

TITLE

**METHODS AND COMPOSITIONS FOR INCREASING NEURONAL ACTIVITY
AND SYNAPTIC PLASTICITY**

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional Application No. 63/626,785 filed on January 30, 2024, the content of which is incorporated herein by reference in its entirety.

TECHNICAL FIELD

[0002] The present disclosure relates generally to the field of cell therapies and, more specifically, to methods and compositions for increasing neuronal activity and synaptic plasticity in a subject in need thereof.

BACKGROUND

[0003] Over the past several decades, mesenchymal stem cell-based (MSC-based) therapies have emerged as a new strategy for treating neurological disorders and injuries such as stroke, traumatic brain injury (TBI), amyotrophic lateral sclerosis (ALS), and spinal cord injury (SCI) [1, 2]. These neurological disorders or injuries often involve the impairment of certain cognitive functions due to disruptions in how neurons communicate. In order to re-establish neuronal communication and promote functional recovery, there must be an increase in neuronal activity and synaptic plasticity that allows for the repair of certain neural pathways or the creation of new neural pathways.

[0004] MSCs have low immunogenicity and can be isolated from different adult and birth tissues and cultured at great expansion capacity [3]. Vandefitemcel, also known as SB623 cells, are a type of human bone marrow-derived MSC cells. Recent studies have shown that intracerebral implantation of vandefitemcel or SB623 cells was safe and could improve a patient's motor functions [4, 5]. However, little is known about the effect of MSCs on the electrophysiology of neurons (and, especially, human neurons) after their implantation or transplantation.

[0005] While neurons for research can come from a variety of sources, one renewable source of human neurons that do not require invasive biopsies of human central nervous system (CNS) tissue is human induced pluripotent stem cells (iPSCs). Human iPSCs can be

1 differentiated into a range of somatic cells including types of neurons such as glutamatergic
2 and GABAergic neurons [6].

3 [0006] Moreover, multielectrode arrays (MEAs) have been used in the past to measure the
4 electrophysiology of neurons [7]. MEAs typically include a special multi-well culture plate
5 with small electrodes embedded at the bottom of each well. Extracellular spontaneous
6 action potentials are recorded from neuronal cells cultured directly on top of the recording
7 electrodes.

8 [0007] Therefore, there is a need for safe and effective cell-based therapies for increasing
9 neuronal activity and synaptic plasticity. Such therapies should be capable of being tested
10 on human iPSC-derived neurons using state-of-the-art MEA measurement systems.

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SUMMARY

13 [0008] In some aspects, disclosed is a method for inducing, triggering, or causing tonic
14 release of glutamate in a subject. The method comprises administering vandefitemcel to a
15 region of the brain of the subject. The vandefitemcel can be cells descended from
16 mesenchymal stem cells (MSCs) transiently-transfected by a polynucleotide encoding a
17 Notch intracellular domain (NICD).

18 [0009] In some aspects, administering vandefitemcel to a region of the brain of the subject
19 further comprises administering the vandefitemcel by intracerebral implantation.

20 [0010] In some aspects, the vandefitemcel can tonically release glutamate to neurons of the
21 brain when implanted via intracerebral implantation.

22 [0011] In some aspects, administering vandefitemcel to a region of the brain of the subject
23 further comprises injecting the vandefitemcel at multiple sites within the region of the
24 brain.

25 [0012] In some aspects, administering vandefitemcel to a region of the brain of the subject
26 further comprises administering the vandefitemcel stereotactically via a burr hole in the
27 skull of the subject.

28 [0013] In some aspects, the region of the brain is the forebrain of the subject including at
29 least one of the cerebrum, the thalamus, and the hypothalamus of the subject.

30 [0014] In some aspects, the region of the brain can be a site of injury or disease.

31 [0015] In some aspects, the region of the brain can be the hippocampus of the subject.

32 [0016] In some aspects, administering vandefitemcel to a region of the brain of the subject
33 further comprises administering the vandefitemcel by parenteral administration.

1 [0017] In some aspects, the vandefitemcel can be suspended in a sterile isotonic crystalloid
2 solution.

3 [0018] In some aspects, administering vandefitemcel to a region of the brain of the subject
4 further comprises administering between about 1.0 million cells and 10.0 million cells.

5 [0019] In some aspects, the vandefitemcel can be made by a method comprising providing
6 a culture of MSCs; contacting the culture of MSCs with the polynucleotide encoding the
7 NICD, wherein the polynucleotide does not encode a full-length Notch protein; selecting
8 cells that comprise the polynucleotide; and further culturing the selected cells in the
9 absence of selection for the polynucleotide.

10 [0020] In some aspects, the MSCs can be human bone marrow-derived cells.

11 [0021] In some aspects, the method can further comprise capturing functional magnetic
12 resonance imaging (fMRI) scans of regions of the brain of the subject, selecting one region
13 of the brain showing neuronal activity based on the fMRI scans, and administering the
14 vandefitemcel to the one region of the brain.

15 [0022] In some aspects, disclosed is a method for increasing synapse formation in neurons.
16 The method can comprise administering vandefitemcel to a region of the brain of the
17 subject comprising the neurons. The vandefitemcel can be cells descended from
18 mesenchymal stem cells (MSCs) transiently-transfected by a polynucleotide encoding a
19 Notch intracellular domain (NICD).

20 [0023] In some aspects, administering the vandefitemcel further comprises administering
21 the vandefitemcel by intracerebral implantation.

22 [0024] In some aspects, the vandefitemcel can tonically release glutamate to the neurons of
23 the brain of the subject after the vandefitemcel is implanted via intracerebral implantation.

24 [0025] In some aspects, administering the vandefitemcel further comprises injecting the
25 vandefitemcel at multiple sites within the region of the brain.

26 [0026] In some aspects, administering the vandefitemcel further comprises administering
27 the vandefitemcel stereotactically via a burr hole in the skull of the subject.

28 [0027] In some aspects, the region of the brain is the forebrain of the subject including at
29 least one of the cerebrum, the thalamus, and the hypothalamus of the subject.

30 [0028] In some aspects, the region of the brain can be a site of injury or disease.

31 [0029] In some aspects, the region of the brain can be a hippocampus of the subject.

32 [0030] In some aspects, administering the vandefitemcel can further comprise
33 administering the vandefitemcel by parenteral administration.

1 [0031] In some aspects, the vandefitemcel can be suspended in a sterile isotonic crystalloid
2 solution.

3 [0032] In some aspects, administering the vandefitemcel can further comprise
4 administering between about 1.0 million cells and 10.0 million cells of the vandefitemcel.

5 [0033] In some aspects, the vandefitemcel can be made by a method comprising providing
6 a culture of MSCs; contacting the culture of MSCs with the polynucleotide encoding the
7 NICD, wherein the polynucleotide does not encode a full-length Notch protein; selecting
8 cells that comprise the polynucleotide; and further culturing the selected cells in the
9 absence of selection for the polynucleotide.

10 [0034] In some aspects, the MSCs can be human bone marrow-derived cells.

11 [0035] In some aspects, the method can further comprise capturing functional magnetic
12 resonance imaging (fMRI) scans of regions of the brain of the subject, selecting one region
13 of the brain showing neuronal activity based on the fMRI scans, and administering the
14 vandefitemcel to the one region of the brain.

15 [0036] In some aspects, a composition for increasing synapse formation in neurons is
16 disclosed. The composition can comprise vandefitemcel in an amount between about 1.0
17 million cells and 10.0 million cells and one or more pharmaceutically acceptable
18 excipients. The vandefitemcel can be produced by modifying mesenchymal stem cells
19 derived from human bone marrow.

20 [0037] In some aspects, the vandefitemcel can be made by a process comprising providing
21 a culture of mesenchymal stem cells (MSCs); contacting the culture of MSCs with a
22 polynucleotide encoding a Notch intracellular domain (NICD), wherein the polynucleotide
23 does not encode a full-length Notch protein; selecting cells that comprise the
24 polynucleotide; and further culturing the selected cells in the absence of selection for the
25 polynucleotide.

26 [0038] In some aspects, the MSCs can be human bone marrow-derived cells.

