

COMPOSITIONS AND METHODS FOR DETECTION OF LUNG CANCER

CROSS-REFERENCE TO RELATED APPLICATIONS

[1] This application claims benefit of U.S. Provisional Application No. 63/297684 filed January 7, 2022, and U.S. Provisional Application No. 63/417299 filed October 18, 2022, the contents of which are hereby incorporated by reference in their entirety.

BACKGROUND

[2] Early detection of cancer greatly increases the chance of successful treatment. However, many cancers including lung cancer still lack effective screening recommendations. Typical challenges for cancer-screening tests include limited sensitivity and specificity. A high rate of false-positive results can be of particular concern, as it can create difficult management decisions for clinicians and patients who would not want to unnecessarily administer (or receive) anti-cancer therapy that may potentially have undesirable side effects. Conversely, a high rate of false-negative results fails to satisfy the purpose of the screening test, as patients who need therapy are missed, resulting in a treatment delay and consequently a reduced possibility of success.

SUMMARY

[3] The present disclosure, among other things, provides insights and technologies for achieving effective lung cancer screening from a biological sample. In some examples, such a biological sample is or comprises a bodily fluid-derived sample, e.g., in some examples a blood-derived sample. In some examples, the present disclosure, among other things, provides insights and technologies that are particularly useful for non-small cell lung cancer screening. In some examples, provided technologies are effective for detection of early stage lung cancers (*e.g.*, in some examples non-small cell lung cancer). In some examples, provided technologies are effective even when applied to populations comprising or consisting of asymptomatic individuals (*e.g.*, due to sufficiently high sensitivity and/or low rates of false positive and/or false negative results). In some examples, provided technologies are effective when applied to populations comprising or consisting of individuals (*e.g.*, asymptomatic individuals) without hereditary risk in developing lung cancer (*e.g.*, in some

examples non-small cell lung cancer). In some examples, provided technologies are effective when applied to populations comprising or consisting of symptomatic individuals (*e.g.*, individuals suffering from one or more symptoms of lung cancer). In some examples, provided technologies are effective when applied to populations comprising or consisting of individuals at risk for lung cancer (*e.g.*, individuals with hereditary and/or life-history associated risk factors for lung cancer). In some examples, provided technologies may be or include one or more compositions (*e.g.*, molecular entities or complexes, systems, cells, collections, combinations, kits, *etc.*) and/or methods (*e.g.*, of making, using, assessing, *etc.*), as will be clear to one skilled in the art reading the disclosure provided herein.

[4] In some examples, the present disclosure identifies the source of a problem with certain prior technologies including, for example, certain conventional approaches to detection and diagnosis of lung cancer. For example, the present disclosure appreciates that many conventional diagnostic assays, *e.g.*, X-ray imaging, sputum testing, low-dose CT scanning, and/or molecular tests based on cell-free nucleic acids, serum biomarkers (*e.g.*, carcinoembryonic antigen (CEA), cytokeratin 19 fragment (CYFRA 21-1), neuron-specific enolase (NSE), progastrin-releasing peptide (ProGRP), and/or squamous cell carcinoma antigen (SCCA)), and/or bulk analysis of extracellular vesicles, can be time-consuming, costly, and/or lacking sensitivity and/or specificity sufficient to provide a reliable and comprehensive diagnostic assessment. In some examples, the present disclosure provides technologies (including systems, compositions, and methods) that solve such problems, among other things, by detecting co-localization of a target biomarker signature of lung cancer in individual nanoparticles having a size range of interest that includes extracellular vesicles, which comprises at least one extracellular vesicle-associated surface biomarker (including, *e.g.*, membrane-bound and/or membrane-associated polypeptides) and at least one target biomarker selected from the group consisting of surface biomarkers, internal biomarkers, and RNA biomarkers. In some examples, the present disclosure provides technologies (including systems, compositions, and methods) that solve such problems, among other things, by detecting such target biomarker signature of lung cancer using a target entity detection approach that was developed by Applicant and described in US2020/0299780 and WO2020180741, which are based on interaction and/or co-localization of at least two or more target entities (*e.g.*, a target biomarker signature) in individual nanoparticles, *e.g.*, extracellular vesicles.

[5] In some examples, extracellular vesicles for detection as described herein can be isolated from a bodily fluid of a subject by a size exclusion-based method. As will be understood by a skilled artisan, in some examples, a size exclusion-based method may provide a sample comprising nanoparticles having a size range of interest that includes nanoparticles. Accordingly, in some examples, provided technologies of the present disclosure encompass detection, in individual nanoparticles having a size range of interest (e.g., in some examples about 30 nm to about 1000 nm) that includes nanoparticles, of co-localization of at least two or more surface biomarkers (e.g., as described herein) that forms a target biomarker signature of lung cancer. A skilled artisan reading the present disclosure will understand that various examples described herein in the context of “nanoparticle(s)” can be also applicable in the context of “nanoparticles” as described herein.

[6] In some examples, the present disclosure, among other things, provides insights that screening of asymptomatic individuals, *e.g.*, regular screening prior to or otherwise in absence of developed symptom(s), can be beneficial, and even important for effective management (*e.g.*, successful treatment) of lung cancer (*e.g.*, in some examples non-small cell lung cancer). In some examples, the present disclosure provides lung cancer screening systems that can be implemented to detect lung cancer (*e.g.*, in some examples non-small cell lung cancer), including early-stage cancer, in some examples in asymptomatic individuals. In some examples, provided technologies are implemented to achieve regular screening of asymptomatic individuals. The present disclosure provides, for example, compositions (*e.g.*, reagents, kits, components, *etc.*), and methods of providing and/or using them, including strategies that involve regular testing of one or more individuals (*e.g.*, symptomatic or asymptomatic individuals). The present disclosure defines usefulness of such systems, and provides compositions and methods for implementing them.

[7] In some examples, provided technologies achieve detection (*e.g.*, early detection, *e.g.*, in asymptomatic individual(s) and/or population(s)) of one or more features (*e.g.*, incidence, progression, responsiveness to therapy, recurrence, *etc.*) of lung cancer, with sensitivity and/or specificity (*e.g.*, rate of false positive and/or false negative results) appropriate to permit useful application of provided technologies to single-time and/or regular (*e.g.*, periodic) assessment. In some examples, provided technologies are useful in conjunction with regular medical examinations, such as but not limited to: physicals, general practitioner

visits, cholesterol/lipid blood tests, diabetes (type 2) screening, colonoscopies, blood pressure screening, thyroid function tests, prostate cancer screening, mammograms, HPV/Pap smears, and/or vaccinations. In some examples, provided technologies are useful in conjunction with treatment regimen(s); in some examples, provided technologies may improve one or more characteristics (*e.g.*, rate of success according to an accepted parameter) of such treatment regimen(s).

[8] In some examples, provided are technologies for use in classifying a subject (*e.g.*, an asymptomatic subject) as having or being susceptible to lung cancer (*e.g.*, in some examples non-small cell lung cancer). In some examples, the present disclosure provides methods or assays for classifying a subject (*e.g.*, an asymptomatic subject) as having or being susceptible to lung cancer (*e.g.*, in some examples non-small cell lung cancer). In some examples, a provided method or assay comprises (a) detecting, in a biological sample (*e.g.*, in some examples a bodily fluid-derived sample such as, *e.g.*, but not limited to a blood-derived sample) from a subject in need thereof, nanoparticles (having a size range of interest that includes extracellular vesicles) expressing a target biomarker signature of lung cancer, the target biomarker signature comprising: at least one extracellular vesicle-associated surface biomarker (including, *e.g.*, membrane-bound and/or membrane-associated polypeptides) and at least one target biomarker selected from the group consisting of: surface biomarkers (as described herein), intravesicular biomarkers (as described herein), and intravesicular RNA biomarkers (as described herein), wherein the surface biomarkers are selected from ADGRF1, ALCAM, ABCC3, ARSL, B3GNT3, B3GNT5, CDCP1, CDH1, CDH3, CD55, CD274 (PD-L1), CEACAM5, CEACAM6, CELSR1, CLDN18, CLDN3, CLDN4, CLDN7, CLIC6, DMBT1, DSG2, EGFR, EPCAM, EPHX3, EVA1A, FAM241B, FOLR1, FXRD3, GALNT14, GJB1, GJB2, GPC4, HAS3, HS6ST2, KDELR3, KRTCAP3, IG1FR, LAMB3, LAPTM4B, LARGE2, LFNG, LSR, MAL2, MANEAL, MET, MSLN, MUC1, MUC21, NRCAM, PIGT, PODXL2, PRRG4, PRSS21, ROS1, SDC1, SERINC2, SEZ6L2, SLC34A2, Lewis X antigen, sialyl Lewis X antigen, sTn antigen, Tn antigen, T antigen, SLC44A4, SLC6A14, SLC7A7, SLC7A11, SMIM22, SMPDL3B, ST14, TACSTD2, TMC4, TMC5, TMEM45B, TMPRSS2, TMPRSS4, TNFRSF10B, TSPAN1, TSPAN8, UCHL1 and combinations thereof; the intravesicular biomarkers are selected from AOC1, C12orf45, CRABP2, CST1, ETV4, FAM83A, FOXA2, HMGB3, LGALS3BP, MIF, NAPSA, PPP1R14D, S100A14, SBK1, SCGB3A2, SFTA2,

SFTPA1, SFTPA2, SFTPB, SPINK1, TGFA, ZC3H11A, and combinations thereof; and the intravesicular RNA (e.g., but not limited to mRNA and noncoding RNA such as, e.g., orphan noncoding RNA, long noncoding RNA, piwi-interacting RNA, microRNA, circular RNA, etc.) biomarkers are selected from ABCC3, AOC1, ARSL, B3GNT3, C12orf45, CDCP1, CDH1, CDH3, CEACAM5, CEACAM6, CELSR1, CLDN18, CLDN3, CLDN4, CLDN7, CLIC6, CRABP2, CST1, DMBT1, DSG2, EPCAM, EPHX3, ETV4, EVA1A, FAM83A, FOLR1, FOXA2, GJB1, GJB2, GPC4, HMGB3, HS6ST2, KDELR3, KRTCAP3, LAMB3, LFNG, LGALS3BP, LSR, MANEAL, MIF, MSLN, MUC1, MUC21, NAPS A, PIGT, PODXL2, PPP1R14D, PRRG4, ROS1, S100A14, SBK1, SCGB3A2, SDC1, SERINC2, SEZ6L2, SFTA2, SFTPA1, SFTPA2, SFTPB, SLC34A2, SLC44A4, SLC6A14, SLC7A7, SMIM22, SMPDL3B, SPINK1, ST14, TGFA, TMC4, TMC5, TMEM45B, TMPRSS2, TMPRSS4, TSPAN1, TSPAN8, ZC3H11A, and combinations thereof; (b) comparing sample information indicative of level of the target biomarker signature-expressing nanoparticles in the biological sample to reference information including a reference threshold level; and (c) classifying the subject as having or being susceptible to lung cancer when the biological sample shows an elevated level of target biomarker signature-expressing nanoparticles relative to a classification cutoff referencing the reference threshold level.

[9] In some examples, provided are technologies for use in classifying a subject (*e.g.*, an asymptomatic subject) as having or being susceptible to lung cancer (*e.g.*, in some examples non-small cell lung cancer). In some examples, the present disclosure provides methods or assays for classifying a subject (*e.g.*, an asymptomatic subject) as having or being susceptible to lung cancer (*e.g.*, in some examples non-small cell lung cancer). In some examples, a provided method or assay comprises (a) detecting, in a biological sample from a subject in need thereof, nanoparticles expressing a target biomarker signature of lung cancer (*e.g.*, in some examples non-small cell lung cancer), the target biomarker signature comprising: at least one nanoparticle-associated surface biomarker and at least one target biomarker selected from the group consisting of: surface biomarkers (as described herein), intravesicular biomarkers (as described herein), and intravesicular RNA biomarkers (as described herein); (b) comparing sample information indicative of level of the target biomarker signature-expressing nanoparticles in the biological sample to reference information including a reference threshold level; and (c) classifying the subject as having or being susceptible to lung

cancer when the biological sample shows an elevated level of target biomarker signature-expressing nanoparticles relative to a classification cutoff referencing the reference threshold level. In some examples, at least one such target biomarker is or comprises a surface biomarker selected from: Sialyl Tn (sTn) antigen, Sialyl-6T antigen (6-sialyl core 1), T antigen, PTK7, TSPAN8, CELSR2, GPC1, ST14, PTPRZ1, GPR87, GJB5, GJB2, RHOV, LYPD3, CLDN7, DSP, SERINC2, ABHD17C, PERP, MPZL2, ITGB4, MEST, GPNMB, SLC35A2, Gb3 (CD77), ITGA6, ABCC5, ATP1B3, JAG1, TMPRSS11D, Sialyl Lewis A antigen (CA19-9), Globo H, Gb5 (SSEA-3), Lactotriaosylceramide, (Lc3), Forssman antigen, ABCA3, ABCC1, ABCC3, ACBD3, ACSL5, ADGRF1, AGER, ALCAM, AP1M2, APH1A, APOO, ATP11A, ATP11B, ATP1B1, ATP6AP2, B3GNT5, B4GALT4, BCAP31, BSPRY, CD109, CD55, CD9, CDC42, CDH1, CDH3, CDKAL1, CEACAM5, CEACAM6, CELSR1, CIP2A, CISD2, CKAP4, CLCA2, CLDN1, CLIC6, CLPTM1L, CLSTN1, CNTN1, CPD, CYP2S1, CYP4F11, CYP4F3, DPY19L1, DSC2, DSC3, DSG2, DSG3, EGFR, EPCAM, EPHB3, FAM241B, FAT2, FBXO45, FERMT1, FOLR1, FXYD3, FZD6, GALNT1, GALNT3, GALNT5, GALNT6, GALNT14, GGCX, GOLM1, GOLPH3L, GRHL2, HACD3, HAS3, IER3IP1, IGSF3, IL1RAP, ITGA2, ITGB6, KLRG2, KPNA2, KRTCAP3, LAD1, LAMB3, LAMC2, LAMP3, LAMTOR2, LAPTM4B, LARGE2, LCLAT1, LPCAT1, LSR, MAGT1, MAL2, MARCKSL1, MET, MGAT1, MSLN, MUC1, MUC4, NCSTN, NECTIN1, NECTIN4, NRAS, NRCAM, NT5E, NUP210, PARL, PEX13, PIGN, PIGT, PLA2G4A, PLCH1, PLEC, PRSS21, PSMD2, PTDSS1, PTGFRN, PTPRF, QSOX1, RAB25, RAB38, RAB6B, RAP2B, RCC2, RIT1, SCAMP3, SDC1, SEL1L3, SHROOM2, SLC7A11, SLC2A1, SLC34A2, SLC35B2, SLC39A11, SMIM22, SMPDL3B, SOAT1, SPAST, SSR1, SSR4, SURF4, SYNGR2, TACSTD2, TESC, TFRC, TMC5, TMC01, TMED2, TMED3, TMEM132A, TMEM33, TMPRSS4, TMTC3, TOMM22, TOR1AIP2, TRAM1, TRPV4, TTC33, UGT1A6, UPK1B, VAMP8, VMA21, VRK2, VWA1, XPR1, XXYLT1, ADAM28, AXL, BSG, CD274, CD47, CLU, DKK1, ERBB3, FLT4, GM3, HGF, IGF1R, IL6, KDR, LAG3, UCHL1, Lewis X, Lewis Y/B antigen, LY6E, NOTCH2, NOTCH3, Phosphatidylserine, TIGIT, TNFRSF10A, TNFRSF10B, TNFSF18, TPBG, VEGFA, Tn antigen, Lewis Y/CD174, Sialyl Lewis X (sLex) (also known as Sialyl SSEA-1 (SLX)), NeuGcGM3, and combinations thereof. In some examples, at least one such target biomarker is or comprises a surface biomarker selected from: ALCAM, BCAP31, CD109, CD274, CD55, CD9, CDH3, CEACAM5, CEACAM6, DSC2, DSC3, EGFR, EPCAM, FOLR1, HACD3,

