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DIAGNOSTIC AND TREATMENT MONITORING BASED ON BLOOD-BRAIN BARRIER DISRUPTION

Abstract

The disclosure provides technologies that permit detection and/or characterization of brain biomarkers (e.g., disease-associated biomarkers) in non-CNS samples, including by liquid biopsy (e.g., blood, serum, etc.). Among other things, the present disclosure demonstrates that ultrasound opening of the blood brain barrier can achieve detectable increases in brain-derived materials (e.g., brain-derived proteins, neuron-derived extracellular vesicles, and/or cell-free DNA) in readily-sampled systemic liquids.

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Background/Summary

CROSS-REFERENCE TO RELATED APPLICATIONS [0001] This application is a U.S. National Stage Application under 37 U.S.C. § 371 of International Application No. PCT/IB2022/000376, filed Jun. 29, 2022, which claims priority to U.S. Provisional Application No. 63/217,543, filed on Jul. 1, 2021, the entire contents of each of which are incorporated herein.

DESCRIPTION OF THE TEXT FILE SUBMITTED ELECTRONICALLY
[0002] The content of the text file submitted electronically herewith is incorporated herein by reference in their entirety: A computer readable format copy of the Sequence Listing (Filename: “INS-124_ST25.txt”; Date created: Dec. 19, 2023; File size: 1,137 bytes).
BACKGROUND
[0003] Liquid biopsy involves the detection and analysis of pathology-derived material in blood without the need for invasive interventions such as open surgery. In cancer, it has the potential to provide a unifying platform for diagnosis, informing treatment selection by detecting resistant or sensitive tumor variants, and disease response monitoring. This approach has made strides for systemic cancers, with the development of several clinically approved assays for circulating tumor DNA (“ctDNA”) and circulating tumor cells, among others. Its use in, e.g., central nervous system (CNS) tumors, however, is severely limited by the blood-brain barrier (“BBB”), which prevents biomarker transit. Even with the altered blood-tumor barrier, there is substantial heterogeneity in permeability that can prevent representation of the tumor in circulation.
[0004] Similarly, diseases of the brain or central nervous system, e.g. Alzheimer's disease and Parkinson's disease, suffer from detection and diagnostic limitation due to the prevent of biomarker transit across the BBB and into the bloodstream.
[0005] Thus, there remains a significant need for improved compositions and methods that enable the sensitive detection of brain-derived biomarkers reflecting the pathology for clinical treatment and decision-making.
SUMMARY
[0006] Accordingly, in various aspects, the present disclosure relates to improved technologies for, e.g., diagnosis, disease typing (e.g., without limitation, determining disease subtype, mutation etiology, and the like), treatment selection, and/or theranosis, particularly of CNS diseases, disorders, conditions, and/or states, based on disruption, e.g. transient disruption, of the BBB.
[0007] In aspects, there is provided a method of diagnosing a disease or disorder of the brain in a human patient or patient population, comprising: (a) selecting a human patient or patient population suspected of having a disease or disorder of the brain or at risk for developing a disease or disorder of the brain; (b) applying an ultrasound beam across the cranium of the human patient or patient population to cause disruption, e.g. transient disruption, of the BBB of the human patient or patient population and to permit the transit of one or more brain-derived biomarkers into the blood; and (c) obtaining a plasma sample from the human patient or patient population; (d) contacting the plasma sample with a detection agent to detect the presence, absence or level of the one or more brain-derived biomarker; and (e) diagnosing the patient as being afflicted or not afflicted with the disease or disorder of the brain based on step (d).

[0008] In aspects, there is provided a method diagnosing a disease or disorder of the brain in a human patient or patient population, comprising: (a) obtaining a plasma sample from the human patient or patient population, the human patient or patient population having been exposed to an ultrasound beam across the cranium to cause disruption, e.g. transient disruption, of the BBB and to permit the transit of one or more brain-derived biomarkers into the blood; (b) contacting the plasma sample with a detection agent to detect the presence, absence or level of the one or more brain-derived biomarker; and (c) diagnosing the patient as being afflicted or not afflicted with the disease or disorder of the brain based on step (b).

[0009] In aspects, there is provided a method diagnosing and treating a disease or disorder of the brain in a patient, comprising: (a) obtaining a plasma sample from the human patient or patient population, the human patient or patient population having been exposed to an ultrasound beam across the cranium to cause disruption, e.g. transient disruption, of the BBB and to permit the transit of one or more brain-derived biomarkers into the blood; (b) detecting one or more brain-derived biomarkers by contacting the plasma sample with a detection agent; (c) diagnosing the patient with a disease or disorder of the brain when the one or more brain-derived biomarkers is in the plasma sample; and (d) administering an effective amount of a treatment agent to the diagnosed patient.

[0010] In aspects, there is provided a method of treating a disease or disorder of the brain in a patient, comprising administering an effective amount of a treatment agent to the patient, the patient having been exposed to an ultrasound beam across the cranium to cause disruption, e.g. transient disruption, of the blood-brain barrier (BBB) and to permit the transit of one or more brain-derived biomarkers into the blood undergone and diagnosed with the disease or disorder of the brain by detection of one or more brain-derived biomarkers in a plasma sample from the human patient.

[0011] In aspects, there is provided a method of evaluating or predicting a response to a treatment agent for a disease or disorder of the brain in a patient, comprising: (a) obtaining a plasma sample from the human patient or patient population, the human patient or patient population: (i) having been exposed to an ultrasound beam across the cranium to cause disruption, e.g. transient disruption, of the BBB and to permit the transit of one or more brain-derived biomarkers into the blood and (ii) undergoing treatment with one or more treatment agents; (b) detecting one or more brain-derived biomarkers by contacting the plasma sample with a detection agent; (c) determining whether the human patient or patient population is responsive, poorly responsive or non-responsive to the treatment agent based on step (b); and (d) administering: (i) an effective amount of the treatment agent to the responsive human patient or patient population or (ii) an alternative treatment agent, an adjunctive treatment agent or a different amount of the treatment agent to the poorly responsive or non-responsive human patient or patient population.

[0012] In aspects, there is provided a method of conducting an adaptive clinical trial for developing a treatment agent, the method comprising: (a) obtaining a treatment agent; (b) enrolling a first patient population and a second patient population into the adaptive clinical trial; (c) systemically administering the treatment agent to the first patient population; (d) systemically administering the treatment agent to the second patient population contemporaneously with an ultrasound beam across the cranium to cause disruption, e.g. transient disruption, of the BBB and allow transit of the treatment agent therethrough; and (e) comparing treatment efficacy of the treatment agent in the first patient population and the second patient population.

[0013] In aspects, there is provided a method of conducting an adaptive clinical trial for developing a treatment agent, the method comprising: (a) obtaining a treatment agent; (b) enrolling a patient population into the adaptive clinical trial; (c) administering the treatment agent to the patient population contemporaneously with an ultrasound beam across the cranium to cause disruption, e.g. transient disruption, of the BBB to permit the transit of one or more brain-derived biomarkers into the blood; (d) measuring the biomarkers in a plasma sample from the patient population; and

(e) adjusting the type of treatment or dose of the treatment agent if the measuring indicates non-efficacious treatment agent effect.
[0014] In aspects, there is provided a method of conducting an adaptive clinical trial for developing a treatment agent, the method comprising: (a) obtaining a treatment agent; (b) adding an imaging tracer to the treatment agent to non-invasively track its biodistribution in the body; (c) enrolling a patient population into the adaptive clinical trial; (d) administering the traced treatment agent to the patient population contemporaneously with an ultrasound beam applied across the cranium to cause disruption, e.g. transient disruption, of the BBB and allow transit of the treatment agent to the target regions; (e) measuring drug delivery to the target brain regions via the non-invasive imaging tracer signal, (f) comparing efficacy of drug delivery of the treatment agent in the patient population to a second patient population receiving a non-traced treatment agent; (g) measuring biomarkers in a plasma sample from the patient population of brain-derived biomarkers released by the disruption of the BBB, specific to the region where treatment agent is being delivered, (h) determining the effect of improved drug delivery across the BBB on clinical outcomes and/or measured biomarkers, optionally pharmacokinetics and/or pharmacodynamics, released from the target region; (i) adjusting the type of treatment or dose of the treatment agent if the measuring indicates non-efficacious treatment agent effect.
[0015] In aspects, there is provided a method of conducting an adaptive clinical trial for developing a treatment agent, the method comprising: (a) obtaining a treatment agent; (b) adding an imaging tracer to the treatment agent to non-invasively track its biodistribution in the body; (c) enrolling a patient population into the adaptive clinical trial; (d) administering the traced treatment agent to the patient population contemporaneously with an ultrasound beam applied across the cranium to cause disruption, e.g. transient disruption, of the BBB and allow transit of the treatment agent to the target regions; (e) measuring drug delivery to the target brain regions via the non-invasive imaging tracer signal, (f) comparing efficacy of improving drug delivery of the treatment agent in the patient population to a second patient population receiving the traced treatment agent without ultrasound BBB disruption; (g) measuring biomarkers in a plasma sample from the patient population of brain-derived biomarkers released by the disruption of the BBB, specific to the region where treatment agent is being delivered, (h) determining the effect of improved drug delivery across the BBB on clinical outcomes and/or measured biomarkers, optionally pharmacokinetics and/or pharmacodynamics, released from the target region; (i) adjusting the type of treatment or dose of the treatment agent if the measuring indicates non-efficacious treatment agent effect.
[0016] The details of one or more examples of the disclosure are set forth in the description below. Other features or advantages of the present disclosure will be apparent from the following drawings, detailed description of several examples, and also from the appended claims. The details of the disclosure are set forth in the accompanying description below. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, illustrative methods and materials are now described. Other features, objects, and advantages of the disclosure will be apparent from the description and from the claims. In the specification and the appended claims, the singular forms also include the plural unless the context clearly dictates otherwise. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs.

Description

BRIEF DESCRIPTION OF THE DRAWING
[0017] The patent or application file contains at least one drawing executed in color. Copies of this

patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0018] FIG. **1**A, FIG. **1**B, FIG. **1**C, FIG. **1**D, and FIG. **1**E are images showing how opening of the blood-brain barrier in large brain regions is achieved with transcranial MR-guided focused ultrasound. FIG. **1**A shows a diagram of a magnetic resonance guided focused ultrasound ("MRgFUS") device and study design with a blood-brain barrier opening ("BBBO") procedures overlapping adjuvant chemotherapy regimen in patients with glioblastoma after surgical resection and concurrent chemoradiation. Blood samples are collected immediately before and after sonication on the same day of the procedure. FIG. **1**B shows images of the lesions (yellow circles) on baseline MRI for each patient. FIG. **1**C shows images how ultrasound can be targeted specifically to an operator-defined contour. The green polygon represents one of many target contours (others not shown). The color map indicates the cavitation dose detected by device upon ultrasound delivery. In FIG. **1**D, the top panel shows increased contrast extravasation in the areas of increased BBB permeability after MRgFUS for P5 (yellow arrow). The bottom panel of FIG. **1**D shows partial restoration of the BBB integrity approximately 24 hours later, in comparison to the top panel and baseline image of the tumor in FIG. **1**B. FIG. **1**E shows images of intensity difference maps and further show increased BBB permeability in P5 within large regions spatially distributed in the tumor and tumor margins. Heterogeneous changes in contrast extravasation is partially due to the underlying tumor.

[0019] FIG. **2**A, FIG. **2**B, FIG. **2**C, FIG. **2**D, FIG. **2**E, and FIG. **2**F are graphs showing how transient blood-brain barrier opening enriches signal of circulating biomarkers. FIG. **2**A is a graph showing how plasma cfDNA concentration was significantly enhanced after noninvasive MRgFUS BBBO. Each data point represents the mean cfDNA concentration for each patient. FIG. **2**B is a graph showing the fold change in plasma cfDNA concentration, which is graphed over the course of adjuvant chemotherapy. Each gray line represents a single patient. The solid black line indicates the group mean at each cycle. FIG. **2**C is a graph showing the concentration of single nucleosome length cfDNA and how it was also increased after BBBO. FIG. **2**D is a graph showing the fold change in plasma cfDNA concentration at each cycle, which is plotted against the volume of BBBO, demonstrating a positive Spearman's correlation between the two variables. Each point represents the data at one cycle. FIG. **2**E is graph showing L1CAM (also known as CD171), and NCAM double-positive extracellular vesicles are increased after BBBO. FIG. **2**F is a graph showing how plasma S100b levels are significantly increased after BBBO. In FIG. **2**C, FIG. **2**E, and FIG. **2**F, measurements were taken pre- and post-BBBO at only one cycle per patient, given availability of samples. In FIG. **2**E, "ndEV" refers to "neuron-derived extracellular vesicle."

[0020] FIG. **3**A, FIG. **3**B, and FIG. **3**C are graphs showing how post-BBBO cfDNA have distinctive disease-specific signature. FIG. **3**A is a graph of principle component analysis showing a separation of the methylation signature of pre- and post-BBBO cfDNA. FIG. **3**B is graph showing the result of a functional enrichment analysis of the differentially hypomethylated probe set based on the Human Protein Atlas. FIG. **3**C is graph showing the results of a principle component analysis of methylation data with cfDNA from non-brain tumor patients post-BBBO as controls suggest disease-specific signatures. In FIG. **3**C, "GBM" refers to glioblastoma.

