



(51) International Patent Classification:

A61K 35/30 (2015.01) C07K 14/16 (2006.01)
C12N 5/071 (2010.01) A61P 31/18 (2006.01)

(21) International Application Number:

PCT/US2024/059659

(22) International Filing Date:

11 December 2024 (11.12.2024)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

63/609,031 12 December 2023 (12.12.2023) US

(71) Applicant: **THE REGENTS OF THE UNIVERSITY OF CALIFORNIA** [US/US]; 1111 Franklin Street, 5th Floor, Oakland, California 94607-5200 (US).

(72) Inventor: **FIELDS, Jerel Adam**; c/o UC San Diego, 9500 Gilman Drive, Mail Code 0910, La Jolla, California 92093 (US).

(74) Agent: **KONSKI, Antoinette F.** et al.; Foley & Lardner LLP, 3000 K Street N.W., Suite 600, Washington, District of Columbia 20007-5109 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO,

RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to non-prejudicial disclosures or exceptions to lack of novelty (Rule 4.17(v))

Published:

- with international search report (Art. 21(3))

(54) Title: NOVEL DIAGNOSTIC PLATFORM FOR BRAIN DISEASE

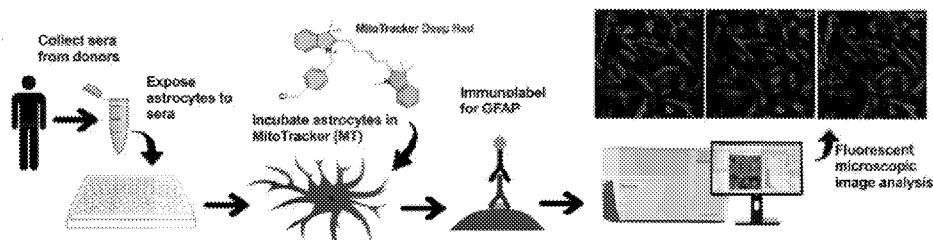


FIG. 1A

(57) Abstract: Applicant provides herein an *in vitro* method to determine if one or more test agents modify metabolic changes in a sample of brain cells, e.g., astrocytes, the method comprising, or consisting essentially of, or consisting of: culturing the sample with sera and/or cerebrospinal fluid (CSF) and the one or more test agents and assaying the sample combined with the sera and/or CSF for metabolic changes.

NOVEL DIAGNOSTIC PLATFORM FOR BRAIN DISEASE

CROSS-REFERENCE TO RELATED PATENT APPLICATIONS

[0001] This application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application No. 63/609,031, filed December 12, 2023, the contents of which are incorporated herein by reference.

STATEMENT OF GOVERNMENT SUPPORT

[0002] This invention was made with government support under Grant No. MH128108, awarded by the National Institutes of Health (“NIH”). The government has certain rights in the invention.

BACKGROUND

[0003] People with human immunodeficiency virus (HIV; PWH) experience significant comorbidities, including psychiatric disorders (ex: depression) and neurological disorders (ex: HIV-associated neurocognitive disorders (HAND)) despite effective antiretroviral therapies (ART) (1-5). Depression occurs in PWH at a rate 2-3x the general population, with some meta-analyses finding that a global average of 39% of PWH experience depression (6). Additionally, PWH are living longer as a result of treatment with ART, leading to an increase in the prevalence of HIV-associated neurologic disorders (7). Low levels of central nervous system (CNS) viral expression and antiretroviral drugs themselves can lead to chronic neuroinflammation and mitochondrial dysfunction (3), pathologies known to occur in a variety of neurologic and psychiatric disorders (8-10). However, the mechanisms underlying HIV and ART-induced psychiatric and neurologic dysfunction are incompletely understood.

[0004] Therapies targeting HIV-associated neurologic disorders, such as HAND, are lacking, and inflammatory depression, the phenotype that potentially underlies HIV-associated depression, is less responsive to antidepressants (32). Thus, new approaches are needed to identify mechanisms of and treatments for HIV- and ART-associated psychiatric and neurologic disorders. A recent study showed that sera collected from bipolar patients, compared to sera from controls, induced dendritic simplification in neurons in culture (33), demonstrating that in vitro cultures exposed to blood specimens from patients suffering from

a psychiatric disorder might reflect and provide a platform to investigate neuropathogenic mechanisms occurring in patients. However, the degree to which sera-induced dendritic simplification varied among the donor samples, suggesting individualized neuropathogenic mechanisms. Thus, a need exists in the art to provide non-invasive techniques to identify specific mechanisms underlying disease in individual patients and lead to patient-specific therapeutic approaches. This disclosure satisfies this need and related advantages as well.

SUMMARY OF THE DISCLOSURE

[0005] Applicant provides herein an *in vitro* method to determine the cognitive status of a subject and/or to determine if one or more test agents modify metabolic changes in a sample of brain cells, for example neurons, microglia, oligodendrocytes and astrocytes, the method comprising, or consisting essentially of, or consisting of: culturing the sample with sera and/or cerebrospinal fluid (CSF) obtained from the subject or patient and optionally the one or more test agents and assaying the sample combined with the sera and/or CSF for metabolic changes. In one aspect, the sera and/or CSF are isolated from a subject that has been assessed from cognitive and/or behavioral changes or abnormalities. A non-limiting example of cognitive and/or behavioral changes comprises depression. In a further aspect, the cognitive and/or behavioral changes comprises neurocognitive impairment.

[0006] Applicant discloses herein a method of screening compounds for drug potential and a method to evaluate potential compounds as treatment for patients. The methods are schematically represented in **FIGS. 1 and 2**.

[0007] In a further aspect of the disclosure, the brain cells, for example neurons, microglia, oligodendrocytes and astrocytes are assayed for metabolic baseline prior to culturing with the sample and/or CSF and/or the test agent.

[0008] In a further aspect, the metabolic baseline and metabolic activity is determined by a method selected from one or more of: fluorescent microscopic image analysis, metabolic assays, mitochondrial activity using MitoTracker or other potentiometric dyes, mitochondrial respiration, and gene expression analysis.

[0009] In a further aspect, the metabolic change is determined by a method selected from one or more of: fluorescent microscopic image analysis, metabolic assays, mitochondrial respiration, and gene expression analysis.

[0010] In another embodiment, the method further comprises, or consists essentially of, or consists of correlating the metabolic change with neurological change.

[0011] In another aspect, the sample of sera and/or CSF are isolated from a vertebrate, for example, is a mammal, optionally a human patient.

[0012] In a further aspect, the test agent is a biologic or a small molecule.

[0013] In a further embodiment, the neurological change comprises depression.

[0014] In a yet further aspect, the method further comprises, or alternatively consists essentially of, or consists of, administering to the vertebrate an agent such as the test agent identified to have a positive correlation to neurological change.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] **FIGS. 1A – 1C:** Schematic of experimental design and analysis. **(FIG. 1A)** Sera was collected from donors ($n=40$). Human astrocytes were exposed to sera for 24 h and then incubated with the potentiometric dye, MitoTracker (MT) and immunolabeled for GFAP (glial fibrillary acidic protein). Astrocytes were then imaged using the high throughput fluorescent microscope image analysis system, Thermo Scientific CellInsight CX5 (CX5). **(FIG. 1B)** Spot detector assay analysis definitions for spot average intensity, spot average area, and spot total area per object; and cell health profiling assay analysis definitions target average intensity. **(FIG. 1C)** Representative images for spot detector for MT and target intensity for GFAP.

[0016] **FIG. 2** exemplifies some diagnostic analysis that can be used to evaluate potential compounds as treatment for patients.

[0017] **FIGS. 3A – 3G:** IL-1 β induces increased MitoTracker measures and GFAP in cultured primary human astrocytes compared to control and human sera collected from people with HIV and minimal – severe depressive symptoms. **(FIG. 3A)** Representative images demonstrating target intensity for GFAP and mitotracker (MT) between control (media + FBS) and 20 ng/mL IL-1 β (in media + FBS). Scale bar=10 μ m. Quantification of

target average intensity between control and 24 h of IL-1 β treatment or 10 % v/v human sera/media minus FBS for **(FIG. 3B)** MT and **(FIG. 3C)** GFAP. **(FIG. 3D)** Representative images demonstrating spot detector for MT between control and IL-1 β . Quantification of **(FIG. 3E)** MT spot average intensity **(FIG. 3F)** MT spot total area/cell and **(FIG. 3G)** MT spot average area between control and 24 h of IL-1 β or human sera treatment. Statistical analysis one-way ANOVA with post-hoc Tukey's * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$ compared to control or IL-1 β (bar). # $p < 0.05$ compared to minimal depressive symptoms via t-tailed *t*-test. $n = 4-6$ biologic replicates (Mean $\pm$ SEM).

[0018] FIG. 4: Heat map demonstrating relationships between clinical data of donors and astrocyte reactivity and mitochondrial activity in 24 h sera-exposed human astrocyte cultures. Correlation matrix demonstrating Pearson *r* correlation coefficients ranging from 1- to -1 and color coded with darker hues representing stronger associations, blue for a positive correlation, white for no correlation, and red for negative correlation. The strongest relationships with GFAP target average intensity occur with age (positive), learning memory (negative), recall memory (negative), and verbal fluency (negative). The strongest relationships with mitotracker (MT) signal measures are with Beck's depression inventory (BDI-II, negative), executive function (positive), speed of information processing (SIP, positive), and working memory (positive). The matrix also illustrates a strong negative relationship between BDI-II and cognitive T-scores and a strong positive correlation between GFAP target average intensity and MT signal measures.

[0019] FIGS. 5A – 5C: A higher burden of depression symptoms is associated with decreased mitochondrial activity in 24 h sera-exposed human astrocyte cultures. Simple linear regression was conducted to analyze the strength of association between BDI (Beck's depression inventory) and **(FIG. 5A)** mitotracker (MT) spot average intensity **(FIG. 5B)** MT spot average area and **(FIG. 5C)** MT target average intensity. Higher BDI-II scores are representative of more severe depressive symptoms and higher MT signal measures are representative of more active mitochondria.

[0020] FIGS. 6A – 6J: Higher cognitive function T-Scores, except for learning T-score, were associated with increased mitochondrial activity in 24 h sera-exposed human astrocyte cultures. Simple linear regression was conducted to analyze the strength of association

between **(FIG. 6A)** global T-score and mitotracker (MT) spot average area **(FIG. 6B)** global T-score and MT spot average intensity **(FIG. 6C)** executive T-score and MT target average intensity **(FIG. 6D)** executive T-score and MT spot average area **(FIG. 6E)** executive T-score and MT spot average intensity **(FIG. 6F)** speed of information processing (SIP) T-score and MT target average intensity **(FIG. 6G)** SIP T-score and MT spot average intensity **(FIG. 6H)** working memory T-score and MT spot average intensity **(FIG. 6I)** working memory T-score and MT spot average intensity **(FIG. 6J)** learning T-score and MT spot total area/object. Higher T-scores are representative of better performance and higher MT signal measures are representative of more active mitochondria.

[0021] FIGS. 7A – 7C: Higher cognitive function T-Scores were associated with decreased astrocyte reactivity in 24 h sera-exposed human astrocyte cultures. Simple linear regression was conducted to analyze the strength of association between GFAP target average intensity and **(FIG. 7A)** learning mean T-score **(FIG. 7B)** recall memory T-score **(FIG. 7C)** verbal fluency T-score. Higher T-scores are representative of better performance and higher GFAP signal intensity is representative of increases in astrocyte reactivity.

[0022] FIGS. 8A – 8C: Increased age is associated with increased mitochondrial activity and astrocyte reactivity in 24 h sera-exposed astrocyte cultures. Simple linear regression was conducted to analyze the strength of association between age and **(FIG. 8A)** mitotracker (MT) target average intensity **(FIG. 8B)** MT spot total area/obj **(FIG. 8C)** GFAP target average intensity. Age represented in years. Higher MT signal measures represent more active mitochondria and higher GFAP signal intensity represents increased astrocyte reactivity.

[0023] FIGS. 9A – 9F: Increased depressive symptoms are associated with reduced oxidative phosphorylation (OCR, SRC) and increased glycolysis (ECAR) in 24 h sera-exposed astrocyte cultures. **(FIG. 9A, FIG. 9C, FIG. 9F)** FBS=control (media + FBS), IL-1 β =20 ng/mL IL-1 β in media + FBS, BDI=human sera 10 % v/v in media without FBS. $n=5/\text{group}$. $*p<0.05$, $****p<0.0001$ compared to FBS, IL-1 β (bar), or BDI= <13 (bar). There is a significant negative association between SRC and BDI-II **(FIG. 9D)**, $p = 0.02$, $R^2 = 0.51$, i.e. SCR decreases with more severe depressive symptoms. One-way ANOVA, post-hoc Tukey's (Mean $\pm$ SEM). **(FIG. 9B, FIG. 9E, FIG. 9F)** Simple linear regression. OCR

(oxygen consumption rate), SRC (spare respiratory capacity), ECAR (extracellular acidification rate) were assessed using a Seahorse extracellular Flux Analyzer. Data were normalized to cell count in each well.

[0024] **FIGS. 10A – 10C:** Antidepressant medication usage had no significant change in mitochondrial activity in 24 h sera-exposed astrocyte cultures. Quantification of **(FIG. 10A)** MT spot average intensity, **(FIG. 10B)** MT spot average area, and **(FIG. 10C)** MT spot total area/obj between antidepressant medication users and nonusers. Statistical analysis was performed using a two-tailed *t*-test. *n*=17–23/group (Mean ± SEM).

[0025] **FIG.11:** Cerebrospinal fluid (CSF) from methamphetamine- people with HIV with neurocognitive impairment (NCI) induces a reduction in mitochondrial activity in human astrocytes compared to CSF from neurocognitively unimpaired PWH. Human astrocytes were incubated with CSF (10% of media) from PWH who were NUI and also those with NCI. After 24 hours, human astrocytes were incubated with MT and then fixed and analyzed by fluorescent microscopy. Worse NCI is associated with reduced MT intensity in human astrocytes. MT average intensity in astrocytes exposed to CSF from PWH without and with NCI. (**p*<0.05; Cohen's *d* = 0.66).

DETAILED DESCRIPTION OF THE DISCLOSURE

[0026] Definitions

[0027] Unless defined otherwise, all technical and scientific terms used herein have the same meanings 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 invention, the preferred methods, devices, and materials are now described. All technical and patent publications cited herein are incorporated herein by reference in their entirety. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

[0028] Throughout and within this application technical and patent literature are referenced by a citation. For certain of these references, the identifying citation is found at the end of this application immediately preceding the claims. All publications are incorporated by reference

into the present disclosure to more fully describe the state of the art to which this disclosure pertains.

[0029] The practice of the present disclosure will employ, unless otherwise indicated, conventional techniques of tissue culture, immunology, molecular biology, microbiology, cell biology and recombinant DNA, which are within the skill of the art. See, e.g., Sambrook and Russell eds. (2001) *Molecular Cloning: A Laboratory Manual*, 3rd edition; the series Ausubel et al. eds. (2007) *Current Protocols in Molecular Biology*; the series *Methods in Enzymology* (Academic Press, Inc., N.Y.); MacPherson et al. (1991) *PCR 1: A Practical Approach* (IRL Press at Oxford University Press); MacPherson et al. (1995) *PCR 2: A Practical Approach*; Harlow and Lane eds. (1999) *Antibodies, A Laboratory Manual*; Freshney (2005) *Culture of Animal Cells: A Manual of Basic Technique*, 5th edition; Gait ed. (1984) *Oligonucleotide Synthesis*; U.S. Patent No. 4,683,195; Hames and Higgins eds. (1984) *Nucleic Acid Hybridization*; Anderson (1999) *Nucleic Acid Hybridization*; Hames and Higgins eds. (1984) *Transcription and Translation; Immobilized Cells and Enzymes* (IRL Press (1986)); Perbal (1984) *A Practical Guide to Molecular Cloning*; Miller and Calos eds. (1987) *Gene Transfer Vectors for Mammalian Cells* (Cold Spring Harbor Laboratory); Makrides ed. (2003) *Gene Transfer and Expression in Mammalian Cells*; Mayer and Walker eds. (1987) *Immunochemical Methods in Cell and Molecular Biology* (Academic Press, London); Herzenberg et al. eds (1996) *Weir's Handbook of Experimental Immunology; Manipulating the Mouse Embryo: A Laboratory Manual*, 3rd edition (Cold Spring Harbor Laboratory Press (2002)); Sohail (ed.) (2004) *Gene Silencing by RNA Interference: Technology and Application* (CRC Press).

