MMEs, about 22 MMEs, about 23 MMEs, about 24 MMEs, about 25 MMEs, about 26 MMEs, about 27 MMEs, about 28 MMEs, about 29 MMEs, about 30 MMEs, about 31 MMEs, about 32 MMEs, about 33 MMEs, about 34 MMEs, about 35 MMEs, about 36 MMEs, about 37 MMEs, about 38 MMEs, about 39 MMEs, about 40-45 MMEs, about 45-50 MMEs, about 50-55 MMEs, about 55-60 MMEs,

about 60-65 MMEs, about 65-70 MMEs, about 70-75 MMEs, about 75-80 MMEs, about 80-85 MMEs, about 85-90 MMEs, about 90-95 MMEs, about 95-100 MMEs, about 100-110 MMEs, about 110-120 MMEs, about 120-130 MMEs, about 130-140 MMEs, about 140-150 MMEs, about 150-160 MMEs, about 160-170 MMEs, about 170-180 MMEs, about 180-190 MMEs, or about 190-200 MMEs. Additionally or alternatively, in some embodiments, the effective amount of the intraoperative opioid analgesic is administered as a series of bolus doses or as a continuous infusion during the tumor resection surgery. In certain embodiments, the effective amount of the intraoperative opioid analgesic is administered to the cancer patient prior to incision. Additionally or alternatively, in some embodiments, the effective amount of the intraoperative opioid analgesic is administered intravenously.

**[00213]** In any and all of the preceding embodiments of the methods disclosed herein, the cancer patient comprises microsatellite instability-high (MSI-H) tumors.

**[00214]** Additionally or alternatively, in some embodiments, the methods of the present technology further comprise administering to the cancer patient an effective amount of a local anesthetic solution via an epidural catheter before, during and/or after the tumor resection surgery. The effective amount of the local anesthetic solution may range from about 0.05%-4% local anesthetic solution in a volume of 1-10 ml per hour when administered via an epidural catheter. In some embodiments, the effective amount of the local anesthetic solution is about 0.05 %, about 0.06 %, about 0.07 %, about 0.08 %, about 0.09 %, about 0.1 %, about 0.15 %, about 0.2 %, about 0.25 %, about 0.3 %, about 0.35 %, about 0.4 %, about 0.45 %, about 0.5 %, about 0.55 %, about 0.6 %, about 0.65 %, about 0.7 %, about 0.75 %, about 0.8 %, about 0.85 %, about 0.9 %, about 0.95 %, about 1.0 %, about 1.1 %, about 1.2 %, about 1.3 %, about 1.4 %, about 1.5 %, about 1.6 %, about 1.7 %, about 1.8 %, about 1.9 %, about 2.0 %, about 2.1 %, about 2.2 %, about 2.3 %, about 2.4 %, about 2.5 %, about 2.6 %, about 2.7 %, about 2.8 %, about 2.9 %, about 3.0 %, about 3.1 %, about 3.2 %, about 3.3 %, about 3.4 %, about 3.5 %, about 3.6 %, about 3.7 %, about 3.8 %, about 3.9 %, or about 4.0 % local anesthetic solution in a volume of about 1 ml per hour, about 1.5 ml per hour, about 2 ml per hour, about 2.5 ml per hour, about 3 ml per hour, about 3.5 ml per hour, about 4 ml per hour, about 4.5 ml per hour, about 5 ml per hour, about 5.5 ml per hour, about 6 ml per hour, about 6.5 ml per hour, about 7 ml per hour, about 7.5 ml per hour, about 8 ml per hour, about 8.5 ml per hour, about 9 ml per hour, about 9.5 ml per hour, or about 10 ml per hour when administered via an epidural catheter. Additionally or alternatively, in some embodiments, the effective amount of the local anesthetic solution is administered as a single injection,

series of bolus doses or as a continuous infusion during the tumor resection surgery. Examples of suitable local anesthetics include, but are not limited to, lidocaine, mepivacaine, prilocaine, bupivacaine, etidocaine, ropivacaine, levobupivacaine, cocaine, procaine, tetracaine, chloroprocaine, and benzocaine.

**[00215]** In other embodiments, the effective amount of the local anesthetic solution may be administered before, during and/or after the tumor resection surgery using any suitable regional anesthesia technique for colon cancer (*e.g.*, transversus abdominis plane (TAP) blocks, Ilioinguinal (II) and iliohypogastric (IH) blocks, Rectus sheath blocks, Transversalis fascia plane blocks, quadratus lumborum blocks). The effective amount of the local anesthetic solution may range from about 0.05%-4% local anesthetic solution in a volume of 10-40 ml when administered using any suitable regional anesthesia technique for colon cancer (*e.g.*, transversus abdominis plane (TAP) blocks, Ilioinguinal (II) and iliohypogastric (IH) blocks, Rectus sheath blocks, Transversalis fascia plane blocks, quadratus lumborum blocks). In some embodiments, the effective amount of the local anesthetic solution is about 0.05 %, about 0.06 %, about 0.07 %, about 0.08 %, about 0.09 %, about 0.1 %, about 0.15 %, about 0.2 %, about 0.25 %, about 0.3 %, about 0.35 %, about 0.4 %, about 0.45 %, about 0.5 %, about 0.55 %, about 0.6 %, about 0.65 %, about 0.7 %, about 0.75 %, about 0.8 %, about 0.85 %, about 0.9 %, about 0.95 %, about 1.0 %, about 1.1 %, about 1.2 %, about 1.3 %, about 1.4 %, about 1.5 %, about 1.6 %, about 1.7 %, about 1.8 %, about 1.9 %, about 2.0 %, about 2.1 %, about 2.2 %, about 2.3 %, about 2.4 %, about 2.5 %, about 2.6 %, about 2.7 %, about 2.8 %, about 2.9 %, about 3.0 %, about 3.1 %, about 3.2 %, about 3.3 %, about 3.4 %, about 3.5 %, about 3.6 %, about 3.7 %, about 3.8 %, about 3.9 %, or about 4.0 % local anesthetic solution in a volume of about 10 ml, about 12.5 ml, about 15 ml, about 17.5 ml, about 20 ml, about 22.5 ml, about 25 ml, about 27.5 ml, about 30 ml, about 32.5 ml, about 35 ml, about 37.5 ml, or about 40 ml when administered using any suitable regional anesthesia technique for colon cancer (*e.g.*, transversus abdominis plane (TAP) blocks, Ilioinguinal (II) and iliohypogastric (IH) blocks, Rectus sheath blocks, Transversalis fascia plane blocks, quadratus lumborum blocks). Examples of suitable local anesthetics include, but are not limited to, lidocaine, mepivacaine, prilocaine, bupivacaine, etidocaine, ropivacaine, levobupivacaine, cocaine, procaine, tetracaine, chloroprocaine, and benzocaine.

**[00216]** Additionally or alternatively, in certain embodiments, the local anesthetic solution may further comprise an opioid (*e.g.*, fentanyl, hydromorphone, morphine, oxycodone, hydrocodone, codeine, meperidine, remifentanyl, or sufentanyl). In some embodiments, the

local anesthetic solution may comprise 0.5 mcg/ml-50 mcg/ml opioid. In certain embodiments, the local anesthetic solution may comprise about 0.5 mcg/ml, about 0.6 mcg/ml, about 0.7 mcg/ml, about 0.8 mcg/ml, about 0.9 mcg/ml, about 1.0 mcg/ml, about 1.5 mcg/ml, about 2.0 mcg/ml, about 2.5 mcg/ml, about 3.0 mcg/ml, about 3.5 mcg/ml, about 4.0 mcg/ml, about 4.5 mcg/ml, about 5.0 mcg/ml, about 5.5 mcg/ml, about 6.0 mcg/ml, about 6.5 mcg/ml, about 7.0 mcg/ml, about 7.5 mcg/ml, about 8.0 mcg/ml, about 8.5 mcg/ml, about 9.0 mcg/ml, about 10 mcg/ml, about 15 mcg/ml, about 20 mcg/ml, about 25 mcg/ml, about 30 mcg/ml, about 35 mcg/ml, about 40 mcg/ml, about 45 mcg/ml, or about 50 mcg/ml.

[00217] Additionally or alternatively, in certain embodiments, the methods of the present technology further comprise administering to the cancer patient an effective amount of a post-operative opioid analgesic after the tumor resection surgery. Examples of post-operative opioid analgesics include, but are not limited to, fentanyl, hydromorphone, morphine, oxycodone, hydrocodone, codeine, meperidine, remifentanyl, or sufentanyl. In some embodiments, the post-operative opioid analgesic and the intraoperative opioid analgesic are the same opioid analgesic or different opioid analgesics. In other embodiments, the effective amount of the post-operative opioid analgesic and the effective amount of the intraoperative opioid analgesic are the same or different. Additionally or alternatively, in some embodiments, the effective amount of the post-operative opioid analgesic is administered to the cancer patient as a bolus of about 0.005 mg to about 100 mg. In some embodiments, the effective amount of the post-operative opioid analgesic is administered to the cancer patient as a bolus of about 0.005 mg, about 0.006 mg, about 0.007 mg, about 0.008 mg, about 0.009 mg, about 0.01 mg, about 0.02 mg, about 0.03 mg, about 0.04 mg, about 0.05 mg, about 0.06 mg, about 0.07 mg, about 0.08 mg, about 0.09 mg, about 0.1 mg, about 0.2 mg, about 0.3 mg, about 0.4 mg, about 0.5 mg, about 0.6 mg, about 0.7 mg, about 0.8 mg, about 0.9 mg, about 1-5 mg, about 5-10 mg, about 1-5 mg, about 5-10 mg, about 1-5 mg, about 5-10 mg, about 1-5 mg, about 5-10 mg, about 10-15 mg, about 15-20 mg, about 20-25 mg, about 25-30 mg, about 30-35 mg, about 35-40 mg, about 40-45 mg, about 45-50 mg, about 50-55 mg, about 55-60 mg, about 60-65 mg, about 65-70 mg, about 70-75 mg, about 75-80 mg, about 80-85 mg, about 85-90 mg, about 90-95 mg, or about 95-100 mg. In other embodiments, the effective amount of the post-operative opioid analgesic may be continuously delivered to the cancer patient at a per hour rate of about 0.01 mg/hr to about 10 mg/hr. In certain embodiments, the effective amount of the post-operative opioid analgesic is continuously delivered to the cancer patient at a per hour rate of about 0.01 mg/hr, about 0.02 mg/hr, about

0.03 mg/hr, about 0.04 mg/hr, about 0.05 mg/hr, about 0.06 mg/hr, about 0.07 mg/hr, about 0.08 mg/hr, about 0.09 mg/hr, about 0.1 mg/hr, about 0.2 mg/hr, about 0.3 mg/hr, about 0.4 mg/hr, about 0.5 mg/hr, about 0.6 mg/hr, about 0.7 mg/hr, about 0.8 mg/hr, about 0.9 mg/hr, about 1 mg/hr, about 1.5 mg/hr, about 2 mg/hr, about 2.5 mg/hr, about 3 mg/hr, about 3.5 mg/hr, about 4 mg/hr, about 4.5 mg/hr, about 5 mg/hr, about 5.5 mg/hr, about 6 mg/hr, about 6.5 mg/hr, about 7 mg/hr, about 7.5 mg/hr, about 8 mg/hr, about 8.5 mg/hr, about 9 mg/hr, about 9.5 mg/hr, or about 10 mg/hr. Additionally or alternatively, in some embodiments, the effective amount of the post-operative opioid analgesic is administered intravenously, orally, or transdermally.

**[00218]** Additionally or alternatively, in any and all embodiments of the methods disclosed herein, the methods of the present technology further comprise administering to the cancer patient an effective amount of one or more non-opioid analgesics selected from among ketamine, dexmedetomidine, a carboxylic acid derivative NSAID, or acetaminophen.

**[00219]** Additionally or alternatively, in some embodiments, the ketamine is administered intravenously. The effective amount of ketamine may be administered intraoperatively, preoperatively, and/or postoperatively.

**[00220]** Additionally or alternatively, in some embodiments, the ketamine is infused at a rate of 0.04 mg/kg/hr-2.5 mg/kg/hr. In some embodiments of the methods disclosed herein, the ketamine is infused at a rate of about 0.04 mg/kg/hr, about 0.05 mg/kg/hr, about 0.06 mg/kg/hr, about 0.07 mg/kg/hr, about 0.08 mg/kg/hr, about 0.09 mg/kg/hr, about 0.1 mg/kg/hr, about 0.2 mg/kg/hr, about 0.3 mg/kg/hr, about 0.4 mg/kg/hr, about 0.5 mg/kg/hr, about 0.6 mg/kg/hr, about 0.7 mg/kg/hr, about 0.8 mg/kg/hr, about 0.9 mg/kg/hr, about 1.0 mg/kg/hr, about 1.1 mg/kg/hr, about 1.2 mg/kg/hr, about 1.3 mg/kg/hr, about 1.4 mg/kg/hr, about 1.5 mg/kg/hr, about 1.6 mg/kg/hr, about 1.7 mg/kg/hr, about 1.8 mg/kg/hr, about 1.9 mg/kg/hr, about 2.0 mg/kg/hr, about 2.1 mg/kg/hr, about 2.2 mg/kg/hr, about 2.3 mg/kg/hr, about 2.4 mg/kg/hr, or about 2.5 mg/kg/hr.

**[00221]** Additionally or alternatively, in some embodiments, the ketamine is administered as a bolus of 0.5 mg/kg -4.5 mg/kg. In some embodiments of the methods disclosed herein, the ketamine is administered as a bolus of about 0.04 mg/kg, about 0.05 mg/kg, about 0.06 mg/kg, about 0.07 mg/kg, about 0.08 mg/kg, about 0.09 mg/kg, about 0.1 mg/kg, about 0.2 mg/kg, about 0.3 mg/kg, about 0.4 mg/kg, about 0.5 mg/kg, about 0.6 mg/kg, about 0.7 mg/kg, about 0.8 mg/kg, about 0.9 mg/kg, about 1.0 mg/kg, about 1.1 mg/kg, about 1.2 mg/kg, about

1.3 mg/kg, about 1.4 mg/kg, about 1.5 mg/kg, about 1.6 mg/kg, about 1.7 mg/kg, about 1.8 mg/kg, about 1.9 mg/kg, about 2.0 mg/kg, about 2.1 mg/kg, about 2.2 mg/kg, about 2.3 mg/kg, about 2.4 mg/kg, about 2.5 mg/kg, about 2.6 mg/kg, about 2.7 mg/kg, about 2.8 mg/kg, about 2.9 mg/kg, about 3.0 mg/kg, about 3.1 mg/kg, about 3.2 mg/kg, about 3.3 mg/kg, about 3.4 mg/kg, about 3.5 mg/kg, about 3.6 mg/kg, about 3.7 mg/kg, about 3.8 mg/kg, about 3.9 mg/kg, about 4.0 mg/kg, about 4.1 mg/kg, about 4.2 mg/kg, about 4.3 mg/kg, about 4.4 mg/kg, or about 4.5 mg/kg.

**[00222]** Additionally or alternatively, in some embodiments, the ketamine is administered intramuscularly at a dose of about 4-13 mg/kg. In certain embodiments of the methods disclosed herein, the ketamine is administered intramuscularly at a dose of 4 mg/kg, 5 mg/kg, 6 mg/kg, 7 mg/kg, 8 mg/kg, 9 mg/kg, 10 mg/kg, 11 mg/kg, 12 mg/kg, or 13 mg/kg. Additionally or alternatively, in some embodiments, the ketamine is administered orally at a dose of about 6-10 mg/kg. In certain embodiments of the methods disclosed herein, the ketamine is administered orally at a dose of 6 mg/kg, 7 mg/kg, 8 mg/kg, 9 mg/kg, or 10 mg/kg.

**[00223]** In any and all of the preceding embodiments of methods disclosed herein, the carboxylic acid derivative NSAID is an acetic acid NSAID or a propionic acid NSAID. Examples of the carboxylic acid derivative NSAID include, but are not limited to, ibuprofen, dexibuprofen, naproxen, fenoprofen, ketoprofen, dexketoprofen, flurbiprofen, oxaprozin, loxoprofen, pelubiprofen, zaltoprofen, indomethacin, tolmetin, sulindac, etodolac, ketorolac, diclofenac, aceclofenac, bromfenac, or nabumeton. In some embodiments, the carboxylic acid derivative NSAID is administered orally, intravenously, or intramuscularly. Additionally or alternatively, in some embodiments, the effective amount of the carboxylic acid derivative NSAID is administered intraoperatively and/or postoperatively. The effective amount of the carboxylic acid derivative NSAID may be administered to the cancer patient during closing of an incision.

**[00224]** Additionally or alternatively, in some embodiments of the methods disclosed herein, the effective amount of the carboxylic acid derivative NSAID is about 10 mg-1500 mg. In some embodiments of the methods disclosed herein, the effective amount of the carboxylic acid derivative NSAID is about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 30 mg, about 35 mg, about 40 mg, about 45 mg, about 50 mg, about 55 mg, about 60 mg, about 65 mg, about 70 mg, about 75 mg, about 80 mg, about 85 mg, about 90 mg, about 95 mg, about 100 mg, about 150 mg, about 200 mg, about 250 mg, about 300 mg, about 350 mg, about 400 mg, about 450 mg, about 500 mg, about 550 mg, about 600 mg, about 650 mg,

about 700 mg, about 750 mg, about 800 mg, about 850 mg, about 900 mg, about 950 mg, about 1000 mg, about 1050 mg, about 1100 mg, about 1150 mg, about 1200 mg, about 1250 mg, about 1300 mg, about 1350 mg, about 1400 mg, about 1450 mg, or about 1500 mg.

**[00225]** In certain embodiments, the effective amount of the carboxylic acid derivative NSAID is administered as a bolus dose during the tumor resection surgery. In a further embodiment, the carboxylic acid derivative NSAID is administered as a bolus of 0.25 mg/kg-10 mg/kg. In some embodiments of the methods disclosed herein, the carboxylic acid derivative NSAID is administered as a bolus of about 0.25 mg/kg, about 0.5 mg/kg, about 0.75 mg/kg, about 1 mg/kg, about 1.5 mg/kg, about 2 mg/kg, about 2.5 mg/kg, about 3 mg/kg, about 3.5 mg/kg, about 4 mg/kg, about 4.5 mg/kg, about 5 mg/kg, about 5.5 mg/kg, about 6 mg/kg, about 6.5 mg/kg, about 7 mg/kg, about 7.5 mg/kg, about 8 mg/kg, about 8.5 mg/kg, about 9 mg/kg, about 9.5 mg/kg, or about 10 mg/kg.

**[00226]** Additionally or alternatively, in some embodiments, the effective amount of the acetaminophen is administered preoperatively, intraoperatively and/or postoperatively. In certain embodiments, the effective amount of the acetaminophen may range from about 80 mg to about 1000 mg. In some embodiments, the effective amount of the acetaminophen is about 80 mg, about 90 mg, about 100 mg, about 110 mg, about 120 mg, about 130 mg, about 140 mg, about 150 mg, about 160 mg, about 170 mg, about 180 mg, about 190 mg, about 200 mg, about 210 mg, about 220 mg, about 230 mg, about 240 mg, about 250 mg, about 260 mg, about 270 mg, about 280 mg, about 290 mg, about 300 mg, about 310 mg, about 320 mg, about 330 mg, about 340 mg, about 350 mg, about 360 mg, about 370 mg, about 380 mg, about 390 mg, about 400 mg, about 410 mg, about 420 mg, about 430 mg, about 440 mg, about 450 mg, about 460 mg, about 470 mg, about 480 mg, about 490 mg, about 500 mg, about 510 mg, about 520 mg, about 530 mg, about 540 mg, about 550 mg, about 560 mg, about 570 mg, about 580 mg, about 590 mg, about 600 mg, about 610 mg, about 620 mg, about 630 mg, about 640 mg, about 650 mg, about 660 mg, about 670 mg, about 680 mg, about 690 mg, about 700 mg, about 710 mg, about 720 mg, about 730 mg, about 740 mg, about 750 mg, about 760 mg, about 770 mg, about 780 mg, about 790 mg, about 800 mg, about 810 mg, about 820 mg, about 830 mg, about 840 mg, about 850 mg, about 860 mg, about 870 mg, about 880 mg, about 890 mg, about 900 mg, about 910 mg, about 920 mg, about 930 mg, about 940 mg, about 950 mg, about 960 mg, about 970 mg, about 980 mg, about 990 mg, or about 1000 mg. Additionally or alternatively, in some embodiments, the effective amount of the acetaminophen may be administered as a series of bolus doses, or as

a continuous infusion during the tumor resection surgery. In some embodiments, the acetaminophen is administered intravenously, or orally.

**[00227]** Additionally or alternatively, in some embodiments, the effective amount of dexmedetomidine may be administered intraoperatively, preoperatively, and/or postoperatively. In some embodiments, the dexmedetomidine is administered intravenously, transdermally, intramuscularly, orally, buccally, or intranasally.

**[00228]** Additionally or alternatively, in some embodiments, the dexmedetomidine is infused at a rate of 0.05 µg/kg/hr to 0.7 µg/kg/hr. In some embodiments of the methods disclosed herein, the dexmedetomidine is infused at a rate of about 0.05 µg/kg/hr, about 0.06 µg/kg/hr, about 0.07 µg/kg/hr, about 0.08 µg/kg/hr, about 0.09 µg/kg/hr, about 0.1 µg/kg/hr, 0.15 µg/kg/hr, about 0.2 µg/kg/hr, about 0.25 µg/kg/hr, about 0.3 µg/kg/hr, about 0.35 µg/kg/hr, about 0.4 µg/kg/hr, about 0.45 µg/kg/hr, about 0.5 µg/kg/hr, about 0.55 µg/kg/hr, about 0.6 µg/kg/hr, about 0.65 µg/kg/hr, or about 0.7 µg/kg/hr.

**[00229]** Additionally or alternatively, in some embodiments, the dexmedetomidine is administered as a bolus of 0.05 µg/kg – 1 µg/kg. In some embodiments of the methods disclosed herein, the dexmedetomidine is administered as a bolus of about 0.05 µg/kg, about 0.06 µg/kg, about 0.07 µg/kg, about 0.08 µg/kg, about 0.09 µg/kg, about 0.1 µg/kg, about 0.15 µg/kg, about 0.2 µg/kg, about 0.25 µg/kg, about 0.3 µg/kg, about 0.35 µg/kg, about 0.4 µg/kg, about 0.45 µg/kg, about 0.5 µg/kg, about 0.55 µg/kg, about 0.6 µg/kg, about 0.65 µg/kg, about 0.7 µg/kg, about 0.75 µg/kg, about 0.8 µg/kg, about 0.85 µg/kg, about 0.9 µg/kg, about 0.95 µg/kg, or about 1.0 µg/kg.

**[00230]** In one aspect, the present disclosure provides a method for selecting a cancer patient undergoing tumor resection surgery for colon cancer for treatment with an intraoperative opioid analgesic or intraoperative non-opioid analgesic comprising (a) assaying gene expression levels of MLH1, MSH2, MSH6, and PMS2 in a biological sample obtained from the cancer patient and (b) administering to the cancer patient an effective amount of an intraoperative opioid analgesic or intraoperative non-opioid analgesic during the tumor resection surgery, wherein the gene expression levels of MLH1, MSH2, MSH6, and PMS2 in the biological sample obtained from the cancer patient are comparable to that observed in a control sample obtained from a healthy subject or a predetermined threshold. In certain embodiments, expression levels of MLH1, MSH2, MSH6, and PMS2 are assayed via next-generation sequencing, PCR, quantitative PCR (qPCR), digital PCR (dPCR),

Reverse transcriptase-PCR (RT-PCR), Northern blotting, microarray, dot or slot blots, *in situ* hybridization, fluorescent *in situ* hybridization (FISH), RNA-seq, Western blotting, enzyme-linked immunosorbent assays (ELISA), dot blotting, immunohistochemistry, immunofluorescence, immunoprecipitation, immunoelectrophoresis, or mass-spectrometry.

5 Additionally or alternatively, in some embodiments, the biological sample comprises genomic DNA, cDNA, RNA, and/or mRNA. Additionally or alternatively, in some embodiments, the cancer patient comprises microsatellite instability-low (MSI-L) or MSS tumors.

**[00231]** In one aspect, the present disclosure provides a method for selecting a cancer  
 10 patient undergoing tumor resection surgery for colon cancer for treatment with an intraoperative opioid analgesic or intraoperative non-opioid analgesic comprising (a) assaying gene expression levels of one or more genes selected from among CCK, NTSR2, IFNG, NTSR1, MLN, LIPN, CXCL8, FCGR3B, PTGS2, CALB2, CXCL10, IL1RN, CCL5, CCL4, IL1B, CCL4L2, FCGR3A, ITIH4, GAD1, CD8A, HTR3A, SLC1A3, LHB, ADRB1,  
 15 CXCR2, HRH2, CCL3, ESR2, CSF2, ENO2, GRM5, CXCR4, PRKCG, CD68, IL10, TLR2, ICAM1, RGS2, CX3CL1 and KCNJ3 in a biological sample obtained from the cancer patient; and (b) administering to the cancer patient an effective amount of an intraoperative opioid analgesic or intraoperative non-opioid analgesic during the tumor resection surgery, wherein the gene expression levels of one or more of CCK, NTSR2, IFNG, NTSR1, MLN, LIPN,  
 20 CXCL8, FCGR3B, PTGS2, CALB2, CXCL10, IL1RN, CCL5, CCL4, IL1B, CCL4L2, FCGR3A, ITIH4, GAD1, CD8A, HTR3A, SLC1A3, LHB, ADRB1, CXCR2, HRH2, CCL3, ESR2, CSF2, ENO2, GRM5, CXCR4, PRKCG, CD68, IL10, TLR2, ICAM1, RGS2, CX3CL1 and KCNJ3 in the biological sample obtained from the cancer patient are comparable relative to a reference sample obtained from a colon cancer subject having  
 25 microsatellite stable (MSS) tumors or a predetermined threshold. In some embodiments, the gene expression levels of at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 31, at  
 30 least 32, at least 33, at least 34, at least 35, at least 36, at least 37, at least 38, at least 39, or 40 genes selected from among CCK, NTSR2, IFNG, NTSR1, MLN, LIPN, CXCL8, FCGR3B, PTGS2, CALB2, CXCL10, IL1RN, CCL5, CCL4, IL1B, CCL4L2, FCGR3A, ITIH4, GAD1, CD8A, HTR3A, SLC1A3, LHB, ADRB1, CXCR2, HRH2, CCL3, ESR2,

CSF2, ENO2, GRM5, CXCR4, PRKCG, CD68, IL10, TLR2, ICAM1, RGS2, CX3CL1 and KCNJ3 in the biological sample obtained from the cancer patient are comparable relative to a reference sample obtained from a colon cancer subject having microsatellite stable (MSS) tumors or a predetermined threshold. In certain embodiments, expression levels of CCK, NTSR2, IFNG, NTSR1, MLN, LIPN, CXCL8, FCGR3B, PTGS2, CALB2, CXCL10, IL1RN, CCL5, CCL4, IL1B, CCL4L2, FCGR3A, ITIH4, GAD1, CD8A, HTR3A, SLC1A3, LHB, ADRB1, CXCR2, HRH2, CCL3, ESR2, CSF2, ENO2, GRM5, CXCR4, PRKCG, CD68, IL10, TLR2, ICAM1, RGS2, CX3CL1 and KCNJ3 are assayed via next-generation sequencing, PCR, quantitative PCR (qPCR), digital PCR (dPCR), Reverse transcriptase-PCR (RT-PCR), Northern blotting, microarray, dot or slot blots, *in situ* hybridization, fluorescent *in situ* hybridization (FISH), RNA-seq, Western blotting, enzyme-linked immunosorbent assays (ELISA), dot blotting, immunohistochemistry, immunofluorescence, immunoprecipitation, immunoelectrophoresis, or mass-spectrometry. Additionally or alternatively, in some embodiments, the biological sample comprises genomic DNA, cDNA, RNA, and/or mRNA. Additionally or alternatively, in some embodiments, the cancer patient comprises microsatellite instability-low (MSI-L) or MSS tumors.

**[00232]** In one aspect, the present disclosure provides a method for selecting a cancer patient undergoing tumor resection surgery for colon cancer for treatment with an intraoperative opioid analgesic or intraoperative non-opioid analgesic comprising (a) assaying gene expression levels of one or more genes selected from among GHRH, RBFOX3, NOS1, KISS1, NPFFR2, GRIA2, SLC6A2, ADCY5, HCRT, OPCML, NPY1R, PPP1R14C, NTRK2, CHAT, LEP, CPB1, NR1I2, ADCY8, EGF, F2, UGT1A8, PLG, GRPR, PYY, DRD1, ABCB1, SCT, NTS, NPFFR1, TH, CYP2B6, HTR2C, TAC1, CCKBR, CALCA, ATP12A, LINC01411, P2RX3, ALB, REN, SLC6A4, KNG1 and DRD2 in a biological sample obtained from the cancer patient; and (b) administering to the cancer patient an effective amount of an intraoperative opioid analgesic or intraoperative non-opioid analgesic during the tumor resection surgery, wherein the gene expression levels of one or more of GHRH, RBFOX3, NOS1, KISS1, NPFFR2, GRIA2, SLC6A2, ADCY5, HCRT, OPCML, NPY1R, PPP1R14C, NTRK2, CHAT, LEP, CPB1, NR1I2, ADCY8, EGF, F2, UGT1A8, PLG, GRPR, PYY, DRD1, ABCB1, SCT, NTS, NPFFR1, TH, CYP2B6, HTR2C, TAC1, CCKBR, CALCA, ATP12A, LINC01411, P2RX3, ALB, REN, SLC6A4, KNG1 and DRD2 in the biological sample obtained from the cancer patient are comparable relative to a reference sample obtained from a colon cancer subject having microsatellite stable (MSS)

tumors or a predetermined threshold. In some embodiments, the gene expression levels of at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 31, at least 32, at least 33, at least 34, at least 35, at least 36, at least 37, at least 38, at least 39, at least 40, at least 41, at least 42, or 43 genes selected from among GHRH, RBFOX3, NOS1, KISS1, NPFFR2, GRIA2, SLC6A2, ADCY5, HCRT, OPCML, NPY1R, PPP1R14C, NTRK2, CHAT, LEP, CPB1, NR1I2, ADCY8, EGF, F2, UGT1A8, PLG, GRPR, PYY, DRD1, ABCB1, SCT, NTS, NPFFR1, TH, CYP2B6, HTR2C, TAC1, CCKBR, CALCA, ATP12A, LINC01411, P2RX3, ALB, REN, SLC6A4, KNG1 and DRD2 in the biological sample obtained from the cancer patient are comparable relative to a reference sample obtained from a colon cancer subject having microsatellite stable (MSS) tumors or a predetermined threshold. In certain embodiments, expression levels of GHRH, RBFOX3, NOS1, KISS1, NPFFR2, GRIA2, SLC6A2, ADCY5, HCRT, OPCML, NPY1R, PPP1R14C, NTRK2, CHAT, LEP, CPB1, NR1I2, ADCY8, EGF, F2, UGT1A8, PLG, GRPR, PYY, DRD1, ABCB1, SCT, NTS, NPFFR1, TH, CYP2B6, HTR2C, TAC1, CCKBR, CALCA, ATP12A, LINC01411, P2RX3, ALB, REN, SLC6A4, KNG1 and DRD2 are assayed via next-generation sequencing, PCR, quantitative PCR (qPCR), digital PCR (dPCR), Reverse transcriptase-PCR (RT-PCR), Northern blotting, microarray, dot or slot blots, *in situ* hybridization, fluorescent *in situ* hybridization (FISH), RNA-seq, Western blotting, enzyme-linked immunosorbent assays (ELISA), dot blotting, immunohistochemistry, immunofluorescence, immunoprecipitation, immunoelectrophoresis, or mass-spectrometry. Additionally or alternatively, in some embodiments, the biological sample comprises genomic DNA, cDNA, RNA, and/or mRNA. Additionally or alternatively, in some embodiments, the cancer patient comprises microsatellite instability-low (MSI-L) or MSS tumors.

**[00233]** In another aspect, the present disclosure provides a method for selecting a cancer patient undergoing tumor resection surgery for colon cancer for treatment with an intraoperative opioid analgesic or intraoperative non-opioid analgesic comprising (a) detecting the expression levels or activity of one or more genes that result (i) in hyperactivation of Wnt pathway and/or (ii) reduced activity of Th1 immune response signature in a biological sample obtained from the cancer patient relative to a predetermined threshold; and (b) administering to the cancer patient an effective amount of an intraoperative

opioid analgesic or intraoperative non-opioid analgesic during the tumor resection surgery. Additionally or alternatively, in some embodiments, the cancer patient comprises at least one mutation that results in hyperactivation of Wnt pathway and/or (ii) reduced activity of Th1 immune response signature. Additionally or alternatively, in some embodiments, the at least one mutation is a frameshift mutation, a missense mutation, a deletion, an insertion, a nonsense mutation, an inversion, or a translocation. The at least one mutation may be detected using any nucleic acid detection assay known in the art such as next-generation sequencing, PCR, real-time quantitative PCR (qPCR), digital PCR (dPCR), Southern blotting, Reverse transcriptase-PCR (RT-PCR), Northern blotting, microarray, dot or slot blots, *in situ* hybridization, or fluorescent *in situ* hybridization (FISH). In some embodiments, the biological sample comprises genomic DNA, cDNA, RNA, and/or mRNA. Additionally or alternatively, in some embodiments, the cancer patient comprises microsatellite instability-low (MSI-L) or MSS tumors.

**[00234]** Additionally or alternatively, in some embodiments, the hyperactivation of Wnt pathway is determined by detecting the expression levels or activity of one or more genes selected from among ADAM17, AXIN1, AXIN2, CCND2, CSNK1E, CTNNB1, CUL1, DKK1, DKK4, DLL1, DVL2, FRAT1, FZD1, FZD8, GNAI1, HDAC11, HDAC2, HDAC5, HEY1, HEY2, JAG1, JAG2, KAT2A, LEF1, MAML1, MYC, NCOR2, NCSTN, NKD1, NOTCH1, NOTCH4, NUMB, PPARD, PSEN2, PTCH1, RBPJ, SKP2, TCF7, TP53, WNT1, WNT5B, and WNT6. In certain embodiments, the hyperactivation of Wnt pathway is determined by detecting the expression levels or activity of at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 31, at least 32, at least 33, at least 34, at least 35, at least 36, at least 37, at least 38, at least 39, at least 40, at least 41, or 42 genes selected from among ADAM17, AXIN1, AXIN2, CCND2, CSNK1E, CTNNB1, CUL1, DKK1, DKK4, DLL1, DVL2, FRAT1, FZD1, FZD8, GNAI1, HDAC11, HDAC2, HDAC5, HEY1, HEY2, JAG1, JAG2, KAT2A, LEF1, MAML1, MYC, NCOR2, NCSTN, NKD1, NOTCH1, NOTCH4, NUMB, PPARD, PSEN2, PTCH1, RBPJ, SKP2, TCF7, TP53, WNT1, WNT5B, and WNT6. In certain embodiments, the expression levels of one or more genes selected from among ADAM17, AXIN1, AXIN2, CCND2, CSNK1E, CTNNB1, CUL1, DKK1, DKK4, DLL1, DVL2, FRAT1, FZD1, FZD8, GNAI1, HDAC11, HDAC2, HDAC5, HEY1, HEY2, JAG1,

JAG2, KAT2A, LEF1, MAML1, MYC, NCOR2, NCSTN, NKD1, NOTCH1, NOTCH4, NUMB, PPARD, PSEN2, PTCH1, RBPJ, SKP2, TCF7, TP53, WNT1, WNT5B, and WNT6 are detected via next-generation sequencing, PCR, quantitative PCR (qPCR), digital PCR (dPCR), Reverse transcriptase-PCR (RT-PCR), Northern blotting, microarray, dot or slot blots, *in situ* hybridization, fluorescent *in situ* hybridization (FISH), RNA-seq, Western blotting, enzyme-linked immunosorbent assays (ELISA), dot blotting, immunohistochemistry, immunofluorescence, immunoprecipitation, immunoelectrophoresis, or mass-spectrometry. Additionally or alternatively, in some embodiments, the biological sample comprises genomic DNA, cDNA, RNA, and/or mRNA.

**[00235]** Additionally or alternatively, in some embodiments, the reduced activity of Th1 immune response signature is determined by detecting the expression levels or activity of one or more genes selected from among IFNG, LTA, APBB2, DOK5, IL12RB2, APOD, ZBTB32, CD38, CSF2, CTLA4, CD70, DPP4, EGFL6, BST2, DUSP5, LRP8, IL22, DGKI, CCL4, GGT1, LRRN3, SYNGR3, ATP9A, BTG3, CMAHP, HBEGF, and SGCB. In certain embodiments, the reduced activity of Th1 immune response signature is determined by detecting the expression levels or activity of at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, or 27 genes selected from among IFNG, LTA, APBB2, DOK5, IL12RB2, APOD, ZBTB32, CD38, CSF2, CTLA4, CD70, DPP4, EGFL6, BST2, DUSP5, LRP8, IL22, DGKI, CCL4, GGT1, LRRN3, SYNGR3, ATP9A, BTG3, CMAHP, HBEGF, and SGCB. In certain embodiments, the expression levels of one or more genes selected from among IFNG, LTA, APBB2, DOK5, IL12RB2, APOD, ZBTB32, CD38, CSF2, CTLA4, CD70, DPP4, EGFL6, BST2, DUSP5, LRP8, IL22, DGKI, CCL4, GGT1, LRRN3, SYNGR3, ATP9A, BTG3, CMAHP, HBEGF, and SGCB are detected via next-generation sequencing, PCR, quantitative PCR (qPCR), digital PCR (dPCR), Reverse transcriptase-PCR (RT-PCR), Northern blotting, microarray, dot or slot blots, *in situ* hybridization, fluorescent *in situ* hybridization (FISH), RNA-seq, Western blotting, enzyme-linked immunosorbent assays (ELISA), dot blotting, immunohistochemistry, immunofluorescence, immunoprecipitation, immunoelectrophoresis, or mass-spectrometry. Additionally or alternatively, in some embodiments, the biological sample comprises genomic DNA, cDNA, RNA, and/or mRNA.

**[00236]** In one aspect, the present disclosure provides a method for reducing the risk of tumor recurrence in a cancer patient undergoing tumor resection surgery for colon cancer comprising administering to the cancer patient an effective amount of an intraoperative opioid analgesic or intraoperative non-opioid analgesic during the tumor resection surgery, wherein the cancer patient does not comprise any alterations in DNA mismatch repair (MMR) system. In some embodiments, mRNA or polypeptide expression levels of MLH1, MSH2, MSH6, and PMS2 in a biological sample obtained from the cancer patient are comparable relative to that observed in a control sample obtained from a healthy subject or a predetermined threshold. Additionally or alternatively, in some embodiments, the mRNA or polypeptide expression levels of one or more of CCK, NTSR2, IFNG, NTSR1, MLN, LIPN, CXCL8, FCGR3B, PTGS2, CALB2, CXCL10, IL1RN, CCL5, CCL4, IL1B, CCL4L2, FCGR3A, ITIH4, GAD1, CD8A, HTR3A, SLC1A3, LHB, ADRB1, CXCR2, HRH2, CCL3, ESR2, CSF2, ENO2, GRM5, CXCR4, PRKCG, CD68, IL10, TLR2, ICAM1, RGS2, CX3CL1 and KCNJ3 in a biological sample obtained from the cancer patient are comparable relative to a reference sample obtained from a colon cancer subject having microsatellite stable (MSS) tumors or a predetermined threshold. Additionally or alternatively, in some embodiments, the mRNA or polypeptide levels of one or more of GHRH, RBFOX3, NOS1, KISS1, NPFFR2, GRIA2, SLC6A2, ADCY5, HCRT, OPCML, NPY1R, PPP1R14C, NTRK2, CHAT, LEP, CPB1, NR1I2, ADCY8, EGF, F2, UGT1A8, PLG, GRPR, PYY, DRD1, ABCB1, SCT, NTS, NPFFR1, TH, CYP2B6, HTR2C, TAC1, CCKBR, CALCA, ATP12A, LINC01411, P2RX3, ALB, REN, SLC6A4, KNG1 and DRD2 in the biological sample obtained from the cancer patient are comparable relative to a reference sample obtained from a colon cancer subject having microsatellite stable (MSS) tumors or a predetermined threshold. Additionally or alternatively, in some embodiments, the cancer patient comprises at least one genetic mutation or altered expression levels or activity of one or more genes that result (i) in hyperactivation of Wnt pathway and/or (ii) reduced activity of Th1 immune response signature. In some embodiments, the hyperactivation of Wnt pathway is determined by detecting the expression levels or activity of one or more genes selected from among ADAM17, AXIN1, AXIN2, CCND2, CSNK1E, CTNNB1, CUL1, DKK1, DKK4, DLL1, DVL2, FRAT1, FZD1, FZD8, GNAI1, HDAC11, HDAC2, HDAC5, HEY1, HEY2, JAG1, JAG2, KAT2A, LEF1, MAML1, MYC, NCOR2, NCSTN, NKD1, NOTCH1, NOTCH4, NUMB, PPARD, PSEN2, PTCH1, RBPJ, SKP2, TCF7, TP53, WNT1, WNT5B, and WNT6. In other embodiments, the reduced activity of Th1 immune response signature is determined by detecting the expression levels or activity of one or more genes selected from among IFNG,

LTA, APBB2, DOK5, IL12RB2, APOD, ZBTB32, CD38, CSF2, CTLA4, CD70, DPP4, EGFL6, BST2, DUSP5, LRP8, IL22, DGKI, CCL4, GGT1, LRRN3, SYNGR3, ATP9A, BTG3, CMAHP, HBEGF, and SGCB. Additionally or alternatively, in some embodiments, the cancer patient comprises microsatellite instability-low (MSI-L) or MSS tumors.

**[00237]** Additionally or alternatively, in some embodiments of the preceding embodiments, genetic mutations or mRNA expression levels are detected via next generation sequencing, RNA-seq, real-time quantitative PCR (qPCR), digital PCR (dPCR), Reverse transcriptase-PCR (RT-PCR), Northern blotting, microarray, dot or slot blots, *in situ* hybridization, or fluorescent *in situ* hybridization (FISH). Additionally or alternatively, in certain  
 10   embodiments, polypeptide expression levels are detected via Western blotting, enzyme-linked immunosorbent assays (ELISA), dot blotting, immunohistochemistry, immunofluorescence, immunoprecipitation, immunoelectrophoresis, or mass-spectrometry.