[0021] FIG. **4** is a schematic showing a MRgFUS-based platform for both enhanced drug delivery and liquid biopsy. FIG. **4** is a concept diagram of a MRgFUS-based platform for both enhancing drug delivery and liquid biopsy to deliver personalized medicine for patients with brain tumors. MRgFUS can be combined with repeated doses of anti-tumor therapy (1), each time providing an opportunity to collect blood samples containing analytes derived from the pathology in the sonicated areas (2). After pre-processing (3) and extraction for specific markers (4), commercially available techniques were used for processing (5, e.g., droplet digital PCR). Biological changes in the pathology might inform a change in anti-tumor therapy (6).

[0022] FIG. **5** shows two images of a DNA electrophoresis of nine paired pre-post BBBO cfDNA

samples. As shown in FIG. **5**, the plasma 0 to 280 bp fragment size cfDNA is increased in each patient after MRgFUS induced BBBO.

[0023] FIG. **6** is a graph showing the fold change in cfDNA compared to time elapsed from completion of sonication. In FIG. **6**, "p" refers to Spearman's correlation.

[0024] FIG. **7**A and FIG. **7**B are graphs showing how the MRgFUS induced enhancement in cfDNA concentration at the initial TMZ cycle is stratified by patients with or without residual tumor on MRI. FIG. **7**A shows level of cfDNA enhancement in two patients with a subtotal resection. FIG. **7**B shows the fold change in plasma cfDNA at the last cycle for each patient normalized to their initial cycle, stratified by patients who did and did not have progressive disease by RANO Criteria.

[0025] FIG. **8** is a graph showing nanoscale flow cytometry data. Nanoscale flow cytometry data of plasma samples from P2 and P7 in FIG. **8** shows the number of L1CAM (vertical axis) and NCAM (horizontal axis) double-positive particles (quadrant B) increases from pre-to post-BBBO.

[0026] FIG. **9** is a graph showing a comparison of differentially methylated probes between post-vs pre-BBBO cfDNA. In FIG. **9**, volcano plot shows differentially methylated probes between post-vs pre-BBBO are 95% more hypomethylated, consistent with a cancer signature. Dots to the left of 0.0 on the x axis of the graph indicate hypomethylated probes in post compared to pre-BBBO samples, and to the right of 0.0, hypermethylated probes. Red dots represent significantly differentially methylated probes that were identified by an absolute difference of mean beta value >0.1 and FDR-corrected $p<0.05$.

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS

[0027] The present invention is based, in part, on the surprising discovery that ultrasound application to the brain enriches the signal of circulating brain-derived biomarkers, demonstrating the potential of the technology to support liquid biopsy for the brain. As disclosed herein, MRgFUS can non-invasively open the BBB, which normally limits therapeutic access to brain regions and also limits shedding of brain pathology-derived biomarkers into the bloodstream for liquid biopsy, and therefore focused ultrasound enhances circulating brain-derived biomarkers, supporting the feasibility of focused ultrasound to aid liquid biopsy.

[0028] Liquid biopsy is promising for detection, monitoring of response, and for detecting the recurrence of cancer. However, the blood-brain barrier ("BBB") limits the transit of biomarker, such as cell-free DNA (cfDNA), into the blood, and their detection by conventional assays. To address this hurdle, MRgFUS is an emerging technology that permits non-invasive access to brain pathology by inducing transient BBB opening (BBBO). MRI-guidance imparts spatially precise and flexible selection of sonicated brain regions.

[0029] Experiments and examples of the present disclosure, among other things, represent the first in-human demonstration that non-invasive, transient opening of the BBB using focused ultrasound technology can enrich circulating brain-derived biomarkers with potential applications in liquid biopsy. As disclosed herein, transcranial focused ultrasound can safely and open, e.g. transiently open, the BBB, providing an opportunity for less-invasive access to brain pathology. Furthermore, precise targeting achieved by present technologies ensures that materials (e.g., biomarkers) released into the blood via the BBB opening, e.g. transient BBB opening, are co-localized with, and thus likely to be associated with, brain pathology (e.g., a tumor or other site of damage, etc.).

Experiments and examples disclosed herein demonstrate, among other things, that focused ultrasound can enrich the signal of circulating brain-derived biomarkers to aid in liquid biopsy. In aspects, there is provided a method of diagnosing a disease or disorder of the brain in a human patient or patient population, comprising: (a) selecting a human patient or patient population; (b) applying an ultrasound beam across the cranium of the human patient or patient population to cause disruption, e.g. transient disruption, of the BBB of the human patient or patient population and to permit the transit of one or more brain-derived biomarkers into the blood; and (c) obtaining a plasma sample from the human patient or patient population; (d) contacting the plasma sample with

a detection agent to detect the presence, absence or level of the one or more brain-derived biomarker; and (e) diagnosing the patient as being afflicted or not afflicted with the disease or disorder of the brain based on step (d).

[0030] In aspects, there is provided a method of diagnosing a disease or disorder of the brain in a human patient or patient population, comprising: (a) selecting a human patient or patient population suspected of having a disease or disorder of the brain or at risk for developing a disease or disorder of the brain; (b) applying an ultrasound beam across the cranium of the human patient or patient population to cause disruption, e.g. transient disruption, of the BBB of the human patient or patient population and to permit the transit of one or more brain-derived biomarkers into the blood; and (c) obtaining a plasma sample from the human patient or patient population; (d) contacting the plasma sample with a detection agent to detect the presence, absence or level of the one or more brain-derived biomarker; and (e) diagnosing the patient as being afflicted or not afflicted with the disease or disorder of the brain based on step (d).

[0031] In aspects, there is provided a method diagnosing a disease or disorder of the brain in a human patient or patient population, comprising: (a) obtaining a plasma sample from the human patient or patient population, the human patient or patient population having been exposed to an ultrasound beam across the cranium to cause disruption, e.g. transient disruption, of the BBB and to permit the transit of one or more brain-derived biomarkers into the blood; (b) contacting the plasma sample with a detection agent to detect the presence, absence or level of the one or more brain-derived biomarker; and (c) diagnosing the patient as being afflicted or not afflicted with the disease or disorder of the brain based on step (b).

[0032] In aspects there is provided a method of evaluating a subtype or mutational basis of a disease or disorder of the brain in a human patient or patient population, comprising: (a) selecting a human patient or patient population; (b) applying an ultrasound beam across the cranium of the human patient or patient population to cause disruption, e.g. transient disruption, of the BBB of the human patient or patient population and to permit the transit of one or more brain-derived biomarkers into the blood; (c) obtaining a plasma sample from the human patient or patient population; (d) contacting the plasma sample with a detection agent to detect the presence, absence or level of the one or more brain-derived biomarkers; and (e) determining subtype or mutational basis of the disease or disorder based on step (b).

[0033] In aspects there is provided a method of evaluating a subtype or mutational basis of a disease or disorder of the brain in a human patient or patient population, comprising: (a) selecting a human patient or patient population suspected of having a disease or disorder of the brain or at risk for developing a disease or disorder of the brain; (b) applying an ultrasound beam across the cranium of the human patient or patient population to cause disruption, e.g. transient disruption, of the BBB of the human patient or patient population and to permit the transit of one or more brain-derived biomarkers into the blood; (c) obtaining a plasma sample from the human patient or patient population; (d) contacting the plasma sample with a detection agent to detect the presence, absence or level of the one or more brain-derived biomarkers; and (e) determining subtype or mutational basis of the disease or disorder based on step (b).

[0034] In aspects there is provided a method of evaluating a subtype or mutational basis of a disease or disorder of the brain in a human patient or patient population, comprising: (a) obtaining a plasma sample from the human patient or patient population, the human patient or patient population having been exposed to an ultrasound beam across the cranium to cause disruption, e.g. transient disruption, of the BBB and to permit the transit of one or more brain-derived biomarkers into the blood; (b) contacting the plasma sample with a detection agent to detect the presence, absence or level of the one or more brain-derived biomarkers; and (c) determining subtype or mutational basis of the disease or disorder based on step (b).

[0035] In aspects, there is provided a method diagnosing and treating a disease or disorder of the brain in a patient, comprising: (a) obtaining a plasma sample from the human patient or patient

population, the human patient or patient population having been exposed to an ultrasound beam across the cranium to cause disruption, e.g. transient disruption, of the BBB and to permit the transit of one or more brain-derived biomarkers into the blood; (b) detecting one or more brain-derived biomarkers by contacting the plasma sample with a detection agent; (c) diagnosing the patient with a disease or disorder of the brain when the one or more brain-derived biomarkers is in the plasma sample; and (d) administering an effective amount of a treatment agent to the diagnosed patient.

[0036] In aspects, there is provided a method of treating a disease or disorder of the brain in a patient, comprising administering an effective amount of a treatment agent to the patient, the patient having been exposed to an ultrasound beam across the cranium to cause disruption, e.g. transient disruption, of the blood-brain barrier (BBB) and to permit the transit of one or more brain-derived biomarkers into the blood undergone and diagnosed with the disease or disorder of the brain by detection of one or more brain-derived biomarkers in a plasma sample from the human patient.

[0037] In aspects, there is provided a method of evaluating or predicting a response to a treatment agent for a disease or disorder of the brain in a patient, comprising: (a) obtaining a plasma sample from the human patient or patient population, the human patient or patient population: (i) having been exposed to an ultrasound beam across the cranium to cause disruption, e.g. transient disruption, of the BBB and to permit the transit of one or more brain-derived biomarkers into the blood and (ii) undergoing treatment with one or more treatment agents; (b) detecting one or more brain-derived biomarkers by contacting the plasma sample with a detection agent; (c) determining whether the human patient or patient population is responsive, poorly responsive or non-responsive to the treatment agent based on step (b); and (d) administering: (i) an effective amount of the treatment agent to the responsive human patient or patient population or (ii) an alternative treatment agent, an adjunctive treatment agent or a different amount of the treatment agent to the poorly responsive or non-responsive human patient or patient population. In embodiments, the results of step (c) direct the administration of step (d). In embodiments, determining can be serial, e.g. relative to a first administration or relative to a previous administration, e.g. to provide a dynamic read out. In embodiments, the technology allows for dose correction, e.g. increasing or decreasing a dose of the treatment agent based on the results of the biomarker data. In embodiments, provided methods comprise performing multiple assessments as described, e.g. prior to initiation of therapy and after initiation of therapy. In embodiments, a method provides an outcome of maintaining therapy as it was before the evaluation or modify (e.g., dosing) of same therapy as compared to before the evaluation; or changing to different therapy as compared to before the evaluation.

[0038] In aspects, there is provided a method of determining progression or lack of progression of disease or disorder of the brain in a human patient or patient population, comprising: (a) selecting a human patient or patient population having a disease or disorder of the brain; (b) applying an ultrasound beam across the cranium of the human patient or patient population to cause disruption, e.g. transient disruption, of the BBB of the human patient or patient population and to permit the transit of one or more brain-derived biomarkers into the blood; and (c) obtaining a plasma sample from the human patient or patient population; (d) contacting the plasma sample with a detection agent to detect the presence, absence or level of the one or more brain-derived biomarker; and (e) determining the progression or lack of progression of the disease or disorder of the brain based on step (d). In embodiments, determining can be serial, e.g. relative to a first time point or relative to a previous time point, e.g. to provide a dynamic read out.

[0039] In aspects, the present disclosure relates to the focused ultrasound-based platform for both enhanced drug delivery and liquid biopsy as depicted in FIG. **4**.

[0040] In aspects, the present disclosure relates to methods that allow, e.g. differentiating radiation necrosis from tumor recurrence. In aspects, the present disclosure relates to methods that allow, e.g.

diagnosing lesions where surgical biopsy is risky or surgical debulking is unnecessary.
[0041] In embodiments, there is provided a method in which patient's tumor sample is characterized for somatic mutations that are specific to the patient's tumor to establish a Tumor Mutational Burden (TMB). In embodiments, a higher TMB indicates increased likelihood that the tumor will respond to immunotherapy. In embodiments, a TMB above a particular threshold, and/or presence of certain mutations, is used as a companion diagnostic for selecting therapy (e.g., immunotherapy and/or other targeted therapies). In embodiments, present methods allow for the creation of a patient-specific signature (e.g., that is or comprises one or more somatic mutations, or a set thereof, and/or a particular TMB level) that can be detected in the blood and, in some embodiments, tracked over time. In embodiments, a higher level of somatic mutation found in blood as described herein indicates a higher tumor burden (e.g., larger tumor size and/or greater number of tumors) in the subject. In embodiments, a lower level of somatic mutation found in blood as described herein indicates a lower tumor burden (e.g., smaller tumor size and/or fewer tumors).
[0042] In embodiments, present technologies avoid false negatives that are resultant from the BBB which normally blocks the transit of biomarkers into the bloodstream.
[0043] In embodiments, provided technologies may be used, for example, to track minimal residual disease status, early recurrence, and/or to monitor response to treatment, for example by detecting a determined signature (e.g., a mutation signature, such as a patient-specific signature as described herein, or another appropriate tumor signature determined in accordance with the present disclosure and/or through use of other technologies). In embodiments, if a tumor is progressing, a signature (e.g. a number of mutations and/or presence of particular mutations) increases. In embodiments, if a tumor is regressing (e.g., in response to treatment), a signature (e.g. a number of mutations and/or presence of particular mutations) clears from the bloodstream, i.e. decreases. If a tumor is pseudo-progressing (e.g., if immune inflammation is creating an appearance on imaging of tumor growth without actual tumor growth, and in some cases despite good treatment response) the signature does not increase. Those skilled in the art, reading the present disclosure, will appreciate that the same workflow can be utilized for a variety of types of signatures—e.g., those including only somatic tumor mutations (and/or numbers thereof), and/or those including features such as methylation patterns and/or other epigenetic modifications in addition or as alternatives to somatic mutations.
[0044] In embodiments, there is provided a method of evaluating and/or predicting a response to a treatment comprising obtaining a plasma sample from a human patient exposed to an ultrasound beam to permit transit of brain-derived biomarkers wherein the brain derived biomarkers are the number of somatic mutations (e.g., of tumor-associated somatic mutations) identified in the bloodstream. In embodiments, a low amount of somatic mutations obtained after BBB disruption indicates minimal residual disease. In embodiments, an amount of somatic mutations obtained after BBB disruption increases over time in association with tumor recurrence during disease monitoring and allows early detection of recurrent disease. In embodiments, a decrease and overall clearance rate of the total number of somatic mutations over time is indicative of successful treatment response (e.g. classification of responders). In embodiments, an increase in total number of somatic mutations over time after treatment initiation is indicative of disease progression and/or treatment resistance (e.g. classification of non-responders). In embodiments, a reduction in number of observed somatic mutations over time despite increase in size of the tumor on imaging is indicative of a diagnosis of pseudo-progression.
[0045] In embodiments, the present methods involve real time monitoring during treatment by, e.g. acoustic response or MRI imaging to assess at least one of safety and efficacy of procedure.
Brain-Derived Biomarkers
[0046] In embodiments, the present disclosure provides technologies relating to evaluation, e.g. without limitation, measuring a presence, absence, or level of one or more brain-derived

