

[0004] The present disclosure provides, *inter alia*, novel methods of enriching EVs from biological fluid samples from mammalian subjects based on presence of cell surface markers, and for optionally further testing the isolated EVs for the presence of additional biomarkers, such as biomarkers that may be found either on the surface or in the lumen of EVs and that may be associated with particular cell types or disease states. EVs shed from tumors, for example, contain tumor-derived proteins and nucleic acids (Rabinowits et al., 2009; Inal et al. 2013). The ability to specifically enrich for these will enable the study of tumor-specific information in liquid biopsy over time and across multiple metastatic foci.

SUMMARY

[0005] This disclosure, in some embodimentexamples, relates to processes of enriching extracellular vesicles (EVs) in a biological fluid sample from a subject. In one aspect, the disclosure provides a process of enriching cell-type specific extracellular vesicles (EVs) in a biological fluid sample from a subject, comprising: a. providing a biological fluid sample from the subject; b. contacting the sample with antibodies specific for a cell surface marker for one or more cell types, optionally wherein the antibodies are attached to a matrix; and c. isolating antibody-bound EVs from unbound EVs in the sample. In some embodimentexamples, the EVs are cell type-specific. In some embodimentexamples, the processes comprise (a) providing a biological fluid sample from the subject; (b) contacting the sample with antibodies specific for a cell surface marker for one or more cell types, optionally wherein the antibodies are attached to a matrix; and (c) isolating antibody-bound EVs from unbound EVs the sample. In some embodimentexample, the sample comprises a volume from 100 μ l to 7 mL, from 200 μ l to 6.5 mL, from 250 μ l to 6.5 mL, from 200 μ l to 6 mL, from 250 μ l to 6 mL, from 200 μ l to 5 mL, from 200 μ l to 4 mL, from 200 μ l to 3 mL, from 200 μ l to 2 mL, from 100 μ l to 1 mL, from 150 μ l to 1 mL, from 200 μ l to 1 mL, from 250 μ l to 1mL, from 200 μ l to 500 μ l, from 200 μ l to 500 μ l, from 200 μ l to 400 μ l, from 250 μ l to 500 μ l, or from 250 μ l to 400 μ l. In any of the above processes, the EVs may comprise EVs from epithelial cells, lung tissue cells, breast tissue cells, liver tissue cells, prostate tissue cells, kidney tissue cells, urinary tract cells, neural cells, tumor cells, solid tumor cells, lung tumor cells, breast tumor cells, liver tumor cells, prostate tumor cells, kidney tumor cells, urinary tumor cells, glioblastoma cells, or amyloid-beta expressing cells. In some embodimentexample, the cell surface marker is an epithelial cell surface marker. In some embodimentexamples, the cell surface markers may be (a) an epithelial cell surface marker, such as EpCAM, EGFR, PSMA, GSP64, CD3, CD49b, CD87, CD95, E-Cadherin CA9, CA12, N-cadherin, OB-cadherin, cadherin-11, a cytokeratin or epithelial membrane antigen (EMA); (b) a lung tissue marker, such as EpCAM, CA9, CA12, DSG3, FAT2, GPR87, KISS1R, LYPD3, SLC7A11, TMPRSS4, CD133, prominin-1, AC133, programmed death-1 receptor (PD1) or FAS; (c) a breast tissue marker, such as E-cadherin, epithelial membrane antigen (EMA), human epidermal growth factor receptor type 2 (Her2/neu), α v β 6 integrin, EpCAM, carcinoembryonic antigen (CEA), folate receptor-alpha (FR- α), urokinase-type plasminogen activator receptor (uPAR) or placental-specific protein 1 (PLAC1); (d) a liver cell marker, such as CD133, Prominin-1,

CD44, EpCAM, delta-like 1 non-canonical Notch ligand 1 (DLK1), ALDH, CD13, CD90, CD24, OV6, ICAM-1, CD34, C-kit, α 2 δ 1, K19, LGR5, GPC3, Annexin A2, CD15, ABC transporters, Nope, DCLK1, ASGPR, CK or CD47; € a glioblastoma biomarker, such as fibronectin, CD63, HSP70, Annexin A2, CD9, CD81, CD44, GRP78, CD133, CD15, a sialoglycoprotein, SLC1A3, PTPRZ1, GPR56, CLU or ALD1A3; (f) a urinary tract cell marker, such as a tetraspanin, CD9, CD81, LAMP-1, CD10, CD24, CD44 or CD63; or (g) an amyloid marker such as beta-amyloid precursor protein (β -APP), presenilin protein PS-1, or proinsulin protein PS-2. In some [embodimentexamples](#), the cell surface marker can be EpCAM.