**[00238]** The intraoperative opioid analgesic may be fentanyl, hydromorphone, morphine, oxycodone, hydrocodone, codeine, meperidine, remifentanyl, or sufentanyl. The effective  
 15   amount of the intraoperative opioid analgesic may range from about 1 MME to about 200 MMEs. In certain embodiments, the effective amount of the intraoperative opioid analgesic is about 1 MME to about 20 MMEs, about 20 MMEs to about 45 MMEs, or about 45 MMEs to about 200 MMEs. In some embodiments, the effective amount of the intraoperative opioid analgesic is about 1 MME, about 2 MMEs, about 3 MMEs, about 4 MMEs, about 5 MMEs,  
 20   about 6 MMEs, about 7 MMEs, about 8 MMEs, about 9 MMEs, about 10 MMEs, about 11 MMEs, about 12 MMEs, about 13 MMEs, about 14 MMEs, about 15 MMEs, about 16 MMEs, about 17 MMEs, about 18 MMEs, about 19 MMEs, about 20 MMEs, about 21 MMEs, about 22 MMEs, about 23 MMEs, about 24 MMEs, about 25 MMEs, about 26 MMEs, about 27 MMEs, about 28 MMEs, about 29 MMEs, about 30 MMEs, about 31 MMEs, about 32 MMEs,  
 25   about 33 MMEs, about 34 MMEs, about 35 MMEs, about 36 MMEs, about 37 MMEs, about 38 MMEs, about 39 MMEs, about 40-45 MMEs, about 45-50 MMEs, about 50-55 MMEs, about 55-60 MMEs, about 60-65 MMEs, about 65-70 MMEs, about 70-75 MMEs, about 75-80 MMEs, about 80-85 MMEs, about 85-90 MMEs, about 90-95 MMEs, about 95-100 MMEs, about 100-110 MMEs, about 110-120 MMEs, about 120-130 MMEs, about 130-140 MMEs, about 140-150 MMEs, about 150-160 MMEs, about 160-170 MMEs, about 170-180 MMEs, about 180-190 MMEs, or about 190-200 MMEs. Additionally or alternatively, in some embodiments, the effective amount of the intraoperative opioid analgesic is administered as a series of bolus doses or as a continuous infusion during the tumor resection  
 30

surgery. In certain embodiments, the effective amount of the intraoperative opioid analgesic is administered to the cancer patient prior to incision. Additionally or alternatively, in some embodiments, the effective amount of the intraoperative opioid analgesic is administered intravenously.

5 **[00239]** Examples of non-opioid analgesics include regional anesthetics, ketamine, dexmedetomidine, a carboxylic acid derivative NSAID, or acetaminophen.

**[00240]** Additionally or alternatively, in some embodiments, the methods of the present technology further comprise administering to the cancer patient an effective amount of a regional anesthetic solution via an epidural catheter before, during and/or after the tumor  
10 resection surgery. The effective amount of the regional anesthetic solution may range from about 0.05%-4% regional anesthetic solution in a volume of 1-10 ml per hour when administered via an epidural catheter. In some embodiments, the effective amount of the regional anesthetic solution is about 0.05 %, about 0.06 %, about 0.07 %, about 0.08 %, about 0.09 %, about 0.1 %, about 0.15 %, about 0.2 %, about 0.25 %, about 0.3 %, about 0.35 %, about 0.4 %, about 0.45 %, about 0.5 %, about 0.55 %, about 0.6 %, about 0.65 %, about 0.7  
15 %, about 0.75 %, about 0.8 %, about 0.85 %, about 0.9 %, about 0.95 %, about 1.0 %, about 1.1 %, about 1.2 %, about 1.3 %, about 1.4 %, about 1.5 %, about 1.6 %, about 1.7 %, about 1.8 %, about 1.9 %, about 2.0 %, about 2.1 %, about 2.2 %, about 2.3 %, about 2.4 %, about 2.5 %, about 2.6 %, about 2.7 %, about 2.8 %, about 2.9 %, about 3.0 %, about 3.1 %, about 3.2 %, about 3.3 %, about 3.4 %, about 3.5 %, about 3.6 %, about 3.7 %, about 3.8 %, about  
20 3.9 %, or about 4.0 % regional anesthetic solution in a volume of about 1 ml per hour, about 1.5 ml per hour, about 2 ml per hour, about 2.5 ml per hour, about 3 ml per hour, about 3.5 ml per hour, about 4 ml per hour, about 4.5 ml per hour, about 5 ml per hour, about 5.5 ml per hour, about 6 ml per hour, about 6.5 ml per hour, about 7 ml per hour, about 7.5 ml per hour, about 8 ml per hour, about 8.5 ml per hour, about 9 ml per hour, about 9.5 ml per hour, or about 10 ml per hour when administered via an epidural catheter. Additionally or alternatively, in some embodiments, the effective amount of the regional anesthetic solution is administered as a single injection, series of bolus doses or as a continuous infusion during the tumor resection surgery. Examples of suitable regional anesthetics include, but are not  
30 limited to, lidocaine, mepivacaine, prilocaine, bupivacaine, etidocaine, ropivacaine, levobupivacaine, cocaine, procaine, tetracaine, chlorprocaine, and benzocaine.

**[00241]** In other embodiments, the effective amount of the regional anesthetic solution may be administered before, during and/or after the tumor resection surgery using any

suitable regional anesthesia technique for colon cancer (*e.g.*, transversus abdominis plane (TAP) blocks, Ilioinguinal (II) and iliohypogastric (IH) blocks, Rectus sheath blocks, Transversalis fascia plane blocks, quadratus lumborum blocks). The effective amount of the regional anesthetic solution may range from about 0.05%-4% regional anesthetic solution in a volume of 10-40 ml when administered using any suitable regional anesthesia technique for colon cancer (*e.g.*, transversus abdominis plane (TAP) blocks, Ilioinguinal (II) and iliohypogastric (IH) blocks, Rectus sheath blocks, Transversalis fascia plane blocks, quadratus lumborum blocks). In some embodiments, the effective amount of the regional anesthetic solution is about 0.05 %, about 0.06 %, about 0.07 %, about 0.08 %, about 0.09 %, about 0.1 %, about 0.15 %, about 0.2 %, about 0.25 %, about 0.3 %, about 0.35 %, about 0.4 %, about 0.45 %, about 0.5 %, about 0.55 %, about 0.6 %, about 0.65 %, about 0.7 %, about 0.75 %, about 0.8 %, about 0.85 %, about 0.9 %, about 0.95 %, about 1.0 %, about 1.1 %, about 1.2 %, about 1.3 %, about 1.4 %, about 1.5 %, about 1.6 %, about 1.7 %, about 1.8 %, about 1.9 %, about 2.0 %, about 2.1 %, about 2.2 %, about 2.3 %, about 2.4 %, about 2.5 %, about 2.6 %, about 2.7 %, about 2.8 %, about 2.9 %, about 3.0 %, about 3.1 %, about 3.2 %, about 3.3 %, about 3.4 %, about 3.5 %, about 3.6 %, about 3.7 %, about 3.8 %, about 3.9 %, or about 4.0 % regional anesthetic solution in a volume of about 10 ml, about 12.5 ml, about 15 ml, about 17.5 ml, about 20 ml, about 22.5 ml, about 25 ml, about 27.5 ml, about 30 ml, about 32.5 ml, about 35 ml, about 37.5 ml, or about 40 ml when administered using any suitable regional anesthesia technique for colon cancer (*e.g.*, transversus abdominis plane (TAP) blocks, Ilioinguinal (II) and iliohypogastric (IH) blocks, Rectus sheath blocks, Transversalis fascia plane blocks, quadratus lumborum blocks). Examples of suitable regional anesthetics include, but are not limited to, lidocaine, mepivacaine, prilocaine, bupivacaine, etidocaine, ropivacaine, levobupivacaine, cocaine, procaine, tetracaine, chloroprocaine, and benzocaine.

**[00242]** Additionally or alternatively, in certain embodiments, the regional anesthetic solution may further comprise an opioid (*e.g.*, fentanyl, hydromorphone, morphine, oxycodone, hydrocodone, codeine, meperidine, remifentanyl, or sufentanyl). In some embodiments, the regional anesthetic solution may comprise 0.5 mcg/ml-50 mcg/ml opioid. In certain embodiments, the regional anesthetic solution may comprise about 0.5 mcg/ml, about 0.6 mcg/ml, about 0.7 mcg/ml, about 0.8 mcg/ml, about 0.9 mcg/ml, about 1.0 mcg/ml, about 1.5 mcg/ml, about 2.0 mcg/ml, about 2.5 mcg/ml, about 3.0 mcg/ml, about 3.5 mcg/ml, about 4.0 mcg/ml, about 4.5 mcg/ml, about 5.0 mcg/ml, about 5.5 mcg/ml, about 6.0 mcg/ml,

about 6.5 mcg/ml, about 7.0 mcg/ml, about 7.5 mcg/ml, about 8.0 mcg/ml, about 8.5 mcg/ml, about 9.0 mcg/ml, about 10 mcg/ml, about 15 mcg/ml, about 20 mcg/ml, about 25 mcg/ml, about 30 mcg/ml, about 35 mcg/ml, about 40 mcg/ml, about 45 mcg/ml, or about 50 mcg/ml.

**[00243]** Additionally or alternatively, in certain embodiments, the methods of the present technology further comprise administering to the cancer patient an effective amount of a post-operative opioid analgesic after the tumor resection surgery. Examples of post-operative opioid analgesics include, but are not limited to, fentanyl, hydromorphone, morphine, oxycodone, hydrocodone, codeine, meperidine, remifentanyl, or sufentanyl. In some embodiments, the post-operative opioid analgesic and the intraoperative opioid analgesic are the same opioid analgesic or different opioid analgesics. In other embodiments, the effective amount of the post-operative opioid analgesic and the effective amount of the intraoperative opioid analgesic are the same or different. Additionally or alternatively, in some embodiments, the effective amount of the post-operative opioid analgesic is administered to the cancer patient as a bolus of about 0.005 mg to about 100 mg. In some embodiments, the effective amount of the post-operative opioid analgesic is administered to the cancer patient as a bolus of about 0.005 mg, about 0.006 mg, about 0.007 mg, about 0.008 mg, about 0.009 mg, about 0.01 mg, about 0.02 mg, about 0.03 mg, about 0.04 mg, about 0.05 mg, about 0.06 mg, about 0.07 mg, about 0.08 mg, about 0.09 mg, about 0.1 mg, about 0.2 mg, about 0.3 mg, about 0.4 mg, about 0.5 mg, about 0.6 mg, about 0.7 mg, about 0.8 mg, about 0.9 mg, about 1-5 mg, about 5-10 mg, about 1-5 mg, about 5-10 mg, about 1-5 mg, about 5-10 mg, about 1-5 mg, about 5-10 mg, about 10-15 mg, about 15-20 mg, about 20-25 mg, about 25-30 mg, about 30-35 mg, about 35-40 mg, about 40-45 mg, about 45-50 mg, about 50-55 mg, about 55-60 mg, about 60-65 mg, about 65-70 mg, about 70-75 mg, about 75-80 mg, about 80-85 mg, about 85-90 mg, about 90-95 mg, or about 95-100 mg. In other embodiments, the effective amount of the post-operative opioid analgesic may be continuously delivered to the cancer patient at a per hour rate of about 0.01 mg/hr to about 10 mg/hr. In certain embodiments, the effective amount of the post-operative opioid analgesic is continuously delivered to the cancer patient at a per hour rate of about 0.01 mg/hr, about 0.02 mg/hr, about 0.03 mg/hr, about 0.04 mg/hr, about 0.05 mg/hr, about 0.06 mg/hr, about 0.07 mg/hr, about 0.08 mg/hr, about 0.09 mg/hr, about 0.1 mg/hr, about 0.2 mg/hr, about 0.3 mg/hr, about 0.4 mg/hr, about 0.5 mg/hr, about 0.6 mg/hr, about 0.7 mg/hr, about 0.8 mg/hr, about 0.9 mg/hr, about 1 mg/hr, about 1.5 mg/hr, about 2 mg/hr, about 2.5 mg/hr, about 3 mg/hr, about 3.5 mg/hr, about 4 mg/hr, about 4.5 mg/hr, about 5 mg/hr, about 5.5 mg/hr, about 6 mg/hr, about

6.5 mg/hr, about 7 mg/hr, about 7.5 mg/hr, about 8 mg/hr, about 8.5 mg/hr, about 9 mg/hr, about 9.5 mg/hr, or about 10 mg/hr. Additionally or alternatively, in some embodiments, the effective amount of the post-operative opioid analgesic is administered intravenously, orally, or transdermally.

5 [00244] Additionally or alternatively, in some embodiments, the ketamine is administered intravenously. The effective amount of ketamine may be administered intraoperatively, preoperatively, and/or postoperatively.

[00245] Additionally or alternatively, in some embodiments, the ketamine is infused at a rate of 0.04 mg/kg/hr-2.5 mg/kg/hr. In some embodiments of the methods disclosed herein,  
10 the ketamine is infused at a rate of about 0.04 mg/kg/hr, about 0.05 mg/kg/hr, about 0.06 mg/kg/hr, about 0.07 mg/kg/hr, about 0.08 mg/kg/hr, about 0.09 mg/kg/hr, about 0.1 mg/kg/hr, about 0.2 mg/kg/hr, about 0.3 mg/kg/hr, about 0.4 mg/kg/hr, about 0.5 mg/kg/hr, about 0.6 mg/kg/hr, about 0.7 mg/kg/hr, about 0.8 mg/kg/hr, about 0.9 mg/kg/hr, about 1.0 mg/kg/hr, about 1.1 mg/kg/hr, about 1.2 mg/kg/hr, about 1.3 mg/kg/hr, about 1.4 mg/kg/hr,  
15 about 1.5 mg/kg/hr, about 1.6 mg/kg/hr, about 1.7 mg/kg/hr, about 1.8 mg/kg/hr, about 1.9 mg/kg/hr, about 2.0 mg/kg/hr, about 2.1 mg/kg/hr, about 2.2 mg/kg/hr, about 2.3 mg/kg/hr, about 2.4 mg/kg/hr, or about 2.5 mg/kg/hr.

[00246] Additionally or alternatively, in some embodiments, the ketamine is administered as a bolus of 0.5 mg/kg -4.5 mg/kg. In some embodiments of the methods disclosed herein,  
20 the ketamine is administered as a bolus of about 0.04 mg/kg, about 0.05 mg/kg, about 0.06 mg/kg, about 0.07 mg/kg, about 0.08 mg/kg, about 0.09 mg/kg, about 0.1 mg/kg, about 0.2 mg/kg, about 0.3 mg/kg, about 0.4 mg/kg, about 0.5 mg/kg, about 0.6 mg/kg, about 0.7 mg/kg, about 0.8 mg/kg, about 0.9 mg/kg, about 1.0 mg/kg, about 1.1 mg/kg, about 1.2 mg/kg, about 1.3 mg/kg, about 1.4 mg/kg, about 1.5 mg/kg, about 1.6 mg/kg, about 1.7 mg/kg, about 1.8 mg/kg, about 1.9 mg/kg, about 2.0 mg/kg, about 2.1 mg/kg, about 2.2 mg/kg, about 2.3 mg/kg,  
25 about 2.4 mg/kg, about 2.5 mg/kg, about 2.6 mg/kg, about 2.7 mg/kg, about 2.8 mg/kg, about 2.9 mg/kg, about 3.0 mg/kg, about 3.1 mg/kg, about 3.2 mg/kg, about 3.3 mg/kg, about 3.4 mg/kg, about 3.5 mg/kg, about 3.6 mg/kg, about 3.7 mg/kg, about 3.8 mg/kg, about 3.9 mg/kg, about 4.0 mg/kg, about 4.1 mg/kg, about 4.2 mg/kg, about 4.3 mg/kg, about 4.4 mg/kg, or  
30 about 4.5 mg/kg.

[00247] Additionally or alternatively, in some embodiments, the ketamine is administered intramuscularly at a dose of about 4-13 mg/kg. In certain embodiments of the methods

disclosed herein, the ketamine is administered intramuscularly at a dose of 4 mg/kg, 5 mg/kg, 6 mg/kg, 7 mg/kg, 8 mg/kg, 9 mg/kg, 10 mg/kg, 11 mg/kg, 12 mg/kg, or 13 mg/kg. Additionally or alternatively, in some embodiments, the ketamine is administered orally at a dose of about 6-10 mg/kg. In certain embodiments of the methods disclosed herein, the ketamine is administered orally at a dose of 6 mg/kg, 7 mg/kg, 8 mg/kg, 9 mg/kg, or 10 mg/kg.

**[00248]** In any and all of the preceding embodiments of methods disclosed herein, the carboxylic acid derivative NSAID is an acetic acid NSAID or a propionic acid NSAID. Examples of the carboxylic acid derivative NSAID include, but are not limited to, ibuprofen, dexibuprofen, naproxen, fenoprofen, ketoprofen, dexketoprofen, flurbiprofen, oxaprozin, loxoprofen, pelubiprofen, zaltoprofen, indomethacin, tolmetin, sulindac, etodolac, ketorolac, diclofenac, aceclofenac, bromfenac, or nabumeton. In some embodiments, the carboxylic acid derivative NSAID is administered orally, intravenously, or intramuscularly. Additionally or alternatively, in some embodiments, the effective amount of the carboxylic acid derivative NSAID is administered intraoperatively and/or postoperatively. The effective amount of the carboxylic acid derivative NSAID may be administered to the cancer patient during closing of an incision.

**[00249]** Additionally or alternatively, in some embodiments of the methods disclosed herein, the effective amount of the carboxylic acid derivative NSAID is about 10 mg-1500 mg. In some embodiments of the methods disclosed herein, the effective amount of the carboxylic acid derivative NSAID is about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 30 mg, about 35 mg, about 40 mg, about 45 mg, about 50 mg, about 55 mg, about 60 mg, about 65 mg, about 70 mg, about 75 mg, about 80 mg, about 85 mg, about 90 mg, about 95 mg, about 100 mg, about 150 mg, about 200 mg, about 250 mg, about 300 mg, about 350 mg, about 400 mg, about 450 mg, about 500 mg, about 550 mg, about 600 mg, about 650 mg, about 700 mg, about 750 mg, about 800 mg, about 850 mg, about 900 mg, about 950 mg, about 1000 mg, about 1050 mg, about 1100 mg, about 1150 mg, about 1200 mg, about 1250 mg, about 1300 mg, about 1350 mg, about 1400 mg, about 1450 mg, or about 1500 mg.

**[00250]** In certain embodiments, the effective amount of the carboxylic acid derivative NSAID is administered as a bolus dose during the tumor resection surgery. In a further embodiment, the carboxylic acid derivative NSAID is administered as a bolus of 0.25 mg/kg-10 mg/kg. In some embodiments of the methods disclosed herein, the carboxylic acid derivative NSAID is administered as a bolus of about 0.25 mg/kg, about 0.5 mg/kg, about 0.75 mg/kg, about 1 mg/kg, about 1.5 mg/kg, about 2 mg/kg, about 2.5 mg/kg, about 3 mg/kg,

about 3.5 mg/kg, about 4 mg/kg, about 4.5 mg/kg, about 5 mg/kg, about 5.5 mg/kg, about 6 mg/kg, about 6.5 mg/kg, about 7 mg/kg, about 7.5 mg/kg, about 8 mg/kg, about 8.5 mg/kg, about 9 mg/kg, about 9.5 mg/kg, or about 10 mg/kg.

**[00251]** Additionally or alternatively, in some embodiments, the effective amount of the acetaminophen is administered preoperatively, intraoperatively and/or postoperatively. In certain embodiments, the effective amount of the acetaminophen may range from about 80 mg to about 1000 mg. In some embodiments, the effective amount of the acetaminophen is about 80 mg, about 90 mg, about 100 mg, about 110 mg, about 120 mg, about 130 mg, about 140 mg, about 150 mg, about 160 mg, about 170 mg, about 180 mg, about 190 mg, about 200 mg, about 210 mg, about 220 mg, about 230 mg, about 240 mg, about 250 mg, about 260 mg, about 270 mg, about 280 mg, about 290 mg, about 300 mg, about 310 mg, about 320 mg, about 330 mg, about 340 mg, about 350 mg, about 360 mg, about 370 mg, about 380 mg, about 390 mg, about 400 mg, about 410 mg, about 420 mg, about 430 mg, about 440 mg, about 450 mg, about 460 mg, about 470 mg, about 480 mg, about 490 mg, about 500 mg, about 510 mg, about 520 mg, about 530 mg, about 540 mg, about 550 mg, about 560 mg, about 570 mg, about 580 mg, about 590 mg, about 600 mg, about 610 mg, about 620 mg, about 630 mg, about 640 mg, about 650 mg, about 660 mg, about 670 mg, about 680 mg, about 690 mg, about 700 mg, about 710 mg, about 720 mg, about 730 mg, about 740 mg, about 750 mg, about 760 mg, about 770 mg, about 780 mg, about 790 mg, about 800 mg, about 810 mg, about 820 mg, about 830 mg, about 840 mg, about 850 mg, about 860 mg, about 870 mg, about 880 mg, about 890 mg, about 900 mg, about 910 mg, about 920 mg, about 930 mg, about 940 mg, about 950 mg, about 960 mg, about 970 mg, about 980 mg, about 990 mg, or about 1000 mg. Additionally or alternatively, in some embodiments, the effective amount of the acetaminophen may be administered as a series of bolus doses, or as a continuous infusion during the tumor resection surgery. In some embodiments, the acetaminophen is administered intravenously, or orally.

**[00252]** Additionally or alternatively, in some embodiments, the effective amount of dexmedetomidine may be administered intraoperatively, preoperatively, and/or postoperatively. In some embodiments, the dexmedetomidine is administered intravenously, transdermally, intramuscularly, orally, buccally, or intranasally.

**[00253]** Additionally or alternatively, in some embodiments, the dexmedetomidine is infused at a rate of 0.05 µg/kg/hr to 0.7 µg/kg/hr. In some embodiments of the methods disclosed herein, the dexmedetomidine is infused at a rate of about 0.05 µg/kg/hr, about 0.06

μg/kg/hr, about 0.07 μg/kg/hr, about 0.08 μg/kg/hr, about 0.09 μg/kg/hr, about 0.1 μg/kg/hr, about 0.15 μg/kg/hr, about 0.2 μg/kg/hr, about 0.25 μg/kg/hr, about 0.3 μg/kg/hr, about 0.35 μg/kg/hr, about 0.4 μg/kg/hr, about 0.45 μg/kg/hr, about 0.5 μg/kg/hr, about 0.55 μg/kg/hr, about 0.6 μg/kg/hr, about 0.65 μg/kg/hr, or about 0.7 μg/kg/hr.

5 [00254] Additionally or alternatively, in some embodiments, the dexmedetomidine is administered as a bolus of 0.05 μg/kg – 1 μg/kg. In some embodiments of the methods disclosed herein, the dexmedetomidine is administered as a bolus of about 0.05 μg/kg, about 0.06 μg/kg, about 0.07 μg/kg, about 0.08 μg/kg, about 0.09 μg/kg, about 0.1 μg/kg, about 0.15 μg/kg, about 0.2 μg/kg, about 0.25 μg/kg, about 0.3 μg/kg, about 0.35 μg/kg, about 0.4  
10 μg/kg, about 0.45 μg/kg, about 0.5 μg/kg, about 0.55 μg/kg, about 0.6 μg/kg, about 0.65 μg/kg, about 0.7 μg/kg, about 0.75 μg/kg, about 0.8 μg/kg, about 0.85 μg/kg, about 0.9 μg/kg, about 0.95 μg/kg, or about 1.0 μg/kg.

[00255] In any and all embodiments of the methods disclosed herein, the cancer patient exhibits stage I, stage II or stage III colon cancer. Additionally or alternatively, in some  
15 embodiments, the cancer patient has been diagnosed with colon adenocarcinoma (COAD). The colon adenocarcinoma may be synchronous or non-synchronous. The tumor surgery may be laparoscopic, robotic or open. In certain embodiments, the tumor surgery is segmental or extended.

[00256] In any of the preceding embodiments of the methods disclosed herein, the cancer  
20 patient has received/is receiving an adjuvant therapy. The adjuvant therapy may be chemotherapy, radiation therapy or chemoradiation therapy.

[00257] In any of the preceding embodiments of the methods disclosed herein, the cancer patient has not received an adjuvant therapy.

[00258] Additionally or alternatively, in some embodiments of the methods disclosed  
25 herein, the patient is human.

[00259] In any and all embodiments of the methods disclosed herein, the biological sample obtained from the cancer patient comprises biopsied tumor tissue, whole blood, plasma, or serum.

## EXAMPLES

30 [00260] The present technology is further illustrated by the following Examples, which should not be construed as limiting in any way. The examples herein are provided to illustrate

advantages of the present technology and to further assist a person of ordinary skill in the art with preparing or using the methods of the present technology. The examples should in no way be construed as limiting the scope of the present technology, as defined by the appended claims. The examples can include or incorporate any of the variations, aspects, or  
5      embodiments of the present technology described above. The variations, aspects, or embodiments described above may also further each include or incorporate the variations of any or all other variations, aspects or embodiments of the present technology.

### **Example 1: Methods for Profiling Lung Cancer Tumors**

#### **Patients**

10      **[00261]**    After institutional review board approval, a prospectively maintained database of 740 patients with primary pathological stage I-III LUAD who underwent a complete (R0) resection from 2010 to 2019 was retrospectively reviewed. Demographic, radiographic, pathologic, genomic and follow-up patient data were reviewed. Predominant invasive LUAD histologic subtype was designated as either lepidic, acinar, papillary, micropapillary, solid or  
15      unknown. The Elixhauser-van Walraven (EvW) score, a well-validated co-morbidity index, was used to quantify patient comorbid status.

**[00262]**    All patients consented to next generation sequencing (NGS; MSK-IMPACT) on their primary tumor. The CONSORT diagram shows exclusion criteria for the patient cohort (**FIG. 4**). Metachronous and synchronous tumors were excluded with metachronous tumors  
20      differentiated from recurrent tumors in accordance with the Martini and Melamed criteria, as previously described in Martini N, Melamed MR. *J Thorac Cardiovasc Surg.* 70(4):606-612 (1975).

#### **MSK-IMPACT Sequencing**

25      **[00263]**    Tumor genomic profiling was performed on all 740 LUAD samples using the MSK-IMPACT platform (described in Cheng DT, *et al.*, *J Mol Diagn.* 17: 251-264 (2015)). Genomic factors of interest were selected for further analysis, including tumor mutational burden (TMB), fraction genome altered (FGA), and all genes altered at  $\geq 5\%$  frequency in the cohort. Also included were the ten canonical oncologic signaling pathways (cell cycle, Hippo, Myc, Notch, Nrf2, Pi3K, RTK/RAS, TGF $\beta$ , p53, and Wnt).

30      **[00264]**    Sequencing breadth of the MSK-IMPACT panel has increased over time resulting in 16, 201, and 583 patients from this cohort sequenced with 341-, 410-, and 468-gene panels, respectively (Cheng DT, *et al.*, *J Mol Diagn.* 17: 251-264 (2015)). Tumor mutation burden

(TMB) was defined as the total number of nonsynonymous coding variants per megabase (Mb) and was normalized by each panel size (0.98, 1.06, 1.22 Mb in the 341-, 410-, 468-gene panels, respectively). The total number of mutations by length of coding region was divided by the total number of panels. Fraction genome altered (FGA) was defined as the number of bases in sequenced genomic segments with log2 copy number fold change  $>0.2$  or  $\leq -0.2$  over the total number of bases in all sequenced segments. Known mutations and copy number alterations which have been described to activate oncogenes or inactivate tumor suppressor genes were identified using the proprietary OncoKB Knowledge Base. This system was necessary to distinguish between those mutations and alterations with known or presumed functional implications against benign variants or those with unknown clinical significance (Chakravarty D *et al.*, *JCO Precision Oncology*. 1:1-84 6 (2017)).

**[00265]** A total of 121 genes were identified *a priori* in the 10 oncogenic signaling pathways. Zhou J *et al.*, *Clinical Cancer Research*. 25(24):7475-7484 (2019). A pathway was considered altered in a tumor if at least one gene within the corresponding pathway template was altered. For analysis of co-occurrence and mutual exclusivity, all genes known to be drivers in LUAD were assessed (Chakravarty D *et al.*, *JCO Precision Oncology*. 1:1-84 6 (2017)). Mutual exclusivity and co-occurrence alterations in genes and oncogenic signaling pathways was assessed using Fisher's exact test and P values were adjusted to correct for multiple comparisons using the false discovery rate (FDR) correction.

#### Intraoperative Analgesic Agents

**[00266]** Doses of fentanyl, hydromorphone, and morphine administered intraoperatively were extracted from electronic anesthesia records, converted to oral morphine milligram equivalents (MMEs), and summed to give total intraoperative dose, where 10 MMEs is equal to 50 mcg fentanyl IV (a standard intraoperative bolus dose) (**FIG. 5A**). Intraoperative analgesic agents included hydromorphone, fentanyl, and morphine, with the majority of patients receiving fentanyl (**FIG. 5A**). Total intraoperative morphine milligram equivalents (MMEs) were evaluated in a continuous dose-dependent manner.

#### Immunohistochemistry on LUAD Samples

**[00267]** Immunohistochemical staining was performed on ten patient samples using a hydrogen peroxidase method. Frozen tissue samples were chosen based on tissue availability from a subset of available tissue in this patient cohort.

[00268] The LUAD samples were run in duplicate against matched adjacent non-tumor lung tissue samples to detect expression levels of the MOR. Tissue samples were homogenized in PBS (10mg in 100µl PBS) and samples were centrifuged at 3000rpm for 15 minutes at which point, the supernatant was removed and run on quantitative sandwich enzyme linked immunosorbent assay (ELISA) kits for the mu opioid receptor (MOR) purchased from MyBioSource, Inc. (San Diego, CA). A preliminary bicinchoninic acid protein assay was performed to quantify total protein in each sample and normalize all samples prior to MOR ELISA analysis. ELISA sensitivities were <7.81pg/ml with intra-assay coefficient of variability (CV) <8% and inter-assay CV <10% precision. Median optical density (OD) levels for all tumor samples were obtained and compared against non-tumor matched controls. Standard OD levels were analyzed on a logistic regression and median sample concentrations were estimated for both the tumor and non-tumor specimens. Standard error of the mean was estimated for tumor and non-tumor MOR sample composite concentrations.

#### Statistical Analysis

[00269] The primary objective of the study was to quantify the association between intraoperative opioid dose and oncologic outcomes. The primary outcome was recurrence-specific survival (RSS). Time to event was determined from the time of surgical resection to the time of first recurrence, otherwise censored at the time of last follow-up. RSS was chosen in place of the alternative recurrence-free survival (RFS), time to recurrence or death from any cause, in order to determine whether opioids and the adjuncts were associated with disease progression in stage I-III LUAD. The secondary outcome was cancer specific survival (OS), which was defined as time to death from any cause.

[00270] To estimate the association between intraoperative opioids and oncologic outcomes, univariable and multivariable Cox proportional hazard regressions were used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs), treating intraoperative MME as a continuous variable and adjunct administration as a categorical variable. For each endpoint (RFS and OS), variables with a p-value of < 0.1 were included in the multivariable model, while retaining some clinically relevant variables. In the case of RSS analysis, patients who died without recurrence were considered censored using a cause-specific hazard model rather than a sub-distribution hazard model; in this etiological framework, the cause-specific hazard is more appropriate as it directly quantifies the hazard among subjects that are actually at risk of developing the event of interest, whereas in the sub-distribution hazard

model individuals that experienced the competing event remain in the risk set. Each model was designed for the OS and RSS outcomes and were adjusted for relevant clinicopathological features. An additional multivariable model was constructed for each genomic factor of interest, for each outcome, by adding to the multivariable model a term for presence of factor alteration and a term for interaction between the factor and opioid dose.

[00271] To better visualize the impact of incremental increases in intraoperative MMEs on outcomes, predicted 5-year OS and RSS estimates, corresponding to a range of MMEs, were generated based on the final Multivariable Cox regression analyses (MVAs) for a representative patient. Survival endpoints were measured from the time of surgery and patients were censored at the time of last follow-up. OS and CSS were estimated using the Kaplan-Meier (KM) approach by MME and compared using log-rank tests. Median follow-up duration was estimated using the Kaplan-Meier (KM) method.

[00272] All univariable models were stratified by pathologic stage. Multivariable Cox regression analyses (MVA) against each outcome were constructed in a backward elimination procedure. The set of factors for each MVA were confirmed by LASSO variable selection procedure. Because of their clinical relevance to oncologic outcomes, pathologic stage and procedure type were included in the MVAs regardless of statistical significance. Intraoperative MMEs, and procedure type were included in the MVA for both survival outcomes. The proportional hazards assumptions were assessed using scaled Schoenfeld residuals. The linearity assumption of continuous variables was assessed using restricted cubic splines in the models for both outcomes. All statistical tests were two-sided with  $p < 0.05$  indicating statistical significance. R 3.6.2 (R Core Team, Vienna, Austria) was used for statistical analyses.

[00273] Genomic factors were selected for further analysis, including tumor mutational burden (TMB), fraction genome altered (FGA), and all genes altered at  $\geq 5\%$  frequency in the cohort (further adjusted by background genomic variability (TMB, FGA)). Also included were the ten canonical oncologic signaling pathways (cell cycle, Hippo, Myc, Notch, Nrf2, PI3K, RTK/RAS, TGF $\beta$ , p53, and Wnt). P-values from all interaction models were adjusted for multiple testing using false discovery rate (FDR) corrections.

#### Predicted MME Curves

[00274] The predicted 5-year overall survival (OS) and recurrence-specific survival (RSS) estimate curves were generated based on the most frequently observed characteristics or

median value for each continuous variable in the MVA (papillary/acinar histologic subtype, median Exlihauser-van Walraven score, lobectomy procedure, median age, and pathologic stage I). These predicted OS and RSS estimates by MME curve were generated for the MVAs without genomic factors as well as for the MVAs with statistically significant effects between genomic factors and intraoperative MMEs. In the case of FGA, the predicted 5-year curves represent 25<sup>th</sup> and 75<sup>th</sup> percentile for the overall cohort.

**[00275]** *Lung Cancer Patient Demographics.* Seven hundred forty patients were included in the study. The majority were female (N=489, 66%) and most were former or current smokers (N=543, 73%). The median age at surgery was 68 years (interquartile range [IQR], 61-73 years) and median Elixhauser-van Walraven (EvW) comorbidity score was 12 (IQR, 7-15).

**[00276]** *Clinicopathologic and Analgesic Variables.* Median intraoperative MMEs received by the overall cohort was 42 (IQR, 30-60 MMEs). Patients who received dexmedetomidine as a surgical adjunct received significantly less intraoperative MMEs (median 40, 30-57) compared to the no adjunct cohort (median 50, IQR 30-61). All patients had pathologically diagnosed LUAD which were differentiated into groups based on histologic subtype: lepidic (N=136, 18%), acinar/papillary (N=441, 60%), micropapillary/solid (N=153, 21%), and unknown (N=10, 1.4%). Four hundred fifty-six patients were pathologic stage I (62%), 157 patients (21%) were stage II and 127 patients (17%) were stage III. Thirteen percent of the cohort (N=96) received induction chemotherapy and most patients received a lobectomy (N=610, 82%). Almost a third (N=227, 31%) received adjuvant therapy, 31 patients (4.2%) received combination chemoradiation, 153 patients (21%) received chemotherapy alone, and 29 patients (3.9%) received radiation therapy. Median follow-up duration was 2.74 years (IQR, 1.76 – 3.82).

### **Example 2: Association between Intraoperative Analgesics and Outcomes in Lung Cancer Patients**

**[00277]** Five-year RSS was 61.8% (95% CI, 54.7 – 69.7%) and OS was 74.4% (95% CI, 68.6 – 80.6%). There were 95 deaths and 160 locoregional or distant recurrence events in this observation window. The linearity assumptions for intraoperative MME were not violated for both RSS (p=0.32) and OS (p=0.35), hence this primary exposure factor was treated as a continuous variable in the analyses. Intraoperative opioid dose was not significantly associated with RSS on either univariable or multivariable analysis.

**Example 3: Interaction of Gene Alterations with Opioid Dose**

[00278] An exploratory analysis was performed to assess any modifying effect of tumor specific genomic alterations, delineated by stage in the oncoprint, on the opioid-survival relationship (**FIG. 1A**). **FIG. 9** shows a loss of function/gain of function plot of the altered oncogenic pathways.

[00279] The fraction of the genome altered (FGA) refers to the percentage of copy number altered regions out of all sequenced regions. FGA may be defined as the number of bases in sequenced genomic segments with log2 copy number fold change  $>0.2$  or  $<-0.2$  over the total number of bases in all sequenced segments. **FIG. 8** shows a distribution of the FGA within the cohort.

[00280] Higher TMB ( $p=0.026$ ) and FGA ( $p=0.044$ ), treated as continuous variables, resulted in even worse OS with increasing opioid dose, though the actual size of the TMB effect estimate was small (**FIG. 1B** and **FIG. 6**).

[00281] When FGA was broke down into 25<sup>th</sup> and 75<sup>th</sup> percentiles for the predicted model curves, higher FGA had a profound interaction with the opioid-OS relationship (**FIG. 1B**). Exemplary minimum, maximum, median, 25th and 75th percentile values of FGA are provided below:

| Min | Q1       | Med     | Q3     | Max    |
|-----|----------|---------|--------|--------|
| 0   | 0.004775 | 0.04225 | 0.1231 | 0.6425 |

[00282] CDKN2A alteration (9% alteration rate (N=64); loss or reduction of function) resulted in worse OS with increasing opioid dose compared to wildtype ( $p=0.052$ ; **FIG. 1B**). CDKN2A alteration rates were significantly higher in pathologic stage II (16%) and stage III (10%) compared to stage I (6%,  $p=0.002$ ; **FIG. 1C**). Over half of the total driver alterations (33 of 61) were homozygous deletions (**FIG. 1A**).

[00283] These results demonstrate that the methods of the present technology are useful for determining whether a patient diagnosed with lung cancer and undergoing tumor resection surgery will benefit from treatment with intraoperative opioid analgesics or opioid-free intraoperative analgesics.

**Example 4: Interaction of Canonical Pathway Alterations with Opioid Dose**

[00284] Alterations in the ten canonical oncogenic pathways as well as the sum total number of pathways altered were also investigated (FIG. 2A). FIG. 7 shows genes altered in oncogenic pathways stratified by pathologic stage.

5 [00285] Alterations in the Wnt pathway (6.2% alteration rate (N=46)) were associated with improved 5-year RSS with increasing opioid exposure (p=0.029) as compared to an unaltered pathway. A similar result was found for the Hippo pathway (1.8% alteration rate (N=13), p=0.040) (FIG. 2B). Notably, Wnt and Hippo pathway alteration rates increased with higher pathologic stage but were not significantly different (p=0.114 and p=0.306, respectively; FIG. 10 2C).

[00286] These results demonstrate that the methods of the present technology are useful for determining whether a patient diagnosed with lung cancer and undergoing tumor resection surgery will benefit from treatment with intraoperative opioid analgesics or opioid-free intraoperative analgesics.

15 **Example 5: Co-occurrence and Mutual Exclusivity of Genes and Pathways**

[00287] Co-occurrences and mutual exclusivity between altered genes and pathways were assessed (FIG. 1D and FIG. 2D). Wnt pathway was found to significantly co-occur with the PI3K pathway (p=0.020), though PI3K interaction with opioid dose was not significantly associated with either RSS or OS outcomes (p=0.127 and p=0.119; FIG. 6). FIGs. 10 and 20 11 show the frequency of the different loss of function/gain of function gene mutations and pathway mutations identified in the LUAD patient cohort, respectively.

[00288] *MOR Expression in Patient LUAD Samples.* Mean MOR concentrations for the selected LUAD samples, as determined by ELISA, were found to be not significantly different from non-tumor matched controls (113.454 pg/ml  $\pm$  21.66473 versus 79.718 pg/ml  $\pm$  8.527698; p=0.18), though seven of the ten patients had significantly higher MOR concentration levels in the tumor sample as compared to the matched adjacent lung sample (FIG. 3). The ELISA results reported here demonstrate that MOR is present on both tumor and normal lung tissue in LUAD, with increased concentrations in tumor specimen – represented in 7 of 10 of the 266 patient samples - consistent with previous results in NSCLC40. These results demonstrate that the expression and variation of MOR on tumor is not in itself sufficient to explain opioid effects. In contrast, FIGs. 1A-1D and 2A-2D show

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that alteration of other genes and pathways interact with opioid exposure to affect the predicted opioid dose-outcome response curve.

[00289] These results demonstrate that the methods of the present technology are useful for determining whether a patient diagnosed with lung cancer and undergoing tumor resection surgery will benefit from treatment with intraoperative opioid analgesics or opioid-free intraoperative analgesics.

### **Example 6: Methods for Profiling Colon Cancer Tumors**

#### *Study population*

[00290] Following institutional review board approval, a retrospective review of a prospectively maintained database was performed to identify patients with stage I–III COAD who underwent curative resection at Memorial Sloan Kettering Cancer Center between March 1, 2010 and December 31, 2018 (follow-up updated in July 2020). Patients were excluded if their MMR subtype was unknown, received neoadjuvant treatment, had a rare histological subtype, or another invasive cancer within 5 years prior to colectomy (**FIG. 15**). MMR subtype was determined proficient if immunohistochemical staining (IHC) identified that the proteins MLH1, MSH2, MSH6, and PMS2 were present in the pretreatment biopsy or the resected specimen (see Vilar E, Gruber SB. *Nat Rev Clin Oncol* 7: 153-162 (2010), which is incorporated by reference herein in its entirety). If one or more of these were absent in IHC, the patient was considered dMMR.

#### *Intraoperative analgesic agents*

[00291] Doses of fentanyl, hydromorphone, and morphine administered intraoperatively were extracted from the electronic anaesthesia records, converted to oral morphine milligram equivalents (MMEs), and summed to give the total intraoperative dose. For reference, 10 MMEs equals 50 mcg IV fentanyl (a common intraoperative bolus dose). Intraoperative administration of ketorolac, ketamine, and dexmedetomidine was also captured. Fentanyl accounted for the overwhelming majority of opioids received (**FIG. 26**).

#### *Statistical Analysis of clinical outcomes*

[00292] The primary outcome was recurrence, either local or distant; death without recurrence was treated as a competing event. Recurrence was calculated from time of surgery to recurrence if a patient experienced the event, until death if a patient experienced the competing event, or it was censored at last follow-up. The relationship between

intraoperative opioids and MMR subtype on recurrence was summarised using cumulative incidence functions and quantified using competing risk regression models. The secondary outcome was overall survival (OS), calculated from time of surgery to death from any cause. The relationship between intraoperative opioids and MMR subtype on OS was summarised using the Kaplan-Meier approach and quantified using Cox proportional hazards regression models. In all models, intraoperative MMEs were treated as a continuous variable, and administration of adjuncts and ketorolac as categorical variables. For multivariable analyses, a set of factors selected a-priori (intraoperative opioid, MMR subtype, adjunct, and ketorolac) were included. Backward regression was used to determine additional adjusting baseline factors, starting with a model including all factors with  $P < 0.1$  in the univariable models for each endpoint. Associations quantified via regression modeling, were presented as hazard ratios and 95% confidence intervals (CI).