IL1RAP, IGF1R, IGSF3, ITGA2, LAMB3, Lewis Y antigen, Lewis X antigen, MARCKSL1, MET, MSLN, MUC1, MUC4, NT5E, Phosphatidylserine, PTGFRN, PTK7, SDC1, Sialyl Lewis X antigen, SLC34A2, Sialyl Tn antigen (sTn antigen), Tn antigen, T antigen, TACSTD2, TNFRSF10B, TFRC, TRPV4, TSPAN8, CD274, CDH1, CNTN1, GOLM1, VWA1, CELSR2, CLDN1, DSG2, DSG3, GPC1, LAMC2, NECTIN1, ST14, CLCA2, PTPRZ1, SLC2A1, LYPD3, Gb3 (CD77), GJB2, MPZL2, RAP2B, ITGB4, DSP, MEST, ITGA6, ABCC5, ATP1B3, JAG1, GPNMB, PERP, TMPRSS11D, Sialyl-6T antigen (6-sialyl core 1), Sialyl-T antigen, Sialyl Lewis A antigen (CA19-9), Globo H, Gb5 (SSEA-3), Lactotriaosylceramide (Lc3), Forssman antigen, and combinations thereof. In some examples, at least one such target biomarker is or comprises an intravesicular biomarker selected from ABRACL, ACP5, ADH7, AGR2, AIF1, AKR1C1, AKR1C2, AKR1C3, ALDH1A1, ALDH3A1, ALDH3B2, ALG1L, AOC1, AP1M2, APOBEC3B, APOBEC3C, ARNTL2, ASF1B, AURKB, BAIAP2L1, BIRC5, C12orf45, C15orf48, C19orf33, C1S, C8orf4, CA9, CALML3, CAPNS2, CBLC, CCL19, CCNB2, CDC20, CDC45, CDCA4, CDCA5, CDK1, CDKN2A, CDKN2B, CENPW, CEP55, CES1, CHMP4C, CNN2, CPA3, CRABP2, CST1, CSTA, CTSC, CTSE, CYP2S1, DPYSL3, EFS, EGLN3, EHF, ELF3, ELF4, ENAH, ESRP1, ETV4, EVPL, FAM129B, FAM60A, FAM83A, FAM83D, FAM83H, FBP1, FERMT1, FOXA2, FOXE1, FOXM1, GBP6, GNA15, GPX2, GRHL2, GSTA1, HCK, HMGB3, HOXB7, ID1, IGF2BP2, IMPA2, IRF6, IVL, JUP, KIAA1522, KIF2C, KIFC1, KLF4, KLF5, KRT13, KRT14, KRT15, KRT16, KRT17, KRT18, KRT19, KRT5, KRT6A, KRT6B, KRT6C, KRT7, KRT8, LGALS3BP, LGALS7B, LSP1, MAGEA4, MAGEA6, MCM2, MDFI, MIF, MYBL2, MYH14, MZB1, NAPSA, NCF2, NNMT, NRARP, NUP210, NUSAP1, OSGIN1, PALLD, PITX1, PKP1, PKP3, PLEK, PLEK2, POSTN, PPP1R14C, PPP1R14D, PRAME, PTPN6, RBP1, RIN2, RIPK4, RPS4Y1, RRM2, S100A11, S100A14, S100A16, S100A2, S100P, SBK1, SCGB3A2, SERPINB13, SERPINB3, SERPINB5, SFTA2, SFTPA1, SFTPA2, SFTPB, SH3BP4, SNAI2, SOX2, SPI1, SPINK1, SPINT1, SPRR1A, SPRR1B, SPRR2A, SPRR2D, SPRR2E, SPRR3, SULF1, SYK, SYTL1, TBC1D2, TEAD2, TEAD3, TFAP2C, TGFA, THBS2, TK1, TOP2A, TP63, TPD52, TPX2, TRIM29, TRIP13, UBE2C, YAP1, ZC3H11A, ZNF217, ZNF750, and combinations thereof. In some examples, at least one such target biomarker is or comprises an intravesicular RNA (*e.g.*, but not limited to mRNA and noncoding RNA such as, *e.g.*, orphan noncoding RNA, long noncoding RNA, piwi-interacting RNA, microRNA, circular RNA, etc.) selected from ABCA3,

ABCC1, ABCC3, ABRACL, ACP5, ADAM23, ADH7, AGR2, AIF1, AKR1C1, AKR1C2, AKR1C3, ALDH1A1, ALDH3A1, ALDH3B2, ALG1L, ANTXR1, AOC1, AP1M2, APOBEC3B, APOBEC3C, AQP3, AREG, ARNTL2, ARSL, ASF1B, ATP8B1, AURKB, B3GNT3, B3GNT5, BAIAP2L1, BCAM, BIK, BIRC5, C12orf45, C15orf48, C19orf33, C1S, C8orf4, CA12, CA9, CALML3, CAPNS2, CBLC, CCL19, CCL5, CCNB2, CD109, CD24, CD53, CD74, CD9, CDC20, CDC42EP1, CDC45, CDCA4, CDCA5, CDCP1, CDH1, CDH3, CDK1, CDKN2A, CDKN2B, CEACAM5, CEACAM6, CELSR1, CENPW, CEP55, CES1, CHMP4C, CLCA2, CLDN1, CLDN18, CLDN3, CLDN4, CLDN7, CLIC6, CNN2, COL17A1, CPA3, CRABP2, CST1, CSTA, CTSC, CTSE, CX3CL1, CXADR, CXCR4, CYBB, CYP2S1, CYP4F11, DAPL1, DMBT1, DPYSL3, DSC2, DSC3, DSG2, DSG3, DSP, EFNA1, EFS, EGFR, EGLN3, EHD2, EHF, ELF3, ELF4, EMP1, EMP2, ENAH, EPCAM, EPHA2, EPHB3, EPHX1, EPHX3, ESRP1, ETV4, EVA1A, EVPL, F11R, F2R, F2RL1, F3, FAM129B, FAM60A, FAM83A, FAM83D, FAM83H, FAT1, FAT2, FBLIM1, FBP1, FCER1G, FERMT1, FGFR2, FGFR3, FOLR1, FOXA2, FOXE1, FOXM1, FXYD3, GALNT3, GBP6, GJA1, GJB1, GJB2, GJB3, GJB5, GJB6, GNA15, GPC1, GPC3, GPC4, GPNMB, GPR87, GPRC5A, GPX2, GRHL2, GSTA1, HAS3, HCK, HMGB3, HOXB7, HS6ST2, ID1, IGF2BP2, IGSF9, IL2RG, IMPA2, IRF6, ITGA2, ITGA6, ITGB4, ITGB6, IVL, JAG2, JUP, KCNS3, KDELR3, KIAA1522, KIF2C, KIFC1, KITLG, KLF4, KLF5, KRT13, KRT14, KRT15, KRT16, KRT17, KRT18, KRT19, KRT5, KRT6A, KRT6B, KRT6C, KRT7, KRT8, KRTCAP3, LAMB3, LAMP3, LAPTM5, LFNG, LGALS3BP, LGALS7B, LRP11, LRRC4, LSP1, LSR, LYPD3, MAGEA4, MAGEA6, MAL2, MANEAL, MAOA, MARCO, MCM2, MDFI, MET, MIF, MMP14, MPZL2, MSLN, MUC1, MUC21, MYBL2, MYH14, MYOF, MZB1, NAPSA, NCF2, NKG7, NNMT, NOTCH3, NRARP, NTRK2, NUP210, NUSAP1, OSGIN1, OSMR, PALLD, PDPN, PDZK1IP1, PECAM1, PERP, PIGR, PIGT, PITX1, PKP1, PKP3, PLEK, PLEK2, PLVAP, PMP22, PODXL2, POSTN, PPL, PPP1R14C, PPP1R14D, PRAME, PROM2, PRRG4, PRSS8, PTGES, PTGFRN, PTPN6, PTPRF, PTPRZ1, RAB25, RAB38, RAET1L, RARRES1, RBP1, RGS1, RHCG, RHOV, RIN2, RIPK4, ROS1, RPS4Y1, RRM2, S100A10, S100A11, S100A14, S100A16, S100A2, S100P, SBK1, SCGB3A2, SCNN1A, SDC1, SERINC2, SERPINB13, SERPINB3, SERPINB5, SEZ6L2, SFTA2, SFTPA1, SFTPA2, SFTPB, SH3BP4, SHISA2, SLC1A5, SLC2A1, SLC34A2, SLC40A1, SLC44A4, SLC6A14, SLC6A8, SLC7A7, SLC7A8, SMIM22, SMPDL3B, SNAI2, SOX2, SPI1, SPINK1, SPINT1, SPINT2, SPRR1A,

SPRR1B, SPRR2A, SPRR2D, SPRR2E, SPRR3, ST14, STEAP1, SULF1, SYK, SYTL1, TACSTD2, TBC1D2, TEAD2, TEAD3, TFAP2C, TGFA, THBD, THBS2, TK1, TM4SF1, TMC4, TMC5, TMEM30B, TMEM45B, TMEM54, TMPRSS11D, TMPRSS11E, TMPRSS2, TMPRSS4, TNFRSF18, TNS4, TOP2A, TP53I11, TP63, TPD52, TPX2, TREM2, TRIM29, TRIP13, TSPAN1, TSPAN13, TSPAN6, TSPAN7, TSPAN8, TUSC3, TYROBP, UBE2C, UPK1B, VAMP8, VANGL2, WLS, YAP1, ZC3H11A, ZNF217, ZNF750, and combinations thereof.

[10] In some examples, provided are technologies for use in classifying a subject (*e.g.*, an asymptomatic subject) as having or being susceptible to lung cancer (*e.g.*, in some examples non-small cell lung cancer such as, *e.g.*, lung adenocarcinoma (LUAD) and/or lung squamous cell carcinoma (LUSC)). In some examples, the present disclosure provides methods or assays for classifying a subject (*e.g.*, an asymptomatic subject) as having or being susceptible to lung cancer (*e.g.*, in some examples non-small cell lung cancer such as, *e.g.*, LUAD and/or LUSC)). In some examples, a provided method or assay comprises (a) detecting, in a biological sample from a subject in need thereof, nanoparticles expressing a target biomarker signature of lung cancer (*e.g.*, in some examples non-small cell lung cancer such as, *e.g.*, LUAD and/or LUSC), the target biomarker signature comprising: at least one nanoparticle-associated surface biomarker and at least one target biomarker selected from the group consisting of: surface biomarkers (as described herein), intravesicular biomarkers (as described herein), and intravesicular RNA biomarkers (as described herein); (b) comparing sample information indicative of level of the target biomarker signature-expressing nanoparticles in the biological sample to reference information including a reference threshold level; and (c) classifying the subject as having or being susceptible to lung cancer (*e.g.*, in some examples non-small cell lung cancer such as, *e.g.*, LUAD and/or LUSC) when the biological sample shows an elevated level of target biomarker signature-expressing nanoparticles relative to a classification cutoff referencing the reference threshold level. In some examples, at least one such target biomarker is or comprises a surface biomarker selected from: ABCA3, ACBD3, ADGRF1, AGER, ALCAM, AP1M2, APH1A, APOO, ATP1B1, ATP6AP2, BCAP31, B3GNT5, BSPRY, CDC42, CDH1, CDH3, CDKAL1, CELSR1, CIP2A, CISD2, CKAP4, CLPTM1L, CLSTN1, CPD, DPY19L1, DSG2, EGFR, EPCAM, FAM241B, FXYD3, FZD6, GALNT1, GALNT3, GALNT5, GALNT6, GALNT14, GGCX, GOLPH3L, GRHL2, HACD3,

IER3IP1, IL1RAP, ITGA2, ITGB6, HAS3, KPNA2, KRTCAP3, LAD1, LAMB3, LAMP3, LAMTOR2, LAPTM4B, LARGE2, LCLAT1, LSR, MAL2, MAGT1, MARCKSL1, MET, MGAT1, MUC4, NCSTN, NECTIN4, NRAS, NRCAM, NUP210, PEX13, PIGN, PIGT, PLEC, PTPRF, PRSS21, SLC7A11, QSOX1, RAB25, RCC2, RIT1, SCAMP3, SDC1, SEL1L3, SHROOM2, SLC35B2, SLC39A11, SMIM22, SOAT1, SPAST, SSR1, SSR4, SURF4, SYNGR2, TACSTD2, TMCO1, TMED2, TMED3, TMEM132A, TMEM33, TMPRSS4, TOMM22, TOR1AIP2, TRAM1, TTC33, VAMP8, VMA21, VRK2, VWA1, XPR1, ADAM28, AXL, BSG, CD274, CD47, CLU, DKK1, FLT4, GM3, HGF, IGF1R, IL6, LAG3, UCHL1, Lewis X, Lewis Y/B antigen, LY6E, NOTCH2, NOTCH3, Phosphatidylserine, TIGIT, TNFRSF10A, TNFRSF10B, TPBG, VEGFA, Tn antigen, Lewis Y/CD174, Sialyl Lewis X (sLex) (also known as Sialyl SSEA-1 (SLX)), NeuGcGM3, and combinations thereof. In some examples, at least one such target biomarker is or comprises a surface biomarker selected from: ALCAM, BCAP31, CD109, CD274, CD55, CD9, CDH3, CEACAM5, CEACAM6, DSC2, DSC3, EGFR, EPCAM, FOLR1, HACD3, IL1RAP, IGF1R, IGSF3, ITGA2, LAMB3, Lewis Y antigen, Lewis X antigen, MARCKSL1, MET, MSLN, MUC1, MUC4, NT5E, Phosphatidylserine, PTGFRN, PTK7, SDC1, Sialyl Lewis X antigen, SLC34A2, Sialyl Tn antigen (sTn antigen), Tn antigen, T antigen, TACSTD2, TNFRSF10B, TFRC, TRPV4, TSPAN8, CD274, CDH1, CNTN1, GOLM1, VWA1, CELSR2, CLDN1, DSG2, DSG3, GPC1, LAMC2, NECTIN1, ST14, CLCA2, PTPRZ1, SLC2A1, LYPD3, Gb3 (CD77), GJB2, MPZL2, RAP2B, ITGB4, DSP, MEST, ITGA6, ABCC5, ATP1B3, JAG1, GPNMB, PERP, TMPRSS11D, Sialyl-6T antigen (6-sialyl core 1), Sialyl-T antigen, Sialyl Lewis A antigen (CA19-9), Globo H, Gb5 (SSEA-3), Lactotriaosylceramide (Lc3), Forssman antigen, and combinations thereof. In some examples, at least one such target biomarker is or comprises an intravesicular biomarker selected from ABRACL, ACP5, AIF1, ALDH1A1, ALG1L, AP1M2, APOBEC3C, ASF1B, AURKB, BAIAP2L1, BIRC5, C12orf45, C15orf48, C19orf33, C1S, C8orf4, CBLC, CCL19, CCNB2, CDC20, CDCA5, CDK1, CDKN2A, CDKN2B, CEP55, CHMP4C, CNN2, CPA3, CRABP2, CST1, CTSC, DPYSL3, EGLN3, EHF, ELF3, ELF4, ENAH, ESRP1, ETV4, EVPL, FAM129B, FAM60A, FAM83A, FAM83H, GNA15, GRHL2, HCK, HMGB3, HOXB7, ID1, IMPA2, KIAA1522, KIF2C, KIFC1, KLF4, KLF5, KRT18, KRT19, KRT8, LGALS3BP, LSP1, MDFI, MIF, MYBL2, MYH14, MZB1, NCF2, NNMT, NUP210, NUSAP1, PALLD, PKP3, PLEK, PLEK2, POSTN, PTPN6, RIN2, RIPK4, RPS4Y1,

RRM2, S100A11, S100A14, S100A16, SBK1, SH3BP4, SPI1, SPINT1, SULF1, SYK, SYTL1, TBC1D2, TEAD2, TEAD3, TFAP2C, TGFA, THBS2, TK1, TOP2A, TPD52, TPX2, TRIP13, UBE2C, YAP1, ZC3H11A, ZNF217, and combinations thereof. In some examples, at least one such target biomarker is or comprises an intravesicular RNA (*e.g.*, but not limited to mRNA and noncoding RNA such as, *e.g.*, orphan noncoding RNA, long noncoding RNA, piwi-interacting RNA, microRNA, circular RNA, etc.) selected from ABCA3, ABRACL, ACP5, AIF1, ALDH1A1, ALG1L, ANTXR1, AP1M2, APOBEC3C, AQP3, AREG, ARSL, ASF1B, ATP8B1, AURKB, BAIAP2L1, BCAM, BIK, BIRC5, C12orf45, C15orf48, C19orf33, C1S, C8orf4, CBLC, CCL19, CCL5, CCNB2, CD24, CD53, CD74, CDC20, CDC42EP1, CDCA5, CDCP1, CDH1, CDH3, CDK1, CDKN2A, CDKN2B, CELSR1, CEP55, CHMP4C, CLDN4, CLDN7, CNN2, CPA3, CRABP2, CST1, CTSC, CX3CL1, CXADR, CXCR4, CYBB, DMBT1, DPYSL3, DSG2, EFNA1, EGFR, EGLN3, EHD2, EHF, ELF3, ELF4, EMP1, EMP2, ENAH, EPCAM, EPHA2, EPHX1, EPHX3, ESRP1, ETV4, EVA1A, EVPL, F11R, F2R, F2RL1, F3, FAM129B, FAM60A, FAM83A, FAM83H, FAT1, FBLIM1, FCER1G, GALNT3, GNA15, GPC4, GRHL2, HCK, HMGB3, HOXB7, HS6ST2, ID1, IL2RG, IMPA2, ITGA2, ITGB4, ITGB6, JAG2, KCNS3, KDELR3, KIAA1522, KIF2C, KIFC1, KITLG, KLF4, KLF5, KRT18, KRT19, KRT8, KRTCAP3, LAMB3, LAMP3, LAPTM5, LFNG, LGALS3BP, LRP11, LSP1, LSR, MAL2, MANEAL, MAOA, MARCO, MDFI, MET, MIF, MMP14, MPZL2, MYBL2, MYH14, MYOF, MZB1, NCF2, NKG7, NNMT, NOTCH3, NUP210, NUSAP1, OSMR, PALLD, PDPN, PDZK1IP1, PECAM1, PIGT, PKP3, PLEK, PLEK2, PLVAP, PMP22, PODXL2, POSTN, PPL, PROM2, PRSS8, PTGES, PTPN6, PTPRF, RAB25, RARRES1, RGS1, RHOV, RIN2, RIPK4, RPS4Y1, RRM2, S100A10, S100A11, S100A14, S100A16, SBK1, SCNN1A, SDC1, SERINC2, SEZ6L2, SH3BP4, SHISA2, SLC1A5, SLC40A1, SLC7A7, SPI1, SPINT1, SPINT2, ST14, STEAP1, SULF1, SYK, SYTL1, TACSTD2, TBC1D2, TEAD2, TEAD3, TFAP2C, TGFA, THBS2, TK1, TM4SF1, TMC4, TMEM30B, TMEM54, TMPRSS11E, TMPRSS4, TNFRSF18, TOP2A, TP53I11, TPD52, TPX2, TREM2, TRIP13, TSPAN1, TSPAN13, TSPAN6, TSPAN7, TUSC3, TYROBP, UBE2C, VAMP8, WLS, YAP1, ZC3H11A, ZNF217, and combinations thereof.

