SYSTEMS, COMPOSITIONS, AND METHODS FOR TARGET ENTITY DETECTION

CROSS-REFERENCE TO RELATED APPLICATIONS

[1] This application claims the benefit of U.S. Provisional Application No. 62/812,878 filed March 01, 2019, and U.S. Provisional Application No. 62/962,722 filed January 17, 2020, the contents of each of which are hereby incorporated herein in their entirety.

BACKGROUND

[2] Early detection of cancer greatly increases the chance of successful treatment. However, many cancers 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

[2.1] The present disclosure, among other things, provides insights and technologies for achieving effective cancer screening. In one aspect, the present disclosure refers to a method comprising: contacting a sample with a first detection probe comprising a first target-binding moiety, a first double-stranded portion and a first-single stranded overhang, and a second detection probe comprising a second target-binding moiety, a second double-stranded portion and a second single-stranded overhang that is complementary to at least a portion of the first single-stranded overhang; wherein the first single-stranded overhang and the second single-stranded overhang hybridize together to form a double-stranded complex when the first and second target-binding moieties are bound to their respective targets; ligating at least one strand of the double-stranded complex to form a ligated template; and detecting the ligated template~~(a) contacting a sample that may comprise a biological entity of interest with at least one set of detection probes, each directed to a target, which set comprises at least a first detection probe for a first target and a second detection probe for a second target, so that a combination comprising the entity of interest and the set of detection probes is generated, wherein the first detection probe comprises a first target-binding moiety and a first oligonucleotide domain coupled to the first target-binding moiety, the first oligonucleotide domain comprising a first double-stranded portion and a first single-stranded overhang extended from one end of the first oligonucleotide domain; wherein the second detection probe comprises a second target-binding moiety and a second oligonucleotide domain coupled to the second target-binding moiety, the second oligonucleotide domain comprising a second double-stranded portion and a second single-stranded overhang extended from one end of the second oligonucleotide domain, wherein the second single-stranded overhang comprises a nucleotide sequence complementary to at least a portion of the first single-stranded overhang and can thereby hybridize to the first single-stranded overhang; and~~

~~wherein the first oligonucleotide domain and the second oligonucleotide domain have a combined length such that, when the first and second targets are simultaneously present on the entity of interest and the probes of the set of detection probes are bound to their respective targets on the entity of interest, the first single-stranded overhang and the second single-stranded overhang can hybridize together; (b) maintaining the combination under conditions that permit binding of the set of detection probes to their respective targets on the entity of interest such that, when the entity of interest comprises the first target and the second target, the first detection probe and the second detection probe bind to the entity of interest to form a double-stranded complex; (c) contacting the double-stranded complex with a nucleic acid ligase to generate a ligated template comprising a strand of the first double-stranded portion and a strand of the second double-stranded portion; and (d) detecting the ligated template, wherein presence of the ligated template is indicative of presence in the sample of the entity of interest comprising the first target and the second target~~.

[2.2] In another example~~aspect~~, the present disclosure refers to a method of detecting cancer, the method comprising steps of: detecting on surfaces of intact extracellular vesicles 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.

[2.3] In another example~~aspect~~, the present disclosure refers to a method comprising steps of contacting a sample comprising exosomes with a set of detection probes that specifically bind to surface biomarkers on the exosomes to detect cancer-associated exosomes in the sample with a specificity within a range of 95% to 100% and sensitivity within a range of 30% to 100%.

[2.4] In another example~~aspect~~, the present disclosure refers to a method comprising steps of: capturing exosomes from a biological sample with a capture agent that selectively interacts with a cancer-specific surface protein 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 protein 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 protein biomarkers.

[2.5] In another example~~aspect~~, the present disclosure refers to a method 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.

[2.6] In another example~~aspect~~, the present disclosure refers to a method 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.

[2.7] In another example~~aspect~~, the present disclosure refers to a method 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.

[2.8] In another example~~aspect~~, the present disclosure refers to a complex comprising: (a) an exosome having at least one target biomarker on its surface; and (b) a first detection probe and a second detection probe each bound to the exosome, wherein each of the first detection probe and the second detection probe comprises: (i) a target binding moiety directed to a target biomarker expressed by the exosome; 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.

[2.9] In another example~~aspect~~, the present disclosure refers to a composition comprising extracellular vesicles from a human blood sample bound to a set of at least two probes, each of which comprises a biomarker binding moiety and an oligonucleotide domain, wherein two or more bound probes are in proximity to one another so that their oligonucleotide domains hybridize to each other to form a ligatable hybrid.