**[00293]** Missing covariate data were addressed using multiple imputation by chained equations with 10 imputed datasets as described by van Buuren S, Groothuis-Oudshoorn K. *J Stat Softw* 45: 1-67 (2011), which is incorporated by reference herein in its entirety. This method is a fully conditional specification, where each factor with missingness is independently modelled using the remaining factors and outcome variables as covariates. The imputation method was polytomous logistic regression for patient race (4% missing), logistic regression for patient ethnicity (1% missing) and any adjuvant chemotherapy (3% missing), proportion odds regression for smoking status (0.7% missing), and predictive mean matching for CEA (12% missing), BMI (0.3% missing), and albumin (0.5% missing). All regression models were fit to each imputed dataset for each outcome, and estimates were pooled using Rubin's rule as described by Rubin DB. *Multiple imputation for nonresponse in surveys*. New York, Wiley (1987), which is incorporated by reference herein in its entirety.

**[00294]** To explore the potential interaction between MMR subtype and MMEs on oncological outcomes, the Kaplan-Meier and cumulative incidence functions were calculated separately for dMMR and pMMR patients, stratified by MME at the median. For each endpoint, the interaction of MMR subtype and continuous MMEs was first considered in a three-factor model (MMR, MMEs and interaction between the two). If  $P < 0.05$  for the interaction term, it was then added to the multivariable model obtained from backward regression to estimate its effect after adjusting for baseline factors. To illustrate the impact of the interaction in the multivariable model, the 5-year predicted probability of an event was estimated across a range of MME values for dMMR and pMMR patients. Each set of

predicted probabilities was calculated for a hypothetical patient with no adjunct and ketorolac use, pT3N0, BMI of 27.5 (median of cohort), and no adjuvant chemotherapy. Interaction was similarly explored for the adjuncts and ketorolac.

[00295] Statistical tests were two-sided with  $P < 0.05$  indicating statistical significance. All analyses were performed using R software version 4.1.1 (R Core Team, Vienna, Austria) with the mice v3.13.0 package for multiple imputation as described by van Buuren S, Groothuis-Oudshoorn K. *J Stat Softw* 45: 1-67 (2011).

#### *Tumour-infiltrating lymphocytes*

[00296] For 1010 (87%) of the 1157 patients in the cohort, surgical pathology reports explicitly noted the presence or absence of increased numbers of TILs. A tumour was classified as having increased TILs if the mean number of lymphocytes per high-powered field was  $\geq 4$ , averaged from five consecutive high-powered fields in an area determined to have the highest concentration of TILs by examination of the entire tumour. The relationships of TILs, intraoperative opioids, and recurrence and OS outcomes were explored using cumulative incidence functions and Kaplan-Meier estimates, respectively.

#### *Tumour transcriptomics*

[00297] The TCGA-COAD cohort (see Cancer Genome Atlas Network, *Nature* 487: 330-337(2012)) was used to study gene expression in tumour versus normal tissue as well as specifically in dMMR (MSI-H) versus pMMR tumours (MSS and MSI-L). For the comparison of tumour versus normal tissue, bulk RNA-sequencing data from 358 patients was employed. Of these, 338 patients in the cohort were further classified based on MSI (61 MSI-H, 60 MSI-L; 217 were MSS).

[00298] Transcriptomics analyses focused on differential expression of opioid receptors and genes broadly related to opioid signalling and function. Differential gene expression analysis was performed using the R package DESeq2 as described by Love MI, et al., *Genome Biol* 15: 550 (2014), which is incorporated by reference herein in its entirety. P-values were adjusted using Benjamini-Hochberg correction for multiple hypothesis testing. An absolute fold change of 2 and an adjusted p-value cut-off of 0.05 were used to assess statistical significance. Gene annotation was performed using the R package biomaRt as described by Durinck S, et al., *Nat Protoc* 4: 1184-1191 (2009), which is incorporated by reference herein in its entirety. The R package EnhancedVolcano (available from [github.com/kevinblighe/EnhancedVolcano](https://github.com/kevinblighe/EnhancedVolcano)) was used to illustrate differential gene

expression. The opioid receptors included the canonical receptors mu (*OPRM1*), delta (*OPRD1*), and kappa (*OPRK1*) as well as receptors known to bind opioids and independently associated with oncological outcomes, i.e. opioid growth factor receptor (*OGFR*) and toll like receptor 4 (*TLR4*). In the absence of well-defined opioid signalling pathways, a list of 430 genes broadly related to opioid signalling and function was generated using Geneshot, a search engine for locating genes related to defined search terms (in this case “opioids”), as described by Lachmann A, et al. *Nucleic Acids Res* 47: W571-W577 (2019), which is incorporated by reference herein in its entirety. For dMMR and pMMR comparison, this list was subsequently refined to only include those genes determined to be differentially expressed between MSI and MSS tumours, representing genes at the intersection of opioid and MMR signalling. This was further divided into lists of up- and downregulated genes, where up and down are in reference to expression in MSI versus MSS, and referred to as “Opioid\*MSI” and “Opioid\*MSS”, respectively.

**[00299]** Single-sample gene set enrichment analysis (SSGSEA) was used to identify pathways and immune cell types with expression correlated with the Opioid\*MSI and Opioid\*MSS lists. Pathways included the 50 “Hallmark” gene lists (representing well-defined biological processes; see Liberzon A, et al., *Cell Syst* 1: 417-425 (2015)), while 25 immune cell types were represented by specific gene signatures (Bindea G, et al., *Immunity* 39: 782-795 (2013)).

**[00300]** *Patient, clinicopathologic, and analgesic variables.* During the study period, 1157 patients met the inclusion criteria (**FIG. 15**). From this group, 875 patients (76%) had pMMR tumours while 282 patients (24%) had dMMR tumours. Clinicopathologic data for the entire cohort and for MMR subtypes are summarised in **FIG. 20**. Median age was 60 years (interquartile range [IQR] 51–70). Most tumours were pT3 (61%) and pN0 (61%). Most surgeries were segmental (94%), lasting a median length of 183 minutes (IQR 137–232), with more than half (58%) performed by robotic approach. Patients received general anaesthesia, which generally involved induction with propofol and maintenance with sevoflurane. Median opioid dose was 60 MMEs (IQR 44–90). A total of 366 patients (32%) received ketamine while 161 (14%) received dexmedetomidine; 227 patients (20%) received ketorolac. Half of the patients (583) did not receive regional anaesthesia, while 269 (23%) received an epidural, 303 (26%) received a transversus abdominis plane (TAP) block, and 2 received both. Patients who received regional anaesthesia also had lower intraoperative MMEs (**FIG. 27**): median opioid dose was 75 MMEs (IQR 53-105) without regional

anaesthesia and 50 MMEs with either epidural (IQR 39-71) or TAP block (IQR 37-68). Median follow-up duration was 3.0 years (95% CI 2.8–3.2), estimated using Reverse Kaplan-Meier.

**Example 7: Associations between Intraoperative Analgesics and Outcomes in Colon Cancer Patients**

*Recurrence*

**[00301]** Recurrence was diagnosed in 126 patients (19 local and 107 distant). The cumulative incidence of recurrence at 3 years and 5 years were 12% (95% CI, 10–14%) and 16% (95% CI, 13–18%), respectively (**FIG. 12A**). This corresponded to 14% (95% CI, 12–17%) and 18% (95% CI, 15–22%) for pMMR patients, and 4.9% (95% CI, 2.4–8.6%) and 7.5% (95% CI, 4.0–12%) for dMMR patients.

**[00302]** Higher intraoperative opioid dose was associated with lower hazard of recurrence on both univariable [HR, 0.93 per 10 MME [95% CI, 0.88–0.98],  $P=0.008$ ) and multivariable (HR, 0.92 per 10 MME [95% CI, 0.87–0.98];  $P=0.007$ ) analyses. The dMMR tumour subtype was associated with lower hazard of recurrence on both univariable (reference pMMR: HR, 0.34 [95% CI, 0.19–0.61],  $P<0.001$ ) and multivariable analyses (HR, 0.38 [95% CI, 0.21–0.68],  $P=0.001$ ) (**FIG. 12A**, inset). Ketamine, dexmedetomidine, and ketorolac were not associated with recurrence in either univariable or multivariable analysis. Adjuvant chemotherapy was associated with lower hazard of recurrence (HR 0.49 [95% CI, 0.27–0.90],  $P=0.022$ ), and factors associated with greater hazard of recurrence were pT3 stage (reference pT1: HR, 4.62, [95% CI, 1.43–14.88],  $P=0.010$ ), pT4 stage (reference pT1: HR, 10.0 [95% CI, 2.94–34.21],  $P<0.001$ ), positive nodal stage (reference pN0: pN1 HR, 2.96 [95% CI, 1.62–5.40],  $P<0.001$ ; pN2 HR 4.34 [95% CI, 2.23–8.44],  $P<0.001$ ), and higher BMI (HR, 1.03 [95% CI, 1.01–1.06],  $P=0.009$ ). Full univariable analysis is found in **FIG. 22** and multivariable analysis in **FIG. 21**.

**[00303]** Initial exploratory analysis on interaction between MMR subtype and MMEs (stratified by below vs. above median) is illustrated in **FIG. 12B**. An 8-fold greater decrease of recurrence was seen at high vs. low opioid dose for dMMR compared with pMMR. This interaction was significant on multivariable analysis ( $P=0.016$ ), illustrated by the predicted 5-year recurrence probability (**FIG. 12C**).

*Overall survival*

[00304] Seventy-six patients died during follow-up, 37 of whom experienced a recurrence before death. Estimated 3-year and 5-year OS probability was 95% (95% CI, 93–96%) and 90% (95% CI, 88–93%), respectively, similar for pMMR and dMMR patients. Intraoperative opioid dose was not associated with OS in either univariable (**FIG. 23**) or multivariable (**FIG. 24**) analysis. Compared with no adjunct, intraoperative ketamine was associated with improved OS on univariable (HR, 0.43 [95% CI, 0.21–0.89],  $P=0.023$ ) but not multivariable analysis. Neither dexmedetomidine nor ketorolac were associated with OS. Other factors associated with worse OS on multivariable analysis were age (HR, 1.09 [95% CI, 1.07–1.12],  $P<0.001$ ), Black race (reference White race: HR, 2.4 [95% CI, 1.09–5.27],  $P=0.030$ ), positive nodal stage (reference pN0: pN1 HR, 1.94 [95% CI, 1.14–3.30],  $P=0.016$ ; pN2 HR, 2.05 [95% CI, 1.05–4.00],  $P=0.035$ ), pT4 stage (reference pT1: HR, 2.92 [95% CI, 1.14–7.48],  $P=0.026$ ), and ASA P3/P4 (reference P1/P2: HR, 2.08 [95% CI, 1.01–4.28],  $P=0.046$ ).

### **Example 8: Exploratory Analyses**

#### *TILs, MMR subtype, and opioid dose*

[00305] The presence or absence of increased TILs was reported in 1010 of 1157 patients (87%); of this group, 360 (36%) had increased TILs. Increased TILs were present in 71% of dMMR tumours but only in 24% of pMMR tumours ( $P<0.001$ ) (**FIG. 13A**).

[00306] Consistent with existing literature, increased TILs were associated with a lower risk of recurrence (**FIG. 13B**). After stratifying by below- vs above-median opioid dose, it was found that patients with increased TILs showed an improved cumulative incidence of recurrence and OS with increased opioids (**FIGs. 16A-16B** and **17A-17B**). Stratification of dMMR and pMMR patients by increased TILs and MMEs showed that dMMR featured greater decrease in recurrence at high vs. low MMEs compared with pMMR, regardless of whether increased TILs were present (**FIG. 13C**) in either.

#### *Differential expression of opioid receptor genes*

[00307] Further exploratory analysis focused on differences in gene expression between dMMR and pMMR tumours. In the TCGA-COAD cohort, opioid receptors were differentially expressed between tumour and normal tissue (**FIG. 18A**: *OPRM1* was downregulated in tumour versus normal, while *OGFR* and *OPRD1* were upregulated; *OPRK1* and *TLR4* were not significantly different). Opioid receptors were not, however, differentially expressed between dMMR (MSI) and pMMR (MSS) tumours (**FIG. 18B**).

*Differential expression of opioid-related genes*

[00308] Of 430 genes broadly related to opioid signalling and function, 83 were differentially expressed (greater than two-fold change with adjusted  $P < 0.05$ ) between dMMR (MSI) and pMMR (MSS) tumours (**FIG. 14A** and **FIG. 25**), representing genes at the intersection of opioid and MMR signalling. These were subdivided into 40 upregulated genes (Opioid\*MSI) and 43 downregulated genes (Opioid\*MSS) in MSI versus MSS tumours. SSGSEA uncovered pathways and cell types with expression correlated with these gene lists (**FIG. 19**). Many of the pathways (and immune cell types) correlated with Opioid\*MSI and with each other were related to Th1-mediated immune responses, while Opioid\*MSS gene expression was correlated with the Wnt signalling pathway (**FIGs. 14B-14D**).

**EQUIVALENTS**

[00309] The present technology is not to be limited in terms of the particular embodiments described in this application, which are intended as single illustrations of individual aspects of the present technology. Many modifications and variations of this present technology can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. Functionally equivalent methods and apparatuses within the scope of the present technology, in addition to those enumerated herein, will be apparent to those skilled in the art from the foregoing descriptions. Such modifications and variations are intended to fall within the scope of the present technology. It is to be understood that this present technology is not limited to particular methods, reagents, compounds compositions or biological systems, which can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

[00310] In addition, where features or aspects of the disclosure are described in terms of Markush groups, those skilled in the art will recognize that the disclosure is also thereby described in terms of any individual member or subgroup of members of the Markush group.

[00311] As will be understood by one skilled in the art, for any and all purposes, particularly in terms of providing a written description, all ranges disclosed herein also encompass any and all possible subranges and combinations of subranges thereof. Any listed range can be easily recognized as sufficiently describing and enabling the same range being broken down into at least equal halves, thirds, quarters, fifths, tenths, *etc.* As a non-limiting example, each range discussed herein can be readily broken down into a lower third, middle third and upper third, *etc.* As will also be understood by one skilled in the art all language

such as “up to,” “at least,” “greater than,” “less than,” and the like, include the number recited and refer to ranges which can be subsequently broken down into subranges as discussed above. Finally, as will be understood by one skilled in the art, a range includes each individual member. Thus, for example, a group having 1-3 cells refers to groups having 1, 2, or 3 cells. 5 Similarly, a group having 1-5 cells refers to groups having 1, 2, 3, 4, or 5 cells, and so forth.

**[00312]** All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety, including all FIGs. and tables, to the extent they are not inconsistent with the explicit teachings of this specification.

## Claims

1. A method for selecting a cancer patient undergoing tumor resection surgery for lung cancer for treatment with an intraoperative opioid analgesic comprising

(a) detecting the presence of at least one mutation in one or more genes that results (i) in hyperactivation of Wnt pathway and/or (ii) reduced activity of the Hippo pathway in a biological sample obtained from the cancer patient; and

(b) administering to the cancer patient an effective amount of an intraoperative opioid analgesic during the tumor resection surgery.

2. The method of claim 1, wherein the one or more genes are selected from the group consisting of NF2, LATS1, FAT1, RNF43, CTNNB1, AXIN2, APC and AMER1.

3. The method of claim 1 or 2, wherein the at least one mutation is a frameshift mutation, a missense mutation, a deletion, an insertion, a nonsense mutation, an inversion, or a translocation.

4. The method of any one of claims 1-3, wherein the at least one mutation is detected via next-generation sequencing, PCR, real-time quantitative PCR (qPCR), digital PCR (dPCR), Southern blotting, Reverse transcriptase-PCR (RT-PCR), Northern blotting, microarray, dot or slot blots, *in situ* hybridization, or fluorescent *in situ* hybridization (FISH).

5. A method for prolonging survival of a cancer patient undergoing tumor resection surgery for lung cancer comprising

administering to the cancer patient an effective amount of an intraoperative opioid analgesic during the tumor resection surgery,

wherein mRNA or polypeptide expression and/or activity levels of one or more of NF2, LATS1, FAT1, RNF43, AXIN2, APC and AMER1 in a biological sample obtained from the cancer patient are reduced compared to that observed in a control sample obtained from a healthy subject or a predetermined threshold.

6. A method for prolonging survival of a cancer patient undergoing tumor resection surgery for lung cancer comprising

administering to the cancer patient an effective amount of an intraoperative opioid analgesic during the tumor resection surgery,

wherein mRNA or polypeptide expression and/or activity levels of CTNNB1 in a biological sample obtained from the cancer patient are elevated compared to that observed in a control sample obtained from a healthy subject or a predetermined threshold.

7. The method of claim 5 or 6, wherein mRNA expression levels are detected via real-time quantitative PCR (qPCR), digital PCR (dPCR), Reverse transcriptase-PCR (RT-PCR), Northern blotting, microarray, dot or slot blots, *in situ* hybridization, or fluorescent *in situ* hybridization (FISH).

8. The method of claim 5 or 6, wherein polypeptide expression levels are detected via Western blotting, enzyme-linked immunosorbent assays (ELISA), dot blotting, immunohistochemistry, immunofluorescence, immunoprecipitation, immunoelectrophoresis, or mass-spectrometry.

9. The method of any one of claims 1-8, wherein the intraoperative opioid analgesic is fentanyl, hydromorphone, morphine, oxycodone, hydrocodone, codeine, meperidine, remifentanyl, or sufentanyl.

10. The method of any one of claims 1-9, wherein the effective amount of the intraoperative opioid analgesic is about 1 MME to about 20 MMEs.

11. The method of any one of claims 1-9, wherein the effective amount of the intraoperative opioid analgesic is about 20 MMEs to about 45 MMEs.

12. The method of any one of claims 1-9, wherein the effective amount of the intraoperative opioid analgesic is about 45 MMEs to about 200 MMEs.

13. The method of any one of claims 1-12, wherein the effective amount of the intraoperative opioid analgesic is administered as a series of bolus doses or as a continuous infusion during the tumor resection surgery.

14. The method of any one of claims 1-13, wherein the effective amount of the intraoperative opioid analgesic is administered to the cancer patient prior to incision.

15. The method of any one of claims 1-14, further comprising administering to the cancer patient an effective amount of a local anesthetic solution that comprises one or more of lidocaine, mepivacaine, prilocaine, bupivacaine, etidocaine, ropivacaine, levobupivacaine, cocaine, procaine, tetracaine, chlorprocaine, or benzocaine, and optionally an opioid.

16. The method of claim 15, wherein the local anesthetic solution is administered via an epidural catheter, via a serratus plane nerve block or via an intercostal nerve block before, during and/or after the tumor resection surgery.

17. The method of any one of claims 1-16, further comprising administering to the cancer patient an effective amount of a post-operative opioid analgesic after the tumor resection surgery.

18. The method of claim 17, wherein the post-operative opioid analgesic is fentanyl, hydromorphone, morphine, oxycodone, hydrocodone, codeine, meperidine, remifentanyl, or sufentanyl.

19. The method of claim 17 or 18, wherein the post-operative opioid analgesic and the intraoperative opioid analgesic are the same or different.

20. The method of any one of claims 17-19, wherein the effective amount of the post-operative opioid analgesic and the effective amount of the intraoperative opioid analgesic are the same or different.

21. A method for selecting a cancer patient undergoing tumor resection surgery for lung cancer for treatment with an opioid-free intraoperative analgesic comprising

(a) (i) detecting the presence of at least one mutation in CDKN2A in a biological sample obtained from the cancer patient, wherein the at least one mutation reduces CDKN2A expression and/or activity levels, and/or

(ii) detecting elevated FGA in a biological sample obtained from the cancer patient compared to a control sample obtained from a healthy subject or a predetermined threshold; and

(b) administering to the cancer patient an effective amount of an opioid-free intraoperative analgesic during the tumor resection surgery.

22. The method of claim 21, wherein the at least one mutation in CDKN2A is a frameshift mutation, a missense mutation, a deletion, an insertion, a nonsense mutation, an inversion, or a translocation.

23. The method of any one of claims 21-22, wherein the at least one mutation is detected via next-generation sequencing, PCR, real-time quantitative PCR (qPCR), digital PCR (dPCR), Southern blotting, Reverse transcriptase-PCR (RT-PCR), Northern blotting, microarray, dot or slot blots, *in situ* hybridization, or fluorescent *in situ* hybridization (FISH).

24. A method for prolonging survival of a cancer patient undergoing tumor resection surgery for lung cancer comprising

administering to the cancer patient an effective amount of an opioid-free intraoperative analgesic during the tumor resection surgery,

5 wherein mRNA or polypeptide expression and/or activity levels of CDKN2A in a biological sample obtained from the cancer patient are reduced compared to that observed in a control sample obtained from a healthy subject or a predetermined threshold.

25. The method of claim 24, wherein mRNA expression levels are detected via real-time quantitative PCR (qPCR), digital PCR (dPCR), Reverse transcriptase-PCR (RT-PCR),  
10 Northern blotting, microarray, dot or slot blots, *in situ* hybridization, or fluorescent *in situ* hybridization (FISH).

26. The method of claim 24 or 25, wherein polypeptide expression levels are detected via Western blotting, enzyme-linked immunosorbent assays (ELISA), dot blotting, immunohistochemistry, immunofluorescence, immunoprecipitation, immunoelectrophoresis,  
15 or mass-spectrometry.

27. A method for prolonging survival of a cancer patient undergoing tumor resection surgery for lung cancer comprising

administering to the cancer patient an effective amount of an opioid-free intraoperative analgesic during the tumor resection surgery,

20 wherein FGA in a biological sample obtained from the cancer patient is elevated compared to that observed in a control sample obtained from a healthy subject or a predetermined threshold.

28. The method of any one of claims 21-23 or 27, wherein FGA is detected via next-generation sequencing.

25 29. The method of any one of claims 21-28, wherein the opioid-free intraoperative analgesic is an amide-type local anesthetic or an ester-type local anesthetic.

30. The method of claim 29, wherein the amide-type local anesthetic is lidocaine, mepivacaine, prilocaine, bupivacaine, etidocaine, ropivacaine, or levobupivacaine.

31. The method of claim 29, wherein the ester-type local anesthetic is cocaine, procaine,  
30 tetracaine, chloroprocaine, or benzocaine.

32. The method of any one of claims 21-31, wherein the opioid-free intraoperative analgesic is administered via an epidural catheter.

33. The method of claim 32, wherein the effective amount of the opioid-free intraoperative analgesic is about 0.05%-4% amide-type or ester-type local anesthetic solution  
5 in a volume of 1-10 ml per hour when administered via the epidural catheter.

34. The method of any one of claims 21-33, wherein the effective amount of the opioid-free intraoperative analgesic is administered as a single injection, series of bolus doses, or as a continuous infusion during the tumor resection surgery.

35. The method of any one of claims 21-31, wherein the opioid-free intraoperative  
10 analgesic is administered via a serratus plane nerve block, or via an intercostal nerve block.

36. The method of any one of claims 21-35, further comprising administering to the cancer patient an effective amount of an opioid-free post-operative analgesic after the tumor resection surgery.

37. The method of claim 36, wherein the opioid-free post-operative analgesic is selected  
15 from the group consisting of lidocaine, mepivacaine, prilocaine, bupivacaine, etidocaine, ropivacaine, levobupivacaine, cocaine, procaine, tetracaine, chloroprocaine, and benzocaine.

38. The method of claim 36 or 37, wherein the opioid-free post-operative analgesic and the opioid-free intraoperative analgesic are the same or different.

39. The method of any one of claims 36-38, wherein the effective amount of the opioid-free  
20 post-operative analgesic and the effective amount of the opioid-free intraoperative analgesic are the same or different.

40. The method of any one of claims 1-39, wherein the cancer patient exhibits stage I, stage II or stage III lung cancer.

41. The method of any one of claims 1-40, wherein the cancer patient has been diagnosed  
25 with lung adenocarcinoma (LUAD).

42. The method of claim 41, wherein the lung adenocarcinoma has a histologic subtype selected from among lepidic, acinar, papillary, micropapillary, solid or unknown.

43. The method of any one of claims 1-42, wherein the cancer patient has received an adjuvant therapy.

44. The method of claim 43, wherein the adjuvant therapy is chemotherapy, lobectomy, radiation therapy or chemoradiation therapy.

45. The method of any one of claims 1-44, wherein the biological sample obtained from the cancer patient comprises biopsied tumor tissue, whole blood, plasma, or serum.

5 46. A method for selecting a cancer patient undergoing tumor resection surgery for colon cancer for treatment with an intraoperative opioid analgesic comprising

(a) assaying gene expression levels of MLH1, MSH2, MSH6, and PMS2 in a biological sample obtained from the cancer patient and

(b) administering to the cancer patient an effective amount of an intraoperative opioid analgesic during the tumor resection surgery,

wherein the gene expression levels of one or more of MLH1, MSH2, MSH6, and PMS2 in the biological sample obtained from the cancer patient are reduced or undetectable compared to that observed in a control sample obtained from a healthy subject or a predetermined threshold.

15 47. A method for selecting a cancer patient undergoing tumor resection surgery for colon cancer for treatment with an intraoperative opioid analgesic comprising

(a) assaying gene expression levels of one or more genes selected from among CCK, NTSR2, IFNG, NTSR1, MLN, LIPN, CXCL8, FCGR3B, PTGS2, CALB2, CXCL10, IL1RN, CCL5, CCL4, IL1B, CCL4L2, FCGR3A, ITIH4, GAD1, CD8A, HTR3A, SLC1A3, LHB, ADRB1, CXCR2, HRH2, CCL3, ESR2, CSF2, ENO2, GRM5, CXCR4, PRKCG, CD68, IL10, TLR2, ICAM1, RGS2, CX3CL1 and KCNJ3 in a biological sample obtained from the cancer patient; and

(b) administering to the cancer patient an effective amount of an intraoperative opioid analgesic during the tumor resection surgery,

25 wherein the gene expression levels of one or more of CCK, NTSR2, IFNG, NTSR1, MLN, LIPN, CXCL8, FCGR3B, PTGS2, CALB2, CXCL10, IL1RN, CCL5, CCL4, IL1B, CCL4L2, FCGR3A, ITIH4, GAD1, CD8A, HTR3A, SLC1A3, LHB, ADRB1, CXCR2, HRH2, CCL3, ESR2, CSF2, ENO2, GRM5, CXCR4, PRKCG, CD68, IL10, TLR2, ICAM1, RGS2, CX3CL1 and KCNJ3 in the biological sample obtained from the cancer patient are elevated relative to a reference sample obtained from a colon cancer subject having microsatellite stable (MSS) tumors or a predetermined threshold.

48. A method for selecting a cancer patient undergoing tumor resection surgery for colon cancer for treatment with an intraoperative opioid analgesic comprising

(a) assaying gene expression levels of one or more genes selected from among GHRH, RBFOX3, NOS1, KISS1, NPFFR2, GRIA2, SLC6A2, ADCY5, HCRT, OPCML, NPY1R, PPP1R14C, NTRK2, CHAT, LEP, CPB1, NR1I2, ADCY8, EGF, F2, UGT1A8, PLG, GRPR, PYY, DRD1, ABCB1, SCT, NTS, NPFFR1, TH, CYP2B6, HTR2C, TAC1, CCKBR, CALCA, ATP12A, LINC01411, P2RX3, ALB, REN, SLC6A4, KNG1 and DRD2 in a biological sample obtained from the cancer patient; and

(b) administering to the cancer patient an effective amount of an intraoperative opioid analgesic during the tumor resection surgery,

wherein the gene expression levels of one or more of GHRH, RBFOX3, NOS1, KISS1, NPFFR2, GRIA2, SLC6A2, ADCY5, HCRT, OPCML, NPY1R, PPP1R14C, NTRK2, CHAT, LEP, CPB1, NR1I2, ADCY8, EGF, F2, UGT1A8, PLG, GRPR, PYY, DRD1, ABCB1, SCT, NTS, NPFFR1, TH, CYP2B6, HTR2C, TAC1, CCKBR, CALCA, ATP12A, LINC01411, P2RX3, ALB, REN, SLC6A4, KNG1 and DRD2 in the biological sample obtained from the cancer patient are reduced relative to a reference sample obtained from a colon cancer subject having microsatellite stable (MSS) tumors or a predetermined threshold.

49. A method for selecting a cancer patient undergoing tumor resection surgery for colon cancer for treatment with an intraoperative opioid analgesic comprising

(a) detecting the expression levels or activity of one or more genes that result (i) in reduced activity of Wnt pathway and/or (ii) hyperactivation of Th1 immune response signature in a biological sample obtained from the cancer patient relative to a predetermined threshold; and

(b) administering to the cancer patient an effective amount of an intraoperative opioid analgesic during the tumor resection surgery.

50. The method of claim 49, wherein the cancer patient comprises at least one mutation that results in reduced activity of Wnt pathway and/or (ii) hyperactivation of Th1 immune response signature.

51. A method for reducing the risk of tumor recurrence in a cancer patient undergoing tumor resection surgery for colon cancer comprising administering to the cancer patient an effective amount of an intraoperative opioid analgesic during the tumor resection surgery,

wherein the cancer patient comprises at least one alteration in DNA mismatch repair (MMR) system.

52. The method of claim 51, wherein mRNA or polypeptide expression levels of one or more of MLH1, MSH2, MSH6, and PMS2 in a biological sample obtained from the cancer patient are reduced or undetectable compared to that observed in a control sample obtained from a healthy subject or a predetermined threshold; and/or

wherein mRNA or polypeptide expression levels of one or more of CCK, NTSR2, IFNG, NTSR1, MLN, LIPN, CXCL8, FCGR3B, PTGS2, CALB2, CXCL10, IL1RN, CCL5, CCL4, IL1B, CCL4L2, FCGR3A, ITIH4, GAD1, CD8A, HTR3A, SLC1A3, LHB, ADRB1, CXCR2, HRH2, CCL3, ESR2, CSF2, ENO2, GRM5, CXCR4, PRKCG, CD68, IL10, TLR2, ICAM1, RGS2, CX3CL1 and KCNJ3 in a biological sample obtained from the cancer patient are elevated relative to a reference sample obtained from a colon cancer subject having microsatellite stable (MSS) tumors or a predetermined threshold; and/or

wherein mRNA or polypeptide levels of one or more of GHRH, RBFOX3, NOS1, KISS1, NPFFR2, GRIA2, SLC6A2, ADCY5, HCRT, OPCML, NPY1R, PPP1R14C, NTRK2, CHAT, LEP, CPB1, NR1I2, ADCY8, EGF, F2, UGT1A8, PLG, GRPR, PYY, DRD1, ABCB1, SCT, NTS, NPFFR1, TH, CYP2B6, HTR2C, TAC1, CCKBR, CALCA, ATP12A, LINC01411, P2RX3, ALB, REN, SLC6A4, KNG1 and DRD2 in the biological sample obtained from the cancer patient are reduced relative to a reference sample obtained from a colon cancer subject having microsatellite stable (MSS) tumors or a predetermined threshold; and/or

wherein the cancer patient comprises at least one genetic mutation or altered expression levels or activity of one or more genes that result (i) in reduced activity of Wnt pathway and/or (ii) hyperactivation of Th1 immune response signature.

53. A method for selecting a cancer patient undergoing tumor resection surgery for colon cancer for treatment with an intraoperative opioid analgesic or intraoperative non-opioid analgesic comprising

(a) assaying gene expression levels of MLH1, MSH2, MSH6, and PMS2 in a biological sample obtained from the cancer patient and

(b) administering to the cancer patient an effective amount of an intraoperative opioid analgesic or intraoperative non-opioid analgesic during the tumor resection surgery,

wherein the gene expression levels of MLH1, MSH2, MSH6, and PMS2 in the biological sample obtained from the cancer patient are comparable to that observed in a control sample obtained from a healthy subject or a predetermined threshold.

54. A method for selecting a cancer patient undergoing tumor resection surgery for colon cancer for treatment with an intraoperative opioid analgesic or intraoperative non-opioid analgesic comprising

(a) assaying gene expression levels of one or more genes selected from among CCK, NTSR2, IFNG, NTSR1, MLN, LIPN, CXCL8, FCGR3B, PTGS2, CALB2, CXCL10, IL1RN, CCL5, CCL4, IL1B, CCL4L2, FCGR3A, ITIH4, GAD1, CD8A, HTR3A, SLC1A3, LHB, ADRB1, CXCR2, HRH2, CCL3, ESR2, CSF2, ENO2, GRM5, CXCR4, PRKCG, CD68, IL10, TLR2, ICAM1, RGS2, CX3CL1 and KCNJ3 in a biological sample obtained from the cancer patient; and

(b) administering to the cancer patient an effective amount of an intraoperative opioid analgesic or intraoperative non-opioid analgesic during the tumor resection surgery,

wherein the gene expression levels of one or more of CCK, NTSR2, IFNG, NTSR1, MLN, LIPN, CXCL8, FCGR3B, PTGS2, CALB2, CXCL10, IL1RN, CCL5, CCL4, IL1B, CCL4L2, FCGR3A, ITIH4, GAD1, CD8A, HTR3A, SLC1A3, LHB, ADRB1, CXCR2, HRH2, CCL3, ESR2, CSF2, ENO2, GRM5, CXCR4, PRKCG, CD68, IL10, TLR2, ICAM1, RGS2, CX3CL1 and KCNJ3 in the biological sample obtained from the cancer patient are comparable relative to a reference sample obtained from a colon cancer subject having microsatellite stable (MSS) tumors or a predetermined threshold.

55. A method for selecting a cancer patient undergoing tumor resection surgery for colon cancer for treatment with an intraoperative opioid analgesic or intraoperative non-opioid analgesic comprising

(a) assaying gene expression levels of one or more genes selected from among GHRH, RBFOX3, NOS1, KISS1, NPFFR2, GRIA2, SLC6A2, ADCY5, HCRT, OPCML, NPY1R, PPP1R14C, NTRK2, CHAT, LEP, CPB1, NR1I2, ADCY8, EGF, F2, UGT1A8, PLG, GRPR, PYY, DRD1, ABCB1, SCT, NTS, NPFFR1, TH, CYP2B6, HTR2C, TAC1, CCKBR, CALCA, ATP12A, LINC01411, P2RX3, ALB, REN, SLC6A4, KNG1 and DRD2 in a biological sample obtained from the cancer patient; and

(b) administering to the cancer patient an effective amount of an intraoperative opioid analgesic or intraoperative non-opioid analgesic during the tumor resection surgery,

wherein the gene expression levels of one or more of GHRH, RBFOX3, NOS1, KISS1, NPFFR2, GRIA2, SLC6A2, ADCY5, HCRT, OPCML, NPY1R, PPP1R14C, NTRK2, CHAT, LEP, CPB1, NR1H2, ADCY8, EGF, F2, UGT1A8, PLG, GRPR, PYY, DRD1, ABCB1, SCT, NTS, NPFFR1, TH, CYP2B6, HTR2C, TAC1, CCKBR, CALCA, ATP12A, LINC01411, P2RX3, ALB, REN, SLC6A4, KNG1 and DRD2 in the biological sample obtained from the cancer patient are comparable relative to a reference sample obtained from a colon cancer subject having microsatellite stable (MSS) tumors or a predetermined threshold.

56. A method for selecting a cancer patient undergoing tumor resection surgery for colon cancer for treatment with an intraoperative opioid analgesic or intraoperative non-opioid analgesic comprising

(a) detecting the expression levels or activity of one or more genes that result (i) in hyperactivation of Wnt pathway and/or (ii) reduced activity of Th1 immune response signature in a biological sample obtained from the cancer patient relative to a predetermined threshold; and

(b) administering to the cancer patient an effective amount of an intraoperative opioid analgesic or intraoperative non-opioid analgesic during the tumor resection surgery.

57. The method of claim 56, wherein the cancer patient comprises at least one mutation that results in hyperactivation of Wnt pathway and/or (ii) reduced activity of Th1 immune response signature.

58. A method for reducing the risk of tumor recurrence in a cancer patient undergoing tumor resection surgery for colon cancer comprising administering to the cancer patient an effective amount of an intraoperative opioid analgesic or intraoperative non-opioid analgesic during the tumor resection surgery, wherein the cancer patient does not comprise any alterations in DNA mismatch repair (MMR) system.

59. The method of claim 58, wherein mRNA or polypeptide expression levels of MLH1, MSH2, MSH6, and PMS2 in a biological sample obtained from the cancer patient are comparable relative to that observed in a control sample obtained from a healthy subject or a predetermined threshold; and/or

wherein mRNA or polypeptide expression levels of one or more of CCK, NTSR2, IFNG, NTSR1, MLN, LIPN, CXCL8, FCGR3B, PTGS2, CALB2, CXCL10, IL1RN, CCL5, CCL4, IL1B, CCL4L2, FCGR3A, ITIH4, GAD1, CD8A, HTR3A, SLC1A3, LHB, ADRB1, CXCR2,

HRH2, CCL3, ESR2, CSF2, ENO2, GRM5, CXCR4, PRKCG, CD68, IL10, TLR2, ICAM1, RGS2, CX3CL1 and KCNJ3 in a biological sample obtained from the cancer patient are comparable relative to a reference sample obtained from a colon cancer subject having microsatellite stable (MSS) tumors or a predetermined threshold; and/or

- 5 wherein mRNA or polypeptide levels of one or more of GHRH, RBFOX3, NOS1, KISS1, NPFFR2, GRIA2, SLC6A2, ADCY5, HCRT, OPCML, NPY1R, PPP1R14C, NTRK2, CHAT, LEP, CPB1, NR1H2, ADCY8, EGF, F2, UGT1A8, PLG, GRPR, PYY, DRD1, ABCB1, SCT, NTS, NPFFR1, TH, CYP2B6, HTR2C, TAC1, CCKBR, CALCA, ATP12A, LINC01411, P2RX3, ALB, REN, SLC6A4, KNG1 and DRD2 in the biological sample  
10 obtained from the cancer patient are comparable relative to a reference sample obtained from a colon cancer subject having microsatellite stable (MSS) tumors or a predetermined threshold; and/or

wherein the cancer patient comprises at least one genetic mutation or altered expression levels or activity of one or more genes that result (i) in hyperactivation of Wnt pathway and/or (ii)  
15 reduced activity of Th1 immune response signature.

60. The method of any one of claims 53-59, wherein the non-opioid analgesics is a regional anesthetic, ketamine, dexmedetomidine, a carboxylic acid derivative NSAID, or acetaminophen.

61. The method of claim 60, wherein the regional anesthetic is lidocaine, mepivacaine,  
20 prilocaine, bupivacaine, etidocaine, ropivacaine, levobupivacaine, cocaine, procaine, tetracaine, chlorprocaine, or benzocaine.

62. The method of claim 60, wherein the carboxylic acid derivative NSAID is ibuprofen, dexibuprofen, naproxen, fenoprofen, ketoprofen, dexketoprofen, flurbiprofen, oxaprozin, loxoprofen, pelubiprofen, zaltoprofen, indomethacin, tolmetin, sulindac, etodolac, ketorolac,  
25 diclofenac, aceclofenac, bromfenac, or nabumeton.

63. The method of any one of claims 46-50, 52-57, or 59-62, wherein the at least one genetic mutation or gene expression levels are detected via next generation sequencing, RNA-seq, real-time quantitative PCR (qPCR), digital PCR (dPCR), Reverse transcriptase-PCR (RT-PCR), Northern blotting, microarray, dot or slot blots, *in situ* hybridization, fluorescent  
30 *in situ* hybridization (FISH), Western blotting, enzyme-linked immunosorbent assays (ELISA), dot blotting, immunohistochemistry, immunofluorescence, immunoprecipitation, immunoelectrophoresis, or mass-spectrometry.

64. The method of any one of claims 46-63, wherein the intraoperative opioid analgesic is fentanyl, hydromorphone, morphine, oxycodone, hydrocodone, codeine, meperidine, remifentanyl, or sufentanyl.

65. The method of any one of claims 46-64, wherein the effective amount of the intraoperative opioid analgesic is about 1 MME to about 20 MMEs.

66. The method of any one of claims 46-64, wherein the effective amount of the intraoperative opioid analgesic is about 20 MMEs to about 45 MMEs.

67. The method of any one of claims 46-64, wherein the effective amount of the intraoperative opioid analgesic is about 45 MMEs to about 200 MMEs.

68. The method of any one of claims 46-67, wherein the effective amount of the intraoperative opioid analgesic is administered as a series of bolus doses or as a continuous infusion during the tumor resection surgery.

69. The method of any one of claims 46-68, wherein the effective amount of the intraoperative opioid analgesic is administered to the cancer patient prior to incision.

70. The method of any one of claims 46-52, further comprising administering to the cancer patient an effective amount of a local anesthetic solution that comprises one or more of lidocaine, mepivacaine, prilocaine, bupivacaine, etidocaine, ropivacaine, levobupivacaine, cocaine, procaine, tetracaine, chlorprocaine, or benzocaine, and optionally an opioid.

71. The method of claim 70, wherein the local anesthetic solution is administered via an epidural catheter, via a transversus abdominis plane (TAP) block, via an Ilioinguinal (II) and iliohypogastric (IH) block, via a rectus sheath block, via a transversalis fascia plane block, or via a quadratus lumborum block before, during and/or after the tumor resection surgery.

72. The method of any one of claims 46-71, further comprising administering to the cancer patient an effective amount of a post-operative opioid analgesic after the tumor resection surgery.

73. The method of claim 72, wherein the post-operative opioid analgesic is fentanyl, hydromorphone, morphine, oxycodone, hydrocodone, codeine, meperidine, remifentanyl, or sufentanyl.

74. The method of claim 72 or 73, wherein the post-operative opioid analgesic and the intraoperative opioid analgesic are the same or different.

75. The method of any one of claims 72-74, wherein the effective amount of the post-operative opioid analgesic and the effective amount of the intraoperative opioid analgesic are the same or different.

76. The method of any one of claims 46-52, further comprise administering to the cancer patient an effective amount of one or more non-opioid analgesics selected from among ketamine, dexmedetomidine, a carboxylic acid derivative NSAID, or acetaminophen.

77. The method of any one of claims 46-76, wherein the cancer patient exhibits stage I, stage II or stage III colon cancer.

78. The method of any one of claims 46-77, wherein the cancer patient has been diagnosed with colon adenocarcinoma (COAD).

79. The method of any one of claims 46-78, wherein the cancer patient has received an adjuvant therapy.

80. The method of claim 79, wherein the adjuvant therapy is chemotherapy, radiation therapy or chemoradiation therapy.

81. The method of any one of claims 46-50, 52-57, or 59-80, wherein the biological sample obtained from the cancer patient comprises biopsied tumor tissue, whole blood, plasma, or serum.