biomarkers. In embodiments, provided technologies relate to detecting and/or quantifying or otherwise characterizing one or more brain-derived biomarkers.
[0047] In embodiments, detection of one or more brain-derived biomarker(s) as described herein is employed in decision-making regarding diagnosis, disease typing (e.g., without limitation, determining disease subtype, mutation etiology, and the like), treatment selection, and/or theranosis, as described herein.
[0048] In embodiments, a brain-derived biomarker detected in accordance with the present disclosure circulates only in the cerebrospinal fluid in the absence of the focused ultrasound. In embodiments, “circulates” means that the biomarker is present at levels that are detectable in samples of the relevant biological fluid.
[0049] In embodiments, a brain-derived biomarker is contained within the brain and/or cerebrospinal fluid and is absent from the blood, without wishing to be bound by any particular theory, presumably due to the BBB. In embodiments, a brain-derived biomarker is contained within the brain and/or cerebrospinal fluid and prevented from transiting to the blood by the BBB. In embodiments, upon application of ultrasound energy as described herein to locally disrupt the BBB, a brain-derived biomarker transits from the cerebrospinal fluid to the blood via the disrupted BBB. In embodiments, a brain-derived biomarker is not substantially perturbed from a tumor cell by the ultrasound application or exposure. For instance, the ultrasound application or exposure allows for transit of free biomarker and does not alter the tissue from which the biomarker is derived. In embodiments, present methods do not target ultrasound at the tumor cells directly but target the tumor microenvironment (TME). In embodiments, present methods do not target ultrasound at tumor pathology, but other brain regions for region specific biomarker analysis, for example neurodegenerative disease, or other functional areas.
[0050] In embodiments, provided technologies target ultrasound at microbubbles, which act as an activator and/or interact with endothelial cells which open the BBB. In embodiments, provided technologies target ultrasound at the microbubbles within blood vessels.
[0051] In embodiments, a brain-derived biomarker is or comprises a disease or drug response biomarker.
[0052] In embodiments, a brain-derived biomarker is or comprises a nucleic acid.
[0053] In embodiments, a nucleic acid is or comprises DNA or RNA. In embodiments, a nucleic acid is or comprises cell-free DNA (cfDNA). In embodiments, a nucleic acid is or comprises circulating tumor DNA (ctDNA). In embodiments, a nucleic acid is or comprises a microRNA.
[0054] In embodiments, a brain derived biomarker is or comprises a protein. In embodiments, a brain derived biomarker is or comprises a cytokine.
[0055] In embodiments, a brain derived biomarker is or comprises a whole cell.
[0056] In embodiments, a brain derived biomarker is or comprises an extracellular vesicle. In embodiments, a extracellular vesicle is or comprises an exosome, ectosome, or large oncosome.
[0057] In embodiments, a brain-derived biomarker is or comprises a metabolite.
[0058] In embodiments, a brain-derived biomarker is or comprises a cancer biomarker. In embodiments, a cancer biomarker is selected from AFP, BCR-ABL, BRCA1/BRCA2, BRAF V600E, CA-125, CA19.9, CEA, EGFR, HER-2, KIT, PSA, PD-1, PD-L1, and $100. In embodiments, a cancer biomarker and cancer is selected from AFP (liver cancer), BCR-ABL (chronic myeloid leukemia), BRCA1/BRCA2 (breast/ovarian cancer), BRAF V600E (melanoma/colorectal cancer), CA-125 (ovarian cancer), CA19.9 (pancreatic cancer), CEA (colorectal cancer), EGFR (non-small-cell lung carcinoma), HER-2 (breast cancer), KIT (gastrointestinal stromal tumor), PSA (prostate specific antigen) (prostate cancer), and S100 (melanoma). In embodiments, a cancer biomarker and cancer is selected from mutations on genes KRAS, p53, EGFR, erbB2 for colorectal, esophageal, liver, and pancreatic cancer; mutations of genes BRCA1 and BRCA2 for breast and ovarian cancer; hypermethylation of MYOD1, CDH1, and CDH13 for cervical cancer; and hypermethylation of p16, p14, and RB1, for oral cancer In

embodiments, a cancer biomarker and cancer is abnormal methylation of tumor suppressor genes p16, CDKN2B, and p14ARF for brain cancer.
[0059] In embodiments, a cancer biomarker is or comprises a Tumor Mutation Burden (TMB) profile. In embodiments, a detection of a high TMB (TMB-H) directs administration of an immunotherapeutic agent, optionally a checkpoint immunotherapy, optionally directed to
[0060] PD-1 or PD-L1. In embodiments, a TMB-H is at least about 10 mutations/megabase based on a sequencing-based detection method, optionally FoundationOne CDx assay In embodiments, a cancer biomarker is or comprises a glioblastoma biomarker. In embodiments, a glioblastoma biomarker is selected from IDH mutations, 1p19q deletion, MGMT promoter methylation, and EGFRvIII amplification. In embodiments, a IDH mutation is R132H.
[0061] In embodiments, a disease biomarker is or comprises an Alzheimer's disease biomarker. In embodiments, a Alzheimer's disease biomarker is selected from amyloid beta protein, tau protein, APOE ε4 variant, APP mutation, PSEN1 mutations, and PSEN2 mutations, methylation signatures and other epigenetic biomarkers on the released brain region-specific cfDNA.
[0062] In embodiments, a disease biomarker is or comprises a Parkinson's disease biomarker. In embodiments, a Parkinson's disease biomarker is selected from alpha-synuclein and GBA mutations, methylation signatures and other epigenetic biomarkers on the released brain region-specific cfDNA.
Disease or Disorder of the Brain
[0063] In embodiments, present disclosure relates to diagnosis, disease typing (e.g., without limitation, determining disease subtype, mutation etiology, and the like), treatment selection, and/or theranosis that concerns various diseases, disorders, conditions, and/or states of the brain.
[0064] In embodiments, a disease or disorder of the brain is or comprises a cancer. In embodiments, a cancer is or comprises a metastatic tumor in the brain. In embodiments, a metastatic tumor in the brain originates in a breast, lung, skin (including melanoma), prostate gland, uterus, kidney or gastrointestinal tract. In embodiments, a tumor is or comprises a primary brain tumor. In embodiments, a primary brain tumor is glial. In embodiments, a glial tumor is one or more of astrocytoma, ependymoma, glioblastoma multiforme (GBM), medulloblastoma, and oligodendroglioma. In embodiments, a primary brain tumor is non-glial. In embodiments, a tumor is a posterior fossa tumor, optionally selected from a cerebellar tumor, pontine tumor, and medulloblastoma (primitive neuroectodermal tumor). In embodiments, a tumor is a cerebral hemisphere tumor. In embodiments, a cancer is or comprises a glioblastoma.
[0065] In embodiments, present method involves BBB disruption and liquid biopsy (e.g., of a liquid other than a CNS liquid—e.g., other than cerebrospinal fluid, and in particular of blood) with use of specific biomarkers, which may, for example, include one or more of proteins, cell free nucleic acid (e.g., cfDNA and/or cfRNA), one or more epigenetic (e.g., methylation) signatures, signatures of “hot” and “cold” tumors, etc. When combined with immunotherapy and/or immune priming adjuvant therapy, the method allows for the simultaneous detection of immune antigen release and immune response/antigen presentation to monitor and guide therapy.
[0066] In embodiments, provided methods, e.g. by virtue of directly acoustic energy to the TME and opening the BBB to systemic immune surveillance, provide for synergistic immune priming, therapeutic drug delivery, and/or region-specific immune monitoring.
[0067] In embodiments, present methods allow for classification of a patient or patient population as having “cold” or “hot” tumors. In embodiments, present methods allow for classification of a patient or patient population as having “cold” or “hot” tumors and directs treatment (e.g. those having “hot” tumors being administered systemic immunotherapies like checkpoint inhibitors while those having “cold tumor” not being administered systemic immunotherapies like checkpoint inhibitors and, optionally, receiving alternative treatment, for example directed at improving the immune response).
[0068] In embodiments, present methods provide serial BBB disruption for therapeutic reasons,

optionally in combination with serial liquid biopsy to analyze release of region-specific and disease-specific biomarkers over time. In some embodiments, such biomarkers can include one or more of proteins and cell free DNA, including tumor-derived DNA, called circulating tumor DNA (ctDNA). ctDNA carries the mutational profile of a tumor, including, for example, somatic mutations and/or epigenetic modifications associated with (and in some cases found only in) the tumor, and this profile can be tracked in the blood in correlation with the amount of tumor present in the region. Absent technologies provided by the present disclosure, such data have typically not been diagnostic for brain tumors because the BBB limits transit of this signal and so it has not been possible to track it for these purposes and/or collection of such data has not provided useful, actionable information, e.g. due to high false negative rates. However, as described herein, with BBB disruption it becomes possible to track these mutations over time in the blood stream. Higher amounts of somatic tumor-specific mutations (and/or other tumor-associated biomarkers) in the bloodstream are associated with higher amounts of tumor in the region being targeted. Conversely, lower amounts of tumor-specific mutations (and/or other tumor-associated biomarkers) detectable in the blood indicate reduction in tumor size which may, for example, relate to the success of a therapy being delivered. Alternatively or additionally, rate of change (i.e., of increase or decrease) of tumor-associated biomarker(s) detectable in blood as described herein can provide useful information indicative of rate of tumor growth or shrinkage.

[0069] Monitoring of increase or decrease, or rate thereof, of tumor-associated biomarker(s) over time provides a way to monitor treatment response, in some cases offering earlier diagnosis of responders and non-responders, which in turn permits faster pivoting to alternative therapies in patients unlikely to receive a benefit before their tumor burden becomes too large, as can happen when awaiting repeated serial imaging alone. Alternatively or additionally, such monitoring can be used as a measure of progression (e.g. tumor growth) versus pseudo-progression (e.g. immune response giving the appearance of increased inflammation and increased size on neuroimaging yet actual underlying tumor response as measured by clearance of the associated ctDNA mutations). Still further alternatively or additionally, such measurements can be used in an adaptive manner to update treatment decisions such as increasing the dosing of immune priming acoustic energy, and/or adding higher doses of thermal and cavitation energy to increase the ability to turn the “cold” tumor “hot.” In some embodiments, tumor-associated biomarkers (e.g., region-specific biomarkers) can be analyzed for cytokine and other protein panel profiles, as well as for methylation profiles to aid in the diagnosis of transition from “cold” to “hot.” In addition to adjusting immune priming doses, addition of therapeutics (including, e.g., high molecular weight therapeutics such as biologic therapeutics—for example antibodies and/or antibody-drug conjugates, specifically including cytotoxic antibody-drug conjugates) to the immune priming regimen can be done in personalized tailored way by monitoring the evolution of specific biomarker (e.g., ctDNA) signals over time (e.g. increase in EGFR or Her2 leading to utilization of cytotoxic payloads tied to these antibodies aimed at these targets) with or without increasing anti-tumor acoustic energies and/or with or without systemically administered immunotherapies from cells to checkpoints to agonist/immune activating therapies.

[0070] In embodiments, a disease or disorder of the brain is or comprises a neurodegenerative disease. In embodiments, a neurodegenerative disease is or comprises Alzheimer's disease.

[0071] In embodiments, technologies disclosed herein allow tracking stages of Alzheimer's disease in a patient or patient population. In embodiments, methods disclosed herein allow tracking stages of Alzheimer's disease in a patient or patient population that is improved relative to tau PET and/or serial invasive CSF sampling. In embodiments, methods disclosed herein allow tracking of Braak stages, describing the gradual regional deposition of hyperphosphorylated tau protein over the course of the disease. Braak stage is strongly associated with cognitive impairment and is part of the hallmark pathological criteria for the diagnosis of AD. Braak stage I and stage II are usually clinically asymptomatic with tau accumulation in the medial temporal lobe (MTL) and

transentorhinal region, followed by the entorhinal cortex in stage II. Clinical symptoms develop in stages III-IV. Stage III is marked by the gradual spread of NFT into the limbic structures including the amygdala and hippocampus, with stage IV noting spread to the thalamus and claustrum. In embodiments, present methods allow for detection of progression or lack of progression between stages. In embodiments, present methods allow detection of response to a treatment agent, e.g. if a human patient or patient population does not progress between the stages.