[11] In some examples, provided are technologies for use in classifying a subject (*e.g.*, an asymptomatic subject) as having or being susceptible to a certain type of lung cancer (*e.g.*, in particular examples lung adenocarcinoma (LUAD)). In some examples, the present

disclosure provides methods or assays for classifying a subject (*e.g.*, an asymptomatic subject) as having or being susceptible to a certain type of lung cancer (*e.g.*, in particular examples LUAD). In some examples, a provided method or assay comprises (a) detecting, in a biological sample from a subject in need thereof, nanoparticles expressing a target biomarker signature of a certain type of lung cancer (*e.g.*, in particular examples LUAD), the target biomarker signature comprising: at least one nanoparticle-associated surface biomarker and at least one target biomarker selected from the group consisting of: surface biomarkers (as described herein), intravesicular biomarkers (as described herein), and intravesicular RNA biomarkers (as described herein); (b) comparing sample information indicative of level of the target biomarker signature-expressing nanoparticles in the biological sample to reference information including a reference threshold level; and (c) classifying the subject as having or being susceptible to a certain type of lung cancer (*e.g.*, in particular examples LUAD) when the biological sample shows an elevated level of target biomarker signature-expressing nanoparticles relative to a classification cutoff referencing the reference threshold level. In some examples, at least one such target biomarker that is particularly useful for classifying a subject as having or being susceptible to LUAD is or comprises a surface biomarker selected from: ABCC3, ACSL5, ATP11A, CD55, CEACAM5, CEACAM6, CLIC6, FOLR1, GOLM1, LPCAT1, MSLN, MUC1, NT5E, PLA2G4A, PLCH1, SLC34A2, SMPDL3B, TESC, TMC5, ERBB3, KDR, and combinations thereof. In some examples, at least one such target biomarker that is particularly useful for classifying a subject as having or being susceptible to LUAD is or comprises an intravesicular biomarker selected from AGR2, AOC1, CTSE, FBP1, FOXA2, KRT7, NAPSA, PPP1R14D, S100P, SCGB3A2, SFTA2, SFTPA1, SFTPA2, SFTPB, SPINK1, and combinations thereof. In some examples, at least one such target biomarker that is particularly useful for classifying a subject as having or being susceptible to LUAD is or comprises an intravesicular RNA (*e.g.*, but not limited to mRNA and noncoding RNA such as, *e.g.*, orphan noncoding RNA, long noncoding RNA, piwi-interacting RNA, microRNA, circular RNA, etc.) selected from ABCC3, AGR2, AOC1, B3GNT3, CEACAM5, CEACAM6, CLDN18, CLDN3, CLIC6, CTSE, FBP1, FOLR1, FOXA2, GJB1, GPRC5A, KRT7, MSLN, MUC1, MUC21, NAPSA, PIGR, PPP1R14D, ROS1, S100P, SCGB3A2, SFTA2, SFTPA1, SFTPA2, SFTPB, SLC34A2, SLC44A4, SLC6A14, SMIM22, SMPDL3B, SPINK1, TMC5, TMEM45B,

TMPRSS2, TSPAN8, and combinations thereof. In some examples, certain aforementioned target biomarkers are particularly useful in differentiating LUAD from LUSC.

[12] In some examples, provided are technologies for use in classifying a subject (*e.g.*, an asymptomatic subject) as having or being susceptible to a certain type of lung cancer (*e.g.*, in particular examples lung squamous cell carcinoma (LUSC)). In some examples, the present disclosure provides methods or assays for classifying a subject (*e.g.*, an asymptomatic subject) as having or being susceptible to a certain type of lung cancer (*e.g.*, in particular examples LUSC). In some examples, a provided method or assay comprises (a) detecting, in a biological sample from a subject in need thereof, nanoparticles expressing a target biomarker signature of a certain type of lung cancer (*e.g.*, in particular examples LUSC), the target biomarker signature comprising: at least one nanoparticle-associated surface biomarker and at least one target biomarker selected from the group consisting of: surface biomarkers (as described herein), intravesicular biomarkers (as described herein), and intravesicular RNA biomarkers (as described herein); (b) comparing sample information indicative of level of the target biomarker signature-expressing nanoparticles in the biological sample to reference information including a reference threshold level; and (c) classifying the subject as having or being susceptible to a certain type of lung cancer (*e.g.*, in particular examples LUSC) when the biological sample shows an elevated level of target biomarker signature-expressing nanoparticles relative to a classification cutoff referencing the reference threshold level. In some examples, at least one such target biomarker that is particularly useful for classifying a subject as having or being susceptible to LUSC is or comprises a surface biomarker selected from: ABCC1, ATP11B, B4GALT4, CD109, CD9, CLCA2, CLDN1, CNTN1, CYP2S1, CYP4F11, CYP4F3, DSC2, DSC3, DSG3, EPHB3, FAT2, FBXO45, FERMT1, IGSF3, KLRG2, LAMC2, NECTIN1, PARL, PSMD2, PTDSS1, PTGFRN, RAB38, RAB6B, RAP2B, SLC2A1, TFRC, TMTC3, TRPV4, UGT1A6, UPK1B, XXYLT1, TNFSF18, and combinations thereof. In some examples, at least one such target biomarker that is particularly useful for classifying a subject as having or being susceptible to LUSC is or comprises an intravesicular biomarker selected from ADH7, AKR1C1, AKR1C2, AKR1C3, ALDH3A1, ALDH3B2, APOBEC3B, ARNTL2, CA9, CALML3, CAPNS2, CDC45, CDCA4, CENPW, CES1, CSTA, CYP2S1, EFS, FAM83D, FERMT1, FOXE1, FOXM1, GBP6, GPX2, GSTA1, IGF2BP2, IRF6, IVL, JUP, KRT13, KRT14, KRT15, KRT16, KRT17, KRT5, KRT6A, KRT6B, KRT6C, LGALS7B,

MAGEA4, MAGEA6, MCM2, NRARP, OSGIN1, PITX1, PKP1, PPP1R14C, PRAME, RBP1, S100A2, SERPINB13, SERPINB3, SERPINB5, SNAI2, SOX2, SPRR1A, SPRR1B, SPRR2A, SPRR2D, SPRR2E, SPRR3, TP63, TRIM29, ZNF750, and combinations thereof. In some examples, at least one such target biomarker that is particularly useful for classifying a subject as having or being susceptible to LUSC is or comprises an intravesicular RNA (*e.g.*, but not limited to mRNA and noncoding RNA such as, *e.g.*, orphan noncoding RNA, long noncoding RNA, piwi-interacting RNA, microRNA, circular RNA, etc.) selected from ABCC1, ADAM23, ADH7, AKR1C1, AKR1C2, AKR1C3, ALDH3A1, ALDH3B2, APOBEC3B, ARNTL2, B3GNT5, CA12, CA9, CALML3, CAPNS2, CD109, CD9, CDC45, CDCA4, CENPW, CES1, CLCA2, CLDN1, COL17A1, CSTA, CYP2S1, CYP4F11, DAPL1, DSC2, DSC3, DSG3, DSP, EFS, EPHB3, FAM83D, FAT2, FERMT1, FGFR2, FGFR3, FOXE1, FOXM1, FXYD3, GBP6, GJA1, GJB2, GJB3, GJB5, GJB6, GPC1, GPC3, GPNMB, GPR87, GPX2, GSTA1, HAS3, IGF2BP2, IGSF9, IRF6, ITGA6, IVL, JUP, KRT13, KRT14, KRT15, KRT16, KRT17, KRT5, KRT6A, KRT6B, KRT6C, LGALS7B, LRRC4, LYPD3, MAGEA4, MAGEA6, MCM2, NRARP, NTRK2, OSGIN1, PERP, PITX1, PKP1, PPP1R14C, PRAME, PRRG4, PTGFRN, PTPRZ1, RAB38, RAET1L, RBP1, RHCG, S100A2, SERPINB13, SERPINB3, SERPINB5, SLC2A1, SLC6A8, SLC7A8, SNAI2, SOX2, SPRR1A, SPRR1B, SPRR2A, SPRR2D, SPRR2E, SPRR3, THBD, TMPRSS11D, TNS4, TP63, TRIM29, UPK1B, VANG2, ZNF750, and combinations thereof. In some examples, certain aforementioned target biomarkers are particularly useful in differentiating LUSC from LUAD.

[13] In some examples, methods or assays described herein may be performed for at least one more additional target biomarker signature (including, *e.g.*, at least one, at least two, at least three, or more additional target biomarker signatures). In some such examples, a classification cutoff may reference additional reference threshold level(s) corresponding to each additional target biomarker signature.

[14] In some examples, a nanoparticle-associated surface biomarker for use in a target biomarker signature of lung cancer used and/or described herein may be or comprise a tumor-specific biomarker and/or a tissue-specific biomarker (*e.g.*, a lung tissue-specific biomarker). In some examples, such a nanoparticle-associated surface biomarker may be or comprise a non-specific marker, *e.g.*, it is present in one or more non-target tumors, and/or in one or more non-target tissues. In some examples, such a nanoparticle-associated surface

biomarker may be or comprise one or more of biomarkers selected from: ALCAM, B3GNT3, CDCP1, CDH1, CDH3, CD55, CD274 (PD-L1), CEACAM5, CEACAM6, CLDN3, CLDN4, DSG2, EGFR, EPCAM, FOLR1, GJB1, GJB2, IG1FR, LAMB3, MET, MSLN, MUC1, PIGT, PODXL2, ROS1, SDC1, SLC34A2, sTn antigen, Tn antigen, T antigen, SMPDL3B, ST14, TACSTD2, TMPRSS4, TNFRSF10B, TSPAN8, and combinations thereof. In some examples, such a nanoparticle-associated surface biomarker may be or comprise SLC34A2, CEACAM5, CEACAM6, and/or EPCAM. In some examples, such a nanoparticle-associated surface biomarker may be or comprise ALCAM, CD55, CDH1, CDH3, CD274 (PD-L1), CEACAM5, CEACAM6, DSG2, EGFR, EPCAM, FOLR1, IG1FR, MET, MSLN, MUC1, SLC34A2, sTn antigen, Tn antigen, T antigen, TACSTD2, TNFRSF10B, or combinations thereof. In some examples, such a nanoparticle-associated surface biomarker may be or comprise a SLC34A2 polypeptide. In some examples, such a nanoparticle-associated surface biomarker may be or comprise a CEACAM5 polypeptide. In some examples, such a nanoparticle-associated surface biomarker may be or comprise a CEACAM6 polypeptide. In some examples, such a nanoparticle-associated surface biomarker may be or comprise an EPCAM polypeptide. In some examples such a nanoparticle-associated surface biomarker may be or comprise a sTn antigen and/or a MUC1 polypeptide.

[15] In some examples, a target biomarker signature of lung cancer comprises a nanoparticle-associated surface biomarker (*e.g.*, ones described herein) and at least one (including, *e.g.*, 1, 2, 3, or more) additional target surface biomarker, which, in some examples, may be or comprise ALCAM, ABCC3, ARSL, B3GNT3, CDCP1, CDH1, CDH3, CD55, CD274 (PD-L1), CEACAM5, CEACAM6, CELSR1, CLDN18, CLDN3, CLDN4, CLDN7, CLIC6, DMBT1, DSG2, EGFR, EPCAM, EPHX3, EVA1A, FOLR1, GJB1, GJB2, GPC4, HS6ST2, IG1FR, KDELR3, KRTCAP3, LAMB3, LFNG, LSR, MANEAL, MET, MSLN, MUC1, MUC21, PIGT, PODXL2, PRRG4, ROS1, SDC1, SERINC2, SEZ6L2, SLC34A2, SLC44A4, SLC6A14, SLC7A7, SMIM22, SMPDL3B, ST14, sTn antigen, Tn antigen, T antigen, TACSTD2, TMC4, TMC5, TMEM45B, TMPRSS2, TMPRSS4, TNFRSF10B, TSPAN1, TSPAN8, or combinations thereof.

[16] In some examples, a nanoparticle-associated surface biomarker for use in a target biomarker signature of lung cancer used and/or described herein may be or comprise a tumor-specific biomarker and/or a tissue-specific biomarker (*e.g.*, a lung tissue-specific

biomarker). In some examples, such a nanoparticle-associated surface biomarker may be or comprise a non-specific marker, *e.g.*, it is present in one or more non-target tumors, and/or in one or more non-target tissues. In some examples, such a nanoparticle-associated surface biomarker may be or comprise one or more of surface biomarkers described herein. In some examples, such a nanoparticle-associated surface biomarker may be or comprise one or more of biomarkers selected from: Sialyl Tn (sTn) antigen, Sialyl-6T antigen (6-sialyl core 1), T antigen, PTK7, TSPAN8, CELSR2, GPC1, ST14, PTPRZ1, GPR87, GJB5, GJB2, RHOV, LYPD3, CLDN7, DSP, SERINC2, ABHD17C, PERP, MPZL2, ITGB4, MEST, GPNMB, SLC35A2, Gb3 (CD77), ITGA6, ABCC5, ATP1B3, JAG1, TMPRSS11D, Sialyl Lewis A antigen (CA19-9), Globo H, Gb5 (SSEA-3), Lactotriaosylceramide, (Lc3), Forssman antigen, ABCA3, ABCC1, ABCC3, ACBD3, ACSL5, ADGRF1, AGER, ALCAM, AP1M2, APH1A, APOO, ATP11A, ATP11B, ATP1B1, ATP6AP2, B3GNT5, B4GALT4, BCAP31, BSPRY, CD109, CD55, CD9, CDC42, CDH1, CDH3, CDKAL1, CEACAM5, CEACAM6, CELSR1, CIP2A, CISD2, CKAP4, CLCA2, CLDN1, CLIC6, CLPTM1L, CLSTN1, CNTN1, CPD, CYP2S1, CYP4F11, CYP4F3, DPY19L1, DSC2, DSC3, DSG2, DSG3, EGFR, EPCAM, EPHB3, FAM241B, FAT2, FBXO45, FERMT1, FOLR1, FXYP3, FZD6, GALNT1, GALNT3, GALNT5, GALNT6, GALNT14, GGCX, GOLM1, GOLPH3L, GRHL2, HACD3, HAS3, IER3IP1, IGSF3, IL1RAP, ITGA2, ITGB6, KLRG2, KPNA2, KRTCAP3, LAD1, LAMB3, LAMC2, LAMP3, LAMTOR2, LAPTM4B, LARGE2, LCLAT1, LPCAT1, LSR, MAGT1, MAL2, MARCKSL1, MET, MGAT1, MSLN, MUC1, MUC4, NCSTN, NECTIN1, NECTIN4, NRAS, NRCAM, NT5E, NUP210, PARL, PEX13, PIGN, PIGT, PLA2G4A, PLCH1, PLEC, PRSS21, PSMD2, PTDSS1, PTGFRN, PTPRF, QSOX1, RAB25, RAB38, RAB6B, RAP2B, RCC2, RIT1, SCAMP3, SDC1, SEL1L3, SHROOM2, SLC7A11, SLC2A1, SLC34A2, SLC35B2, SLC39A11, SMIM22, SMPDL3B, SOAT1, SPAST, SSR1, SSR4, SURF4, SYNGR2, TACSTD2, TESC, TFRC, TMC5, TMC01, TMED2, TMED3, TMEM132A, TMEM33, TMPRSS4, TMTC3, TOMM22, TOR1AIP2, TRAM1, TRPV4, TTC33, UGT1A6, UPK1B, VAMP8, VMA21, VRK2, VWA1, XPR1, XXYL1, ADAM28, AXL, BSG, CD274, CD47, CLU, DKK1, ERBB3, FLT4, GM3, HGF, IGF1R, IL6, KDR, LAG3, UCHL1, Lewis X, Lewis Y/B antigen, LY6E, NOTCH2, NOTCH3, Phosphatidylserine, TIGIT, TNFRSF10A, TNFRSF10B, TNFSF18, TPBG, VEGFA, Tn antigen, Lewis Y/CD174, Sialyl Lewis X (sLex) (also known as Sialyl SSEA-1 (SLX)), NeuGcGM3, and combinations thereof.

[17] In some examples, such a nanoparticle-associated surface biomarker may be or comprise a surface biomarker selected from: ALCAM, BCAP31, CD109, CD274, CD55, CD9, CDH3, CEACAM5, CEACAM6, DSC2, DSC3, EGFR, EPCAM, FOLR1, HACD3, IL1RAP, IGF1R, IGSF3, ITGA2, LAMB3, Lewis Y antigen, Lewis X antigen, MARCKSL1, MET, MSLN, MUC1, MUC4, NT5E, Phosphatidylserine, PTGFRN, PTK7, SDC1, Sialyl Lewis X antigen, SLC34A2, Sialyl Tn antigen (sTn antigen), Tn antigen, T antigen, TACSTD2, TNFRSF10B, TFRC, TRPV4, TSPAN8, CD274, CDH1, CNTN1, GOLM1, VWA1, CELSR2, CLDN1, DSG2, DSG3, GPC1, LAMC2, NECTIN1, ST14, CLCA2, PTPRZ1, SLC2A1, LYPD3, Gb3 (CD77), GJB2, MPZL2, RAP2B, ITGB4, DSP, MEST, ITGA6, ABCC5, ATP1B3, JAG1, GPNMB, PERP, TMPRSS11D, Sialyl-6T antigen (6-sialyl core 1), Sialyl-T antigen, Sialyl Lewis A antigen (CA19-9), Globo H, Gb5 (SSEA-3), Lactotriaosylceramide (Lc3), Forssman antigen, and combinations thereof.