82. The method of any one of claims 46-81, wherein the tumor surgery is laparoscopic, robotic or open.

FIG. 1A

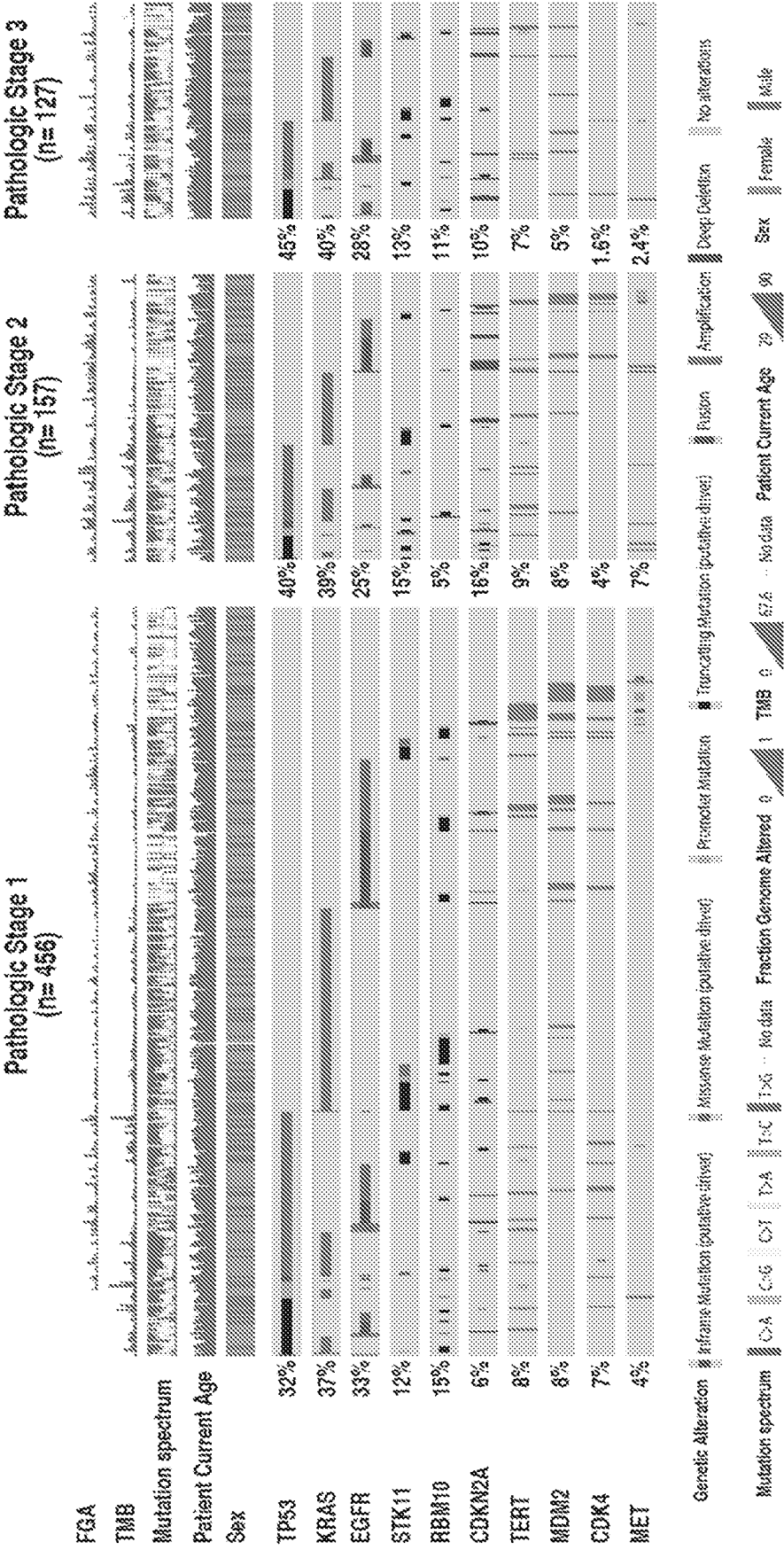


FIG. 1B

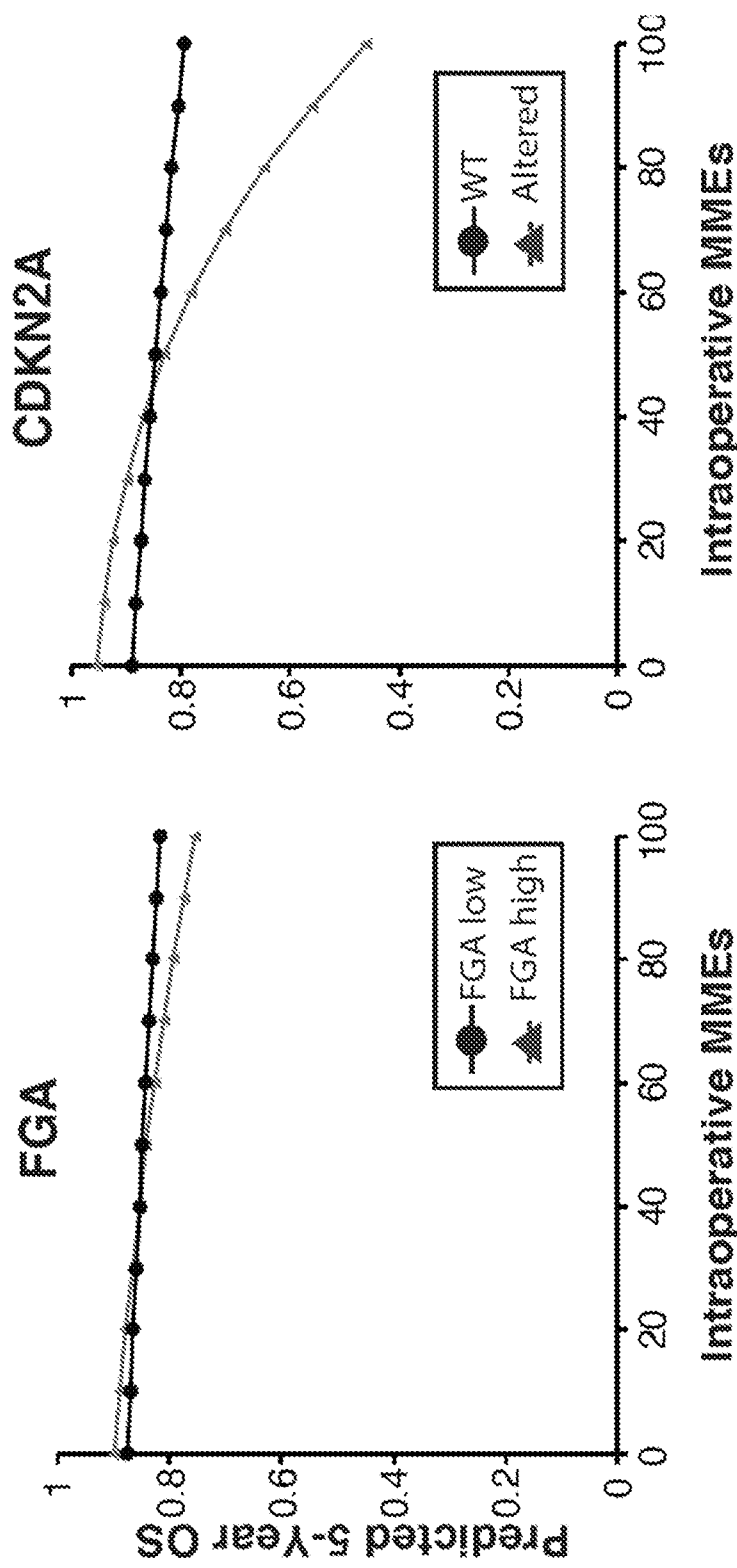


FIG. 1C

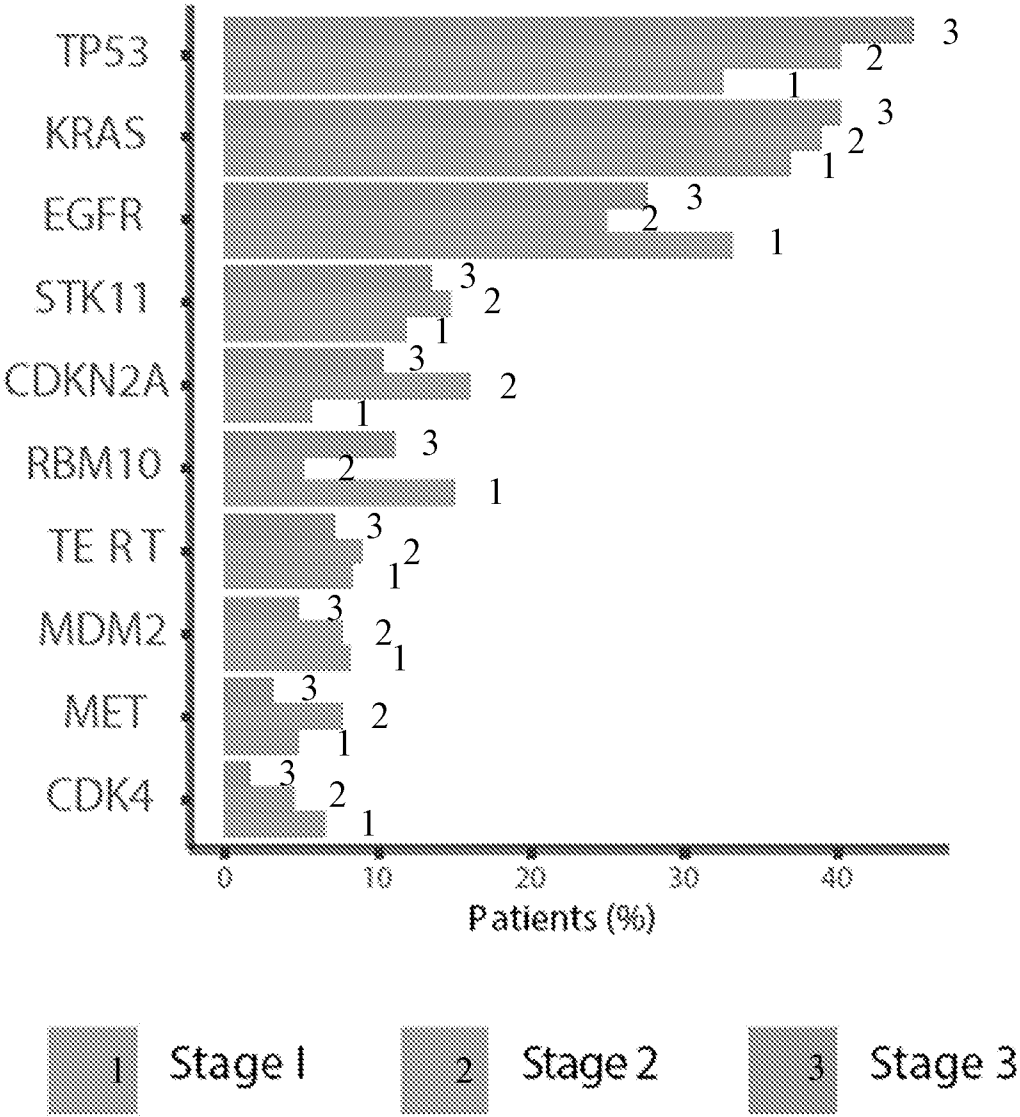


FIG. 1D

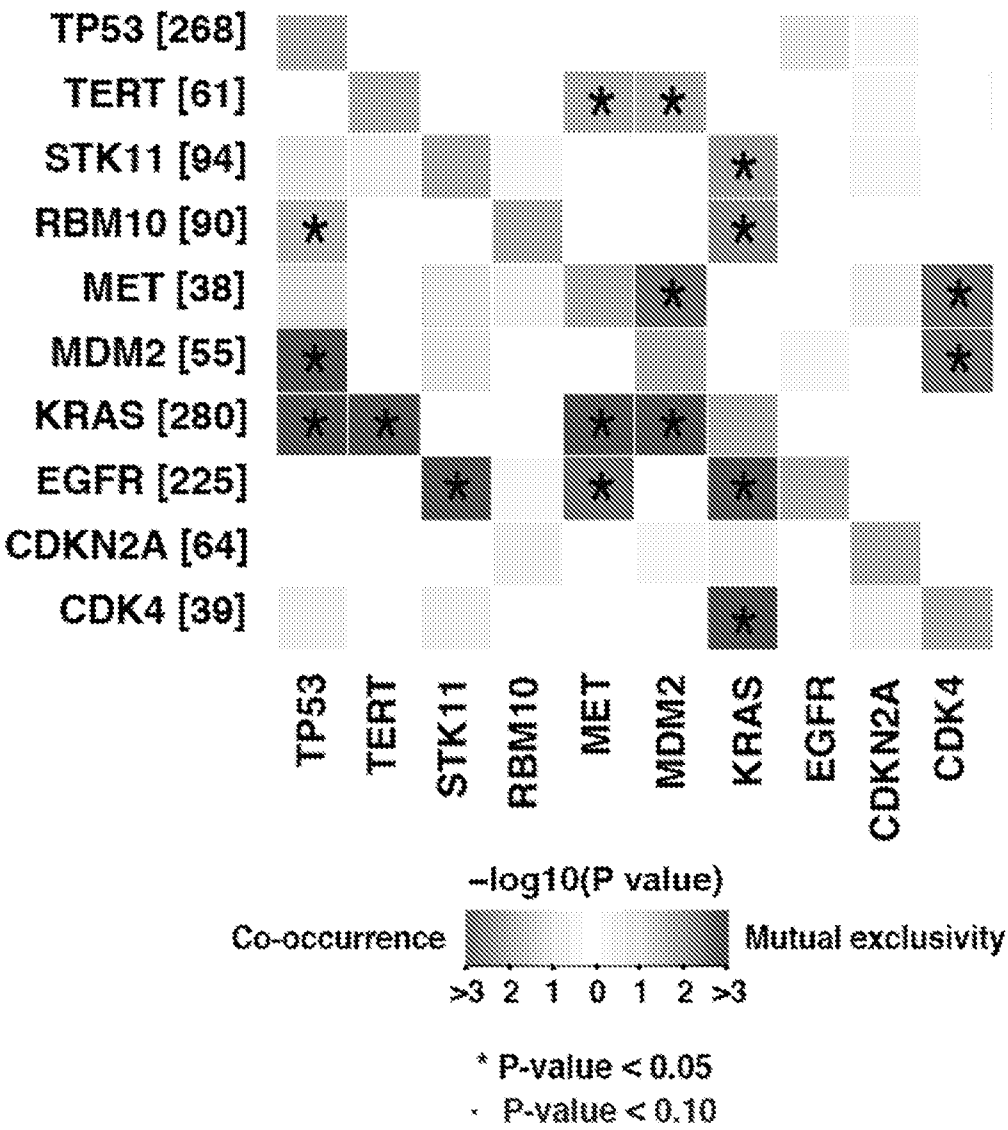


FIG. 2A

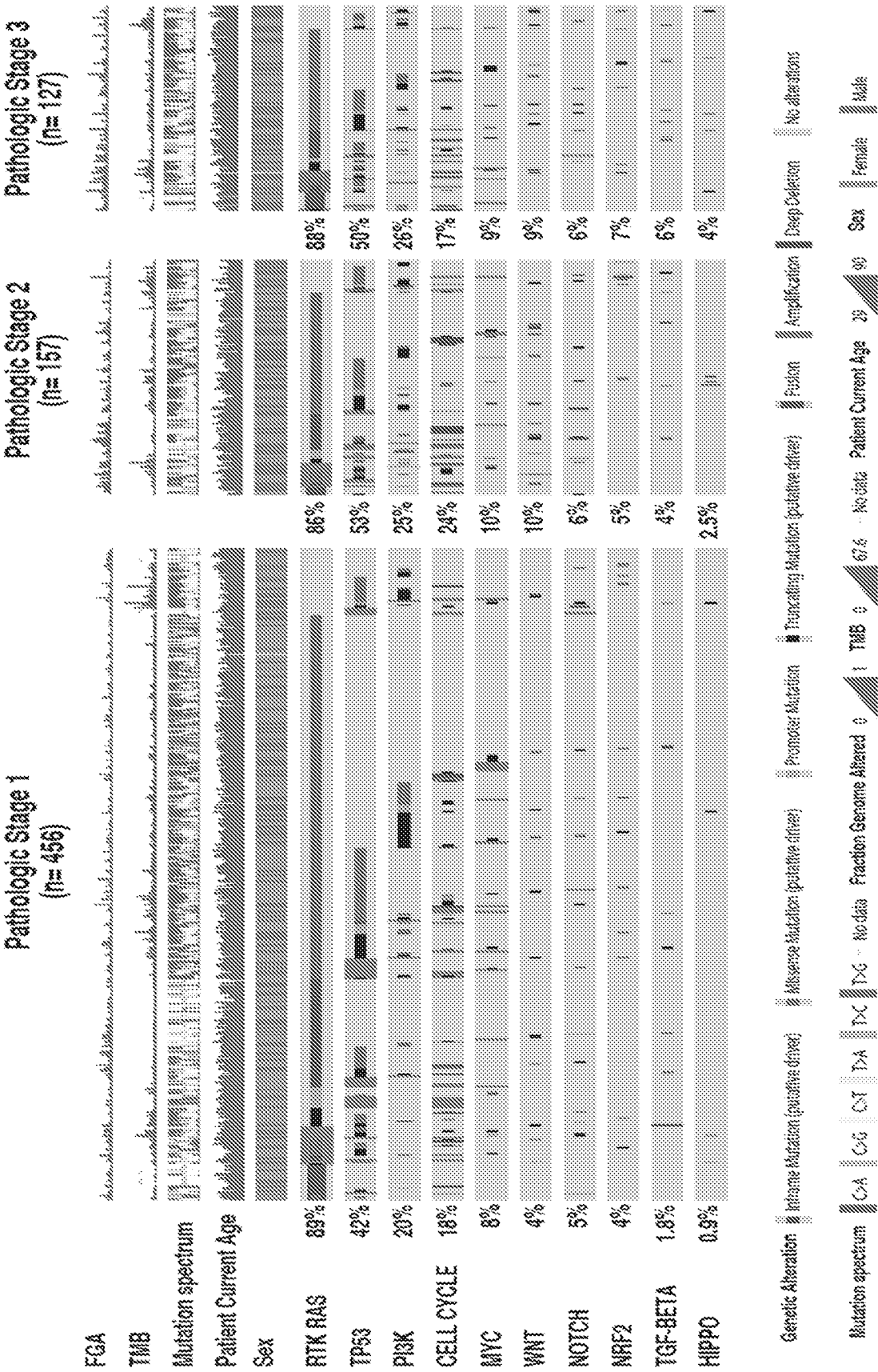


FIG. 2B

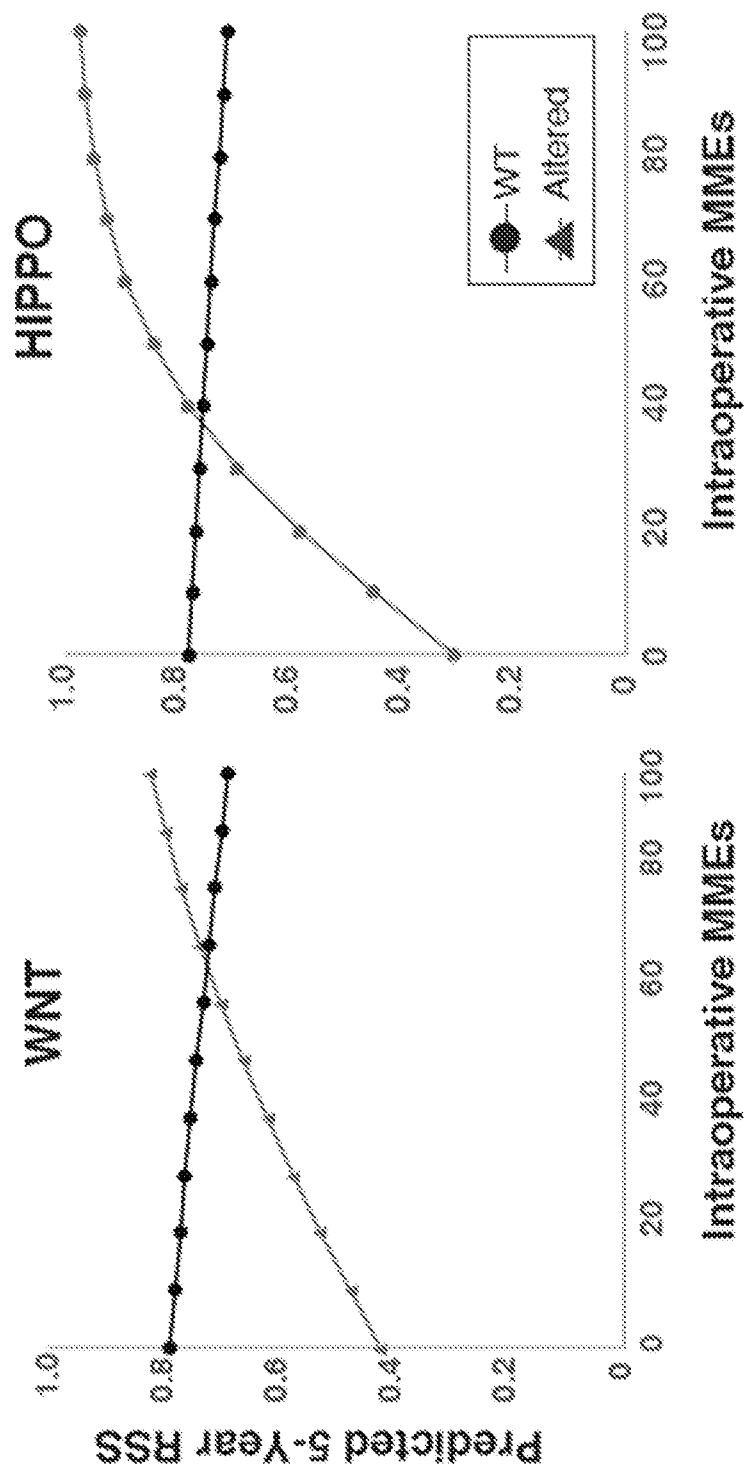


FIG. 2C

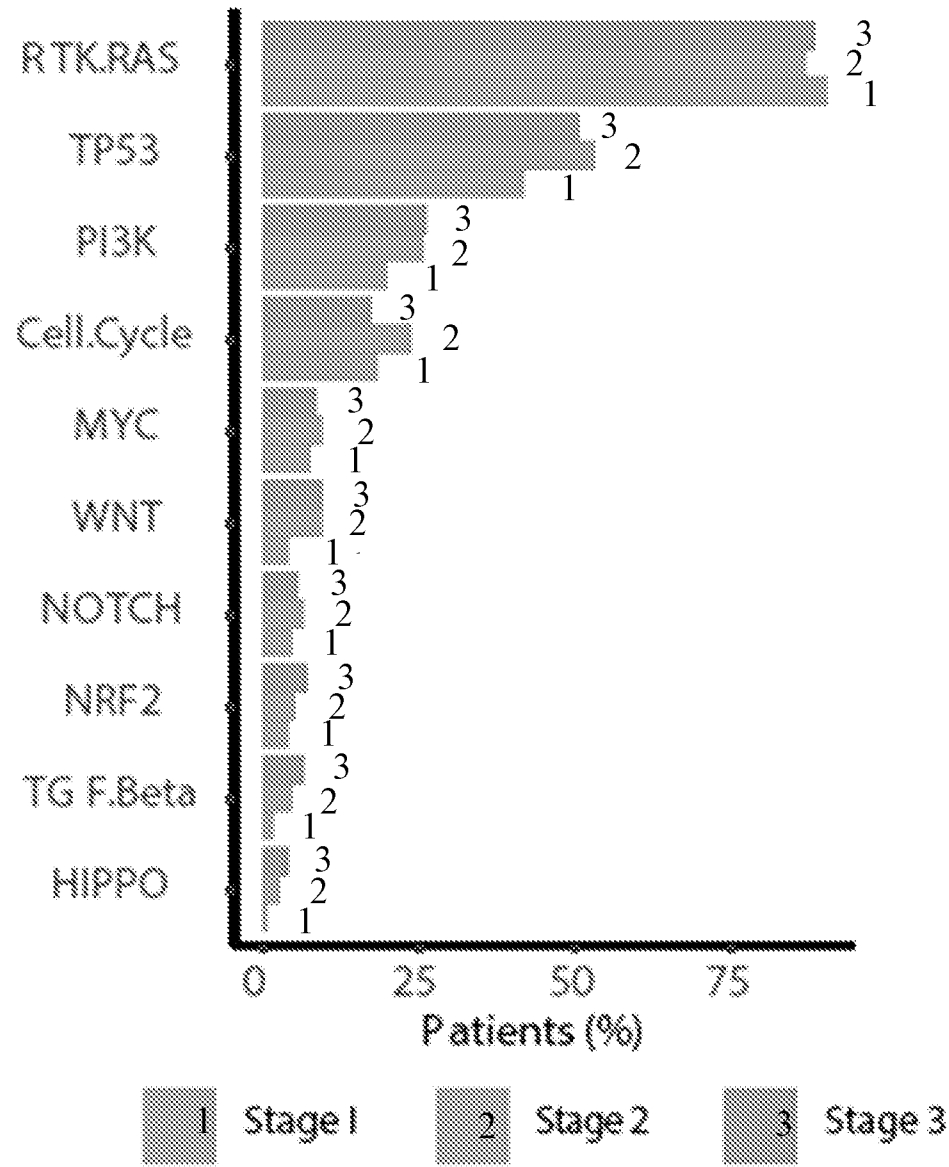


FIG. 2D

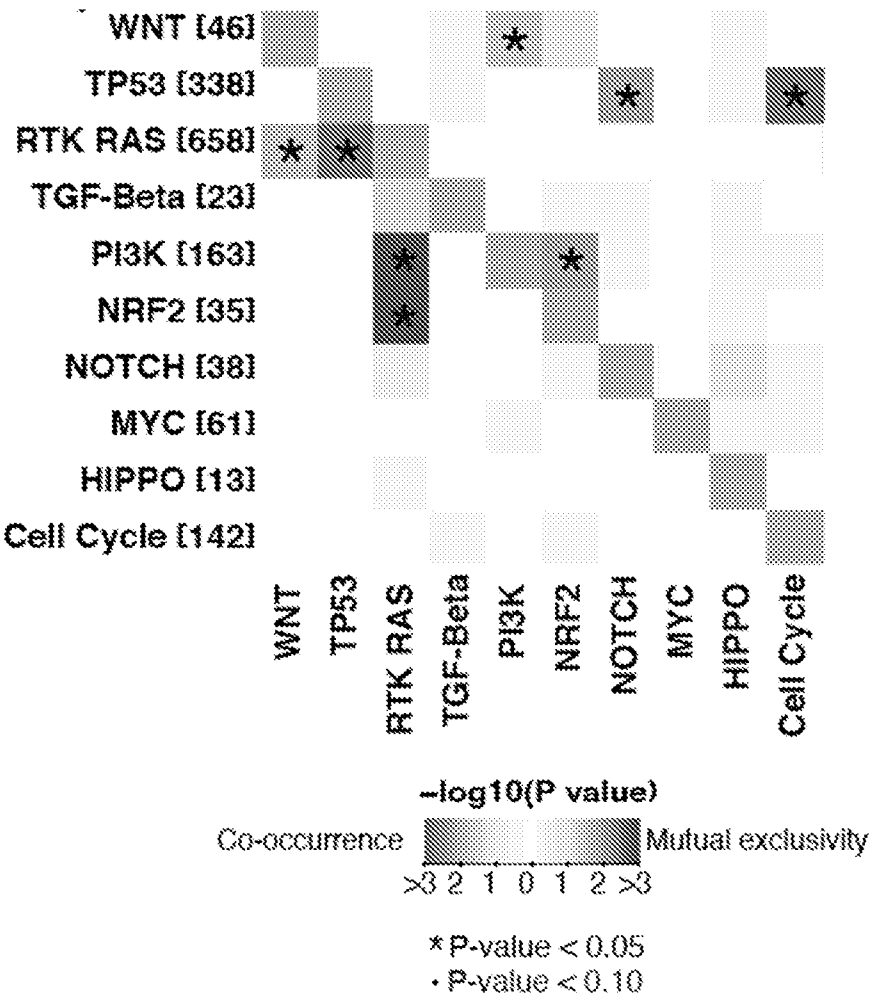


FIG. 3

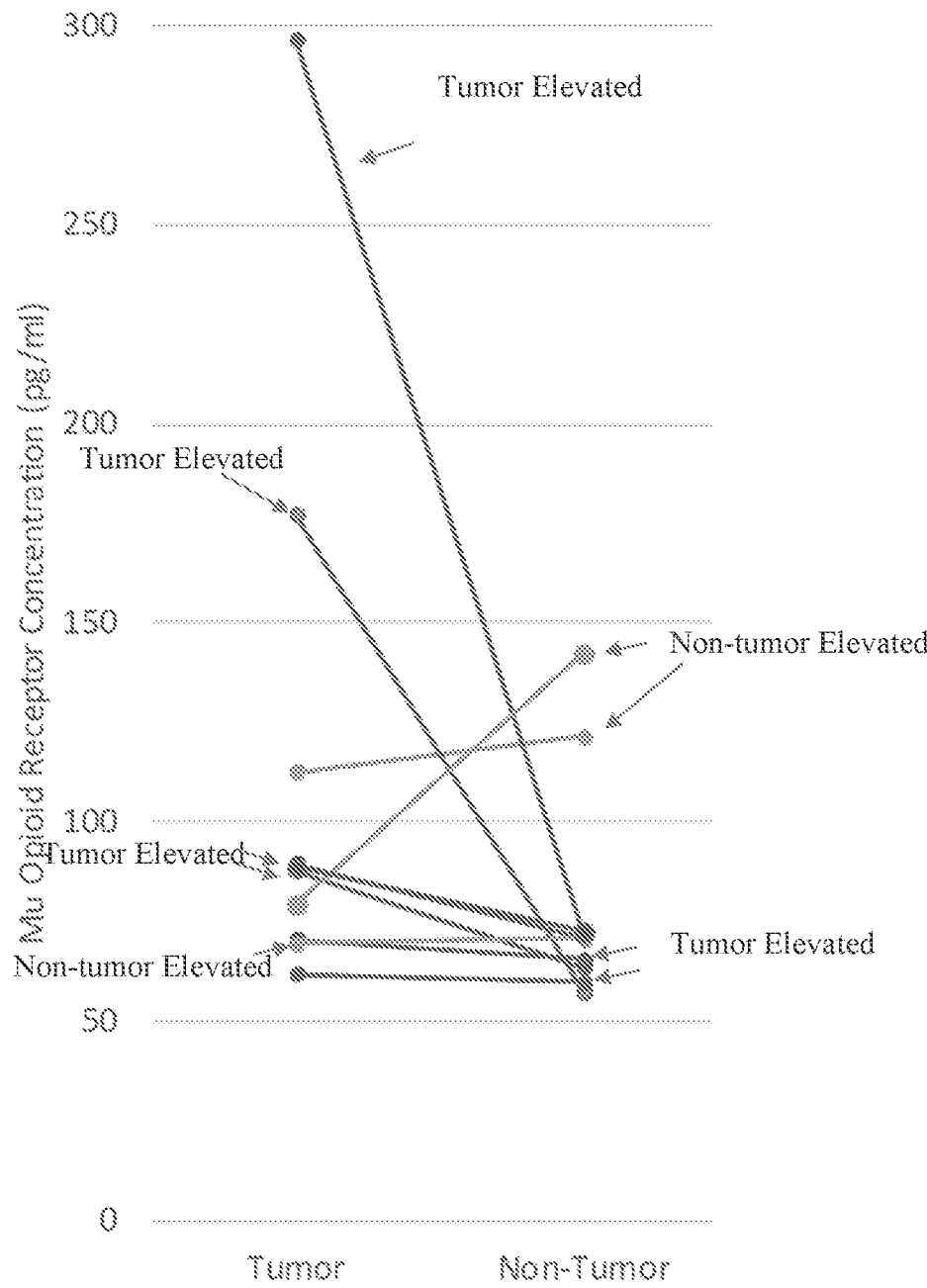
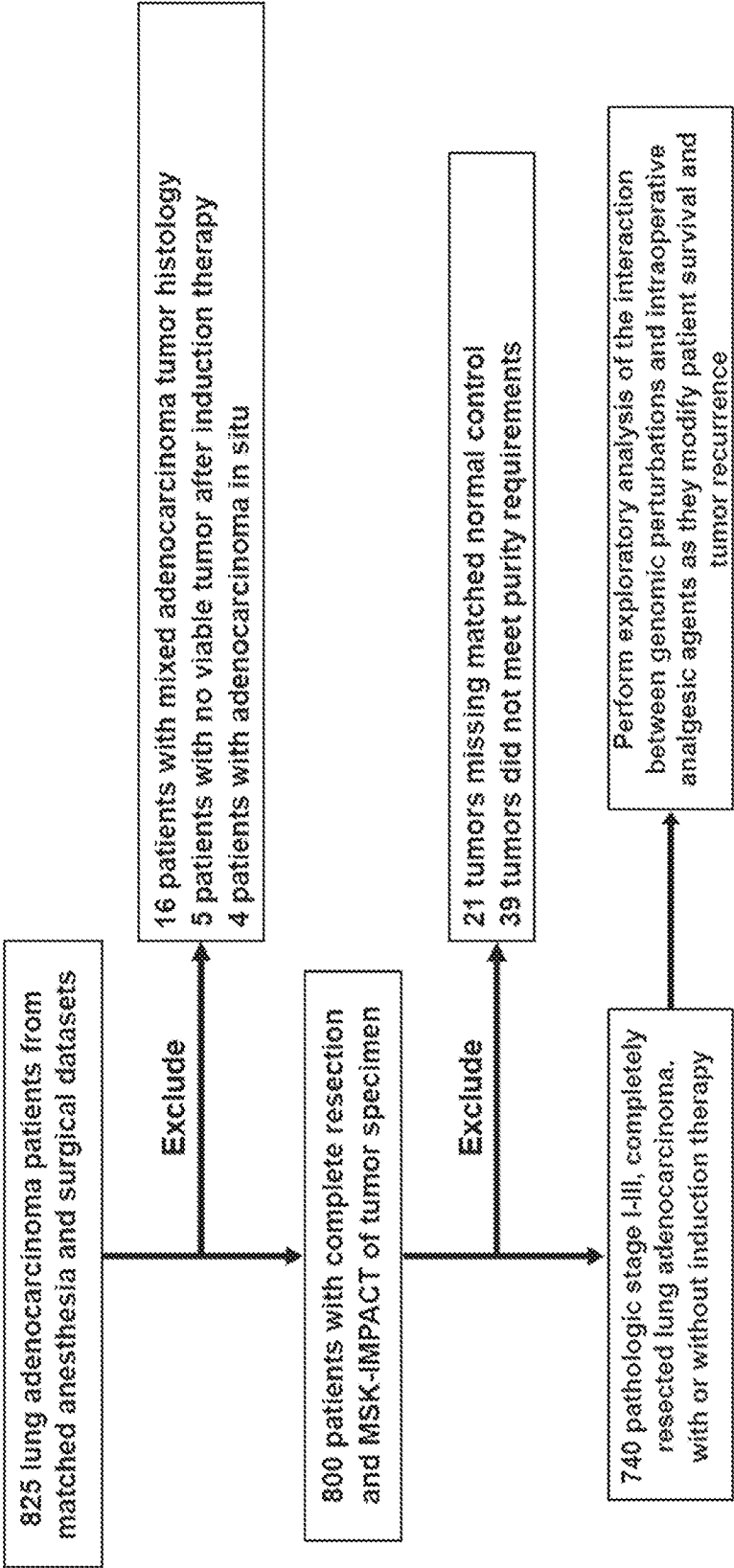
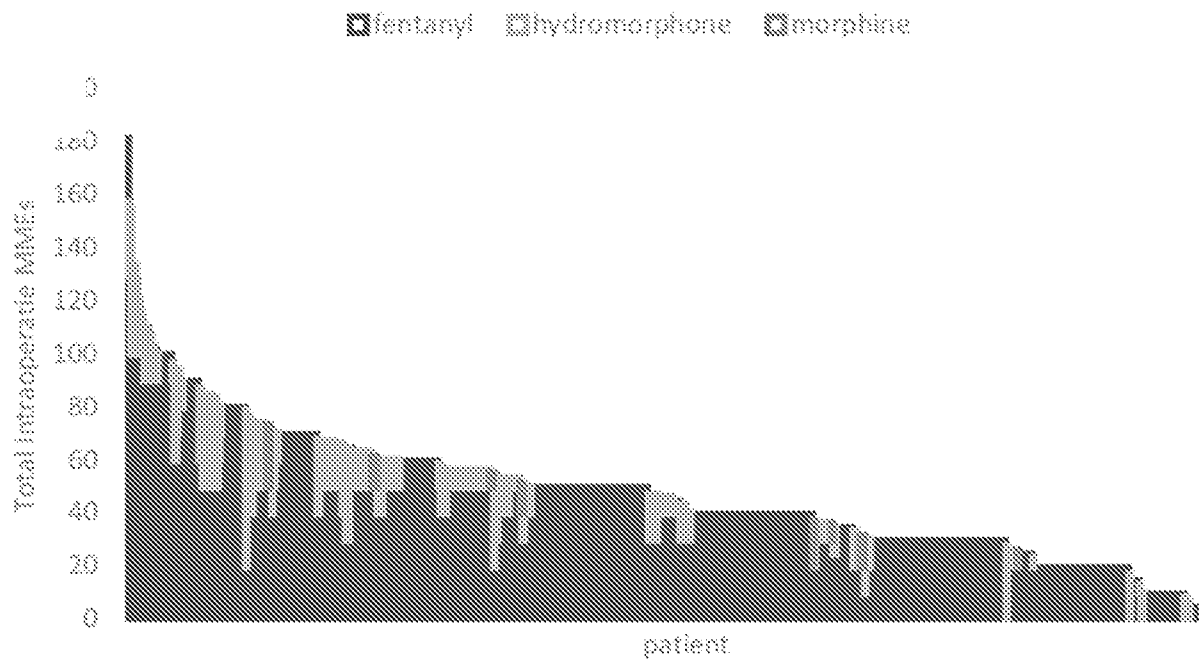


FIG. 4



**FIG. 5A**



**FIG. 5B**

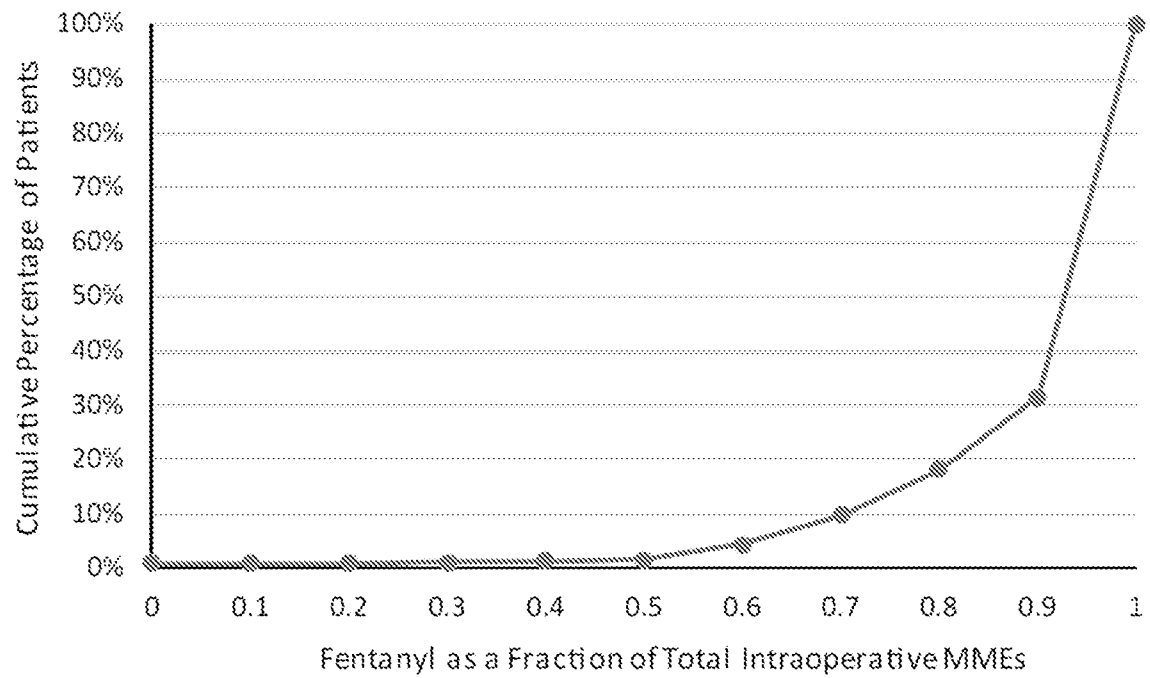


FIG. 6

| Genomic<br>Factor  | Overall Survival |             |          | Recurrence-Specific Survival |               |          |
|--------------------|------------------|-------------|----------|------------------------------|---------------|----------|
|                    | Estimate         | 95% CI      | <i>P</i> | Estimate                     | 95% CI        | <i>P</i> |
| TMB                | 0.005            | 0.001-0.009 | 0.026    | 0.001                        | 0.003-0.004   | 0.757    |
| FGA                | 0.447            | 0.011-0.883 | 0.044    | 0.097                        | 0.2594-0.4540 | 0.593    |
| Cell cycle         | 0.115            | 0.071-0.301 | 0.224    | 0.095                        | 0.0363-0.2271 | 0.156    |
| Hippo              | -0.012           | 0.330-0.306 | 0.942    | -0.440                       | 0.8591-0.0209 | 0.040    |
| Myc                | -0.130           | 0.430-0.170 | 0.395    | -0.207                       | 0.4629-0.0482 | 0.112    |
| Notch              | 0.043            | 0.144-0.230 | 0.653    | -0.120                       | 0.348-0.1073  | 0.300    |
| Nrf2               | 0.121            | 0.099-0.341 | 0.281    | 0.092                        | 0.098-0.2823  | 0.342    |
| Pi3K               | -0.104           | 0.238-0.030 | 0.127    | -0.090                       | 0.204-0.0232  | 0.119    |
| RTK/RAS            | 0.431            | 0.131-0.166 | 0.817    | 0.055                        | 0.0813-0.1908 | 0.372    |
| TGFβ               | -0.121           | 0.434-0.191 | 0.446    | 0.004                        | 0.3182-0.3267 | 0.979    |
| p53                | -0.049           | 0.201-0.103 | 0.525    | 0.008                        | 0.1078-0.1244 | 0.889    |
| Wnt                | 0.147            | 0.037-0.331 | 0.118    | -0.191                       | 0.3634-0.0194 | 0.029    |
| No. of<br>pathways | -0.006           | 0.065-0.053 | 0.834    | -0.021                       | 0.0643-0.0224 | 0.344    |
| CDK4               | 0.043            | 0.363-0.449 | 0.837    | 0.156                        | 0.1793-0.4908 | 0.362    |
| CDKN2A             | -0.157           | 0.001-0.434 | 0.052    | 0.101                        | 0.0563-0.2591 | 0.208    |
| EGFR               | -0.157           | 0.346-0.032 | 0.104    | 0.086                        | 0.0392-0.2111 | 0.178    |
| KRAS               | -0.004           | 0.138-0.130 | 0.956    | -0.088                       | 0.2046-0.0288 | 0.177    |
| MDM2               | 0.023            | 0.261-0.306 | 0.876    | 0.130                        | 0.0872-0.3476 | 0.241    |
| MET                | 0.100            | 0.402-0.601 | 0.696    | 0.019                        | 0.3052-0.3432 | 0.908    |
| RBM10              | -0.036           | 0.263-0.191 | 0.755    | -0.028                       | 0.2209-0.1651 | 0.777    |
| STK11              | -0.038           | 0.212-0.137 | 0.674    | -0.111                       | 0.2789-0.0566 | 0.194    |
| TERT               | -0.200           | 0.513-0.112 | 0.209    | -0.064                       | 0.2296-0.1009 | 0.445    |
| TP53               | -0.014           | 0.154-0.126 | 0.843    | -0.008                       | 0.1177-0.1022 | 0.891    |