[2.10] In another example~~aspect~~, the present disclosure refers to a complex comprising: (a) an exosome comprising a cancer-associated target biomarker signature; and (b) at least a first detection probe and a second detection probe each bound to the exosome, wherein each of the detection probes comprise: (i) a target binding moiety directed; to 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 at least partially complementary.

[2.11] In yet another example~~aspect~~, the present disclosure refers to a set of probes, each of which comprises: (a) a biomarker binding moiety that specifically binds to a surface biomarker on extracellular vesicles from cancer cells; and (b) an oligonucleotide domain, wherein the oligonucleotide domains of probes within the set are arranged and constructed so that, when the probes are bound to their target biomarkers, their oligonucleotide domains hybridize to one another to form a ligatable hybrid only when the target biomarkers are in proximity to one another.

CLAIMS

What is claimed is:

1. A method comprising:

~~(a)~~ contacting a sample with ~~that may comprise a biological entity of interest with at least one set of detection probes, each directed to a target, which set comprises at least a first detection probe for a first target and a second detection probe for a second target, so that a combination comprising the entity of interest and the set of detection probes is generated,~~

~~wherein the~~ a first detection probe comprising ~~comprises~~ a first target-binding moiety, a ~~and a first oligonucleotide domain coupled to the first target-binding moiety, the first oligonucleotide domain comprising a~~ first double-stranded portion and a first ~~-~~single ~~-~~stranded overhang, and a ~~extended from one end of the first oligonucleotide domain;~~

~~wherein the~~ second detection probe comprising~~comprises~~ a second target-binding moiety, ~~and a second oligonucleotide domain coupled to the second target-binding moiety, the second oligonucleotide domain comprising~~ a second double-stranded portion and a second single-stranded overhang that is~~extended from one end of the second oligonucleotide domain, wherein the second single-stranded overhang comprises a nucleotide sequence~~ complementary to at least a portion of the first single-stranded overhang; wherein the first single-stranded overhang and ~~can thereby hybridize to~~ the second~~first~~ single-stranded overhang hybridize together to form a double-stranded complex~~; and wherein the first oligonucleotide domain and the second oligonucleotide domain have a combined length such that,~~ when the first and second target-binding moieties~~targets are simultaneously present on the entity of interest and the probes of the set of detection probes~~ are bound to their respective targets~~; on the entity of interest, the first single-stranded overhang and the second single-stranded overhang can hybridize together;~~

~~(b)~~ ligating at least one strand of ~~maintaining the combination under conditions that permit binding of the set of detection probes to their respective targets on the entity of interest such that, when the entity of interest comprises the first target~~

and the second target, the first detection probe and the second detection probe bind to the entity of interest to form a double-stranded complex;

(c) contacting the double-stranded complex with a nucleic acid ligase to generate a ligated template comprising a strand of the first double-stranded complexportion and a strand of the second double-stranded portion to form a ligated template; and

(d) detecting the ligated template, wherein presence of the ligated template is indicative of presence in the sample of the entity of interest comprising the first target and the second target.

2. The method of claim 1, wherein the detecting comprises performing amplification of the ligated template and detecting the presence of anthe amplification product, optionally wherein the amplification is or comprises quantitative polymerase chain reaction.

3. The method of claim 1 or 2, further comprising immobilizing extracellular vesicleswherein from the sampleentity of interest is immobilized on a solid substrate prior to contacting the sample, optionally wherein the solid substrate is or comprises a bead.

4. The method of any one of claims 1-3, wherein method does not comprise, prior to the contacting of step (c), contacting the double-stranded complex with a connector oligonucleotide that associates the first oligonucleotide with the second oligonucleotide domain.

5. The method of claim 4, wherein the connector oligonucleotide hybridizes to at least a portion of the first oligonucleotide domain and at least a portion of the second oligonucleotide domain.

6.4. The method of any one of claims 1-53, wherein the first target-binding moiety and/or the second target-binding moiety comprise(s) an antibody agent.

7.5. The method of any one of claims 1-4,6, wherein the set of detection probes further comprisingcomprises contacting the sample with an additional detection probe for a third target, the additional detection probe comprising a third target-binding moiety, and a

third ~~oligonucleotide domain coupled to the third target-binding moiety, the third oligonucleotide domain comprising a~~ double-stranded portion, and a third single-stranded overhang ~~extended from each end of the third oligonucleotide domain~~.