[0006] In some [embodimentexamples](#), wherein the antibodies are attached to a matrix, the matrix may comprise particles such as beads, pellets, or chips, which are optionally magnetic. In some [embodimentexamples](#), the biological fluid sample may comprise tears, saliva, lymph fluid, urine, serum, cerebral spinal fluid, pleural effusion, ascites, or plasma. In some [embodimentexamples](#), the sample comprises serum or plasma.

[0007] In any of the above processes, the subject may be a human. In some [embodimentexamples](#), the subject may be a cancer subject or suspected cancer subject. In some [embodimentexamples](#), the cancer is a solid tumor. In some [embodimentexamples](#), the cancer is breast cancer, triple negative breast cancer, lung cancer, NSCLC, SCLC, liver cancer, urinary tract cancer, bladder cancer, brain cancer, or glioblastoma. In some [embodimentexamples](#), the subject may be an infectious disease subject or suspected infectious disease subject. In some [embodimentexamples](#), the subject may be an inflammatory disease subject or suspected inflammatory disease subject. In some [embodimentexamples](#), where the subject is a cancer subject or suspected cancer subject, the cell surface marker is EpCAM.

[0008] In some [embodimentexamples](#), the subject may be an amyloid disease subject or suspected amyloid disease subject.

[0009] In some cases, the methods may further comprise isolating total extracellular vesicles (Evs) from the sample, such as by membrane capture or differential ultracentrifugation, determining total protein levels in the isolated total extracellular vesicles, and optionally comparing the total protein levels from the total Evs to levels of cell surface marker in the Evs.

[0010] In some [embodimentexamples](#), isolation of antibody bound Evs from unbound Evs is through attachment of antibodies to a matrix. In some [embodimentexamples](#), isolation of the antibody bound Evs from unbound Evs comprises separating the matrix from supernatant in the sample, such as by allowing the matrix to precipitate by gravity or centrifugation.

[0011] In some [embodimentexamples](#), the processes may include contacting the antibody bound Evs with a detection agent specific for a second biomarker. In some [embodimentexamples](#), the antibody

bound EVs are resuspended in lysis buffer prior to being contacted with the detection agent specific for the second biomarker. In some [embodimentexamples](#), the detection agent is an antibody. In some [embodimentexamples](#), the second biomarker is a protein, a polynucleotide (e.g. an RNA molecule), a lipid, a drug, or a drug metabolite. In some [embodimentexample](#), the subject has cancer or is suspected of having cancer, and where the second biomarker is a polypeptide expressed or overexpressed in tumor cells, and/or wherein the second biomarker is a cytokine, cytokine receptor, or an immune checkpoint regulator. In some [embodimentexamples](#), the second biomarker is one or more of PD-L1, SCF, IL-3, GM-CSF, G-CSF, M-CSF, TNF-alpha, IL-2, IL-5, TMB, CTLA-4, ICOS, 4-1BB (CD137), PD-1, CTLA-4, LAG-3, Tim-3, CD39, IFN-β, IFN-γ, IL-2, IL-10, TGF-β, CCR10, CXCR4, CCR7, sMICA, IL-8, IDO1, GBP1, class II MHC molecules, CXCL9, CXCL10 (IP-10), CXCL11, IL-6, CCL4, CCL5, IFNGR1, IFNGR2, JAK2, IRF1, IFIT1, IFIT2, MTAP, miR3, SOCA1, PIAS4, GZMA, GZMB, PRF1, HLA-DQA1, HLA-DRB1, IFNG, STAT1, ICAM1-5, VCAM-1, JAK1, JAK2, CCR5, HLA-DRA, CXCR6, TIGIT, CD27, CD274, PDCD1LG2 (PD-L2), LAG3, NKG7, PSMB10, CMKLR1, CD8A, CD8B, HLA-DB1, HLA-E, CD276, CD3D, CD3E, CD3G, CD247, ZAP70, CD2, CD28, ICOS, IL12Rb1, GZMM, FLTSLG, IL-15, Eotazin, GRO-1, VEGF, HGF, AFP, BCR-ABL, BRCA1/BRCA2, B-Raf V600E, CA-125, CA19-9, CEA, EGFR, HER-2/neu, KIT, PSA, S100, KRAS, UGT1A1 or CD20.