[0030] All numerical designations, e.g., pH, temperature, time, concentration, and molecular weight, including ranges, are approximations which are varied (+) or (-) by increments of 0.1 or 1.0, where appropriate. It is to be understood, although not always explicitly stated that all numerical designations are preceded by the term “about.” It also is to be understood, although not always explicitly stated, that the reagents described herein are merely exemplary and that equivalents of such are known in the art.

[0031] As used in the specification and claims, the singular form “a”, “an” and “the” include plural references unless the context clearly dictates otherwise. For example, the term “a cell” includes a plurality of cells, including mixtures thereof.

[0032] As used herein, the term “comprising” or “comprises” is intended to mean that the compositions and methods include the recited elements, but not excluding others.

“Consisting essentially of” when used to define compositions and methods, shall mean excluding other elements of any essential significance to the combination for the stated purpose. Thus, a composition consisting essentially of the elements as defined herein would not exclude trace contaminants from the isolation and purification method and pharmaceutically acceptable carriers, such as phosphate buffered saline, preservatives and the like. “Consisting of” shall mean excluding more than trace elements of other ingredients and substantial method steps for administering the compositions of this invention or process steps to produce a composition or achieve an intended result. Embodiments defined by each of these transition terms are within the scope of this disclosure.

[0033] The term “isolated” as used herein with respect to nucleic acids, such as DNA or RNA, refers to molecules separated from other DNAs or RNAs, respectively that are present in the natural source of the macromolecule. The term “isolated nucleic acid” is meant to include nucleic acid fragments which are not naturally occurring as fragments and would not be found in the natural state. The term “isolated” is also used herein to refer to polypeptides, proteins and/or host cells that are isolated from other cellular proteins and is meant to encompass both purified and recombinant polypeptides. In other embodiments, the term “isolated” means separated from constituents, cellular and otherwise, in which the cell, tissue, polynucleotide, peptide, polypeptide, protein, antibody or fragment(s) thereof, which are normally associated in nature. For example, an isolated cell is a cell that is separated from tissue or cells of dissimilar phenotype or genotype. As is apparent to those of skill in the art, a non-naturally occurring polynucleotide, peptide, polypeptide, protein, antibody or fragment(s) thereof, does not require “isolation” to distinguish it from its naturally occurring counterpart.

[0034] “Homology” or “identity” or “similarity” refers to sequence similarity between two peptides or between two nucleic acid molecules. Homology can be determined by comparing

a position in each sequence which may be aligned for purposes of comparison. When a position in the compared sequence is occupied by the same base or amino acid, then the molecules are homologous at that position. A degree of homology between sequences is a function of the number of matching or homologous positions shared by the sequences. An “unrelated” or “non-homologous” sequence shares less than 40% identity, or alternatively less than 25% identity, with one of the sequences of the present disclosure.

[0035] A polynucleotide or polynucleotide region (or a polypeptide or polypeptide region) has a certain percentage (for example, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99%) of “sequence identity” to another sequence means that, when aligned, that percentage of bases (or amino acids) are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in Ausubel et al. eds. (2007) Current Protocols in Molecular Biology. Preferably, default parameters are used for alignment. One alignment program is BLAST, using default parameters. In particular, programs are BLASTN and BLASTP, using the following default parameters: Genetic code = standard; filter = none; strand = both; cutoff = 60; expect = 10; Matrix = BLOSUM62; Descriptions = 50 sequences; sort by = HIGH SCORE; Databases = non-redundant, GenBank + EMBL + DDBJ + PDB + GenBank CDS translations + SwissProtein + SPupdate + PIR. Details of these programs can be found at the following Internet address: ncbi.nlm.nih.gov/cgi-bin/BLAST.

[0036] An equivalent or biological equivalent nucleic acid, polynucleotide or oligonucleotide or peptide is one having at least 80 % sequence identity, or alternatively at least 85 % sequence identity, or alternatively at least 90 % sequence identity, or alternatively at least 92 % sequence identity, or alternatively at least 95 % sequence identity, or alternatively at least 97 % sequence identity, or alternatively at least 98 % sequence identity to the reference nucleic acid, polynucleotide, oligonucleotide or peptide.

[0037] “Detectable label”, “label”, “detectable marker” or “marker” are used interchangeably, including, but not limited to radioisotopes, fluorochromes, chemiluminescent compounds, dyes, and proteins, including enzymes. Detectable labels can also be attached to a polynucleotide, polypeptide, antibody or composition described herein.

[0038] Examples of suitable fluorescent labels include, but are not limited to, fluorescein, rhodamine, tetramethylrhodamine, eosin, erythrosin, coumarin, methyl-coumarins, pyrene, Malacite green, stilbene, Lucifer Yellow, Cascade Blue™, and Texas Red. Other suitable optical dyes are described in the Haugland, Richard P. (1996) Handbook of Fluorescent Probes and Research Chemicals (6th ed.).

[0039] In some embodiments, the fluorescent label is functionalized to facilitate covalent attachment to a cellular component present in or on the surface of the cell or tissue such as a cell surface marker. Suitable functional groups, include, but are not limited to, isothiocyanate groups, amino groups, haloacetyl groups, maleimides, succinimidyl esters, and sulfonyl halides, all of which may be used to attach the fluorescent label to a second molecule. The choice of the functional group of the fluorescent label will depend on the site of attachment to either a linker, the agent, the marker, or the second labeling agent.

[0040] As used herein, a purification label or marker refers to a label that may be used in purifying the molecule or component that the label is conjugated to, such as an epitope tag (including but not limited to a Myc tag, a human influenza hemagglutinin (HA) tag, a FLAG tag), an affinity tag (including but not limited to a glutathione-S transferase (GST), a poly-histidine (His) tag, calmodulin binding protein (CBP), or maltose-binding protein (MBP)), or a fluorescent tag.

[0041] The term “propagates” or “expand” means to grow a cell or population of cells. The term “growing” also refers to the proliferation of cells in the presence of supporting media, nutrients, growth factors, support cells, or any chemical or biological compound necessary for obtaining the desired number of cells or cell type.

[0042] The term “culturing” refers to the *in vitro* propagation of cells or organisms on or in media of various kinds. It is understood that the descendants of a cell grown in culture may not be completely identical (i.e., morphologically, genetically, or phenotypically) to the parent cell.

[0043] A population of cells intends a collection of more than one cell that is identical (clonal) or non-identical in phenotype and/or genotype. A substantially homogenous population of cells is a population having at least 70 %, or alternatively at least 75 %, or alternatively at least 80%, or alternatively at least 85%, or alternatively at least 90 %, or

alternatively at least 95 %, or alternatively at least 98% identical phenotype, as measured by pre-selected markers.

[0044] The term "stem cell" refers to a cell that is in an undifferentiated or partially differentiated state and has the capacity for self-renewal or to generate differentiated progeny or both. Self-renewal is defined as the capability of a stem cell to proliferate and give rise to more such stem cells, while maintaining its developmental potential (i.e., totipotent, pluripotent, multipotent, etc.). The term "somatic stem cell" is used herein to refer to any stem cell derived from non-embryonic tissue, including fetal, juvenile, and adult tissue. Natural somatic stem cells have been isolated from a wide variety of adult tissues including blood, bone marrow, brain, olfactory epithelium, skin, pancreas, skeletal muscle, and cardiac muscle. Exemplary naturally occurring somatic stem cells include, but are not limited to, mesenchymal stem cells (MSCs) and neural or neuronal stem cells (NSCs). In some embodiments, the stem or progenitor cells can be embryonic stem cells or an induced pluripotent stem cell (iPSC). In some embodiments, the stem or progenitor cells are hematopoietic stem cells (HSCs).

[0045] A "precursor" or "progenitor cell" intends to mean cells that have a capacity to differentiate into a specific type of cell. A progenitor cell may be a stem cell. A progenitor cell may also be more specific than a stem cell. A progenitor cell may be unipotent or multipotent. Compared to adult stem cells, a progenitor cell may be in a later stage of cell differentiation. An example of progenitor cell includes, without limitation, a progenitor nerve cell.

[0046] As used herein, a "pluripotent cell" defines a less differentiated cell that can give rise to at least two distinct (genotypically or phenotypically or both) further differentiated progeny cells. In another aspect, a "pluripotent cell" includes an Induced Pluripotent Stem Cell (iPSC) which is an artificially derived stem cell from a non-pluripotent cell, typically an adult somatic cell, that has historically been produced by inducing expression of one or more stem cell specific genes. Such stem cell specific genes include, but are not limited to, the family of octamer transcription factors, i.e. Oct-3/4; the family of Sox genes, i.e., Sox1, Sox2, Sox3, Sox 15 and Sox 18; the family of Klf genes, i.e. Klf1, Klf2, Klf4 and Klf5; the family of Myc genes, i.e. c-myc and L-myc; the family of Nanog genes, i.e., OCT4, NANOG and

REX1; or LIN28. Examples of iPSCs are described in Takahashi et al. (2007) Cell advance online publication 20 November 2007; Takahashi & Yamanaka (2006) Cell 126:663–76; Okita et al. (2007) Nature 448:260-262; Yu et al. (2007) Science advance online publication 20 November 2007; and Nakagawa et al. (2007) Nat. Biotechnol. Advance online publication 30 November 2007.

[0047] An “induced pluripotent cell” intends embryonic-like cells reprogrammed to the immature phenotype from adult cells. Various methods are known in the art, e.g., "A simple new way to induce pluripotency: Acid." Nature, 29 January 2014 and available at [sciencedaily.com/releases/2014/01/140129184445](https://www.sciencedaily.com/releases/2014/01/140129184445), last accessed on February 5, 2014 and U.S. Patent Application Publication No. 2010/0041054. Human iPSCs also express stem cell markers and can generate cells characteristic of all three germ layers. Exemplary iPSCs for use in the methods of this invention include astrocyte cell cultures that have been differentiated from the patient's fibroblast-generated induced pluripotent stem cells. This serves to match the sera to a model for brain cells that are isogenic with the patient's brain cells. Commercially available iPSC-derived human astrocytes are available from iXCells, Cat. #40-HU-008

(<https://www.bing.com/search?q=iXCells&form=ANNH01&ref=60cee204ae794b0991620da6dc4f4e76&pc=U53>, last accessed on December 8, 2024) and Creative Bioarray, Cat. No. CSC-7836W, <https://www.creative-bioarray.com/Human-Astrocytes-HA-CSC-7836W-item-1891.htm>, last accessed December 8, 2024).

[0048] A neural stem cell is a cell that can be isolated from the adult central nervous systems of mammals, including humans. They have been shown to generate neurons, migrate and send out axonal and dendritic projections and integrate into pre-existing neuronal circuits and contribute to normal brain function. Reviews of research in this area are found in Miller (2006) The Promise of Stem Cells for Neural Repair, Brain Res. Vol. 1091(1):258-264; Pluchino et al. (2005) Neural Stem Cells and Their Use as Therapeutic Tool in Neurological Disorders, Brain Res. Brain Res. Rev., Vol. 48(2):211-219; and Goh, et al. (2003) Adult Neural Stem Cells and Repair of the Adult Central Nervous System, J. Hematother. Stem Cell Res., Vol. 12(6):671-679.

[0049] An “astrocyte” is a subtype of glial cells that make up the majority of cells in the human central nervous system (CNS). They perform metabolic, structural, homeostatic, and neuroprotective tasks such as clearing excess neurotransmitters, stabilizing and regulating the blood-brain barrier, and promoting synapse formation.

[0050] A “neuron” intends a cell that functionally sends messages over the body. As used herein, unless specifically recited they intend sensory neurons, motor neurons and interneurons. Alternatively, they intend unipolar, bipolar, multipolar and pseudounipolar neurons.

[0051] A “microglia” intends a brain cell that regulates brain development. See for example, Colonna and Butovsky, *Annu. Rev. Immunol.* (2017) Apr. 26:35:441-468.

[0052] “Differentiation” describes the process whereby an unspecialized cell acquires the features of a specialized cell such as a heart, liver, or muscle cell. “Directed differentiation” refers to the manipulation of stem cell culture conditions to induce differentiation into a particular cell type. “Dedifferentiated” defines a cell that reverts to a less committed position within the lineage of a cell. As used herein, the term “differentiates or differentiated” defines a cell that takes on a more committed (“differentiated”) position within the lineage of a cell. As used herein, “a cell that differentiates into a mesodermal (or ectodermal or endodermal) lineage” defines a cell that becomes committed to a specific mesodermal, ectodermal, or endodermal lineage, respectively. Examples of cells that differentiate into a mesodermal lineage or give rise to specific mesodermal cells include, but are not limited to, cells that are adipogenic, leiomyogenic, chondrogenic, cardiogenic, dermatogenic, hematopoietic, hemangiogenic, myogenic, nephrogenic, urogenitogenic, osteogenic, pericardiogenic, or stromal.

[0053] As used herein, the “lineage” of a cell defines the heredity of the cell, i.e. its predecessors and progeny. The lineage of a cell places the cell within a hereditary scheme of development and differentiation.

[0054] A “multi-lineage stem cell” or “multipotent stem cell” refers to a stem cell that reproduces itself and at least two further differentiated progeny cells from distinct developmental lineages. The lineages can be from the same germ layer (i.e. mesoderm, ectoderm or endoderm), or from different germ layers. An example of two progeny cells

with distinct developmental lineages from differentiation of a multi-lineage stem cell is a myogenic cell and an adipogenic cell (both are of mesodermal origin yet give rise to different tissues). Another example is a neurogenic cell (of ectodermal origin) and adipogenic cell (of mesodermal origin).

[0055] As used herein, a “pluripotent cell” defines a less differentiated cell that can give rise to at least two distinct (genotypically and/or phenotypically) further differentiated progeny cells. In another aspect, a “pluripotent cell” includes an Induced Pluripotent Stem Cell (iPSC) which is an artificially derived stem cell from a non-pluripotent cell, typically an adult somatic cell, that has historically been produced by inducing expression of one or more stem cell specific genes. Such stem cell specific genes include, but are not limited to, the family of octamer transcription factors, i.e. Oct-3/4; the family of Sox genes, i.e., Sox1, Sox2, Sox3, Sox 15 and Sox 18; the family of Klf genes, i.e. Klf1, Klf2, Klf4 and Klf5; the family of Myc genes, i.e. c-myc and L-myc; the family of Nanog genes, i.e., OCT4, NANOG and REX1; or LIN28. Examples of iPSCs are described in Takahashi et al. (2007) Cell advance online publication 20 November 2007; Takahashi & Yamanaka (2006) Cell 126:663–76; Okita et al. (2007) Nature 448:260-262; Yu et al. (2007) Science advance online publication 20 November 2007; and Nakagawa et al. (2007) Nat. Biotechnol. Advance online publication 30 November 2007. Exemplary iPSCs for use in the methods of this invention include astrocyte cell cultures that have been differentiated from the patient’s fibroblast-generated induced pluripotent stem cells. This serves to match the sera to a model for brain cells that are isogenic with the patient’s brain cells. Commercially available iPSC-derived human astrocytes are available from iXCells, Cat. #40-HU-008 (<https://www.bing.com/search?q=iXCells&form=ANNH01&ref=60cee204ae794b0991620da6dc4f4e76&pc=U53>, last accessed on December 8, 2024) and Creative Bioarray, Cat. No. CSC-7836W, <https://www.creative-bioarray.com/Human-Astrocytes-HA-CSC-7836W-item-1891.htm>, last accessed December 8, 2024).

[0056] A “composition” is intended to mean a combination of active polypeptide, polynucleotide or antibody and another compound or composition, inert (e.g. a detectable label) or active (e.g. a gene delivery vehicle)

[0057] A “pharmaceutical composition” is intended to include the combination of an active polypeptide, polynucleotide or antibody with a carrier, inert or active such as a solid support, making the composition suitable for diagnostic or therapeutic use *in vitro*, *in vivo* or *ex vivo*.