[18] In some examples, a target biomarker signature of lung cancer comprises a nanoparticle-associated surface biomarker (*e.g.*, ones described herein) and at least one (including, *e.g.*, 1, 2, 3, or more) additional target surface biomarker, which, in some examples, may be or comprise Sialyl Tn (sTn) antigen, Sialyl-6T antigen (6-sialyl core 1), T antigen, PTK7, TSPAN8, CELSR2, GPC1, ST14, PTPRZ1, GPR87, GJB5, GJB2, RHOV, LYPD3, CLDN7, DSP, SERINC2, ABHD17C, PERP, MPZL2, ITGB4, MEST, GPNMB, SLC35A2, Gb3 (CD77), ITGA6, ABCC5, ATP1B3, JAG1, TMPRSS11D, Sialyl Lewis A antigen (CA19-9), Globo H, Gb5 (SSEA-3), Lactotriaosylceramide, (Lc3), Forssman antigen, ABCA3, ABCC1, ABCC3, ACBD3, ACSL5, ADGRF1, AGER, ALCAM, AP1M2, APH1A, APOO, ATP11A, ATP11B, ATP1B1, ATP6AP2, B3GNT5, B4GALT4, BCAP31, BSPRY, CD109, CD55, CD9, CDC42, CDH1, CDH3, CDKAL1, CEACAM5, CEACAM6, CELSR1, CIP2A, CISD2, CKAP4, CLCA2, CLDN1, CLIC6, CLPTM1L, CLSTN1, CNTN1, CPD, CYP2S1, CYP4F11, CYP4F3, DPY19L1, DSC2, DSC3, DSG2, DSG3, EGFR, EPCAM, EPHB3, FAM241B, FAT2, FBXO45, FERMT1, FOLR1, FXYP3, FZD6, GALNT1, GALNT3, GALNT5, GALNT6, GALNT14, GGCX, GOLM1, GOLPH3L, GRHL2, HACD3, HAS3, IER3IP1, IGSF3, IL1RAP, ITGA2, ITGB6, KLRG2, KPNA2, KRTCAP3, LAD1, LAMB3, LAMC2, LAMP3, LAMTOR2, LAPTM4B, LARGE2, LCLAT1, LPCAT1, LSR, MAGT1, MAL2, MARCKSL1, MET, MGAT1, MSLN, MUC1, MUC4, NCSTN, NECTIN1, NECTIN4, NRAS, NRCAM, NT5E, NUP210, PARL, PEX13, PIGN, PIGT, PLA2G4A, PLCH1, PLEC,

PRSS21, PSMD2, PTDSS1, PTGFRN, PTPRF, QSOX1, RAB25, RAB38, RAB6B, RAP2B, RCC2, RIT1, SCAMP3, SDC1, SEL1L3, SHROOM2, SLC7A11, SLC2A1, SLC34A2, SLC35B2, SLC39A11, SMIM22, SMPDL3B, SOAT1, SPAST, SSR1, SSR4, SURF4, SYNGR2, TACSTD2, TESC, TFRC, TMC5, TMC01, TMED2, TMED3, TMEM132A, TMEM33, TMPRSS4, TMTC3, TOMM22, TOR1AIP2, TRAM1, TRPV4, TTC33, UGT1A6, UPK1B, VAMP8, VMA21, VRK2, VWA1, XPR1, XXYL1, ADAM28, AXL, BSG, CD274, CD47, CLU, DKK1, ERBB3, FLT4, GM3, HGF, IGF1R, IL6, KDR, LAG3, UCHL1, Lewis X, Lewis Y/B antigen, LY6E, NOTCH2, NOTCH3, Phosphatidylserine, TIGIT, TNFRSF10A, TNFRSF10B, TNFSF18, TPBG, VEGFA, Tn antigen, Lewis Y/CD174, Sialyl Lewis X (sLex) (also known as Sialyl SSEA-1 (SLX)), NeuGcGM3, and combinations thereof. In some examples, at least one (including, *e.g.*, 1, 2, 3, or more) additional target surface biomarker may be or comprise a surface biomarker selected from: ALCAM, BCAP31, CD109, CD274, CD55, CD9, CDH3, CEACAM5, CEACAM6, DSC2, DSC3, EGFR, EPCAM, FOLR1, HACD3, IL1RAP, IGF1R, IGSF3, ITGA2, LAMB3, Lewis Y antigen, Lewis X antigen, MARCKSL1, MET, MSLN, MUC1, MUC4, NT5E, Phosphatidylserine, PTGFRN, PTK7, SDC1, Sialyl Lewis X antigen, SLC34A2, Sialyl Tn antigen (sTn antigen), Tn antigen, T antigen, TACSTD2, TNFRSF10B, TFRC, TRPV4, TSPAN8, CD274, CDH1, CNTN1, GOLM1, VWA1, CELSR2, CLDN1, DSG2, DSG3, GPC1, LAMC2, NECTIN1, ST14, CLCA2, PTPRZ1, SLC2A1, LYPD3, Gb3 (CD77), GJB2, MPZL2, RAP2B, ITGB4, DSP, MEST, ITGA6, ABCC5, ATP1B3, JAG1, GPNMB, PERP, TMPRSS11D, Sialyl-6T antigen (6-sialyl core 1), Sialyl-T antigen, Sialyl Lewis A antigen (CA19-9), Globo H, Gb5 (SSEA-3), Lactotriaosylceramide (Lc3), Forssman antigen, and combinations thereof.

[19] In some examples, a target biomarker signature of lung cancer comprises a nanoparticle-associated surface biomarker (*e.g.*, ones described herein) and at least one target intravesicular RNA (*e.g.*, but not limited to mRNA and noncoding RNA such as, *e.g.*, orphan noncoding RNA, long noncoding RNA, piwi-interacting RNA, microRNA, circular RNA, etc.) biomarker, which, in some examples, may be or comprise at least one RNA transcript (*e.g.*, mRNA transcript) encoded by a human gene as follows: ABCC3, AOC1, ARSL, B3GNT3, C12orf45, CDCP1, CDH1, CDH3, CEACAM5, CEACAM6, CELSR1, CLDN18, CLDN3, CLDN4, CLDN7, CLIC6, CRABP2, CST1, DMBT1, DSG2, EPCAM, EPHX3, ETV4, EVA1A, FAM83A, FOLR1, FOXA2, GJB1, GJB2, GPC4, HMGB3, HS6ST2, KDELR3, KRTCAP3,

LAMB3, LFNG, LGALS3BP, LSR, MANEAL, MIF, MSLN, MUC1, MUC21, NAPSA, PIGT, PODXL2, PPP1R14D, PRRG4, ROS1, S100A14, SBK1, SCGB3A2, SDC1, SERINC2, SEZ6L2, SFTA2, SFTPA1, SFTPA2, SFTPB, SLC34A2, SLC44A4, SLC6A14, SLC7A7, SMIM22, SMPDL3B, SPINK1, ST14, TGFA, TMC4, TMC5, TMEM45B, TMPRSS2, TMPRSS4, TSPAN1, TSPAN8, ZC3H11A, or combinations thereof.

[20] In some examples, a target biomarker signature of lung cancer comprises a nanoparticle-associated surface biomarker (*e.g.*, ones described herein) and at least one target intravesicular RNA (*e.g.*, but not limited to mRNA and noncoding RNA such as, *e.g.*, orphan noncoding RNA, long noncoding RNA, piwi-interacting RNA, microRNA, circular RNA, etc.) biomarker, which, in some examples, may be or comprise at least one RNA transcript (*e.g.*, mRNA transcript) encoded by a human gene as follows: ABCA3, ABCC1, ABRACL, ACP5, ADAM23, ADH7, AGR2, AIF1, AKR1C1, AKR1C2, AKR1C3, ALDH1A1, ALDH3A1, ALDH3B2, ALG1L, ANTXR1, AP1M2, APOBEC3B, APOBEC3C, AQP3, AREG, ARNTL2, ASF1B, ATP8B1, AURKB, B3GNT5, BAIAP2L1, BCAM, BIK, BIRC5, C15orf48, C19orf33, C1S, C8orf4, CA12, CA9, CALML3, CAPNS2, CBLC, CCL19, CCL5, CCNB2, CD109, CD24, CD53, CD74, CD9, CDC20, CDC42EP1, CDC45, CDCA4, CDCA5, CDCP1, CDH1, CDH3, CDK1, CDKN2A, CDKN2B, CEACAM5, CEACAM6, CELSR1, CENPW, CEP55, CES1, CHMP4C, CLCA2, CLDN1, CLDN4, CLDN7, CNN2, COL17A1, CPA3, CRABP2, CSTA, CTSC, CTSE, CX3CL1, CXADR, CXCR4, CYBB, CYP2S1, CYP4F11, DAPL1, DPYSL3, DSC2, DSC3, DSG2, DSG3, DSP, EFNA1, EFS, EGFR, EGLN3, EHD2, EHF, ELF3, ELF4, EMP1, EMP2, ENAH, EPCAM, EPHA2, EPHB3, EPHX1, ESRP1, EVPL, F11R, F2R, F2RL1, F3, FAM129B, FAM60A, FAM83D, FAM83H, FAT1, FAT2, FBLIM1, FBP1, FCER1G, FERMT1, FGFR2, FGFR3, FOXE1, FOXM1, FXYD3, GALNT3, GBP6, GJA1, GJB2, GJB3, GJB5, GJB6, GNA15, GPC1, GPC3, GPNMB, GPR87, GPRC5A, GPX2, GRHL2, GSTA1, HAS3, HCK, HOXB7, ID1, IGF2BP2, IGSF9, IL2RG, IMPA2, IRF6, ITGA2, ITGA6, ITGB4, ITGB6, IVL, JAG2, JUP, KCNS3, KIAA1522, KIF2C, KIFC1, KITLG, KLF4, KLF5, KRT13, KRT14, KRT15, KRT16, KRT17, KRT18, KRT19, KRT5, KRT6A, KRT6B, KRT6C, KRT7, KRT8, KRTCAP3, LAMP3, LAPTM5, LGALS7B, LRP11, LRRC4, LSP1, LSR, LYPD3, MAGEA4, MAGEA6, MAL2, MAOA, MARCO, MCM2, MDFI, MET, MMP14, MPZL2, MUC1, MYBL2, MYH14, MYOF, MZB1, NCF2, NKG7, NNMT, NOTCH3, NRARP, NTRK2, NUP210, NUSAP1, OSGIN1, OSMR, PALLD, PDPN, PDZK1IP1, PECAM1, PERP, PIGR,

PIGT, PITX1, PKP1, PKP3, PLEK, PLEK2, PLVAP, PMP22, POSTN, PPL, PPP1R14C, PRAME, PROM2, PRRG4, PRSS8, PTGES, PTGFRN, PTPN6, PTPRF, PTPRZ1, RAB25, RAB38, RAET1L, RARRES1, RBP1, RGS1, RHCG, RHOV, RIN2, RIPK4, RPS4Y1, RRM2, S100A10, S100A11, S100A14, S100A16, S100A2, S100P, SCNN1A, SDC1, SERINC2, SERPINB13, SERPINB3, SERPINB5, SEZ6L2, SH3BP4, SHISA2, SLC1A5, SLC2A1, SLC34A2, SLC40A1, SLC6A8, SLC7A8, SNAI2, SOX2, SPI1, SPINT1, SPINT2, SPRR1A, SPRR1B, SPRR2A, SPRR2D, SPRR2E, SPRR3, ST14, STEAP1, SULF1, SYK, SYTL1, TACSTD2, TBC1D2, TEAD2, TEAD3, TFAP2C, THBD, THBS2, TK1, TM4SF1, TMC4, TMEM30B, TMEM54, TMPRSS11D, TMPRSS11E, TMPRSS4, TNFRSF18, TNS4, TOP2A, TP53I11, TP63, TPD52, TPX2, TREM2, TRIM29, TRIP13, TSPAN1, TSPAN13, TSPAN6, TSPAN7, TUSC3, TYROBP, UBE2C, UPK1B, VAMP8, VANGL2, WLS, YAP1, ZC3H11A, ZNF217, ZNF750, or combinations thereof.

[21] In some examples, a target biomarker signature of lung cancer comprises a nanoparticle-associated surface biomarker (*e.g.*, ones described herein) and at least one additional target intravesicular biomarker, which, in some examples, may be or comprise at least one polypeptide encoded by a human gene as follows: AOC1, C12orf45, CRABP2, CST1, ETV4, FAM83A, FOXA2, HMGB3, LGALS3BP, MIF, NAPSA, PPP1R14D, S100A14, SBK1, SCGB3A2, SFTA2, SFTPA1, SFTPA2, SFTPB, SPINK1, TGFA, ZC3H11A, or combinations thereof. In some examples, a target biomarker signature comprises at least one nanoparticle-associated surface biomarker, which is or comprises a SCL34A2 polypeptide and/or a CEACAM5 polypeptide; and at least one target biomarker SLC34A2, CEACAM5, CEACAM6 and/or EPCAM.

[22] In some examples, a target biomarker signature of lung cancer comprises a nanoparticle-associated surface biomarker (*e.g.*, ones described herein) and at least one additional target intravesicular biomarker, which, in some examples, may be or comprise at least one polypeptide encoded by a human gene as follows: ABRACL, ACP5, ADH7, AGR2, AIF1, AKR1C1, AKR1C2, AKR1C3, ALDH1A1, ALDH3A1, ALDH3B2, ALG1L, AP1M2, APOBEC3B, APOBEC3C, ARNTL2, ASF1B, AURKB, BAIAP2L1, BIRC5, C15orf48, C19orf33, C1S, C8orf4, CA9, CALML3, CAPNS2, CBLC, CCL19, CCNB2, CDC20, CDC45, CDCA4, CDCA5, CDK1, CDKN2A, CDKN2B, CENPW, CEP55, CES1, CHMP4C, CNN2, CPA3, CRABP2, CSTA, CTSC, CTSE, CYP2S1, DPYSL3, EFS, EGLN3, EHF, ELF3, ELF4,

ENAH, ESRP1, EVPL, FAM129B, FAM60A, FAM83D, FAM83H, FBP1, FERMT1, FOXE1, FOXM1, GBP6, GNA15, GPX2, GRHL2, GSTA1, HCK, HOXB7, ID1, IGF2BP2, IMPA2, IRF6, IVL, JUP, KIAA1522, KIF2C, KIFC1, KLF4, KLF5, KRT13, KRT14, KRT15, KRT16, KRT17, KRT18, KRT19, KRT5, KRT6A, KRT6B, KRT6C, KRT7, KRT8, LGALS7B, LSP1, MAGEA4, MAGEA6, MCM2, MDFI, MYBL2, MYH14, MZB1, NCF2, NNMT, NRARP, NUP210, NUSAP1, OSGIN1, PALLD, PITX1, PKP1, PKP3, PLEK, PLEK2, POSTN, PPP1R14C, PRAME, PTPN6, RBP1, RIN2, RIPK4, RPS4Y1, RRM2, S100A11, S100A14, S100A16, S100A2, S100P, SERPINB13, SERPINB3, SERPINB5, SH3BP4, SNAI2, SOX2, SPI1, SPINT1, SPRR1A, SPRR1B, SPRR2A, SPRR2D, SPRR2E, SPRR3, SULF1, SYK, SYTL1, TBC1D2, TEAD2, TEAD3, TFAP2C, THBS2, TK1, TOP2A, TP63, TPD52, TPX2, TRIM29, TRIP13, UBE2C, YAP1, ZC3H11A, ZNF217, ZNF750, or combinations thereof.

[23] In some examples, a reference threshold level for use in a provided method or assay described herein is determined by levels of target biomarker signature-expressing nanoparticles observed in comparable samples from a population of non-lung cancer subjects.

[24] In some examples, a nanoparticle-associated surface biomarker included in a target biomarker signature may be detected using affinity agents (*e.g.*, but not limited to antibody-based agents). In some examples, a nanoparticle-associated surface biomarker may be detected using a capture assay comprising an antibody-based agent. For example, in some examples, a capture assay for detecting the presence of a nanoparticle-associated surface biomarker in a nanoparticle may involve contacting a biological sample comprising nanoparticles with a capture agent directed to such a nanoparticle-associated surface biomarker. In some examples, such a capture agent may comprise a binding moiety directed to a nanoparticle-associated surface biomarker (*e.g.*, ones described herein), which may be optionally conjugated to a solid substrate. Without limitations, an exemplary capture agent for a nanoparticle-associated surface biomarker may be or comprising a solid substrate (*e.g.*, a magnetic bead) and a binding moiety (*e.g.*, an antibody agent) directed to a nanoparticle-associated surface biomarker.

[25] In some examples, a target biomarker included in a target biomarker signature may be detected using appropriate methods known in the art, which may vary with types of analytes to be detected (*e.g.*, surface analytes vs. intravesicular analytes; and/or polypeptides and/or glycoforms vs. carbohydrates vs. RNAs). For example, a person skilled in the art, reading

the present disclosure, will appreciate that a surface biomarker and/or an intravesicular biomarker may be detected using affinity agents (e.g., antibody-based agents) in some examples, while in some examples, an intravesicular RNA (e.g., but not limited to mRNA and noncoding RNA such as, e.g., orphan noncoding RNA, long noncoding RNA, piwi-interacting RNA, microRNA, circular RNA, etc.) biomarker may be detected using nucleic acid-based agents, e.g., using quantitative reverse transcription PCR.

[26] For example, in some examples where a target biomarker is or comprises a surface biomarker and/or an intravesicular marker, such a target biomarker may be detected involving a proximity ligation assay, e.g., following a capture assay (e.g., ones as described herein) to capture nanoparticles that display a nanoparticle-associated surface biomarker (e.g., ones as used and/or described herein). In some examples, such a proximity ligation assay may comprise contacting a biological sample comprising nanoparticles with a set of detection probes, each directed to a target biomarker, which set comprises at least two distinct detection probes, so that a combination comprising the nanoparticles and the set of detection probes is generated, wherein the two detection probes each comprise: (i) a binding moiety directed to a surface biomarker and/or an intravesicular biomarker; and (ii) an oligonucleotide domain coupled to the binding moiety, the oligonucleotide domain comprising a double-stranded portion and a single-stranded overhang portion extended from one end of the oligonucleotide domain. Such single-stranded overhang portions of the detection probes are characterized in that they can hybridize with each other when the detection probes are bound to the same nanoparticle. Such a combination comprising the nanoparticles and the set of detection probes is then maintained under conditions that permit binding of the set of detection probes to their respective targets on the nanoparticles such that their oligonucleotide domains are in close enough proximity to anneal to form a double-stranded complex. Such a double-stranded complex can be detected by contacting the double-stranded complex with a nucleic acid ligase to generate a ligated template; and detecting the ligated template. In some examples, a ligated template can be detected using quantitative PCR. The presence of such a ligated template is indicative of presence of nanoparticles that are positive for a target biomarker signature of lung cancer. While such a proximity ligation assay may perform better, e.g., with higher specificity and/or sensitivity, than other existing proximity ligation assays, a person skilled in the art reading the present disclosure

will appreciate that other forms of proximity ligation assays that are known in the art may be used instead.