27 [0039] In some aspects, the MSCs can be transiently-transfected with a plasmid vector
28 comprising the polynucleotide encoding the NICD.

29 [0040] In some aspects, the one or more pharmaceutically acceptable excipients can
30 comprise at least one of buffers, proteins, stabilizers, and preservatives.

31 [0041] In some aspects, the vandefitemcel can be suspended in a sterile isotonic crystalloid
32 solution.

33 [0042] In some aspects, the one or more pharmaceutically acceptable excipients can
34 comprise carriers or diluents.

1 [0043] In some aspects, a method of inducing, triggering, or causing the release of
2 glutamate in a subject is disclosed. The method can comprise capturing functional
3 magnetic resonance imaging (fMRI) scans of regions of the brain of the subject, selecting a
4 region of the brain showing neuronal activity based on the fMRI scans, and administering
5 vandefitemcel to the region of the brain selected. The vandefitemcel can be cells descended
6 from mesenchymal stem cells (MSCs) transiently-transfected by a polynucleotide encoding
7 a Notch intracellular domain (NICD). The vandefitemcel can release glutamate after being
8 administered via intracerebral implantation.

9 [0044] In some aspects, a method of increasing synapse formation in neurons is disclosed.
10 The method can comprise capturing functional magnetic resonance imaging (fMRI) scans
11 of regions of the brain of the subject, selecting a region of the brain showing neuronal
12 activity based on the fMRI scans, and administering vandefitemcel to the region of the
13 brain selected comprising the neurons. The vandefitemcel can be cells descended from
14 mesenchymal stem cells (MSCs) transiently-transfected by a polynucleotide encoding a
15 Notch intracellular domain (NICD). The vandefitemcel can release glutamate to the
16 neurons after being administered via intracerebral implantation.

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BRIEF DESCRIPTION OF THE DRAWINGS

19 [0045] Figs. 1A-1D are graphs illustrating the electrophysiological activity and viability of
20 separate cultures of vandefitemcel and iFbNs over an eight-week period using a
21 multielectrode array (MEA) system.

22 [0046] Fig. 1E are raster plots illustrating electrophysiological activity and network burst
23 development at four weeks and eight weeks for iFbN cell cultures at different cell densities.

24 [0047] Fig. 2A are reproductions of immunocytochemistry stains of a coculture of
25 vandefitemcel and iPSC-derived forebrain neuron (iFbN) cells.

26 [0048] Fig. 2B is a graph illustrating the presence of vandefitemcel in cocultures of
27 vandefitemcel and iFbN cells. The presence of vandefitemcel was confirmed by an increase
28 in the resistance measured between the cells and the MEA electrodes.

29 [0049] Fig. 2C is a bar chart illustrating that at two weeks of coculture, an increase in
30 neuronal excitability was detected in the iFbN and vandefitemcel coculture versus the iFbN
31 culture alone based on the difference in the number of spikes observed.

32 [0050] Fig. 2D is a bar chart illustrating that at four weeks of coculture, the addition of
33 vandefitemcel to the iFbN cells increased the number of network bursts in a cell density-
34 dependent manner.

1 [0051] Fig. 2E are graphs illustrating an increase in glial fibrillary acidic protein (GFAP)
2 cells in the coculture of iFbN cells with vandefitemcel compared to the iFbN control
3 culture. Fig. 2E also illustrates that the 5-ethynyl-2'-deoxyuridine (EdU) populations were
4 comparable for both the iFbN and vandefitemcel coculture and the iFbN control culture.

5 [0052] Figs. 3A-3B are bar charts illustrating that after vandefitemcel was added to iFbN
6 cells, the coculture of vandefitemcel and iFbN generated significantly more spikes and
7 network bursts than the culture of iFbN cells alone. Also, Figs. 3A-3B illustrate that
8 cocultures of conditioned medium of vandefitemcel and iFbN also generated more spikes
9 and network bursts than iFbN cells alone. Furthermore, cocultures of human astrocytes and
10 iFbN also generated more spikes and network bursts than iFbN cells alone.

11 [0053] Fig. 3C are raster plots illustrating electrophysiological activity and network burst
12 development at four weeks of culture across MEA electrodes for iFbN cells alone and
13 cocultures of vandefitemcel and iFbN, conditioned medium of vandefitemcel and iFbN,
14 and human astrocytes and iFbN.

15 [0054] Fig. 4A are reproductions of various immunocytochemistry (ICC) stains of a culture
16 of iFbNs at thirty days of culture maturation.

17 [0055] Fig. 4B is a bar chart illustrating that, at day three of coculture, vandefitemcel
18 promoted high levels of spike activity in glutamatergic neurons, when compared to spike
19 activity in either the glutamatergic neurons alone or the glutamatergic neurons and human
20 astrocytes cocultures.

21 [0056] Fig. 4C is a graph illustrating that the spike activity in glutamatergic neurons
22 promoted by vandefitemcel kept increasing for two weeks while human astrocytes (hA)
23 slightly promoted spike activity in glutamatergic neurons starting at week three.

24 [0057] Fig. 4D is a graph illustrating that vandefitemcel induced significant network burst
25 activity in the first week of coculture with glutamatergic neurons while glutamatergic
26 neurons in coculture with human astrocytes (GluN+hA) did not show network bursts until
27 week four.

28 [0058] Fig. 4E are raster plots illustrating the electrophysiological activity of cocultures of
29 glutamatergic neurons and vandefitemcel over time and progressive network burst
30 development across electrodes on the MEA system.

31 [0059] Figs. 4F and 4G are graphs illustrating that vandefitemcel promoted spikes and
32 network bursts of GABAergic neurons in week two and week four of coculture when
33 compared to GABAergic neurons alone or in cocultures of GABAergic neurons and human
34 astrocytes.

1 [0060] Fig. 4H are raster plots illustrating the electrophysiological activity and network
2 burst development of cocultures of GABAergic neurons and vandeitemcel over time and
3 progressive network burst development across electrodes on the MEA system.

4 [0061] Fig. 5A is a bar chart illustrating that at two days of culture, the increased levels of
5 conditioned medium (CM) glutamate were cell density-dependent in cultures of both
6 vandeitemcel and human astrocytes (hA), but the levels of glutamate in CM of
7 vandeitemcel were about 10-fold higher than that in the CM of hA.

8 [0062] Fig. 5B is a graph illustrating that, at five days of culture, the levels of glutamate in
9 CM of vandeitemcel increased 4.9-fold while the levels of glutamate in CM of hA
10 increased 2.7-fold.

11 [0063] Fig. 5C is a bar chart illustrating that after incubation with 5 μ M and 50 μ M of a
12 glutaminase inhibitor (CB-839), the levels of glutamate in the CM of vandeitemcel were
13 reduced 1.5-fold and 2.0-fold, respectively, compared to the control of vandeitemcel
14 incubated with dimethylsulfoxide (DMSO).

15 [0064] Fig. 5D is a bar chart illustrating that incubation with CB-839 did not alter the
16 viability of vandeitemcel.

17 [0065] Figs. 5E and 5F are bar charts illustrating that after one week of coculture, the
18 inhibition of glutaminase in vandeitemcel significantly reduced spike activity and network
19 bursts in the cocultures of glutamatergic neurons and vandeitemcel compared to the
20 control culture of vandeitemcel incubated with DMSO.

21 [0066] Figs. 5G and 5H are bar charts illustrating that astrocytes reduced the number of
22 spikes and network bursts that would have otherwise been induced by vandeitemcel on
23 glutamatergic neurons.

24 [0067] Fig. 5I is a schematic diagram showing vandeitemcel promoting an increase in
25 neuronal activity and synaptic plasticity via tonic glutamate release.

27 DETAILED DESCRIPTION

28 [0068] The below terms are defined as follows for purposes of this disclosure.

29 [0069] Definitions

30 [0070] The terms “administration” and “administering” refer to the physical introduction of
31 a composition comprising a therapeutic agent to a subject, using any of the various
32 methods and delivery systems known to those skilled in the art. For example, routes of
33 administration for vandeitemcel can include intracerebral, intrathecal, or other parenteral
34 routes of administration, for example by injection or infusion. The phrase “parenteral

1 administration” as used herein means modes of administration other than enteral and
2 topical administration, usually by injection and infusion. Administering can also be
3 performed, for example, once, a plurality of times, and/or over one or more extended
4 periods, and can be a therapeutically effective dose or a subtherapeutic dose.