[0058] As used herein, the term “pharmaceutically acceptable carrier” encompasses any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, and emulsions, such as an oil/water or water/oil emulsion, and various types of wetting agents. The compositions also can include stabilizers and preservatives. For examples of carriers, stabilizers and adjuvants, see Martin (1975) Remington’s Pharm. Sci., 15th Ed. (Mack Publ. Co., Easton).

[0059] A “subject,” “individual” or “patient” is used interchangeably herein, and refers to a vertebrate, preferably a mammal, more preferably a human. Mammals include, but are not limited to, murines, rats, rabbit, simians, bovines, ovine, porcine, canines, feline, farm animals, sport animals, pets, equine, and primate, particularly human. Besides being useful for human treatment, the present invention is also useful for veterinary treatment of companion mammals, exotic animals and domesticated animals, including mammals, rodents, and the like which is susceptible to neurodegenerative disease. In one embodiment, the mammals include horses, dogs, and cats. In another embodiment of the present invention, the human is an adolescent or infant under the age of eighteen years of age.

[0060] “Treating” or “treatment” of a disease includes: (1) preventing the disease, i.e., causing the clinical symptoms of the disease not to develop in a patient that may be predisposed to the disease but does not yet experience or display symptoms of the disease; (2) inhibiting the disease, i.e., arresting or reducing the development of the disease or its clinical symptoms; or (3) relieving the disease, i.e., causing regression of the disease or its clinical symptoms.

[0061] The term “suffering” as it related to the term “treatment” refers to a patient or individual who has been diagnosed with or is predisposed to infection or a disease incident to infection. A patient may also be referred to being “at risk of suffering” from a disease because of active or latent infection. This patient has not yet developed characteristic disease pathology.

[0062] An “effective amount” is an amount sufficient to effect beneficial or desired results. An effective amount can be administered in one or more administrations, applications or dosages. Such delivery is dependent on a number of variables including the time period for which the individual dosage unit is to be used, the bioavailability of the therapeutic agent, the route of administration, etc. It is understood, however, that specific dose levels of the therapeutic agents of the present invention for any particular subject depends upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, and diet of the subject, the time of administration, the rate of excretion, the drug combination, and the severity of the particular disorder being treated and form of administration. Treatment dosages generally may be titrated to optimize safety and efficacy. Typically, dosage-effect relationships from *in vitro* and/or *in vivo* tests initially can provide useful guidance on the proper doses for patient administration. In general, one will desire to administer an amount of the compound that is effective to achieve a serum level commensurate with the concentrations found to be effective *in vitro*. Determination of these parameters is well within the skill of the art. These considerations, as well as effective formulations and administration procedures are well known in the art and are described in standard textbooks. Consistent with this definition, as used herein, the term “therapeutically effective amount” is an amount sufficient to inhibit RNA virus replication *ex vivo*, *in vitro* or *in vivo*.

[0063] As used herein, the term “contacting” means direct or indirect binding or interaction between two or more molecules or other entities. A particular example of direct interaction is binding. A particular example of an indirect interaction is where one entity acts upon an intermediary molecule, which in turn acts upon the second referenced entity. Contacting as used herein includes in solution, in solid phase, *in vitro*, *ex vivo*, in a cell and *in vivo*. Contacting *in vivo* can be referred to as administering, or administration.

[0064] The term administration shall include without limitation, administration by oral, parenteral (e.g., intramuscular, intraperitoneal, intravenous, ICV, intracisternal injection or infusion, subcutaneous injection, or implant), by inhalation spray nasal, vaginal, rectal, sublingual, urethral (e.g., urethral suppository), intracranial, or topical routes of administration (e.g., gel, ointment, cream, aerosol, etc.) and can be formulated, alone or together, in suitable dosage unit formulations containing conventional non-toxic

pharmaceutically acceptable carriers, adjuvants, excipients, and vehicles appropriate for each route of administration. The invention is not limited by the route of administration, the formulation or dosing schedule.

[0065] A “neurodegenerative disease or disorder” is a disease or phenotype characterized by degeneration of the nervous system, especially the neurons in the CNS. Non-limiting examples of such include a central nervous system (CNS) disease or disorder. A CNS disease or disorder is a group of neurological disorders that affect the structure or function of the brain or spinal cord, and that may result in degeneration of one or more parts of the brain or spinal cord. Non-limiting examples include Huntington’s disease (HD), Alzheimer’s disease, Parkinson’s disease, traumatic brain injury, stroke, autoimmune disorders such as multiple sclerosis, primary or secondary progressive multiple sclerosis, relapsing remitting multiple sclerosis, brain inflammation, Bell’s palsy, cervical spondylosis, carpal tunnel syndrome, brain or spinal cord tumors, peripheral neuropathy, Guillain-Barre syndrome, amyotrophic lateral sclerosis, and Huntington chorea. To treat or ameliorate the symptoms of a CNS injury intends to improve the patient’s nerve function reduce the adverse effect of inherited or acquired disease, injury or a disorder. The symptoms and course of the disease are known to the skilled artisan, see, hopkinsmedicine.org/healthlibrary/conditions/nervous_system_disorders/overview_of_nervous_system_disorders_85,P00799, accessed on May 21, 2018.

[0066] As used herein, the term “cognitive decline and depression” intends a reduction in the subjective ability to think and reason, loss of appetite, failure to experience joy, and/or a deepening or decrease in the subject’s mood. Levels of depression can be determined using Beck Depression Inventor II, having a scoring of 0-13: minimal depressive symptoms, 14-19 mild depressive symptoms; 20-28: moderate depressive symptoms; and 29-63: severe depressive symptoms.

[0067] Cerebrospinal fluid (CSF) intends the plasma contained within the ventricles of the brain and the subarachnoid spaces of the cranium and spine. It is a clear body fluid found within the tissue that surrounds the brain and spinal cord of all vertebrates. It can be isolated by a lumbar puncture or spinal tap.

[0068] The term “sera (plural for serum) intends the portion of the blood that is the watery portion of an animal fluid remaining after coagulation. It is the portion of blood that is liquid fraction of whole blood that is collected after the blood is allowed to clot. The clot is removed by centrifugation and the resulting supernatant, designated serum, is removed or isolated.

[0069] “Beck Depressive Inventory” is a self-report questionnaire used to measure the severity of depression. (BDI). See <https://www.talkspace.com/mental-health/conditions/articles/beck-depression-inventory/> for information on the use and scoring of BDI (last accessed on December 11, 2023).

[0070] Modes For Carrying Out the Disclosure

[0071] Applicant designed experiments exposing cultured human astrocytes to human sera from patients that had been assessed for cognitive and behavioral abnormalities. After finding strong correlations between metabolic phenotype or mitochondrial activity in the patients’ astrocytes and the cognitive and depressive symptoms in the patients from which the sera was collected, Applicant tested possible therapeutic compounds to treat one or more patient, focusing first on those that may block or induce metabolic pathways to determine how the human sera may be affecting brain cells *in vivo*.

[0072] Without being bound by theory, Applicant hypothesized that metabolic dysfunction in astrocytes exposed to patient sera or cerebrospinal fluid (CSF) reflects depressive symptoms and neurocognitive impairments in patients from whom the sera and/or CSF were collected. Applicant therefore provides herein a method that combines patient sera and/or CSF with brain cells for example neurons, microglia, oligodendrocytes or astrocytes as an assay to assess cognitive state (e.g., depression and/or cognitive impairment) as well as to test for new chemical entities and treatments and/or to repurpose FDA-approved compounds that could be used to treat the patients’ neurological symptoms. In one aspect, the cells are astrocytes. It also provides a method to quantitatively determine a subject’s or patient’s depression or cognitive impairment that is particularly useful when the patient or subject is unable to communicate that to the health care professional.

[0073] Thus, in one embodiment, provided herein is an *in vitro* method to determine the cognitive state (e.g., depression and/or cognitive depression) of a subject or patient, the

method comprising, or consisting essentially of, or consisting of culturing brain cells, for example neurons, microglia, oligodendrocytes or astrocytes with sera and/or cerebrospinal fluid (CSF) and optionally the one or more test agents (e.g., a biologic or a small molecule) and assaying the brain cells, for example neurons, microglia, oligodendrocytes or astrocytes combined with the sera and/or CSF for metabolic changes, e.g., by assessing mitochondrial activity of brain cells, for example neurons, microglia, oligodendrocytes or astrocytes using for example MitoTrack (MT) signal changes. In one aspect, the cells are astrocytes. In another aspect, a control sample with of the brain cells, for example neurons, microglia, oligodendrocytes or astrocytes cultured in sera are assessed for mitochondrial activity or MT signal changes. In one aspect, the cells are astrocytes. In a further aspect, the sera and/or CSF are isolated from a subject that has been assessed for cognitive and/or behavioral changes or abnormalities. In one aspect, if and/or after the metabolic change is present or has been determined, then in a further aspect, the metabolic change is correlated with neurological change. Applicant has shown that there is a strong relationship between mitochondrial activity (e.g., as determined by MitoTrack (MT) signal) and depression and/or cognitive impairment or decline. In one aspect, the MT signal data is normalized, e.g., by dividing the MT signal value from the subject's sample with a control sample wherein the astrocytes are cultured or incubated with fetal bovine sera in the absence of patient sample and/or test agent.

[0074] In one aspect, a MT signal that is below the MT spot average intensity in the astrocytes incubated in fetal bovine sera indicates an increased risk of that subject having a BDI-II score of over 25 and therefore suffering from severe depression. For those subjects, an effective amount of an antidepressant can be administered, e.g., SSRI antidepressants such as Citalopram, Fluvoxamine, Escitalopram, Paroxetine, Fluoxetine, or Sertraline, or alternatively, an SNRI antidepressant such as Duloxetine, Levomilnacipran, Venlafaxine, Desvenlafaxine, or a TCA such as Amitriptyline, or Amoxapine. For other patients, these therapies or other therapies can be administered, depending on the severity of the depression and/or cognitive impairment.

[0075] One can further add to the culture one or more agents or drugs to be tested to the brain cells, for example neurons, microglia, oligodendrocytes or astrocytes to determine of the agent has an effect on mitochondrial activity and/or the MT (MT signal) as raw data or

normalized, as described above. In one aspect, the cells are astrocytes. A possible therapeutic is a test agent that increases mitochondrial activity increases in the culture.

[0076] As used herein, the terms “subject” and “patient” intend any vertebrate animal, e.g., amphibians, birds, mammals, and fish. In one aspect, the patient is a mammal, e.g., a human, a canine, a feline, a bovine, a simian, an ovine, a rat or mouse. In one aspect, the patient is a human. In a further aspect, the patient is a mammal with an HIV-like disease. In a further aspect, the patient is a human patient diagnosed with HIV. When a patient’s cells, e.g., an astrocyte derived from a patient iPSC, the method can be useful to determine a personalized therapy for the patient. In one aspect the drug is beta-hydroxybutyrate which is commercially available from various vendors (e.g., Labcorp, CPT: 82010, <https://www.labcorp.com/tests/503610/hydroxybutyrate>, last accessed on December 8, 2024.) The method also can be combined with quantitative measures of one or more of interleukin 1, interleukin 6, and tumor necrosis factor alpha (TNF- α). In a yet further aspect of the disclosure, the methods as described herein the metabolic baseline of the astrocytes are assayed for metabolic baseline prior to culturing with the sample, and/or CSF. Non-limiting examples of methods to determine or evaluate the metabolic baseline comprises, or consists essentially of, or yet further consists of one or more of: fluorescent microscopic image analysis, metabolic assays, mitochondrial activity using potentiometric dyes (such as MitoTracker), mitochondrial respiration, and gene expression analysis. Exemplary methods are described and shown herein.

[0077] In one aspect of the above method, the cognitive and/or behavioral changes in the subject comprise, or consist essentially of, or yet further consist of, depression and/or neurocognitive impairment or decline.

[0078] In one aspect, the method comprises, or consists essentially of, or yet further consists of exposing brain cells, for example neurons, microglia, oligodendrocytes or astrocytes, e.g., human astrocytes and/or iPSC cells to sera and/or CSF from one or more vertebrate subjects, such as human patients, with varying degrees of depressive symptomatology. In one aspect, the cells are astrocytes. MitoTrackerTM Deep Red FM (MTDR) can be used to visualize mitochondrial activity as an indicator of brain cell brain cells, for example neurons, microglia, oligodendrocytes and astrocytes metabolic changes. In one example, astrocytes

were visualized using the high-throughput fluorescent microscopy and image analyses platform, Thermo Scientific CellInsight CX5 (CX5). More severe depression, as indexed by higher BDI-II scores, was associated with lower MT signal quantity. Overall, better cognitive function, as assessed by neuropsychiatric testing t-scores, correlated with increased MT signal measures. Consistent with the MT data, higher BDI-II scores were associated with decreased in oxygen consumption rate and increased extracellular acidification rate in astrocytes.

[0079] Applicant next administered 2-deoxyglucose or beta-hydroxybutyrate to the astrocyte plus sera combination to inhibit glycolysis and promote mitochondrial activity. Overall, these findings show that 2-deoxyglucose or beta-hydroxybutyrate promote mitochondrial activity in cultured human astrocytes and reverse sera-induced decreases in mitochondrial activity that correlated with worse depressive symptoms and worse cognitive function, suggesting a mechanism to maintain mitochondrial and astrocyte homeostasis that can be detected peripherally. Therefore, this combination of in-vitro cultures and patient bio specimens provides a model to identify neuropathogenic mechanisms and test personalized therapeutics for neurologic and psychiatric disorders.

[0080] Applicant shows herein that sera and cerebrospinal fluid (CSF) from patients with depression or cognitive impairment induce phenotypic changes in brain cells, and the quantitative measures of these phenotypic changes strongly correlate with quantitative measures of depressive or cognitive symptoms in the patients. The depressive and cognitive symptoms in patients are quantified by Beck Depression Inventory and Global Deficit Score (as determined by a neuropsychological battery). Cell cultures models for the major brain cell types (astrocytes [SVG p12 cells; ATCC, cat# CRL8621), neurons (ReNcell CX Human Neural Progenitor Cell Line; MilliporeSigma, cat# SCC007), microglia (HMC3; ATCC, cat# CRL3304), or oligodendrocytes (MilliporeSigma, cat# SCC163) are incubated with patient sera and CSF at a concentration of 10% of the culture media to induce phenotypic changes in the brain cells as compared to control cells incubated in fetal bovine sera (the standard sera for the cell cultures). Phenotypic analyses of the brain cell cultures include 1) metabolic changes as measured by microscopic analysis of potentiometric dyes (Mitotracker is one such potentiometric dye [ThermoFisher cat# M22426]) that permeate and fluoresce in active mitochondria, quantification of extracellular flux of oxygen and protons (H⁺) in brain cell

culture media, measures of the metabolic molecule adenosine triphosphate; and 2) changes in gene expression, particularly inflammatory and metabolic gene expression. These phenotypic changes indicate depressive and cognitive symptoms in the patients and serve as personalized (patient-specific) disease models to identify potential mechanisms of the depressive and cognitive symptoms, and test drug compounds to reverse the phenotypic changes and indicate testable therapeutics for the patients.

[0081] Experimental Methods

[0082] Experiment No. 1

[0083] In one aspect, Applicant sought to determine if in vitro exposure of astrocytes to sera collected from PWH could induce alterations in astrocyte reactivity and mitochondrial activity and if such changes correlate with depressive symptoms or cognitive function. Sera from PWH likely contains peripherally derived inflammatory and hormonal factors as well as brain-derived biomarkers of psychiatric or neurologic disorders. To the best of Applicant's knowledge, this is the first study that combines sera samples from PWH with in vitro brain cell models to investigate mechanisms underlying depression and cognitive function in this population. Thus, combining human sera with brain cells can provide valuable insights into Cpersonalized mechanisms leading to psychiatric and neurological complications in PWH.

Methods and Materials

Study Population

[0084] The inclusion criteria for this study were HIV individuals on ART with viral suppression. Sera from a total of 40 participants with HIV from the HIV Neurobehavioral Research Program [Institutional Review Board (IRB) #080323] were tested, 20 of which were from PWH who had lifetime or current major depressive disorder (MDD) diagnoses and 20 from PWH who did not have MDD diagnoses. Study participants underwent complete neuromedical evaluation, and routine clinical measures were assessed in blood. All studies adhered to the ethical guidelines of the National Institutes of Health and the University of California, San Diego.