[0072] In embodiments, a neurodegenerative disease is or comprises Parkinson's disease. In embodiments, methods disclosed herein allow for early diagnosis and treatment of Parkinson's disease. α-Synuclein is a presynaptic neuronal protein that is linked genetically and neuropathologically to Parkinson's disease. Its aberrant cytotoxic aggregation causes neuronal death and the symptoms of Parkinson's disease occur when this process involves the substantia nigra. In embodiments, methods disclosed herein allow tracking of stages of disease, e.g. lesions initially occur in the medulla oblongata and dorsal motor nucleus of the glossopharyngeal and vagal nerves and anterior olfactory nucleus (Braak Stage 1). The disease process in the brain stem pursues an ascending course with little interindividual variation. Spread into the pontine tegmentum occurs in Braak Stage 2 and arrival in the midbrain occurs in Braak Stage 3, in particular in the pars compacta of the substantia nigra. Braak Stage 4 involves the basal prosencephalon and temporal mesocortex (trasentorhinal region), with Braak Stage 5 and 6 involving the neocortex, with high order sensory association and prefrontal areas. First order sensory association/premotor areas and primary sensory/motor fields then follow suit. Tracking the regional spread of the disease and targeting the toxicity conferred by these aggregations may lead to novel therapeutic strategies not only in PD, but also in other related neurodegenerative conditions called synucleinopathies. In embodiments, present methods allow for detection of progression or lack of progression between stages. In embodiments, present methods allow detection of response to a treatment agent, e.g. if a human patient or patient population does not progress between the stages.

[0073] In embodiments, a disease or disorder of the brain is an anxiety disorder, for example, selected from generalized anxiety disorders, social phobias, specific phobias, panic disorders, obsessive compulsive disorder (OCD) and post-traumatic stress disorder (PTSD).

[0074] In embodiments, a disease or disorder of the brain is or comprises addiction.

[0075] In embodiments, a disease or disorder of the brain is or comprises bipolar disorder.

[0076] In embodiments, a disease or disorder of the brain is or comprises a behavior disorder selected from oppositional defiant disorder (ODD), conduct disorder (CD) and attention deficit hyperactivity disorder (ADHD).

[0077] In embodiments, a disease or disorder of the brain is or comprises depression.

[0078] In embodiments, a disease or disorder of the brain is or comprises an eating disorder selected from anorexia, bulimia nervosa and other binge eating disorders.

[0079] In embodiments, a disease or disorder of the brain is or comprises epilepsy.

[0080] In embodiments, a disease or disorder of the brain is or comprises an autoimmune disease.

[0081] In embodiments, a disease or disorder of the brain is or comprises multiple sclerosis.

[0082] In embodiments, a disease or disorder of the brain is or comprises dementia or cognitive dysfunction.

[0083] In embodiments, a disease or disorder of the brain is or comprises concussion or other traumatic brain injury.

Patient Characteristics

[0084] In embodiments, present disclosure relates to diagnosis, disease typing (e.g., without limitation, determining disease subtype, mutation etiology, and the like), treatment selection, and/or theranosis in or for human patients.

[0085] In embodiments, a human patient or patient population is afflicted with, or suspected to be afflicted with a disease or disorder of the brain or at risk for developing a disease or disorder of the

brain, e.g. any one of those described herein.
[0086] In embodiments, a human patient or patient population is afflicted with, or suspected to be afflicted with a cancer or at risk for developing a cancer. In embodiments, a cancer is or comprises any one of those described herein.
[0087] In embodiments, a cancer is or comprises an intracranial tumor.
[0088] In embodiments, a cancer is or comprises a metastatic tumor in the brain. In embodiments, a metastatic tumor in the brain originates in a breast, lung, skin (including melanoma), prostate gland, uterus, kidney or gastrointestinal tract.
[0089] In embodiments, a tumor is or comprises a primary brain tumor. In embodiments, a primary brain tumor is glial. In embodiments, a glial tumor is one or more of astrocytoma, ependymoma, glioblastoma multiforme (GBM), medulloblastoma, and oligodendroglioma. In embodiments, a primary brain tumor is non-glial. In embodiments, a brain tumor is a posterior fossa tumor, optionally selected from a cerebellar tumor, pontine tumor, and medulloblastoma (primitive neuroectodermal tumor). In embodiments, a brain tumor is a cerebral hemisphere tumor. In embodiments, a cancer is a glioblastoma.
[0090] In embodiments, a human patient or patient population is afflicted with, or suspected to be afflicted with a neurodegenerative disease or at risk for developing a neurodegenerative disease. In embodiments, a neurodegenerative disease is or comprises Alzheimer's disease. In embodiments, a neurodegenerative disease is or comprises Parkinson's disease.
[0091] In embodiments, a human patient or patient population is healthy and the present methods find use in general monitoring of brain health.
Features of the Ultrasound Beam/Application Thereof
[0092] Various embodiments of the present disclosure relate to disruption of the BBB through the application of an ultrasound beam across the cranium of the human patient or patient population.
[0093] In embodiments, the disruption, permeabilization or opening of the BBB is “transient”, meaning, in embodiments, non-permanent or temporary disruption, permeabilization, or opening of the BBB.
[0094] In embodiments, an ultrasound beam is or comprises a focused ultrasound beam. In embodiments, an ultrasound beam is or comprises a guided ultrasound beam.
[0095] In embodiments, an ultrasound beam is or comprises a focused ultrasound beam which is narrow (e.g. about 1-10 mm, or about 1-5 mm, or about 1-3 mm, or about 1 mm, or about 2 mm, or about 3 mm, or about 4 mm, or about 5 mm, or about 6 mm, or about 7 mm, or about 8 mm, or about 9 mm, or about 10 mm) or wide (e.g. about 1-10 cm, or about 1-5 cm, or about 1-3 cm, or about 1 cm, or about 2 cm, or about 3 cm, or about 4 cm, or about 5 cm, or about 6 cm, or about 7 cm, or about 8 cm, or about 9 cm, or about 10 cm).
[0096] In embodiments, an ultrasound beam is or comprises a focused, magnetic resonance-guided ultrasound beam (MRgFUS). In embodiments, an ultrasound beam is or comprises a focused, computerized tomography (CT)-guided ultrasound beam. In embodiments, an ultrasound beam is or comprises a focused, positron emission tomography (PET)-guided ultrasound beam. In embodiments, an ultrasound beam is or comprises a focused, positron emission tomography (PET)-guided ultrasound beam. In embodiments, an ultrasound beam is or comprises a focused, stereotactically-navigated guided ultrasound beam based on registration to prior scan.
[0097] In embodiments, a focused ultrasound beam is applied directly to the human patient's cranium, e.g. using a helmet-shaped ultrasound transducer.
[0098] 20) In embodiments, a focused ultrasound beam is applied at a center frequency of about 180 to about 230 kHz, e.g. about 220 KHz.
[0099] In embodiments, an ultrasound beam is applied at a maximum power of up to about 30 W, e.g. about 4 W to about 30 W, about 6 W to about 30 W, about 8 W to about 30 W, about 10 W to about 30 W, or about 10 W to about 20 W, or about 20 W to about 30 W.
[0100] 25 In embodiments in which the ultrasound beam does not go through nominal skull, beam

power is reduced and power range is about 0.5 W to about 15 W, e.g. about 0.5 W, or about 5 W, or about 10 W, or about 15 W.
[0101] In embodiments, an ultrasound beam is delivered in about 3 mm sonication subspots separated by about 2.5-3 mm for continuous and/or slightly-overlapping delivery of In embodiments, an ultrasound beam is 1-2 V*s target dose, which is, optionally, monitored in real-time via measured Webers of magnetic flux, i.e. electromotive force of one Volt induced per second.
[0102] In embodiments, a treatment duration is at least about 10 minutes, or at least about 20 minutes or at least about 30 minutes or at least about 40 minutes or at least about 50 minutes or at least about 60 minutes or at least about 70 minutes or at least about 80 minutes, or at least about 90 minutes, or at least about 120 minutes, or at least about 150 minutes, or at least about 180 minutes. In embodiments, a treatment duration is about 100 minutes, or about 110 minutes, or about 120 minutes, or about 130 minutes, or about 140 minutes, or about 150 minutes, or about 160 minutes.
[0103] In embodiments, an ultrasound beam is applied for at least about 10 seconds, or at least about 20 seconds, or at least about 30 seconds, or at least about 40 seconds, or at least about 50 seconds, or at least about 60 seconds.
[0104] In embodiments, an ultrasound beam is applied for about 10 seconds, or about 20 seconds, or about 30 seconds, or about 40 seconds, or about 50 seconds, or about 60 seconds.
[0105] In embodiments, an ultrasound beam is applied in pulses.
[0106] In embodiments, an ultrasound beam is applied at a power of at least about 5W, or at least about 10 W, or at least about 15W, or at least about 20W, or at least about 25W.
[0107] In embodiments, an ultrasound beam is applied at a power of about 10W, or about 15W, or about 20W.
[0108] In embodiments, an ultrasound beam is applied via a computationally generating a protocol for targeting of at least one site, as described in WO 2019/002943, the entire contents of which are incorporated by reference.
[0109] In embodiments, present methods employ microbubble-enhanced ultrasound procedures that employ an ultrasound system, a monitoring system and a microbubble administration system as described in WO 2019/116097, the entire contents of which are incorporated by reference.
[0110] In embodiments, an ultrasound beam targets one or more of the frontal lobe, parietal lobe, temporal lobe, occipital lobe, and cerebellum. In embodiments, an ultrasound beam targets the supratentorial region of the brain. In embodiments, an ultrasound beam targets the infratentorial region of the brain. In embodiments, an ultrasound beam targets the insula. In embodiments, an ultrasound beam targets the brainstem. In embodiments, an ultrasound beam targets the pons.
[0111] In embodiments, an ultrasound beam targets the posterior fossa. In embodiments, an ultrasound beam targets the corticomedullary gray/white junction. In embodiments, an ultrasound beam targets the meninges.
[0112] In embodiments, present disclosure relates to real-time monitoring during treatment by e.g. acoustic response or MRI imaging. In embodiments, real-time monitoring allows for detection of safety and/or efficacy of the present methods.
Contacting/Detecting/Detection Agents
[0113] In embodiments, present disclosure relates to measuring a presence, absence, or level of a biomarker by use of one or more detection agents.
[0114] In embodiments, a biomarker is detected within about 10 minutes to about 60 minutes, or about 20 minutes to about 40 minutes after completion of the BBB disruption. In embodiments, a biomarker is detected within about 10 minutes, or about 20 minutes, or about 30 minutes, or about 40 minutes, or about 50 minutes, or about 60 minutes after completion of the BBB disruption
[0115] In embodiments, detecting comprises measuring a presence, absence, or level of a epigenetic pattern or profile. In embodiments, detecting comprises measuring a presence, absence, or level of a methylation pattern or signature. In embodiments, detecting comprises chromatin

immunoprecipitation (ChIP) or bisulfite modification. In embodiments, detecting comprises measuring a presence, absence, or level of a histone modification.
[0116] In embodiments, detecting comprises measuring a presence, absence, or level of a mutant version of the biomarker. In embodiments, detecting comprises measuring a presence, absence, or level of a tumor-associated mutation. In embodiments, detecting comprises measuring a presence, absence, or level of a mutational burden. In embodiments, detecting comprises measuring a presence, absence, or level of a polymorphism. In embodiments, detecting comprises measuring a presence, absence, or level of a SNPs. In embodiments, detecting comprises measuring a presence, absence, or level of a copy number aberration, optionally as compared to a reference or parental genome. In embodiments, detecting comprises measuring a presence, absence, or level of a microsatellite instability.
[0117] In embodiments, a detection agent is or comprises a primer or probe. In embodiments, a detection agent is or comprises labeled probe, optionally fluorescently or radiolabeled. In embodiments, detecting comprises a primer-based detection method. In embodiments, detecting comprises a probe-based detection method.
[0118] In embodiments, detecting comprises polymerase chain reaction (PCR), reverse transcriptase polymerase chain reaction (RT-PCR), quantitative PCR (qPCR), multiplex polymerase chain reaction, nested polymerase chain reaction, hot start polymerase chain reaction, long-range PCR, assembly polymerase chain reaction, asymmetric polymerase chain reaction, digital droplet PCT (ddPCR) or BEAMing (beads, emulsion, amplification, and magnetics, see, e.g. Z. Cheng et al., “An emulsion digital PCR quantitative method based on microbeads and micropillar array chip,” 2017 19th International Conference on Solid-State Sensors, Actuators and Microsystems (TRANSDUCERS), 2017, pp. 575-578).
[0119] In embodiments, detecting comprises a recombinase polymerase amplification (RPA), a loop-mediated amplification (LAMP), or a helicase-dependent amplification (HDA).
[0120] In embodiments, detecting comprises RNA or DNA gel electrophoresis or Southern or Northern blotting.
[0121] In embodiments, detecting comprises a microarray-based assay.
[0122] In embodiments, detecting comprises a hybridization technique, optionally selected from solution hybridization, capillary hybridization, hybridization to nucleic acid arrays, optionally macroarrays, microarrays or high-density oligonucleotide arrays (Gene Chips). In embodiments, detecting comprises DNA sequencing.
[0123] In embodiments, a detection agent is or comprises an antibody. In embodiments, detecting comprises an antibody-based detection method. In embodiments, detecting comprises immunohistochemical staining, western blotting, in-cell western, immunofluorescent staining, ELISA, and fluorescent activating cell sorting (FACS). In embodiments, a detection agent is or comprises an antibody specific for an epigenetic signature and/or nucleosome-protein adduct. In embodiments, a detection agent is or comprises an antibody specific for a mutant protein.
[0124] In embodiments, detecting comprises spectroscopy, optionally mass spectroscopy.
Diagnosing
[0125] In embodiments, present methods, in part or in whole, relate to diagnosis.
[0126] In embodiments, diagnosing comprises prediction of disease onset or progression. In embodiments, diagnosing comprises prediction or monitoring of progression or pseudoprogression.
[0127] In embodiments, diagnosing comprises prediction or monitoring of response to treatment.
[0128] In embodiments, diagnosing comprises measuring of minimal residual disease status. In embodiments, diagnosing comprises prediction of recurrence.
[0129] In embodiments, a disease or disorder of the brain is a glioblastoma and the brain-derived biomarker is selected from IDH mutations, 1p19q deletion, MGMT promoter methylation, and EGFRvIII amplification.
[0130] In embodiments, a neurodegenerative disease is Alzheimer's disease and the brain-derived