[0012] The processes described above may also have one or more of the following characteristics: (a) the sample is not subjected to chromatography either before or after contacting the sample with the antibodies; (b) the sample is not treated before contacting the sample with the antibodies other than optionally to remove particles above 1 μm, above 2 μm, above 5 μm, above 10 μm, or above 20 μm in diameter and/or to remove cellular bodies and debris larger than EVs; (c) the EVs are not subjected to chromatography either before or after contacting the sample with the antibodies; (d) the antibodies are attached to a matrix, and the matrix is not a filter, an ion-exchange medium, or a membrane; or (e) the antibodies are attached to a matrix and the matrix is not charged.

[0013] In some processes herein, isolation of cell-type specific EVs from the sample consists essentially of (a) contacting the sample with antibodies specific for the cell surface marker for the one or more cell types (optionally wherein the antibodies are attached to the matrix), and (b) isolating the antibody-bound EVs from unbound EVs the sample.

[0014] This disclosure, in some [embodimentexamples](#), also relates to processes of enriching cell-type specific extracellular vesicles (EVs) from a subject, comprising: (a) providing a biological fluid sample from the subject; (b) contacting the sample with antibodies specific for a cell surface

marker, optionally wherein the antibodies are attached to a matrix; (c) isolating antibody-bound EVs from unbound EVs the sample; (d) optionally resuspending the antibody-bound EVs in a lysis buffer; and (e) contacting the EVs with a detection agent specific for a second biomarker.

In one aspect, the disclosure provides a process of enriching cell-type specific extracellular vesicles (EVs) from a subject, comprising: a. providing a biological fluid sample from the subject; b. contacting the sample with antibodies specific for a cell surface marker, optionally wherein the antibodies are attached to a matrix; c. isolating antibody-bound EVs from unbound EVs in the sample; d. optionally resuspending the antibody-bound EVs in a lysis buffer; and e. contacting the EVs with a detection agent specific for a second biomarker; optionally wherein the cell surface marker is: i. an epithelial cell surface marker, optionally wherein the epithelial cell surface marker is EpCAM, EGFR, PSMA, GSP64, CD3, CD49b, CD87, CD95, E-Cadherin CA9, CA12, N-cadherin, OB-cadherin, cadherin-11, a cytokeratin or epithelial membrane antigen (EMA); ii. a lung tissue marker, optionally wherein the lung tissue marker is EpCAM, CA9, CA12, DSG3, FAT2, GPR87, KISS1R, LYPD3, SLC7A11, TMPRSS4, CD133, prominin-1, AC133, programmed death-1 receptor (PD1) or FAS; iii. breast tissue marker, optionally wherein the breast tissue marker is E-cadherin, epithelial membrane antigen (EMA), human epidermal growth factor receptor type 2 (Her2/neu), $\alpha v \beta 6$ integrin, EpCAM, carcinoembryonic antigen (CEA), folate receptor-alpha (FR- α), urokinase-type plasminogen activator receptor (uPAR) or placental-specific protein 1 (PLAC1); iv. a liver cell marker, optionally wherein the liver cell marker is CD133, Prominin-1, CD44, EpCAM, delta-like 1 non-canonical Notch ligand 1 (DLK1), ALDH, CD13, CD90, CD24, OV6, ICAM-1, CD34, C-kit, $\alpha 2 \delta 1$, K19, LGR5, GPC3, Annexin A2, CD15, ABC transporters, Nope, DCLK1, ASGPR, CK or CD47; v. a glioblastoma biomarker, optionally wherein the glioblastoma biomarker is fibronectin, CD63, HSP70, Annexin A2, CD9, CD81, CD44, GRP78, CD133, CD15, a sialoglycoprotein, SLC1A3, PTPRZ1, GPR56, CLU or ALD1A3; vi. a urinary tract cell marker, optionally wherein the urinary tract cell marker is a tetraspanin, CD9, CD81, LAMP-1, CD10, CD24, CD44 or CD63; or vii. an amyloid marker, optionally wherein the amyloid marker is beta-amyloid precursor protein (β -APP), presenilin protein PS-1, or proinsulin protein PS-2. In some **embodimentexamples**, the sample comprises a volume from 100 μ L to 7 mL, from 200 μ L to 6.5 mL, from 250 μ L to 6.5 mL, from 200 μ L to 6 mL, from 250 μ L to 6 mL, from 200 μ L to 5 mL, from 200 μ L to 4 mL, from 200 μ L to 3 mL, from 200 μ L to 2 mL, from 100 μ L to 1 mL, from 150 μ L to 1 mL, from 200 μ L to 1 mL, from 250 μ L to 1 mL, from 200 μ L to 500 μ L, from 200 μ L to 500 μ L, from 200 μ L to 400 μ L, from 250 μ L to 500 μ L, or from 250 μ L to 400 μ L. In some of these processes, the EVs may be EVs from epithelial cells, lung tissue cells, breast tissue cells, liver tissue cells, prostate tissue cells, kidney tissue cells, urinary tract cells, neural cells, tumor cells, solid tumor cells, lung tumor cells, breast tumor cells, liver tumor cells, prostate tumor cells, kidney tumor cells, urinary tumor cells, glioblastoma cells, or amyloid-beta expressing cells. In some **embodimentexamples**, the cell surface marker is an epithelial cell surface marker.