Psychiatric Assessments

[0085] Participants were evaluated for lifetime (any point in one's lifetime) and current (last 30 days) MDD and substance use disorder (dependence or abuse) diagnoses using the Composite International Diagnostic Interview (CIDI) (34), a computerized psychodiagnostics clinical interview based on the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV), as study methodology was developed prior to the release of the DSM-5. In addition to the diagnostic evaluation, participants completed the Beck Depression Inventory-II (BDI-II) (34), to measure depressive symptoms experienced in the past two weeks. Possible range of the BDI-II score is from 0 to 63, with the total score 0-13 considered no to minimal depressive symptoms, 14-19 mild, 20-28 moderate, and 29-63 severe. In order to preserve statistical power in primary analyses, Applicant chose to model current depression with the total BDI-II score instead of MDD diagnosis, as only 10 total study participants had a current MDD diagnosis. Domain-specific BDI-II scores reflecting cognitive (possible range: 0 to 27), affective (possible range: 0 to 12), and somatic (possible range: 0 to 24) symptoms of depression were computed based on a previous factor analysis of the BDI-II in 1,583 PWH (34).

Neuropsychological Assessments

[0086] Neuropsychological evaluation was performed as previously described (34-36). Briefly, seven neurocognitive domains were assessed, including executive function, motor skill, processing speed, episodic memory, attention/working memory, language, and visual perception. Raw test scores were transformed into normally distributed T-scores that were adjusted for demographic variables, including age, education, gender, and race, based on normative samples of HIV participants and were then averaged across all tests to obtain a global cognitive T-score and within domains to obtain cognitive domain-specific T-scores.

Neuromedical Assessment

[0087] Baseline demographic data such as age and sex were collected. Medical comorbidities and medications were determined by interview. Additional HIV disease-related variables were collected. These included a history of Acquired Immunodeficiency Syndrome (AIDS), estimated duration of HIV disease (years), current CD4+ T cell count,

nadir CD4+ T cell count, and duration of ART use (years). HIV RNA level was measured in plasma by RT-PCR (Abbott Diagnostics; lower limit of quantitation 50 copies/mL).

Human Astrocytes

[0088] This study was approved by the University of California, San Diego Human Research Protections Program and deemed IRB exempt (Federal-wide Assurance #00000021 and Institutional Review Board #IORG0000210 [7 March 2019]). All data presented for this study used astrocytes that were from a differentiated cell line originally generated prior to 5 June 2019 (as per NIH NOT-OT-19-128) from fetal human brain tissue from terminated pregnancy between 12 and 16 weeks of gestation, as previously described (37). Donors gave written informed consent for research use of the cells and tissue. The experiments were repeated and findings were corroborated in SVG p12 astrocyte cultures (ATCC, cat. No. CRL- CRL8621).

Treatment of Astrocytes and High-throughput Fluorescent Microscopic Analyses of Astrocytes Exposed to Patient Sera.

[0089] As depicted in **FIG. 1A**, primary human fetal astrocytes were cultured in one 96-well plate at 5,000 cells/well one day prior to treatment. Astrocyte cell cultures were treated in duplicate with sera from 40 PWH. Sera was separated from participant blood using serum separator tubes (BD, cat. No. 367988) and used to treat primary human astrocytes, making up 10% of the total volume of culture media minus fetal bovine sera (FBS). In parallel on the same plate, astrocytes were treated with culture media containing FBS without interleukin-1 β (IL-1 β) (negative control) or with IL-1 β at 20ng/mL (positive control, prototypical inflammatory stimuli). Mitochondrial activity was visualized using MitoTrackerTM Deep Red (MT) FM (Invitrogen, cat. no. M22426). After incubating with sera for 24 hours at 37 °C, astrocyte cultures were incubated with MT at 250 nM for 45 minutes followed by two washes with 1X PBS and fixation in 4% paraformaldehyde (PFA) at 4 °C for 20 minutes. Fixed astrocytes were incubated in blocking buffer (5% BSA and 0.2% Triton X-100 in PBS) for one hour at room temperature before incubating overnight at 4 °C with primary antibody, GFAP 1:500 (Sigma-Aldrich; cat. no. G3893). After three washes with PBS astrocytes were incubated in secondary antibody, Alexa Fluor Goat anti-Mouse 488 1:500 (Invitrogen, cat. No. A11001), for 30 minutes. The cells were then stained with blue fluorescent stain, DAPI,

1:10000, for 5 minutes and then washed in PBS three times, and imaged and analyzed using Thermo Scientific CellInsight CX5 (CX5) imaging platform (**FIG. 1B**). Images using three channels were captured for 4 fields of view per well, and this imaging was repeated three more times using different fields of view per well with every scan. Cell Health Profiling Assay was performed to analyze MT and GFAP. A threshold intensity was selected and applied to all wells to identify these targets. MT signal was assessed using the Spot Detector Assay function on the CX5 Cell Insight platform. Data were analyzed as follows: i. Spot average intensity = total intensity of all pixels within all spots imaged in a well divided by the total area of all spots imaged in a well (i.e. average individual mitochondria intensity per well), ii. Spot average area = total area (μm^2) of all spots imaged in a well divided by the number of spots imaged in a well (i.e. average individual mitochondria size per well), iii. Spot total area per object = total area (μm^2) of all spots in a well divided by the number of cells in a well (i.e. average mitochondrial area per cell per well), iv. Target average intensity = average intensity of all pixels within the target mask (outline around cell) (i.e. average intensity of MT or GFAP per cell). This experiment was repeated with SVG p12 (ATCC, cat. No. CRL- CRL8621) cultured in two 96-well plates at 10,000 cells/well (data not shown).

Extracellular flux analyses

[0090] To determine if the observed changes in the MT readouts were consistent with metabolic changes in astrocytes, human sera samples with minimal depressive symptoms (BDI <13) and severe depressive symptoms (BDI > 25) that fell close to the linear regression line were chosen for further bioenergetic analysis (n = 5/group). Astrocytes were split into a Seahorse plate at 3×10^4 cells/well and exposed to sera (10% of media volume) for 24 h. Cultures were incubated in a non-CO₂ incubator at 37 °C to equilibrate for approximately 30 minutes prior to assay. Baseline measurements of oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were taken prior to the addition of oligomycin (2 μM), followed by a titrated concentration of FCCP, and then rotenone (500 nM) together with antimycin (1 μM) (Sigma-Aldrich, cat no. A8674). After each addition of mitochondrial inhibitor, three readings were taken before injection of the subsequent inhibitor. Maximum oxygen consumption rate was recorded after two consecutive injections of 250 nM FCCP. Spare respiratory capacity (SRC) was calculated by subtracting the basal OCR from the

maximum OCR. ECAR was automatically calculated and recorded by the Seahorse XFe96 software. Rates were calculated by the Seahorse analyzer, reported as pM O₂/minute and log of H⁺ production rate, respectively. Samples were run in biological replicates of five in three independent experiments.

Statistical Analysis

[0091] Statistical analysis was performed using GraphPad Prism 9 and R. For IL-1 β treatments and experiments evaluating the effects of sera by BDI-II category (minimal – severe), statistical significance was tested using two-sample t-tests or one-way ANOVA with post-hoc Tukey’s pairwise comparisons. Comparisons between MT signal measures and current antidepressant use were done by two-tailed t-test. For the extracellular flux analyses, statistical significance was tested with one-way ANOVA with post-hoc Tukey’s. Simple linear regression was performed to determine the strength of the association between MT signal measures, GFAP intensity, or extracellular flux analyses and clinical measures, including BDI-II scores, cognitive T-scores, or age. R² is used to measure the effect size for each association. MT signal measures and GFAP intensity were normalized to control astrocytes that were treated with media containing FBS. Separate multivariable linear models adjusted for the covariates age, sex, ethnicity, metabolic syndrome, height, duration, and total ART exposure were used to assess the influence of these covariates on the magnitude and significance of the tested associations (data not shown). To visually assess how MT and GFAP signals relate to clinical data from sera donors, the data were plotted with the use of a correlation matrix.

Results

Clinical Characteristics

[0092] Demographic and clinical data are illustrated in **Table 1**. The majority of participants were male. Mean age, duration of ART, and CD4 count do not differ significantly between minimal, mild, moderate, and severe depression groups. Of the 20 individuals with a lifetime diagnosis of MDD, six had a diagnosis of MDD prior to HIV infection. A total of 23 individuals was currently taking antidepressants. One individual had a substance use disorder, specifically cannabis use disorder.

Table 1. Clinical characteristics of human cohort.				
BDI scores	None/Minimal (n=20)	Depressed		
		Mild (n=8)	Moderate (n=5)	Severe (n=7)
Demographics				
Sex (f/m)	2/18	1/7	1/4	1/6
Years of Age	49.7 ± 12.1	48.4 ± 13.7	47.4 ± 16.0	45.7 ± 6.3
HIV Disease Characteristics				
Nadir CD4 count	188.45 ± 138.96	179.75 ± 130.64	78.6 ± 93.81	274.14 ± 357.07
Current CD4 count	724.2 ± 207.9	657.9 ± 415.2	570.4 ± 313.8	551.8 ± 331.4
Duration of infection (years)	16.96 ± 8.94	16.27 ± 7.2	14.69 ± 10.61	16.64 ± 8.36
Duration on ART regimen (years)	10.4 ± 5.5	11.2 ± 7.9	15.4 ± 15.8	12.3 ± 6.4
AIDS ever (Y/N)	11/7	5/3	5/0	4/3
Clinical Characteristics				
Current Antidepressants	10/20	5/8	2/5	6/7
MDD Before/After HIV Diagnosis (Before/After)	2/7	2/5	0/5	2/3
Current Substance Use Disorder	0/20	0/8	0/5	1/7

IL-1 β induces increased MitoTracker measures and GFAP in cultured primary human astrocytes compared to control and human sera collected from people with HIV and minimal – severe depressive symptoms.

[0093] IL-1 β induces an 80% and 40% increase in GFAP and MT Target Average Intensity, respectively, compared to control (media + FBS) (FIGS. 3A-3C). IL-1 β significantly increases MT target average intensity compared to human sera collected from PWH with minimal ($p < 0.001$), mild ($p < 0.001$), moderate ($p < 0.01$), or severe depressive symptoms ($p < 0.0001$) (FIG. 3B). IL-1 β significantly increases GFAP target average intensity compared to human sera collected from PWH with minimal ($p < 0.0001$), mild ($p < 0.0001$), moderate ($p < 0.01$), or severe depressive symptoms ($p < 0.0001$) (FIG. 3C). Sera from PWH with minimal depressive symptoms significantly increases MT target average intensity compared to control ($p < 0.05$) and sera from PWH with severe depressive symptoms ($p < 0.05$) (FIG. 3B). IL-1 β increases MT spots throughout astrocyte cell bodies and processes (FIG. 3D), and quantities of spot total area, average area, and average intensity by 100%, 30%, and 35%, respectively, compared to control (FIGS. 3E-3G). IL-1 β significantly increases MT spot average intensity compared to human sera collected from PWH with minimal ($p < 0.05$), mild ($p < 0.01$), moderate ($p < 0.05$), or severe depressive symptoms ($p < 0.001$) (FIG. 3E). Sera from PWH with minimal depressive symptoms significantly increases MT spot average intensity compared to control ($p < 0.05$) and sera from PWH with severe depressive symptoms ($p < 0.05$) (FIG. 3E). Compared to control MT spot total area was significantly increased by human sera collected from PWH and minimal ($p < 0.05$) or mild depressive symptoms ($p < 0.05$) (FIG. 3F). MT spot average area was significantly increased by human sera from PWH and minimal depressive symptoms ($p < 0.05$) compared to control (FIG. 3G).

Heat map demonstrating relationships between clinical data of donors and astrocyte reactivity and mitochondrial activity in 24h sera-exposed human astrocyte cultures.

[0094] Correlation matrix analysis (FIG. 4) indicates that the strongest relationships with GFAP target average intensity occur with age (positive), learning memory (negative), recall memory (negative), and verbal fluency (negative). The strongest relationships with MT signal measures are with BDI-II (negative), executive function (positive), speed of

Information processing (SIP)(positive), and working memory (positive). The matrix also illustrates a strong negative relationship between BDI-II and cognitive T-scores and a strong positive correlation between GFAP target average intensity and MT signal measures.

A higher burden of depression symptoms was associated with decreased mitochondria activity in sera-exposed astrocyte cultures.

[0095] MT spot average intensity in astrocyte cultures decreased with increasing BDI-II score (FIG. 5A; $p = 0.03$; $R^2 = 0.11$). MT spot average area in astrocyte cultures decreased with increasing BDI-II score (FIG. 5B; $p = 0.12$; $R^2 = 0.06$). MT target average intensity in astrocyte cultures decreased with increasing BDI-II score (FIG. 5C; $p = 0.056$; $R^2 = 0.09$).

Higher cognitive function T-Scores, except for Learning T-Score, were associated with increased mitochondrial activity in sera-exposed astrocyte cultures.

[0096] MT spot average area (FIG. 6A; $p = 0.10$; $R^2 = 0.07$) and intensity (FIG. 6B; $p = 0.01$; $R^2 = 0.15$) in astrocyte cultures increased with increasing global T-score. MT Target average intensity (FIG. 6C; $p = 0.08$; $R^2 = 0.08$), spot average area (FIG. 6D; $p = 0.07$; $R^2 = 0.08$), and spot average intensity (FIG. 6E; $p = 0.004$; $R^2 = 0.20$) in astrocyte cultures increased with increasing Executive T-score. MT target average intensity (FIG. 6F; $p = 0.06$; $R^2 = 0.09$) and MT Spot Average Intensity (FIG. 6G; $p = 0.006$; $R^2 = 0.19$) in astrocyte cultures increased with SIP T-Score. MT Spot Average Area (FIG. 6H; $p = 0.02$; $R^2 = 0.13$) and MT Spot Average Intensity (FIG. 6I; $p = 0.001$; $R^2 = 0.26$) in astrocyte cultures increased with Working Memory T-Scores. MT Spot Average Area (FIG. 6J; $p = 0.04$; $R^2 = 0.10$) in astrocyte cultures decreased with Learning Memory T-Scores.

Higher cognitive function T-Scores were associated with decreased astrocyte reactivity in sera-exposed astrocyte cultures.

[0097] GFAP Target Average Intensity in astrocyte cultures decreased with increasing Learning Mean T-Score (FIG. 7A; $p = 0.04$; $R^2 = 0.11$), Recall Memory T-Score (FIG. 7B; $p = 0.07$; $R^2 = 0.09$) and Verbal Fluency T-Score (FIG. 7C; $p = 0.06$; $R^2 = 0.09$).

Increased age is associated with increased mitochondrial activity and astrocyte reactivity in sera-exposed astrocyte cultures.

[0098] MT Target Average Intensity (**FIG. 8A**; $p = 0.06$; $R^2 = 0.08$) and MT Spot Total Area (**FIG. 8B**; $p = 0.04$; $R^2 = 0.10$) in astrocyte cultures increased with increasing age. GFAP Target Average Intensity (**FIG. 8C**; $p = 0.02$; $R^2 = 0.13$) in astrocyte cultures increased with increasing age. Multivariable models showed results consistent with the results of univariable analyses (data not shown).

Increased depressive symptoms were associated with decreased oxidative phosphorylation and increased glycolytic activity in sera-exposed astrocytes.

[0099] OCR and SRC are measures of oxidative phosphorylation. ECAR is a measure of glycolytic activity. Sera from PWH with $BDI > 25$ induced a 10% reduction in OCR (**FIG. 9A**) a 35% reduction in SRC (**FIG. 9C**; $p = 0.052$), and a 10% increase in ECAR (**FIG. 9E**; $p < 0.05$) in astrocytes compared to sera from PWH with $BDI < 13$. IL-1 β significantly increases OCR (**FIG. 9A**, $p < 0.01$), SRC (**FIG. 9C**, $p < 0.0001$), and ECAR (**FIG. 9E**, $p < 0.05$) compared to control (media + FBS). There is a non-significant negative correlation between OCR and BDI-II (**FIG. 9B**, $p = 0.19$, $R^2 = 0.20$), i.e. OCR decreases with more severe depressive symptoms. There is a significant negative association between SRC and BDI-II (**FIG. 9D**, $p = 0.02$, $R^2 = 0.51$), i.e. SCR decreases with more severe depressive symptoms. There is a significant positive correlation between ECAR and BDI-II (**FIG. 9F**, $p = 0.04$, $R^2 = 0.78$), i.e. ECAR increases with more severe depressive symptoms.