5 **[0071]** The terms “implantation” and “transplantation” are used to denote the introduction
6 of exogenous cells (e.g., vandefitemcel or SB623 cells) into a subject or patient. Exogenous
7 cells can be autologous (i.e., obtained from the subject) or allogeneic (i.e., obtained from an
8 individual other than the subject).

9 **[0072]** An “isotonic crystalloid solution” is a solution containing water-soluble
10 electrolytes. Such a solution contains the same amount of electrolytes as plasma.

11 **[0073]** “Mesenchymal cells” refer to cells of mesenchymal tissue (e.g., chondroblasts,
12 chondrocytes, osteoblasts, osteocytes, adipocytes) and their precursors and include, for
13 example, fibroblasts (e.g., human foreskin fibroblasts), MSCs (as defined herein) and cells
14 derived from MSCs such as, for example, vandefitemcel, as defined herein.

15 **[0074]** “MSCs” (“mesenchymal stem cells”) refer to adherent, non-hematopoietic
16 pluripotent cells obtained from bone marrow. These cells are variously known as
17 mesenchymal stem cells, mesenchymal stromal cells, marrow adherent stromal cells,
18 marrow adherent stem cells and bone marrow stromal cells. MSCs can also be obtained
19 from, e.g., umbilical cord blood, adipose tissue, dental pulp, Wharton's jelly, and various
20 types of connective tissue. MSCs can be obtained by selecting (e.g., by growth in culture)
21 adherent cells (i.e., cells that adhere to tissue culture plastic) from bone marrow. To obtain
22 MSC populations having a sufficient number of cells for use in therapy, populations of
23 adherent cells are expanded in culture after selecting for adherence. Expansion in culture
24 also enriches MSCs, since contaminating cells (such as monocytes) do not proliferate under
25 the culture conditions. Exemplary disclosures of MSCs are provided in U.S. Patent
26 Publication No. 2003/0003090 and the literature [8]. Methods for isolating and purifying
27 MSCs can be found, for example, in U.S. Pat. No. 5,486,359 and the literature [9, 10].
28 Human MSCs are commercially available (e.g., BioWhittaker, Walkersville, Md.) or can
29 be obtained from donors by, e.g., bone marrow aspiration, followed by culture and
30 selection for adherent bone marrow cells. See, e.g., WO 2005/100552. MSCs can also be
31 isolated from umbilical cord blood [11, 12]. Additional sources of MSCs include, for
32 example, adipose tissue, dental pulp, and Wharton's jelly.

1 [0075] “Network burst” refers to a collective or synchronous/near-synchronous behavior of
2 neurons comprised of alternating periods of relatively fast spiking or spike behavior
3 followed by inactivity. Also referred to as population bursting.

4 [0076] “Neuronal spike” or “spike” refers to an action potential or a brief electrical
5 impulse that can be captured or recorded when neurons fire. Spikes can be picked up by
6 electrodes such as electrodes of a microelectrode array.

7 [0077] A “neurodegenerative disorder,” “neurological disorder,” or “neurological disease”
8 refers to disorders or diseases in which cells of the central nervous system (CNS) stop
9 working, die, or are compromised in some manner. These types of diseases or disorders can
10 include, but are not limited to, Alzheimer’s disease, Parkinson’s disease, Huntington’s
11 disease, amyotrophic lateral sclerosis (ALS), traumatic brain injury (TBI), ischemic stroke,
12 hemorrhagic stroke, chronic stroke, or spinal cord injury.

13 [0078] The “Notch protein” (e.g., Notch 1 protein) is a transmembrane receptor, found in
14 all metazoans, that influences cell differentiation through intracellular signaling. Contact of
15 the Notch extracellular domain (e.g., the extracellular domain of the Notch 1 protein) with
16 a Notch ligand (e.g., Delta, Serrate, Jagged) results in two proteolytic cleavages of the
17 Notch protein, the second of which is catalyzed by a γ -secretase and releases the Notch
18 intracellular domain (NICD) into the cytoplasm. In the mouse Notch protein, this cleavage
19 occurs between amino acids gly1743 and val1744. The NICD translocates to the nucleus,
20 where it acts as a transcription factor, recruiting additional transcriptional regulatory
21 proteins (e.g., MAM, histone acetylases) to relieve transcriptional repression of various
22 target genes (e.g., Hes 1). Additional details and information regarding Notch signaling can
23 be found in the literature [13, 14, 15].

24 [0079] The term “pharmaceutically acceptable excipient” refers to an excipient for
25 administration of a pharmaceutical agent. These “excipients” refer to any substance other
26 than the active pharmaceutical agent. Examples of pharmaceutically acceptable excipients
27 can include, but are not limited to, buffers, proteins, carbohydrates, oleochemicals,
28 petrochemicals, stabilizers, preservatives, fillers, diluents, binders, viscosity agents,
29 coatings, disintegrants, colorants, and lubricants.

30 [0080] The terms “vandefitemcel,” vandefitemcel cells,” “SB623,” and “SB623 cells” refer
31 to populations of cells obtained following transient expression of an exogenous Notch
32 intracellular domain (NICD) in MSCs. For example, a population of vandefitemcel or
33 SB623 cells can be obtained by transient transfection of MSCs with a plasmid vector
34 comprising sequences encoding a NICD (e.g., from the human Notch 1 protein) but not

1 encoding a full-length Notch protein followed by selection (e.g., with G418). The selected
2 cells can be further cultured in a standard culture medium, optionally supplemented with a
3 serum, in the absence of any added growth factors or differentiation factors (other than
4 those which may be present in the serum, if serum is present in the culture medium).
5 Vandefitemcel can be derived from human (allogeneic) bone marrow MSCs by transient
6 transfection of human bone marrow MSCs with NICD (e.g., the human Notch1
7 intracellular domain (NICD1)), followed by selection, and subsequent expansion. This
8 process produces a cell population that is different than the parental MSCs [5, 6, 7].
9 Vandefitemcel or SB623 cells have also been referred to as descendants of NICD
10 transiently-transfected MSCs (“DNTT-MSCs”).

11 **[0081]** The term “transiently-transfected” refers to methods of introducing foreign DNA or
12 RNA into cells for temporary expression of the introduced gene(s). Transient transfection
13 influences short-term gene expression, lasting for a few hours to several days.

14 **[0082]** The terms “tonic release of glutamate” and “tonically release glutamate” refer to the
15 non-vesicular release of glutamate from neurons or glial cells into the extracellular space.
16 Tonic glutamate release can help maintain or modulate a subject’s baseline neuronal
17 activity and help maintain or modulate a subject’s synaptic plasticity.

18 **[0083]** “Therapeutically effective” means the amount of an agent required to provide a
19 meaningful patient benefit or promote disease regression evidenced by a decrease in
20 severity of disease symptoms, an increase in frequency and duration of disease symptom-
21 free periods, or a prevention of impairment or disability due to the disease affliction. The
22 therapeutically effective amount of an agent can be evaluated using a variety of methods
23 known to the skilled practitioner such as a practitioner in the field of neurology.

24 **[0084] Preparation of vandefitemcel**

25 **[0085]** The vandefitemcel administered are allogeneic cells descended from mesenchymal
26 stem cells transiently-transfected by a polynucleotide encoding a Notch intracellular
27 domain (NICD). The cells can be made by a method comprising providing a culture of the
28 mesenchymal stem cells, contacting the culture of mesenchymal stem cells with the
29 polynucleotide encoding an NICD (where the polynucleotide does not encode a full-length
30 Notch protein), selecting cells that comprise the polynucleotide, and further culturing the
31 selected cells in the absence of selection for the polynucleotide. The mesenchymal stem
32 cells can be human bone marrow-derived cells.