~~8.~~6. The method of any one of claims 1-5, further comprising~~7, wherein the set of detection probes comprises~~ contacting the sample with 2-20 detection probes each for a specific target, each of the detection probes comprising a target-binding moiety, ~~and an oligonucleotide domain coupled to the target-binding moiety, the oligonucleotide domain comprising~~ a double-stranded portion, and a single-stranded overhang ~~extended from at least one end of the oligonucleotide domain~~.

~~9.~~7. The method of any one of claims 1-6,~~8, wherein the set of detection probes~~ further comprising~~comprises~~ contacting the sample with a control probe, wherein the control probe is characterized in that binding of the control probe to ~~the entity of interest~~ a control reference inhibits generation of a ligated template and/or inhibits amplification of a ligated template from a non-target region~~biological entity, optionally wherein the control probe is configured to bind to a control reference~~.

~~10.~~8. The method of claim 1, wherein the sample~~biological entity of interest~~ ~~is or~~ comprises a population of extracellular vesicles, optionally wherein at least one of the extracellular vesicles expresses~~expreses~~ a target biomarker signature for a disease, disorder, or condition, for example, wherein the disease, disorder, or condition is cancer, optionally

wherein the cancer is selected from the group consisting of bladder cancer, brain cancer, breast cancer, cervical cancer, chronic lymphocytic leukemia, chronic myeloid leukemia, colorectal cancer, endometrial cancer, esophageal cancer, gastrointestinal cancer, Hodgkin lymphoma, kidney cancer, liver cancer, lung cancer, multiple myeloma, non-Hodgkin lymphoma, ovarian cancer, pancreatic cancer, prostate cancer, sarcomas, skin cancer, and stomach cancer.

~~11.~~9. The method of claim 1, wherein the sample ~~is or~~ comprises a blood-derived sample from a subject.

~~12.~~10. The method of claim 9~~11~~, wherein the subject has at least one or more of the following characteristics:

a. (i) an asymptomatic subject who is susceptible to cancer;

(ii) a subject with a family history of cancer;

(iii) a subject determined to have one or more germline mutations in one or more cancer-associated genes;

(iv) an elderly subject;

(v) a subject with one or more non-specific symptoms of cancer;

(vi) a subject recommended for periodic cancer screening;

(vii) a subject diagnosed with an imaging-confirmed mass;

(viii) a subject at hereditary risk for cancer before undergoing a risk-reducing surgical intervention;

(ix) a subject with a benign tumor; and

(x) a subject who has been previously treated for cancer.

b. the sample has been subjected to size exclusion chromatography to isolate nanoparticles having a size range of interest that includes extracellular vesicles; or

c. the method is used in combination with one or more of the following diagnostic assays:

(i) the subject's annual physical examination;

(ii) a cancer screening test;

(iii) a genetic assay to screen blood plasma for genetic mutations in circulating tumor DNA and/or protein biomarkers linked to cancer;

(iv) an assay involving immunofluorescent staining to identify cell phenotype and marker expression, followed by amplification and analysis by next-generation sequencing; and

(v) germline and somatic mutation assays, or assays involving cell-free tumor DNA, liquid biopsy, serum protein and cell-free DNA, and/or circulating tumor cells.

~~13.~~11. The method of claim ~~8~~10, wherein the target biomarker signature comprises

(i) a first target biomarker comprising an extracellular vesicle-associated membrane-bound polypeptide; and

(ii) a second target biomarker comprising a target biomarker selected from the group consisting of: surface protein biomarkers, intravesicular protein biomarkers, and intravesicular RNA biomarkers.

~~14.~~12. The method of claim 1, further comprising performing a capture assay prior to contacting the sample.

~~step (a).~~

~~15.~~13. The method of claim 12~~14~~, wherein the capture assay involves contacting the sample

with a capture agent comprising a target-capture moiety that binds to an extracellular vesicle-associated membrane-bound polypeptide.