Antidepressant medication use was not related to alterations in MT signal in sera-exposed astrocyte cultures.

[0100] MT Spot Average Intensity (**FIG. 10A**; $p = 0.9974$), MT Spot Average Area (**FIG. 10B**; $p = 0.3346$), and MT Spot Total Area/Obj (**FIG. 10C**; $p = 0.9420$) in cultured astrocytes had no significant difference between treatment with sera from antidepressant medication users and nonusers.

Experimental Discussion

[0101] To the best of Applicant's knowledge, this is the first study to test the effects of sera from PWH with varying degrees of depressive symptoms and cognitive function on

mitochondrial activity and GFAP expression in cultured human astrocytes. Overall, the data shows that sera from PWH with worse depressive symptoms (as measured by BDI-II scores) leads to decreases in mitochondrial activity, decreases in oxidative phosphorylation, and increases in glycolytic activity in cultured human astrocytes. These alterations in mitochondrial activity are not attributable to antidepressant usage. Additionally, sera from PWH with higher cognitive T-scores leads to increases in mitochondrial activity and decreases in astrocyte reactivity. Both increases in mitochondrial activity and astrocyte reactivity are associated with older age. The properties of sera from PWH to induce changes in astrocyte reactivity and mitochondrial metabolic activity may either be due to the presence of circulating factors derived directly from the CNS (ex: exosomes, metabolites) or from factors in the periphery that possess the ability to regulate astrocyte homeostasis through a signaling mechanism that may involve the BBB. In either case, such compounds likely occur at lower concentrations in the periphery than in the CNS.

[0102] There is mounting evidence for the role of inflammation in depression (7). Peripheral levels of inflammatory cytokines are elevated in people with MDD (38), and the inflammasome pathway, which leads to IL1 β activation, has been implicated both in depression (39) and HIV-associated neurological dysfunction (40, 41). The inflammatory association is particularly important in PWH because elevated levels of inflammation are associated with depressive symptoms and chronic inflammation occurs in PWH despite viral suppression with ART (34, 42, 43). Neuroinflammation is associated with increases in GFAP, reflective of increases in astrocyte reactivity (11, 12, 14, 16). Reactive astrocytes produce inflammatory cytokines and undergo morphologic changes, leading to increased energy demands. Thus, neuroinflammation is also associated with an increase in astrocyte mitochondrial oxidative phosphorylation(24, 25).

[0103] Therefore, Applicant hypothesized that higher depressive symptoms would be associated with increases in both GFAP and mitochondrial activity as is seen with the prototypical inflammatory stimuli IL-1 β . Although there was an overall positive correlation between GFAP intensity and mitochondrial activity, the observation that decreases in mitochondrial activity were associated with higher depression symptoms absent changes in GFAP was somewhat unexpected.

[0104] Of note, only six individuals in this study carried a diagnosis of MDD prior to HIV infection (**Table 1**). It is possible that for the individuals whose HIV infection preceded their depression, their depression is due to a virally related pathogenic mechanism. Chronic inflammation in PWH, even while virally suppressed with ART, is thought to contribute to depression in PWH (51).

[0105] This study also examined the associations between cognitive T-scores and in vitro sera-induced changes to mitochondrial activity and GFAP reactivity. Overall, the results demonstrate that higher cognitive T-scores are associated with increases in mitochondrial activity and decreases in astrocyte reactivity. In other words, lower mitochondrial activity is associated with lower cognitive T-scores. Of note, lower mitochondrial activity was also associated with higher depressive symptoms, and higher depressive symptoms were correlated with lower cognitive function.

[0106] The increases in GFAP intensity that occur in the astrocytes treated with sera derived from individuals with lower cognitive T-scores may suggest that an inflammatory stimulus is present in the sera. Previous studies have shown that increases in GFAP intensity coincide with increases in mitochondrial activity (26, 27, 52), an overall correlation that holds true in this study. Therefore, it may appear counterintuitive that in the context of cognition function, lower cognitive T-scores are associated higher GFAP intensity but lower mitochondrial activity. It is possible that the astrocytes are being directed toward a more glycolytic state in a homeostatic attempt to provide metabolic support for impaired neurons that may be present in individuals with lower cognitive T-scores (29, 31). Additionally, although astrocytes are often canonically presented as increasing oxidative phosphorylation following an inflammatory stimulus and can be simplistically divided into A1 (inflammatory) and A2 (neuroprotective) phenotypes, the reality is that astrocyte phenotypes are expressed on a more nuanced spectrum (20, 53-55). This may be a factor in the current studies in which individual patient sera is being used to treat astrocytes, and likely represents a more complex paradigm than simple IL-1 β stimulation.

[0107] This study also examined the associations between age and in vitro sera-induced changes to mitochondrial activity and GFAP reactivity. Age is associated with elevated levels of inflammation in PWH(8). Therefore, it is unsurprising that increases in GFAP and

mitochondrial activity were associated with older age, reflecting the pattern seen in vitro with the inflammatory stimulus IL-1 β . This is opposite to what was seen with the cognitive T-score data. It is possible that homeostatic mechanisms capable of inducing a metabolic switch to the more neuronally supportive glycolytic astrocyte phenotype become impaired with age.

[0108] Within the statistically significant linear regression models the R^2 values ranged from 0.06 to 0.25, implying additional variables beyond depression, cognitive T-scores, and age likely influence changes to mitochondria and GFAP signal measures. Controlling for covariates did not substantially change the results seen in the simple linear regression (data not shown). It is likely that individual variability accounts for the lower R^2 values, which is not unexpected in clinic samples and consistent with in vitro studies demonstrating differential effects of sera from bipolar patients on in vitro dendritic sprouting (33). Pathophysiologic variability amongst people with depression is known to occur. For example, biomarkers of inflammation are elevated in some people with depression but not others, suggesting that inflammation may be only one component of a complex interaction of pathways involved in depressive symptoms. Additionally, the methods used in this study serve as a high throughput screening tool to identify associations between cellular processes and clinical measures. It allows for future studies to validate associations using more in-depth methods such as mitochondrial bioenergetic analyses, and to identify mechanistic differences between individuals who fall along the regression slope versus outlying individuals.

[0109] As stated previously, inflammation has been implicated in a variety of psychiatric and neurologic disorders, including depression and HAND (8-10, 43, 56, 57). However, clinical trials targeting such mechanisms have shown minimal effectiveness across populations. This has led to increased emphasis on personalized medicine to use genetics to identify therapies best suited for particular individuals. However, while providing powerful insights into disease, genetics does not explain all variability in any given population. Thus, it would be useful to identify tools that can disentangle inflammation-associated depression or cognitive symptoms from depression or cognitive symptoms associated with other neuropathogenic mechanisms. In vitro models that expose neuroinflammatory cells such as astroglia to patient sera samples may be useful in both identifying patients with inflammation-associated

symptoms of depression or cognitive dysfunction, and for testing therapeutic strategies in vitro.

[0110] Experiment No. 2

[0111] Treatment of human Astrocytes and High-throughput Fluorescent Microscopic Analyses of Astrocytes Exposed to Patient Cerebrospinal fluid.

[0112] The experiments were performed SVG p12 astrocyte cultures (ATCC, cat. No. CRL-8621).

[0113] SVG p12 astrocytes were cultured in a 96-well plate at 10,000 cells/well one day prior to treatment. Astrocyte cell cultures were treated in duplicate with sera from 40 PWH. CSF was thawed and used to treat SVGP12 astrocytes, making up 10% of the total volume of culture media. Mitochondrial activity was visualized using MitoTracker™ Deep Red (MT) FM (Invitrogen, cat. no. M22426). After incubating with CSF for 24 hours at 37 °C, astrocyte cultures were incubated with MT at 250 nM for 45 minutes followed by two washes with 1X PBS and fixation in 4% paraformaldehyde (PFA) at 4 °C for 20 minutes. Fixed astrocytes were incubated in blocking buffer (5% BSA and 0.2% Triton X-100 in PBS) for one hour at room temperature before incubating overnight at 4 °C with primary antibody, GFAP 1:500 (Sigma-Aldrich; cat. no. G3893). After three washes with PBS astrocytes were incubated in secondary antibody, Alexa Fluor Goat anti-Mouse 488 1:500 (Invitrogen, cat. No. A11001), for 30 minutes. The cells were then stained with blue fluorescent stain, DAPI, 1:10000, for 5 minutes and then washed in PBS three times, and imaged and analyzed using Thermo Scientific CellInsight CX5 (CX5) imaging platform (**FIG. 11**). Images using three channels were captured for 4 fields of view per well, and this imaging was repeated three more times using different fields of view per well with every scan. Cell Health Profiling Assay was performed to analyze MT and GFAP. A threshold intensity was selected and applied to all wells to identify these targets. MT signal was assessed using the Spot Detector Assay function on the CX5 Cell Insight. Data were analyzed as follows: i. Spot average intensity = total intensity of all pixels within all spots imaged in a well divided by the total area of all spots imaged in a well (i.e. average individual mitochondria intensity per well), ii. Spot average area = total area (μm^2) of all spots imaged in a well divided by the number of spots imaged in a well (i.e. average individual mitochondria size per well), iii. Spot total area per

object = total area (μm^2) of all spots in a well divided by the number of cells in a well (i.e. average mitochondrial area per cell per well), iv. Target average intensity = average intensity of all pixels within the target mask (outline around cell) (i.e. average intensity of MT or GFAP per cell).

[0114] Equivalents

[0115] It is to be understood that while the invention has been described in conjunction with the above embodiments, that the foregoing description and examples are intended to illustrate and not limit the scope of the invention. Other aspects, advantages and modifications within the scope of the invention will be apparent to those skilled in the art to which the invention pertains.

[0116] Unless otherwise defined, 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 invention belongs. All nucleotide sequences provided herein are presented in the 5' to 3' direction.

[0117] The inventions illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms “comprising”, “including,” “containing”, etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed.

[0118] Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification, improvement and variation of the inventions embodied therein herein disclosed may be resorted to by those skilled in the art, and that such modifications, improvements and variations are considered to be within the scope of this invention. The materials, methods, and examples provided here are representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention.

[0119] The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

[0120] In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

[0121] All publications, patent applications, patents, and other references mentioned herein are expressly incorporated by reference in their entirety, to the same extent as if each were incorporated by reference individually. In case of conflict, the present specification, including definitions, will control.

[0122] Embodiments

[0123] 1. An *in vitro* method to determine if one or more test agents modify metabolic changes in a sample of brain cells, the method comprising culturing the sample with sera and/or cerebrospinal fluid (CSF) and the one or more test agents and assaying the sample combined with the sera and/or CSF for metabolic changes.

[0124] 2. An *in vitro* method to determine the cognitive status of a subject, the method comprising culturing a sample of brain cells astrocytes with sera and/or cerebrospinal fluid (CSF) isolated from the subject and assaying the sample combined with the sera and/or CSF for metabolic changes.

[0125] 3. The method of Embodiment 2, further comprising adding one or more test agents to the sample combined with the sera and/or CSF and assaying the sample for metabolic changes.

[0126] 4. The method of any one of Embodiments 1-3, further comprising determining the metabolic status of the brain cells by determining the mitochondrial activity of the astrocytes cultured in the absence of one or more of the sera, the CSF and/or the test agents.

[0127] 5. The method of any one of Embodiments 1-4, further comprising determining the metabolic status of the brain cells by determining the mitochondrial activity of the brain cells cultured in the absence of one or more of the sera, the CSF and/or the test agents.

[0128] 6. The method of any one of Embodiments 1-5, wherein the metabolic changes are assayed by a method comprising determining mitochondrial activity of the brain cells in the sample after culture with the sera and/or CSF.

[0129] 7. The method of any one of Embodiments 1-6, where low mitochondrial activity of the brain cells in the culture indicates cognitive decline, cognitive impairment and/or behavioral changes or abnormalities.

[0130] 8. The method of any one of Embodiments 1-7, wherein the sera and/or CSF are isolated from a subject that has been assessed from cognitive and/or behavioral changes or abnormalities.

[0131] 9. The method of Embodiment 8, wherein the cognitive and/or behavioral changes comprises depression.

[0132] 10. The method of Embodiment 8, wherein the cognitive and/or behavioral changes comprises neurocognitive impairment.

[0133] 11. The method of any one of Embodiments 1-10, wherein the brain cells are assayed for metabolic baseline prior to culturing with the sample and/or CSF.

[0134] 12. The method of Embodiment 11, wherein the metabolic baseline is determined by a method selected from one or more of: fluorescent microscopic image analysis, metabolic assays, mitochondrial activity using potentiometric dyes, MitoTracker, mitochondrial respiration, and gene expression analysis.

[0135] 13. The method of any one of Embodiments 1-12, metabolic change is determined by a method selected from one or more of: fluorescent microscopic image analysis, metabolic assays, mitochondrial activity using potentiometric dyes, MitoTracker, mitochondrial respiration, and gene expression analysis.

[0136] 14. The method of any of Embodiments 1-13, further comprising, or consisting essentially of, or consisting of correlating the metabolic change with neurological change.

[0137] 15. The method of any of Embodiments 1-14, wherein the sample of sera and/or CSF is isolated from a vertebrate.

[0138] 16. The method of Embodiment 15, wherein the vertebrate is a mammal, optionally a human patient.

[0139] 17. The method of any of Embodiments 1 or 3-16, wherein the test agent is a biologic or a small molecule.

[0140] 18. The method of any one of Embodiments 1-17, further comprising administering to the vertebrate an agent to treat the neurological change.

[0141] 19. The method of Embodiment 18, wherein the agent is an antidepressant.

[0142] 20. The method of any one of Embodiments 1-19, wherein the brain cells are selected from neurons, microglia, oligodendrocytes or astrocytes.

[0143] The method of any one of Embodiments 1-20, wherein the brain cells comprise or consist essentially of or consist of astrocytes.

References

1. Uwishema O, Ayoub G, Badri R, Onyeaka H, Berjaoui C, Karabulut E, et al. Neurological disorders in HIV: Hope despite challenges. *Immun Inflamm Dis*. 2022;10(3):e591.
2. Heaton RK, Franklin DR, Jr., Deutsch R, Letendre S, Ellis RJ, Casaletto K, et al. Neurocognitive change in the era of HIV combination antiretroviral therapy: the longitudinal CHARTER study. *Clin Infect Dis*. 2015;60(3):473-80.
3. Bhatia NS, Chow FC. Neurologic Complications in Treated HIV-1 Infection. *Curr Neurol Neurosci Rep*. 2016;16(7):62.
4. Bhatia MS, Munjal S. Prevalence of Depression in People Living with HIV/AIDS Undergoing ART and Factors Associated with it. *J Clin Diagn Res*. 2014;8(10):WC01-4.
5. Bhatia R, Ryscavage P, Taiwo B. Accelerated aging and human immunodeficiency virus infection: emerging challenges of growing older in the era of successful antiretroviral therapy. *J Neurovirol*. 2012;18(4):247-55.
6. Uthman OA, Magidson JF, Safren SA, Nachega JB. Depression and adherence to antiretroviral therapy in low-, middle- and high-income countries: a systematic review and meta-analysis. *Curr HIV/AIDS Rep*. 2014;11(3):291-307.
7. Beurel E, Toups M, Nemeroff CB. The Bidirectional Relationship of Depression and Inflammation: Double Trouble. *Neuron*. 2020;107(2):234-56.
8. Mackiewicz MM, Overk C, Achim CL, Masliah E. Pathogenesis of age-related HIV neurodegeneration. *J Neurovirol*. 2019;25(5):622-33.
9. Fields JA, Ellis RJ. HIV in the cART era and the mitochondrial: immune interface in the CNS. *Int Rev Neurobiol*. 2019;145:29-65.
10. Onyango IG. Modulation of mitochondrial bioenergetics as a therapeutic strategy in Alzheimer's disease. *Neural Regen Res*. 2018;13(1):19-25.
11. Tavazzi E, Morrison D, Sullivan P, Morgello S, Fischer T. Brain inflammation is a common feature of HIV-infected patients without HIV encephalitis or productive brain infection. *Curr HIV Res*. 2014;12(2):97-110.