[27] In some examples where a target biomarker is or comprises an intravesicular RNA (*e.g.*, but not limited to mRNA and noncoding RNA such as, *e.g.*, orphan noncoding RNA, long noncoding RNA, piwi-interacting RNA, microRNA, circular RNA, etc.) marker, such a target biomarker may be detected involving a nucleic acid detection assay. In some examples, an exemplary nucleic acid detection assay may be or comprise reverse-transcription PCR.

[28] In some examples where a target biomarker is or comprises an intravesicular biomarker (*e.g.*, an intravesicular biomarker and/or an intravesicular RNA (*e.g.*, but not limited to mRNA and noncoding RNA such as, *e.g.*, orphan noncoding RNA, long noncoding RNA, piwi-interacting RNA, microRNA, orphan noncoding RNA, long noncoding RNA, piwi-interacting RNA, etc.) biomarker), such a target biomarker may be detected involving, prior to a detection assay (*e.g.*, a proximity ligation assay as described herein), a sample treatment (*e.g.*, fixation and/or permeabilization) to expose such biomarker(s) within nanoparticles for subsequent detection.

[29] The present disclosure, among other things, recognizes that detection of a single lung cancer-associated serum protein or a plurality of lung cancer-associated biomarkers based on a bulk sample (*e.g.*, a bulk sample of extracellular vesicles), rather than at a resolution of a single extracellular vesicle, typically does not provide sufficient specificity and/or sensitivity in determination of whether a subject from whom the sample is obtained is likely to be suffering from or susceptible to lung cancer. The present disclosure, among other things, provides technologies, including systems, compositions, and/or methods, that solve such problems, including for example by specifically requiring that individual nanoparticles having a size range of interest that includes extracellular vesicles for detection be characterized by presence of a target biomarker signature comprising a combination of at least one or more extracellular vesicle-associated surface biomarkers and at least one or more target biomarkers. In particular examples, the present disclosure teaches technologies that require such individual nanoparticles be characterized by presence (*e.g.*, by expression) of such a target biomarker signature of lung cancer, while nanoparticles that do not comprise the target biomarker signature do not produce a detectable signal (*e.g.*, a level that is above a reference level, *e.g.*, by at least

10% or more, where in some examples, a reference level may be a level observed in a negative control sample, such as a sample in which individual nanoparticles comprising such a target biomarker signature are absent).

[30] As will be understood by a skilled artisan, in some examples, a sample comprising extracellular vesicles may also comprise nanoparticles having a size range of interest that includes extracellular vesicles. Thus, in some examples, provided technologies of the present disclosure in the context of extracellular vesicles are also applicable to detection of nanoparticles having a size range interest that includes extracellular vesicles. Accordingly, in some examples, the present disclosure, among other things, provides technologies for detection, in individual nanoparticles having a size range of interest (e.g., in some examples about 30 nm to about 1000 nm) that includes extracellular vesicles, of co-localization of at least two or more surface biomarkers (e.g., as described herein) that forms a target biomarker signature of lung cancer.

[31] In one aspect, the present disclosure provides for a method comprising steps of: (a) detecting, in a biological sample obtained from a subject, biological entities expressing a first target biomarker signature, the first target biomarker signature comprising: at least one surface biomarker and at least one target biomarker selected from the group consisting of: surface biomarkers, intravesicular biomarkers, and intravesicular RNA biomarkers; (b) comparing sample information indicative of level of the first target biomarker signature-expressing biological entities in the biological sample to reference information including a first reference threshold level; and (c) classifying the subject as having or being susceptible to lung cancer when the biological sample shows an elevated level of first target biomarker signature-expressing biological entities relative to a classification cutoff referencing the first reference threshold level. In another aspect, the present disclosure provides for a method comprising steps of: (a) detecting, in a biological sample obtained from a subject, biological entities having a size within the range of about 30 nm to about 1000 nm and expressing a first target biomarker signature, the first target biomarker signature comprising: at least one surface biomarker and at least one target biomarker, wherein the target biomarker is or comprises a surface biomarker, wherein the target biomarker signature comprises a biomarker combination as listed in Tables 4A or 4B;

(b) comparing sample information indicative of level of the first target biomarker signature-expressing biological entities in the biological sample to reference information including a first reference threshold level; and (c) classifying the subject as having or being susceptible to lung cancer when the biological sample shows an elevated level of first target biomarker signature-expressing biological entities relative to a classification cutoff referencing the first reference threshold level. In some examples, the present disclosure describes a method comprising steps of: (a) providing or obtaining a sample comprising nanoparticles having a size within the range of about 30 nm to about 1000 nm, which are isolated from a bodily fluid-derived sample (e.g., a blood-derived sample) of a subject; (b) detecting on surfaces of the nanoparticles co-localization of at least two surface biomarkers whose combined expression level has been determined to be associated with lung cancer, wherein the surface biomarkers are selected from (i) polypeptides encoded by human genes as follows: *PTK7*, *TSPAN8*, *CELSR2*, *GPC1*, *ST14*, *PTPRZ1*, *GPR87*, *GJB5*, *GJB2*, *RHOV*, *LYPD3*, *CLDN7*, *DSP*, *SERINC2*, *ABHD17C*, *PERP*, *MPZL2*, *ITGB4*, *MEST*, *GPNMB*, *SLC35A2*, *Gb3 (CD77)*, *ITGA6*, *ABCC5*, *ATP1B3*, *JAG1*, *TMPRSS11D*, *ABCA3*, *ABCC1*, *ABCC3*, *ACBD3*, *ACSL5*, *ADGRF1*, *AGER*, *ALCAM*, *AP1M2*, *APH1A*, *APOO*, *ATP11A*, *ATP11B*, *ATP1B1*, *ATP6AP2*, *B3GNT5*, *B4GALT4*, *BCAP31*, *BSPRY*, *CD109*, *CD55*, *CD9*, *CDC42*, *CDH1*, *CDH3*, *CDKAL1*, *CEACAM5*, *CEACAM6*, *CELSR1*, *CIP2A*, *CISD2*, *CKAP4*, *CLCA2*, *CLDN1*, *CLIC6*, *CLPTM1L*, *CLSTN1*, *CNTN1*, *CPD*, *CYP2S1*, *CYP4F11*, *CYP4F3*, *DPY19L1*, *DSC2*, *DSC3*, *DSG2*, *DSG3*, *EGFR*, *EPCAM*, *EPHB3*, *FAM241B*, *FAT2*, *FBXO45*, *FERMT1*, *FOLR1*, *FXYD3*, *FZD6*, *GALNT1*, *GALNT3*, *GALNT5*, *GALNT6*, *GALNT14*, *GGCX*, *GOLM1*, *GOLPH3L*, *GRHL2*, *HACD3*, *HAS3*, *IER3IP1*, *IGSF3*, *IL1RAP*, *ITGA2*, *ITGB6*, *KLRG2*, *KPNA2*, *KRTCAP3*, *LAD1*, *LAMB3*, *LAMC2*, *LAMP3*, *LAMTOR2*, *LAPTM4B*, *LARGE2*, *LCLAT1*, *LPCAT1*, *LSR*, *MAGT1*, *MAL2*, *MARCKSL1*, *MET*, *MGAT1*, *MSLN*, *MUC1*,

MUC4, NCSTN, NECTIN1, NECTIN4, NRAS, NRCAM, NT5E, NUP210, PARL, PEX13, PIGN, PIGT, PLA2G4A, PLCH1, PLEC, PRSS21, PSMD2, PTDSS1, PTGFRN, PTPRF, QSOX1, RAB25, RAB38, RAB6B, RAP2B, RCC2, RIT1, SCAMP3, SDC1, SEL1L3, SHROOM2, SLC7A11, SLC2A1, SLC34A2, SLC35B2, SLC39A11, SMIM22, SMPDL3B, SOAT1, SPAST, SSR1, SSR4, SURF4, SYNGR2, TACSTD2, TESC, TFRC, TMC5, TMCO1, TMED2, TMED3, TMEM132A, TMEM33, TMPRSS4, TMTC3, TOMM22, TOR1AIP2, TRAM1, TRPV4, TTC33, UGT1A6, UPK1B, VAMP8, VMA21, VRK2, VWA1, XPR1, XXYL1, ADAM28, AXL, BSG, CD274, CD47, CLU, DKK1, ERBB3, FLT4, GM3, HGF, IGF1R, IL6, KDR, LAG3, UCHL1, NOTCH2, NOTCH3, TIGIT, TNFRSF10A, TNFRSF10B, TNFSF18, TPBG, VEGFA, and combinations thereof; and/or (ii) carbohydrate-dependent markers as follows: Sialyl Tn (sTn) antigen, Sialyl-6T antigen (6-sialyl core 1), T antigen, Phosphatidylserine, Tn antigen, Sialyl Lewis A antigen (CA19-9), Globo H, Gb5 (SSEA-3), Lactotriaosylceramide, (Lc3), Forssman antigen, Lewis X, Lewis Y/B antigen, LY6E, Lewis Y/CD174, Sialyl Lewis X (sLex) (also known as Sialyl SSEA-1 (SLX)), NeuGcGM3, and combinations thereof; (c) comparing the detected co-localization level with the determined level; and (d) classifying the subject as having or being susceptible to lung cancer when the detected co-localization level is at or above the determined level.

[32] In some examples, the first surface biomarker and the second surface biomarker(s) are each independently selected from: (i) polypeptides encoded by human genes as follows: *PTK7, TSPAN8, CELSR2, GPC1, ST14, PTPRZ1, GPR87, GJB5, GJB2, RHOV, LYPD3, CLDN7, DSP, SERINC2, ABHD17C, PERP, MPZL2, ITGB4, MEST, GPNMB, SLC35A2, Gb3 (CD77), ITGA6, ABCC5, ATP1B3, JAG1, TMPRSS11D, ABCA3, ABCC1, ABCC3, ACBD3, ACSL5, ADGRF1, AGER, ALCAM, AP1M2, APH1A, APOO, ATP11A, ATP11B, ATP1B1, ATP6AP2, B3GNT5, B4GALT4, BCAP31, BSPRY, CD109, CD55, CD9, CDC42, CDH1, CDH3, CDKAL1, CEACAM5, CEACAM6, CELSR1, CIP2A, CISD2, CKAP4, CLCA2, CLDN1, CLIC6, CLPTM1L, CLSTN1, CNTN1, CPD, CYP2S1, CYP4F11, CYP4F3, DPY19L1, DSC2, DSC3, DSG2, DSG3, EGFR, EPCAM, EPHB3, FAM241B, FAT2, FBXO45, FERMT1, FOLR1, FXYD3, FZD6, GALNT1, GALNT3, GALNT5, GALNT6, GALNT14, GGCX, GOLM1, GOLPH3L, GRHL2, HACD3, HAS3, IER3IP1, IGSF3, IL1RAP, ITGA2, ITGB6, KLRG2, KPNA2, KRTCAP3, LAD1, LAMB3, LAMC2, LAMP3, LAMTOR2, LAPTM4B, LARGE2, LCLAT1, LPCAT1, LSR, MAGT1, MAL2, MARCKSL1, MET, MGAT1, MSLN, MUC1,*

MUC4, NCSTN, NECTIN1, NECTIN4, NRAS, NRCAM, NT5E, NUP210, PARL, PEX13, PIGN, PIGT, PLA2G4A, PLCH1, PLEC, PRSS21, PSMD2, PTDSS1, PTGFRN, PTPRF, QSOX1, RAB25, RAB38, RAB6B, RAP2B, RCC2, RIT1, SCAMP3, SDC1, SEL1L3, SHROOM2, SLC7A11, SLC2A1, SLC34A2, SLC35B2, SLC39A11, SMIM22, SMPDL3B, SOAT1, SPAST, SSR1, SSR4, SURF4, SYNGR2, TACSTD2, TESC, TFRC, TMC5, TMCO1, TMED2, TMED3, TMEM132A, TMEM33, TMPRSS4, TMTC3, TOMM22, TOR1AIP2, TRAM1, TRPV4, TTC33, UGT1A6, UPK1B, VAMP8, VMA21, VRK2, VWA1, XPR1, XXYL1, ADAM28, AXL, BSG, CD274, CD47, CLU, DKK1, ERBB3, FLT4, GM3, HGF, IGF1R, IL6, KDR, LAG3, UCHL1, NOTCH2, NOTCH3, TIGIT, TNFRSF10A, TNFRSF10B, TNFSF18, TPBG, VEGFA, and combinations thereof; and/or (ii) carbohydrate-dependent markers as follows: Sialyl Tn (sTn) antigen, Sialyl-6T antigen (6-sialyl core 1), T antigen, Phosphatidylserine, Tn antigen, Sialyl Lewis A antigen (CA19-9), Globo H, Gb5 (SSEA-3), Lactotriaosylceramide, (Lc3), Forssman antigen, Lewis X, Lewis Y/B antigen, LY6E, Lewis Y/CD174, Sialyl Lewis X (sLex) (also known as Sialyl SSEA-1 (SLX)), NeuGcGM3, and combinations thereof.

[33] In some examples, the first surface biomarker and the second surface biomarker(s) are each independently selected from: ALCAM, BCAP31, CD109, CD274, CD55, CD9, CDH3, CEACAM5, CEACAM6, DSC2, DSC3, EGFR, EPCAM, FOLR1, HACD3, IL1RAP, IGF1R, IGSF3, ITGA2, LAMB3, Lewis Y antigen, Lewis X antigen, MARCKSL1, MET, MSLN, MUC1, MUC4, NT5E, Phosphatidylserine, PTGFRN, PTK7, SDC1, Sialyl Lewis X antigen, SLC34A2, Sialyl Tn antigen (sTn antigen), Tn antigen, T antigen, TACSTD2, TNFRSF10B, TFRC, TRPV4, TSPAN8, CD274, CDH1, CNTN1, GOLM1, VWA1, CELSR2, CLDN1, DSG2, DSG3, GPC1, LAMC2, NECTIN1, ST14, CLCA2, PTPRZ1, SLC2A1, LYPD3, Gb3 (CD77), GJB2, MPZL2, RAP2B, ITGB4, DSP, MEST, ITGA6, ABCC5, ATP1B3, JAG1, GPNMB, PERP, TMPRSS11D, Sialyl-6T antigen (6-sialyl core 1), Sialyl-T antigen, Sialyl Lewis A antigen (CA19-9), Globo H, Gb5 (SSEA-3), Lactotriaosylceramide (Lc3), Forssman antigen, and combinations thereof.

[34] Accordingly, in some examples, technologies provided herein can be useful for detection of incidence or recurrence of lung cancer in a subject and/or across a population of subjects. In some examples, a target biomarker signature may be selected for detection of lung cancer. In some examples, a target biomarker signature may be selected for detection

of a specific category of lung cancer, including, *e.g.*, but not limited to lung adenocarcinoma, small cell lung cancer, squamous and transitional cell lung cancer, large cell lung cancer, non-small cell carcinoma, other specified carcinomas, sarcomas, and other specified types of lung cancer as known in the art (see, *e.g.*, SEER Cancer Statistics Review 1975-2017). In some examples, technologies provided herein can be used periodically (*e.g.*, every year) to screen a human subject or across a population of human subjects for early-stage lung cancer or lung cancer recurrence.

[35] In some examples, a subject that is amenable to technologies provided herein for detection of incidence or recurrence of lung cancer may be an asymptomatic human subject and/or across an asymptomatic population. Such an asymptomatic subject may be a subject who has a family history of lung cancer, who has been previously treated for lung cancer, who is at risk of lung cancer recurrence after cancer treatment, who is in remission after lung cancer treatment, and/or who has been previously or periodically screened for the presence of lung cancer by chest X-ray, sputum analysis, low dose CT, and/or the presence of at least one lung cancer serum biomarker, *e.g.*, but not limited to CEA, CYFRA 21-1, NSE, ProGRP, and/or SCCA serum proteins. In some examples, such an asymptomatic subject may be a subject who is determined to have a normal medical diagnosis result from, *e.g.*, chest X-ray, sputum analysis, low dose CT analysis, or serum CEA, CYFRA 21-1, NSE, ProGRP, and/or SCCA levels. In some examples, such an asymptomatic subject may be a subject who is determined to have an abnormal medical diagnosis result from, *e.g.*, chest X-ray, sputum analysis, low dose CT analysis, and/or a serum level of CEA, CYFRA 21-1, NSE, ProGRP, and/or SCCA level when compared to results as typically observed in non-lung cancer subjects and/or normal healthy subjects. Alternatively, in some examples, an asymptomatic subject may be a subject who has not been previously screened for lung cancer, who has not been diagnosed for lung cancer, and/or who has not previously received lung cancer therapy.

[36] In some examples, a subject or population of subjects may be selected based on one or more characteristics such as age, race, geographic location, genetic history, medical history, personal and/or medical history (*e.g.*, smoking, alcohol, drugs, carcinogenic agents, diet, obesity, diabetes, physical activity, sun exposure, radiation exposure, exposure to infectious agents such as viruses, and/or occupational hazard).

[37] In some examples, technologies provided herein can be useful for selecting surgery or therapy for a subject who is suffering from or susceptible to lung cancer. In some examples, a lung cancer therapy and/or an adjunct therapy can be selected in light of findings based on technologies provided herein.

[38] In some examples, technologies provided herein can be useful for monitoring and/or evaluating efficacy of therapy administered to a subject (*e.g.*, a lung cancer subject).