33 **[0086]** As previously discussed, vandefitemcel can be obtained from marrow-adherent
34 stromal cells, also known as MSCs, by transiently expressing the intracellular domain of

1 the Notch protein in the MSCs. Transient expression of the Notch intracellular domain
2 (e.g., the NICD from the human Notch 1 protein) in an MSC can be sufficient to convert a
3 population of MSCs into a population of vanderfitemcel. Additional treatment with growth
4 and/or differentiation factors is not required. Thus, a population of MSCs can be converted
5 to a population of vanderfitemcel by transient transfection of MSCs with a plasmid vector
6 comprising sequences encoding an NICD (but not encoding full-length Notch protein),
7 followed by selection for cells comprising the vector and further culture of the selected
8 cells in serum-containing medium, in the absence of exposure to additional growth and/or
9 differentiation factors. See, for example, U.S. Pat. No. 7,682,825; U.S. Pat. No. 8,945,919;
10 and WO 2009/023251; the contents of which are incorporated herein by reference in their
11 entireties for the purposes of describing isolation of mesenchymal stem cells and
12 conversion of mesenchymal stem cells to vanderfitemcel (also referred to as “neural
13 precursor cells” and “neural regenerating cells” in those documents).

14 **[0087]** In this disclosure, any polynucleotide encoding a Notch intracellular domain (e.g., a
15 plasmid vector) can be used, and any method for the selection and enrichment of
16 transfected cells can be used. For example, MSCs can be transfected with a vector
17 containing sequences encoding a Notch intracellular domain (e.g., the human Notch 1
18 intracellular domain) and also containing sequences encoding a selection marker (e.g., drug
19 resistance; e.g., resistance to G418). In some instances, two plasmid vectors, one
20 containing sequences encoding a Notch intracellular domain and the other containing
21 sequences encoding a drug resistance marker, can be used for transfection of MSCs. In
22 these instances, selection is achieved, after transfection of a cell culture with the vector or
23 vectors, by adding a selective agent (e.g., G418) to the cell culture in an amount sufficient
24 to kill cells that do not comprise the vector but spare cells that do. Absence of selection
25 entails the removal of said selective agent or reduction of its concentration to a level that
26 does not kill cells that do not comprise the vector. Following selection (e.g., for seven
27 days) the selective agent can be removed and the cells can be further cultured (e.g., for two
28 passages) in serum-containing culture medium.

29 **[0088]** It is also possible, depending on the nature of the selection marker and/or the
30 concentration of the selective agent used, that not every cell that lacks a vector encoding a
31 selection marker will be killed during the selection process. For example, a selective agent
32 may inhibit growth of a cell not comprising the selection marker and, after removal of the
33 selective agent, the cell may recover and resume growth.

1 [0089] Preparation of vandefitemcel thus involves transient expression of an exogenous
2 Notch intracellular domain in an MSC. To this end, MSCs can be transfected with a
3 plasmid vector comprising sequences encoding a Notch intracellular domain (e.g., the
4 human Notch 1 intracellular domain) wherein said sequences do not encode a full-length
5 Notch protein. All such sequences are known and readily available to those of skill in the
6 art [14].

7 [0090] Similar information is available for Notch proteins and nucleic acids from
8 additional species, including rat, *Xenopus*, *Drosophila*, and human [16, 17]. Additional
9 information can also be found in NCBI Reference Sequence No.

10 NM_017167, SwissProt P46531, SwissProt Q01705, and GenBank CAB40733. The
11 foregoing references are incorporated by reference in their entireties for the purpose of
12 disclosing the amino acid sequence of the full-length Notch protein and the amino acid
13 sequence of the Notch intracellular domain in a number of different species.

14 [0091] In some instances, vandefitemcel can be prepared by introducing, into MSCs, a
15 nucleic acid comprising sequences encoding a Notch intracellular domain such that the
16 MSCs do not express exogenous Notch extracellular domain. Such can be accomplished,
17 for example, by transfecting MSCs with a plasmid vector comprising sequences encoding a
18 Notch intracellular domain wherein said sequences do not encode a full-length Notch
19 protein.

20 [0092] Additional details on the preparation of vandefitemcel or SB623 cells, and methods
21 for making cells with properties similar to those of vandefitemcel or SB623 cells which can
22 be used in the methods disclosed herein, can be found in the literature [18] and U.S. Pat.
23 No. 7,682,825; U.S. Pat. No. 8,945,919; and U.S. Pat. No. 9,441,199, the contents of which
24 are incorporated herein by reference in their entireties for the purposes of describing
25 alternative methods for the preparation of, vandefitemcel, and for providing methods for
26 making cells with properties similar to those of vandefitemcel.

27 [0093] The vandefitemcel used for the experiments disclosed herein were stored in liquid
28 nitrogen. For cocultures, except where indicated, the vandefitemcel cells were thawed,
29 washed, and plated directly. For glutaminase inhibition assays, vandefitemcel (or SB623)
30 cells were cultured after thawing in minimum essential medium-alpha (α -MEM) (from
31 CORNING, Corning, NY) supplemented with 10% of fetal bovine serum (FBS). In
32 cocultures with neurons or for glutamate assay, vandefitemcel (or SB623) cells were kept
33 in BrainPhys™ medium supplemented with NeuroCult™ SM1 neuronal supplement. To
34 assess the levels of glutamate release by the vandefitemcel cells into conditioned medium,

1 Gibco GlutaMAX™ (100X; 200 mM L-alanyl-L-glutamine from Thermo Fisher Scientific,
2 Waltham, MA) was added (final concentration 2 mM) to vandeitemcel cells cultured in
3 BrainPhys™ medium supplemented with NeuroCult™ SM1 neuronal supplement and
4 levels of glutamate were assessed after two days.

5 **[0094] Transfection**

6 **[0095]** Methods for introduction of exogenous DNA into cells (i.e., transfection), and
7 selection of transfected cells, are known in the art [19, 20].

8 **[0096] Preparation of iPSC-derived forebrain neurons (iFbNs)**

9 **[0097]** Human episomal iPSCs (e.g., Gibco™ A18945 hiPSC from Thermo Fisher
10 Scientific, Waltham, MA) were grown using a StemFlex™ Medium Kit (from Thermo
11 Fisher Scientific) in six-well tissue culture plates pre-coated with Geltrex™ hESC-
12 Qualified (from Thermo Fisher Scientific) and passaged with ReLeSR™ (from Stem Cells
13 Technologies, Vancouver, Canada). To generate forebrain neurons, iPSCs were
14 differentiated into neural progenitor cells using STEMdiff™ Forebrain Neuron
15 Differentiation Kit (from Stem Cells Technologies, Vancouver, Canada). After a single cell
16 dissociation procedure, iPSCs were seeded on six-well tissue culture plates pre-coated with
17 15 µg/mL of poly-L-ornithine (from Sigma-Aldrich, St. Louis, MO) and 20 µg/mL of
18 laminin (Sigma-Aldrich, St. Louis, MO) and differentiated using a monolayer culture
19 system in StemDiff™ Neural Induction Medium plus SMADi (both from Stem Cells
20 Technologies, Vancouver, Canada) following neural induction procedures (Stem Cells
21 Technologies, Vancouver, Canada). Neural progenitor cells were passaged to a new six-
22 well tissue culture plate pre-coated with 15 µg/mL of poly-L-ornithine and 20 µg/mL of
23 laminin and then differentiated into forebrain neurons using the STEMdiff™ Forebrain
24 Neuron Differentiation Kit for one week. After differentiation, forebrain neurons were
25 matured using the STEMdiff™ forebrain neuron maturation kit (from Stem Cells
26 Technologies, Vancouver, Canada) for two weeks. Forebrain neuronal cultures were then
27 kept in BrainPhys™ medium supplemented with NeuroCult™ SM1 neuronal supplement
28 (from Stem Cells Technologies, Vancouver, Canada). All cells were grown in humidified
29 sterile environments at 37°C and 5% CO₂.