biomarker is selected from amyloid beta protein, tau protein, APOE 84 variant, APP mutation, PSEN1 mutations, and PSEN2 mutations.
[0131] In embodiments, a neurodegenerative disease is Parkinson's disease and the brain-derived biomarker is selected from alpha-synuclein and GBA mutations.
Treatment Agents
[0132] In embodiments, present disclosure relates, in part, to methods that assist with selection of treatment agents for a patient afflicted with a disease or disorder. In embodiments, present disclosure relates, in part, to methods that allow for the determination of efficacy of one or more treatment agents against a disease or disorder.
[0133] In embodiments, a treatment agent is a small interference RNA (siRNA), a microRNA (miRNA) inhibitor, or an antisense RNA.
[0134] In embodiments, a treatment agent is or comprises a small molecule. In embodiments, a treatment agent is or comprises a chemotherapy. In embodiments, a chemotherapy is or comprises an alkylating agent. In embodiments, an alkylating agent is selected from altretamine, bendamustine, busulfan, carboplatin, carmustine, chlorambucil, cisplatin, cyclophosphamide, dacarbazine, ifosfamide, lomustine, mechlorethamine, melphalan, oxaliplatin, temozolomide, thiotepa, and trabectedin. In embodiments, an alkylating agent is or comprises temozolomide.
[0135] In embodiments, a treatment agent is or comprises biologic agent. In embodiments, a treatment agent is or comprises a full length antibody or an antibody format. In embodiments, an antibody format is selected from a single-chain antibody (scFv), a microprotein (cysteine knot protein, knottin), a DARPin; a Tetranectin; an Affibody; a Transbody; an Anticalin; an AdNectin; an Affilin; a Microbody; a plastic antibody; a single-domain antibody, a recombinant heavy-chain-only antibody (VHH), a shark heavy-chain-only antibody (VNAR), a phylomer; a stradobody; a maxibody; an evibody; a fynomer, an armadillo repeat protein, a Kunitz domain, an avimer, an atrimer, a probody, an immunobody, a triomab, a troybody; a pepbody; a vaccibody, a UniBody; an Affimer, a DuoBody, a Fv, a Fab, a Fab′, and a F(ab′).sub.2. In embodiments, a treatment agent is or comprises a monoclonal antibody. In embodiments, a treatment agent is or comprises a bispecific antibody. In embodiments, a treatment agent is or comprises an antibody-drug conjugate.
[0136] In embodiments, a treatment agent is or comprises a whole cell agent. In embodiments, a whole cell agent is selected from a chimeric antigen receptor (CAR) T cell therapy, a tumor-infiltrating lymphocyte (TIL) therapy, an engineered T cell receptor (TCR) therapy, a natural killer (NK) cell therapy, and a dendritic cell therapy.
[0137] In embodiments, present methods involve use of an imaging tracer that is detectable, for example via radio-labeled PET (or alternatively by MRI, CT or X-ray contrast agent) and is added to a treatment agent of interest to develop a theranostic that can be noninvasively monitored for biodistribution and drug delivery to the target regions of the brain. In embodiments, focused ultrasound is used as a noninvasive device treatment to enhance baseline drug delivery. In embodiments, this is done via the targeted delivery of precise ultrasonic acoustic energy through the skin and skull to specific regions of the brain, in combination with intravenously-delivered microbubble actuators, to permeabilize, e.g. transiently permeabilize, the BBB for the purpose of improved therapeutic delivery to this region. The ultrasound can be focused under direct MR-guidance, or alternatively via stereotactic guidance using a recent imaging scan and/or based on anatomic surface landmarks for localization.
[0138] In embodiments, volumetric voxel-based analyses is used to confirm and quantify the amount of treatment agent that has been delivered to the target tissue, and to confirm avoidance of exposure of tissue outside of the targeted area.
[0139] In embodiments, a treatment agent is associated with or conjugated to an imaging tracer. In embodiments, an imaging tracer is radiolabeled. In embodiments, a radiolabel is selected from Indium-111 (.sup.111In), Fluorine-18 (.sup.18F), and Carbon-11 (.sup.11C). In embodiments, an imaging tracer is radiolabeled position emission tomography (PET) ligand. In embodiments, a

treatment agent is associated with or conjugated to an imaging tracer is selected from florbetapir, florbetaben, flutemetamol, [.sup.18F]T-807, and [.sup.18F]THK-5351.

[0140] In embodiments, treatment agents are used in a treatment that occurs before, after, or concurrent with after craniectomy.

Microbubbles

[0141] The present disclosure relates, in part, to the present ultrasound applications in conjunction with microbubbles. Microbubbles are typically gas-filled, typically air or perfluorocarbon, and oscillate and vibrate when a sonic energy field is applied and may reflect ultrasound waves. This distinguishes the microbubbles from surrounding tissues. In embodiments, because gas bubbles in liquid lack stability and would therefore quickly dissolve, microbubbles are encapsulated with a solid shell. In embodiments, a shell is made from either a lipid or a protein. Materials having a hydrophilic outer layer to interact with the bloodstream and a hydrophobic inner layer to house the gas molecules are the most thermodynamically stable. In embodiments, air, sulfur hexafluoride, and perfluorocarbon gases all can serve as the composition of the microbubble interior.

[0142] In embodiments, present methods comprise administration of one or more microbubble compositions or the human patient or patient population has undergone administration with one or more microbubble compositions immediately before and/or during the application of the ultrasound beam.

[0143] In embodiments, one or more microbubble compositions are administered immediately before and/or during the application of the ultrasound beam.

[0144] In embodiments, one or more microbubble compositions are administered contemporaneously with the application of the ultrasound beam.

[0145] In embodiments, microbubble compositions comprise one or more lipid-based microspheres. In embodiments, microbubble compositions are perflutren lipid microspheres. In embodiments, microbubble compositions comprise (R)-hexadecanoic acid, 1-[(phosphonoxy) methyl]-1,2-ethanediyl ester, monosodium salt (DPPA); (R)-4-hydroxy-N,N,Ntrimethyl-10-oxo-7-[(1-oxohexadecyl)oxy]-3,4,9-trioxa-4-phosphapentacosan-1-aminium, 4-oxide, inner salt (DPPC); and (R)-α-[6-hydroxy-6-20) oxido-9-[(1-oxohexadecyl)oxy]-5,7, 11-trioxa-2-aza-6-phosphahexacos-1-yl]-ω-methoxypoly (ox-1,2-ethanediyl), monosodium salt; (N-(methoxypolyethylene glycol 5000 carbamoyl)-1,2-dipalmitoyl-sn-glycero-3-phosphatidylethanolamine, monosodium salt, MPEG5000 DPPE). In embodiments, microbubble compositions are lipid-coated echogenic microbubbles filled with octafluoropropane gas. In embodiments, microbubble compositions comprise octafluoropropane encapsulated in an outer lipid shell comprising (R)-hexadecanoic acid, 1-[(phosphonoxy) methyl]-1,2-ethanediyl ester, monosodium salt (DPPA); (R)-4-hydroxy-N,N,Ntrimethyl-10-oxo-7-[(1-oxohexadecyl)oxy]-3,4,9-trioxa-4-phosphapentacosan-1-aminium, 4-oxide, inner salt (DPPC); and (R)-α-[6-hydroxy-6-oxido-9-[(1-oxohexadecyl)oxy]-5,7,11-trioxa-2-aza-6-phosphahexacos-1-yl]-ω-methoxypoly (ox-1,2-ethanediyl), monosodium salt; (N-(methoxypolyethylene glycol 5000 carbamoyl)-1,2-dipalmitoyl-sn-glycero-3-phosphatidylethanolamine, monosodium salt, MPEG5000 DPPE).

[0146] In embodiments, microbubble compositions are administered to the patient no more than 60, or 30, or 20, or 10 minutes before the application of the ultrasound beam.

[0147] In embodiments, microbubble compositions are administered to the patient throughout the method.

[0148] In embodiments, microbubble compositions are administered by systemic injection, bolus injection or slow diffusion injection. In embodiments, microbubble compositions are administered by systemic infusion. In embodiments, microbubble compositions are administered by continuous intravenous infusion. In embodiments, microbubble compositions are administered by continuous intravenous infusion of a mixture about 1×10.sup.10 to about 6×10.sup.10 microsphere in a carrier. In embodiments, microbubble compositions are administered by continuous intravenous infusion of a mixture about 1×10.sup.10 to about 6×10.sup.10 microsphere in a saline carrier. In embodiments,

microbubble compositions are administered by continuous intravenous infusion of a mixture about 1×10.sup.10 to about 6×10.sup.10 microsphere in about 250 mL of saline carrier. In embodiments, microbubble compositions are administered by continuous intravenous infusion of about 5×10.sup.7 to about 3×10.sup.8/mL. In embodiments, microbubble compositions are continuously infused at a rate of about 1 to about 3 mL/minute during the application of the ultrasound beam.

[0149] In embodiments, present disclosure relates, in part, to the present ultrasound applications in conjunction with alternatives microbubbles, such as nanodroplets or nanoparticles, e.g. gold nanoparticles. Embodiments relating to microbubbles apply equally to nanodroplets or nanoparticles, e.g. gold nanoparticles.

Ultrasound Targets

[0150] In embodiments, application of the ultrasound beam targets at least one region of the brain.

[0151] In embodiments, application of the ultrasound beam targets at least two regions of the brain.

[0152] In embodiments, application of the ultrasound beam targets at least three regions of the brain.

[0153] In embodiments, application of the ultrasound beam targets at least one, or two, or three regions of the brain contemporaneously.

Clinical Trial Design

[0154] In embodiments, present technology relates to or finds use in clinical trial design.

[0155] In embodiments, present technology relates to or finds use in post-approval clinical trial studies and/or in identification of specific patients or patient populations for treatment.

[0156] In embodiments, present technology allows for classification of responders. In embodiments, present technology allows for classification of non-responders.

[0157] In embodiments, present methods provide for companion diagnostics, molecular subtyping, pharmacodynamics and/or disease monitoring. In embodiments, this information allows for clinical trial design which allows for mitigation of late-stage clinical trial failures, e.g. due to poor and unpredictable drug delivery, while also addressing the need for brain region-specific molecular biomarkers.

[0158] In embodiments, present methods provide for solutions to the poor yield/diagnostic sensitivity and high false negative rate of liquid biopsy for brain diseases.

[0159] In embodiments, a tumor is anatomically-targeted on the basis of neuroimaging features such as contrast-enhancing mass lesion and other neuroimaging abnormalities (e.g. T2, perfusion, DWI, etc,) and/or known resection cavity, as well as newer modalities such as molecular imaging and PET. An initial liquid biopsy blood sample is obtained during first BBB disruption to achieve a tumor-specific liquid biopsy molecular profile. This provides diagnostic data in terms of actionable mutations that can be used for treatment selection (e.g. EGFR mutation detection for selection of anti-EGFR agent, etc.). It also provides a baseline signature of tumor-specific mutations as a signal of residual disease status that can be tracked as a signature in the future for monitoring recurrence, progression vs. pseudoprogression.

[0160] In embodiments, Alzheimer's disease is the disease of interest and pathologic areas of the brain are anatomically-targeted on the basis of neuroimaging features such as anti-Amyloid PET and/or anti-Tau PET. An initial liquid biopsy blood sample is obtained during first BBB disruption targeted to this region to achieve a disease-specific and site-specific molecular profile to track treatment response over time. Initiation of therapy may occur (e.g. IVIG, or specific antibody therapy like anti-AB (e.g. Aducanumab) or anti-Tau can be initiated), with and without BBB disruption, with clinical responses evaluated in terms of neurocognitive outcomes, clearance of plaques on PET, and region-specific pharmacodynamic responses measured on serial liquid biopsy analyses.

[0161] In embodiments, Parkinson's disease is the disease of interest and pathologic areas of the brain are anatomically-targeted on the basis of neuroimaging based on location or if molecularly-guided imaging becomes available (e.g. anti-alpha-Synuclein PET). An initial liquid biopsy blood

sample is obtained during first BBB disruption targeted to this region to achieve a disease-specific and site-specific molecular profile to track treatment response over time. Initiation of therapy (e.g. IVIG, or specific antibody therapy like anti-alpha-Synuclein therapy can be initiated) may occur, with and without BBB disruption, with clinical responses evaluated in terms of neurocognitive outcomes, neuroimaging response and region-specific pharmacodynamic responses measured on serial liquid biopsy analyses.

[0162] In embodiments, there is provided a method for conducting adaptive clinical trials to develop a therapeutic drug device product in situations where therapeutic delivery was rate-limiting in the drug-only arm. The method, in embodiments, comprises using a noninvasive drug delivery device as a modification during an adaptive clinical trial, wherein the drug delivery device is able to deliver more therapeutic across the BBB to the targeted brain region or pathology of interest. Other methods set out herein involve using voxel-based radiolabel analyses to monitor improvements in drug delivery noninvasively, and the timed collection of region-specific biomarker data in the bloodstream related to the process of temporary BBB disruption that can further be used in the adaptive clinical trial design to monitor treatment response and perform population enrichment.