[39] In some examples, the present disclosure provides technologies for managing patient care, *e.g.*, for one or more individual subjects and/or across a population of subjects. To give but a few examples, in some examples, the present disclosure provides technologies that may be utilized in screening (*e.g.*, temporally or incidentally motivated screening and/or non-temporally or incidentally motivated screening, *e.g.*, periodic screening such as annual, semi-annual, bi-annual, or with some other frequency). For example, in some examples, provided technologies for use in temporally motivated screening can be useful for screening one or more individual subjects or across a population of subjects (*e.g.*, asymptomatic subjects) who are older than a certain age (*e.g.*, over 30, 35, 40, 45, 50, 55, 60, 65, 70, or older). For example, in some examples, provided technologies for use in temporally motivated screening can be useful for screening one or more individual subjects or across a population of subjects (*e.g.*, asymptomatic subjects) who have a cigarette pack-year history greater than a certain number (*e.g.*, 5 pack years, 10 pack years, 15 pack years, 20 pack years, 25 pack years, 30 pack years, and/or greater than 35 pack years; 1 pack year is equal to 1 pack of cigarettes smoked per day for one year, while 2 packs smoked per day for one year would equal 2 pack years, or ½ pack smoked per day for two years would equal 1 pack years, etc.). In some examples, provided technologies for use in incidentally motivated screening can be useful for screening individual subjects who may have experienced an incident or event that motivates screening for lung cancer as described herein. For example, in some examples, an incidental motivation relating to determination of one or more indicators of cancer or susceptibility thereto may be or comprise, *e.g.*, an incident based on their family history (*e.g.*, a close relative such as blood-related relative was previously diagnosed for lung cancer), identification of one or more risk factors associated with lung cancer (*e.g.*, life history risk factors including but not limited to, *e.g.*, smoking, alcohol, diet, obesity, occupational hazard, *etc.*) and/or prior incidental findings from genetic tests (*e.g.*, genome sequencing), and/or imaging diagnostic tests (*e.g.*, X-ray, ultrasound, computerized tomography

(CT), low dose CT, and/or magnetic resonance imaging (MRI) scans), development of one or more signs or symptoms characteristic of lung cancer (*e.g.*, abnormal imaging results, and/or symptoms potentially indicative of lung cancer, *etc.*).

[40] In some examples, provided technologies for managing patient care can inform treatment and/or payment (*e.g.*, reimbursement for treatment) decisions and/or actions. For example, in some examples, provided technologies can provide determination of whether individual subjects have one or more indicators of incidence or recurrence of lung cancer, thereby informing physicians and/or patients when to initiate therapy in light of such findings. Additionally or alternatively, in some examples, provided technologies can inform physicians and/or patients of treatment selection, *e.g.*, based on findings of specific responsiveness biomarkers (*e.g.*, lung cancer responsiveness biomarkers). In some examples, provided technologies can provide determination of whether individual subjects are responsive to current treatment, *e.g.*, based on findings of changes in one or more levels of molecular targets associated with lung cancer, thereby informing physicians and/or patients of efficacy of such therapy and/or decisions to maintain or alter therapy in light of such findings.

[41] In some examples, provided technologies can inform decision making relating to whether health insurance providers reimburse (or not), *e.g.*, for (1) screening itself (*e.g.*, reimbursement available only for periodic/regular screening or available only for temporally and/or incidentally motivated screening); and/or for (2) initiating, maintaining, and/or altering therapy in light of findings by provided technologies. For example, in some examples, the present disclosure provides methods relating to (a) receiving results of a screening as described herein and also receiving a request for reimbursement of the screening and/or of a particular therapeutic regimen; (b) approving reimbursement of the screening if it was performed on a subject according to an appropriate schedule or response to a relevant incident and/or approving reimbursement of the therapeutic regimen if it represents appropriate treatment in light of the received screening results; and, optionally (c) implementing the reimbursement or providing notification that reimbursement is refused. In some examples, a therapeutic regimen is appropriate in light of received screening results if the received screening results detect a biomarker that represents an approved biomarker for the relevant therapeutic regimen (*e.g.*, as may be noted in a prescribing information label and/or via an approved companion diagnostic). Alternatively or additionally, the present disclosure contemplates reporting systems (*e.g.*,

implemented via appropriate electronic device(s) and/or communications system(s)) that permit or facilitate reporting and/or processing of screening results, and/or of reimbursement decisions as described herein.

[42] Some examples provided herein relate to systems and kits for use in provided technologies. In some examples, a system or kit may comprise detection agents for a tumor biomarker signature of lung cancer (*e.g.*, ones described herein). In some examples, such a system or kit may comprise a capture agent for an extracellular vesicle-associated surface biomarker present in nanoparticles associated with lung cancer (*e.g.*, ones used and/or described herein); and (b) at least one or more detection agents directed to one or more target biomarkers of a target biomarker signature of lung cancer, which may be or comprise additional surface biomarker(s) (*e.g.*, ones as used and/or described herein), intravesicular biomarker(s) (*e.g.*, ones as used and/or described herein), and/or intravesicular RNA (*e.g.*, but not limited to mRNA and noncoding RNA such as, *e.g.*, orphan noncoding RNA, long noncoding RNA, piwi-interacting RNA, microRNA, circular RNA, etc.) biomarker(s) (*e.g.*, ones as used and/or described herein).

[43] In some examples, a capture agent included in a system and/or kit may comprise a binding moiety directed to an extracellular vesicle-associated surface biomarker (*e.g.*, ones described herein). In some examples, such a binding moiety may be conjugated to a solid substrate, which in some examples may be or comprise a solid substrate. In some examples, such a solid substrate may be or comprise a magnetic bead. In some examples, an exemplary capture agent included in a provided system and/or kit may be or comprise a solid substrate (*e.g.*, a magnetic bead) and an affinity reagent (*e.g.*, but not limited to an antibody agent) directed to an extracellular vesicle-associated surface biomarker conjugated thereto.

[44] In some examples where a target biomarker includes a surface biomarker and/or an intravesicular biomarker, a system and/or kit may include detection agents for performing a proximity ligation assay (*e.g.*, ones as described herein). In some examples, such detection agents for performing a proximity ligation assay may comprise a set of detection probes, each directed to a target biomarker of a target biomarker signature, which set comprises at least two detection probes, wherein the two detection probes each comprise: (i) a polypeptide-binding moiety directed to a target biomarker; and (ii) an oligonucleotide domain coupled to the binding moiety, the oligonucleotide domain comprising a double-stranded portion and a single-stranded overhang portion extended from one end of the oligonucleotide domain, wherein the

single-stranded overhang portions of the detection probes are characterized in that they can hybridize to each other when the detection probes are bound to the same nanoparticle.

[45] In some examples, a provided system and/or kit may comprise a plurality (*e.g.*, 2, 3, 4, 5, or more) of sets of detection probes, each set of which comprises two or more (*e.g.*, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more) detection probes. In some examples, at least one set of detection probes may be directed to detection for lung cancer. For example, in some examples, a provided system and/or kit may comprise at least one set for detection probes for detection of lung cancer and at least one set of detection probes for detection of a different cancer (*e.g.*, pancreatic cancer). In some examples, two or more detection probes may be directed to different categories of lung cancer, including, *e.g.*, but not limited to lung adenocarcinoma lung cancer, small cell lung cancer, squamous and transitional cell lung cancer, Large cell lung cancer, non-small cell carcinoma lung cancer, other specified carcinoma lung cancer, sarcoma lung cancer, and other specified types of lung cancer as known in the art (see, *e.g.*, SEER Cancer Statistics Review 1975-2017). In some examples, two or more sets may be directed to detection of lung cancer of different categories. In some examples, two or more sets may be directed to detection of lung cancer of the same category. In some examples, two or more sets may be directed to detection of lung cancer of different stages. In some examples, two or more sets may be directed to detection of lung cancer of the same stage.

[46] In some examples, detection probes in a provided kit may be provided as a single mixture in a container. In some examples, multiple sets of detection probes may be provided as individual mixtures in separate containers. In some examples, each detection probe is provided individually in a separate container.

[47] In some examples where a target biomarker includes an intravesicular RNA (*e.g.*, but not limited to mRNA and noncoding RNA such as, *e.g.*, orphan noncoding RNA, long noncoding RNA, piwi-interacting RNA, microRNA, circular RNA, etc.) biomarker, such a system and/or kit may include detection agents for performing a nucleic acid detection assay. In some examples, such a system and/or kit may include detection agents for performing a quantitative reverse-transcription PCR, for example, which may comprise primers directed to intravesicular RNA (*e.g.*, but not limited to mRNA and noncoding RNA such as, *e.g.*, orphan

noncoding RNA, long noncoding RNA, piwi-interacting RNA, microRNA, circular RNA, etc.) target(s).

[48] A skilled artisan reading the present disclosure will understand that a system or kit for detection of extracellular vesicles can also be employed to detect nanoparticles having a size range of interest that includes extracellular vesicles. Accordingly, in some examples, a system or kit may comprise (i) a capture agent for a first surface biomarker of a lung cancer-associated biomarker signature (e.g., as described herein) present on the surface of nanoparticles having a size range of interest that includes extracellular vesicles; and (ii) at least one or more detection agents directed to a second surface biomarker of the lung cancer-specific biomarker signature. In some examples, such nanoparticles have a size within the range of about 30 nm to about 1000 nm.

[49] In some examples, the present disclosure describes a kit for detection of lung cancer comprising: (a) a capture agent comprising a target-capture moiety directed to a first surface biomarker; and (b) at least one set of detection probes, which set comprises at least two detection probes each directed to a second surface biomarker, wherein the detection probes each comprise: (i) a target binding moiety directed at the second surface biomarker; and (ii) an oligonucleotide domain coupled to the target binding moiety, the oligonucleotide domain comprising a double-stranded portion and a single-stranded overhang portion extended from one end of the oligonucleotide domain, wherein the single-stranded overhang portions of the at least two detection probes are characterized in that they can hybridize to each other when the at least two detection probes are bound to the same nanoparticle having a size within the range of about 30 nm to about 1000 nm; wherein at least the first surface biomarker and the second surface biomarker form a target biomarker signature determined to be associated with lung cancer, and wherein the first and second surface biomarkers are each independently selected from: (i) polypeptides encoded by human genes as follows: *PTK7*, *TSPAN8*, *CELSR2*, *GPC1*, *ST14*, *PTPRZ1*, *GPR87*, *GJB5*, *GJB2*, *RHOV*, *LYPD3*, *CLDN7*, *DSP*, *SERINC2*, *ABHD17C*, *PERP*, *MPZL2*, *ITGB4*, *MEST*, *GPNUMB*, *SLC35A2*, *Gb3 (CD77)*, *ITGA6*, *ABCC5*, *ATP1B3*, *JAG1*, *TMPRSS11D*, *ABCA3*, *ABCC1*, *ABCC3*, *ACBD3*, *ACSL5*, *ADGRF1*, *AGER*, *ALCAM*, *AP1M2*, *APH1A*, *APOO*, *ATP11A*, *ATP11B*, *ATP1B1*, *ATP6AP2*, *B3GNT5*, *B4GALT4*, *BCAP31*, *BSPRY*, *CD109*, *CD55*, *CD9*, *CDC42*, *CDH1*, *CDH3*, *CDKAL1*, *CEACAM5*, *CEACAM6*, *CELSR1*, *CIP2A*, *CISD2*, *CKAP4*, *CLCA2*, *CLDN1*, *CLIC6*, *CLPTM1L*, *CLSTN1*, *CNTN1*, *CPD*, *CYP2S1*,

CYP4F11, CYP4F3, DPY19L1, DSC2, DSC3, DSG2, DSG3, EGFR, EPCAM, EPHB3, FAM241B, FAT2, FBXO45, FERMT1, FOLR1, FXYD3, FZD6, GALNT1, GALNT3, GALNT5, GALNT6, GALNT14, GGCX, GOLM1, GOLPH3L, GRHL2, HACD3, HAS3, IER3IP1, IGSF3, IL1RAP, ITGA2, ITGB6, KLRG2, KPNA2, KRTCAP3, LAD1, LAMB3, LAMC2, LAMP3, LAMTOR2, LAPTM4B, LARGE2, LCLAT1, LPCAT1, LSR, MAGT1, MAL2, MARCKSL1, MET, MGAT1, MSLN, MUC1, MUC4, NCSTN, NECTIN1, NECTIN4, NRAS, NRCAM, NT5E, NUP210, PARL, PEX13, PIGN, PIGT, PLA2G4A, PLCH1, PLEC, PRSS21, PSMD2, PTDSS1, PTGFRN, PTPRF, QSOX1, RAB25, RAB38, RAB6B, RAP2B, RCC2, RIT1, SCAMP3, SDC1, SEL1L3, SHROOM2, SLC7A11, SLC2A1, SLC34A2, SLC35B2, SLC39A11, SMIM22, SMPDL3B, SOAT1, SPAST, SSR1, SSR4, SURF4, SYNGR2, TACSTD2, TESC, TFRC, TMC5, TMCO1, TMED2, TMED3, TMEM132A, TMEM33, TMPRSS4, TMTCC3, TOMM22, TOR1AIP2, TRAM1, TRPV4, TTC33, UGT1A6, UPK1B, VAMP8, VMA21, VRK2, VWA1, XPR1, XXYL1, ADAM28, AXL, BSG, CD274, CD47, CLU, DKK1, ERBB3, FLT4, GM3, HGF, IGF1R, IL6, KDR, LAG3, UCHL1, NOTCH2, NOTCH3, TIGIT, TNFRSF10A, TNFRSF10B, TNFSF18, TPBG, VEGFA, and combinations thereof; and/or (ii) carbohydrate-dependent markers as follows: Sialyl Tn (sTn) antigen, Sialyl-6T antigen (6-sialyl core 1), T antigen, Phosphatidylserine, Tn antigen, Sialyl Lewis A antigen (CA19-9), Globo H, Gb5 (SSEA-3), Lactotriaosylceramide, (Lc3), Forssman antigen, Lewis X, Lewis Y/B antigen, LY6E, Lewis Y/CD174, Sialyl Lewis X (sLex) (also known as Sialyl SSEA-1 (SLX)), NeuGcGM3, and combinations thereof.

[50] In some examples, the first surface biomarker and the second surface biomarker(s) are each independently selected from: (i) polypeptides encoded by human genes as follows: *PTK7, TSPAN8, CELSR2, GPC1, ST14, PTPRZ1, GPR87, GJB5, GJB2, RHOF, LYPD3, CLDN7, DSP, SERINC2, ABHD17C, PERP, MPZL2, ITGB4, MEST, GPNMB, SLC35A2, Gb3 (CD77), ITGA6, ABCC5, ATP1B3, JAG1, TMPRSS11D, ABCA3, ABCC1, ABCC3, ACBD3, ACSL5, ADGRF1, AGER, ALCAM, AP1M2, APH1A, APOO, ATP11A, ATP11B, ATP1B1, ATP6AP2, B3GNT5, B4GALT4, BCAP31, BSPRY, CD109, CD55, CD9, CDC42, CDH1, CDH3, CDKAL1, CEACAM5, CEACAM6, CELSR1, CIP2A, CISD2, CKAP4, CLCA2, CLDN1, CLIC6, CLPTM1L, CLSTN1, CNTN1, CPD, CYP2S1, CYP4F11, CYP4F3, DPY19L1, DSC2, DSC3, DSG2, DSG3, EGFR, EPCAM, EPHB3, FAM241B, FAT2, FBXO45, FERMT1, FOLR1, FXYD3, FZD6, GALNT1, GALNT3, GALNT5, GALNT6, GALNT14, GGCX,*

GOLM1, GOLPH3L, GRHL2, HACD3, HAS3, IER3IP1, IGSF3, IL1RAP, ITGA2, ITGB6, KLRG2, KPNA2, KRTCAP3, LAD1, LAMB3, LAMC2, LAMP3, LAMTOR2, LAPTM4B, LARGE2, LCLAT1, LPCAT1, LSR, MAGT1, MAL2, MARCKSL1, MET, MGAT1, MSLN, MUC1, MUC4, NCSTN, NECTIN1, NECTIN4, NRAS, NRCAM, NT5E, NUP210, PARL, PEX13, PIGN, PIGT, PLA2G4A, PLCH1, PLEC, PRSS21, PSMD2, PTDSS1, PTGFRN, PTPRF, QSOX1, RAB25, RAB38, RAB6B, RAP2B, RCC2, RIT1, SCAMP3, SDC1, SEL1L3, SHROOM2, SLC7A11, SLC2A1, SLC34A2, SLC35B2, SLC39A11, SMIM22, SMPDL3B, SOAT1, SPAST, SSR1, SSR4, SURF4, SYNGR2, TACSTD2, TESC, TFRC, TMC5, TMCO1, TMED2, TMED3, TMEM132A, TMEM33, TMPRSS4, TMTC3, TOMM22, TOR1AIP2, TRAM1, TRPV4, TTC33, UGT1A6, UPK1B, VAMP8, VMA21, VRK2, VWA1, XPR1, XXYL1, ADAM28, AXL, BSG, CD274, CD47, CLU, DKK1, ERBB3, FLT4, GM3, HGF, IGF1R, IL6, KDR, LAG3, UCHL1, NOTCH2, NOTCH3, TIGIT, TNFRSF10A, TNFRSF10B, TNFSF18, TPBG, VEGFA, and combinations thereof; and/or (ii) carbohydrate-dependent markers as follows: Sialyl Tn (sTn) antigen, Sialyl-6T antigen (6-sialyl core 1), T antigen, Phosphatidylserine, Tn antigen, Sialyl Lewis A antigen (CA19-9), Globo H, Gb5 (SSEA-3), Lactotriaosylceramide, (Lc3), Forssman antigen, Lewis X, Lewis Y/B antigen, LY6E, Lewis Y/CD174, Sialyl Lewis X (sLex) (also known as Sialyl SSEA-1 (SLX)), NeuGcGM3, and combinations thereof.