30 **[0098] Preparation of human glutamatergic neurons, human GABAergic neurons,**
31 **and human astrocytes**

32 **[0099]** Human glutamatergic neurons (e.g., iCell GlutaNeurons™) and human GABAergic
33 neurons (e.g., iCell GABANeurons™) were obtained from FUJIFILM Cellular Dynamics
34 FUJIFILM Cellular Dynamics Inc. (Madison, WI) and directly cultured on multielectrode

1 array (MEA) plates/wells pre-coated with 15 µg/mL of poly-L-ornithine (from Sigma-
2 Aldrich, St. Louis, MO) and 20 µg/mL of laminin (from Sigma-Aldrich, St. Louis, MO) in
3 BrainPhys™ medium supplemented with NeuroCult™ SM1 neuronal supplement. Human
4 fetal-derived astrocytes (from ScienceCell Research Laboratories Carlsbad, CA) were
5 seeded on T-75 flasks pre-coated with 10 µg/mL of poly-L-lysine (from Sigma-Aldrich, St.
6 Louis, MO) and expanded in astrocytes medium supplemented with 2% fetal bovine serum
7 (FBS) and 1% of astrocytes supplement (all from Science Cell Research Laboratories).
8 Human astrocytes were kept in BrainPhys™ medium supplemented with NeuroCult™
9 SM1 neuronal supplement prior to cocultures with neurons or for use in glutamate assays.

10 **[0100] Multielectrode Array (MEA) System**

11 **[0101]** Wells/plates of CytoView MEA 24 (from Axion Biosystems, Atlanta, GA) were
12 treated with 30-50 µL of 15 µg/mL of poly-L-ornithine for two hours or overnight at 37°C
13 and 5% CO₂. The wells were washed twice with phosphate-buffered saline (PBS) and once
14 with Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 and
15 4- (2-Hydroxyethyl)piperazine-1-ethane-sulfonic acid (DMEM/F12-HEPES) (from Stem
16 Cells Technologies, Vancouver, Canada). Wells were coated with 30-50 µL of 20 µg/mL of
17 laminin (from Sigma-Aldrich, St. Louis, MO) for two hours or overnight at 37°C and 5%
18 CO₂. Laminin was removed and neurons were immediately seeded and incubated for 30-45
19 minutes at 37°C and 5% CO₂. Then fresh medium was added to reach a volume of 400 µL
20 per well. Half of the medium volume was changed every two to three days. Forebrain
21 neurons (iFbNs), 3 x 10⁴ or 5 x 10⁴ cells per well, were cultured in BrainPhys™
22 supplemented with maturation supplements (from Stem Cells Technologies, Vancouver,
23 Canada) for two weeks. After two weeks, the medium was changed to BrainPhys™
24 medium supplemented with NeuroCult™ SM1 neuronal supplement. To coculture
25 vandeitemcel cells or human astrocytes with iPSC-derived forebrain neurons (iFbNs), the
26 medium was transferred into a 24-well plate leaving about 10-20 µL in the inner well of the
27 MEA plates. The vandeitemcel (or SB623) cells or astrocytes were added in cell solution
28 of 10-20 µL and incubated for 45 minutes at 37°C and 5% CO₂. After 30-45 minutes of
29 incubation, 1:1 fresh medium and saved conditioned medium were added to reach a volume
30 of 400 µL per well. Half of the medium volume was changed every three or four days.

31 **[0102]** Glutamatergic or GABAergic neurons, 9 x 10⁴ cells per well or 1 x 10⁵ cells per
32 well, were plated in 25 µL volume cell in BrainPhys™ medium supplemented with
33 NeuroCult™ SM1 neuronal supplement and incubated for 30-45 minutes at 37°C and 5%
34 CO₂. For coculturing, vandeitemcel (or SB623) cells or astrocytes were cocultured by

1 adding 10-20 μL of cell solution with 1.5×10^4 cells per well following a new incubation
2 for 45 minutes at 37°C and 5% CO_2 . Fresh medium was added to reach a volume of 400 μL
3 per well and cells incubated at 37°C and 5% CO_2 . Half of the medium volume was changed
4 every three or four days.

5 **[0103]** To isolate the presynaptic activity on MEAs with iPbNs after 50 days of culture, 50
6 μM of the broad glutamate receptor antagonist Kynurenic acid (from R&D Systems,
7 Minneapolis, MN) was added at 90 seconds from the start of the recording. Spike activity
8 was assessed to detect the blockage of glutamate receptor activity.

9 **[0104]** MEA recordings were performed at 37°C and 5% CO_2 using Maestro Edge (from
10 Axion Biosystems, Atlanta, GA). The medium was changed at least two hours before the
11 recordings. Before recordings, levels of CO_2 were adjusted to 5%. The duration of each
12 recording was five minutes using AxIS Navigator 3.6.2 with configuration module Neural
13 Real-Time; Spontaneous + Viability from Axion Biosystems with minimum spike rate for
14 active electrodes of five spikes/minute.

15 **[0105] Flow Cytometry**

16 **[0106]** iPSC-derived forebrain neuronal cultures were dissociated from multielectrode
17 wells into single cell suspension(s). For this, cultures were washed once with phosphate-
18 buffer saline (PBS) (from Thermo Fisher Scientific, Waltham, MA) and incubated with
19 TrypLE™ express (from Thermo Fisher Scientific, Waltham, MA) for 5 minutes at 37°C
20 and 5% CO_2 . Cells were recovered in BrainPhys™ medium and washed 2x followed by 30
21 minutes of fixation with 500 μL of eBioscience™ IC fixation buffer (from Thermo Fisher
22 Scientific, Waltham, MA) in PBS at room temperature. After fixation, the cells were
23 washed twice for 5 minutes at 300 g with 2 mL of eBioscience™ Permeabilization Buffer
24 (from Thermo Fisher Scientific, Waltham, MA). The cells were incubated with a blocking
25 solution containing 5% normal goat serum (from Jackson ImmunoResearch Inc., West
26 Grove, PA) + 2% bovine serum albumin (BSA) in eBioscience™ Permeabilization buffer
27 for 15 minutes at room temperature using an orbital shaker protected from light. Without
28 washing the cells, the conjugated antibody was added directly to cells for detection of
29 intracellular antigen and incubated for 30 minutes at room temperature using an orbital
30 shaker and protected from light. The conjugated antibodies used were REA Control
31 Antibody REAfinity™ and GFAP Antibody REAfinity™ (both from Miltenyi Biotec, San
32 Jose, CA). The cells were washed two times for 5 minutes at 300 g centrifugation with
33 eBioscience™ Permeabilization buffer and resuspended in eBioscience™ Flow Cytometer
34 Staining Buffer (from Thermo Fisher Scientific, Waltham, MA). For the proliferation

1 assay, Click-iT® 5-ethynyl-2'-deoxyuridine (EdU) Flow Cytometry Assay Kits (from
2 Thermo Fisher Scientific, Waltham, MA) were used. EdU was added for 24 hours. Flow
3 cytometry was performed using MACSQuant® Analyzer 10 Flow Cytometer and data
4 were analyzed using MACSQuantify™ (both from Miltenyi Biotec, San Jose, CA).

5 **[0107] Immunocytochemistry Stains**

6 **[0108]** iFbNs and vandefitemcel (or SB623) cells were cocultured in Nunc™ Lab-Tek™ II
7 CC2 chamber slides previously coated with poly-L-ornithine and laminin. Cells were
8 washed with PBS (from Thermo Fisher Scientific, Waltham, MA), followed by 15 minutes
9 of incubation with 4% paraformaldehyde at room temperature. For staining, the samples
10 were blocked with 5% normal goat serum (from Jackson ImmunoResearch Laboratories
11 Inc., West Grove, PA) in PBS with a nonionic surfactant (e.g., 0.3% Triton X-100 from
12 Sigma-Aldrich) for 30 min at room temperature. The primary antibodies used were rabbit
13 anti-GFP (1:200; from Abcam, Fremont, CA), rabbit anti-GFAP (1:1000; from Agilent
14 DAKO), rabbit anti-Olig2 (1:100; from Cell Signaling, Danvers, MA), chicken anti-MAP2
15 (1:1000; from Abcam, Fremont, CA), rabbit anti-synaptophysin (1:500; from Abcam,
16 Fremont, CA), mouse anti-VGLUT1 (1:1000) and rabbit anti-GABA (1:100; from Sigma-
17 Aldrich, St. Louis, MO). Cells were washed three times with PBS with nonionic surfactant
18 (e.g., 0.3% Triton X-100 from Sigma-Aldrich, St. Louis, MO) and incubated with
19 respective secondary antibodies overnight at 4°C. The secondary antibodies used were
20 Alexa Fluor 488-conjugated goat anti-rabbit (1:1000; Invitrogen, Waltham, MA), Alexa
21 Fluor™ 546-conjugated goat anti-mouse (1:1000; from Invitrogen, Waltham, MA), Alexa
22 Fluor™ 647-conjugated goat anti-chicken (1:1000; from Invitrogen, Waltham, MA).
23 Followed by washing three times with PBS with a nonionic surfactant (e.g., 0.3% Triton X-
24 100 for 5 minutes each. The slides were mounted with Fluoromount-G™ mounting
25 medium, with 4',6-diamidino-2-phenylindole (DAPI) (from Thermo Fisher Scientific,
26 Waltham, MA). Fluorescence images were recorded using a digital camera attached to a
27 fluorescence microscope (from Leica DMi8, Wetzlar, Germany). Image processing was
28 performed by Fiji-ImageJ software (from National Institute of Health; NIH). Antibody
29 specificity and sample backgrounds were tested by using only secondary antibodies for
30 each slice stained.