[0015] In some of these processes, the cell surface marker is: (a) an epithelial cell surface marker, such as EpCAM, EGFR, PSMA, GSP64, CD3, CD49b, CD87, CD95, E-Cadherin CA9, CA12, N-cadherin, OB-cadherin, cadherin-11, a cytokeratin or epithelial membrane antigen (EMA); (b) a lung tissue marker, such as EpCAM, CA9, CA12, DSG3, FAT2, GPR87, KISS1R, LYPD3, SLC7A11, TMPRSS4, CD133, prominin-1, AC133, programmed death-1 receptor (PD1) or FAS; (c) a breast tissue marker, such as E-cadherin, epithelial membrane antigen (EMA), human epidermal growth factor receptor type 2 (Her2/neu), $\alpha v \beta 6$ integrin, EpCAM, carcinoembryonic antigen (CEA), folate receptor-alpha (FR- α), urokinase-type plasminogen activator receptor (uPAR) or placental-specific protein 1 (PLAC1); (d) a liver cell marker, such as CD133, Prominin-1, CD44, EpCAM, delta-like 1 non-canonical Notch ligand 1 (DLK1), ALDH, CD13, CD90, CD24, OV6, ICAM-1, CD34, C-kit, $\alpha 2 \delta 1$, K19, LGR5, GPC3, Annexin A2, CD15, ABC transporters, Nope, DCLK1, ASGPR, CK or CD47; (e) a glioblastoma biomarker, such as fibronectin, CD63, HSP70, Annexin A2, CD9, CD81, CD44, GRP78, CD133, CD15, a sialoglycoprotein, SLC1A3, PTPRZ1, GPR56, CLU or ALD1A3; (f) a urinary tract cell marker, such as a tetraspanin, CD9, CD81, LAMP-1, CD10, CD24, CD44 or CD63; or (g) an amyloid marker such as beta-amyloid precursor protein (β -APP), presenilin protein PS-1, or proinsulin protein PS-2.

[0016] In some of the above **embodimentexamples**, the antibodies are attached to a matrix and the matrix comprises particles such as beads, pellets, or chips, which are optionally magnetic. In some **embodimentexamples**, the biological fluid sample comprises tears, saliva, lymph fluid, urine,

serum, cerebral spinal fluid, pleural effusion, ascites, or plasma. In some [embodimentexamples](#), the sample is serum or plasma.

[0017] In some of the above processes, the subject is human. In some [embodimentexamples](#), the subject may be a cancer subject or suspected cancer subject. In some [embodimentexamples](#), the cancer is a solid tumor. In some [embodimentexamples](#), the cancer is breast cancer, triple negative breast cancer, lung cancer, NSCLC, SCLC, liver cancer, urinary tract cancer, bladder cancer, brain cancer, or glioblastoma. In some [embodimentexamples](#), the subject may be an infectious disease subject or suspected infectious disease subject.

[0018] In some of the above processes, the cell surface marker comprises EpCAM, EGFR, PSMA, GSP64, CD3, CD49b, CD87, CD95, E-Cadherin CA9, CA12, N-cadherin, OB-cadherin, cadherin-11, cytokeratins and epithelial membrane antigen (EMA). Some processes may further include a second biomarker that comprises one or more of IL-4, IL-5, IL-8, IL-10, IL-35, IFN- α , IFN- γ , CXCL10 (IP-10), C reactive protein, hemagglutinin, virus RNA, virus DNA, viral proteins (such as hepatitis B surface antigen, and HIV p24 antigen), bacterial RNA, bacterial DNA, a bacterial protein, a surface immunoglobulin or a receptor for C3 complement component. In some [embodimentexamples](#), the cell surface marker is EpCAM.