[51] In some examples, the first surface biomarker and the second surface biomarker(s) are each independently selected from: ALCAM, BCAP31, CD109, CD274, CD55, CD9, CDH3, CEACAM5, CEACAM6, DSC2, DSC3, EGFR, EPCAM, FOLR1, HACD3, IL1RAP, IGF1R, IGSF3, ITGA2, LAMB3, Lewis Y antigen, Lewis X antigen, MARCKSL1, MET, MSLN, MUC1, MUC4, NT5E, Phosphatidylserine, PTGFRN, PTK7, SDC1, Sialyl Lewis X antigen, SLC34A2, Sialyl Tn antigen (sTn antigen), Tn antigen, T antigen, TACSTD2, TNFRSF10B, TFRC, TRPV4, TSPAN8, CD274, CDH1, CNTN1, GOLM1, VWA1, CELSR2, CLDN1, DSG2, DSG3, GPC1, LAMC2, NECTIN1, ST14, CLCA2, PTPRZ1, SLC2A1, LYPD3, Gb3 (CD77), GJB2, MPZL2, RAP2B, ITGB4, DSP, MEST, ITGA6, ABCC5, ATP1B3, JAG1, GPNMB, PERP, TMPRSS11D, Sialyl-6T antigen (6-sialyl core 1), Sialyl-T antigen, Sialyl Lewis A antigen (CA19-9), Globo H, Gb5 (SSEA-3), Lactotriaosylceramide (Lc3), Forssman antigen, and combinations thereof.

[52] In some examples, a provided system and/or kit may comprise at least one chemical reagent, *e.g.*, to process a sample and/or nanoparticles (including, *e.g.*, in some examples extracellular vesicles) therein. In some examples, a provided system and/or kit may comprise at least one chemical reagent to process nanoparticles (including, *e.g.*, in some examples extracellular vesicles) in a sample, including, *e.g.*, but not limited to a fixation agent, a permeabilization agent, and/or a blocking agent. In some examples, a provided system and/or kit may comprise a nucleic acid ligase and/or a nucleic acid polymerase. In some examples, a provided system and/or kit may comprise one or more primers and/or probes. In some examples, a provided system and/or kit may comprise one or more pairs of primers, for example for PCR, *e.g.*, quantitative PCR (qPCR) reactions. In some examples, a provided system and/or kit may comprise one or more probes such as, for example, hydrolysis probes which may in some examples be designed to increase the specificity of qPCR (*e.g.*, TaqMan probes). In some examples, a provided system and/or kit may comprise one or more multiplexing probes, for example as may be useful when simultaneous or parallel qPCR reactions are employed (*e.g.*, to facilitate or improve readout).

[53] In some examples, a provided system and/or kit can be used for screening (*e.g.*, regular screening) and/or other assessment of individuals (*e.g.*, asymptomatic or symptomatic subjects) for detection (*e.g.*, early detection) of lung cancer. In some examples, a provided system and/or kit can be used for screening and/or other assessment of individuals susceptible to lung cancer (*e.g.*, individuals with a known genetic, environmental, or experiential risk, *etc.*). In some examples, provided system and/or kits can be used for monitoring recurrence of lung cancer in a subject who has been previously treated. In some examples, provided systems and/or kits can be used as a companion diagnostic in combination with a therapy for a subject who is suffering from lung cancer. In some examples, provided systems and/or kits can be used for monitoring or evaluating efficacy of a therapy administered to a subject who is suffering from lung cancer. In some examples, provided systems and/or kits can be used for selecting a therapy for a subject who is suffering from lung cancer. In some examples, provided systems and/or kits can be used for making a therapy decision and/or selecting a therapy for a subject with one or more symptoms (*e.g.*, non-specific symptoms) associated with lung cancer.

[54] Complexes formed by performing methods described herein and/or using systems and/or kits described herein are also within the scope of disclosure. For example, in some examples, a complex comprising: (a) a nanoparticle expressing a target biomarker signature, at least two of which include at least one nanoparticle-associated surface biomarker and at least one target biomarker selected from the group consisting of: surface biomarkers, intravesicular biomarkers, and intravesicular RNA biomarkers, wherein the surface biomarkers are selected from ADGRF1, ALCAM, ABCC3, ARSL, B3GNT3, B3GNT5, CDCP1, CDH1, CDH3, CD55, CD274 (PD-L1), CEACAM5, CEACAM6, CELSR1, CLDN18, CLDN3, CLDN4, CLDN7, CLIC6, DMBT1, DSG2, EGFR, EPCAM, EPHX3, EVA1A, FAM241B, FOLR1, FXYD3, GALNT14, GJB1, GJB2, GPC4, HAS3, HS6ST2, KDELR3, KRTCAP3, IG1FR, LAMB3, LAPTM4B, LARGE2, Lewis X antigen, LFNG, LSR, MAL2, MANEAL, MET, MSLN, MUC1, MUC21, NRCAM, PIGT, PODXL2, PRRG4, PRSS21, ROS1, SDC1, SERINC2, SEZ6L2, SLC34A2, sTn antigen, Tn antigen, T antigen, SLC44A4, SLC6A14, SLC7A7, SLC7A11, SMIM22, SMPDL3B, ST14, TACSTD2, TMC4, TMC5, TMEM45B, TMPRSS2, TMPRSS4, TNFRSF10B, TSPAN1, TSPAN8, UCHL1, and combinations thereof; intravesicular biomarkers are selected from: AOC1, C12orf45, CRABP2, CST1, ETV4, FAM83A, FOXA2, HMGB3, LGALS3BP, MIF, NAPSA, PPP1R14D, S100A14, SBK1, SCGB3A2, SFTA2, SFTPA1, SFTPA2, SFTPB, SPINK1, TGFA, ZC3H11A, and combinations thereof; and the intravesicular RNA (*e.g.*, but not limited to mRNA and noncoding RNA such as, *e.g.*, orphan noncoding RNA, long noncoding RNA, piwi-interacting RNA, microRNA, circular RNA, etc.) biomarkers are selected from ABCC3, AOC1, ARSL, B3GNT3, C12orf45, CDCP1, CDH1, CDH3, CEACAM5, CEACAM6, CELSR1, CLDN18, CLDN3, CLDN4, CLDN7, CLIC6, CRABP2, CST1, DMBT1, DSG2, EPCAM, EPHX3, ETV4, EVA1A, FAM83A, FOLR1, FOXA2, GJB1, GJB2, GPC4, HMGB3, HS6ST2, KDELR3, KRTCAP3, LAMB3, LFNG, LGALS3BP, LSR, MANEAL, MIF, MSLN, MUC1, MUC21, NAPSA, PIGT, PODXL2, PPP1R14D, PRRG4, ROS1, S100A14, SBK1, SCGB3A2, SDC1, SERINC2, SEZ6L2, SFTA2, SFTPA1, SFTPA2, SFTPB, SLC34A2, SLC44A4, SLC6A14, SLC7A7, SMIM22, SMPDL3B, SPINK1, ST14, TGFA, TMC4, TMC5, TMEM45B, TMPRSS2, TMPRSS4, TSPAN1, TSPAN8, ZC3H11A, and combinations thereof, wherein the nanoparticle is immobilized onto a solid substrate comprising a binding moiety directed to such a nanoparticle-associated surface biomarker. In some examples, such a complex further comprises at least two detection probes directed to at least one target biomarker

of a target biomarker signature present in the nanoparticle, wherein each detection probe is bound to a respective target biomarker and each comprises: (i) a binding moiety directed to the target biomarker; and (ii) an oligonucleotide domain coupled to the binding moiety, the oligonucleotide domain comprising a double-stranded portion and a single-stranded overhang portion extended from one end of the oligonucleotide domain, wherein the single-stranded overhang portions of the detection probes are hybridized to each other.

[55] In some examples, a nanoparticle-associated surface biomarker present in a nanoparticle that forms a complex comprises one or more of surface biomarkers described herein. In some examples, such a nanoparticle-associated surface biomarker may be or comprise one or more of biomarkers selected from: ALCAM, B3GNT3, CDCP1, CDHA1, CDH3, CD55, CD274 (PD-L1), CEACAM5, CEACAM6, CLDN3, CLDN4, DSG2, EGFR, EPCAM, FOLR1, GJB1, GJB2, IG1FR, LAMB3, MET, MSLN, MUC1, PIGT, PODXL2, ROS1, SDC1, SLC34A2, SMPDL3B, ST14, sTn antigen, Tn antigen, T antigen, TACSTD2, TMPRSS4, TSPAN8, TNFRSF10B, and combinations thereof. In some examples, such a nanoparticle-associated surface biomarker and/or a surface biomarker included in a target biomarker signature may be or comprise a SLC34A2 polypeptide. In some examples, such a nanoparticle-associated surface biomarker and/or a surface biomarker included in a target biomarker signature may be or comprise a CEACAM5 polypeptide. In some examples, such an nanoparticle-associated surface biomarker and/or a surface biomarker included in a target biomarker signature may be or comprise a CEACAM6 polypeptide. In some examples, such a nanoparticle-associated surface biomarker and/or a surface biomarker included in a target biomarker signature may be or comprise an EPCAM polypeptide. In some examples, such a nanoparticle-associated surface biomarker and/or a surface biomarker included in a target biomarker signature may be or comprise an ALCAM polypeptide. In some examples, such a nanoparticle-associated surface biomarker and/or a surface biomarker included in a target biomarker signature may be or comprise a CD55 polypeptide. In some examples, such a nanoparticle-associated surface biomarker and/or a surface biomarker included in a target biomarker signature may be or comprise a CDH1 polypeptide. In some examples, such a nanoparticle-associated surface biomarker and/or a surface biomarker included in a target biomarker signature may be or comprise a CDH3 polypeptide. In some examples, such a nanoparticle-associated surface biomarker and/or a surface biomarker included in a target

biomarker signature may be or comprise a CD274 (PD-L1) polypeptide. In some examples, such a nanoparticle-associated surface biomarker and/or a surface biomarker included in a target biomarker signature may be or comprise a DSG2 polypeptide. In some examples, such a nanoparticle-associated surface biomarker and/or a surface biomarker included in a target biomarker signature may be or comprise a EGFR polypeptide. In some examples, such a nanoparticle-associated surface biomarker and/or a surface biomarker included in a target biomarker signature may be or comprise a FOLR1 polypeptide. In some examples, such a nanoparticle-associated surface biomarker and/or a surface biomarker included in a target biomarker signature may be or comprise a IG1FR polypeptide. In some examples, such a nanoparticle-associated surface biomarker and/or a surface biomarker included in a target biomarker signature may be or comprise a MET polypeptide. In some examples, such a nanoparticle-associated surface biomarker and/or a surface biomarker included in a target biomarker signature may be or comprise a MSLN polypeptide. In some examples, such a nanoparticle-associated surface biomarker and/or a surface biomarker included in a target biomarker signature may be or comprise a MUC1 polypeptide. In some examples, such a nanoparticle-associated surface biomarker and/or a surface biomarker included in a target biomarker signature may be or comprise a sTn antigen polypeptide glycosylation. In some examples, such a nanoparticle-associated surface biomarker and/or a surface biomarker included in a target biomarker signature may be or comprise a Tn antigen polypeptide glycosylation. In some examples, such a nanoparticle-associated surface biomarker and/or a surface biomarker included in a target biomarker signature may be or comprise a T antigen polypeptide glycosylation. In some examples, such a nanoparticle-associated surface biomarker and/or a surface biomarker included in a target biomarker signature may be or comprise a TACSTD2 polypeptide. In some examples, such a nanoparticle-associated surface biomarker and/or a surface biomarker included in a target biomarker signature may be or comprise a TNFRSF10B polypeptide.

[56] In some examples, a nanoparticle-associated surface biomarker and/or a surface biomarker included in a target biomarker signature may be or comprise a MUC1 polypeptide. In some examples, a nanoparticle-associated surface biomarker and/or a surface biomarker included in a target biomarker signature may be or comprise a Lewis Y antigen. In some examples, a nanoparticle-associated surface biomarker and/or a surface

biomarker included in a target biomarker signature may be or comprise a sLex antigen. In some examples, a nanoparticle-associated surface biomarker and/or a surface biomarker included in a target-biomarker signature may be or comprise a T antigen. In some examples, a nanoparticle-associated surface biomarker and/or a surface biomarker included in a target-biomarker signature may be or comprise a Tn antigen.

[57] Also within the scope of the present disclosure is a complex comprising: a nanoparticle having a size range of interest that includes nanoparticles, and comprising a lung cancer-specific biomarker signature, which includes at least two surface biomarkers described herein, wherein the nanoparticle is immobilized onto a solid substrate comprising a binding moiety directed to a first surface biomarker of a lung cancer-specific biomarker signature. In some examples, such a complex is also bound to at least two detection probes each directed to a surface biomarker (which can be the same or different surface biomarker(s)) of the lung cancer-specific biomarker signature, wherein each detection probe is bound to a respective surface biomarker and each comprises: (i) a binding moiety directed to the surface biomarker; and (ii) an oligonucleotide domain coupled to the binding moiety, the oligonucleotide domain comprising a double-stranded portion and a single-stranded overhang portion extended from one end of the oligonucleotide domain, wherein the single-stranded overhang portions of the detection probes are hybridized to each other.

[58] In some examples, the present disclosure describes a complex comprising: (a) a nanoparticle having a size within the range of about 30 nm to about 1000 nm and comprising at least a first surface biomarker and a second surface biomarker on its surface, which combination is determined to be a target biomarker signature for lung cancer, wherein the first surface biomarker and the second surface biomarker are each independently selected from: (i) polypeptides encoded by human genes as follows: *PTK7*, *TSPAN8*, *CELSR2*, *GPC1*, *ST14*, *PTPRZ1*, *GPR87*, *GJB5*, *GJB2*, *RHOV*, *LYPD3*, *CLDN7*, *DSP*, *SERINC2*, *ABHD17C*, *PERP*, *MPZL2*, *ITGB4*, *MEST*, *GPNMB*, *SLC35A2*, *Gb3 (CD77)*, *ITGA6*, *ABCC5*, *ATP1B3*, *JAG1*, *TMPRSS11D*, *ABCA3*, *ABCC1*, *ABCC3*, *ACBD3*, *ACSL5*, *ADGRF1*, *AGER*, *ALCAM*, *AP1M2*, *APH1A*, *APOO*, *ATP11A*, *ATP11B*, *ATP1B1*, *ATP6AP2*, *B3GNT5*, *B4GALT4*, *BCAP31*, *BSPRY*, *CD109*, *CD55*, *CD9*, *CDC42*, *CDH1*, *CDH3*, *CDKAL1*, *CEACAM5*, *CEACAM6*, *CELSR1*, *CIP2A*, *CISD2*, *CKAP4*, *CLCA2*, *CLDN1*, *CLIC6*, *CLPTM1L*, *CLSTN1*, *CNTN1*, *CPD*, *CYP2S1*, *CYP4F11*, *CYP4F3*, *DPY19L1*, *DSC2*, *DSC3*, *DSG2*, *DSG3*, *EGFR*, *EPCAM*, *EPHB3*,

FAM241B, FAT2, FBXO45, FERMT1, FOLR1, FXYD3, FZD6, GALNT1, GALNT3, GALNT5, GALNT6, GALNT14, GGCX, GOLM1, GOLPH3L, GRHL2, HACD3, HAS3, IER3IP1, IGSF3, IL1RAP, ITGA2, ITGB6, KLRG2, KPNA2, KRTCAP3, LAD1, LAMB3, LAMC2, LAMP3, LAMTOR2, LAPTM4B, LARGE2, LCLAT1, LPCAT1, LSR, MAGT1, MAL2, MARCKSL1, MET, MGAT1, MSLN, MUC1, MUC4, NCSTN, NECTIN1, NECTIN4, NRAS, NRCAM, NT5E, NUP210, PARL, PEX13, PIGN, PIGT, PLA2G4A, PLCH1, PLEC, PRSS21, PSMD2, PTDSS1, PTGFRN, PTPRF, QSOX1, RAB25, RAB38, RAB6B, RAP2B, RCC2, RIT1, SCAMP3, SDC1, SEL1L3, SHROOM2, SLC7A11, SLC2A1, SLC34A2, SLC35B2, SLC39A11, SMIM22, SMPDL3B, SOAT1, SPAST, SSR1, SSR4, SURF4, SYNGR2, TACSTD2, TESC, TFRC, TMC5, TMC01, TMED2, TMED3, TMEM132A, TMEM33, TMPRSS4, TMTC3, TOMM22, TOR1AIP2, TRAM1, TRPV4, TTC33, UGT1A6, UPK1B, VAMP8, VMA21, VRK2, VWA1, XPR1, XXYL1, ADAM28, AXL, BSG, CD274, CD47, CLU, DKK1, ERBB3, FLT4, GM3, HGF, IGF1R, IL6, KDR, LAG3, UCHL1, NOTCH2, NOTCH3, TIGIT, TNFRSF10A, TNFRSF10B, TNFSF18, TPBG, VEGFA, and combinations thereof; and/or (ii) carbohydrate-dependent markers as follows: Sialyl Tn (sTn) antigen, Sialyl-6T antigen (6-sialyl core 1), T antigen, Phosphatidylserine, Tn antigen, Sialyl Lewis A antigen (CA19-9), Globo H, Gb5 (SSEA-3), Lactotriaosylceramide, (Lc3), Forssman antigen, Lewis X, Lewis Y/B antigen, LY6E, Lewis Y/CD174, Sialyl Lewis X (sLex) (also known as Sialyl SSEA-1 (SLX)), NeuGcGM3, and combinations thereof; (b) a solid substrate comprising a target-capture moiety directed to the first surface biomarker; wherein the target-capture moiety binds to the first surface biomarker of the nanoparticle such that the nanoparticle is immobilized on the solid substrate; and (c) at least a first detection probe and a second detection probe each bound to the nanoparticle, wherein each detection probe comprises: (i) a target binding moiety directed to the second surface biomarker; and (ii) an oligonucleotide domain coupled to the target binding moiety, the oligonucleotide domain comprising a double-stranded portion and a single-stranded overhang portion extended from one end of the oligonucleotide domain, wherein the single-stranded overhang portions of the first and second detection probes are hybridized to each other.