12. Abdelhak A, Foschi M, Abu-Rumeileh S, Yue JK, D'Anna L, Huss A, et al. Blood GFAP as an emerging biomarker in brain and spinal cord disorders. *Nat Rev Neurol*. 2022;18(3):158-72.
13. Kulbe JR, Jain S, Nelson LD, Korley FK, Mukherjee P, Sun X, et al. Association of day-of-injury plasma glial fibrillary acidic protein concentration and six-month posttraumatic stress disorder in patients with mild traumatic brain injury. *Neuropsychopharmacology*. 2022;47(13):2300-8.
14. Ellis RJ, Fan Y, Grelotti D, Tang B, Letendre S, He JJ. Astrocyte Activation is A Potential Mechanism Underlying Depressed Mood and Apathy in People with HIV. *J Neurol Psychol*. 2022;9(1).
15. Steinacker P, Al Shweiki MR, Oeckl P, Graf H, Ludolph AC, Schonfeldt-Lecuona C, et al. Glial fibrillary acidic protein as blood biomarker for differential diagnosis and severity of major depressive disorder. *J Psychiatr Res*. 2021;144:54-8.
16. Michel M, Fiebich BL, Kuzior H, Meixensberger S, Berger B, Maier S, et al. Increased GFAP concentrations in the cerebrospinal fluid of patients with unipolar depression. *Transl Psychiatry*. 2021;11(1):308.
17. Davis S, Thomas A, Perry R, Oakley A, Kalaria RN, O'Brien JT. Glial fibrillary acidic protein in late life major depressive disorder: an immunocytochemical study. *J Neurol Neurosurg Psychiatry*. 2002;73(5):556-60.
18. Beard E, Lengacher S, Dias S, Magistretti PJ, Finsterwald C. Astrocytes as Key Regulators of Brain Energy Metabolism: New Therapeutic Perspectives. *Front Physiol*. 2021;12:825816.
19. Guo H, Fan Z, Wang S, Ma L, Wang J, Yu D, et al. Astrocytic A1/A2 paradigm participates in glycogen mobilization mediated neuroprotection on reperfusion injury after ischemic stroke. *J Neuroinflammation*. 2021;18(1):230.
20. Sofroniew MV. Astrocyte Reactivity: Subtypes, States, and Functions in CNS Innate Immunity. *Trends Immunol*. 2020;41(9):758-70.
21. Khakh BS, Deneen B. The Emerging Nature of Astrocyte Diversity. *Annu Rev Neurosci*. 2019;42:187-207.

22. Abbott NJ, Ronnback L, Hansson E. Astrocyte-endothelial interactions at the blood-brain barrier. *Nat Rev Neurosci.* 2006;7(1):41-53.
23. Gendelman H, Lipton S, Tardieu M, Bukrinsky M, Nottet H. The neuropathogenesis of HIV-1 infection. *JLeukocyte Biol.* 1994;56:389-98.
24. Yin F, Sancheti H, Patil I, Cadenas E. Energy metabolism and inflammation in brain aging and Alzheimer's disease. *Free Radic Biol Med.* 2016;100:108-22.
25. Jiang T, Cadenas E. Astrocytic metabolic and inflammatory changes as a function of age. *Aging Cell.* 2014;13(6):1059-67.
26. Swinton MK, Carson A, Telese F, Sanchez AB, Soontornniyomkij B, Rad L, et al. Mitochondrial biogenesis is altered in HIV+ brains exposed to ART: Implications for therapeutic targeting of astroglia. *Neurobiol Dis.* 2019;130:104502.
27. Fields JA, Swinton MK, Carson A, Soontornniyomkij B, Lindsay C, Han MM, et al. Tenofovir disoproxil fumarate induces peripheral neuropathy and alters inflammation and mitochondrial biogenesis in the brains of mice. *Sci Rep.* 2019;9(1):17158.
28. Cotto B, Natarajaseenivasan K, Langford D. Astrocyte activation and altered metabolism in normal aging, age-related CNS diseases, and HAND. *J Neurovirol.* 2019;25(5):722-33.
29. Natarajaseenivasan K, Cotto B, Shanmughapriya S, Lombardi AA, Datta PK, Madesh M, et al. Astrocytic metabolic switch is a novel etiology for Cocaine and HIV-1 Tat-mediated neurotoxicity. *Cell Death Dis.* 2018;9(4):415.
30. Sivalingam K, Cirino TJ, McLaughlin JP, Samikkannu T. HIV-Tat and Cocaine Impact Brain Energy Metabolism: Redox Modification and Mitochondrial Biogenesis Influence NRF Transcription-Mediated Neurodegeneration. *Mol Neurobiol.* 2021;58(2):490-504.
31. Samikkannu T, Atluri VS, Nair MP. HIV and Cocaine Impact Glial Metabolism: Energy Sensor AMP-activated protein kinase Role in Mitochondrial Biogenesis and Epigenetic Remodeling. *Sci Rep.* 2016;6:31784.

32. Haroon E, Daguanno AW, Woolwine BJ, Goldsmith DR, Baer WM, Wommack EC, et al. Antidepressant treatment resistance is associated with increased inflammatory markers in patients with major depressive disorder. *Psychoneuroendocrinology*. 2018;95:43-9.
33. Wollenhaupt-Aguiar B, Pfaffenseller B, Chagas VS, Castro MAA, Passos IC, Kauer-Sant'Anna M, et al. Reduced Neurite Density in Neuronal Cell Cultures Exposed to Serum of Patients with Bipolar Disorder. *Int J Neuropsychopharmacol*. 2016;19(10).
34. Saloner R, Savini N, Letendre SL, Moore DJ, Montoya JL. Neopterin Relates to Lifetime Depression in Older Adults With HIV on Suppressive Antiretroviral Therapy. *J Acquir Immune Defic Syndr*. 2022;89(4):454-61.
35. Heaton RK, Clifford DB, Franklin DR, Jr., Woods SP, Ake C, Vaida F, et al. HIV-associated neurocognitive disorders persist in the era of potent antiretroviral therapy: CHARTER Study. *Neurology*. 2010;75(23):2087-96.
36. Sundermann EE, Saloner R, Rubtsova A, Nguyen AL, Letendre S, Moore RC, et al. The association between benzodiazepine use and greater risk of neurocognitive impairment is moderated by medical burden in people with HIV. *J Neurovirol*. 2022.
37. Fields J, Ghorpade A. C/EBPbeta regulates multiple IL-1beta-induced human astrocyte inflammatory genes. *J Neuroinflammation*. 2012;9(1):177.
38. Kohler CA, Freitas TH, Maes M, de Andrade NQ, Liu CS, Fernandes BS, et al. Peripheral cytokine and chemokine alterations in depression: a meta-analysis of 82 studies. *Acta Psychiatr Scand*. 2017;135(5):373-87.
39. Herman FJ, Pasinetti GM. Principles of inflammasome priming and inhibition: Implications for psychiatric disorders. *Brain Behav Immun*. 2018;73:66-84.
40. Chivero ET, Guo ML, Periyasamy P, Liao K, Callen SE, Buch S. HIV-1 Tat Primes and Activates Microglial NLRP3 Inflammasome-Mediated Neuroinflammation. *J Neurosci*. 2017;37(13):3599-609.
41. Sil S, Niu F, Chivero ET, Singh S, Periyasamy P, Buch S. Role of Inflammasomes in HIV-1 and Drug Abuse Mediated Neuroinflammation. *Cells*. 2020;9(8).

42. Rubin LH, Langenecker SA, Phan KL, Keating SM, Neigh GN, Weber KM, et al. Remitted depression and cognition in HIV: The role of cortisol and inflammation. *Psychoneuroendocrinology*. 2020;114:104609.
43. Mudra Rakshasa-Loots A, Whalley HC, Vera JH, Cox SR. Neuroinflammation in HIV-associated depression: evidence and future perspectives. *Mol Psychiatry*. 2022;27(9):3619-32.
44. Cotto B, Natarajanseenivasan K, Langford D. HIV-1 infection alters energy metabolism in the brain: Contributions to HIV-associated neurocognitive disorders. *Prog Neurobiol*. 2019;181:101616.
45. Muraleedharan R, Gawali MV, Tiwari D, Sukumaran A, Oatman N, Anderson J, et al. AMPK-Regulated Astrocytic Lactate Shuttle Plays a Non-Cell-Autonomous Role in Neuronal Survival. *Cell Rep*. 2020;32(9):108092.
46. Rose J, Brian C, Pappa A, Panayiotidis MI, Franco R. Mitochondrial Metabolism in Astrocytes Regulates Brain Bioenergetics, Neurotransmission and Redox Balance. *Front Neurosci*. 2020;14:536682.
47. Deitmer JW, Theparambil SM, Ruminot I, Noor SI, Becker HM. Energy Dynamics in the Brain: Contributions of Astrocytes to Metabolism and pH Homeostasis. *Front Neurosci*. 2019;13:1301.
48. Miller AH, Haroon E, Raison CL, Felger JC. Cytokine targets in the brain: impact on neurotransmitters and neurocircuits. *Depress Anxiety*. 2013;30(4):297-306.
49. Felger JC, Li L, Marvar PJ, Woolwine BJ, Harrison DG, Raison CL, et al. Tyrosine metabolism during interferon-alpha administration: association with fatigue and CSF dopamine concentrations. *Brain Behav Immun*. 2013;31:153-60.
50. Capuron L, Pagnoni G, Drake DF, Woolwine BJ, Spivey JR, Crowe RJ, et al. Dopaminergic mechanisms of reduced basal ganglia responses to hedonic reward during interferon alfa administration. *Arch Gen Psychiatry*. 2012;69(10):1044-53.
51. Del Guerra FB, Fonseca JL, Figueiredo VM, Ziff EB, Konkiewitz EC. Human immunodeficiency virus-associated depression: contributions of immuno-inflammatory,

monoaminergic, neurodegenerative, and neurotrophic pathways. *J Neurovirol.* 2013;19(4):314-27.

52. Liu CY, Yang Y, Ju WN, Wang X, Zhang HL. Emerging Roles of Astrocytes in Neuro-Vascular Unit and the Tripartite Synapse With Emphasis on Reactive Gliosis in the Context of Alzheimer's Disease. *Frontiers in cellular neuroscience.* 2018;12:193.

53. Liddelow SA, Barres BA. Reactive Astrocytes: Production, Function, and Therapeutic Potential. *Immunity.* 2017;46(6):957-67.

54. Liddelow SA, Guttenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L, et al. Neurotoxic reactive astrocytes are induced by activated microglia. *Nature.* 2017;541(7638):481-7.

55. Khakh BS, Sofroniew MV. Diversity of astrocyte functions and phenotypes in neural circuits. *Nat Neurosci.* 2015;18(7):942-52.

56. Todd BP, Chimenti MS, Luo Z, Ferguson PJ, Bassuk AG, Newell EA. Traumatic brain injury results in unique microglial and astrocyte transcriptomes enriched for type I interferon response. *J Neuroinflammation.* 2021;18(1):151.

57. Anwar S, Rivest S. Alzheimer's disease: microglia targets and their modulation to promote amyloid phagocytosis and mitigate neuroinflammation. *Expert Opin Ther Targets.* 2020;24(4):331-44.

WHAT IS CLAIMED IS:

1. An *in vitro* method to determine if one or more test agents modify metabolic changes in a sample of brain cells, the method comprising culturing the sample with sera and/or cerebrospinal fluid (CSF) and the one or more test agents and assaying the sample combined with the sera and/or CSF for metabolic changes.
2. An *in vitro* method to determine the cognitive status of a subject, the method comprising culturing a sample of brain cells astrocytes with sera and/or cerebrospinal fluid (CSF) isolated from the subject and assaying the sample combined with the sera and/or CSF for metabolic changes.
3. The method of claim 2, further comprising adding one or more test agents to the sample combined with the sera and/or CSF and assaying the sample for metabolic changes.
4. The method of any one of claims 1-3, further comprising determining the metabolic status of the brain cells by determining the mitochondrial activity of the astrocytes cultured in the absence of one or more of the sera, the CSF and/or the test agents.
5. The method of any one of claims 1-4, further comprising determining the metabolic status of the brain cells by determining the mitochondrial activity of the brain cells cultured in the absence of one or more of the sera, the CSF and/or the test agents.
6. The method of any one of claims 1-5, wherein the metabolic changes are assayed by a method comprising determining mitochondrial activity of the brain cells in the sample after culture with the sera and/or CSF.
7. The method of any one of claims 1-6, where low mitochondrial activity of the brain cells in the culture indicates cognitive decline, cognitive impairment and/or behavioral changes or abnormalities.
8. The method of any one of claims 1-7, wherein the sera and/or CSF are isolated from a subject that has been assessed from cognitive and/or behavioral changes or abnormalities.

9. The method of claim 8, wherein the cognitive and/or behavioral changes comprises depression.
10. The method of claim 8, wherein the cognitive and/or behavioral changes comprises neurocognitive impairment.
11. The method of any one of claims 1-10, wherein the brain cells are assayed for metabolic baseline prior to culturing with the sample and/or CSF.
12. The method of claim 11, wherein the metabolic baseline is determined by a method selected from one or more of: fluorescent microscopic image analysis, metabolic assays, mitochondrial activity using potentiometric dyes, MitoTracker, mitochondrial respiration, and gene expression analysis.
13. The method of any one of claims 1-12, metabolic change is determined by a method selected from one or more of: fluorescent microscopic image analysis, metabolic assays, mitochondrial activity using potentiometric dyes, MitoTracker, mitochondrial respiration, and gene expression analysis.
14. The method of any of claims 1-13, further comprising, or consisting essentially of, or consisting of correlating the metabolic change with neurological change.
15. The method of any of claims 1-14, wherein the sample of sera and/or CSF is isolated from a vertebrate.
16. The method of claim 15, wherein the vertebrate is a mammal, optionally a human patient.
17. The method of any of claims 1 or 3-16, wherein the test agent is a biologic or a small molecule.
18. The method of any one of claims 1-17, further comprising administering to the vertebrate an agent to treat the neurological change.
19. The method of claim 18, wherein the agent is an antidepressant.

20. The method of any one of claims 1-19, wherein the brain cells are selected from neurons, microglia, oligodendrocytes or astrocytes.