[0019] In some of the above [embodimentexamples](#), the subject may be an inflammatory disease subject or suspected inflammatory disease subject. In some [embodimentexamples](#), the second biomarker comprises one or more of TNF- α , IL-16, IL-17, IL-21, IL-22, IL-33, CD86, CD80, CRP, IL-1, IL-1 α , IL-1 β , IL-2, IL-6, IL-8, IL-12, IFN- γ , serum amyloid (SAA), COX2, NK- κ B, CCL2, CXCL10, CCL2, CXCL5, CXCL9, CXCL6, MMP-7, MMP-2, MMP-9 or CSFIR; and optionally, wherein the cell surface marker is an epithelial cell surface marker, such as EpCAM, EGFR, PSMA, GSP64, CD3, CD49b, CD87, CD95, E-Cadherin CA9, CA12, N-cadherin, OB-cadherin, cadherin-11, a cytokeratin or epithelial membrane antigen (EMA).

[0020] In some [embodimentexamples](#), the subject may be an amyloid disease subject or suspected amyloid disease subject. In some such [embodimentexamples](#), the cell surface marker is β -APP, PS-1, or PS-2, and/or wherein the second biomarker is one or more of CD3, CD4, CD8, CD40L, CD45RO, CD45RA, sCD40L, Fas/CD95, CD14, APP, CD19, CD69, IL-2, IFN- α , sCD40, CD11b, CD14, Iba1, CD11b, CD11c, RCA-1, MHCII (including HLA-DR), ferritin, IL-1 α , CD68, CD163, Ricinus communis agglutinin-1 (RCA-1), translocator protein (TSPO), triggering receptor expressed on myeloid cells 2 (TREM2), CR3 or CD33.

[0021] Some of the processes above further comprise isolating total extracellular vesicles (EVs) from the sample, such as by membrane capture or differential ultracentrifugation, determining total protein levels in the isolated total extracellular vesicles, and optionally

comparing the total protein levels from the total EVs to levels of cell surface marker in the EVs.

In some [embodimentexamples](#), isolation of antibody bound EVs from unbound EVs is through attachment of the antibodies to a matrix. In some [embodimentexamples](#), isolation of the antibody bound EVs from unbound EVs comprises separating the matrix from supernatant in the sample, such as by allowing the matrix to precipitate by gravity or centrifugation.

[0022] In some cases, the above processes have one or more of the following characteristics: (a) the sample is not subjected to chromatography either before or after contacting the sample with the antibodies; (b) the sample is not treated before contacting the sample with the antibodies other than optionally to remove particles above 1 μm , above 2 μm , above 5 μm , above 10 μm , or above 20 μm in diameter from the sample and/or to remove cellular bodies and debris larger than EVs; (c) the EVs are not subjected to chromatography either before or after contacting the sample with the antibodies; (d) the antibodies are attached to a matrix, and the matrix is not a filter, an ion-exchange medium, or a membrane; or (e) the antibodies are attached to a matrix and the matrix is not charged.

[0023] In some [embodimentexamples](#), the isolation of the cell-type specific EVs from the sample may consist essentially of (a) contacting the sample with antibodies specific for the cell surface marker for the one or more cell types (optionally wherein the antibodies are attached to a matrix), (b) and isolating the antibody-bound EVs from unbound EVs the sample, (c) resuspending the antibody-bound EVs in a lysis buffer, and (d) contacting the EVs with a detection agent specific for a second biomarker.

[0024] In some [embodimentexamples](#), the detection agent is an antibody. In some [embodimentexamples](#), the second biomarker may be a protein, a polynucleotide (e.g. an RNA molecule), a lipid, a drug, or a drug metabolite.