[59] In some examples, the first surface biomarker and the second surface biomarker(s) are each independently selected from: (i) polypeptides encoded by human genes as follows: *PTK7, TSPAN8, CELSR2, GPC1, ST14, PTPRZ1, GPR87, GJB5, GJB2, RHOV, LYPD3, CLDN7, DSP, SERINC2, ABHD17C, PERP, MPZL2, ITGB4, MEST, GPNMB,*

SLC35A2, Gb3 (CD77), ITGA6, ABCC5, ATP1B3, JAG1, TMPRSS11D, ABCA3, ABCC1, ABCC3, ACBD3, ACSL5, ADGRF1, AGER, ALCAM, AP1M2, APH1A, APOO, ATP11A, ATP11B, ATP1B1, ATP6AP2, B3GNT5, B4GALT4, BCAP31, BSPRY, CD109, CD55, CD9, CDC42, CDH1, CDH3, CDKAL1, CEACAM5, CEACAM6, CELSR1, CIP2A, CISD2, CKAP4, CLCA2, CLDN1, CLIC6, CLPTM1L, CLSTN1, CNTN1, CPD, CYP2S1, CYP4F11, CYP4F3, DPY19L1, DSC2, DSC3, DSG2, DSG3, EGFR, EPCAM, EPHB3, FAM241B, FAT2, FBXO45, FERMT1, FOLR1, FXYD3, FZD6, GALNT1, GALNT3, GALNT5, GALNT6, GALNT14, GGCX, GOLM1, GOLPH3L, GRHL2, HACD3, HAS3, IER3IP1, IGSF3, IL1RAP, ITGA2, ITGB6, KLRG2, KPNA2, KRTCAP3, LAD1, LAMB3, LAMC2, LAMP3, LAMTOR2, LAPTM4B, LARGE2, LCLAT1, LPCAT1, LSR, MAGT1, MAL2, MARCKSL1, MET, MGAT1, MSLN, MUC1, MUC4, NCSTN, NECTIN1, NECTIN4, NRAS, NRCAM, NT5E, NUP210, PARL, PEX13, PIGN, PIGT, PLA2G4A, PLCH1, PLEC, PRSS21, PSMD2, PTDSS1, PTGFRN, PTPRF, QSOX1, RAB25, RAB38, RAB6B, RAP2B, RCC2, RIT1, SCAMP3, SDC1, SEL1L3, SHROOM2, SLC7A11, SLC2A1, SLC34A2, SLC35B2, SLC39A11, SMIM22, SMPDL3B, SOAT1, SPAST, SSR1, SSR4, SURF4, SYNGR2, TACSTD2, TESC, TFRC, TMC5, TMCO1, TMED2, TMED3, TMEM132A, TMEM33, TMPRSS4, TMTC3, TOMM22, TOR1AIP2, TRAM1, TRPV4, TTC33, UGT1A6, UPK1B, VAMP8, VMA21, VRK2, VWA1, XPR1, XXYL1, ADAM28, AXL, BSG, CD274, CD47, CLU, DKK1, ERBB3, FLT4, GM3, HGF, IGF1R, IL6, KDR, LAG3, UCHL1, NOTCH2, NOTCH3, TIGIT, TNFRSF10A, TNFRSF10B, TNFSF18, TPBG, VEGFA, and combinations thereof; and/or (ii) carbohydrate-dependent markers as follows: Sialyl Tn (sTn) antigen, Sialyl-6T antigen (6-sialyl core 1), T antigen, Phosphatidylserine, Tn antigen, Sialyl Lewis A antigen (CA19-9), Globo H, Gb5 (SSEA-3), Lactotriaosylceramide, (Lc3), Forssman antigen, Lewis X, Lewis Y/B antigen, LY6E, Lewis Y/CD174, Sialyl Lewis X (sLex) (also known as Sialyl SSEA-1 (SLX)), NeuGcGM3, and combinations thereof.

[60] In some examples, the first surface biomarker and the second surface biomarker(s) are each independently selected from: ALCAM, BCAP31, CD109, CD274, CD55, CD9, CDH3, CEACAM5, CEACAM6, DSC2, DSC3, EGFR, EPCAM, FOLR1, HACD3, IL1RAP, IGF1R, IGSF3, ITGA2, LAMB3, Lewis Y antigen, Lewis X antigen, MARCKSL1, MET, MSLN, MUC1, MUC4, NT5E, Phosphatidylserine, PTGFRN, PTK7, SDC1, Sialyl Lewis X antigen, SLC34A2, Sialyl Tn antigen (sTn antigen), Tn antigen, T antigen, TACSTD2, TNFRSF10B, TFRC, TRPV4, TSPAN8, CD274, CDH1, CNTN1, GOLM1, VWA1, CELSR2,

CLDN1, DSG2, DSG3, GPC1, LAMC2, NECTIN1, ST14, CLCA2, PTPRZ1, SLC2A1, LYPD3, Gb3 (CD77), GJB2, MPZL2, RAP2B, ITGB4, DSP, MEST, ITGA6, ABCC5, ATP1B3, JAG1, GPNMB, PERP, TMPRSS11D, Sialyl-6T antigen (6-sialyl core 1), Sialyl-T antigen, Sialyl Lewis A antigen (CA19-9), Globo H, Gb5 (SSEA-3), Lactotriaosylceramide (Lc3), Forssman antigen, and combinations thereof.

[61] These, and other examples encompassed by the present disclosure, are described in more detail below and in the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[62] **FIG. 1** is a schematic diagram illustrating an exemplary workflow of profiling individual nanoparticles, e.g., extracellular vesicles. The figure shows purification of EVs from plasma using size exclusion chromatography (SEC) and immunoaffinity capture of EVs displaying a specific EV-associated surface marker (**Panel A**); detection of co-localized target markers (e.g., intravesicular biomarkers or surface biomarkers) on captured EVs using a target entity detection assay according to some embodiments described herein (**Panel B**).

[63] **FIG. 2** is a schematic diagram illustrating a target entity detection assay according to some embodiments described herein. In some embodiments, a target entity detection assay uses a combination of detection probes, which combination is specific for detection of cancer. In some embodiments, a duplex system includes a first detection probe for a target biomarker 1 and a second detection probe for a target biomarker 2 are added to a sample comprising a biological entity (e.g., extracellular vesicle). In some embodiments, detection probes each comprise a target binding moiety (e.g., an affinity agent such as, e.g., an antibody agent against a target biomarker) coupled to an oligonucleotide domain, which comprises a double-stranded portion and a single-stranded overhang extended from one end of the oligonucleotide domain. A detection signal is generated when distinct target binding moieties (e.g., affinity agents such as, e.g., antibody agents against target biomarker 1 and target biomarker 2, respectively) of the first and second detection probes are localized to the same biological entity (e.g., an extracellular vesicle) in close proximity such that the corresponding single-stranded overhangs hybridize to each other, thus allowing ligation of their oligonucleotide domains to occur. For example, a control entity (e.g., a biological entity from a healthy subject sample) does not express one or both of target biomarker 1 and target biomarker 2, so no

CLAIMS

What is claimed is:

1. A method comprising steps of:

(a) detecting, in a biological sample obtained from a subject, biological entities expressing a first target biomarker signature, the first target biomarker signature comprising:

at least one surface biomarker and

at least one target biomarker selected from the group consisting of: surface biomarkers, intravesicular biomarkers, and intravesicular RNA biomarkers;

(b) comparing sample information indicative of level of the first target biomarker signature-expressing biological entities in the biological sample to reference information including a first reference threshold level; and

(c) classifying the subject as having or being susceptible to lung cancer when the biological sample shows an elevated level of first target biomarker signature-expressing biological entities relative to a classification cutoff referencing the first reference threshold level.

2. The method of claim 1, wherein the steps of (b) and (c) are repeated for at least a second target biomarker signature, and wherein the classification cutoff references the first reference threshold level and at least a second reference threshold level corresponding to the at least a second target biomarker signature.

3. The method of any one of claims 1-2, wherein the surface biomarker is or comprises ALCAM, BCAP31, CD109, CD274, CD55, CD9, CDH3, CEACAM5, CEACAM6, DSC2, DSC3, EGFR, EPCAM, FOLR1, HACD3, IL1RAP, IGF1R, IGSF3, ITGA2, LAMB3, Lewis Y antigen, Lewis X antigen, MARCKSL1, MET, MSLN, MUC1, MUC4, NT5E, Phosphatidylserine, PTGFRN, PTK7, SDC1, Sialyl Lewis X antigen, SLC34A2, Sialyl Tn antigen, Tn antigen, T antigen, TACSTD2, TNFRSF10B, TFRC, TRPV4, TSPAN8, CD274, CDH1, CNTN1, GOLM1, VWA1, CELSR2, CLDN1, DSG2, DSG3, GPC1, LAMC2, NECTIN1, ST14, CLCA2, PTPRZ1, SLC2A1, LYPD3, Gb3 (CD77), GJB2, MPZL2, RAP2B, ITGB4, DSP, MEST, ITGA6, ABCC5, ATP1B3, JAG1, GPNMB, PERP, TMPRSS11D, Sialyl-6T antigen (6-sialyl core 1), Sialyl-T antigen, Sialyl Lewis A antigen (CA19-9), Globo H, Gb5 (SSEA-3), Lactotriaosylceramide (Lc3), Forssman antigen, or combinations thereof.

4. The method of any one of claims 1-3, wherein the first and/or second target biomarker signature comprises at least one surface biomarker and at least two target biomarkers selected from the group consisting of: surface biomarkers, intravesicular biomarkers, and intravesicular RNA biomarkers, wherein:

the surface biomarkers are selected from ALCAM, BCAP31, CD109, CD274, CD55, CD9, CDH3, CEACAM5, CEACAM6, DSC2, DSC3, EGFR, EPCAM, FOLR1, HACD3, IL1RAP, IGF1R, IGSF3, ITGA2, LAMB3, Lewis Y antigen, Lewis X antigen, MARCKSL1, MET, MSLN, MUC1, MUC4, NT5E, Phosphatidylserine, PTGFRN, PTK7, SDC1, Sialyl Lewis X antigen, SLC34A2, Sialyl Tn antigen (sTn antigen), Tn antigen, T antigen, TACSTD2, TNFRSF10B, TFRC, TRPV4, TSPAN8, CD274, CDH1, CNTN1, GOLM1, VWA1, CELSR2, CLDN1, DSG2, DSG3, GPC1, LAMC2, NECTIN1, ST14, CLCA2, PTPRZ1, SLC2A1, LYPD3, Gb3 (CD77), GJB2, MPZL2, RAP2B, ITGB4, DSP, MEST, ITGA6, ABCC5, ATP1B3, JAG1, GPNMB, PERP, TMPRSS11D, Sialyl-6T antigen (6-sialyl core 1), Sialyl-T antigen, Sialyl Lewis A antigen (CA19-9), Globo H, Gb5 (SSEA-3), Lactotriaosylceramide (Lc3), Forssman antigen, and combinations thereof;

the intravesicular biomarkers are selected from AOC1, C12orf45, CRABP2, CST1, ETV4, FAM83A, FOXA2, HMGB3, LGALS3BP, MIF, NAPSA, PPP1R14D, S100A14, SBK1, SCGB3A2, SFTA2, SFTPA1, SFTPA2, SFTPB, SPINK1, TGFA, ZC3H11A, and combinations thereof; and

the intravesicular RNA biomarkers are selected from RNA transcripts (e.g., mRNA transcripts) encoded by human genes as follows: ABCC3, AOC1, ARSL, B3GNT3, C12orf45, CDCP1, CDH1, CDH3, CEACAM5, CEACAM6, CELSR1, CLDN18, CLDN3, CLDN4, CLDN7, CLIC6, CRABP2, CST1, DMBT1, DSG2, EPCAM, EPHX3, ETV4, EVA1A, FAM83A, FOLR1, FOXA2, GJB1, GJB2, GPC4, HMGB3, HS6ST2, KDEL3, KRTCAP3, LAMB3, LFNG, LGALS3BP, LSR, MANEAL, MIF, MSLN, MUC1, MUC21, NAPSA, PIGT, PODXL2, PPP1R14D, PRRG4, ROS1, S100A14, SBK1, SCGB3A2, SDC1, SERINC2, SEZ6L2, SFTA2, SFTPA1, SFTPA2, SFTPB, SLC34A2, SLC44A4, SLC6A14, SLC7A7, SMIM22, SMPDL3B, SPINK1, ST14, TGFA, TMC4, TMC5, TMEM45B, TMPRSS2, TMPRSS4, TSPAN1, TSPAN8, ZC3H11A, and combinations thereof.

5. A method comprising steps of:

(a) detecting, in a biological sample obtained from a subject, biological entities having a size within the range of about 30 nm to about 1000 nm and expressing a first target biomarker signature, the first target biomarker signature comprising:

at least one surface biomarker and

at least one target biomarker, wherein the target biomarker is or comprises a surface biomarker, wherein the target biomarker signature comprises a biomarker combination as listed in Tables 4A or 4B;

(b) comparing sample information indicative of level of the first target biomarker signature-expressing biological entities in the biological sample to reference information including a first reference threshold level; and

(c) classifying the subject as having or being susceptible to lung cancer when the biological sample shows an elevated level of first target biomarker signature-expressing biological entities relative to a classification cutoff referencing the first reference threshold level.

6. The method of any one of claims 1-5, wherein the first or second reference threshold level is determined by levels of target biomarker signature-expressing biological entities observed in comparable samples from a population of non-cancer subjects.

7. The method of any one of claims 1-6, wherein the biological sample has been subjected to size exclusion chromatography to isolate (*e.g.*, directly from the biological sample) biological entities having a size range of interest that includes extracellular vesicles.

8. The method of any one of claims 1-7, wherein the step of detecting comprises a capture assay.

9. The method of claim 8, wherein the capture agent is or comprises a solid substrate comprising the target-capture moiety conjugated thereto.

10. The method of any one of claims 8-9, wherein the target-capture moiety is or comprises an antibody agent.

11. The method of any one of claims 1-10, wherein the step of detecting comprises a capture assay and a detection assay, the capture assay being performed prior to the detection assay.

12. The method of claim 11, wherein when the first and/or second target biomarker signature comprises at least one surface biomarker and/or intravesicular biomarker, and the detection assay involves an immunoassay (including, *e.g.*, immuno-PCR, and/or proximity ligation assay) and a proximity ligation assay, wherein the proximity ligation assay comprises the steps of:

(a) contacting the target biomarker signature-expressing biological entities that express the at least one surface biomarker with a set of detection probes, each directed to a target biomarker of the target biomarker signature, which set comprises at least two detection probes, so that a combination comprising the biological entities and the set of detection probes is generated,

wherein the detection probes each comprise:

(i) a target binding moiety directed to the target biomarker of the target biomarker signature; and

(ii) an oligonucleotide domain coupled to the target binding moiety, the oligonucleotide domain comprising a double-stranded portion and a single-stranded overhang portion extended from one end of the oligonucleotide domain,

wherein the single-stranded overhang portions of the detection probes are characterized in that they can hybridize to each other when the detection probes are bound to the same biological entity,

(b) maintaining the combination under conditions that permit binding of the set of detection probes to their respective targets on the biological entities such that the at least two detection probes can bind to the same biological entity that express the target biomarker signature to form a double-stranded complex;

(c) contacting the double-stranded complex with a nucleic acid ligase to generate a ligated template; and

(d) detecting the ligated template, wherein presence of the ligated template is indicative of presence in the biological sample of the target biomarker signature-expressing biological entities; and

(e) optionally repeating steps a through d at least one additional time using an orthogonal target biomarker signature.

13. The method of any one of claims 10-12, wherein the target-capture moiety of the capture assay is or comprises at least one antibody agent directed to the at least one surface biomarker.

14. The method of any one of claims 1-13, wherein the method is used in combination with one or more of the following diagnostic assays:

- (i) the subject's annual physical examination;
- (ii) a chest imaging (*e.g.*, X-ray, CT scan, or low-dose CT scan);
- (iii) sputum cytology;
- (iv) a genetic assay to screen blood plasma for genetic mutations in circulating tumor DNA and/or protein biomarkers linked to cancer; and
- (v) an assay involving immunofluorescent staining to identify cell phenotype and marker expression, followed by amplification and analysis by next-generation sequencing.

15. The method of any one of claims 1-14 for detecting cancer, the method comprising steps of: detecting on surfaces of intact biological entities from a human blood sample co-localization of at least two biomarkers whose combined expression level has been determined to be associated with cancer; comparing the detected co-localization level with the determined level; and detecting cancer when the detected co-localization level is at or above the determined level.

16. The method of any one of claims 1-14, comprising steps of: capturing exosomes from a biological sample with a capture agent that selectively interacts with a cancer-specific surface biomarker on the exosomes; and contacting the captured exosomes with at least one set of at least two detection probes that each selectively interacts with a surface biomarker on the exosomes; and detecting a product formed when the at least two detection probes of the set are in sufficiently close proximity, such detection indicating co-localization of the surface biomarkers.

17. The method of any one of claims 1-14, comprising steps of: contacting a sample comprising exosomes with a set of probes that specifically bind to surface biomarkers on the exosomes to

detect cancer-associated exosomes in the sample, wherein: (i) each probe in the set comprises a target binding moiety directed to a surface biomarker on the exosomes; and (ii) the set comprises at least one capture probe and at least two detection probes, wherein each detection probe further comprises a detection moiety.

18. The method of any one of claims 1-14, comprising steps of: performing a proximity assay that detects a surface biomarker signature on exosomes from a human subject, the step of performing being performed a period of time after a performance of a prior assay to detect the surface biomarker signature on exosomes from the human subject; and comparing results of the performed assay with those of the prior assay.

19. The method of any one of claims 1-14, comprising steps of: contacting exosomes with at least two detection probes, wherein each detection probe comprises (i) a binding moiety; and (ii) an oligonucleotide entity, wherein the binding moiety is the same and the oligonucleotide entities complement one another.

20. The method of any one of claims 1-14, comprising detecting marker proximity on exosome surfaces, including an improvement that comprises contacting the exosomes with at least a pair of binding agents that each comprise a binding moiety and a proximity moiety, wherein the binding moieties are the same and the proximity moieties complement one another; and detecting an interaction between the proximity moieties.