FIG. 7

|    | Pathway    | Gene    | Total     | I         | II        | III       |
|----|------------|---------|-----------|-----------|-----------|-----------|
| 1  | Cell Cycle | CCND1   | 8 (1.08)  | 6 (0.81)  | 1 (0.14)  | 1 (0.14)  |
| 2  | Cell Cycle | CCND2   | 4 (0.54)  | 3 (0.41)  | 0 (0)     | 1 (0.14)  |
| 3  | Cell Cycle | CCND3   | 2 (0.27)  | 0 (0)     | 2 (0.27)  | 0 (0)     |
| 4  | Cell Cycle | CCNE1   | 6 (0.81)  | 5 (0.68)  | 0 (0)     | 1 (0.14)  |
| 5  | Cell Cycle | CDK4    | 39 (5.27) | 30 (4.05) | 7 (0.95)  | 2 (0.27)  |
| 6  | Cell Cycle | CDK6    | 1 (0.14)  | 0 (0)     | 1 (0.14)  | 0 (0)     |
| 7  | Cell Cycle | CDKN1B  | 7 (0.95)  | 3 (0.41)  | 3 (0.41)  | 1 (0.14)  |
| 8  | Cell Cycle | CDKN2A  | 64 (8.65) | 26 (3.51) | 25 (3.38) | 13 (1.76) |
| 9  | Cell Cycle | CDKN2B  | 29 (3.92) | 9 (1.22)  | 12 (1.62) | 8 (1.08)  |
| 10 | Cell Cycle | CDKN2C  | 1 (0.14)  | 1 (0.14)  | 0 (0)     | 0 (0)     |
| 11 | Cell Cycle | RB1     | 21 (2.84) | 15 (2.03) | 1 (0.14)  | 5 (0.68)  |
| 12 | Hippo      | FAT1    | 8 (1.08)  | 2 (0.27)  | 3 (0.41)  | 3 (0.41)  |
| 13 | Hippo      | LATS1   | 1 (0.14)  | 0 (0)     | 0 (0)     | 1 (0.14)  |
| 14 | Hippo      | NF2     | 4 (0.54)  | 2 (0.27)  | 1 (0.14)  | 1 (0.14)  |
| 15 | Myc        | MAX     | 12 (1.62) | 6 (0.81)  | 4 (0.54)  | 2 (0.27)  |
| 16 | Myc        | MGA     | 27 (3.65) | 14 (1.89) | 6 (0.81)  | 7 (0.95)  |
| 17 | Myc        | MYC     | 23 (3.11) | 15 (2.03) | 6 (0.81)  | 2 (0.27)  |
| 18 | Notch      | CREBSP  | 7 (0.95)  | 5 (0.68)  | 0 (0)     | 2 (0.27)  |
| 19 | Notch      | EP300   | 3 (0.41)  | 0 (0)     | 3 (0.41)  | 0 (0)     |
| 20 | Notch      | FBXW7   | 2 (0.27)  | 0 (0)     | 1 (0.14)  | 1 (0.14)  |
| 21 | Notch      | KDMAA   | 5 (0.68)  | 4 (0.54)  | 0 (0)     | 1 (0.14)  |
| 22 | Notch      | NCOR1   | 4 (0.54)  | 2 (0.27)  | 2 (0.27)  | 0 (0)     |
| 23 | Notch      | NOTCH1  | 3 (0.41)  | 2 (0.27)  | 0 (0)     | 1 (0.14)  |
| 24 | Notch      | NOTCH2  | 1 (0.14)  | 1 (0.14)  | 0 (0)     | 0 (0)     |
| 25 | Notch      | NOTCH3  | 3 (0.41)  | 2 (0.27)  | 1 (0.14)  | 0 (0)     |
| 26 | Notch      | NOTCH4  | 6 (0.81)  | 2 (0.27)  | 2 (0.27)  | 2 (0.27)  |
| 27 | Notch      | SPEN    | 4 (0.54)  | 3 (0.41)  | 1 (0.14)  | 0 (0)     |
| 28 | Nrf2       | KEAP1   | 34 (4.59) | 18 (2.43) | 8 (1.08)  | 8 (1.08)  |
| 29 | Nrf3       | NFE2L2  | 1 (0.14)  | 0 (0)     | 0 (0)     | 1 (0.14)  |
| 30 | P3K        | AKT1    | 1 (0.14)  | 0 (0)     | 1 (0.14)  | 0 (0)     |
| 31 | P3K        | AKT2    | 3 (0.41)  | 2 (0.27)  | 0 (0)     | 1 (0.14)  |
| 32 | P3K        | INPP4B  | 3 (0.41)  | 3 (0.41)  | 0 (0)     | 0 (0)     |
| 33 | P3K        | MTOR    | 3 (0.41)  | 3 (0.41)  | 0 (0)     | 0 (0)     |
| 34 | P3K        | PIK3CA  | 29 (3.92) | 16 (2.16) | 5 (0.68)  | 8 (1.08)  |
| 35 | P3K        | PIK3CB  | 2 (0.27)  | 1 (0.14)  | 1 (0.14)  | 0 (0)     |
| 36 | P3K        | PIK3R1  | 6 (0.81)  | 3 (0.41)  | 2 (0.27)  | 1 (0.14)  |
| 37 | P3K        | PIK3R2  | 1 (0.14)  | 1 (0.14)  | 0 (0)     | 0 (0)     |
| 38 | P3K        | PPP2R1A | 3 (0.41)  | 0 (0)     | 1 (0.14)  | 2 (0.27)  |
| 39 | P3K        | PTEN    | 12 (1.62) | 6 (0.81)  | 3 (0.41)  | 3 (0.41)  |

FIG. 7 (Contd.)

|    | Pathway | Gene   | Total       | I           | II        | III       |
|----|---------|--------|-------------|-------------|-----------|-----------|
| 40 | PI3K    | RHEB   | 1 (0.14)    | 0 (0)       | 1 (0.14)  | 0 (0)     |
| 41 | PI3K    | PICTOR | 5 (0.68)    | 3 (0.41)    | 1 (0.14)  | 1 (0.14)  |
| 42 | PI3K    | STK11  | 94 (12.7)   | 54 (7.3)    | 23 (3.11) | 17 (2.3)  |
| 43 | PI3K    | TSC1   | 5 (0.68)    | 3 (0.41)    | 2 (0.27)  | 0 (0)     |
| 44 | PI3K    | TSC2   | 4 (0.54)    | 2 (0.27)    | 1 (0.14)  | 1 (0.14)  |
| 45 | RTK/RAS | ABL1   | 1 (0.14)    | 1 (0.14)    | 0 (0)     | 0 (0)     |
| 46 | RTK/RAS | ALK    | 17 (2.3)    | 9 (1.22)    | 4 (0.54)  | 4 (0.54)  |
| 47 | RTK/RAS | BRAF   | 30 (4.05)   | 16 (2.16)   | 9 (1.22)  | 5 (0.68)  |
| 48 | RTK/RAS | CBL    | 1 (0.14)    | 0 (0)       | 0 (0)     | 1 (0.14)  |
| 49 | RTK/RAS | EGFR   | 225 (30.41) | 151 (20.41) | 39 (5.27) | 35 (4.73) |
| 50 | RTK/RAS | ERBB2  | 30 (4.05)   | 16 (2.16)   | 6 (0.81)  | 8 (1.08)  |
| 51 | RTK/RAS | ERBB3  | 5 (0.68)    | 4 (0.54)    | 1 (0.14)  | 0 (0)     |
| 52 | RTK/RAS | FGFR1  | 1 (0.14)    | 0 (0)       | 0 (0)     | 1 (0.14)  |
| 53 | RTK/RAS | FGFR4  | 3 (0.41)    | 2 (0.27)    | 1 (0.14)  | 0 (0)     |
| 54 | RTK/RAS | FLT3   | 2 (0.27)    | 2 (0.27)    | 0 (0)     | 0 (0)     |
| 55 | RTK/RAS | KIT    | 2 (0.27)    | 1 (0.14)    | 1 (0.14)  | 0 (0)     |
| 56 | RTK/RAS | KRAS   | 280 (37.84) | 168 (22.7)  | 61 (8.24) | 51 (6.89) |
| 57 | RTK/RAS | MAP2K1 | 6 (0.81)    | 3 (0.41)    | 2 (0.27)  | 1 (0.14)  |
| 58 | RTK/RAS | MAPK1  | 1 (0.14)    | 1 (0.14)    | 0 (0)     | 0 (0)     |
| 59 | RTK/RAS | MET    | 38 (5.14)   | 22 (2.97)   | 12 (1.62) | 4 (0.54)  |
| 60 | RTK/RAS | NF1    | 28 (3.78)   | 17 (2.3)    | 4 (0.54)  | 7 (0.95)  |
| 61 | RTK/RAS | NRAS   | 6 (0.81)    | 3 (0.41)    | 2 (0.27)  | 1 (0.14)  |
| 62 | RTK/RAS | NTRK1  | 6 (0.81)    | 2 (0.27)    | 1 (0.14)  | 3 (0.41)  |
| 63 | RTK/RAS | PDGFRA | 2 (0.27)    | 1 (0.14)    | 1 (0.14)  | 0 (0)     |
| 64 | RTK/RAS | PTPN11 | 1 (0.14)    | 1 (0.14)    | 0 (0)     | 0 (0)     |
| 65 | RTK/RAS | RAC1   | 2 (0.27)    | 1 (0.14)    | 1 (0.14)  | 0 (0)     |
| 66 | RTK/RAS | RAF1   | 4 (0.54)    | 4 (0.54)    | 0 (0)     | 0 (0)     |
| 67 | RTK/RAS | RASA1  | 6 (0.81)    | 2 (0.27)    | 2 (0.27)  | 2 (0.27)  |
| 68 | RTK/RAS | RET    | 14 (1.89)   | 11 (1.49)   | 1 (0.14)  | 2 (0.27)  |
| 69 | RTK/RAS | RIT1   | 2 (0.27)    | 1 (0.14)    | 1 (0.14)  | 0 (0)     |
| 70 | RTK/RAS | ROS1   | 6 (0.81)    | 2 (0.27)    | 1 (0.14)  | 3 (0.41)  |
| 71 | RTK/RAS | SPRED1 | 1 (0.14)    | 1 (0.14)    | 0 (0)     | 0 (0)     |
| 72 | TGFβ    | SMAD2  | 1 (0.14)    | 1 (0.14)    | 0 (0)     | 0 (0)     |
| 73 | TGFβ    | SMAD3  | 1 (0.14)    | 0 (0)       | 0 (0)     | 1 (0.14)  |
| 74 | TGFβ    | SMAD4  | 16 (2.16)   | 5 (0.68)    | 6 (0.81)  | 5 (0.68)  |
| 75 | TGFβ    | TGFBR1 | 3 (0.41)    | 2 (0.27)    | 0 (0)     | 1 (0.14)  |
| 76 | TGFβ    | TGFBR2 | 2 (0.27)    | 0 (0)       | 1 (0.14)  | 1 (0.14)  |
| 77 | TP53    | ATM    | 19 (2.57)   | 7 (0.95)    | 9 (1.22)  | 3 (0.41)  |
| 78 | TP53    | CHEK2  | 1 (0.14)    | 0 (0)       | 0 (0)     | 1 (0.14)  |
| 79 | TP53    | MDM2   | 55 (7.43)   | 37 (5)      | 12 (1.62) | 6 (0.81)  |

**FIG. 7 (Contd.)**

|    | Pathway | Gene   | Total       | I        | II        | III      |
|----|---------|--------|-------------|----------|-----------|----------|
| 80 | TP53    | MDM4   | 5 (0.68)    | 4 (0.54) | 1 (0.14)  | 0 (0)    |
| 81 | TP53    | TP53   | 268 (36.22) | 148 (20) | 63 (8.51) | 57 (7.7) |
| 82 | Wnt     | AMER1  | 6 (0.81)    | 3 (0.41) | 2 (0.27)  | 1 (0.14) |
| 83 | Wnt     | APC    | 17 (2.3)    | 7 (0.95) | 4 (0.54)  | 6 (0.81) |
| 84 | Wnt     | AXIN2  | 3 (0.41)    | 2 (0.27) | 1 (0.14)  | 0 (0)    |
| 85 | Wnt     | CTNNB1 | 20 (2.7)    | 7 (0.95) | 9 (1.22)  | 4 (0.54) |
| 86 | Wnt     | RNF43  | 1 (0.14)    | 0 (0)    | 0 (0)     | 1 (0.14) |

FIG. 8

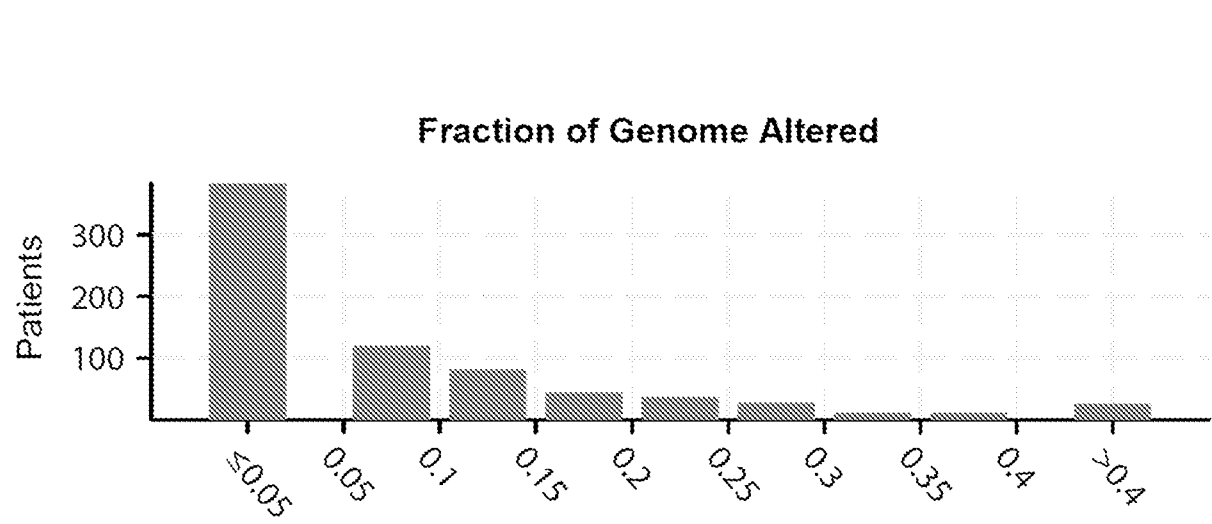


FIG. 9

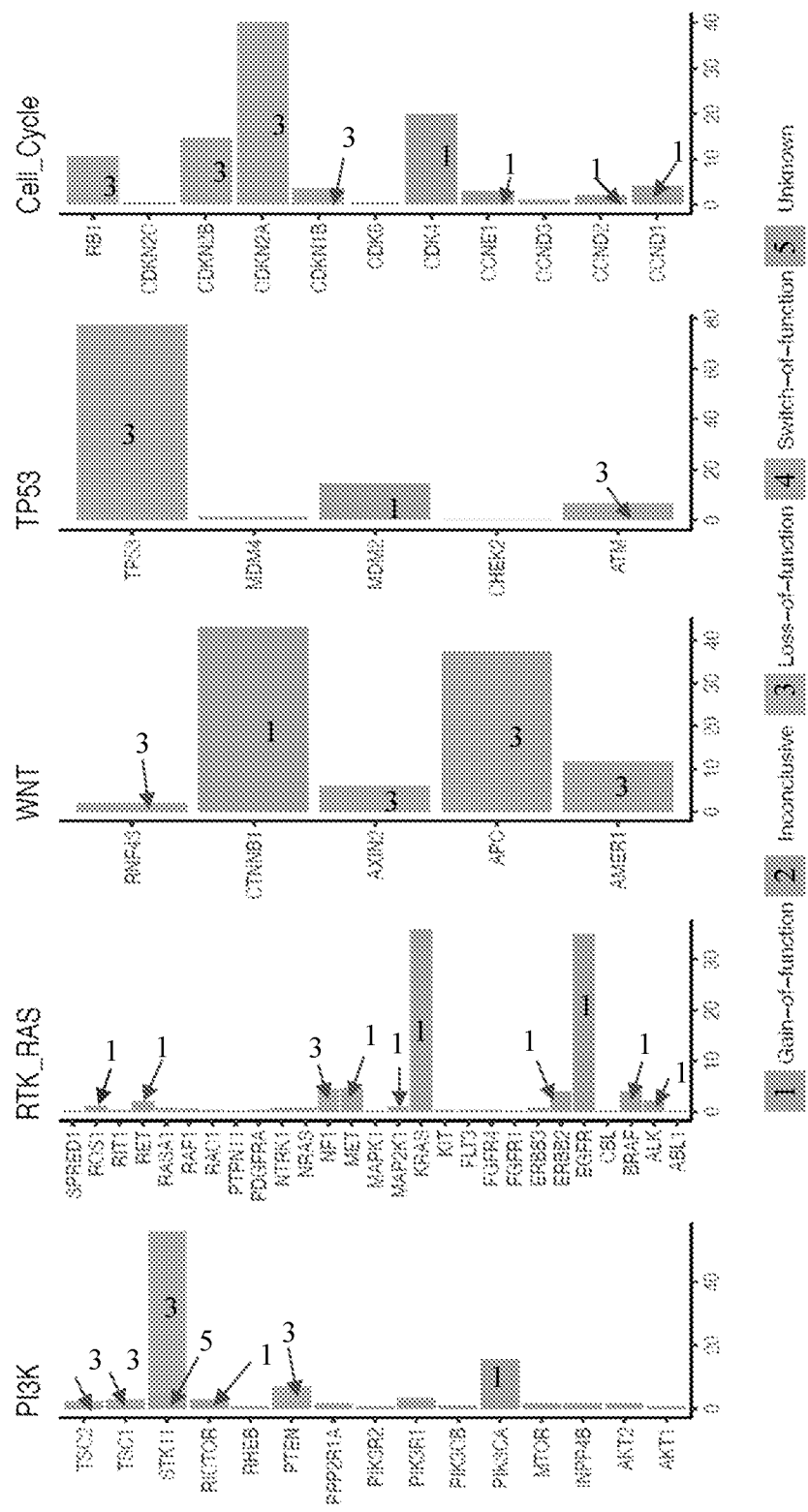
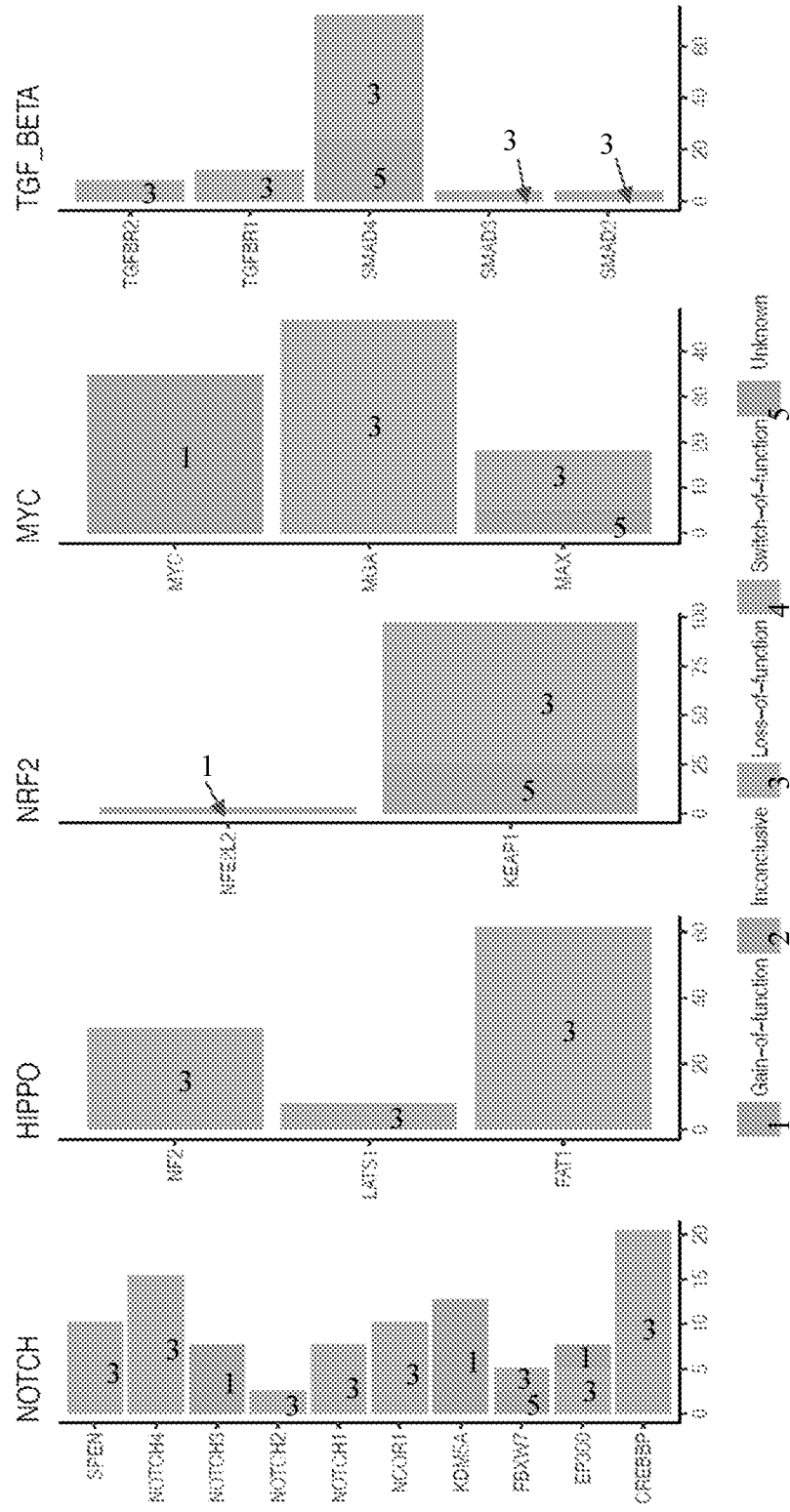


FIG. 9 (Contd.)



**FIG. 10**

| gene   | mut_effect       | freq | total_mut | percent  |
|--------|------------------|------|-----------|----------|
| CDK4   | Gain-of-function | 39   | 39        | 100      |
| CDK4   | Inconclusive     | 0    | 39        | 0        |
| CDK4   | Loss-of-function | 0    | 39        | 0        |
| CDK4   | Unknown          | 0    | 39        | 0        |
| CDKN2A | Gain-of-function | 0    | 79        | 0        |
| CDKN2A | Inconclusive     | 0    | 79        | 0        |
| CDKN2A | Loss-of-function | 79   | 79        | 100      |
| CDKN2A | Unknown          | 0    | 79        | 0        |
| EGFR   | Gain-of-function | 276  | 278       | 99.28058 |
| EGFR   | Inconclusive     | 1    | 278       | 0.359712 |
| EGFR   | Loss-of-function | 0    | 278       | 0        |
| EGFR   | Unknown          | 1    | 278       | 0.359712 |
| KRAS   | Gain-of-function | 285  | 285       | 100      |
| KRAS   | Inconclusive     | 0    | 285       | 0        |
| KRAS   | Loss-of-function | 0    | 285       | 0        |
| KRAS   | Unknown          | 0    | 285       | 0        |
| MET    | Gain-of-function | 37   | 37        | 100      |
| MET    | Inconclusive     | 0    | 37        | 0        |
| MET    | Loss-of-function | 0    | 37        | 0        |
| MET    | Unknown          | 0    | 37        | 0        |
| RBM10  | Gain-of-function | 0    | 92        | 0        |
| RBM10  | Inconclusive     | 0    | 92        | 0        |
| RBM10  | Loss-of-function | 92   | 92        | 100      |
| RBM10  | Unknown          | 0    | 92        | 0        |
| STK11  | Gain-of-function | 0    | 97        | 0        |
| STK11  | Inconclusive     | 0    | 97        | 0        |
| STK11  | Loss-of-function | 89   | 97        | 91.75258 |
| STK11  | Unknown          | 8    | 97        | 8.247423 |
| TERT   | Gain-of-function | 61   | 61        | 100      |
| TERT   | Inconclusive     | 0    | 61        | 0        |
| TERT   | Loss-of-function | 0    | 61        | 0        |
| TERT   | Unknown          | 0    | 61        | 0        |
| TP53   | Gain-of-function | 0    | 298       | 0        |
| TP53   | Inconclusive     | 0    | 298       | 0        |
| TP53   | Loss-of-function | 298  | 298       | 100      |
| TP53   | Unknown          | 0    | 298       | 0        |
| MDM2   | Gain-of-function | 55   | 55        | 100      |
| MDM2   | Loss-of-function | 0    | 55        | 0        |

**FIG. 11**

| gene   | mut_effect       | freq | pathway    | path_tot | percent  |
|--------|------------------|------|------------|----------|----------|
| AKT1   | Gain-of-function | 1    | PI3K       | 173      | 0.578035 |
| ALK    | Gain-of-function | 17   | RTK_RAS    | 792      | 2.146465 |
| AMER1  | Gain-of-function | 0    | WNT        | 51       | 0        |
| APC    | Gain-of-function | 0    | WNT        | 51       | 0        |
| ATM    | Gain-of-function | 0    | TP53       | 384      | 0        |
| AXIN2  | Gain-of-function | 0    | WNT        | 51       | 0        |
| BRAF   | Gain-of-function | 30   | RTK_RAS    | 792      | 3.787879 |
| CBL    | Gain-of-function | 0    | RTK_RAS    | 792      | 0        |
| CDK4   | Gain-of-function | 39   | Cell_Cycle | 197      | 19.79695 |
| CDKN1B | Gain-of-function | 0    | Cell_Cycle | 197      | 0        |
| CDKN2A | Gain-of-function | 0    | Cell_Cycle | 197      | 0        |
| CDKN2C | Gain-of-function | 0    | Cell_Cycle | 197      | 0        |
| CHEK2  | Gain-of-function | 0    | TP53       | 384      | 0        |
| CREBBP | Gain-of-function | 0    | NOTCH      | 39       | 0        |
| CTNNB1 | Gain-of-function | 22   | WNT        | 51       | 43.13725 |
| EGFR   | Gain-of-function | 276  | RTK_RAS    | 792      | 34.84848 |
| EP300  | Gain-of-function | 1    | NOTCH      | 39       | 2.564103 |
| ERBB2  | Gain-of-function | 31   | RTK_RAS    | 792      | 3.914141 |
| ERBB3  | Gain-of-function | 5    | RTK_RAS    | 792      | 0.631313 |
| FAT1   | Gain-of-function | 0    | HIPPO      | 13       | 0        |
| FBXW7  | Gain-of-function | 0    | NOTCH      | 39       | 0        |
| FLT3   | Gain-of-function | 2    | RTK_RAS    | 792      | 0.252525 |
| INPP4B | Gain-of-function | 0    | PI3K       | 173      | 0        |
| KEAP1  | Gain-of-function | 0    | NRF2       | 35       | 0        |
| KRAS   | Gain-of-function | 285  | RTK_RAS    | 792      | 35.98485 |
| LATS1  | Gain-of-function | 0    | HIPPO      | 13       | 0        |
| MAP2K1 | Gain-of-function | 7    | RTK_RAS    | 792      | 0.883838 |
| MAX    | Gain-of-function | 0    | MYC        | 66       | 0        |
| MET    | Gain-of-function | 37   | RTK_RAS    | 792      | 4.671717 |
| MGA    | Gain-of-function | 0    | MYC        | 66       | 0        |
| MTOR   | Gain-of-function | 3    | PI3K       | 173      | 1.734104 |
| NCOR1  | Gain-of-function | 0    | NOTCH      | 39       | 0        |
| NF1    | Gain-of-function | 0    | RTK_RAS    | 792      | 0        |
| NF2    | Gain-of-function | 0    | HIPPO      | 13       | 0        |
| NFE2L2 | Gain-of-function | 1    | NRF2       | 35       | 2.857143 |
| NOTCH1 | Gain-of-function | 0    | NOTCH      | 39       | 0        |
| NOTCH2 | Gain-of-function | 0    | NOTCH      | 39       | 0        |
| NOTCH3 | Gain-of-function | 3    | NOTCH      | 39       | 7.692308 |
| NOTCH4 | Gain-of-function | 0    | NOTCH      | 39       | 0        |

**FIG. 11 (Contd.)**

| gene    | mut_effect       | freq | pathway    | path_tot | percent  |
|---------|------------------|------|------------|----------|----------|
| NRAS    | Gain-of-function | 6    | RTK_RAS    | 792      | 0.757576 |
| NTRK1   | Gain-of-function | 6    | RTK_RAS    | 792      | 0.757576 |
| PIK3CA  | Gain-of-function | 27   | PI3K       | 173      | 15.60694 |
| PIK3CB  | Gain-of-function | 2    | PI3K       | 173      | 1.156069 |
| PIK3R1  | Gain-of-function | 0    | PI3K       | 173      | 0        |
| PIK3R2  | Gain-of-function | 0    | PI3K       | 173      | 0        |
| PPP2R1A | Gain-of-function | 0    | PI3K       | 173      | 0        |
| PTEN    | Gain-of-function | 0    | PI3K       | 173      | 0        |
| PTPN11  | Gain-of-function | 1    | RTK_RAS    | 792      | 0.126263 |
| RAC1    | Gain-of-function | 2    | RTK_RAS    | 792      | 0.252525 |
| RAF1    | Gain-of-function | 4    | RTK_RAS    | 792      | 0.505051 |
| RASA1   | Gain-of-function | 0    | RTK_RAS    | 792      | 0        |
| RB1     | Gain-of-function | 0    | Cell_Cycle | 197      | 0        |
| RIT1    | Gain-of-function | 2    | RTK_RAS    | 792      | 0.252525 |
| RNF43   | Gain-of-function | 0    | WNT        | 51       | 0        |
| ROS1    | Gain-of-function | 8    | RTK_RAS    | 792      | 1.010101 |
| SMAD2   | Gain-of-function | 0    | TGF_BETA   | 25       | 0        |
| SMAD3   | Gain-of-function | 0    | TGF_BETA   | 25       | 0        |
| SMAD4   | Gain-of-function | 0    | TGF_BETA   | 25       | 0        |
| SPEN    | Gain-of-function | 0    | NOTCH      | 39       | 0        |
| SPRED1  | Gain-of-function | 0    | RTK_RAS    | 792      | 0        |
| STK11   | Gain-of-function | 0    | PI3K       | 173      | 0        |
| TGFBR1  | Gain-of-function | 0    | TGF_BETA   | 25       | 0        |
| TGFBR2  | Gain-of-function | 0    | TGF_BETA   | 25       | 0        |
| TP53    | Gain-of-function | 0    | TP53       | 384      | 0        |
| TSC1    | Gain-of-function | 0    | PI3K       | 173      | 0        |
| TSC2    | Gain-of-function | 0    | PI3K       | 173      | 0        |
| AKT1    | Inconclusive     | 0    | PI3K       | 173      | 0        |
| ALK     | Inconclusive     | 0    | RTK_RAS    | 792      | 0        |
| AMER1   | Inconclusive     | 0    | WNT        | 51       | 0        |
| APC     | Inconclusive     | 0    | WNT        | 51       | 0        |
| ATM     | Inconclusive     | 0    | TP53       | 384      | 0        |
| AXIN2   | Inconclusive     | 0    | WNT        | 51       | 0        |
| BRAF    | Inconclusive     | 0    | RTK_RAS    | 792      | 0        |
| CBL     | Inconclusive     | 0    | RTK_RAS    | 792      | 0        |
| CDK4    | Inconclusive     | 0    | Cell_Cycle | 197      | 0        |
| CDKN1B  | Inconclusive     | 0    | Cell_Cycle | 197      | 0        |
| CDKN2A  | Inconclusive     | 0    | Cell_Cycle | 197      | 0        |
| CDKN2C  | Inconclusive     | 0    | Cell_Cycle | 197      | 0        |

**FIG. 11 (Contd.)**

| gene    | mut_effect   | freq | pathway | path_tot | percent  |
|---------|--------------|------|---------|----------|----------|
| CHEK2   | Inconclusive | 0    | TP53    | 384      | 0        |
| CREBBP  | Inconclusive | 0    | NOTCH   | 39       | 0        |
| CTNNB1  | Inconclusive | 0    | WNT     | 51       | 0        |
| EGFR    | Inconclusive | 1    | RTK_RAS | 792      | 0.126263 |
| EP300   | Inconclusive | 0    | NOTCH   | 39       | 0        |
| ERBB2   | Inconclusive | 0    | RTK_RAS | 792      | 0        |
| ERBB3   | Inconclusive | 0    | RTK_RAS | 792      | 0        |
| FAT1    | Inconclusive | 0    | HIPPO   | 13       | 0        |
| FBXW7   | Inconclusive | 0    | NOTCH   | 39       | 0        |
| FLT3    | Inconclusive | 0    | RTK_RAS | 792      | 0        |
| INPP4B  | Inconclusive | 0    | PI3K    | 173      | 0        |
| KEAP1   | Inconclusive | 0    | NRF2    | 35       | 0        |
| KRAS    | Inconclusive | 0    | RTK_RAS | 792      | 0        |
| LATS1   | Inconclusive | 0    | HIPPO   | 13       | 0        |
| MAP2K1  | Inconclusive | 0    | RTK_RAS | 792      | 0        |
| MAX     | Inconclusive | 0    | MYC     | 66       | 0        |
| MET     | Inconclusive | 0    | RTK_RAS | 792      | 0        |
| MGA     | Inconclusive | 0    | MYC     | 66       | 0        |
| MTOR    | Inconclusive | 0    | PI3K    | 173      | 0        |
| NCOR1   | Inconclusive | 0    | NOTCH   | 39       | 0        |
| NF1     | Inconclusive | 0    | RTK_RAS | 792      | 0        |
| NF2     | Inconclusive | 0    | HIPPO   | 13       | 0        |
| NFE2L2  | Inconclusive | 0    | NRF2    | 35       | 0        |
| NOTCH1  | Inconclusive | 0    | NOTCH   | 39       | 0        |
| NOTCH2  | Inconclusive | 0    | NOTCH   | 39       | 0        |
| NOTCH3  | Inconclusive | 0    | NOTCH   | 39       | 0        |
| NOTCH4  | Inconclusive | 0    | NOTCH   | 39       | 0        |
| NRAS    | Inconclusive | 0    | RTK_RAS | 792      | 0        |
| NTRK1   | Inconclusive | 0    | RTK_RAS | 792      | 0        |
| PIK3CA  | Inconclusive | 0    | PI3K    | 173      | 0        |
| PIK3CB  | Inconclusive | 0    | PI3K    | 173      | 0        |
| PIK3R1  | Inconclusive | 0    | PI3K    | 173      | 0        |
| PIK3R2  | Inconclusive | 0    | PI3K    | 173      | 0        |
| PPP2R1A | Inconclusive | 0    | PI3K    | 173      | 0        |
| PTEN    | Inconclusive | 0    | PI3K    | 173      | 0        |
| PTPN11  | Inconclusive | 0    | RTK_RAS | 792      | 0        |
| RAC1    | Inconclusive | 0    | RTK_RAS | 792      | 0        |
| RAF1    | Inconclusive | 0    | RTK_RAS | 792      | 0        |
| RASA1   | Inconclusive | 0    | RTK_RAS | 792      | 0        |

**FIG. 11 (Contd.)**

| gene   | mut_effect       | freq | pathway    | path_tot | percent  |
|--------|------------------|------|------------|----------|----------|
| RB1    | Inconclusive     | 0    | Cell_Cycle | 197      | 0        |
| RIT1   | Inconclusive     | 0    | RTK_RAS    | 792      | 0        |
| RNF43  | Inconclusive     | 0    | WNT        | 51       | 0        |
| ROS1   | Inconclusive     | 0    | RTK_RAS    | 792      | 0        |
| SMAD2  | Inconclusive     | 0    | TGF_BETA   | 25       | 0        |
| SMAD3  | Inconclusive     | 0    | TGF_BETA   | 25       | 0        |
| SMAD4  | Inconclusive     | 0    | TGF_BETA   | 25       | 0        |
| SPEN   | Inconclusive     | 0    | NOTCH      | 39       | 0        |
| SPRED1 | Inconclusive     | 0    | RTK_RAS    | 792      | 0        |
| STK11  | Inconclusive     | 0    | PI3K       | 173      | 0        |
| TGFBR1 | Inconclusive     | 0    | TGF_BETA   | 25       | 0        |
| TGFBR2 | Inconclusive     | 0    | TGF_BETA   | 25       | 0        |
| TP53   | Inconclusive     | 0    | TP53       | 384      | 0        |
| TSC1   | Inconclusive     | 0    | PI3K       | 173      | 0        |
| TSC2   | Inconclusive     | 0    | PI3K       | 173      | 0        |
| AKT1   | Loss-of-function | 0    | PI3K       | 173      | 0        |
| ALK    | Loss-of-function | 0    | RTK_RAS    | 792      | 0        |
| AMER1  | Loss-of-function | 6    | WNT        | 51       | 11.76471 |
| APC    | Loss-of-function | 19   | WNT        | 51       | 37.2549  |
| ATM    | Loss-of-function | 25   | TP53       | 384      | 6.510417 |
| AXIN2  | Loss-of-function | 3    | WNT        | 51       | 5.882353 |
| BRAF   | Loss-of-function | 0    | RTK_RAS    | 792      | 0        |
| CBL    | Loss-of-function | 1    | RTK_RAS    | 792      | 0.126263 |
| CDK4   | Loss-of-function | 0    | Cell_Cycle | 197      | 0        |
| CDKN1B | Loss-of-function | 7    | Cell_Cycle | 197      | 3.553299 |
| CDKN2A | Loss-of-function | 79   | Cell_Cycle | 197      | 40.10152 |
| CDKN2C | Loss-of-function | 1    | Cell_Cycle | 197      | 0.507614 |
| CHEK2  | Loss-of-function | 1    | TP53       | 384      | 0.260417 |
| CREBBP | Loss-of-function | 8    | NOTCH      | 39       | 20.51282 |
| CTNNB1 | Loss-of-function | 0    | WNT        | 51       | 0        |
| EGFR   | Loss-of-function | 0    | RTK_RAS    | 792      | 0        |
| EP300  | Loss-of-function | 2    | NOTCH      | 39       | 5.128205 |
| ERBB2  | Loss-of-function | 0    | RTK_RAS    | 792      | 0        |
| ERBB3  | Loss-of-function | 0    | RTK_RAS    | 792      | 0        |
| FAT1   | Loss-of-function | 8    | HIPPO      | 13       | 61.53846 |
| FBXW7  | Loss-of-function | 1    | NOTCH      | 39       | 2.564103 |
| FLT3   | Loss-of-function | 0    | RTK_RAS    | 792      | 0        |
| INPP4B | Loss-of-function | 3    | PI3K       | 173      | 1.734104 |
| KEAP1  | Loss-of-function | 25   | NRF2       | 35       | 71.42857 |

**FIG. 11 (Contd.)**

| gene    | mut_effect       | freq | pathway    | path_tot | percent  |
|---------|------------------|------|------------|----------|----------|
| KRAS    | Loss-of-function | 0    | RTK_RAS    | 792      | 0        |
| LATS1   | Loss-of-function | 1    | HIPPO      | 13       | 7.692308 |
| MAP2K1  | Loss-of-function | 0    | RTK_RAS    | 792      | 0        |
| MAX     | Loss-of-function | 9    | MYC        | 66       | 13.63636 |
| MET     | Loss-of-function | 0    | RTK_RAS    | 792      | 0        |
| MGA     | Loss-of-function | 31   | MYC        | 66       | 46.9697  |
| MTOR    | Loss-of-function | 0    | PI3K       | 173      | 0        |
| NCOR1   | Loss-of-function | 4    | NOTCH      | 39       | 10.25641 |
| NF1     | Loss-of-function | 34   | RTK_RAS    | 792      | 4.292929 |
| NF2     | Loss-of-function | 4    | HIPPO      | 13       | 30.76923 |
| NFE2L2  | Loss-of-function | 0    | NRF2       | 35       | 0        |
| NOTCH1  | Loss-of-function | 3    | NOTCH      | 39       | 7.692308 |
| NOTCH2  | Loss-of-function | 1    | NOTCH      | 39       | 2.564103 |
| NOTCH3  | Loss-of-function | 0    | NOTCH      | 39       | 0        |
| NOTCH4  | Loss-of-function | 6    | NOTCH      | 39       | 15.38462 |
| NRAS    | Loss-of-function | 0    | RTK_RAS    | 792      | 0        |
| NTRK1   | Loss-of-function | 0    | RTK_RAS    | 792      | 0        |
| PIK3CA  | Loss-of-function | 0    | PI3K       | 173      | 0        |
| PIK3CB  | Loss-of-function | 0    | PI3K       | 173      | 0        |
| PIK3R1  | Loss-of-function | 3    | PI3K       | 173      | 1.734104 |
| PIK3R2  | Loss-of-function | 1    | PI3K       | 173      | 0.578035 |
| PPP2R1A | Loss-of-function | 3    | PI3K       | 173      | 1.734104 |
| PTEN    | Loss-of-function | 11   | PI3K       | 173      | 6.358382 |
| PTPN11  | Loss-of-function | 0    | RTK_RAS    | 792      | 0        |
| RAC1    | Loss-of-function | 0    | RTK_RAS    | 792      | 0        |
| RAF1    | Loss-of-function | 0    | RTK_RAS    | 792      | 0        |
| RASA1   | Loss-of-function | 6    | RTK_RAS    | 792      | 0.757576 |
| RB1     | Loss-of-function | 21   | Cell_Cycle | 197      | 10.6599  |
| RIT1    | Loss-of-function | 0    | RTK_RAS    | 792      | 0        |
| RNF43   | Loss-of-function | 1    | WNT        | 51       | 1.960784 |
| ROS1    | Loss-of-function | 0    | RTK_RAS    | 792      | 0        |
| SMAD2   | Loss-of-function | 1    | TGF_BETA   | 25       | 4        |
| SMAD3   | Loss-of-function | 1    | TGF_BETA   | 25       | 4        |
| SMAD4   | Loss-of-function | 12   | TGF_BETA   | 25       | 48       |
| SPEN    | Loss-of-function | 4    | NOTCH      | 39       | 10.25641 |
| SPRED1  | Loss-of-function | 1    | RTK_RAS    | 792      | 0.126263 |
| STK11   | Loss-of-function | 89   | PI3K       | 173      | 51.44509 |
| TGFBR1  | Loss-of-function | 3    | TGF_BETA   | 25       | 12       |
| TGFBR2  | Loss-of-function | 2    | TGF_BETA   | 25       | 8        |