[0163] In aspects, there is provided a method of conducting an adaptive clinical trial for developing a treatment agent, the method comprising: (a) obtaining a treatment agent; (b) enrolling a first patient population and a second patient population into the adaptive clinical trial; (c) systemically administering the treatment agent to the first patient population; (d) systemically administering the treatment agent to the second patient population contemporaneously with an ultrasound beam across the cranium to cause disruption, e.g. transient disruption, of the BBB and allow transit of the treatment agent therethrough; and (e) comparing treatment efficacy of the treatment agent in the first patient population and the second patient population.

[0164] In aspects, there is provided a method of conducting an adaptive clinical trial for developing a treatment agent, the method comprising: (a) obtaining a treatment agent; (b) enrolling a patient population into the adaptive clinical trial; (c) administering the treatment agent to the patient population contemporaneously with an ultrasound beam across the cranium to cause disruption, e.g. transient disruption, of the BBB to permit the transit of one or more brain-derived biomarkers into the blood; (d) measuring the biomarkers in a plasma sample from the patient population; and (e) adjusting the type of treatment or dose of the treatment agent if the measuring indicates non-efficacious treatment agent effect.

[0165] In aspects, there is provided a method of conducting an adaptive clinical trial for developing a treatment agent, the method comprising: (a) obtaining a treatment agent; (b) adding an imaging tracer to the treatment agent to non-invasively track its biodistribution in the body; (c) enrolling a patient population into the adaptive clinical trial; (d) administering the traced treatment agent to the patient population contemporaneously with an ultrasound beam applied across the cranium to cause disruption, e.g. transient disruption, of the BBB and allow transit of the treatment agent to the target regions; (e) measuring drug delivery to the target brain regions via the non-invasive imaging tracer signal, (f) comparing efficacy of drug delivery of the treatment agent in the patient population to a second patient population receiving a non-traced treatment agent; (g) measuring biomarkers in a plasma sample from the patient population of brain-derived biomarkers released by the disruption of the BBB, specific to the region where treatment agent is being delivered, (h) determining the effect of improved drug delivery across the BBB on clinical outcomes and/or measured biomarkers, optionally pharmacokinetics and/or pharmacodynamics, released from the target region; and (i) adjusting the type of treatment or dose of the treatment agent if the measuring indicates non-efficacious treatment agent effect.

[0166] In embodiments, the imaging tracer is radiolabeled In embodiments, the radiolabel is selected from Indium-111 (.sup.111In), Fluorine-18 (.sup.18F), and Carbon-11 (.sup.11C). In embodiments, the imaging tracer is radiolabeled position emission tomography (PET) ligand.

[0167] In embodiments, present methods find use in an Umbrella Protocol and/or Basket Trial design. In embodiments, new sub-study baskets are launched for each new treatment agent being studied, allowing multiple investigational drugs and combinations of drugs to be tested within a target disease population. The present methods, e.g. permeabilization of the BBB for enhanced drug delivery and/or enhanced blood access to brain-derived biomarkers, allow for assessing for improvement in drug delivery, resultant improved clinical response on primary endpoints, measurement of site-specific pharmacodynamic signals of response as measured via the transient release of target-specific biomarkers into the bloodstream for liquid biopsy in the context of individual or Umbrella/Basket trials.

[0168] For instance, In Master Protocols, a new Sub-Study Basket is created for every new therapeutic agent being tested. Agents are labeled with an imaging tracer to become a theranostic (e.g. radio-labeled PET tracer). Patients are evaluated against controls, and by comparing the relative effects of the drug with and without optimized drug delivery. In one arm, patients receive external acoustic treatment to permeabilize, e.g. transiently permeabilize, the BBB to enhance theranostic delivery to specific brain regions. In the other arm, patients receive drug only. Analyses of brain region-specific molecular signals for pharmacodynamic monitoring can be done at the same time as BBB permeabilization via timed liquid biopsy. If patients demonstrate a significant increase in drug delivery over the baseline drug delivery, the drug-device combination arm can be advanced through regulatory approval as a new drug-device care pathway.

[0169] In embodiments, present methods enhance brain region-specific drug delivery of a therapeutic agent as a comparative arm in a clinical trial and allow for assessing of the relative contribution of drug delivery on clinical outcomes, and provide a potential salvage mechanism for efficacy based on enhanced drug delivery.

Samples

[0170] In embodiments, present methods involve a variety of biological samples. Certain embodiments above refer to blood or plasma samples, but, in embodiments, a sample of mucus or saliva may substitute for the blood or plasma. Certain embodiments above refer to blood or plasma samples, but, in embodiments, a sample of lymph may substitute for the blood or plasma.

[0171] In embodiments, a sample is not a CSF sample.

Kits

[0172] In embodiments, present disclosure provides kits that can simplify the undertaking any method described herein. An illustrative kit of the invention comprises any treatment agent described herein in unit dosage form and/or a device described herein, e.g. a device for applying an ultrasound beam across the cranium of the human patient, and/or one or more microbubbles described herein in unit dosage form.

[0173] In embodiments, the unit dosage form is a container, such as a pre-filled syringe, which can be sterile, containing any composition described herein and a pharmaceutically acceptable carrier, diluent, excipient, or vehicle.

[0174] The kit can further comprise a label or printed instructions instructing the use of any composition described herein.

[0175] As used herein, the word “include,” and its variants, is intended to be non-limiting, such that recitation of items in a list is not to the exclusion of other like items that may also be useful in the materials, compositions, devices, and methods of this technology. Similarly, the terms “can” and “may” and their variants are intended to be non-limiting, such that recitation that an embodiment can or may comprise certain elements or features does not exclude other embodiments of the present technology that do not contain those elements or features. Although the open-ended term “comprising,” as a synonym of terms such as including, containing, or having, is used herein to describe and claim the disclosure, the present technology, or embodiments thereof, may alternatively be described using more limiting terms such as “consisting of” or “consisting essentially of” the recited ingredients.

[0176] Unless defined otherwise, all technical and scientific terms herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Although any methods and materials, similar or equivalent to those described herein, can be used in the practice or testing of the present disclosure, the preferred methods and materials are described herein. All publications, patents, and patent publications cited are incorporated by reference herein in their entirety for all purposes. This disclosure is further illustrated by the following non-limiting examples.

EXAMPLES

[0177] In the following examples, nine patients were treated in a prospective single-arm, open-label trial to investigate serial MRgFUS and adjuvant temozolomide combination in patients with glioblastoma. Blood samples were collected within the hours before and after sonication, with control samples from non-brain tumor patients undergoing BBB opening alone.

[0178] The BBB restricts transport both into and out of the brain, which can present complications both for delivery of agents (e.g., therapeutic and/or diagnostic agents) to the brain, and for release of agents (e.g., disease markers, evidence of therapeutic benefit or lack thereof, etc.) from the brain. This restriction of release means, in part, that markers of brain activity and/or status (e.g., presence of disease) typically are not readily detected other than by direct sampling of the CNS.

[0179] Work described herein represents the first-in-human demonstration that MRgFUS opening of the BBB in glioblastoma patients results in detectable levels of marker(s) such as brain-derived proteins, neuron-derived extracellular vesicles, and cell-free DNA in systemic samples (e.g., blood).

[0180] Differential analysis confirms that detected cell-free DNA is brain-derived and disease-specific.

[0181] Thus, the present disclosure demonstrates that, focused ultrasound can achieve release of brain-derived biomarkers into systemic fluids, thereby permitting, for example, detection and/or characterization of disease presence, status, and/or response to therapy through analysis of non-CNS sample(s) (e.g., blood, serum, lymph, etc.). The present Examples specifically demonstrate surprising effectiveness of MRgFUS applied to neuro-oncology patients to permit assessment of brain-derived markers (specifically including cfDNA) in blood. Thus, the present Examples demonstrates that MRgFUS permits established liquid biopsy technologies to be applied in neuroncological contexts.

[0182] The Examples herein demonstrate that MRgFUS acutely enhanced plasma cfDNA (2.6±1.2 fold, $p<0.01$, Wilcoxon signed-rank test), neuron-derived extracellular vesicles (3.2±1.9 fold, $p<0.01$), and brain specific protein S100b (1.4±0.2 fold, $p<0.01$). Further comparison of the cfDNA methylation profiles suggests a signature that is disease and post-BBB opening specific. The Examples herein further demonstrate that, after MRgFUS-mediated opening of the BBB, levels of mutant isocitrate dehydrogenase I (IDH1) also increased (in diseased, but nor normal subjects).

[0183] Collectively, the Examples herein demonstrate that MRgFUS mediated enrichment of-brain-derived biomarkers, demonstrating that this technology can render brain diseases amenable to various liquid biopsy (e.g., blood, serum, etc.) assessments.

Example 1: Large Volume BBB Opening of the Tumor and Peritumoral Regions

[0184] In this Example, nine patients with WHO grade IV glioblastoma (“GBM”) were enrolled in a single-arm trial investigating serial MRgFUS BBBO to enhance the delivery of adjuvant temozolomide therapy. All patients had undergone surgical resection, some gross total resection, and concurrent chemoradiation. The status of molecular markers was collected from clinical pathology at the time of surgery (see Table 1, below). Notably, IDH1-R132H status by immunohistochemistry was wildtype in all except patient 6 (P6).

TABLE-US-00001 TABLE 1 Patient demographics Eloquent Extent of Patient Age Gender Location resection MGMT promoter IDH1-R132H P1 49 F Yes GTR NA Wildtype P2 52 M No GTR Methylated Wildtype P3 56 F No GTR Unmethylated Wildtype P4 35 M Yes STR NA

Wildtype P5 56 F Yes STR Unmethylated Wildtype P6 42 F No GTR NA Mutation P7 40 F Yes GTR Unmethylated Wildtype P8 36 F Yes GTR Unmethylated Wildtype P9 68 M No GTR Methylated Wildtype

[0185] The MRgFUS procedures were performed using a hemispheric array device (ExAblate, InSightec), overlapping the first day of each five-day temozolomide course (FIG. **1**A, FIG. **1**B). Each regimen was prescribed monthly. In total, 38 procedures were performed, with average 7.8±6.0 cm.sup.3 (range 0.8-23.1 cm.sup.3) of tissue treated within sonication time of 111±39 minutes. In tailoring the target with MRI guidance, the sonication of peri-tumor or peri-cavity regions was prioritized, as well as regions with FLAIR hyperintensities, to improve temozolomide penetration to presumably abnormal tissue (FIG. **1**C). Ultrasound delivery was automated based on real-time monitoring of the acoustic emissions.

[0186] Immediately following the procedure, T1-weighted MRI visualized additional areas of gadobutrol (604 Da) enhancement, indicative of successful BBBO. Decreases in the enhancement the next day in all cases demonstrate partial or complete restoration of the BBB permeability (FIG. **1**D). Maps of intensity differences show the spatial distribution of BBBO to envelope the tumor, which was feasible even for large, deep-seated lesions (FIG. **1**E). The MRgFUS treatments were overall well-tolerated in all patients without any serious adverse events (see Table 2 below).

TABLE-US-00002 TABLE 2 MRgFUS related adverse events over a total of 38 procedures Adverse event Severity N (%) Intra-procedure Headache Mild to moderate 8 (21%) Presyncope Mild to moderate 2 (5%) Nausea/vomiting Mild to moderate 5 (13%) Agitation Mild 2 (5%) Hypotension Mild 2 (5%) Post-procedure, transient Pin site tenderness or Mild to moderate 11 (29%) edema T2* GRE hypointensity Mild 2 (5%)

Example 2: Transient BBB Opening Increases the Concentration of Liquid Biopsy Analytes

[0187] In this Example, blood samples were collected within three hours prior to the first sonication (e.g., during patient preparation), and on average 34 minutes after the last sonication. Plasma cfDNA concentration was acutely elevated after BBBO by 2.6±1.2-fold (from 7.0±3.3 ng/ml to 16.3±5.2 ng/ml of plasma, $p<0.01$, FIG. **2**A). Values from individual cycles are listed in Table 3 below.

TABLE-US-00003 TABLE 3 Plasma cfDNA concentrations pre- and post-BBBO at individual cycles. Sonication Pre-BBBO Post-BBBO volume (cm.sup.3) (ng/mL plasma) (ng/mL plasma) Patient 1 Cycle 1 3.2 10.9 13.1 Cycle 2 3.5 9.0 17.7 Cycle 3 3.5 8.0 6.9 Cycle 4 4.8 10.8 23.3 Patient 2 Cycle 1 3.5 3.9 4.6 Cycle 2 2.5 5.7 9.4 Cycle 3 3.2 3.1 6.9 Cycle 4 5.5 3.0 11.0 Cycle 5 5.1 3.7 12.8 Cycle 6 5.9 4.8 10.3 Patient 3 Cycle 1 0.8 11.0 8.3 Cycle 2 2.0 7.7 29.6 Cycle 3 3.2 13.1 26.0 Cycle 4 3.9 7.9 28.7 Patient 4 Cycle 1 3.9 2.4 5.2 Cycle 2 4.7 6.3 23.0 Cycle 3 6.3 — 23.7 Patient 5 Cycle 1 7.5 5.3 8.5 Cycle 2 9.8 5.6 15.0 Cycle 3 6.7 6.6 9.5 Cycle 4 3.5 6.0 14.0 Patient 6 Cycle 1 5.9 5.7 22.3 Cycle 2 6.3 3.3 22.7 Cycle 3 7.9 4.8 13.5 Cycle 4 8.7 3.8 15.9 Cycle 5 7.9 4.1 9.2 Cycle 6 12.1 5.8 9.2 Patient 7 Cycle 1 6.3 7.2 21.8 Cycle 2 7.9 10.7 30.0 Cycle 3 7.9 5.0 13.3 Cycle 4 14.2 4.1 16.8 Patient 8 Cycle 1 19.2 4.1 11.7 Cycle 2 21.2 3.8 12.2 Cycle 3 20.3 4.9 11.6 Cycle 4 23.1 3.8 7.1 Cycle 5 22.0 4.2 11.2 Patient 9 Cycle 1 4.9 13.7 17.1 Cycle 2 6.2 13.2 28.8

[0188] Up to a 7-fold increase in yield was measured. The cfDNA enhancement was consistently observed longitudinally through the adjuvant temozolomide cycles (FIG. **2**B). To assess the quality and fragmentation pattern of the cfDNA, DNA electrophoresis was performed on one paired samples from each patient, and the results showed the 0 to 280 bp fragments increased from 4.9±3.9 to 17.0±14.9 ng/ml of plasma ($p<0.01$) (FIG. **2**C, FIG. **5**).