[0025] In some cases, the subject has cancer or is suspected of having cancer and the processes include a second biomarker that is a polypeptide expressed or overexpressed in tumor cells. In some such [embodimentexamples](#), the processes further include a second biomarker that is a cytokine, cytokine receptor, or an immune checkpoint regulator. In some such [embodimentexamples](#), the second biomarker is one or more of PD-L1, SCF, IL-3, GM-CSF, G-CSF, M-CSF, TNF-alpha, IL-2, IL-5, TMB, CTLA-4, ICOS, 4-1BB (CD137), PD-1, CTLA-4, LAG-3, Tim-3, CD39, IFN- β , IFN- γ , IL-2, IL-10, TGF- β , CCR10, CXCR4, CCR7, sMICA, IL-8, IDO1, GBP1, class II MHC molecules, CXCL9, CXCL10 (IP-10), CXCL11, IL-6, CCL4, CCL5, IFNGR1, IFNGR2, JAK2, IRF1, IFIT1, IFIT2, MTAP, miR3, SOCA1, PIAS4, GZMA, GZMB, PRF1, HLA-DQA1, HLA-DRB1, IFNG, STAT1, ICAM1-5, VCAM-1, JAK1, JAK2, CCR5, HLA-DRA, CXCR6, TIGIT, CD27, CD274, PDCD1LG2 (PD-L2), LAG3, NKG7, PSMB10, CMKLR1,

CD8A, CD8B, HLA-DB1, HLA-E, CD276, CD3D, CD3E, CD3G, CD247, ZAP70, CD2, CD28, ICOS, IL12Rb1, GZMM, FLTSLG, IL-15, Eotazin, GRO-1, VEGF, HGF, AFP, BCR-ABL, BRCA1/BRCA2, B-Raf V600E, CA-125, CA19-9, CEA, EGFR, HER-2/neu, KIT, PSA, S100, KRAS, UGT1A1 or CD20. In some embodiment examples, the second biomarker comprises PD-L1. In some embodiment examples, the tumor cell surface marker is EpCAM and the second biomarker comprises PD-L1.

[0026] This disclosure also relates to processes of identifying tumor-derived extracellular vesicles (EVs) in a subject, comprising: (a) providing a plasma sample from the subject, optionally wherein the sample is from 100 μ l to 1 mL, 200 μ l to 1 mL, from 250 μ l to 1 mL, from 100 μ l to 500 μ l, from 200 μ l to 500 μ l, from 200 μ l to 400 μ l, from 250 μ l to 500 μ l, or from 250 μ l to 400 μ l; (b) contacting the plasma sample with antibodies specific for EpCAM, optionally wherein the antibodies are attached to a matrix; (c) isolating EpCAM antibody-bound EVs from unbound EVs the sample; (d) optionally resuspending the EpCAM antibody-bound EVs in a lysis buffer; and (e) contacting the EpCAM antibody-bound EVs with an antibody specific for PD-L1 and optionally determining the level of PD-L1 in the EpCAM antibody-bound EVs.