**FIG. 11 (Contd.)**

| gene   | mut_effect         | freq | pathway    | path_tot | percent  |
|--------|--------------------|------|------------|----------|----------|
| TP53   | Loss-of-function   | 298  | TP53       | 384      | 77.60417 |
| TSC1   | Loss-of-function   | 5    | PI3K       | 173      | 2.890173 |
| TSC2   | Loss-of-function   | 4    | PI3K       | 173      | 2.312139 |
| AKT1   | Switch-of-function | 0    | PI3K       | 173      | 0        |
| ALK    | Switch-of-function | 0    | RTK_RAS    | 792      | 0        |
| AMER1  | Switch-of-function | 0    | WNT        | 51       | 0        |
| APC    | Switch-of-function | 0    | WNT        | 51       | 0        |
| ATM    | Switch-of-function | 0    | TP53       | 384      | 0        |
| AXIN2  | Switch-of-function | 0    | WNT        | 51       | 0        |
| BRAF   | Switch-of-function | 0    | RTK_RAS    | 792      | 0        |
| CBL    | Switch-of-function | 0    | RTK_RAS    | 792      | 0        |
| CDK4   | Switch-of-function | 0    | Cell_Cycle | 197      | 0        |
| CDKN1B | Switch-of-function | 0    | Cell_Cycle | 197      | 0        |
| CDKN2A | Switch-of-function | 0    | Cell_Cycle | 197      | 0        |
| CDKN2C | Switch-of-function | 0    | Cell_Cycle | 197      | 0        |
| CHEK2  | Switch-of-function | 0    | TP53       | 384      | 0        |
| CREBBP | Switch-of-function | 0    | NOTCH      | 39       | 0        |
| CTNNB1 | Switch-of-function | 0    | WNT        | 51       | 0        |
| EGFR   | Switch-of-function | 0    | RTK_RAS    | 792      | 0        |
| EP300  | Switch-of-function | 0    | NOTCH      | 39       | 0        |
| ERBB2  | Switch-of-function | 0    | RTK_RAS    | 792      | 0        |
| ERBB3  | Switch-of-function | 0    | RTK_RAS    | 792      | 0        |
| FAT1   | Switch-of-function | 0    | HIPPO      | 13       | 0        |
| FBXW7  | Switch-of-function | 0    | NOTCH      | 39       | 0        |
| FLT3   | Switch-of-function | 0    | RTK_RAS    | 792      | 0        |
| INPP4B | Switch-of-function | 0    | PI3K       | 173      | 0        |
| KEAP1  | Switch-of-function | 0    | NRF2       | 35       | 0        |
| KRAS   | Switch-of-function | 0    | RTK_RAS    | 792      | 0        |
| LATS1  | Switch-of-function | 0    | HIPPO      | 13       | 0        |
| MAP2K1 | Switch-of-function | 0    | RTK_RAS    | 792      | 0        |
| MAX    | Switch-of-function | 0    | MYC        | 66       | 0        |
| MET    | Switch-of-function | 0    | RTK_RAS    | 792      | 0        |
| MGA    | Switch-of-function | 0    | MYC        | 66       | 0        |
| MTOR   | Switch-of-function | 0    | PI3K       | 173      | 0        |
| NCOR1  | Switch-of-function | 0    | NOTCH      | 39       | 0        |
| NF1    | Switch-of-function | 0    | RTK_RAS    | 792      | 0        |
| NF2    | Switch-of-function | 0    | HIPPO      | 13       | 0        |
| NFE2L2 | Switch-of-function | 0    | NRF2       | 35       | 0        |
| NOTCH1 | Switch-of-function | 0    | NOTCH      | 39       | 0        |

**FIG. 11 (Contd.)**

| gene    | mut_effect         | freq | pathway    | path_tot | percent  |
|---------|--------------------|------|------------|----------|----------|
| NOTCH2  | Switch-of-function | 0    | NOTCH      | 39       | 0        |
| NOTCH3  | Switch-of-function | 0    | NOTCH      | 39       | 0        |
| NOTCH4  | Switch-of-function | 0    | NOTCH      | 39       | 0        |
| NRAS    | Switch-of-function | 0    | RTK_RAS    | 792      | 0        |
| NTRK1   | Switch-of-function | 0    | RTK_RAS    | 792      | 0        |
| PIK3CA  | Switch-of-function | 0    | PI3K       | 173      | 0        |
| PIK3CB  | Switch-of-function | 0    | PI3K       | 173      | 0        |
| PIK3R1  | Switch-of-function | 0    | PI3K       | 173      | 0        |
| PIK3R2  | Switch-of-function | 0    | PI3K       | 173      | 0        |
| PPP2R1A | Switch-of-function | 0    | PI3K       | 173      | 0        |
| PTEN    | Switch-of-function | 0    | PI3K       | 173      | 0        |
| PTPN11  | Switch-of-function | 0    | RTK_RAS    | 792      | 0        |
| RAC1    | Switch-of-function | 0    | RTK_RAS    | 792      | 0        |
| RAF1    | Switch-of-function | 0    | RTK_RAS    | 792      | 0        |
| RASA1   | Switch-of-function | 0    | RTK_RAS    | 792      | 0        |
| RB1     | Switch-of-function | 0    | Cell_Cycle | 197      | 0        |
| RIT1    | Switch-of-function | 0    | RTK_RAS    | 792      | 0        |
| RNF43   | Switch-of-function | 0    | WNT        | 51       | 0        |
| ROS1    | Switch-of-function | 0    | RTK_RAS    | 792      | 0        |
| SMAD2   | Switch-of-function | 0    | TGF_BETA   | 25       | 0        |
| SMAD3   | Switch-of-function | 0    | TGF_BETA   | 25       | 0        |
| SMAD4   | Switch-of-function | 0    | TGF_BETA   | 25       | 0        |
| SPEN    | Switch-of-function | 0    | NOTCH      | 39       | 0        |
| SPRED1  | Switch-of-function | 0    | RTK_RAS    | 792      | 0        |
| STK11   | Switch-of-function | 0    | PI3K       | 173      | 0        |
| TGFBR1  | Switch-of-function | 0    | TGF_BETA   | 25       | 0        |
| TGFBR2  | Switch-of-function | 0    | TGF_BETA   | 25       | 0        |
| TP53    | Switch-of-function | 0    | TP53       | 384      | 0        |
| TSC1    | Switch-of-function | 0    | PI3K       | 173      | 0        |
| TSC2    | Switch-of-function | 0    | PI3K       | 173      | 0        |
| AKT1    | Unknown            | 0    | PI3K       | 173      | 0        |
| ALK     | Unknown            | 1    | RTK_RAS    | 792      | 0.126263 |
| AMER1   | Unknown            | 0    | WNT        | 51       | 0        |
| APC     | Unknown            | 0    | WNT        | 51       | 0        |
| ATM     | Unknown            | 0    | TP53       | 384      | 0        |
| AXIN2   | Unknown            | 0    | WNT        | 51       | 0        |
| BRAF    | Unknown            | 0    | RTK_RAS    | 792      | 0        |
| CBL     | Unknown            | 0    | RTK_RAS    | 792      | 0        |
| CDK4    | Unknown            | 0    | Cell_Cycle | 197      | 0        |

**FIG. 11 (Contd.)**

| gene    | mut_effect | freq | pathway    | path_tot | percent  |
|---------|------------|------|------------|----------|----------|
| CDKN1B  | Unknown    | 0    | Cell_Cycle | 197      | 0        |
| CDKN2A  | Unknown    | 0    | Cell_Cycle | 197      | 0        |
| CDKN2C  | Unknown    | 0    | Cell_Cycle | 197      | 0        |
| CHEK2   | Unknown    | 0    | TP53       | 384      | 0        |
| CREBBP  | Unknown    | 0    | NOTCH      | 39       | 0        |
| CTNNB1  | Unknown    | 0    | WNT        | 51       | 0        |
| EGFR    | Unknown    | 1    | RTK_RAS    | 792      | 0.126263 |
| EP300   | Unknown    | 0    | NOTCH      | 39       | 0        |
| ERBB2   | Unknown    | 0    | RTK_RAS    | 792      | 0        |
| ERBB3   | Unknown    | 1    | RTK_RAS    | 792      | 0.126263 |
| FAT1    | Unknown    | 0    | HIPPO      | 13       | 0        |
| FBXW7   | Unknown    | 1    | NOTCH      | 39       | 2.564103 |
| FLT3    | Unknown    | 0    | RTK_RAS    | 792      | 0        |
| INPP4B  | Unknown    | 0    | PI3K       | 173      | 0        |
| KEAP1   | Unknown    | 9    | NRF2       | 35       | 25.71429 |
| KRAS    | Unknown    | 0    | RTK_RAS    | 792      | 0        |
| LATS1   | Unknown    | 0    | HIPPO      | 13       | 0        |
| MAP2K1  | Unknown    | 0    | RTK_RAS    | 792      | 0        |
| MAX     | Unknown    | 3    | MYC        | 66       | 4.545455 |
| MET     | Unknown    | 0    | RTK_RAS    | 792      | 0        |
| MGA     | Unknown    | 0    | MYC        | 66       | 0        |
| MTOR    | Unknown    | 0    | PI3K       | 173      | 0        |
| NCOR1   | Unknown    | 0    | NOTCH      | 39       | 0        |
| NF1     | Unknown    | 1    | RTK_RAS    | 792      | 0.126263 |
| NF2     | Unknown    | 0    | HIPPO      | 13       | 0        |
| NFE2L2  | Unknown    | 0    | NRF2       | 35       | 0        |
| NOTCH1  | Unknown    | 0    | NOTCH      | 39       | 0        |
| NOTCH2  | Unknown    | 0    | NOTCH      | 39       | 0        |
| NOTCH3  | Unknown    | 0    | NOTCH      | 39       | 0        |
| NOTCH4  | Unknown    | 0    | NOTCH      | 39       | 0        |
| NRAS    | Unknown    | 0    | RTK_RAS    | 792      | 0        |
| NTRK1   | Unknown    | 0    | RTK_RAS    | 792      | 0        |
| PIK3CA  | Unknown    | 0    | PI3K       | 173      | 0        |
| PIK3CB  | Unknown    | 0    | PI3K       | 173      | 0        |
| PIK3R1  | Unknown    | 3    | PI3K       | 173      | 1.734104 |
| PIK3R2  | Unknown    | 0    | PI3K       | 173      | 0        |
| PPP2R1A | Unknown    | 0    | PI3K       | 173      | 0        |
| PTEN    | Unknown    | 1    | PI3K       | 173      | 0.578035 |
| PTPN11  | Unknown    | 0    | RTK_RAS    | 792      | 0        |

**FIG. 11 (Contd.)**

| gene   | mut_effect       | freq | pathway    | path_tot | percent  |
|--------|------------------|------|------------|----------|----------|
| RAC1   | Unknown          | 0    | RTK_RAS    | 792      | 0        |
| RAF1   | Unknown          | 0    | RTK_RAS    | 792      | 0        |
| RASA1  | Unknown          | 0    | RTK_RAS    | 792      | 0        |
| RB1    | Unknown          | 0    | Cell_Cycle | 197      | 0        |
| RIT1   | Unknown          | 0    | RTK_RAS    | 792      | 0        |
| RNF43  | Unknown          | 0    | WNT        | 51       | 0        |
| ROS1   | Unknown          | 0    | RTK_RAS    | 792      | 0        |
| SMAD2  | Unknown          | 0    | TGF_BETA   | 25       | 0        |
| SMAD3  | Unknown          | 0    | TGF_BETA   | 25       | 0        |
| SMAD4  | Unknown          | 6    | TGF_BETA   | 25       | 24       |
| SPEN   | Unknown          | 0    | NOTCH      | 39       | 0        |
| SPRED1 | Unknown          | 0    | RTK_RAS    | 792      | 0        |
| STK11  | Unknown          | 8    | PI3K       | 173      | 4.624277 |
| TGFBR1 | Unknown          | 0    | TGF_BETA   | 25       | 0        |
| TGFBR2 | Unknown          | 0    | TGF_BETA   | 25       | 0        |
| TP53   | Unknown          | 0    | TP53       | 384      | 0        |
| TSC1   | Unknown          | 0    | PI3K       | 173      | 0        |
| TSC2   | Unknown          | 0    | PI3K       | 173      | 0        |
| ABL1   | Gain-of-function | 1    | RTK_RAS    | 792      | 0.126263 |
| CDKN2B | Gain-of-function | 0    | Cell_Cycle | 197      | 0        |
| RET    | Gain-of-function | 16   | RTK_RAS    | 792      | 2.020202 |
| ABL1   | Loss-of-function | 0    | RTK_RAS    | 792      | 0        |
| CDKN2B | Loss-of-function | 29   | Cell_Cycle | 197      | 14.72081 |
| RET    | Loss-of-function | 0    | RTK_RAS    | 792      | 0        |
| AKT2   | Gain-of-function | 3    | PI3K       | 173      | 1.734104 |
| CCND1  | Gain-of-function | 8    | Cell_Cycle | 197      | 4.060914 |
| CCND2  | Gain-of-function | 4    | Cell_Cycle | 197      | 2.030457 |
| CCND3  | Gain-of-function | 2    | Cell_Cycle | 197      | 1.015228 |
| CCNE1  | Gain-of-function | 6    | Cell_Cycle | 197      | 3.045685 |
| CDK6   | Gain-of-function | 1    | Cell_Cycle | 197      | 0.507614 |
| FGFR1  | Gain-of-function | 1    | RTK_RAS    | 792      | 0.126263 |
| FGFR4  | Gain-of-function | 3    | RTK_RAS    | 792      | 0.378788 |
| KDM5A  | Gain-of-function | 5    | NOTCH      | 39       | 12.82051 |
| KIT    | Gain-of-function | 2    | RTK_RAS    | 792      | 0.252525 |
| MAPK1  | Gain-of-function | 1    | RTK_RAS    | 792      | 0.126263 |
| MDM2   | Gain-of-function | 55   | TP53       | 384      | 14.32292 |
| MDM4   | Gain-of-function | 5    | TP53       | 384      | 1.302083 |
| MYC    | Gain-of-function | 23   | MYC        | 66       | 34.84848 |
| PDGFRA | Gain-of-function | 2    | RTK_RAS    | 792      | 0.252525 |

**FIG. 11 (Contd.)**

| <b>gene</b> | <b>mut_effect</b> | <b>freq</b> | <b>pathway</b> | <b>path_tot</b> | <b>percent</b> |
|-------------|-------------------|-------------|----------------|-----------------|----------------|
| RHEB        | Gain-of-function  | 1           | PI3K           | 173             | 0.578035       |
| RICTOR      | Gain-of-function  | 5           | PI3K           | 173             | 2.890173       |
| AKT2        | Loss-of-function  | 0           | PI3K           | 173             | 0              |
| CCND1       | Loss-of-function  | 0           | Cell_Cycle     | 197             | 0              |
| CCND2       | Loss-of-function  | 0           | Cell_Cycle     | 197             | 0              |
| CCND3       | Loss-of-function  | 0           | Cell_Cycle     | 197             | 0              |
| CCNE1       | Loss-of-function  | 0           | Cell_Cycle     | 197             | 0              |
| CDK6        | Loss-of-function  | 0           | Cell_Cycle     | 197             | 0              |
| FGFR1       | Loss-of-function  | 0           | RTK_RAS        | 792             | 0              |
| FGFR4       | Loss-of-function  | 0           | RTK_RAS        | 792             | 0              |
| KDM5A       | Loss-of-function  | 0           | NOTCH          | 39              | 0              |
| KIT         | Loss-of-function  | 0           | RTK_RAS        | 792             | 0              |
| MAPK1       | Loss-of-function  | 0           | RTK_RAS        | 792             | 0              |
| MDM2        | Loss-of-function  | 0           | TP53           | 384             | 0              |
| MDM4        | Loss-of-function  | 0           | TP53           | 384             | 0              |
| MYC         | Loss-of-function  | 0           | MYC            | 66              | 0              |
| PDGFRA      | Loss-of-function  | 0           | RTK_RAS        | 792             | 0              |
| RHEB        | Loss-of-function  | 0           | PI3K           | 173             | 0              |
| RICTOR      | Loss-of-function  | 0           | PI3K           | 173             | 0              |

FIG. 12A

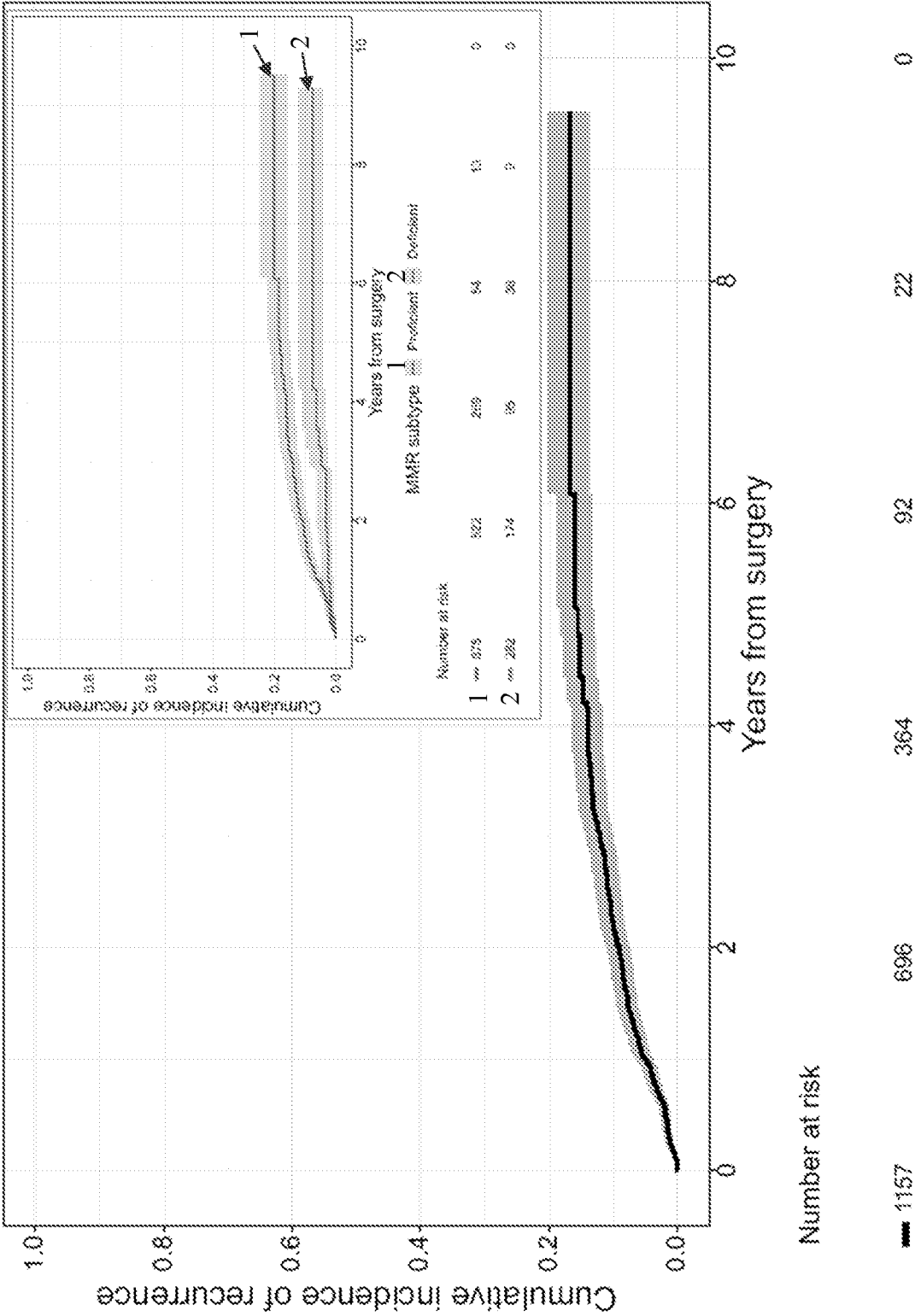


FIG. 12B

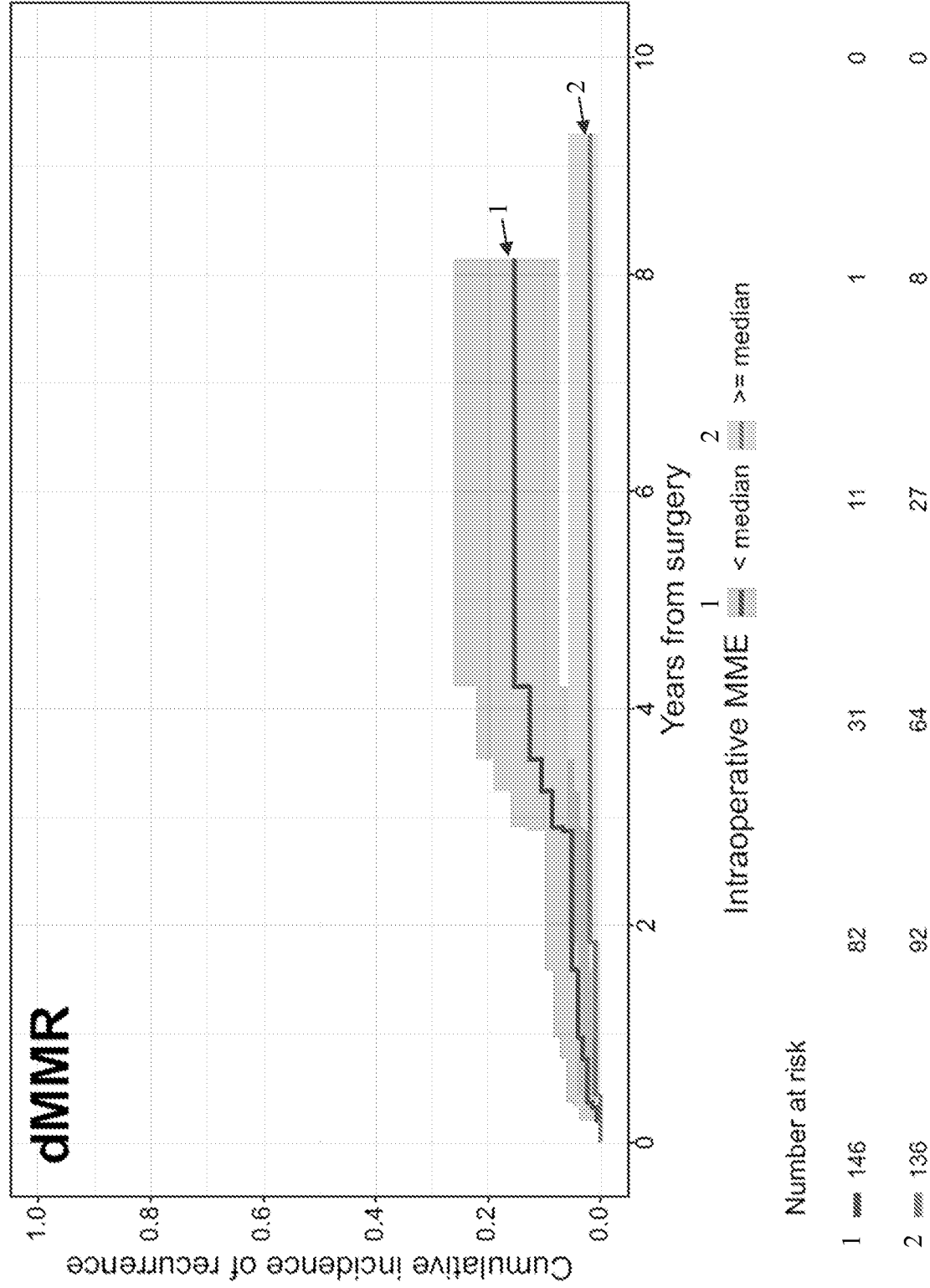


FIG. 12B (Contd.)

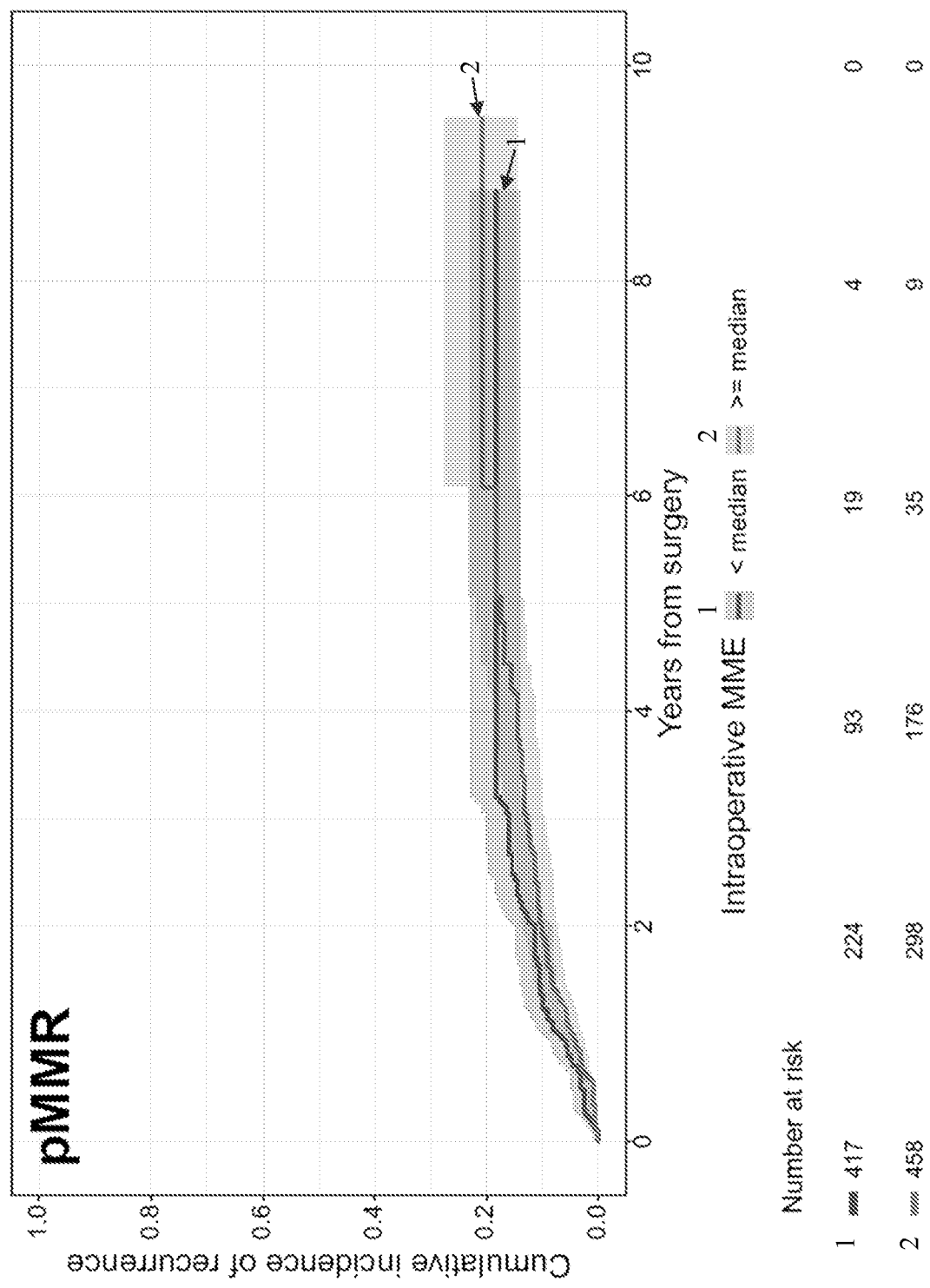


FIG. 12C

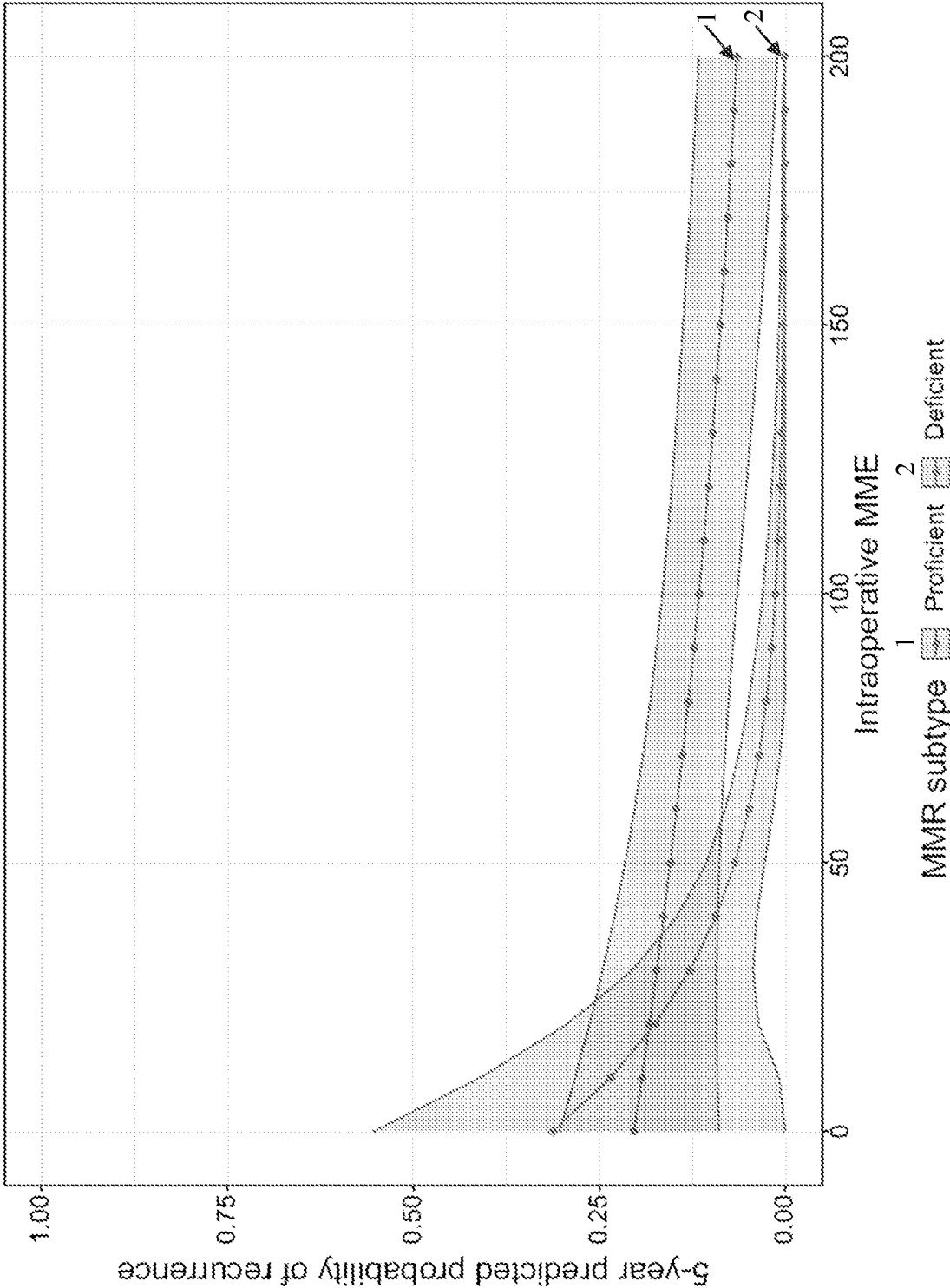


FIG. 13A

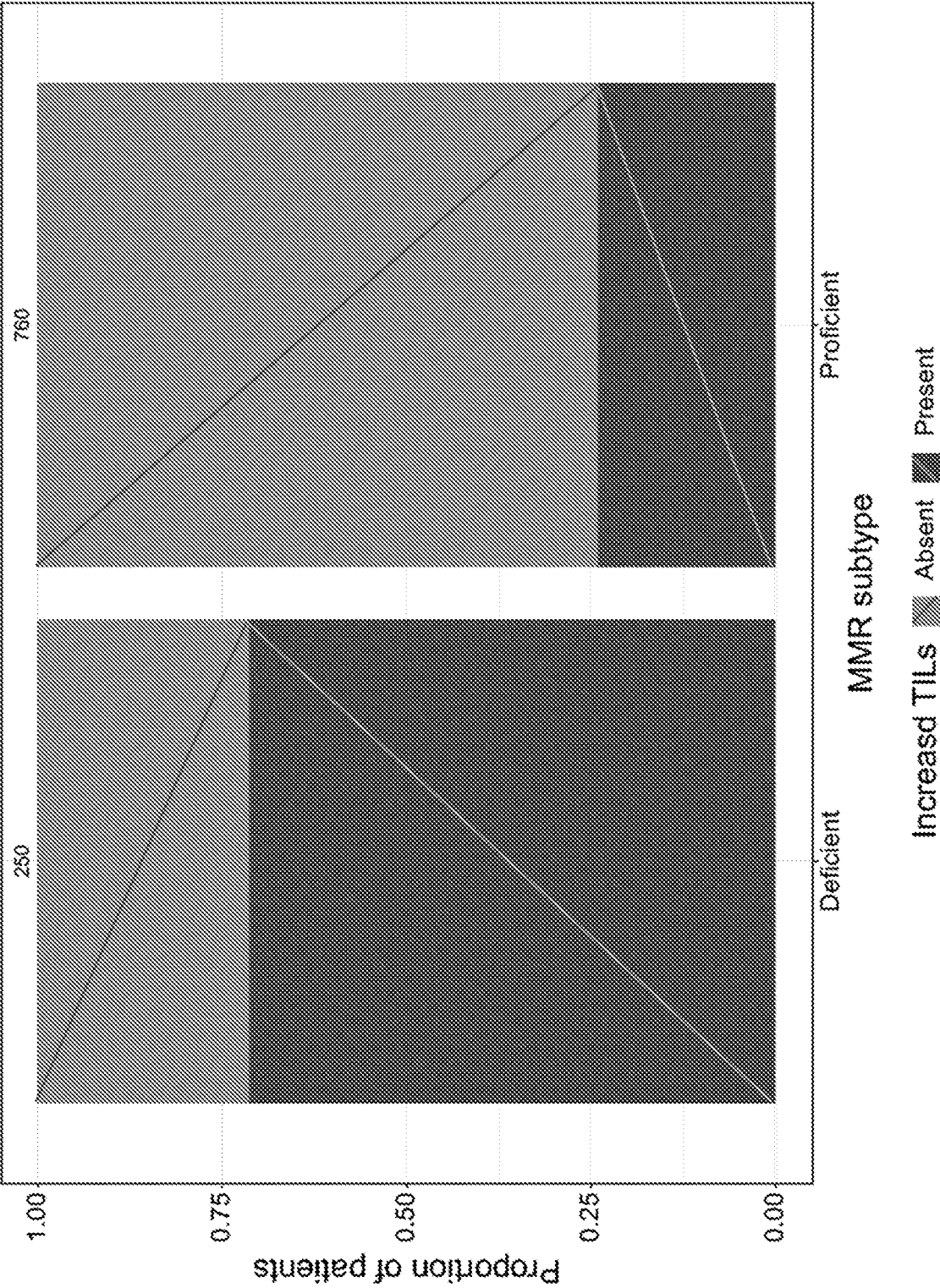


FIG. 13B

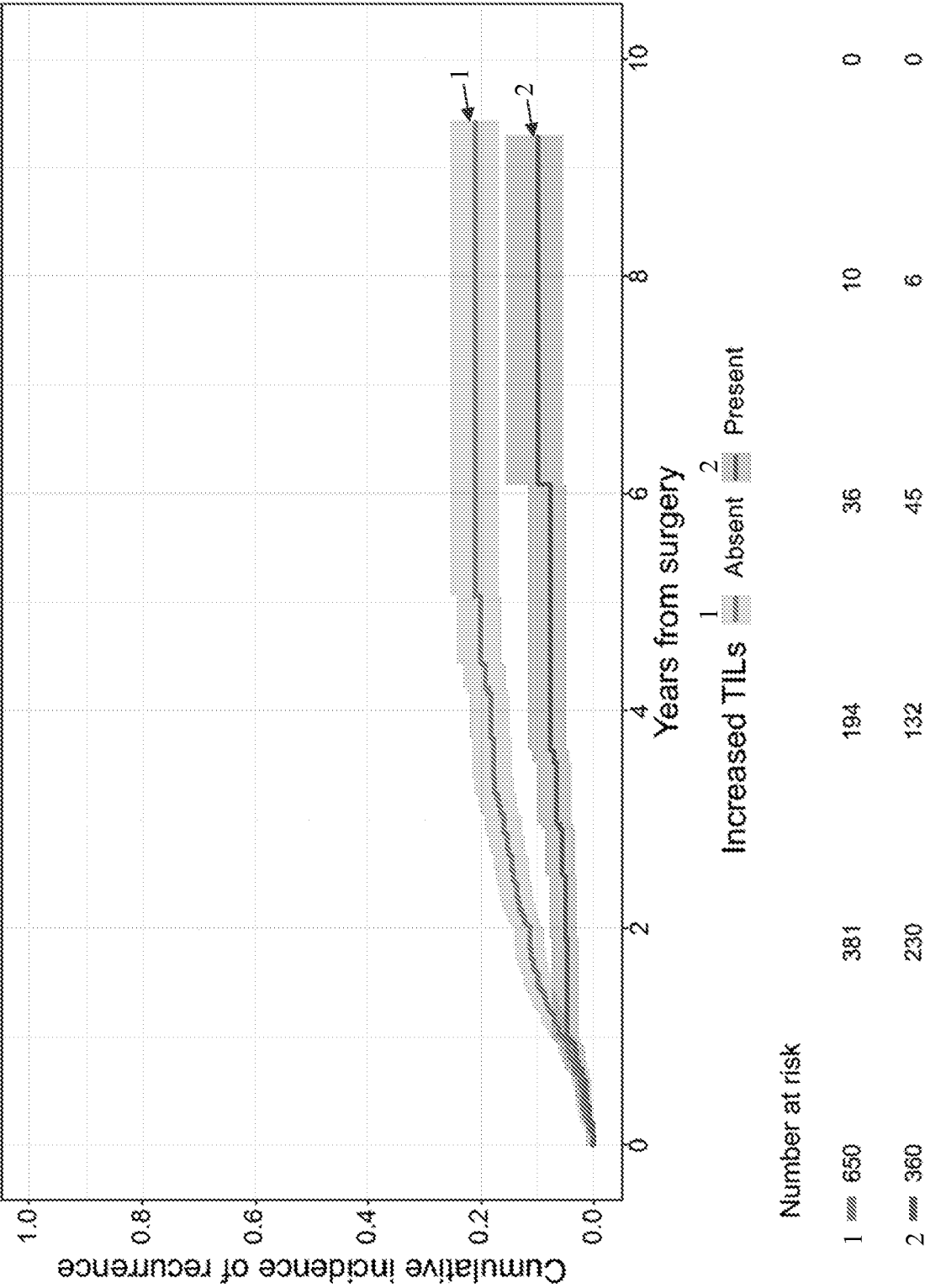


FIG. 13C

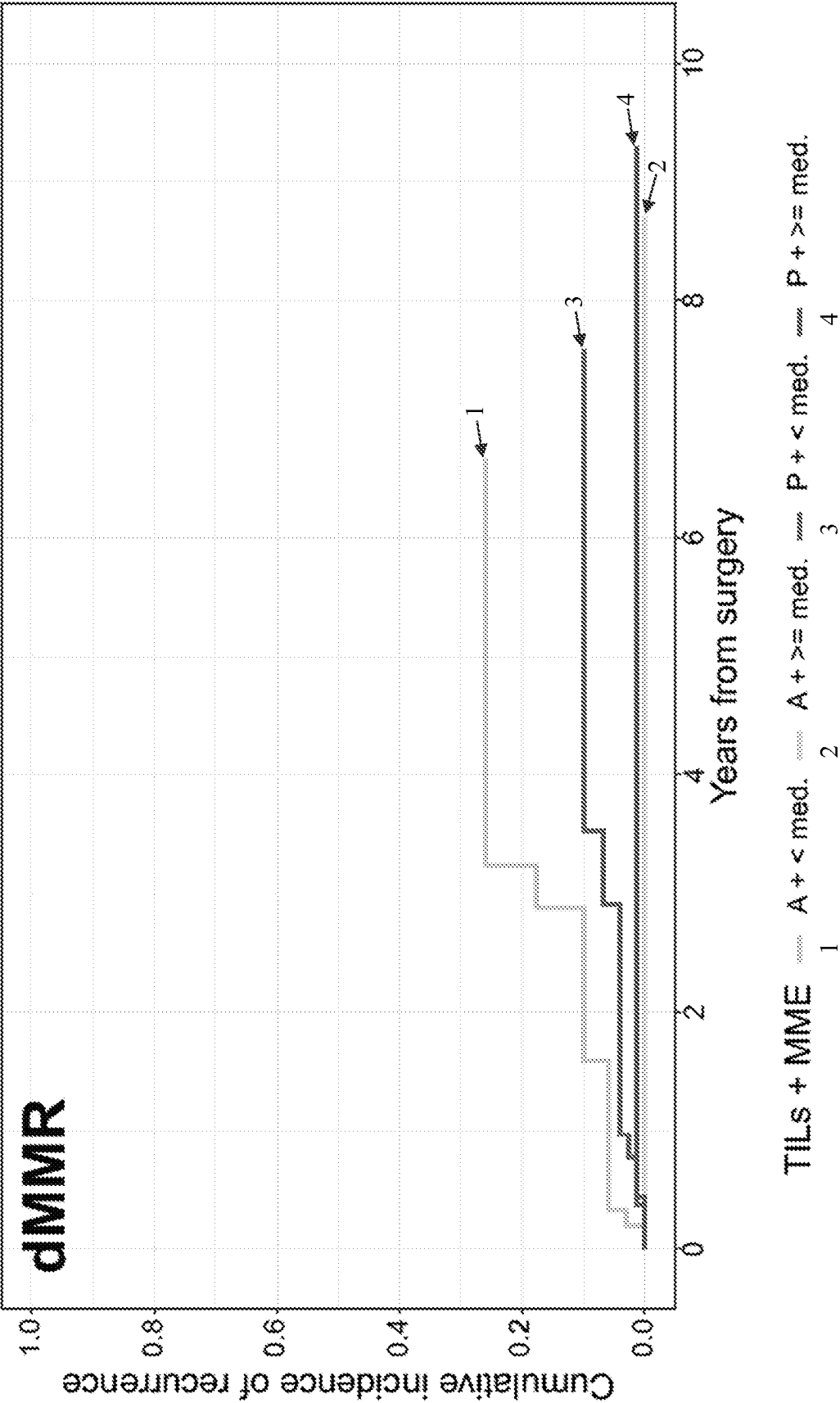


FIG. 13C (Contd.)