31 **[0109] Determination of Glutamate Concentration**

32 **[0110]** The concentration of glutamate in conditioned medium was determined using the
33 Glutamate Assay Kit from Sigma-Aldrich (St. Louis, MO) and following the protocol
34 provided by the manufacturer. Cells were seeded at different cell densities on a 24-well or a

1 96-well plate pre-coated with poly-D-lysine and laminin in BrainPhys™ medium
2 supplemented with NeuroCult™ SM1 neuronal supplement. Cells were kept at 37°C and
3 5% CO₂. Approximately 50 µL of conditioned medium was collected at different time
4 points. Media collected were stored at -20°C until measurement of glutamate
5 concentrations. 50 µL of conditioned medium for each sample was used to measure the
6 concentration of glutamate. The concentration of glutamate in conditioned medium was
7 measured during five days of culture in time course experiments. Absorbance was
8 measured after 30 minutes of incubation on SpectraMax® iD3 Multi-mode Microplate
9 Reader (from Molecular Devices, San Jose, CA).

10 **[0111] Viability Assays**

11 **[0112]** Approximately 50 µL of conditioned medium (CM) was used to determine the
12 glutamate concentration and cell viability. For this assay, PrestoBlue™ high-sensitivity
13 (HS) Cell Viability Reagent (from Thermo Fisher Scientific, Waltham, MA) was used and
14 the reaction absorbance of each sample was read after 2 hours of incubation following the
15 manual procedure provided by the manufacturer.

16 **[0113] Inhibition of Glutaminase**

17 **[0114]** Vandefitemcel (or SB623) cells were cultured with α-MEM supplemented with
18 10% fetal bovine serum (FBS) for 16-24 hours at 37°C and 5% CO₂. Next, vandefitemcel
19 (or SB623) cells were treated with two different concentrations of the glutaminase inhibitor
20 Telaglenastat (CB-839; from Selleckchem, Houston TX): 5 µM and 50 µM for two or three
21 days. vandefitemcel (or SB623) cells were washed two times and cryopreserved for a
22 subsequent coculture with glutamatergic neurons for the MEA experiments. In parallel, 1.0
23 x 10⁴ vandefitemcel (or SB623) cells were incubated with CB-839 for 2 days at 37°C and
24 5% CO₂. Cells were washed two times with PBS and cultured in BrainPhys™ medium
25 supplemented with NeuroCult™ SM1 neuronal supplement for three days at 37°C and 5%
26 CO₂. The levels of glutamate in conditioned medium were assessed.

27 **[0115] Statistical Analysis**

28 **[0116]** Statistical analyses were performed using GraphPad® Prism version 9.4.1 (from
29 GraphPad Software, San Diego, California, USA). Correlation analysis was evaluated
30 using Spearman's correlation coefficient with a 95% confidence interval. The significance
31 of differences between groups was determined by one-way ANOVA followed by Holm-
32 Sidak's post hoc test, paired t-test, or Mann-Whitney test (two-tailed), as indicated in the
33 figure legends. Comparisons of multiple groups and time points were performed using
34 analysis of variance (ANOVA) followed by Tukey's or Sidak's post hoc tests, or mixed-

1 effects models with Geisser-Greenhouse correction and Tukey's post hoc tests. All tests
2 were two-tailed unless otherwise specified.

3 **[0117] Electrophysiological Activity and Viability of Vandefitemcel and iFbNs**

4 **[0118]** To determine whether vandefitemcel (or SB623 cells) exhibit neuron-like
5 electrophysiological behavior, the electrophysiological activities of vandefitemcel (or
6 SB623 cells) and iFbNs were evaluated as single/separate cultures in serum-free
7 neurophysiological conditions using the Axion Biosystem MEA system [21]. As shown in
8 Figs. 1A-1C, during eight weeks of culture, vandefitemcel (or SB623 cells) at a cell density
9 of 2.5×10^4 cells did not exhibit any spontaneous neuron-like electrophysiological activity
10 as evidenced by the number of spikes, active electrodes, and network bursts. However,
11 around three weeks of culture, iFbNs (at cell densities of both 3.0×10^4 cells and 5.0×10^4
12 cells) showed spontaneous neuron-like electrophysiological activity as evidenced by the
13 number of spikes and active electrodes (see Figs. 1A and 1B). iFbNs also exhibited
14 spontaneous neuronal oscillations in the form of network bursts starting at around four to
15 five weeks of cell culture (see Fig. 1C). When iFbN cells were plated at a higher cell
16 density, they exhibited earlier and more pronounced neuronal oscillations when compared
17 to cells plated at a lower cell density, although the difference was not statistically
18 significant (see Fig. 1E).

19 **[0119]** The viability of vandefitemcel (or SB623 cells) and iFbN cells were evaluated in
20 neurophysiological conditions using the impedance module available within the Axion
21 Biosystem MEA system. Resistance (in ohms, Ω) was measured between the cells and the
22 electrodes. In suspension, vandefitemcel (or SB623 cells) were large (around $17.2 \mu\text{m}$
23 diameter) and 100% confluent, covering all 16 electrodes. In contrast, iFbN cells in
24 suspension were smaller (around $10.2 \mu\text{m}$ diameter) and formed cell clusters
25 interconnected with cellular projections when attached to MEA wells. These particular
26 hallmarks distinguish vandefitemcel (or SB623 cells) and iFbN cells and resulted in
27 significantly different resistances, as seen in the first week of culture (see Fig. 1D).
28 Although the resistance assessed using the MEA system indicated that vandefitemcel (or
29 SB623 cells) survived in BrainPhys™ medium, there was a significant reduction in
30 resistance in the first three weeks of culture (32.6%), and a continuous drop that reached
31 58.8% by the eighth week of culture. On the other hand, it was observed that in both low
32 and high cell densities, iFbN cells exhibited increased resistance in the second and third
33 weeks (14% and 28.9% respectively), compared to the resistance observed in the first week
34 in culture (see Fig. 1D). In the third week, and upon withdrawn of maturation supplements,

1 the resistance in iFbN cultures slightly dropped and then remained stable until five-weeks
2 of culture, when the resistance started to increase again. This increased resistance seen in
3 the iFbN cell cultures suggests the presence of progenitor cells in these neuronal cultures.
4 Indeed, at five weeks of culture, 2.25% of iFbN cell cultures were EdU-positive after 24
5 hours of EdU labeling (see Fig. 2C). Despite the increase in resistance in iFbN cell
6 cultures, there was no correlation between resistance and spike counts at low or high iFbN
7 cell seeding (Spearman's correlation, $r = 0.0665$ and $r = -0.1189$, respectively) indicating
8 that the increase in neuronal activity could not be explained by more cells occurring within
9 the MEA system.

10 [0120] Fig. 1E are raster plots of iFbN cells plated at cell densities of 3.0×10^4 cells and
11 5.0×10^4 cells representing the electrophysiological activity of such cells in the form of
12 spikes and network bursts. Single spikes are represented in the plots by black boxes,
13 electrode bursts are represented in the plots by white boxes, and network bursts are
14 represented by broken-line boxes.