~~16. A method of detecting cancer, the method comprising steps of:~~

~~detecting on surfaces of intact extracellular vesicles 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.~~

~~17. The method of claim 1, wherein the cancer is selected from the group of cancers consisting of acute lymphocytic leukemia, acute myeloid leukemia, bile duct cancer, bladder cancer, brain cancer, breast cancer, cervical cancer, chronic lymphocytic leukemia, chronic myeloid leukemia, colorectal cancer, endometrial cancer, esophageal cancer, gastrointestinal cancer, Hodgkin lymphoma, kidney cancer, liver cancer, lung cancer, multiple myeloma, non-Hodgkin lymphoma, ovarian cancer, pancreatic cancer, prostate cancer, sarcomas, skin cancer, and stomach cancer, optionally wherein:~~

~~a) the cancer is colorectal cancer;~~

~~b) the cancer is lung cancer;~~

~~c) the cancer is breast cancer;~~

d) the cancer is ovarian cancer;

e) the step of detecting on surfaces comprises analyzing extracellular vesicles that have been separated from other components of the sample by affinity capture targeting a cancer-specific biomarker on their surfaces;

f) the step of detecting on surfaces comprises contacting the extracellular vesicles with at least one set of detection probes, each directed to a target biomarker, which set comprises at least a first detection probe for a first target biomarker and a second detection probe for a second target biomarker, for example wherein:

i) the first detection probe comprises a first target-binding moiety and a first oligonucleotide domain coupled to the first target-binding moiety, the first oligonucleotide domain comprising a first double-stranded portion and a first single-stranded overhang extended from one end of the first oligonucleotide domain; and

wherein the second detection probe comprises a second target-binding moiety and a second oligonucleotide domain coupled to the second target-binding moiety, the second oligonucleotide domain comprising a second double-stranded portion and a second single-stranded overhang extended from one end of the second oligonucleotide domain, wherein the second single-stranded overhang comprises a nucleotide sequence complementary to at least a portion of the first single-stranded overhang and can thereby hybridize to the first single-stranded overhang, optionally wherein:

A) the first single-stranded overhang and/or the second single-stranded overhang are four nucleotides in length, for example wherein the first single-stranded overhang or the second single-stranded overhang has a nucleotide sequence of GAGT; or

B) the first oligonucleotide domain and the second oligonucleotide domain have a combined length such that, when the first and second target biomarkers are simultaneously present on the extracellular vesicles and the probes of the set of detection probes are bound to their respective target biomarkers on the extracellular vesicles, the first single-stranded overhang and the second single-stranded overhang can hybridize together, forming a double-stranded complex,

for example further comprising contacting the double-stranded complex with a nucleic acid ligase to generate a ligated template comprising a strand of the first double-stranded portion and a strand of the second double-stranded portion, for example wherein the nucleic acid ligase is or comprises a DNA ligase (e.g., T4 or T7 DNA ligase); or

ii) the first target biomarker and the second target biomarker are the same target biomarker;

g) the step of detecting cancer further comprises a step of amplifying a product that is associated with the co-localization, and detecting the presence of the amplified product, optionally wherein the step of amplifying is or comprises quantitative polymerase chain reaction; or

h) the step of detecting on surfaces comprises immobilizing extracellular vesicles on a solid substrate, optionally wherein:

i) the solid substrate is or comprises a bead, for example wherein the bead is a magnetic bead; or

ii) the solid substrate is or comprises a surface, for example wherein the surface is a capture surface of a filter, a matrix, a membrane, a plate, a tube, and/or a well.

18. A method comprising steps of:

contacting a sample comprising exosomes with a set of detection probes that specifically bind to surface biomarkers on the exosomes to detect cancer-associated exosomes in the sample with a specificity within a range of 95% to 100% and sensitivity within a range of 30% to 100%.

19. The method of claim 18, wherein:

a) the sample comprising exosomes is a human blood-derived sample;

b) specificity is evaluated relative to that of a target-binding moiety for one or more other potential target entities;

c) the set of detection probes is selected for detection of cancer such that it provides a positive predictive value greater than about 10%; or

d) a sample is determined to be positive for cancer-associated exosomes if its detection level is at or above a reference threshold level that is set to achieve a specificity within a range of 95% to 100%, optionally wherein the reference threshold is about 2-3 standard

deviations above the mean detection level observed in a control sample, for example wherein the control sample is a blood-derived sample from a human subject who is not diagnosed with cancer; ore) the cancer is selected from the group of cancers consisting of acute lymphocytic leukemia, acute myeloid leukemia, bile duct cancer, bladder cancer, brain cancer, breast cancer, cervical cancer, chronic lymphocytic leukemia, chronic myeloid leukemia, colorectal cancer, endometrial cancer, esophageal cancer, gastrointestinal cancer, Hodgkin lymphoma, kidney cancer, liver cancer, lung cancer, multiple myeloma, non-Hodgkin lymphoma, ovarian cancer, pancreatic cancer, prostate cancer, sarcomas, skin cancer, and stomach cancer.