[0189] MRgFUS-induced cfDNA increase appeared to share a positive correlation with treated volume (Spearman's correlation 0.33, $p=0.04$, FIG. **2**D), and a weaker negative correlation with time elapsed from sonication (Spearman's correlation −0.22, $p=0.20$, FIG. **6**). The level of cfDNA enhancement in two patients with subtotal resection was not different from the others (FIG. **7**A), but there was a trend of greater enhancement with tumor progression (FIG. **7**B).

[0190] The Examples herein demonstrated that a transient BBBO increases brain-derived biomarkers, specifically neuron-derived extracellular vesicles ("ndEV") and S100 calcium-binding protein B (S100b). The latter is primarily expressed by astrocytes. The former is robustly and specifically marked by NCAM and L1CAM surface proteins, and provides a diagnostic platform that can dynamically reflect and track neuropathological changes in vivo. From one randomly selected cycle from each patient, a 3.2±1.9-fold ($p<0.01$, FIG. **2**E) increase was observed in double-positive NCAM and L1CAM particles using nanoscale flow cytometry (FIG. **8**), and a 1.4±0.2-fold ($p<0.01$, FIG. **2**F) in S100b.

Example 3: Post-BBBO cfDNA have Distinctive Disease-Specific Signature

[0191] DNA methylation profiling is a useful tool for classification of CNS tumors and their subtypes. In this Example, unsupervised classification of methylation microarray data of pre- and post-BBBO cfDNA shows a clear separation of the two groups by principle component 1, explaining for 25% of the variability (FIG. **3**A). It was necessary to pool pre-BBBO samples collected from multiple cycles due to low cfDNA yield, while it was feasible to perform post-BBBO analysis on single sessions. Direct comparison of the beta values found 95% (330 of 346) of the differentially methylated probes in the post-BBBO samples were hypomethylated compared to pre-BBBO, consistent with a cancer signature (FIG. **9**).

[0192] A gene set enrichment analysis was performed on the group of hypomethylated probes using g: Profiler. Based on protein expression data from the Human Protein Atlas, the set was significantly enriched for glia and neuron as well as immunologic cells (FIG. **3**B). Of biological processes, enriched terms consisted of immune activation, vesicle-mediated transport, regulation of cell communication and DNA-binding transcription factor activity.

[0193] To determine whether the methylation signature of post-BBBO samples was unique to GBM patients, the experiments incorporated control plasma samples provided by two non-brain tumor patients with a history of Alzheimer's undergoing MRgFUS BBBO alone. Principle component analysis showed a clustering consistent with post-BBBO samples having differentiated signatures amongst patients with different neurological disorders (FIG. **3**C).

[0194] IDH1 mutational status is an important molecular classification and prognostication factor for high-grade gliomas. Only one GBM tumor out of nine was immunopositive for the R132H mutation. Targeted analysis of two different cycles with droplet digital PCR (ddPCR), a highly sensitive technique, found 3.5 and 5 IDH1-R132H mutant copies per 10 ng cfDNA, which is considered a positive readout. These measurements represent a 2 to 3-fold increase from 1.6 mutant copies measured in the same input pre-BBBO cfDNA. Negative controls, from two patients with IDH1 wildtype tumors post-BBBO, had in comparison 1.7±0.1 mutant copies per 10 ng cfDNA.

[0195] The results disclosed herein are the first-in-human proof-of concept studies documenting that MRgFUS enriches the signal of circulating brain-derived biomarkers, demonstrating the ability of the technology to support liquid biopsy assessment (e.g., via blood samples) for the brain.

[0196] The BBB physically limits the quantity of tumor analytes in the circulation. The Examples herein demonstrate for the first time in human patients that transcranial low-frequency MRgFUS can enrich the signal of circulating brain-derived biomarkers, specifically proteins, cfDNA, and extracellular vesicles ("EVs"), to help overcome this limitation. The Examples herein demonstrate a significant increase in cfDNA yield post-MRgFUS BBBO that bear a disease-specific methylation signature unique to brain tumor patients after BBBO. The Examples herein demonstrate an increased signal in clinically actionable plasma IDH1-R132H mutant copies, (e.g., in a single patient harboring the IDH1-R132H tumor mutation). The Examples herein demonstrate the practical feasibility of repeated large volume BBBO in patients over the longitudinal course of adjuvant chemotherapy, supporting the flexibility of the technology when combined with liquid biopsy for disease monitoring.

Methods

[0197] The Examples included demonstrate, among other things, that focused ultrasound enabled

liquid biopsy enriches the signal of circulating biomarkers in patients with brain tumors. The work described herein was part of a prospective single-arm, open-label trial designed to investigate the safety and feasibility of serial MRgFUS and adjuvant temozolomide combination in patients with WHO grade IV glioblastoma (GBM). Details of the inclusion and exclusion criteria can be found in Table 4, below.

TABLE-US-00004 TABLE 4 Study Inclusion and exclusion criteria Inclusion Exclusion 18-80 years of age ≥25% increase in contrast enhancement Underwent surgical of the lesion on MRI at time of enrollment resection relative to first postoperative MRI Pathology diagnosis Evidence of increasing intracranial of WHO grade pressure IV glioblastoma Receiving bevacizumab therapy Completed concurrent Undergoing other concurrent experimental temozolomide therapies e.g. chemotherapy wafer, viral chemoradiation therapy, immunotherapies were excluded without complications Recent (<2 weeks) intracranial and deemed eligible for hemorrhage maintenance temozolomide Increased risk of bleeding, e.g. therapy anticoagulation or antiplatelet therapy Karnofsky score > 70 Contraindication to MRI, gadolinium-based ASA 1-3 contrast, and ultrasound contrast agent Definity ® TIA within the last 1 month, documented cerebral infarction within the last 12 months Cerebral or systemic vasculopathy Severe hypertension, cardiac and pulmonary disorders

[0198] Blood samples were collected from all patients for exploratory analysis. Non-brain tumor control samples were provided by the initial patients enrolled in the MRgFUS BBBO for Alzheimer's disease (“AD”) study.

[0199] Cancer patients underwent the procedure on the first day of every cycle, approximately thirty minutes after ingesting oral temozolomide prescribed by the study neuro-oncologist (FIG. **1**A). Procedures were performed with the ExAblate Neuro hemispheric device (InSightec, Israel) coupled with GE 3-Tesla MRI. The device consists of a hemi-spherical dome with 1024 individual 220 KHz transducer elements that enable precise transcranial MRgFUS delivery. Specifically, the treatment volumes were prescribed by the neurosurgeon by outlining any non-enhancing tumor and a 1-centimeter peritumoral non-enhancing margin on 4 mm interval axial MRIs (FIG. **1**B, FIG. **1**C). Each contour was then filled in by the device software with sonication points separated by 2.5 mm, with approximately 7 mm out-of-plane depth (FIG. **1**C). Contours were kept to less than 20 spots. Ultrasound delivery was performed contour-by-contour in a serial fashion, and was automated using on real-time acoustic emission. In the case of control patients with AD, widespread regions including the hippocampus and parietal lobe were treated by targeting in a similar fashion. Upon completion of the procedure, patients were allowed to leave once meeting routine discharge criteria. Standard-of-care procedures (e.g. MRI) and RANO assessments continued as usual. The procedures ended for a patient (i.e. study exit) when adjuvant TMZ was no longer indicated (e.g. progressive disease, or drug toxicity).

T1-Weighted MRI Analysis

[0200] BBBO was assessed by contrast-enhanced T1-weighted MRIs, which was validated against histological markers of BBB integrity. Intensity difference maps were calculated from same and next day scans by brain extraction, rigid-body co-registration, masking to the region of interest, rescaling to 0 and 1, and subtraction (FMRIB software library v6.0, Advanced Normalization Tools v2.1).

Blood Collection and s100b Measurement

[0201] For all patients, blood was collected immediately prior to, and following, each procedure in EDTA-coated tubes (BD, 366643). Within 30 minutes of collection, plasma tubes were centrifuged at 800 g for 10 minutes at 4° C., then 12,000 g for 10 minutes at room temperature. Clarified supernatant was aliquoted into DNA Lo-Bind tubes (Eppendorf, 22431021) for storage at −80° C. Sample for any analysis will have undergone at the most one freeze-thaw cycle. S100b protein levels were measured via enzyme-linked immunosorbent assay kit (Sigma-Aldrich, EZHS100B-33K) as per manufacturer instructions.

cfDNA Extraction

[0202] The MagMAX Cell-Free DNA Isolation kit (Applied Biosystems, A29319) was used to extract cfDNA. DNA concentration was measured by Qubit 3.0 Fluorometer with Qubit dsDNA HS Assay Kit (Invitrogen, Q32854), and the fragmentation pattern was assessed using the Agilent 2100 Bioanalyzer with Agilent High Sensitive DNA Kit, following manufacturer's instructions.

Methylation Profile Analysis

[0203] 100-200 ng of extracted cfDNA, which was necessarily pooled for pre-BBBO samples, were bisulfite-converted using EZ DNA Methylation kit (Zymo Research, D5002) following the manufacturer's protocol. Effective conversion was confirmed by qPCR amplification of converted and unconverted beta-actin (see Table 5, below).

TABLE-US-00005 TABLE 5 Primers used for bisulfite-conversion quality control qPCR Converted beta-actin Unconverted beta-actin forward: 5′-TGG TGA TGG AGG forward: 5′-TGG TGA TGG AGG AGG CTC AGG TTT AGT AAG T-3′ AGC AAG T-3′ (SEQ ID NO: 1) (SEQ ID NO: 3) reverse: 5′-AAC CAA TAA AAC CTA reverse: 5′-AGC CAA TGG GAC CTG CTC CTC CTC CCT TAA-3′ CTC CCT TGA-3′ (SEQ ID NO: 2) (SEQ ID NO: 4)

[0204] Bisulfite-converted DNA was evaluated for methylation profiling using Illumina MethylationEPIC 850k array (Illumina, USA). Preprocessing with background subtraction adjustment was performed using GenomeStudio (Illumina, USA). Raw data files were processed using the minifi package (Bioconductor) in R and normalized. Probes that overlap with known single nucleotide polymorphisms, and probes that map to X and Y chromosomes were filtered out. Methylation values were exported as beta values. For unsupervised clustering, we performed a principle component analysis on the top 100,000 most variably methylated probes based on mean absolute deviation. Furthermore, differentially methylated probes between pre- and post-BBBO samples were identified by an absolute difference of mean beta value >0.1 and FDR-corrected $p<0.05$. Functional enrichment analysis was conducted using g: Profiler tool.

Plasma Extracellular Vesicles

[0205] NCAM (CD56) and L1CAM (CD171) positive EVs were measured by mixing and incubating 20 μL of plasma with Alexa Fluor 647 mouse anti-human CD56 (2 μL, BD Biosciences #557711) and PE mouse anti-human CD171 (2 μL, BD Biosciences #564193) at room temperature. Isotype controls were prepared similarly with Alexa Fluor 647 mouse IgG1 κ isotype control (2 μL, BD Biosciences #57714), and PE Mouse IgG2a κ isotype control (2 μL, BD Biosciences #553457). A plasma sample from one cycle in every patient (Table 6, below) was diluted by 1:30 in sterile medical grade water and measured in duplicates using a nanoscale flow cytometer (Apogee Flow Systems Inc. A60Micro-Plus) calibrated with Apogee calibration bead mix. Data analysis was performed using Apogee Histogram Software v2020.

TABLE-US-00006 TABLE 6 The cycle from which plasma extracellular vesicles were measured in each patient. Patient Cycle number 1 1 2 6 3 3 4 — 5 4 6 5 7 3 8 6 9 2

Digital Droplet PCR

[0206] Frequency of isocitrate dehydrogenase 1 (IDH1) R132H mutations in plasma-derived cfDNA was assessed using a Bio-Rad QX200 ddPCR system. The ddPCR reaction was performed in a 20 μl volume containing 10 ng of isolated plasma cfDNA or IDH1 R132H immunopositive tumor DNA (positive control), 10 μL of ddPCR Supermix for Probes (No dUTP; Bio-Rad, cat. #186-3023) and 1 μL of Human FAM IDH1 p.R132H c.395G>A ddPCR Mutation Detection Assay (Bio-Rad, Assay ID dHsaMDV2010055). After mixing by vortexing and a spin down, droplets were generated using a QX200 Droplet Generator. Droplets were pipetted into a 96-well PCR plate, which was sealed and cfDNA samples amplified in the C1000 Touch Thermal Cycler (Bio-Rad) using the manufacturer recommended protocol. The fluorescent signal in each droplet was read with a QX200 Droplet Reader. Mutant and wild-type droplets were quantified using QuantaSoft analysis software ver. 1.7.4.0917 (Bio-Rad).