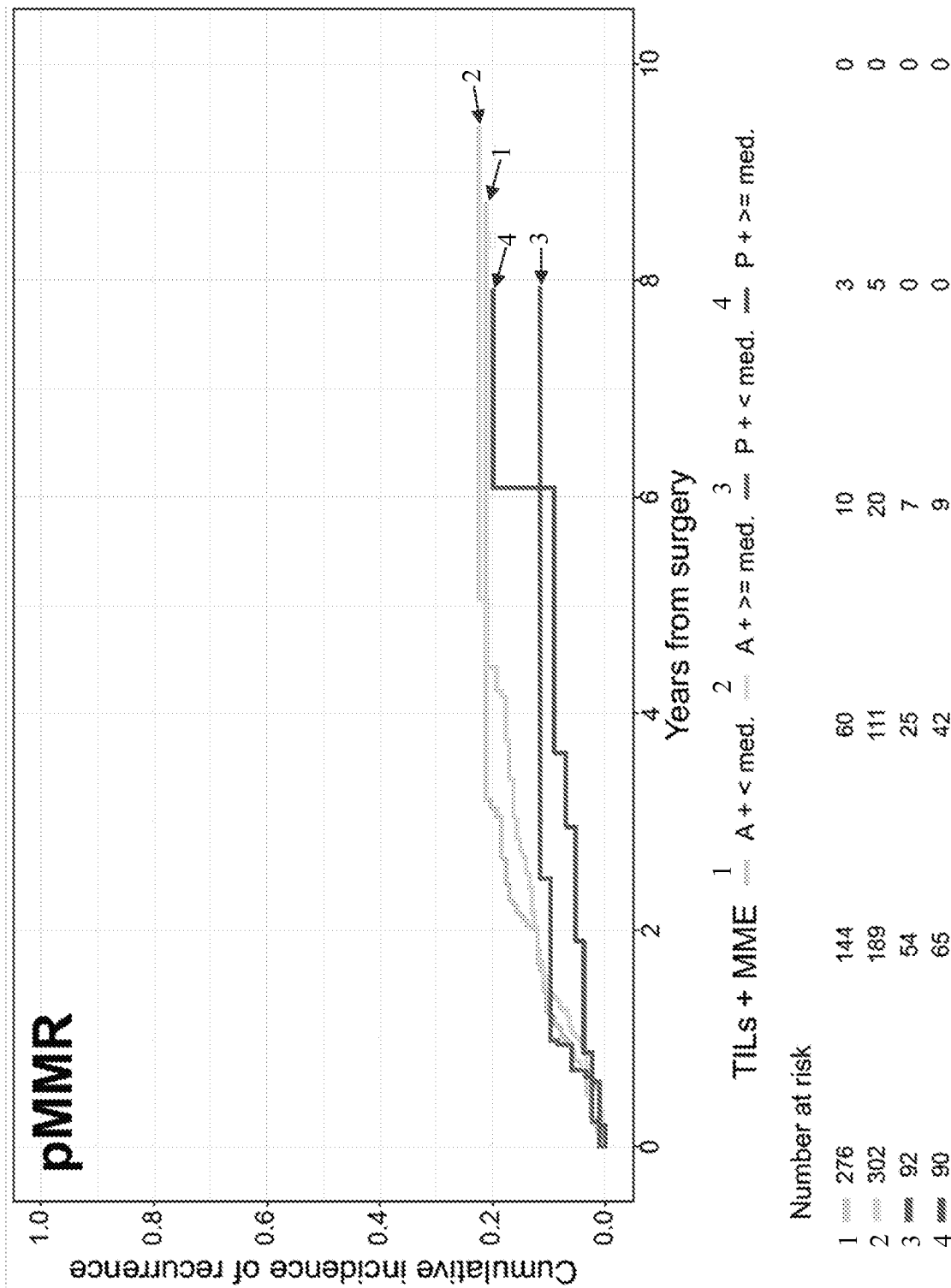




FIG. 14B

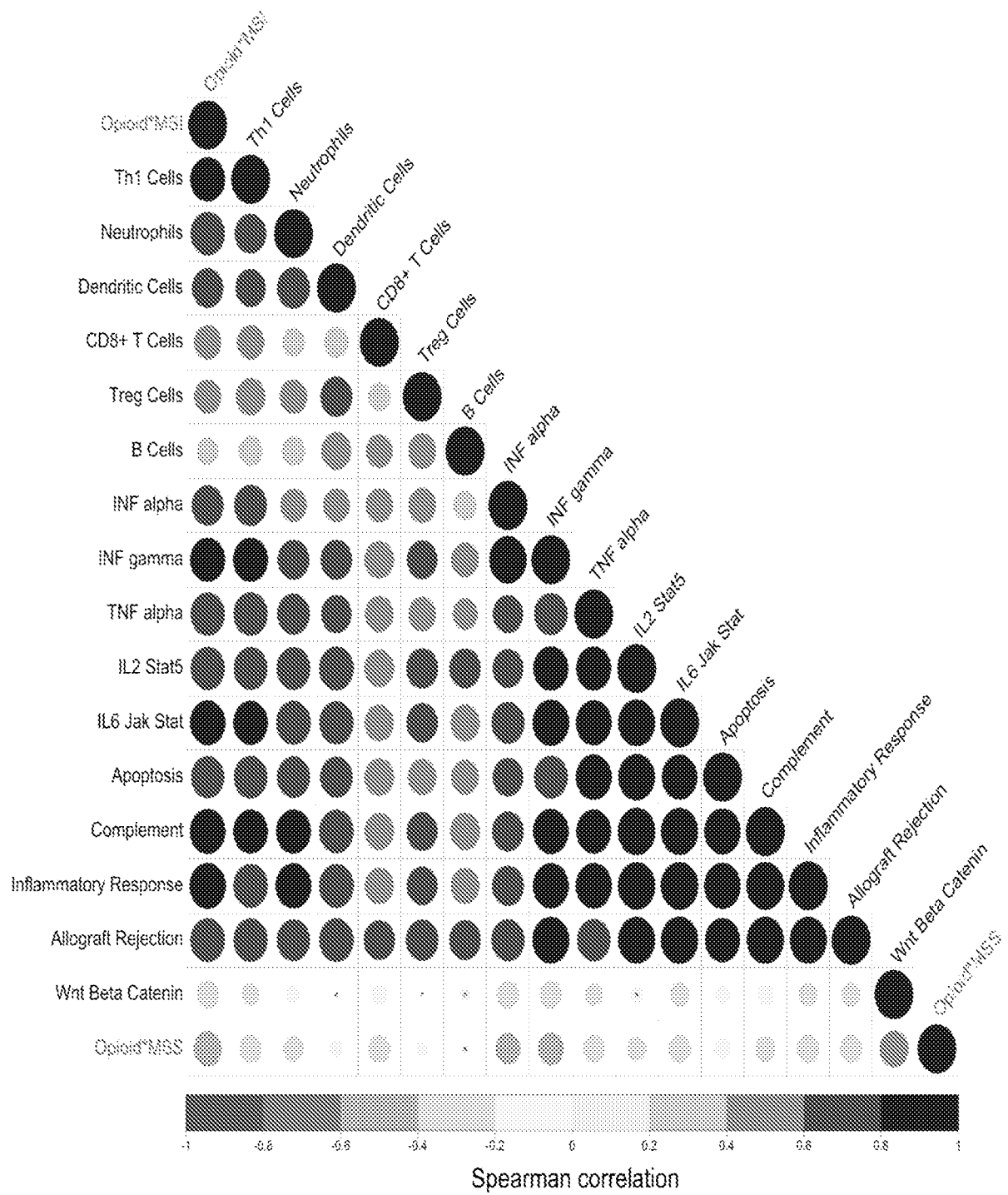


FIG. 14C

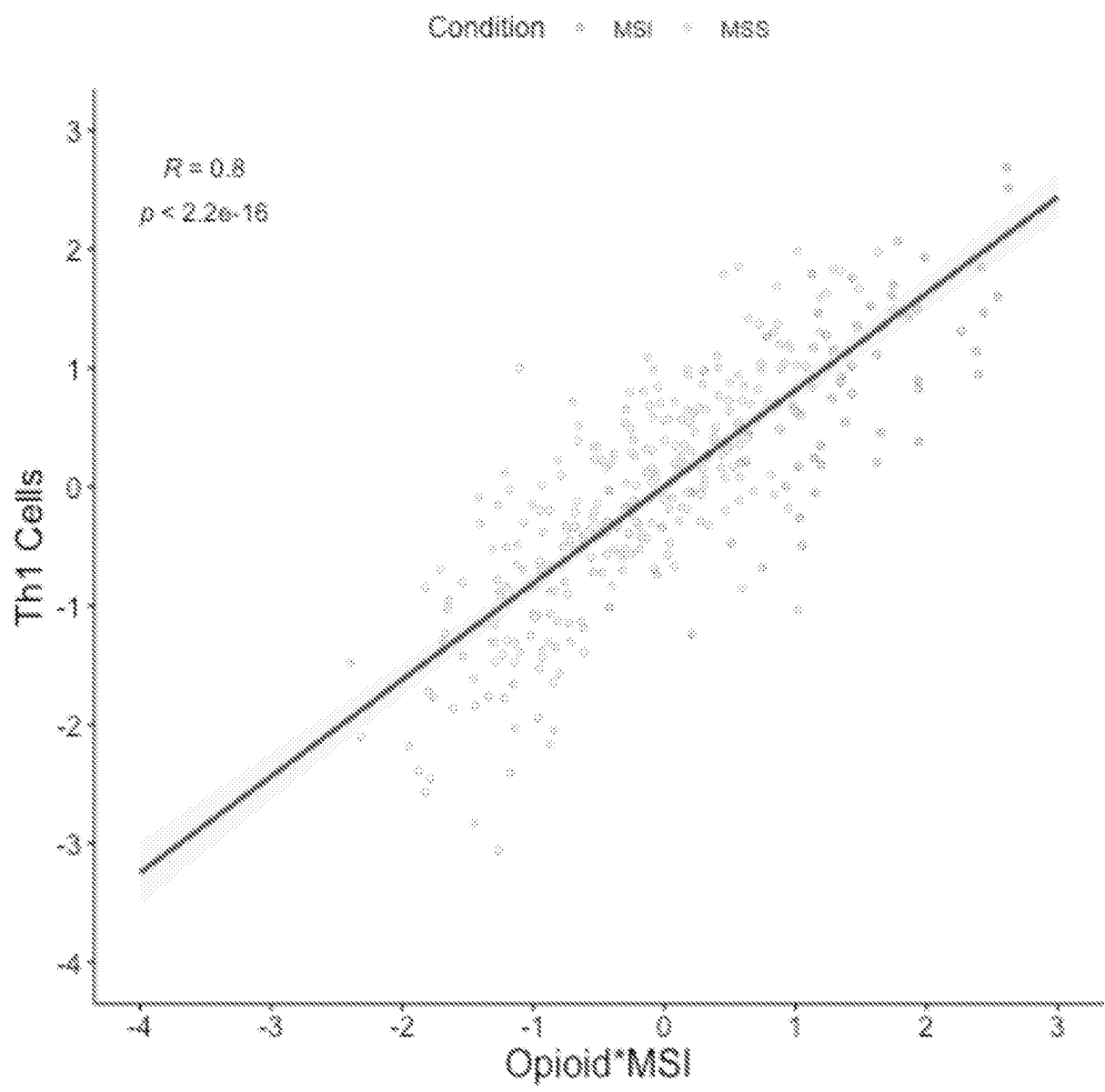


FIG. 14D

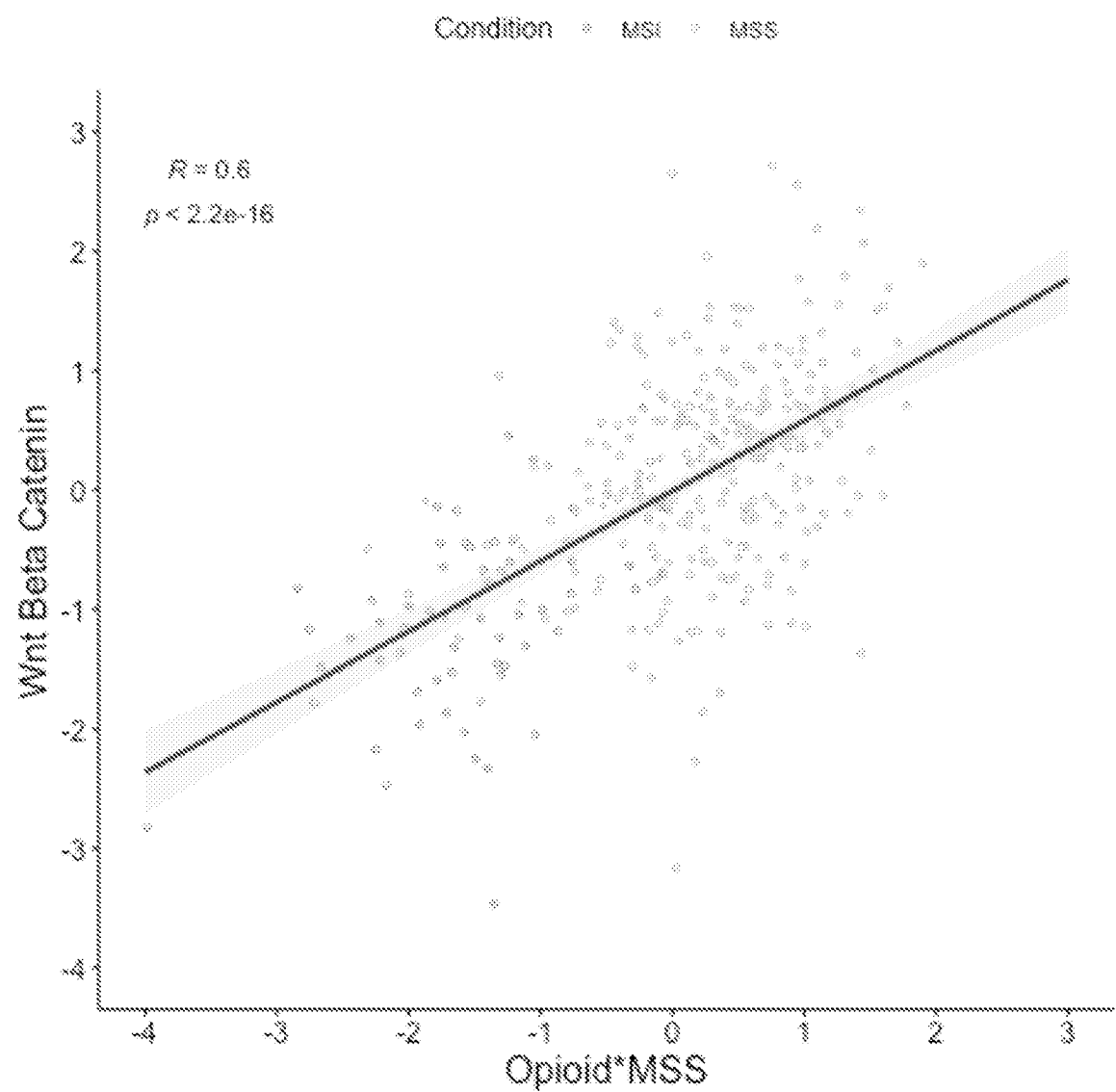


FIG. 15

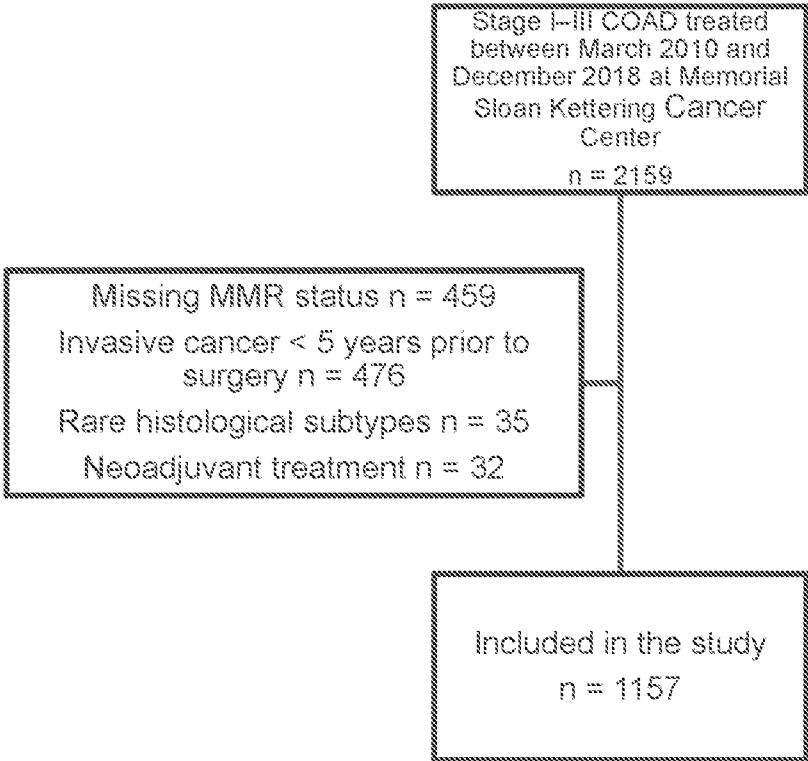


FIG. 16A

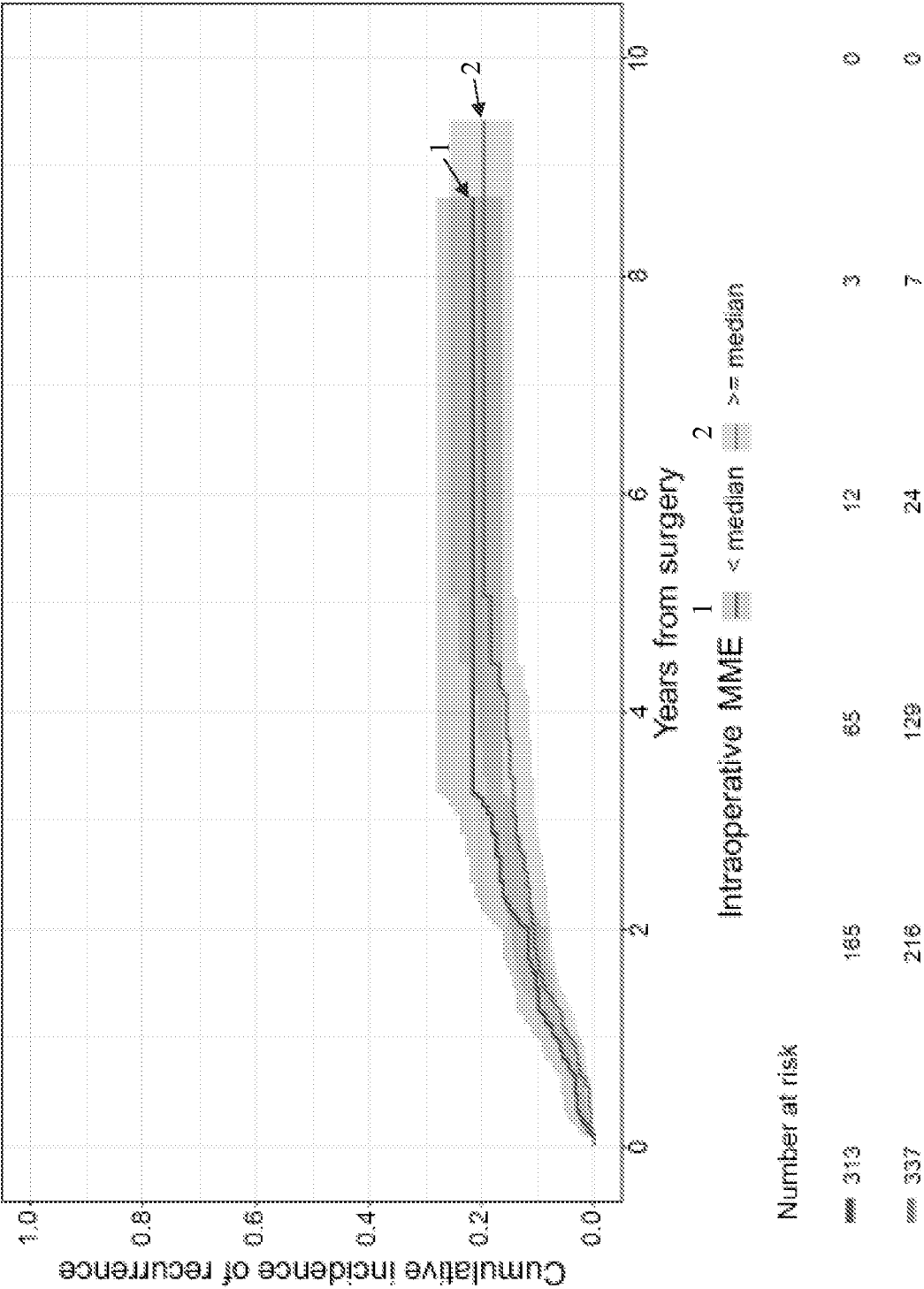


FIG. 16B

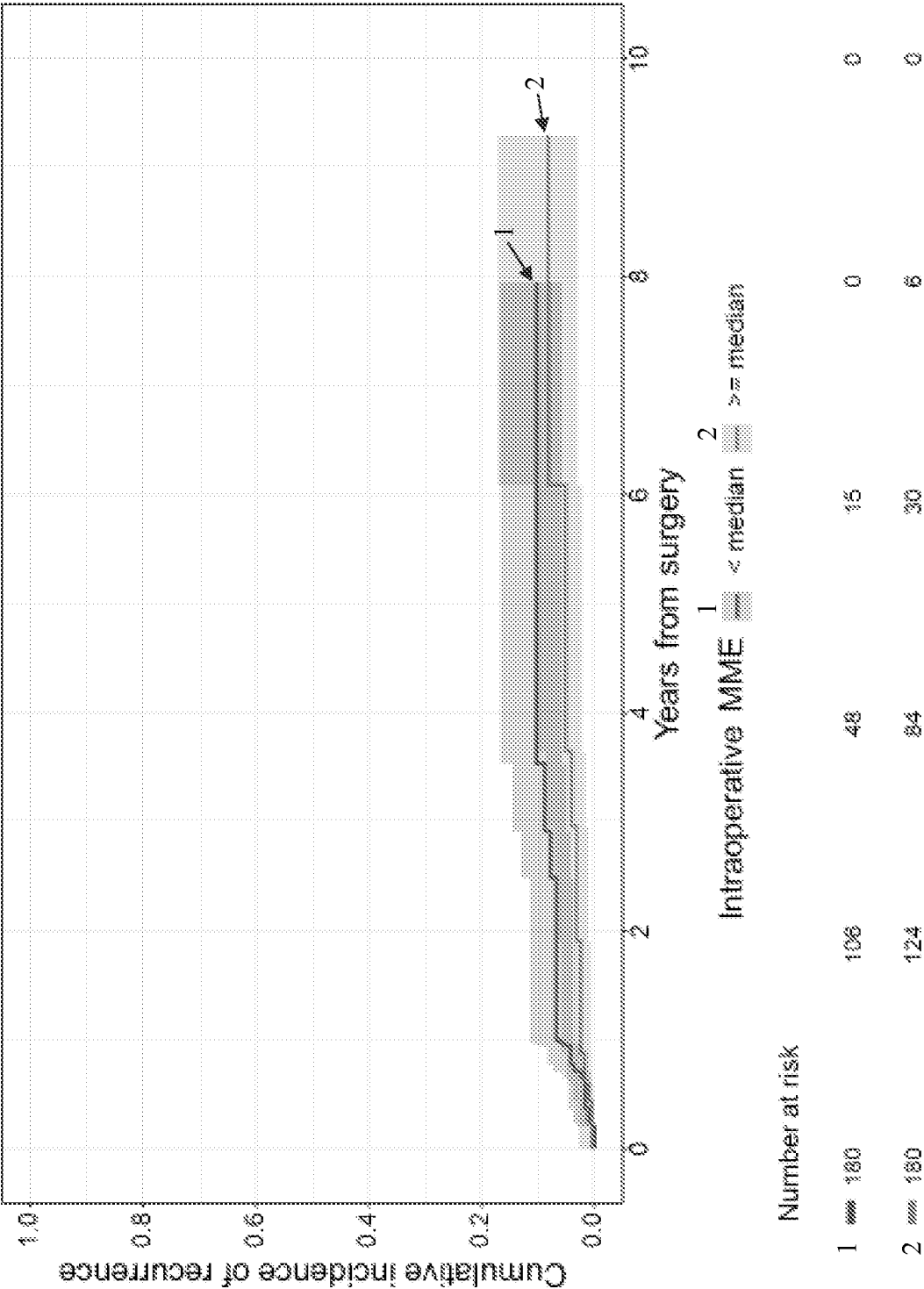


FIG. 17A

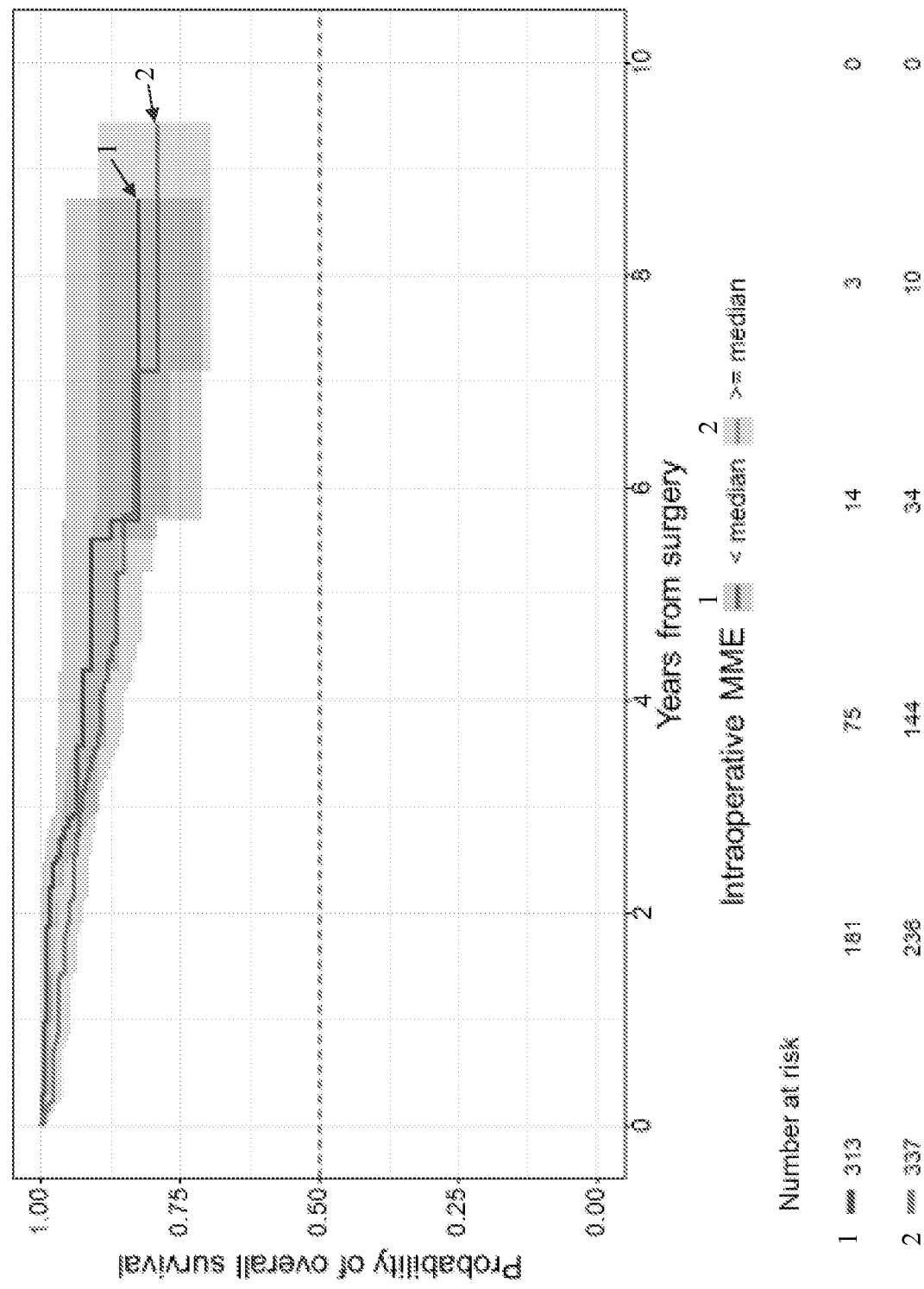


FIG. 17B

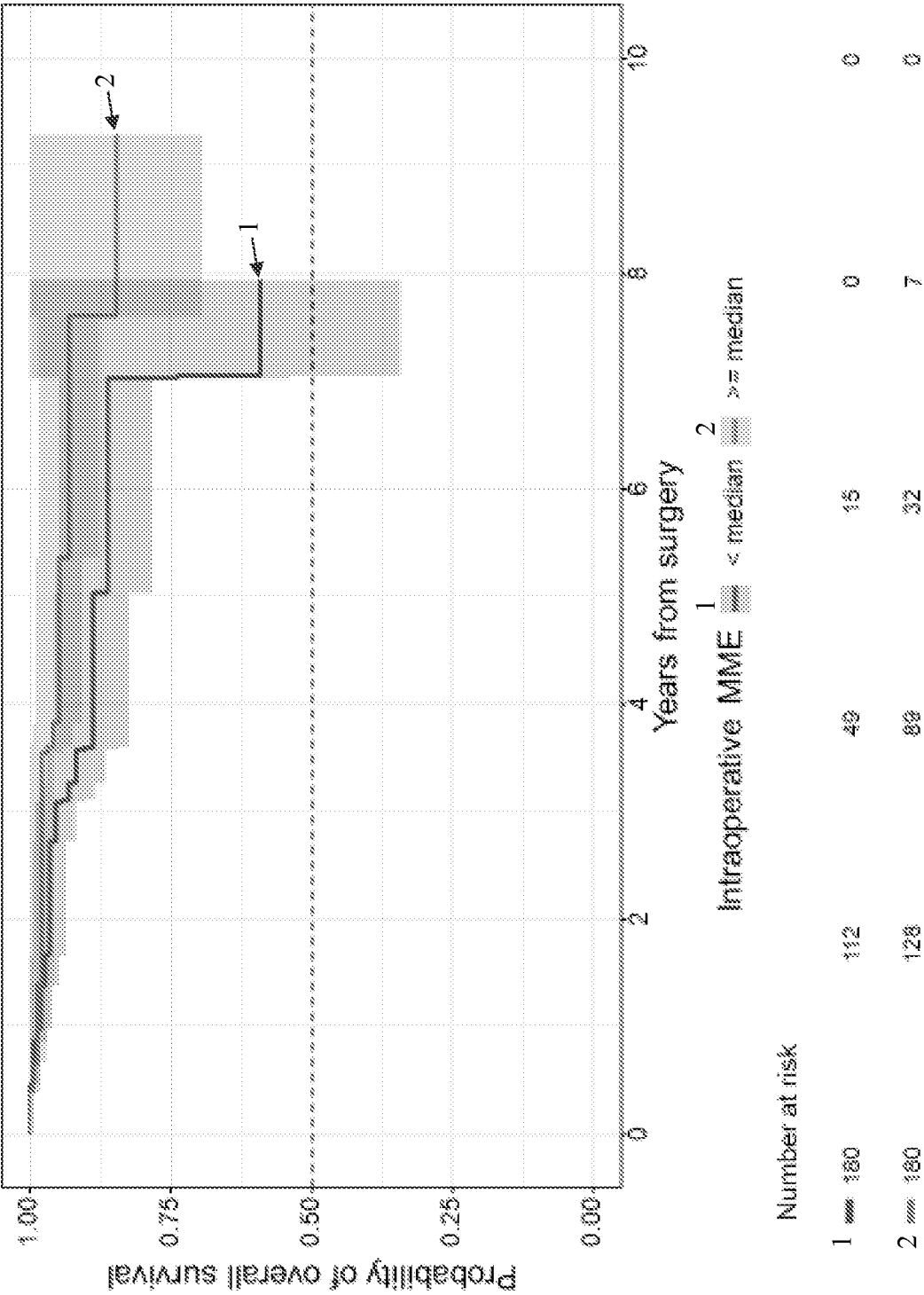


FIG. 18A

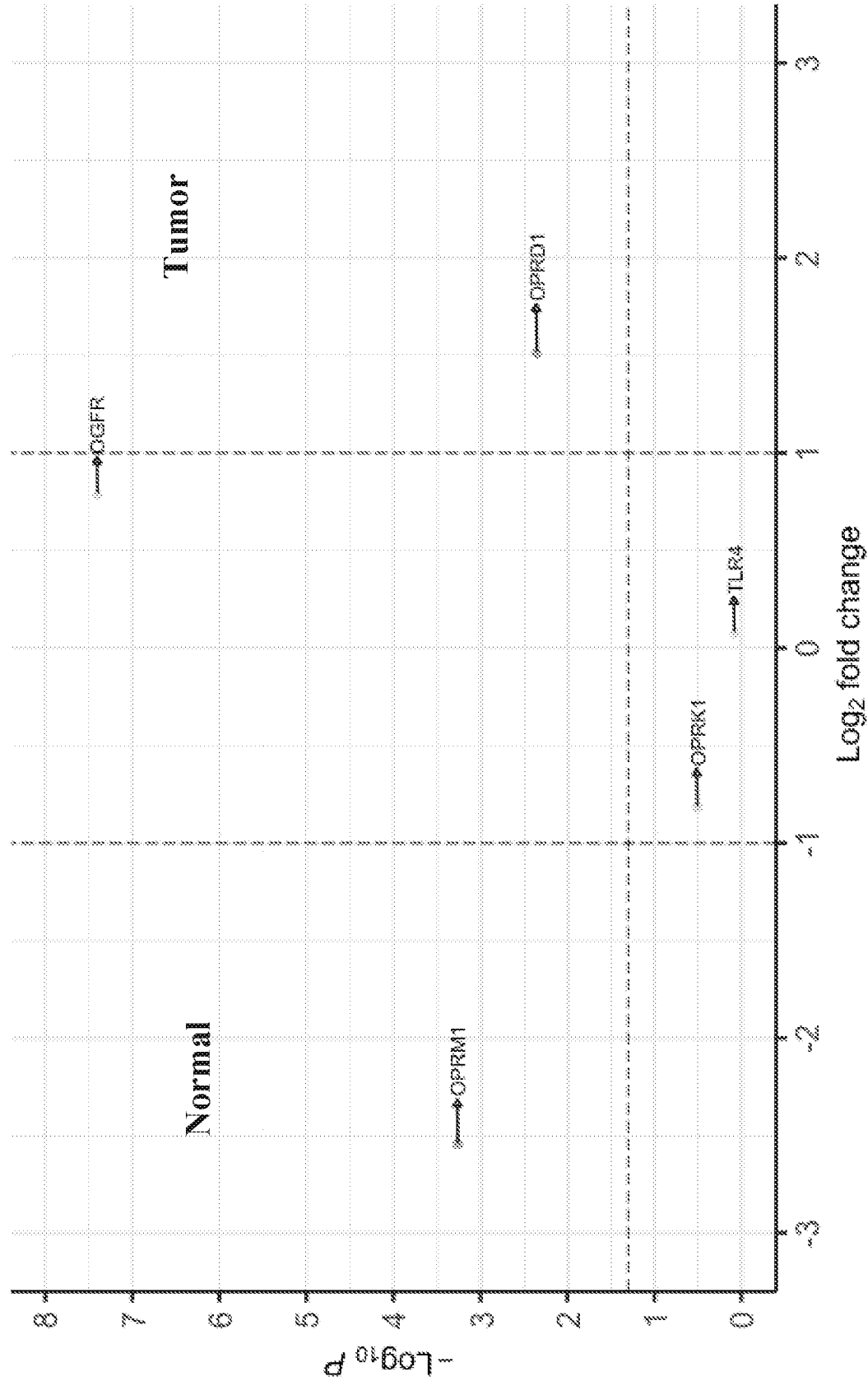


FIG. 18B

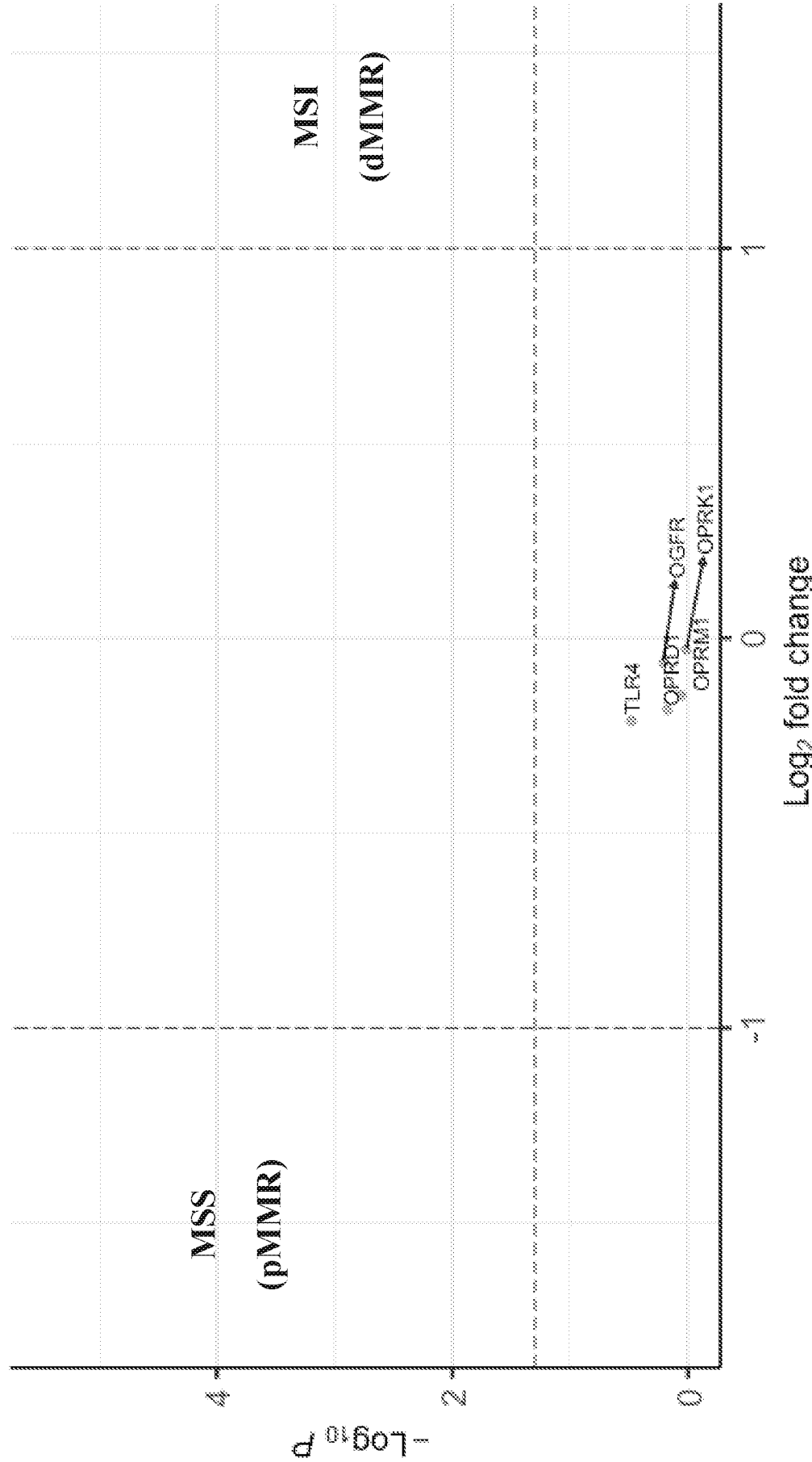


FIG. 19

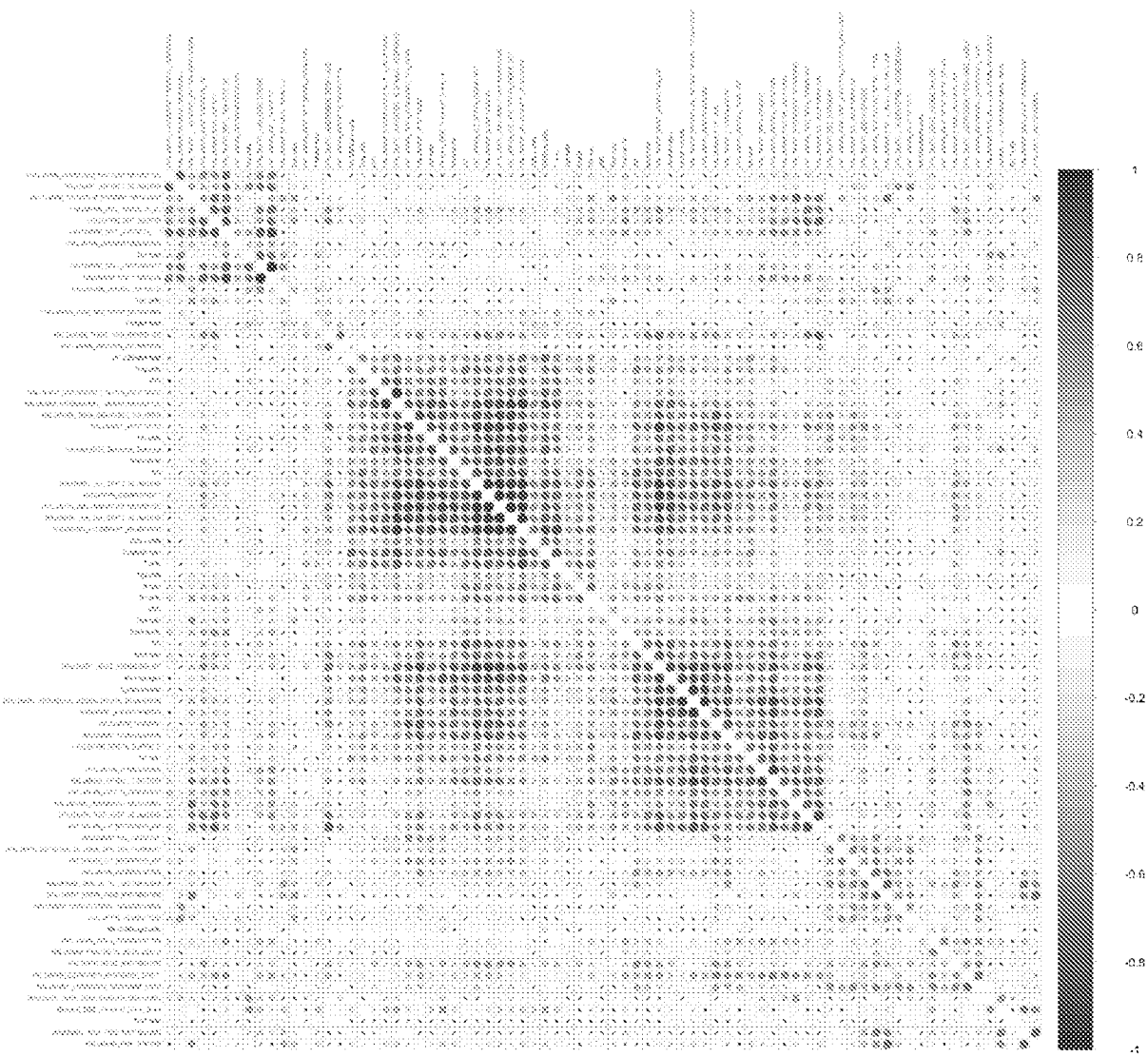


FIG. 20

| Characteristic              | MMR                               |                              |                             | P-value          |
|-----------------------------|-----------------------------------|------------------------------|-----------------------------|------------------|
|                             | Overall,<br>N = 1157 <sup>†</sup> | Proficient,<br>N = 875 (76%) | Deficient,<br>N = 282 (24%) |                  |
| <b>Intraoperative NMEs</b>  |                                   |                              |                             | <b>0.5</b>       |
| Median (IQR)                | 60 (44, 90)                       | 62 (47, 90)                  | 59 (40, 92)                 |                  |
| [Range]                     | [0, 473]                          | [0, 323]                     | [0, 473]                    |                  |
| <b>Adjunct</b>              |                                   |                              |                             | <b>0.025</b>     |
| None                        | 630 (54%)                         | 437 (52%)                    | 173 (61%)                   |                  |
| Dexmedetomidine             | 161 (14%)                         | 136 (15%)                    | 31 (11%)                    |                  |
| Ketamine                    | 166 (12%)                         | 288 (33%)                    | 78 (28%)                    |                  |
| <b>Ketorolac use</b>        |                                   |                              |                             | <b>0.5</b>       |
| No ketorolac                | 930 (80%)                         | 699 (80%)                    | 231 (82%)                   |                  |
| Yes ketorolac               | 227 (20%)                         | 176 (20%)                    | 51 (18%)                    |                  |
| <b>Regional anesthesia</b>  |                                   |                              |                             | <b>0.006</b>     |
| None                        | 583 (50%)                         | 429 (49%)                    | 154 (55%)                   |                  |
| Epidural                    | 269 (23%)                         | 200 (23%)                    | 69 (24%)                    |                  |
| TAP block                   | 303 (26%)                         | 246 (28%)                    | 57 (20%)                    |                  |
| Both                        | 2 (0.2%)                          | 0 (0%)                       | 2 (0.7%)                    |                  |
| <b>Patient age (years)</b>  |                                   |                              |                             | <b>&lt;0.001</b> |
| Median (IQR)                | 60 (51, 70)                       | 58 (50, 68)                  | 66 (53, 77)                 |                  |
| [Range]                     | [24, 96]                          | [25, 96]                     | [24, 96]                    |                  |
| <b>Sex</b>                  |                                   |                              |                             | <b>0.14</b>      |
| Male                        | 594 (51%)                         | 460 (53%)                    | 134 (48%)                   |                  |
| Female                      | 563 (49%)                         | 415 (47%)                    | 148 (52%)                   |                  |
| <b>Race</b>                 |                                   |                              |                             | <b>0.002</b>     |
| White                       | 937 (84%)                         | 688 (82%)                    | 249 (91%)                   |                  |
| Black                       | 67 (6.0%)                         | 58 (6.9%)                    | 9 (3.3%)                    |                  |
| Other                       | 105 (9.5%)                        | 90 (11%)                     | 15 (5.5%)                   |                  |
| Unknown                     | 48                                | 59                           | 9                           |                  |
| <b>Ethnicity</b>            |                                   |                              |                             | <b>0.6</b>       |
| Hispanic                    | 70 (6.1%)                         | 51 (5.9%)                    | 19 (6.8%)                   |                  |
| Not Hispanic                | 1071 (94%)                        | 810 (94%)                    | 261 (93%)                   |                  |
| Unknown                     | 16                                | 14                           | 2                           |                  |
| <b>Pathological T stage</b> |                                   |                              |                             | <b>0.2</b>       |
| 1                           | 153 (13%)                         | 112 (13%)                    | 41 (15%)                    |                  |
| 2                           | 161 (14%)                         | 120 (14%)                    | 41 (15%)                    |                  |