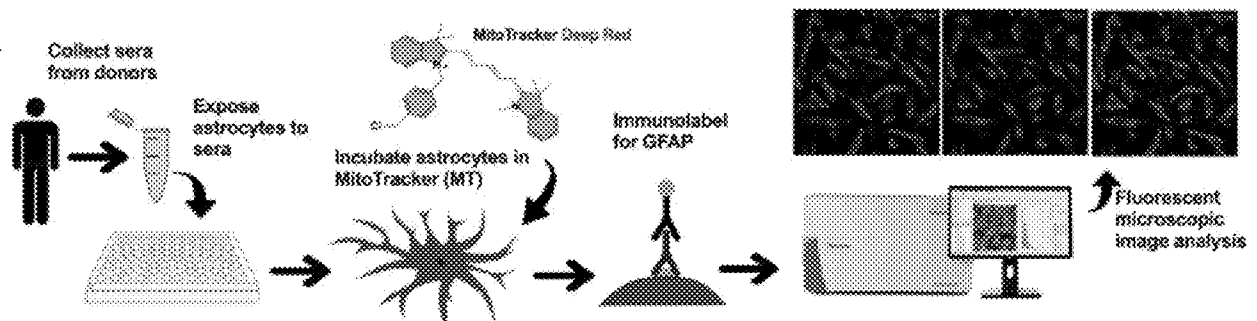


FIG. 1A

Spot Detector Assay Analysis Definitions:

- i. **Spot Average Intensity:** Total intensity of all pixels within all the spots in a well divided by total area of all spots in a well
- ii. **Spot Average Area:** Total area (μm^2) of all spots in a well divided by the number of spots in a well
- iii. **Spot Total Area Per Object:** Total area (μm^2) of all spots in a well divided by number of cells in a well

Cell Health Profiling Assay Analysis Definitions:

- iv. **Target Average Intensity:** Average intensity of all pixels within target mask (outline around cell)

FIG. 1B

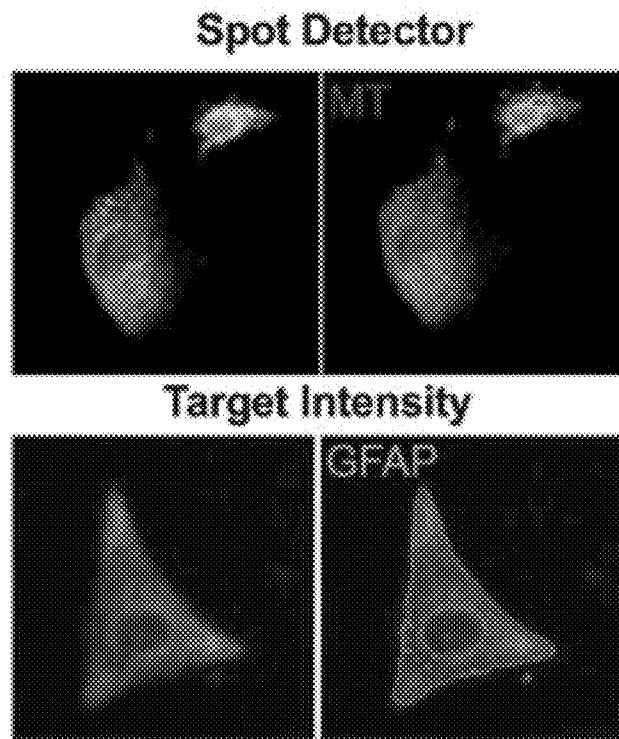
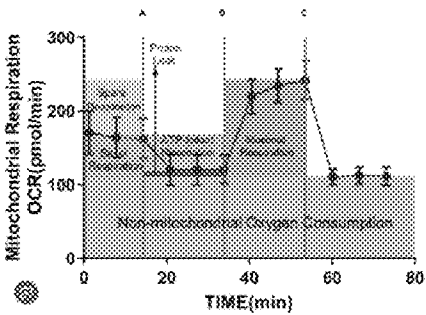


FIG. 1C

Fluorescent
microscopic
image analysis



Metabolic assays



Gene expression
analysis

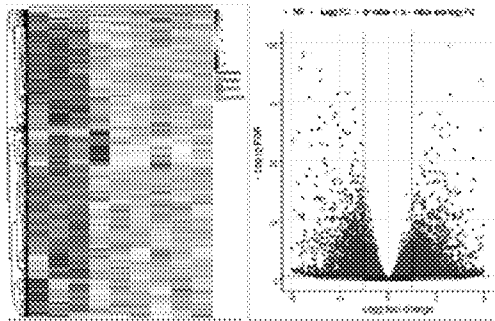
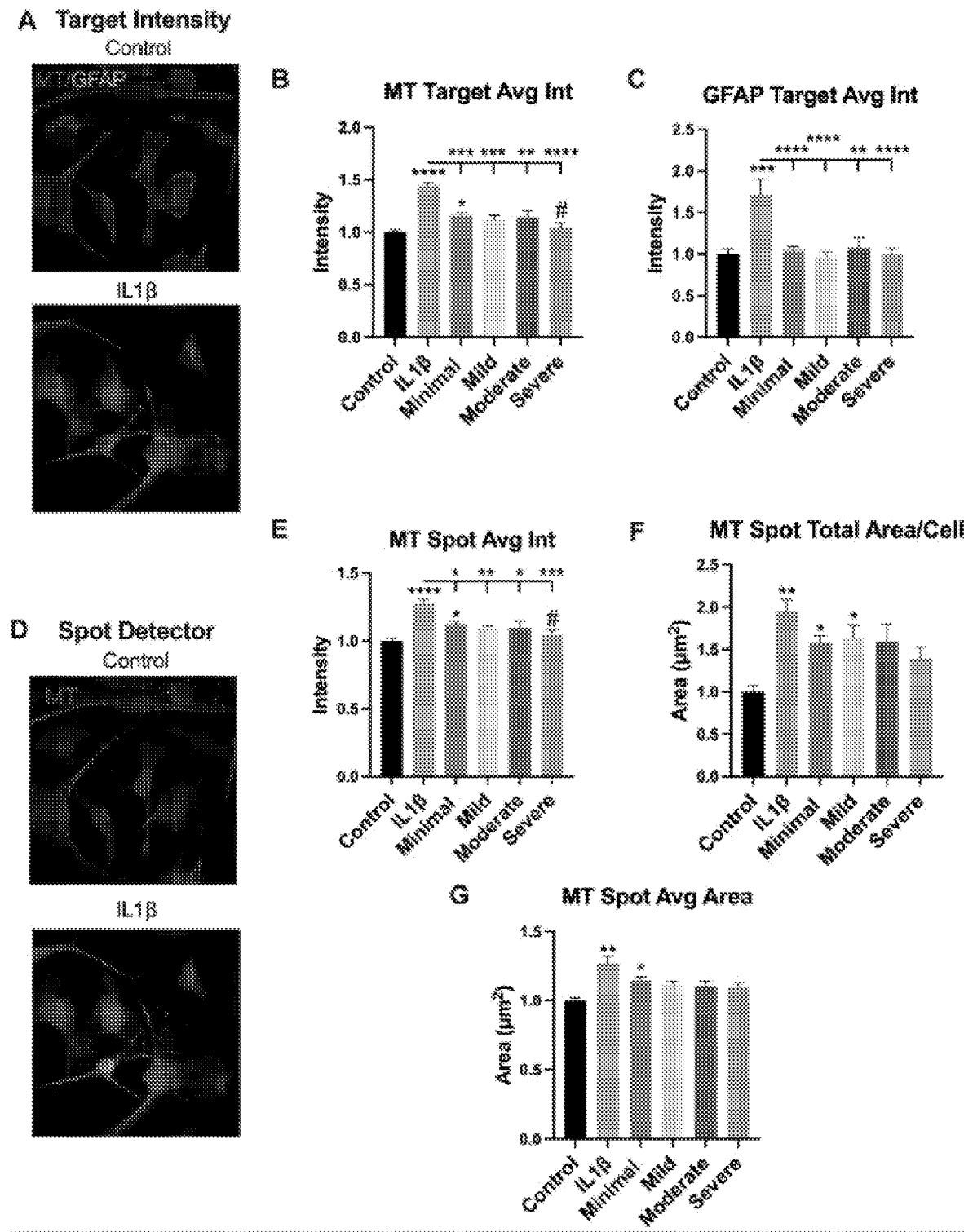


FIG. 2



FIGS. 3A – 3G

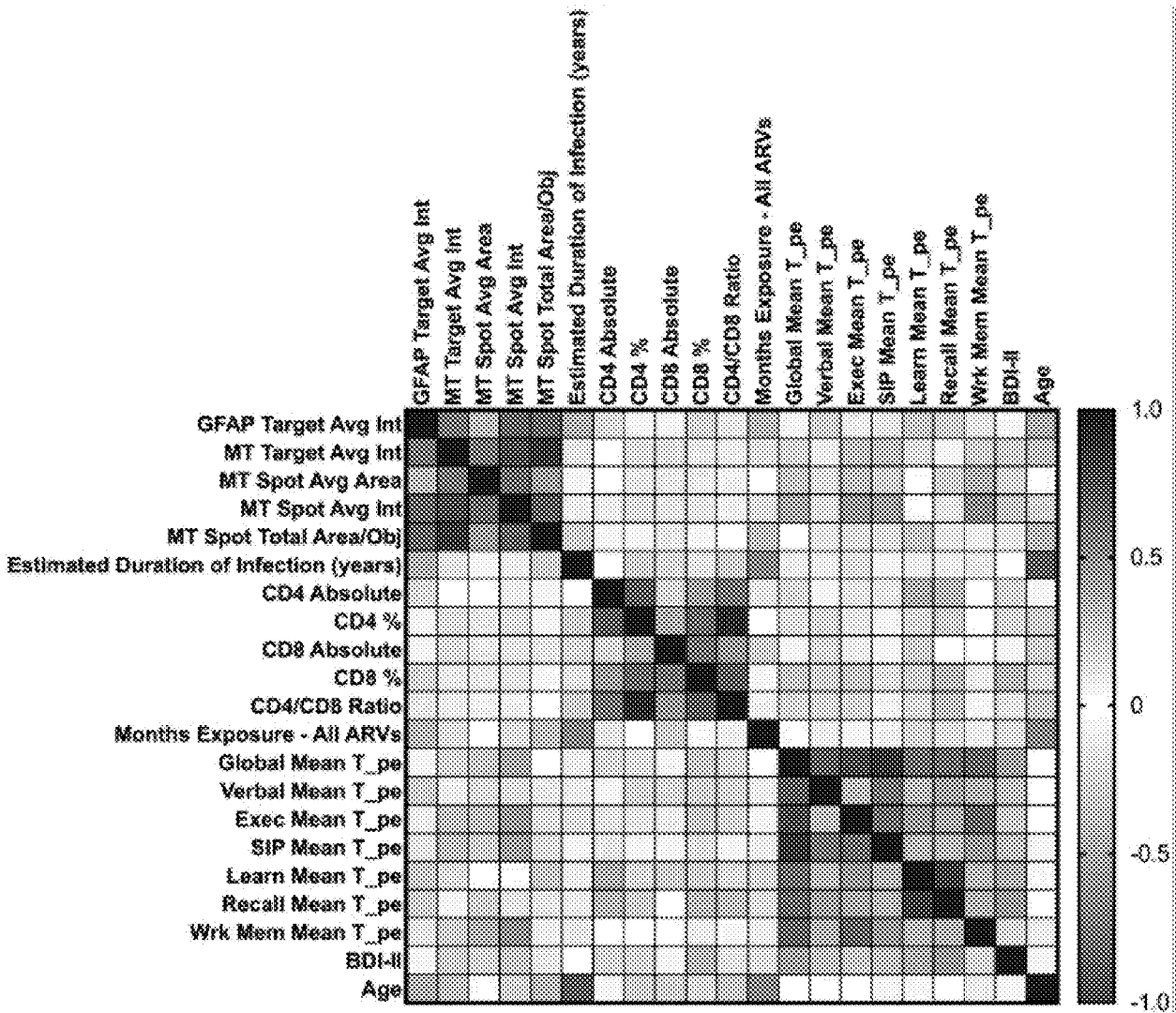
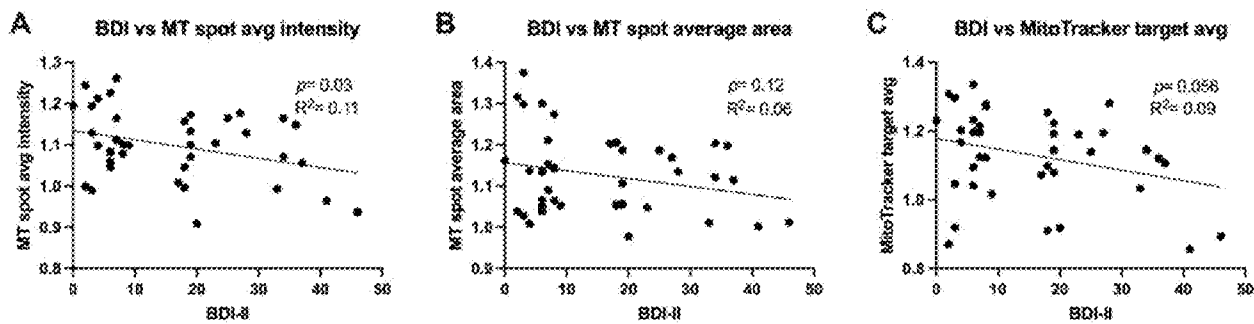
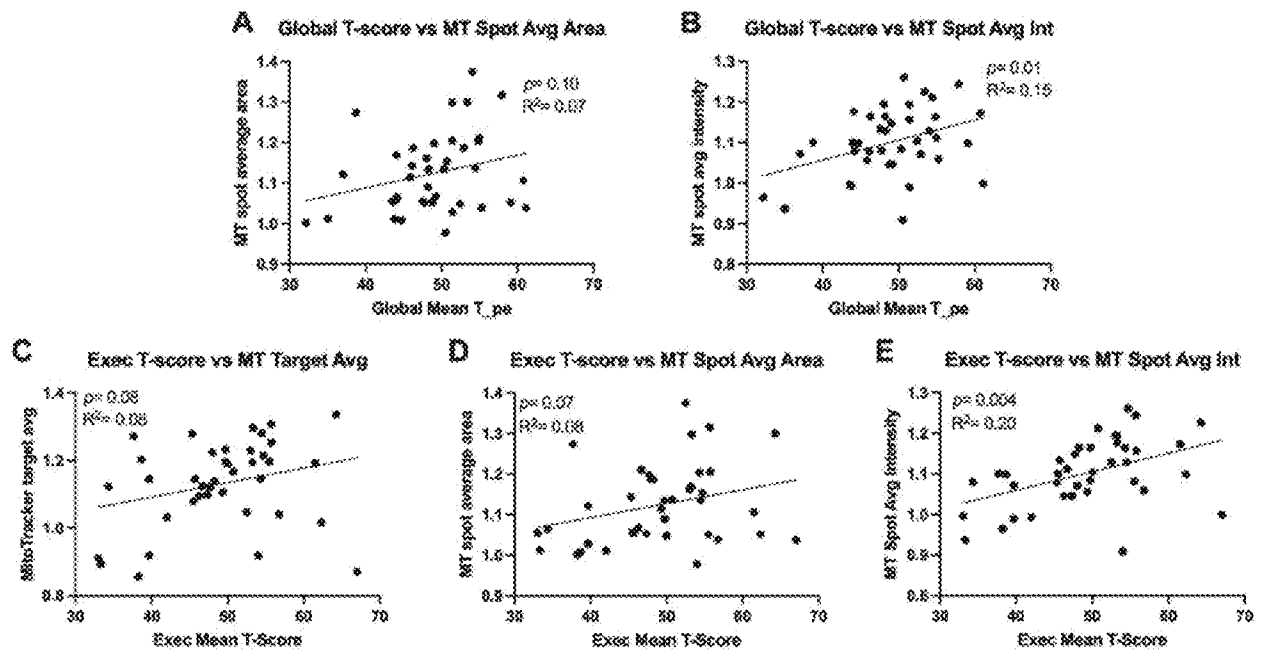