Statistical Analysis
[0207] Descriptive statistics were used to summarize results, but wherever possible all data points were presented. Pairwise data were compared using Wilcoxon signed-rank test, with an alpha of 0.05.
EQUIVALENTS
[0208] While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features herein set forth and as follows in the scope of the appended claims.
[0209] Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific embodiments described specifically herein. Such equivalents are intended to be encompassed in the scope of the following claims.
INCORPORATION BY REFERENCE
[0210] All patents and publications referenced herein are hereby incorporated by reference in their entireties.
[0211] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention.
[0212] As used herein, all headings are simply for organization and are not intended to limit the disclosure in any manner. The content of any individual section may be equally applicable to all sections.

Claims

1.-**3**. (canceled)
4. A method of evaluating a subtype or mutational basis of a disease or disorder of the brain in a human patient, comprising: (a) obtaining a plasma sample from the human patient, the human patient having been exposed to an ultrasound beam across the cranium to cause disruption of the blood-brain barrier (BBB) and to permit the transit of one or more brain-derived biomarkers into the blood; (b) contacting the plasma sample with a detection agent to detect the presence, absence or level of the one or more brain-derived biomarkers; and (c) determining a subtype or mutational basis of the disease or disorder based on step (b).
5.-**7**. (canceled)
8. The method of claim 4, wherein the brain-derived biomarker: circulates in the cerebrospinal fluid in the absence of the ultrasound, transits from the cerebrospinal fluid to the blood via the disrupted BBB, is not perturbed from a tissue by the ultrasound application or exposure, is a disease or drug response biomarker, and/or is selected from a cancer biomarker; an abnormal methylation of a tumor suppressor gene selected from p16, CDKN2B, and p14ARF for brain cancer; a glioblastoma biomarker; an Alzheimer's disease biomarker; a Parkinson's disease biomarker, a glioblastoma biomarker, and abnormal methylation of a tumor suppressor gene selected from p16, CDKN2B, and p14ARF for brain cancer.
9.-**12**. (canceled)
13. The method of claim 8, wherein: the cancer biomarker is selected from AFP, BCR-ABL, BRCA1/BRCA2, BRAF V600E, CA-125, CA19.9, CEA, EGFR, HER-2, KIT, PSA, PD-1, PD-L1, and S100, the cancer biomarker and cancer is selected from AFP (liver cancer), BCR-ABL (chronic myeloid leukemia), BRCA1/BRCA2 (breast/ovarian cancer), BRAF V600E (melanoma/colorectal cancer), CA-125 (ovarian cancer), CA19.9 (pancreatic cancer), CEA (colorectal cancer), EGFR (non-small-cell lung carcinoma), HER-2 (breast cancer), KIT (gastrointestinal stromal tumor), PSA

(prostate specific antigen) (prostate cancer), and $100 (melanoma), or the cancer biomarker and cancer is selected from mutations on genes KRAS, p53, EGFR, erbB2 for colorectal, esophageal, liver, and pancreatic cancer: mutations of genes BRCA1 and BRCA2 for breast and ovarian cancer; hypermethylation of MYOD1, CDH1, and CDH13 for cervical cancer; and hypermethylation of p16, p14, and RB1, for oral cancer.

14.-**25**. (canceled)

26. The method of claim 4, wherein the brain-derived biomarker is; a nucleic acid selected from DNA, which is optionally cell-free DNA (cfDNA) or RNA, which is optionally microRNA, a protein, which is optionally a cytokine, a whole cell, extracellular vesicle selected from an exosome, ectosome, or large oncosome, or a metabolite.

27.-**35**. (canceled)

36. The method of claim 4, wherein the disease or disorder of the brain is a cancer; a neurodegenerative disease; an anxiety disorders selected from generalized anxiety disorders, social phobias, specific phobias, panic disorders, obsessive compulsive disorder (OCD) and post-traumatic stress disorder (PTSD): addiction; bipolar disorder; a behavior disorder selected from oppositional defiant disorder (ODD), conduct disorder (CD) and attention deficit hyperactivity disorder (ADHD); depression; an eating disorder selected from anorexia, bulimia nervosa and other binge eating disorders; epilepsy; an autoimmune disease; multiple sclerosis; or a disease or disorder of the brain is selected from dementia and cognitive dysfunction.

37. The method of claim 36, wherein the cancer is a metastatic tumor in the brain.

38. (canceled)

39. The method of claim 36, wherein the cancer is a primary brain tumor.

40. The method of claim 39, wherein the primary brain tumor is glial, optionally wherein the primary brain tumor is glial, optionally wherein the glial tumor is one or more of astrocytoma, ependymoma, glioblastoma multiforme (GBM), medulloblastoma, and oligodendroglioma.

41. (canceled)

42. The method of claim 39, wherein the primary brain tumor is non-glial.

43. The method of claim 36, wherein: the brain cancer is a posterior fossa tumor, optionally selected from a cerebellar tumor, pontine tumor, and medulloblastoma (primitive neuroectodermal tumor) and/or a cerebral hemisphere tumor, or the cancer is a glioblastoma.

44.-**46**. (canceled)

47. The method of claim 4, wherein the neurodegenerative disease is Alzheimer's disease or Parkinson's disease.

48.-**58**. (canceled)

59. The method of claim 4, wherein the human patient is afflicted with, suspected to be afflicted with, or at risk for developing a cancer or a neurodegenerative disease.

60.-**71**. (canceled)

72. The method of claim 4, wherein the ultrasound beam is a focused, and/or a guided ultrasound beam selected from a magnetic resonance-guided ultrasound beam (MRgFUS), a computerized tomography (CT)-guided ultrasound beam, a positron emission tomography (PET)-guided ultrasound beam, or a stereotactically-navigated guided ultrasound beam based on registration to prior scan.

73.-**77**. (canceled)

78. The method of claim 4, wherein the ultrasound beam is applied: directly to the human patient's cranium using a helmet-shaped ultrasound transducer, at a center frequency of about 220 KHz, at a maximum power of up to about 30 Watts, for at least about 10 seconds, or at least about 20 seconds, or at least about 30 seconds, or at least about 40 seconds, or at least about 50 seconds, or at least about 60 seconds, for about 10 seconds, or about 20 seconds, or about 30 seconds, or about 40 seconds, or about 50 seconds, or about 60 seconds, in pulses, at a power of at least about 5W, or at least about 10 W, or at least about 15W, or at least about 20W, or at least about 25W, at a power

of about 10W, or about 15W, or about 20W, and/or to: at least one region of the brain; at least two regions of the brain; at least three regions of the brain; and/or at least one, or two, or three regions of the brain contemporaneously.

79.-**85**. (canceled)

86. The method of claim 4, wherein the ultrasound beam targets one or more of the frontal lobe, parietal lobe, temporal lobe, occipital lobe, and cerebellum, the supratentorial region of the brain, the infratentorial region of the brain, the insula, the brainstem, the pons, the posterior fossa, the corticomedullary gray/white junction, and/or the meninges.

87.-**94**. (canceled)

95. The method of claim 4, wherein the detecting comprises: measuring a presence, absence, or level of an epigenetic pattern or profile; measuring a presence, absence, or level of a methylation pattern or signature; chromatin immunoprecipitation (ChIP) or bisulfite modification; measuring a presence, absence, or level of a histone modification; measuring a presence, absence, or level of a mutant version of the biomarker: measuring a presence, absence, or level of a tumor-associated mutation; measuring a presence, absence, or level of a mutational burden; measuring a presence, absence, or level of a polymorphism; measuring a presence, absence, or level of a SNPs; measuring a presence, absence, or level of a copy number aberration, optionally as compared to a reference or parental genome; measuring a presence, absence, or level of a microsatellite instability; an primer-based detection method; an probe-based detection method; polymerase chain reaction (PCR), reverse transcriptase polymerase chain reaction (RT-PCR), quantitative PCR (qPCR), multiplex polymerase chain reaction, nested polymerase chain reaction, hot start polymerase chain reaction, long-range PCR, assembly polymerase chain reaction, asymmetric polymerase chain reaction, or digital droplet PCT; a recombinase polymerase amplification (RPA), a loop-mediated amplification (LAMP), or a helicase-dependent amplification (HDA); RNA or DNA gel electrophoresis or Southern or Northern blotting; a microarray-based assay; a hybridization technique, optionally selected from solution hybridization, capillary hybridization, hybridization to nucleic acid arrays, optionally macroarrays, microarrays or high-density oligonucleotide arrays (Gene Chips); DNA sequencing: an antibody-based detection method; spectroscopy, optionally mass spectroscopy; and/or immunohistochemical staining, western blotting, in-cell western, immunofluorescent staining, ELISA, and fluorescent activating cell sorting (FACS).

96.-**105**. (canceled)

106. The method of claim 4, wherein the detection agent is: a primer or probe, optionally a labeled probe, optionally fluorescently or radiolabeled; an antibody: an antibody specific for an epigenetic signature and/or nucleosome-protein adduct; and/or an antibody specific for a mutant protein.

107.-**121**. (canceled)

122. The method of claim 4, wherein the diagnosing comprises prediction of disease onset or progression, progression or pseudoprogression, response to treatment, measuring of minimal residual disease status, and/or prediction of recurrence.

123.-**126**. (canceled)

127. The method of claim 4, wherein: the disease or disorder of the brain is a glioblastoma and the brain-derived biomarker is selected from IDH mutations, 1p19q deletion, MGMT promoter methylation, and EGFRvIII amplification; the disease or disorder of the brain is Alzheimer's disease and the brain-derived biomarker is selected from amyloid beta protein, tau protein, APOE &4 variant, APP mutation, PSEN1 mutations, and PSEN2 mutations; and/or the disease or disorder of the brain is Parkinson's disease and the brain-derived biomarker is selected from alpha-synuclein and GBA mutations.

128.-**148**. (canceled)

149. The method of claim 4, wherein the method further comprises administration of one or more microbubble compositions or the human patient has undergone administration with one or more microbubble compositions immediately before and/or during the application of the ultrasound

beam, wherein: the one or more microbubble compositions have been administered immediately before and/or during the application of the ultrasound beam, or contemporaneously with the application of the ultrasound beam; the microbubble compositions comprise one or more lipid-based microspheres; the microbubble compositions are perflutren lipid microspheres; microbubble compositions comprise (R)-hexadecanoic acid, 1-[(phosphonoxy)methyl]-1,2-ethanediyl ester, monosodium salt (DPPA); (R)-4-hydroxy-N,N,Ntrimethyl-10-oxo-7-[(1-oxohexadecyl)oxy]-3,4,9-trioxa-4-phosphapentacosan-1-aminium, 4-oxide, inner salt (DPPC); and (R)-α-[6-hydroxy-6-oxido-9-[(1-oxohexadecyl)oxy]-5,7,11-trioxa-2-aza-6-phosphahexacos-1-yl]-ω-methoxypoly (ox-1,2-ethanediyl), monosodium salt; (N-(methoxypolyethylene glycol 5000 carbamoyl)-1,2-dipalmitoyl-sn-glycero-3-phosphatidylethanolamine, monosodium salt, MPEG5000 DPPE); the microbubble compositions are lipid-coated echogenic microbubbles filled with octafluoropropane gas; the microbubble compositions comprise octafluoropropane encapsulated in an outer lipid shell comprising (R)-hexadecanoic acid, 1-[(phosphonoxy)methyl]-1,2-ethanediyl ester, monosodium salt (DPPA); (R)-4-hydroxy-N,N,Ntrimethyl-10-oxo-7-[(1-oxohexadecyl)oxy]-3,4,9-trioxa-4-phosphapentacosan-1-aminium, 4-oxide, inner salt (DPPC); and (R)-α-[6-hydroxy-6-oxido-9-[(1-oxohexadecyl)oxy]-5,7,11-trioxa-2-aza-6-phosphahexacos-1-yl]-ω-methoxypoly (ox-1,2-ethanediyl), monosodium salt; (N-(methoxypolyethylene glycol 5000 carbamoyl)-1,2-dipalmitoyl-sn-glycero-3-phosphatidylethanolamine, monosodium salt, MPEG5000 DPPE); the microbubble compositions have been administered to the patient no more than 60, or 30, or 20, or 10 minutes before the application of the ultrasound beam; the microbubble compositions have been administered to the patient throughout the method; the microbubble compositions have been administered by systemic injection, bolus injection or slow diffusion injection; the microbubble compositions have been administered by systemic infusion; the microbubble compositions have been administered by continuous intravenous infusion; the microbubble compositions have been administered by continuous intravenous infusion of a mixture about 1×10.sup.10 to about 6×10.sup.10 microsphere in a carrier; the microbubble compositions have been administered by continuous intravenous infusion of a mixture about 1×10.sup.10 to about 6×10.sup.10 microsphere in a saline carrier; the microbubble compositions have been administered by continuous intravenous infusion of a mixture about 1×10.sup.10 to about 6×10.sup.10 microsphere in about 250 mL of saline carrier; the microbubble compositions have been administered by continuous intravenous infusion of about 5×10.sup.7 to about 3×10.sup.8/mL; and/or the microbubble compositions are continuously infused at a rate of about 1 to about 3 mL/minute during the application of the ultrasound beam.

150.-**176**. (canceled)