20. A method comprising steps of:

capturing exosomes from a biological sample with a capture agent that selectively interacts with a cancer-specific surface protein 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 protein 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 protein biomarkers.

21. The method of claim 20, wherein:

a) the at least one set of detection probes, collectively, detects a biomarker signature indicative of cancer;

b) the capture agent is a target-binding moiety;

c) the capture agent is an antibody, optionally wherein the antibody is coupled to a solid substrate, for example wherein:

i) the solid substrate is or comprises a bead, for example wherein the bead is a magnetic bead; or

ii) the solid substrate is or comprises a surface, optionally wherein the surface is a capture surface of a filter, a matrix, a membrane, a plate, a tube, and/or a well;

d) the step of capturing comprises immobilizing the exosomes on a solid substrate, optionally wherein:

i) the solid substrate is or comprises a bead; or

ii) the solid substrate is or comprises a surface, optionally wherein the surface is a capture surface of a filter, a matrix, a membrane, a plate, a tube, and/or a well;

e) the cancer is selected from the group of cancers consisting of acute lymphocytic leukemia, acute myeloid leukemia, bile duct cancer, bladder cancer, brain cancer, breast cancer, cervical cancer, chronic lymphocytic leukemia, chronic myeloid leukemia, colorectal cancer, endometrial cancer, esophageal cancer, gastrointestinal cancer, Hodgkin lymphoma, kidney cancer, liver cancer, lung cancer, multiple myeloma, non-Hodgkin lymphoma, ovarian cancer, pancreatic cancer, prostate cancer, sarcomas, skin cancer, and stomach cancer; or

f) the set of detection probes comprises two detection probes, optionally wherein the detection probes of the set of detection probes each comprise a target-binding moiety and an oligonucleotide domain comprising a double-stranded portion and a single-stranded overhang extended from one end of the oligonucleotide domain.

22. A method 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.

23. The method of claim 22, wherein:

a) at least two of the target binding moieties bind to the same epitope, optionally wherein the at least two binding moieties that bind the same epitope are the same moiety;

b) the target binding moiety of each of the detection probes binds the same epitope, optionally wherein the binding moieties that bind the same epitope are the same moiety;

c) each of the detection moieties is an oligonucleotide and the at least two detection probes include at least one pair of detection probes whose oligonucleotides hybridize to one another if and only if the detection probes are in close proximity to one another, optionally wherein the hybridization generates a ligatable nucleic acid; or

d) each probe of the set includes a target binding moiety that binds to a different epitope.

~~24. A method 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.~~

~~25. The method of claim 24, wherein:~~

~~a) presence or level of the surface biomarker signature is indicative of presence or risk of cancer;~~

~~b) the step of comparing comprises determining a change in presence or level of the surface biomarker signature;~~

~~c) the human subject is receiving or has received therapy and the change is indicative of the subject's response to the therapy, optionally wherein the human subject's response to the therapy determines whether a change in the therapy is needed;~~

~~d) the period of time is at least six months;~~

~~e) the human subject is an asymptomatic human subject;~~

~~f) the human subject has a family history of cancer;~~

~~g) the human subject has been previously treated for cancer;~~

~~h) the human subject is at risk for cancer;~~

~~i) the human subject is at risk for cancer recurrence after cancer treatment;~~

~~j) the human subject is in remission after cancer treatment;~~

~~k) the human subject was previously diagnosed to be suffering from or susceptible to cancer; or~~

~~l) the human subject is a human subject determined to be suffering from cancer,~~ ~~optionally wherein the method further comprises determining whether the human subject is likely to respond to a therapy based on the comparison between results of the performed assay with those of the prior assay.~~

~~26. A method 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.

27. The method of claim 26, wherein the step of contacting comprises contacting exosomes with at least a first detection probe and a second detection probe, optionally wherein the first detection probe comprises a first oligonucleotide entity comprising a first double-stranded portion and a first single-stranded overhang extended from one end of the oligonucleotide entity, and

the second detection probe comprises a second oligonucleotide entity comprising a second double-stranded portion and a second single-stranded overhang extended from one end of the oligonucleotide entity

for example wherein the first single-stranded overhang and/or the second single-stranded overhang are four nucleotides in length, for example wherein the first single-stranded overhang or the second single-stranded overhang has a nucleotide sequence of GAGT.