In one aspect, the disclosure provides a process of identifying tumor-derived extracellular vesicles (EVs) in a subject, comprising: a. providing a plasma sample from the subject, optionally wherein the sample is from 100 μ l to 1 mL, 200 μ l to 1 mL, from 250 μ l to 1 mL, from 100 μ l to 500 μ l, from 200 μ l to 500 μ l, from 200 μ l to 400 μ l, from 250 μ l to 500 μ l, or from 250 μ l to 400 μ l; b. contacting the plasma sample with antibodies specific for EpCAM, optionally wherein the antibodies are attached to a matrix; c. isolating EpCAM antibody-bound EVs from unbound EVs in the sample; d. optionally resuspending the EpCAM antibody-bound EVs in a lysis buffer; and e. contacting the EpCAM antibody-bound EVs with an antibody specific for PD-L1 and optionally determining the level of PD-L1 in the EpCAM antibody-bound EVs; optionally wherein the subject is a human having or suspected of having breast cancer, triple negative breast cancer, lung cancer, NSCLC, SCLC, liver cancer, urinary tract cancer, bladder cancer, brain cancer, or glioblastoma. This disclosure also relates to processes of determining treatment for a subject with cancer, comprising: (a) providing a plasma sample from the subject, optionally wherein the sample is 100 μ l to 1 mL, from 200 μ l to 1 mL, from 250 μ l to 1 mL, from 100 μ l to 500 μ l, from 200 μ l to 500 μ l, from 200 μ l to 400 μ l, from 250 μ l to 500 μ l, or from 250 μ l to 400 μ l; (b) contacting the plasma sample with antibodies specific for EpCAM, optionally wherein the antibodies are attached to a matrix; (c) isolating antibody-bound extracellular vesicles (EVs) from unbound EVs the sample; (d) optionally resuspending the EpCAM antibody-bound EVs in a lysis buffer; (e) contacting the EVs with an antibody specific for PD-L1; (f) determining the level of PD-L1 in the EpCAM antibody-bound EVs; and (g) determining whether the subject should receive treatment with an immune checkpoint inhibitor (e.g. a PD-1 or PD-L1 inhibitor) based on the determined level of PD-L1 in the EpCAM antibody-bound EVs, wherein increases in PD-L1 levels correlate with need for treatment with the inhibitor. In one aspect, the disclosure provides a process of determining treatment for a subject with cancer, comprising: a. providing a plasma sample from the subject, optionally wherein the sample is 100 μ l to 1 mL, from 200 μ l to 1 mL, from 250 μ l to 1 mL, from 100 μ l to 500 μ l, from 200 μ l to 500 μ l, from 200 μ l to 400 μ l, from 250 μ l to 500 μ l, or from 250 μ l to 400 μ l; b. contacting the plasma sample with antibodies specific for EpCAM, optionally wherein the antibodies are attached to a matrix; c. isolating antibody-bound extracellular vesicles (EVs) from unbound EVs in the sample; d. optionally resuspending the EpCAM antibody-bound EVs in a lysis buffer; e. contacting the EVs with an antibody specific for PD-L1; f. determining the level of PD-L1 in the EpCAM antibody-bound EVs; g. determining whether the subject should receive treatment with an immune checkpoint inhibitor (e.g. a PD-1 or PD-L1 inhibitor) based on the determined level of PD-L1 in the EpCAM antibody-bound EVs, wherein increases in PD-L1 levels correlate with need for treatment with the inhibitor; optionally wherein the treatment is chosen from: i. a PD-L1 inhibitor, optionally wherein the PD-L1 inhibitor is atezolimumab, durvalumab, avelumab, envafolimab, BMS-936559, CK-301, CS-1001, SHR-1316, CBT-502, or BGB-A333; ii. a PD-1 inhibitor, optionally wherein the PD-1 inhibitor is nivolumab, pembrolizumab, cemiplimab, spartalizumab, camrelizumab, sintilimab, tislelizumab, toripalimab, AMP-224, or AMP-514; or iii. a CTLA-4 inhibitor, optionally wherein the CTLA-4 inhibitor is ipilimumab. In some embodiment examples, these processes may include determining whether the subject should receive a PD-L1 inhibitor such as atezolimumab, durvalumab, avelumab, envafolimab, BMS-936559, CK-301, CS-1001, SHR-1316, CBT-502, or BGB-A333. In some embodiment examples, the process may include determining whether the subject should receive a PD-1 inhibitor such as nivolumab, pembrolizumab, cemiplimab, spartalizumab, camrelizumab, sintilimab, tislelizumab, toripalimab, AMP-224, or AMP-514, or a CTLA-4 inhibitor such as ipilimumab. In some cases, the subject is a human having or suspected of having breast cancer, triple negative breast cancer,

lung cancer, NSCLC, SCLC, liver cancer, urinary tract cancer, bladder cancer, brain cancer, or glioblastoma. In some **embodimentexamples**, the process further comprises administering an immune checkpoint inhibitor such as a PD-1 or PD-L1 inhibitor to the subject.

[0027] In any of the processes described above, prior to contacting the sample with the antibodies specific to the cell surface marker, the sample can have been treated to remove particles above 1 μm , above 2 μm , above 5 μm , above 10 μm , or above 20 μm in diameter and/or to remove cellular bodies and debris larger than EVs.

[0028] In any of the processes described above, the EVs can have a particle size of less than 1 micron, such as 40 nm to less than 1 micron, 40-500 nm, 40-300 nm, 100-400 nm, or 100-300 nm, or have a mean particle size of 150-200 nm.