15 [0121] Taken together, vandefitemcel (or SB623 cells) survived in the MEA system but
16 did not exhibit neuron-like electrophysiological activities, while iFbN cells exhibited some
17 electrophysiological activities as evidenced by increasing spike counts, active electrodes,
18 and neuronal network bursts in the Axion Biosystem MEA system.

20 EXAMPLES

21

22 [0122] The examples below are given so as to illustrate the practice of various
23 embodiments of the present disclosure. They are not intended to limit or define the entire
24 scope of this disclosure. It should be appreciated that the disclosure is not limited to the
25 particular embodiments described and illustrated herein but includes all modifications and
26 variations falling within the scope of the disclosure as defined in the appended
27 embodiments.

28 [0123] Example 1: Coculturing Vandefitemcel with iFbN Cells Results in Increased 29 Neuronal Activity and Synapse Formation in a Cell Density-Dependent Manner

30 [0124] Figs. 2A-2E illustrate that vandefitemcel (or SB623 cells, shown as SB in Figs. 2A-
31 2E) promotes neuronal activity/excitability and synapse formation while in coculture with
32 iFbN cells. Fig. 2A are immunocytochemistry (ICC) stains of a coculture of vandefitemcel
33 and iFbN cells where the iFbN cells express the neuronal marker MAP2, the vandefitemcel
34 (or SB623 cells) express green fluorescent protein (GFP), and cell nuclei are stained with

1 4',6-diamidino-2-phenylindole (DAPI). iFbN cells were plated at a cell density of 5×10^4
2 cells and after 14 days of maturation, vandefitemcel were added at two cell densities ($2.0 \times$
3 10^3 cells and 7.0×10^3 cells). As shown in Fig. 2A, after thirty days of coculture,
4 vandefitemcel cells (shown as SB-GFP) were detected within a culture of MAP2 positive
5 forebrain neurons.

6 **[0125]** Fig. 2B illustrates that the presence of the vandefitemcel (or SB623 cells, indicated
7 as "SB" in the figures) was confirmed by an increase in the resistance measured between
8 the cells and the MEA electrodes measured only one day after the beginning of the
9 coculture at the higher cell density (7.0×10^3 cells) of vandefitemcel. At the higher cell
10 density (7.0×10^3 cells) of vandefitemcel, an increase in resistance was observed starting in
11 the fourth week of coculture.

12 **[0126]** To evaluate whether vandefitemcel affected the neuronal electrical activity of
13 iFbNs, the number of spikes in the iFbN control culture (shown as "iFbN" in Fig. 2C) was
14 compared to the number of spikes in the cocultures of iFbN and vandefitemcel of different
15 cell densities (shown as "iFbN+SB (2k)" for the coculture of iFbN cells with 2.0×10^3 cells
16 of vandefitemcel and shown as "iFbN+SB (7k)" for the coculture of iFbN cells with $7.0 \times$
17 10^3 cells of vandefitemcel in Fig. 2C). Fig. 2C illustrates that at two weeks of coculture, an
18 increase in neuronal excitability was detected in the cocultures of iFbN and vandefitemcel
19 coculture versus the iFbN culture based on the difference in the number of spikes observed.
20 As shown in Fig. 2C, the number of spikes, or the degree of neuronal excitability, increased
21 in a cell density-dependent manner. The difference in the number of spikes between the
22 iFbN control culture and the iFbN+SB (7k) coculture was statistically significant (one-way
23 ANOVA Holm-Sidak, $**p < 0.005$).

24 **[0127]** To determine whether the increased neuronal activity promoted by the
25 vandefitemcel (or SB623 cells) also increased synapse formation, the number of network
26 bursts was also counted for the culture of iFbN cells (shown as "iFbN" in Fig. 2D), the
27 coculture of iFbN cells with 2.0×10^3 cells of vandefitemcel (shown as "iFbN+SB (2k)"),
28 and the coculture of iFbN cells with 7.0×10^3 cells of vandefitemcel (shown as "iFbN+SB
29 (7k)"). Fig. 2D illustrates that at four weeks of coculture, the addition of vandefitemcel to
30 the iFbNs also increased the number of network bursts in a cell density-dependent manner.
31 The spike and network burst counts continued to rise over the next eight weeks of
32 coculture, remaining distinguishable from the control of just iFbN cells. The difference in
33 the number of network bursts between the iFbN control culture and either of the iFbN+SB

1 cocultures was statistically significant (one-way ANOVA Holm-Sidak, * $p < 0.05$;
2 ** $p < 0.005$).

3 **[0128]** It was previously reported that vandefitemcel (or SB623 cells) promoted
4 neurogenesis in an embryonic rat neural culture [22, 23]. Fig. 2E shows an increase in glial
5 fibrillary acidic protein (GFAP+) cells in the coculture of iFbN cells with 7.0×10^3 cells of
6 vandefitemcel (shown as “iFbN + SB (7k)” in Fig. 2E) compared to the iFbN control
7 culture (shown as “iFbN” in Fig. 2E). Fig. 2E is a flow cytometer analysis showing that the
8 GFAP+ population in the iFbN + SB (7k) coculture was ~28.2% compared to ~15.82% in
9 the iFbN control culture. The EdU+ populations were comparable for both the iFbN + SB
10 (7k) coculture and the iFbN control culture. This suggests that the vandefitemcel (or SB623
11 cells) promoted more glial differentiation in the forebrain neurons.

12 **[0129] Example 2: Coculturing Vandefitemcel with iFbN Cells Results in More**
13 **Neuronal Activity than Coculturing iFbN Cells with Astrocytes and Molecules**
14 **Secreted by Vandefitemcel Increase Neuronal Activity**

15 **[0130]** Cocultures of vandefitemcel (or SB623 cells) and iFbNs were compared to
16 cocultures of human astrocytes (hA) and iFbNs to investigate whether the increased effect
17 on neuronal activity was caused by extra cells in the MEA system. Also, iFbNs were
18 cultured with conditioned medium (CM) of vandefitemcel to evaluate whether secreted
19 factors could have a similar effect on neuronal activity. Conditioned medium was collected
20 every day from vandefitemcel (or SB623 cells) cultured in BrainPhys™ medium and
21 immediately added to cultures of iFbN cells when medium was replaced. iFbN cells were
22 cultured for two weeks before other cells were added; at two weeks, vandefitemcel,
23 conditioned medium of vandefitemcel, and human astrocytes were added and spikes and
24 network bursts were counted in each case.

25 **[0131]** As shown in Figs. 3A-3C, in the subsequent two weeks after vandefitemcel was
26 added to the iFbN cells, the coculture of vandefitemcel and iFbN (shown in Figs. 3A-3C as
27 “iFbN+SB”) generated significantly more spikes (see Fig. 3A, one-way ANOVA Holm-
28 Sidak, ** $p < 0.01$) and network bursts (see Fig. 3B, one-way ANOVA Holm-Sidak,
29 * $p < 0.05$, and Fig. 3C) than the iFbN cells alone (shown in Figs. 3A-3C as “iFbN”).
30 Moreover, it can be seen in Figs. 3A-3C that cocultures of conditioned medium of
31 vandefitemcel and iFbN (shown in Figs. 3A-3C as “iFbN+CM”) also generated more
32 spikes and network bursts than the iFbN cells alone (although not to the same extent as the
33 iFbN+SB). Furthermore, cocultures of human astrocytes and iFbN (shown in Figs. 3A-3C

1 as “iFbN+hA”) also generated more spikes and network bursts than the iFbN cells alone
2 (although not to the same extent as the iFbN+SB).

3 **[0132]** Fig. 3C are raster plots illustrating the electrophysiological activity of cultures of
4 iFbN alone and cocultures of iFbN with vandefitemcel, conditioned medium (CM) of
5 vandefitemcel, and human astrocytes (hA) at four weeks of culture across electrodes of the
6 MEA system. Single spikes are represented in the plots by black boxes, electrode bursts are
7 represented in the plots by white boxes, and network bursts are represented by broken-line
8 boxes. Altogether, these results suggest that molecules secreted from vandefitemcel
9 resulted in an increase in neuronal activity but the presence of vandefitemcel cells in
10 culture significantly augmented this effect.