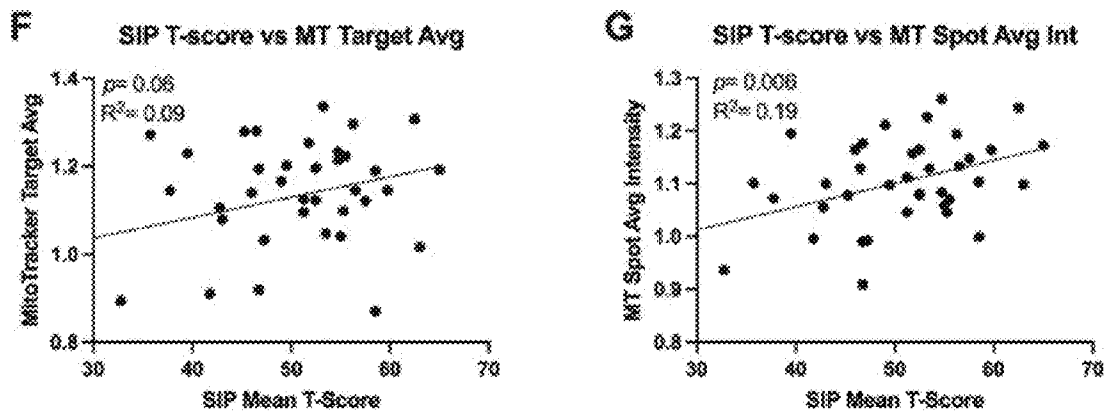
FIG. 4



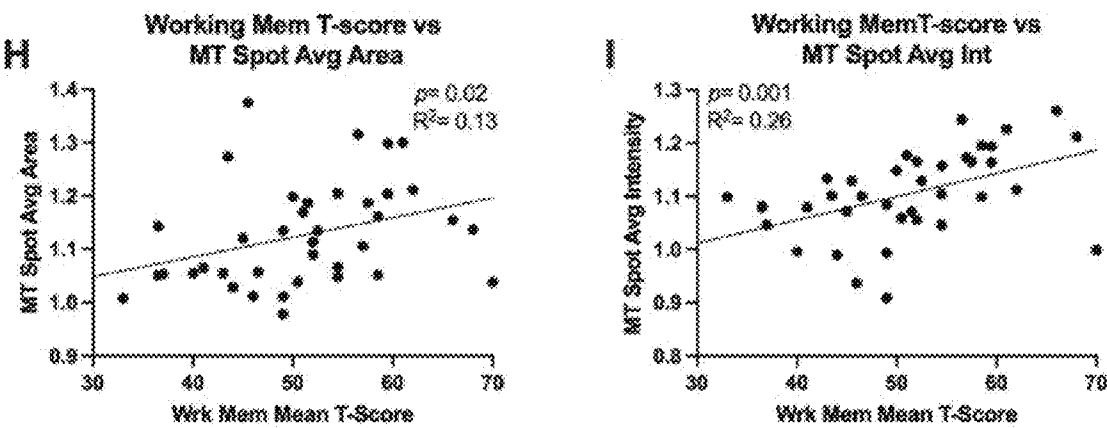
FIGS. 5A – 5C



FIGS. 6A – 6E



FIGS. 6F – 6G



FIGS. 6H – 6I

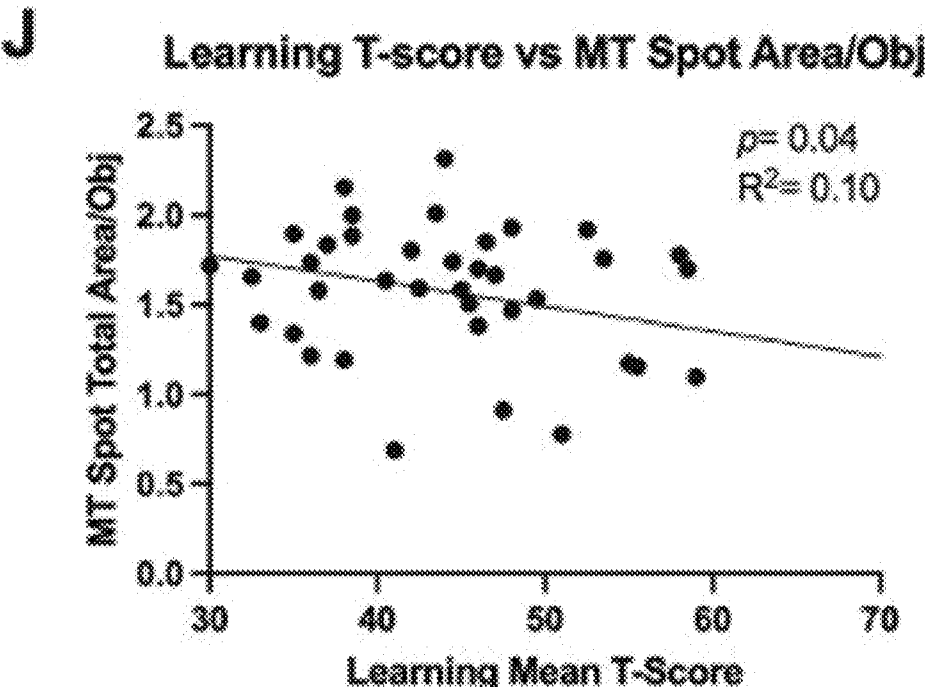
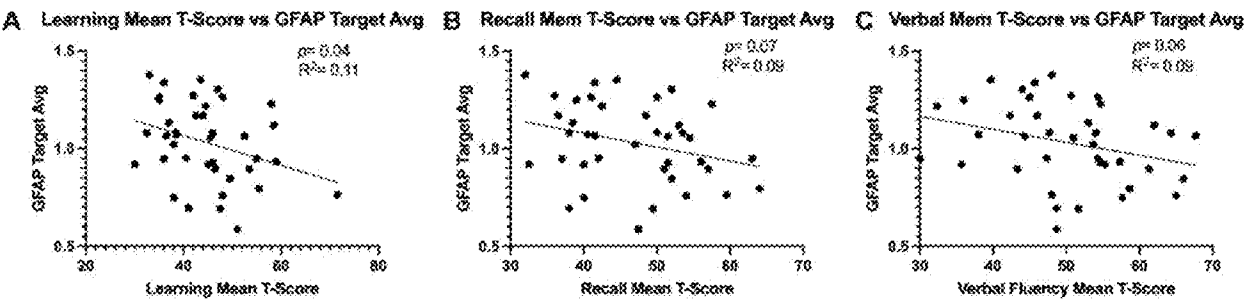
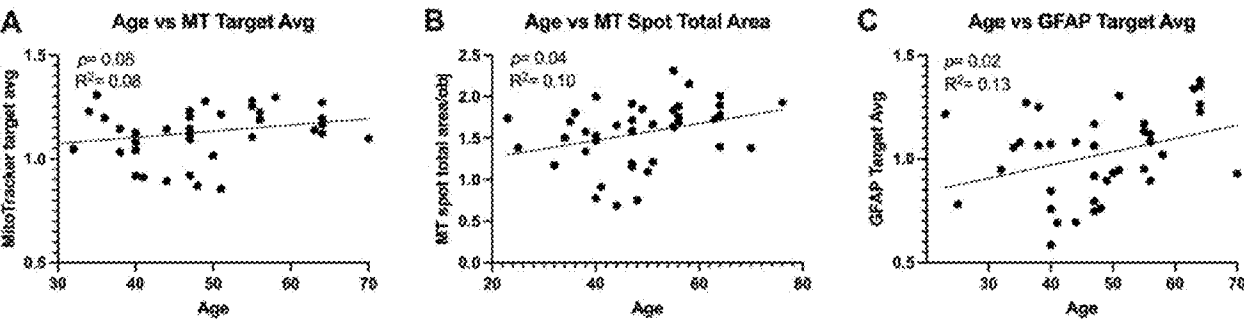


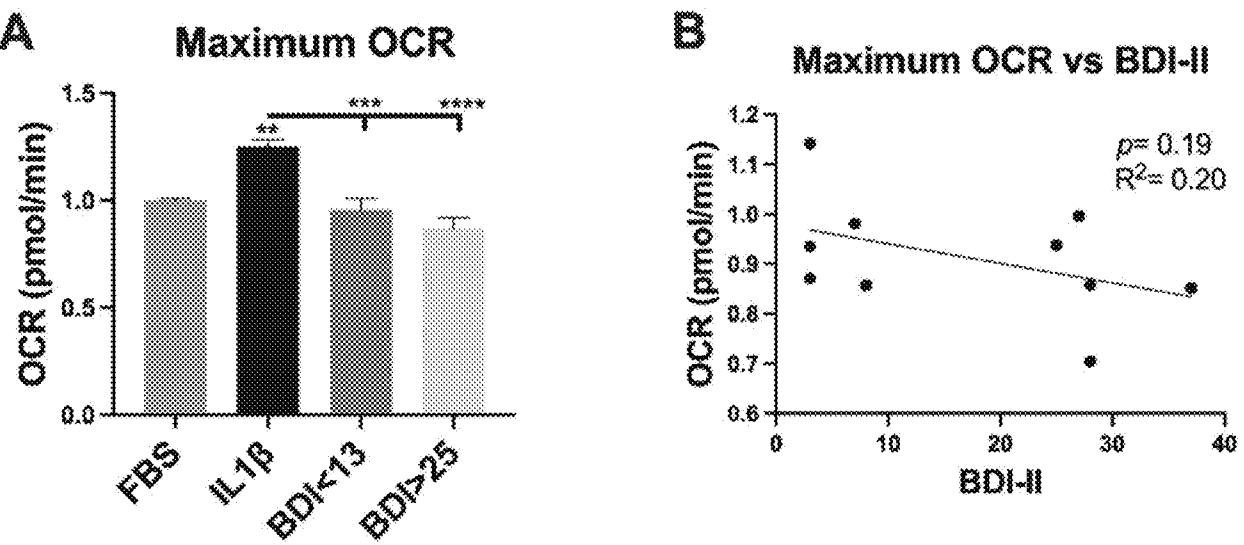
FIG. 6J



FIGS. 7A – 7C

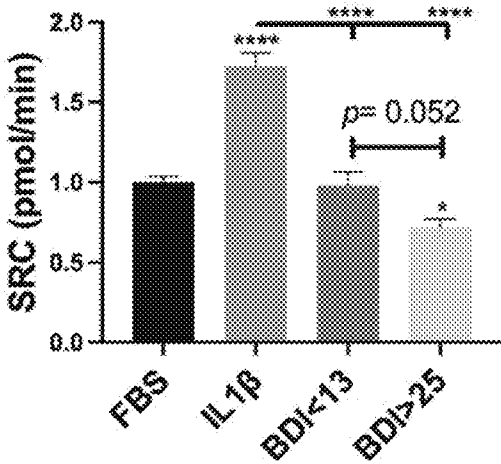


FIGS. 8A – 8C

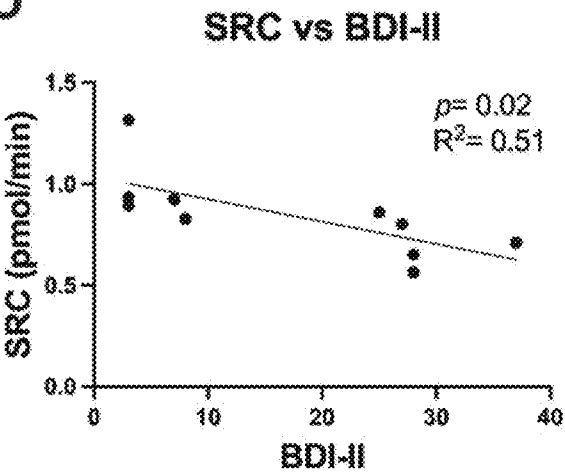


FIGS. 9A – 9B

C Spare Respiratory Capacity

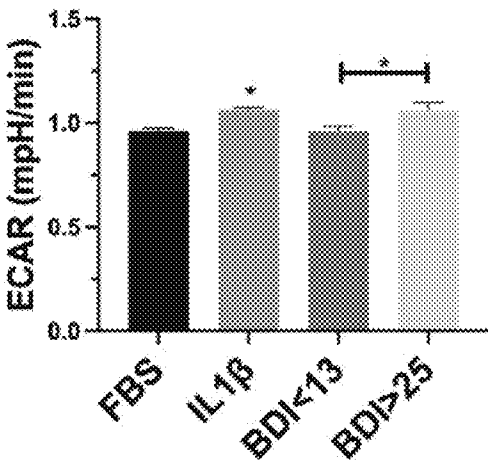


D

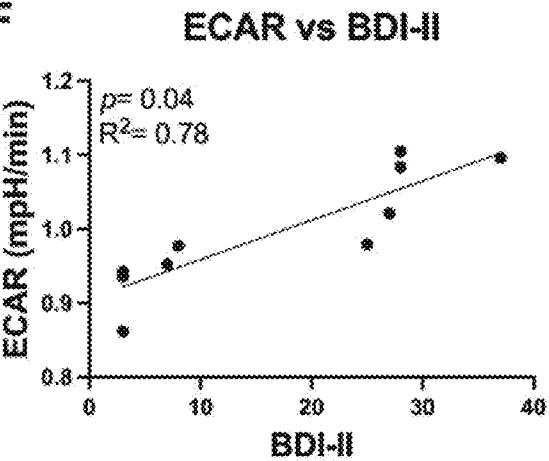


FIGS. 9C – 9D

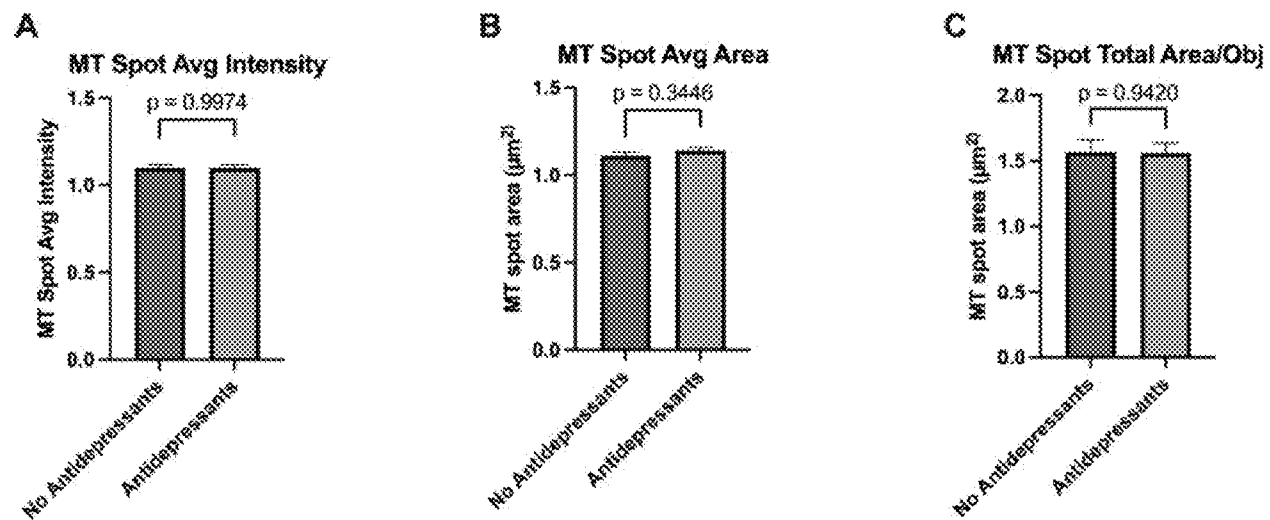
E Maximum ECAR



F



FIGS. 9E – 9F



FIGS. 10A – 10C

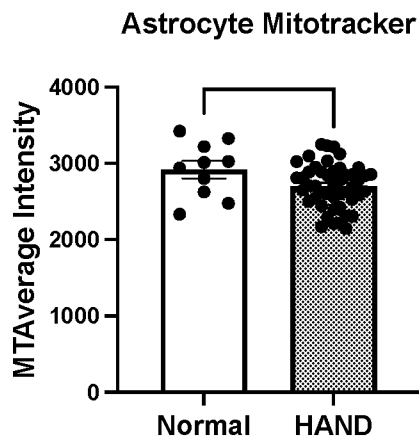


FIG. 11

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/059659

A. CLASSIFICATION OF SUBJECT MATTERIPC: **A61K 35/30** (2025.01); *C12N 5/071* (2025.01); *C07K 14/16* (2025.01); *A61P 31/18* (2025.01)CPC: **C12N 5/069**; *C07K 14/155*

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

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

See Search History Document

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

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KAUR et al., Contemporary Antiretroviral Therapy Dysregulates Iron Transport and Augments Mitochondrial Dysfunction in HIV-Infected Human Microglia and Neural-Lineage Cells. <i>Int. J. Mol. Sci.</i> 31 July 2023, Vol. 24, 12242. Abstract; pg 15, last para; pg 16, para 3; Figure 2 legend	1-4
A	COTTO et al., HIV infection alters energy metabolism in the brain: contributions to HIV-associated neurocognitive disorders. <i>Prog Neurobiol.</i> October 2019, Vol. 181: 101616. Abstract	1-4

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

* Special categories of cited documents:

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

“D” document cited by the applicant in the international application

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

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

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

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

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

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

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

“&” document member of the same patent family

Date of the actual completion of the international search

25 January 2025 (25.01.2025)

Date of mailing of the international search report

06 February 2025 (06.02.2025)

Name and mailing address of the ISA/US

**COMMISSIONER FOR PATENTS
MAIL STOP PCT, ATTN: ISA/US
P.O. Box 1450
Alexandria, VA 22313-1450
UNITED STATES OF AMERICA**

Authorized officer

KARI RODRIQUEZFacsimile No. **571-273-8300**Telephone No. **PCT Help Desk: 571-272-4300**

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/059659

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: **5-20**
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).