[0029] The present disclosure also provides kits for enriching or identifying cell-type specific extracellular vesicles (EVs) in a biological fluid sample from a subject. In one aspect, the disclosure provides a kit for enriching or identifying cell-type specific extracellular vesicles (EVs) in a biological fluid sample from a subject, the kit comprising antibodies specific for a cell surface marker attached to a matrix, and optionally further comprising: (a) one or more detection reagents for detection of a second biomarker, (b) one or more buffers for resuspending antibody-bound EVs and/or for detection of a second biomarker, and (c) instructions for use in enriching EVs from a biological fluid sample of a subject; optionally wherein the cell surface marker is: i. an epithelial cell surface marker, optionally wherein the epithelial cell surface marker is EpCAM, EGFR, PSMA, GSP64, CD3, CD49b, CD87, CD95, E-Cadherin CA9, CA12, N-cadherin, OB-cadherin, cadherin-11, a cytokeratin or epithelial membrane antigen (EMA); ii. a lung tissue marker, optionally wherein the lung tissue marker is EpCAM, CA9, CA12, DSG3, FAT2, GPR87, KISS1R, LYPD3, SLC7A11, TMPRSS4, CD133, prominin-1, AC133, programmed death-1 receptor (PD1) or FAS; iii. a breast tissue marker, optionally wherein the breast tissue marker is E-cadherin, epithelial membrane antigen (EMA), human epidermal growth factor receptor type 2 (Her2/neu), $\alpha\text{v}\beta 6$ integrin, EpCAM, carcinoembryonic antigen (CEA), folate receptor-alpha (FR- α), urokinase-type plasminogen activator receptor (uPAR) or placental-specific protein 1 (PLAC1); iv. a liver cell marker, optionally wherein the liver cell marker is CD133, Prominin-1, CD44, EpCAM, delta-like 1 non-canonical Notch ligand 1 (DLK1), ALDH, CD13, CD90, CD24, OV6, ICAM-1, CD34, C-kit, $\alpha 2\delta 1$, K19, LGR5, GPC3, Annexin A2, CD15, ABC transporters, Nope, DCLK1, ASGPR, CK or CD47; v. a glioblastoma biomarker, optionally wherein the glioblastoma biomarker is fibronectin, CD63, HSP70, Annexin A2, CD9, CD81, CD44, GRP78, CD133, CD15, a sialoglycoprotein, SLC1A3, PTPRZ1, GPR56, CLU or ALD1A3; vi. a urinary tract cell marker, optionally wherein the urinary tract cell marker is a tetraspanin, CD9, CD81, LAMP-1, CD10, CD24, CD44 or CD63; or vii. an amyloid marker, optionally wherein the amyloid marker is beta-amyloid precursor protein (β -APP), presenilin protein PS-1, or proinsulin protein PS-2. In some **embodimentexamples**, the kits may comprise antibodies specific for a cell surface marker attached to a matrix, and optionally further comprising: (a) one or more detection reagents for detection of a second biomarker, (b) one or more buffers for resuspending antibody-bound EVs and/or for detection of a second biomarker, and (c) instructions for use in enriching EVs from a biological fluid sample of a subject. In some **embodimentexamples**, the matrix comprises particles such as beads, pellets, or chips, which are optionally magnetic. In some **embodimentexamples**, the kits may enrich or identify EVs from epithelial cells, lung tissue cells, breast tissue cells, liver tissue cells, prostate tissue cells, kidney tissue cells, urinary tract cells, neural cells, tumor cells, solid tumor cells, lung tumor cells, breast tumor cells, liver tumor cells, prostate tumor cells, kidney tumor cells, urinary tumor cells, glioblastoma cells, or amyloid-beta expressing cells.

[0030] In some of the kits described herein, the cell surface marker is: (a) an epithelial cell surface marker, such as EpCAM, EGFR, PSMA, GSP64, CD3, CD49b, CD87, CD95, E-Cadherin CA9, CA12, N-cadherin, OB-cadherin, cadherin-11, a cytokeratin or epithelial membrane antigen (EMA); (b) a lung tissue marker, such as EpCAM, CA9, CA12, DSG3, FAT2, GPR87, KISS1R, LYPD3, SLC7A11, TMPRSS4, CD133, prominin-1, AC133, programmed death-1 receptor (PD1) or FAS; (c) a breast tissue marker, such as E-cadherin, epithelial membrane antigen (EMA), human epidermal growth factor receptor type 2 (Her2/neu), $\alpha\text{v}\beta 6$ integrin, EpCAM, carcinoembryonic antigen (CEA), folate receptor-alpha (FR- α), urokinase-type plasminogen activator receptor (uPAR) or placental-specific protein 1 (PLAC1); (d) a liver cell marker, such as CD133, Prominin-1, CD44, EpCAM, delta-like 1 non-canonical Notch ligand 1 (DLK1), ALDH, CD13, CD90, CD24, OV6, ICAM-1, CD34, C-kit, $\alpha 2\delta 1$, K19, LGR5, GPC3, Annexin A2, CD15, ABC transporters, Nope, DCLK1, ASGPR, CK or CD47; (e)