**FIG. 20 (Contd.)**

| Characteristic               | MMR                               |                              |                             | P-value          |
|------------------------------|-----------------------------------|------------------------------|-----------------------------|------------------|
|                              | Overall,<br>N = 1157 <sup>†</sup> | Proficient,<br>N = 875 (76%) | Deficient,<br>N = 282 (24%) |                  |
| 3                            | 701 (61%)                         | 528 (60%)                    | 173 (62%)                   |                  |
| 4                            | 142 (12%)                         | 117 (13%)                    | 25 (8.9%)                   |                  |
| <b>Pathological N stage</b>  |                                   |                              |                             | <b>&lt;0.001</b> |
| 0                            | 706 (61%)                         | 497 (57%)                    | 209 (74%)                   |                  |
| 1                            | 313 (27%)                         | 261 (30%)                    | 52 (18%)                    |                  |
| 2                            | 158 (12%)                         | 117 (13%)                    | 21 (7.4%)                   |                  |
| <b>Tumour location</b>       |                                   |                              |                             | <b>&lt;0.001</b> |
| Right                        | 579 (50%)                         | 370 (42%)                    | 209 (74%)                   |                  |
| Left                         | 488 (42%)                         | 443 (51%)                    | 45 (16%)                    |                  |
| Mid-transverse               | 90 (7.8%)                         | 62 (7.1%)                    | 28 (9.9%)                   |                  |
| <b>CEA (ng/ml)</b>           |                                   |                              |                             | <b>0.3</b>       |
| Median (IQR)                 | 3 (2, 6)                          | 3 (2, 6)                     | 3 (2, 6)                    |                  |
| [Range]                      | [0, 1360]                         | [0, 1360]                    | [1, 836]                    |                  |
| Unknown                      | 144                               | 106                          | 38                          |                  |
| <b>Conversion</b>            |                                   |                              |                             | <b>&gt;0.9</b>   |
| No                           | 1088 (94%)                        | 823 (94%)                    | 265 (94%)                   |                  |
| Yes                          | 69 (6.0%)                         | 52 (5.9%)                    | 17 (6.0%)                   |                  |
| <b>Synchronous COAD</b>      |                                   |                              |                             | <b>0.6</b>       |
| No                           | 1122 (97%)                        | 850 (97%)                    | 272 (96%)                   |                  |
| Yes                          | 35 (3.0%)                         | 25 (2.9%)                    | 10 (3.5%)                   |                  |
| <b>Type of resection</b>     |                                   |                              |                             | <b>0.003</b>     |
| Open                         | 192 (17%)                         | 137 (16%)                    | 55 (20%)                    |                  |
| Laparoscopic                 | 298 (26%)                         | 209 (24%)                    | 89 (32%)                    |                  |
| Robotic                      | 667 (58%)                         | 529 (60%)                    | 138 (49%)                   |                  |
| <b>Extent of resection</b>   |                                   |                              |                             | <b>0.001</b>     |
| Extended                     | 69 (6.0%)                         | 41 (4.7%)                    | 28 (9.9%)                   |                  |
| Segmental                    | 1088 (94%)                        | 834 (95%)                    | 254 (90%)                   |                  |
| <b>Adjuvant chemotherapy</b> |                                   |                              |                             | <b>&lt;0.001</b> |
| No                           | 610 (54%)                         | 405 (48%)                    | 205 (75%)                   |                  |
| Yes                          | 511 (46%)                         | 443 (52%)                    | 68 (25%)                    |                  |
| Unknown                      | 36                                | 27                           | 9                           |                  |
| <b>Surgery time (mins)</b>   |                                   |                              |                             | <b>&lt;0.001</b> |
| Median (IQR)                 | 183 (137, 232)                    | 186 (144, 239)               | 168 (126, 232)              |                  |
| [Range]                      | [49, 620]                         | [49, 620]                    | [58, 600]                   |                  |

**FIG. 20 (Contd.)**

| Characteristic                | MMR                               |                              |                             | P-value          |
|-------------------------------|-----------------------------------|------------------------------|-----------------------------|------------------|
|                               | Overall,<br>N = 1157 <sup>1</sup> | Proficient,<br>N = 875 (76%) | Deficient,<br>N = 282 (24%) |                  |
| <b>Smoking history</b>        |                                   |                              |                             | <b>0.7</b>       |
| Never used                    | 656 (57%)                         | 502 (58%)                    | 154 (53%)                   |                  |
| Past smoker                   | 401 (35%)                         | 298 (34%)                    | 103 (37%)                   |                  |
| Current smoker                | 92 (8.0%)                         | 69 (7.9%)                    | 23 (8.2%)                   |                  |
| Unknown                       | 3                                 | 6                            | 2                           |                  |
| <b>Albumin (g/dL)</b>         |                                   |                              |                             | <b>&lt;0.001</b> |
| Median (IQR)                  | 4.20 (3.90, 4.40)                 | 4.20 (4.00, 4.40)            | 4.10 (3.80, 4.30)           |                  |
| [Range]                       | [1.70, 5.20]                      | [1.70, 5.20]                 | [2.40, 4.90]                |                  |
| Unknown                       | 6                                 | 3                            | 3                           |                  |
| <b>ASA</b>                    |                                   |                              |                             | <b>0.024</b>     |
| P1/P2                         | 401 (35%)                         | 319 (36%)                    | 82 (29%)                    |                  |
| P3/P4                         | 756 (65%)                         | 556 (64%)                    | 200 (71%)                   |                  |
| <b>Van Walraven score</b>     |                                   |                              |                             | <b>0.8</b>       |
| Median (IQR)                  | 12.0 (8.0, 12.0)                  | 12.0 (8.0, 12.0)             | 12.0 (7.0, 12.0)            |                  |
| [Range]                       | [0.0, 46.0]                       | [0.0, 46.0]                  | [0.0, 33.0]                 |                  |
| <b>BMI (kg/m<sup>2</sup>)</b> |                                   |                              |                             | <b>0.4</b>       |
| Median (IQR)                  | 27.5 (24.3, 32.4)                 | 27.7 (24.3, 32.7)            | 27.4 (24.2, 31.4)           |                  |
| [Range]                       | [15.8, 62.5]                      | [15.8, 62.5]                 | [16.5, 53.9]                |                  |
| Unknown                       | 3                                 | 2                            | 1                           |                  |
| <b>EBL (ml)</b>               |                                   |                              |                             | <b>0.016</b>     |
| EBL < 100ml                   | 834 (72%)                         | 615 (70%)                    | 219 (78%)                   |                  |
| EBL ≥ 100ml                   | 323 (28%)                         | 260 (30%)                    | 63 (22%)                    |                  |

Abbreviations: MMR, mismatch repair; MMEs, oral morphine milligram equivalents; IQR, interquartile range; CEA, carcinoembryonic antigen; COAD, colon adenocarcinoma; ASA, American Society of Anesthesiologists; BMI, body mass index; EBL, estimated blood loss.

<sup>1</sup> c("Median (IQR)", "[Range]"); n (%)

<sup>2</sup> Wilcoxon rank sum test; Pearson's chi-squared test

**FIG. 21**

| Characteristic               | HR   | 95% CI      | P-value          |
|------------------------------|------|-------------|------------------|
| Intraoperative MMEs (per 10) | 0.92 | 0.87, 0.98  | <b>0.007</b>     |
| MMR subtype                  |      |             |                  |
| Proficient                   | —    | —           |                  |
| Deficient                    | 0.38 | 0.21, 0.68  | <b>0.001</b>     |
| Adjunct                      |      |             |                  |
| None                         | —    | —           |                  |
| Dexmedetomidine              | 1.02 | 0.56, 1.87  | >0.9             |
| Ketamine                     | 0.90 | 0.59, 1.38  | 0.6              |
| Ketorolac use                |      |             |                  |
| No ketorolac                 | —    | —           |                  |
| Yes ketorolac                | 1.41 | 0.93, 2.15  | 0.10             |
| Pathological T stage         |      |             |                  |
| 1                            | —    | —           |                  |
| 2                            | 1.13 | 0.25, 5.00  | 0.9              |
| 3                            | 4.62 | 1.43, 14.88 | <b>0.010</b>     |
| 4                            | 10.0 | 2.94, 34.21 | <b>&lt;0.001</b> |
| Pathological N stage         |      |             |                  |
| 0                            | —    | —           |                  |
| 1                            | 2.96 | 1.62, 5.40  | <b>&lt;0.001</b> |
| 2                            | 4.34 | 2.23, 8.44  | <b>&lt;0.001</b> |
| BMI (kg/m <sup>2</sup> )     | 1.03 | 1.01, 1.06  | <b>0.009</b>     |
| Adjuvant chemotherapy        |      |             |                  |
| No                           | —    | —           |                  |
| Yes                          | 0.49 | 0.27, 0.90  | <b>0.022</b>     |

Abbreviations: HR, hazard ratio; CI, confidence interval; MMR, mismatch repair; MME, oral morphine milligram equivalents; IS, in situ/intramucosal; BMI, body mass index

**FIG. 22**

| Characteristic               | HR   | 95% CI      | P-value          |
|------------------------------|------|-------------|------------------|
| Intraoperative MMEs (per 10) | 0.93 | 0.88, 0.98  | <b>0.008</b>     |
| MMR subtype                  |      |             |                  |
| Proficient                   | —    | —           |                  |
| Deficient                    | 0.34 | 0.19, 0.61  | <b>&lt;0.001</b> |
| Adjunct                      |      |             |                  |
| None                         | —    | —           |                  |
| Dexmedetomidine              | 1.21 | 0.66, 2.23  | 0.5              |
| Ketamine                     | 1.17 | 0.79, 1.75  | 0.4              |
| Ketorolac use                |      |             |                  |
| No ketorolac                 | —    | —           |                  |
| Yes ketorolac                | 1.40 | 0.93, 2.12  | 0.10             |
| Patient age (years)          | 1.01 | 1.00, 1.03  | 0.068            |
| Sex                          |      |             |                  |
| Male                         | —    | —           |                  |
| Female                       | 0.98 | 0.69, 1.38  | 0.9              |
| Race                         |      |             |                  |
| White                        | —    | —           |                  |
| Black                        | 1.79 | 1.00, 3.21  | 0.050            |
| Other                        | 0.93 | 0.49, 1.76  | 0.8              |
| Ethnicity                    |      |             |                  |
| Hispanic                     | —    | —           |                  |
| Not Hispanic                 | 1.54 | 0.63, 3.75  | 0.3              |
| Pathological T stage         |      |             |                  |
| 1                            | —    | —           |                  |
| 2                            | 1.23 | 0.28, 5.44  | 0.8              |
| 3                            | 5.38 | 1.71, 16.92 | <b>0.004</b>     |
| 4                            | 13.2 | 4.09, 42.49 | <b>&lt;0.001</b> |

**FIG. 22 (Contd.)**

| Characteristic        | HR   | 95% CI     | P-value |
|-----------------------|------|------------|---------|
| Pathological N stage  |      |            |         |
| 0                     | —    | —          |         |
| 1                     | 2.41 | 1.59, 3.66 | <0.001  |
| 2                     | 4.41 | 2.84, 6.86 | <0.001  |
| Tumour location       |      |            |         |
| Right                 | —    | —          |         |
| Left                  | 0.79 | 0.54, 1.14 | 0.2     |
| Mid-transverse        | 0.91 | 0.47, 1.75 | 0.8     |
| CEA (ng/ml)           |      |            |         |
| CEA ≤ 5               | —    | —          |         |
| CEA > 5               | 1.78 | 1.21, 2.62 | 0.004   |
| Conversion            |      |            |         |
| No                    | —    | —          |         |
| Yes                   | 1.47 | 0.80, 2.73 | 0.2     |
| Synchronous COAD      |      |            |         |
| No                    | —    | —          |         |
| Yes                   | 1.06 | 0.38, 2.95 | >0.9    |
| Type of resection     |      |            |         |
| Open                  | —    | —          |         |
| Laparoscopic          | 0.64 | 0.39, 1.06 | 0.083   |
| Robotic               | 0.75 | 0.48, 1.17 | 0.2     |
| Extent of resection   |      |            |         |
| Extended              | —    | —          |         |
| Segmental             | 1.15 | 0.53, 2.49 | 0.7     |
| Adjuvant chemotherapy |      |            |         |
| No                    | —    | —          |         |
| Yes                   | 2.14 | 1.48, 3.10 | <0.001  |
| Surgery time (hours)  | 0.99 | 0.86, 1.15 | >0.9    |

**FIG. 22 (Contd.)**

| Characteristic           | HR   | 95% CI     | P-value |
|--------------------------|------|------------|---------|
| Smoking history          |      |            |         |
| Never used               | —    | —          |         |
| Past smoker              | 0.98 | 0.67, 1.44 | >0.9    |
| Current smoker           | 1.52 | 0.84, 2.73 | 0.2     |
| Albumin (g/dL)           | 0.65 | 0.40, 1.06 | 0.083   |
| ASA                      |      |            |         |
| P1/P2                    | —    | —          |         |
| P3/P4                    | 1.27 | 0.87, 1.86 | 0.2     |
| Van Walraven score       | 1.05 | 1.02, 1.08 | <0.001  |
| BMI (kg/m <sup>2</sup> ) | 1.03 | 1.01, 1.06 | 0.019   |
| EBL (ml)                 |      |            |         |
| EBL <100                 | —    | —          |         |
| EBL ≥ 100                | 1.42 | 0.99, 2.05 | 0.056   |

Abbreviations: HR, hazard ratio; CI, confidence interval; MMR, mismatch repair; MMEs, oral morphine milligram equivalents; CEA, carcinoembryonic antigen; COAD, colon adenocarcinoma; ASA, American Society of Anesthesiologists; BMI, body mass index; EBL, estimated blood loss.

**FIG. 23**

| Characteristic               | HR   | 95% CI     | P-value          |
|------------------------------|------|------------|------------------|
| Intraoperative MMEs (per 10) | 0.98 | 0.93, 1.03 | 0.4              |
| MMR subtype                  |      |            |                  |
| Proficient                   | —    | —          |                  |
| Deficient                    | 1.12 | 0.67, 1.88 | 0.7              |
| Adjunct                      |      |            |                  |
| None                         | —    | —          |                  |
| Dexmedetomidine              | 0.59 | 0.18, 1.98 | 0.4              |
| Ketamine                     | 0.43 | 0.21, 0.89 | <b>0.023</b>     |
| Ketorolac use                |      |            |                  |
| No ketorolac                 | —    | —          |                  |
| Yes ketorolac                | 0.79 | 0.41, 1.51 | 0.5              |
| Patient age (years)          | 1.08 | 1.06, 1.10 | <b>&lt;0.001</b> |
| Sex                          |      |            |                  |
| Male                         | —    | —          |                  |
| Female                       | 0.85 | 0.54, 1.35 | 0.5              |
| Race                         |      |            |                  |
| White                        | —    | —          |                  |
| Black                        | 2.27 | 1.11, 4.66 | <b>0.025</b>     |
| Other                        | 0.59 | 0.19, 1.85 | 0.4              |
| Ethnicity                    |      |            |                  |
| Hispanic                     | —    | —          |                  |
| Not Hispanic                 | 4.22 | 0.57, 31.4 | 0.2              |
| Pathological T stage         |      |            |                  |
| 1                            | —    | —          |                  |
| 2                            | 0.50 | 0.12, 2.03 | 0.3              |
| 3                            | 1.17 | 0.49, 2.79 | 0.7              |
| 4                            | 3.96 | 1.60, 9.78 | <b>0.003</b>     |
| Pathological N stage         |      |            |                  |
| 0                            | —    | —          |                  |
| 1                            | 1.53 | 0.91, 2.54 | 0.10             |
| 2                            | 1.75 | 0.93, 3.29 | 0.082            |

**FIG. 23 (Contd.)**

| Characteristic        | HR   | 95% CI     | P-value          |
|-----------------------|------|------------|------------------|
| Tumour location       |      |            |                  |
| Right                 | —    | —          |                  |
| Left                  | 0.73 | 0.45, 1.19 | 0.2              |
| Mid-transverse        | 1.05 | 0.47, 2.38 | 0.9              |
| CEA (ng/ml)           |      |            |                  |
| CEA ≤ 5               | —    | —          |                  |
| CEA > 5               | 1.73 | 1.07, 2.79 | <b>0.026</b>     |
| Conversion            |      |            |                  |
| No                    | —    | —          |                  |
| Yes                   | 0.67 | 0.24, 1.87 | 0.4              |
| Synchronous COAD      |      |            |                  |
| No                    | —    | —          |                  |
| Yes                   | 0.82 | 0.20, 3.44 | 0.8              |
| Type of resection     |      |            |                  |
| Open                  | —    | —          |                  |
| Laparoscopic          | 0.49 | 0.28, 0.86 | <b>0.014</b>     |
| Robotic               | 0.38 | 0.21, 0.67 | <b>0.001</b>     |
| Extent of resection   |      |            |                  |
| Extended              | —    | —          |                  |
| Segmental             | 0.84 | 0.38, 1.88 | 0.7              |
| Adjuvant chemotherapy |      |            |                  |
| No                    | —    | —          |                  |
| Yes                   | 0.85 | 0.54, 1.35 | 0.5              |
| Surgery time (hours)  | 0.76 | 0.61, 0.94 | <b>0.013</b>     |
| Smoking history       |      |            |                  |
| Never used            | —    | —          |                  |
| Past smoker           | 2.34 | 1.42, 3.84 | <b>0.001</b>     |
| Current smoker        | 2.30 | 1.07, 4.93 | <b>0.033</b>     |
| Albumin (g/dL)        | 0.31 | 0.19, 0.50 | <b>&lt;0.001</b> |

**FIG. 23 (Contd.)**

| Characteristic           | HR   | 95% CI     | P-value |
|--------------------------|------|------------|---------|
| ASA                      |      |            |         |
| P1/P2                    | —    | —          |         |
| P3/P4                    | 4.16 | 2.11, 8.19 | <0.001  |
| Van Walraven score       | 1.08 | 1.04, 1.12 | <0.001  |
| BMI (kg/m <sup>2</sup> ) | 1.02 | 0.98, 1.05 | 0.4     |
| EBL (ml)                 |      |            |         |
| EBL < 100                | —    | —          |         |
| EBL ≥ 100                | 1.07 | 0.65, 1.74 | 0.8     |

Abbreviations: HR, hazard ratio; CI, confidence interval; MMR, mismatch repair; MMEs, oral morphine milligram equivalents; CEA, carcinoembryonic antigen; COAD, colon adenocarcinoma; ASA, American Society of Anesthesiologists; BMI, body mass index; EBL, estimated blood loss.

**FIG. 24**

| Characteristic               | HR   | 95% CI     | P-value          |
|------------------------------|------|------------|------------------|
| Intraoperative MMEs (per 10) | 1.02 | 0.96, 1.08 | 0.6              |
| MMR subtype                  |      |            |                  |
| Proficient                   | —    | —          |                  |
| Deficient                    | 0.75 | 0.43, 1.31 | 0.3              |
| Adjunct                      |      |            |                  |
| None                         | —    | —          |                  |
| Dexmedetomidine              | 0.54 | 0.16, 1.85 | 0.3              |
| Ketamine                     | 0.60 | 0.27, 1.30 | 0.2              |
| Ketorolac use                |      |            |                  |
| No ketorolac                 | —    | —          |                  |
| Yes ketorolac                | 1.24 | 0.62, 2.48 | 0.5              |
| Patient age (years)          | 1.09 | 1.07, 1.12 | <b>&lt;0.001</b> |
| Race                         |      |            |                  |
| White                        | —    | —          |                  |
| Black                        | 2.40 | 1.09, 5.27 | <b>0.030</b>     |
| Other                        | 0.43 | 0.13, 1.39 | 0.2              |
| Pathological N stage         |      |            |                  |
| 0                            | —    | —          |                  |
| 1                            | 1.94 | 1.14, 3.30 | <b>0.016</b>     |
| 2                            | 2.05 | 1.05, 4.00 | <b>0.035</b>     |
| Pathological T stage         |      |            |                  |
| 1                            | —    | —          |                  |
| 2                            | 0.39 | 0.09, 1.66 | 0.2              |
| 3                            | 0.80 | 0.32, 1.98 | 0.6              |
| 4                            | 2.92 | 1.14, 7.48 | <b>0.026</b>     |
| ASA                          |      |            |                  |
| P1/P2                        | —    | —          |                  |
| P3/P4                        | 2.08 | 1.01, 4.28 | <b>0.046</b>     |

Abbreviations: HR, hazard ratio; CI, confidence interval; MMR, mismatch repair; MMEs, oral morphine milligram equivalents; ASA, American Society of Anesthesiologists

**FIG. 25**

| Opioid*MSI |                    |            |            |
|------------|--------------------|------------|------------|
| Gene       | log2 (fold change) | P          | adjusted P |
| CCK        | 4.1806373          | 6.29E-21   | 5.29E-19   |
| NTSR2      | 2.153295327        | 3.20E-06   | 2.21E-05   |
| IFNG       | 1.944678082        | 7.32E-17   | 3.38E-15   |
| NTSR1      | 1.94352515         | 3.32E-09   | 4.14E-08   |
| MLN        | 1.915480295        | 0.00078525 | 0.00301226 |
| LIPN       | 1.879282829        | 9.44E-09   | 1.09E-07   |
| CXCL8      | 1.864063595        | 1.97E-15   | 7.38E-14   |
| FCGR3B     | 1.781165703        | 5.31E-11   | 9.18E-10   |
| PTGS2      | 1.730939879        | 2.23E-15   | 8.27E-14   |
| CALB2      | 1.682757804        | 3.32E-09   | 4.15E-08   |
| CXCL10     | 1.633104513        | 1.05E-12   | 2.45E-11   |
| IL1RN      | 1.630502736        | 1.82E-17   | 9.25E-16   |
| CCL5       | 1.571841724        | 6.97E-21   | 5.81E-19   |
| CCL4       | 1.564431461        | 1.97E-22   | 2.04E-20   |
| IL1B       | 1.530245505        | 6.69E-14   | 1.94E-12   |
| CCL4L2     | 1.443545443        | 2.55E-15   | 9.39E-14   |
| FCGR3A     | 1.428472701        | 1.15E-13   | 3.22E-12   |
| ITIH4      | 1.419568663        | 2.04E-14   | 6.47E-13   |
| GAD1       | 1.414199356        | 1.78E-09   | 2.34E-08   |
| CD8A       | 1.400310783        | 1.76E-14   | 5.65E-13   |
| HTR3A      | 1.39396387         | 7.05E-06   | 4.49E-05   |
| SLC1A3     | 1.348718867        | 1.26E-11   | 2.44E-10   |
| LHB        | 1.326129654        | 3.34E-09   | 4.16E-08   |
| ADRB1      | 1.319803504        | 2.19E-08   | 2.36E-07   |
| CXCR2      | 1.286320163        | 1.16E-08   | 1.31E-07   |
| HRH2       | 1.282833457        | 6.45E-09   | 7.64E-08   |
| CCL3       | 1.278148013        | 9.26E-13   | 2.20E-11   |
| ESR2       | 1.225604335        | 5.37E-20   | 3.84E-18   |
| CSF2       | 1.224946213        | 8.17E-07   | 6.42E-06   |
| ENO2       | 1.214957342        | 4.80E-19   | 2.97E-17   |
| GRM5       | 1.201053673        | 0.00124208 | 0.00448855 |
| CXCR4      | 1.201006702        | 1.71E-14   | 5.51E-13   |
| PRKCG      | 1.117443629        | 0.00013298 | 0.00062615 |
| CD68       | 1.109963439        | 4.16E-12   | 8.84E-11   |
| IL10       | 1.108808264        | 7.52E-09   | 8.81E-08   |
| TLR2       | 1.0551997          | 8.12E-13   | 1.96E-11   |
| ICAM1      | 1.042733483        | 2.07E-16   | 9.02E-15   |
| RGS2       | 1.018236141        | 6.29E-11   | 1.07E-09   |
| CX3CL1     | 1.009829302        | 1.30E-10   | 2.09E-09   |
| KCNJ3      | 1.005705167        | 0.00087241 | 0.00330228 |

**FIG. 25 (Contd.)**

| Opioid*MSS |                    |            |            |
|------------|--------------------|------------|------------|
| Gene       | log2 (fold change) | P          | adjusted P |
| GHRH       | -1.059688649       | 0.01313726 | 0.03448099 |
| RBFOX3     | -1.095009494       | 1.04E-05   | 6.39E-05   |
| NOS1       | -1.162385795       | 0.00032925 | 0.00140032 |
| KISS1      | -1.178660702       | 3.75E-08   | 3.87E-07   |
| NPFFR2     | -1.183416145       | 4.37E-05   | 0.00023033 |
| GRIA2      | -1.183795048       | 0.0011007  | 0.00403944 |
| SLC6A2     | -1.193562015       | 0.01071192 | 0.02899708 |
| ADCY5      | -1.228618071       | 5.27E-10   | 7.66E-09   |
| HCRT       | -1.239615649       | 1.20E-05   | 7.27E-05   |
| OPCML      | -1.283391485       | 3.11E-08   | 3.26E-07   |
| NPY1R      | -1.327172697       | 4.32E-06   | 2.89E-05   |
| PPP1R14C   | -1.352150249       | 2.43E-14   | 7.58E-13   |
| NTRK2      | -1.398726232       | 3.59E-06   | 2.45E-05   |
| CHAT       | -1.472084679       | 0.00617002 | 0.01812689 |
| LEP        | -1.47814619        | 0.00026299 | 0.00114877 |
| CPB1       | -1.485637004       | 0.00152782 | 0.00538647 |
| NR1I2      | -1.515889217       | 1.46E-25   | 2.26E-23   |
| ADCY8      | -1.516130372       | 0.00888025 | 0.02474023 |
| EGF        | -1.5538379         | 2.12E-06   | 1.53E-05   |
| F2         | -1.564479651       | 1.21E-07   | 1.13E-06   |
| UGT1A8     | -1.658840371       | 1.54E-09   | 2.06E-08   |
| PLG        | -1.720879516       | 0.0003683  | 0.00154572 |
| GRPR       | -1.850055363       | 8.03E-10   | 1.14E-08   |
| PYY        | -1.852553829       | 3.28E-07   | 2.82E-06   |
| DRD1       | -1.958495354       | 2.21E-13   | 5.84E-12   |
| ABCB1      | -1.973835685       | 8.60E-22   | 8.13E-20   |
| SCT        | -2.043166685       | 3.32E-14   | 1.01E-12   |
| NTS        | -2.213179636       | 4.89E-08   | 4.93E-07   |
| NPFFR1     | -2.256102899       | 3.06E-36   | 1.95E-33   |
| TH         | -2.257592668       | 7.30E-19   | 4.42E-17   |
| CYP2B6     | -2.272433639       | 5.58E-25   | 7.94E-23   |
| HTR2C      | -2.288635244       | 0.012927   | 0.03403024 |
| TAC1       | -2.423462701       | 9.78E-13   | 2.31E-11   |
| CCKBR      | -2.440924019       | 3.85E-06   | 2.61E-05   |
| CALCA      | -2.617088943       | 1.10E-12   | 2.56E-11   |
| ATP12A     | -2.663040168       | 7.47E-11   | 1.26E-09   |
| LINC01411  | -2.688917451       | 1.86E-13   | 4.99E-12   |
| P2RX3      | -2.746442083       | 2.74E-12   | 6.00E-11   |
| ALB        | -2.856699546       | 3.75E-09   | 4.62E-08   |
| REN        | -2.900660672       | 4.69E-22   | 4.58E-20   |
| SLC6A4     | -3.579423441       | 5.85E-38   | 4.40E-35   |
| KNG1       | -3.727811358       | 2.51E-37   | 1.71E-34   |
| DRD2       | -3.802734388       | 3.81E-37   | 2.55E-34   |

FIG. 26A

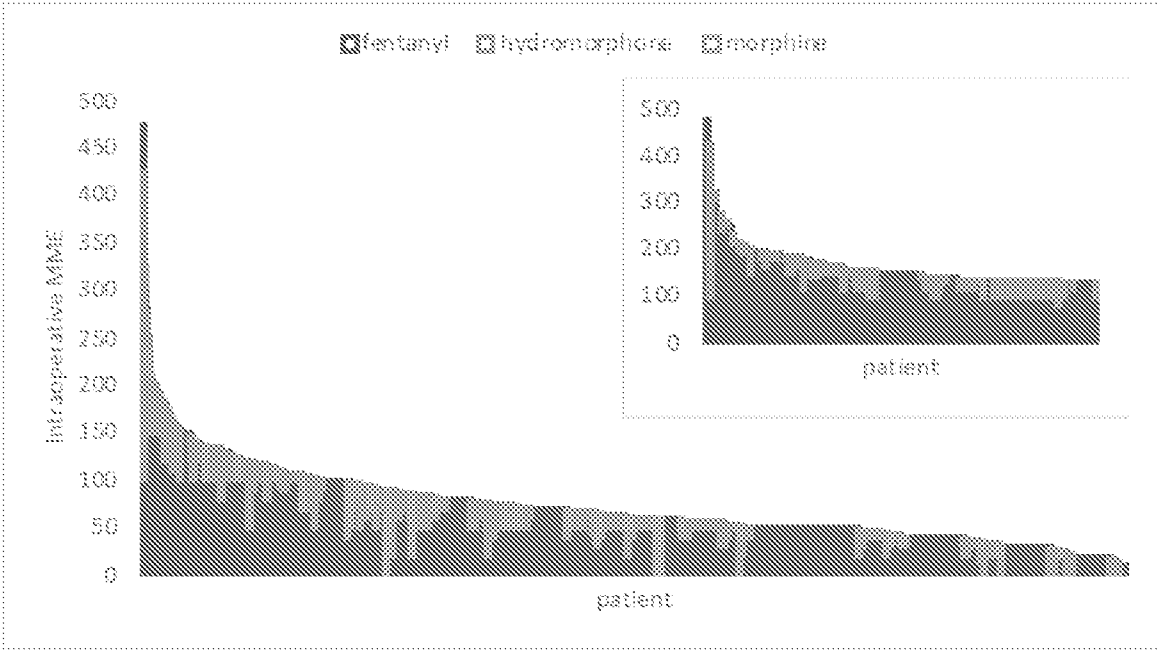


FIG. 26B

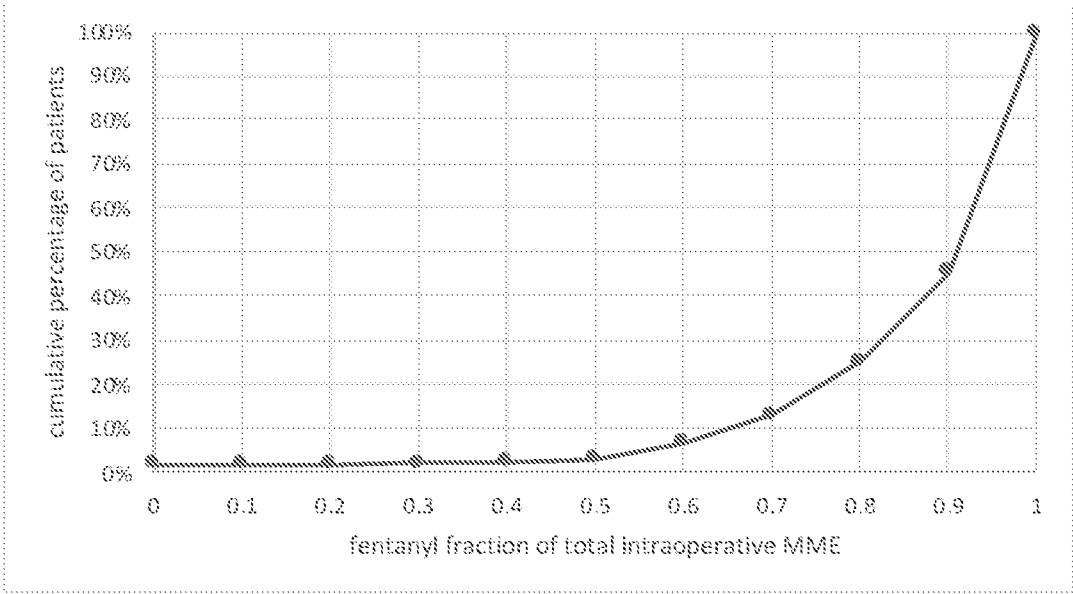


FIG. 27

| Characteristic      | None, N = 583 | Epidural, N = 269 | TAP, N = 303 | Epidural + TAP, N = 2 |
|---------------------|---------------|-------------------|--------------|-----------------------|
| Intraoperative MMEs |               |                   |              |                       |
| Median (IQR)        | 75 (53, 105)  | 50 (39, 71)       | 50 (37, 68)  | 67 (62, 72)           |
| [Range]             | 10, 323       | 0, 473            | 0, 180       | 58, 76                |

Abbreviations: TAP, Transversus Abdominis Plane.

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US22/20502

## A. CLASSIFICATION OF SUBJECT MATTER

IPC - C12Q 1/6883; A61K 31/485; A61P 35/04; G01N 33/574; C12Q 1/6806; C12Q 1/6851 (2022.01)

CPC - C12Q 1/6883; A61K 31/485; A61P 35/04; G01N 33/574; C12N 15/1003; C12Q 1/6806; C12Q 1/6851; G01N 33/5011

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

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

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

See Search History document

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

See Search History document

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                   | Relevant to claim No. |
|-----------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| Y         | CATA. "A retrospective analysis of the effect of intraoperative opioid dose on cancer recurrence after non-small cell lung cancer resection" 900-908. Cancer Medicine. . 21 January 2014; page 901, column 1, paragraphs 2-4; page 906, column 2, paragraph 2; DOI: 10.1002/cam4.236 | 1-3                   |
| Y         | OHGAKI. "APC mutations are infrequent but present in human lung cancer" 197-203. Cancer Letters. . April 2004; Abstract; DOI: 10.1016/j.canlet.2003.10.020                                                                                                                           | 1-3                   |
| Y         | US 2021/0008048 A1 (DANA-FARBER CANCER INSTITUTE, INC.) 14 January 2021; Paragraph [0008], [0030], [0055], [0126], [0183], [0444], [0463]                                                                                                                                            | 5-8                   |
| Y         | MACKLER. "Cellular adaptation to opiates alters ion-channel mRNA levels" 385-389. Neurobiology. . January 1994; page 385, column 1, paragraph 1; page 386, column 1, paragraph 2; page 387, column 1, paragraph 2; page 389, column 1, paragraph 1; DOI: 10.1073/pnas.91.1.385       | 5-8                   |
| Y         | KING. "Morphine treatment accelerates sarcoma-induced bone pain, bone loss, and spontaneous fracture in a murine model of bone cancer" 154-168. Pain. . November 2007; page 5, paragraph 3; page 11, paragraph 2; page 24, paragraph 1; DOI: 10.1016/j.pain.2007.06.026.             | 8                     |
| A         | WO 2019/055234 A1 (MASSACHUSETTS INSTITUTE OF TECHNOLOGY) 21 March 2019; Entire Document                                                                                                                                                                                             | 1-3, 5-8              |
| A         | MONTAGNA. "Intraoperative opioids are associated with improved recurrence-free survival in triple-negative breast cancer" 367-376. British Journal of Anaesthesia. . October 2020; Entire Document; DOI: 10.1016/j.bja.2020.10.021                                                   | 1-3, 5-8              |

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

## \* Special categories of cited documents:

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

"D" document cited by the applicant in the international application

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

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

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

Date of the actual completion of the international search

20 July 2022 (20.07.2022)

Date of mailing of the international search report

AUG 10 2022

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents  
P.O. Box 1450, Alexandria, Virginia 22313-1450  
Facsimile No. 571-273-8300

Authorized officer

Shane Thomas

Telephone No. PCT Helpdesk: 571-272-4300

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US22/20502

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

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

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 4, 9-20, 28-45, 63-69, 72-75, 77-82  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

This International Searching Authority found multiple inventions in this international application, as follows:  
-\*\*\*-Please See Supplemental Page-\*\*\*-

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  
Group I, claims 1-3 and 5-8

### Remark on Protest

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

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US22/20502

\*\*\*-Continued From Box No. III: Observations where unity of invention is lacking-\*\*\*

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

Group I, claims 1-3 and 5-8 is directed to methods for treatment and care of cancer patients undergoing tumor resection surgery for lung cancer comprising administering to the cancer patient an effective amount of an intraoperative opioid analgesic during the tumor resection surgery.

Group II, claims 21-27 is directed to methods for treatment and care of cancer patients undergoing tumor resection surgery for lung cancer comprising administering to the cancer patient an effective amount of an opioid-free intraoperative analgesic during the tumor resection surgery.

Group III, claims 46-62, 70-71, and 76 is directed to methods for treatment and care of cancer patients undergoing tumor resection surgery for colon cancer.

The inventions listed as Groups I-III do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the special technical features of Group I include mutations in genes that result in hyperactivation of Wnt pathway and/or reduced activity of the Hippo pathway, mRNA or polypeptide expression or activity levels of one or more of NF2, LATS1, FAT1, RNF43, AXIN2, APC, AMER1, or CTNNB1, not present in Groups II-III; the special technical features of Group II include detecting the presence of at least one mutation in CDKN2A that reduces CDKN2A expression and/or activity levels, and/or detecting elevated FGA, not present in Groups I or III; and the special technical features of Group III include a cancer patient undergoing tumor resection surgery for colon cancer, and expression levels or activity of one or more genes that result in reduced activity of Wnt pathway and/or hyperactivation of Th1 immune response signature, and at least one alteration in DNA mismatch repair system, not present in Groups I-II.

Groups I-III share the technical features including: methods for selecting, prolonging survival of, or reducing the risk of tumor recurrence in a cancer patient undergoing tumor resection surgery, comprising administering to the cancer patient an effective amount of an intraoperative analgesic during the tumor resection surgery, and determining levels of expression and/or activity of genes in a biological sample from the patient as compared to a control/reference sample or a predetermined threshold.

Groups I-II share the additional technical feature: lung cancer.

Groups II-III share the additional technical feature: an opioid-free intraoperative analgesic.

Groups I and III share the additional technical feature: an intraoperative opioid analgesic.

However, these shared technical features are previously disclosed by the publication entitled "A retrospective analysis of the effect of intraoperative opioid dose on cancer recurrence after non-small cell lung cancer resection" by Cata, et al. (hereinafter "Cata").

Cata discloses methods for selecting (selection of patients; page 901, column 1, paragraph 3), prolonging survival of (high intraoperative doses of opioids would be associated with decreased recurrence and overall free survivals; page 901, column 1, paragraph 2), or reducing the risk of tumor recurrence (high intraoperative doses of opioids would be associated with decreased recurrence and overall free survivals; page 901, column 1, paragraph 2) in a cancer patient undergoing tumor resection surgery (cancer patients undergoing resection surgery; page 901, column 1, paragraph 3), comprising administering to the cancer patient an effective amount of an intraoperative analgesic during the tumor resection surgery (administration of analgesics during surgery; page 901, column 1, paragraph 4), and determining levels of expression and/or activity of genes in a biological sample from the patient as compared to a control/reference sample or a predetermined threshold (increased (compared to a control) expression of MOR in patients with cancer; page 905, column 1, paragraph 1); lung cancer (patients with non-small cell lung cancer; page 901, column 1, paragraph 3); an opioid-free intraoperative analgesic (regional analgesia techniques (opioid-free); page 900, column 2, paragraph 1); and an intraoperative opioid analgesic (opioid-based anesthesia; page 900, column 2, paragraph 1).

Since none of the special technical features of the Groups I-III inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Cata reference, unity of invention is lacking.