28. A complex comprising:

(a) an exosome having at least one target biomarker on its surface; and (b) a first detection probe and a second detection probe each bound to the exosome, wherein each of the first detection probe and the second detection probe comprises:

(i) a target binding moiety directed to a target biomarker expressed by the exosome; 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.

29. The complex of claim 28, wherein:

a) the first detection probe and the second detection probe are each bound to the same target biomarker;

b) the first detection probe and the second detection probe are each bound to different target biomarkers;

c) the formation of the complex is indicative of a cancer-associated exosome; or

d) the exosome was isolated from a blood sample taken from a subject, optionally wherein the subject is a human subject, for example wherein the human subject:

i) is an asymptomatic human subject;

ii) has a family history of cancer;

iii) has been previously treated for cancer;

iv) is at risk for cancer;

v) is at risk for cancer recurrence after cancer treatment;

vi) is in remission after cancer treatment;

vii) was previously diagnosed to be suffering from or susceptible to cancer; or

viii) is a human subject determined to be suffering from cancer.

30. A composition comprising extracellular vesicles from a human blood sample bound to a set of at least two probes, each of which comprises a biomarker binding moiety and an oligonucleotide domain, wherein two or more bound probes are in proximity to one another so that their oligonucleotide domains hybridize to each other to form a ligatable hybrid.

31. The composition of claim 30, wherein:

a) the set of probes comprises 2 probes;

b) at least two of the probes of the set have the same biomarker binding moiety;

c) at least two of the probes of the set have a different biomarker binding moiety; or

d) each of the oligonucleotide domains of each of the probes of the set of probes comprises a double-stranded portion and a single-stranded overhang extended from one end of the oligonucleotide domain, optionally wherein the single-stranded overhangs are four nucleotides in length, for example wherein the single-stranded overhang of the oligonucleotide domain of one of the probes has a nucleotide sequence of GAGT.

32. A complex comprising:

(a) an exosome comprising a cancer-associated target biomarker signature; and

(b) at least a first detection probe and a second detection probe each bound to the exosome, wherein each of the detection probes comprise:

(i) a target binding moiety directed; to 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 at least partially complementary.

33. A set of probes, each of which comprises:

(a) a biomarker binding moiety that specifically binds to a surface biomarker on extracellular vesicles from cancer cells; and

(b) an oligonucleotide domain,

wherein the oligonucleotide domains of probes within the set are arranged and constructed so that, when the probes are bound to their target biomarkers, their oligonucleotide domains hybridize to one another to form a ligatable hybrid only when the target biomarkers are in proximity to one another.

34. The set of probes of claim 33, wherein:

a) the set of probes comprises 2-25 probes;

b) the surface biomarker on extracellular vesicles from cancer cells is a biomarker for a cancer selected from the group of cancers consisting of acute lymphocytic leukemia, acute myeloid leukemia, bile duct cancer, bladder cancer, brain cancer, breast cancer, cervical cancer, chronic lymphocytic leukemia, chronic myeloid leukemia, colorectal cancer, endometrial cancer, esophageal cancer, gastrointestinal cancer, Hodgkin lymphoma, kidney cancer, liver cancer, lung cancer, multiple myeloma, non-Hodgkin lymphoma, ovarian cancer, pancreatic cancer, prostate cancer, sarcomas, skin cancer, and stomach cancer;

c) the set of probes is for use in screening a subject for cancer, optionally wherein:

i) the cancer is early-stage cancer;

ii) the subject is an asymptomatic human subject, for example wherein:

A) the asymptomatic human subject has a family history of cancer;

B) the asymptomatic human subject has been previously treated for cancer;

C) the asymptomatic human subject is at risk of cancer recurrence after cancer treatment; or

D) the asymptomatic human subject is in remission after cancer treatment; or

iii) the subject was previously screened for cancer;

d) the set of probes is for use in monitoring tumor recurrence in a subject who has been treated for cancer;

e) the set of probes is for use as a companion diagnostic in combination with cancer treatment;

f) the set of probes is for use in monitoring or evaluating efficacy of a therapy administered to a subject in need thereof; or

g) the set of probes is for use in selecting a therapy for a subject in need thereof.