11 **[0133] Example 3: Coculturing Vandefitemcel with Glutamatergic Neurons Results in**
12 **More Neuronal Activity**

13 **[0134]** Neuronal network bursts, or neuronal oscillations, are generated by intrinsic
14 neuronal connections between excitatory and inhibitory neurons. Fig. 4A are
15 immunocytochemistry (ICC) stains of a culture of iFbNs, at thirty days of culture
16 maturation, showing the neurons expressing the neuronal marker MAP2 as well as
17 excitatory vesicular glutamate transporter (vGLUT1) and inhibitory γ -aminobutyric acid
18 (GABA). Cell nuclei were stained with DAPI (scale: 50 μ m). The ICC stains showed
19 abundant expression of vGLUT1, indicating the presence of excitatory neurons. This
20 observation was confirmed by a reduction in the spike activity when kynurenic acid (50
21 μ M), a broad glutamate receptor antagonist, was added to the iFbN culture.

22 **[0135]** To investigate the effects of vandefitemcel (or SB623) cells on an excitatory
23 neuronal population, vandefitemcel was cocultured with an enriched population of iPSC-
24 derived glutamatergic neurons (hereinafter, “glutamatergic neurons”). Unlike the iFbNs
25 previously used in these experiments, the glutamatergic neurons were mature at the first
26 day of culture, and did not need two weeks of maturation. Therefore, glutamatergic neurons
27 and vandefitemcel were cocultured together on day 1. The effect of vandefitemcel on
28 glutamatergic neurons (shown in Figs. 4B-4E as “GluN+SB”) was compared to that of
29 glutamatergic neurons alone (shown in Figs. 4B-4D as “GluN”) or glutamatergic neurons
30 in coculture with human astrocytes (shown in Figs. 4B-4D as “GluN+hA”) at the same cell
31 density as that of vandefitemcel (or SB623 cells).

32 **[0136]** Fig. 4B illustrates that on day three of coculture, vandefitemcel promoted high
33 levels of spike activity in glutamatergic neurons, when compared to spike activity in either
34 the glutamatergic neurons alone or the glutamatergic neurons and human astrocytes

1 cocultures (one-way ANOVA Holm-Sidak, **** $p < 0.0001$). Fig. 4C illustrates that this
2 spike activity kept increasing for two weeks, while human astrocytes (hA) slightly
3 promoted spike activity in coculture with glutamatergic neurons starting at week three
4 (two-way ANOVA Tukey, * $p < 0.05$; ** $p < 0.001$; **** $p < 0.0001$). Fig. 4D illustrates that
5 vandefitemcel (or SB623 cells) induced more network burst activity in the first week of
6 coculture with glutamatergic neurons (GluN+SB), while glutamatergic neurons in coculture
7 with human astrocytes (GluN+hA) did not show network bursts until week four (two-way
8 ANOVA Tukey, * $p < 0.05$; ** $p < 0.005$; *** $p < 0.001$; **** $p < 0.0001$). Figs. 4D and 4E also
9 illustrate that network bursts in cocultures of glutamatergic neurons with vandefitemcel
10 (GluN+SB) reduced after the first week, indicating the presence of an inhibitory input in
11 this culture. Figs. 4C and 4D illustrate that a culture of glutamatergic neurons alone (GluN)
12 had lower spike activity compared to cocultures with either human astrocytes (GluN+hA)
13 or vandefitemcel (GluN+SB) and failed to form network bursts for five weeks of culture.
14 **[0137]** Fig. 4E are raster plots illustrating the electrophysiological activity of the GluN+SB
15 coculture and progressive network burst development across electrodes on the MEA
16 system. On day three (D3), there were many spikes observed in the GluN+SB coculture.
17 On day six (D6), many spikes and network bursts were observed in the GluN+SB
18 coculture. On day 27 (D27), there was a drop in spike counts and network burst counts,
19 indicating the input of inhibitory synapses between network bursts. Single spikes are
20 represented in the plots by black boxes, electrode bursts are represented in the plots by
21 white boxes, and network bursts are represented by broken-line boxes.
22 **[0138]** To investigate the effects of vandefitemcel (or SB623) cells on GABAergic
23 neurons, the same experiments were repeated using an enriched population of iPSC-derived
24 GABAergic neurons (hereinafter, "GABAergic neurons"). As shown in Figs. 4F and 4G,
25 vandefitemcel (or SB623 cells) promoted spikes and network bursts of GABAergic
26 neurons in week two and week four of coculture when compared to either in GABAergic
27 neurons alone (shown as "GABA-N") or in cocultures of GABAergic neurons and human
28 astrocytes (shown as "GABA-N+hA") (two-way ANOVA Tukey, * $p < 0.05$; ** $p < 0.005$;
29 **** $p < 0.0001$). While the number of network bursts induced by vandefitemcel in
30 coculture with GABAergic neurons reached a similar level to that observed in coculture
31 with glutamatergic neurons, it took longer to reach this peak (see Figs. 4E and 4H). Like
32 glutamatergic neurons, GABAergic neurons alone did not exhibit network bursts,
33 indicating that supporting cells are required to promote electrical activity in GABAergic
34 neurons. Interestingly, a decrease in resistance at week one of culture was observed,

1 followed by recovery at week two, and then a continuous decline until week five. This
2 pattern mirrors the observations in vandefitemcel (or SB623 cells) cultured alone (see Fig.
3 1D). These findings indicate that vandefitemcel (or SB623 cells) induced an earlier, and
4 higher, spike activity in enriched glutamatergic neurons, suggesting that SB623 cells play a
5 role in modulating the synaptic activity of excitatory neurons.

6 **[0139]** Fig. 4H are raster plots illustrating the electrophysiological activity of the GABA-
7 N+SB coculture and progressive network burst development across electrodes on the MEA
8 system. There was no spike activity on day three of the GABA-N +SB623 coculture (not
9 shown). On day six (D6), many spikes were observed in the GABA-N+SB coculture. On
10 day 27 (D27), many spikes and network bursts were observed in the GABA-N+SB
11 coculture. Single spikes are represented in the plots by black boxes, electrode bursts are
12 represented in the plots by white boxes, and network bursts are represented by broken-line
13 boxes.

14 **[0140] Example 4: Vandefitemcel Increases Neuronal Activity and Promotes Synaptic**
15 **Plasticity by Tonic Glutamate Release**

16 **[0141]** Glutamate, the main excitatory neurotransmitter in the mammalian nervous system,
17 is involved in synapse formation [24]. We first looked at the amount of glutamate released
18 by vandefitemcel by comparing the levels of glutamate in conditioned medium (CM) of
19 vandefitemcel (or SB623 cells) with that in the CM of human astrocytes (hA).
20 Vandefitemcel (or SB623 cells) and human astrocytes were cultured in BrainPhys™
21 medium for five days at different cell densities.

22 **[0142]** Fig. 5A shows that at two days of culture, the increased levels of CM glutamate
23 were cell density-dependent in cultures of both cell types, but the levels of glutamate in
24 CM of vandefitemcel (or SB623 cells, shown as “SB” in Fig. 5A) were about 10-fold
25 higher than that in the CM of human astrocytes (shown as “hA” in Fig. 5A) (two-way
26 ANOVA Holm-Sidak, *** $p < 0.001$; **** $p < 0.0001$).

27 **[0143]** Fig. 5B shows that at five days of culture, the levels of glutamate in CM of
28 vandefitemcel (or SB623 cells, shown as “SB” in Fig. 5B) increased 4.9-fold, while the
29 levels of glutamate in CM of human astrocytes (shown as “hA” in Fig. 5B) increased 2.7-
30 fold, indicating that tonic glutamate release by vandefitemcel was higher than that in
31 human astrocytes (two-way ANOVA Holm-Sidak, * $p < 0.05$; ** $p < 0.005$; *** $p < 0.001$).

32 **[0144]** Glutamate is the catalysis of glutamine by glutaminase. To explore whether
33 glutaminase plays a role in the glutamate release by vandefitemcel (or SB623 cells),
34 glutamine was added to the BrainPhys™ medium and the levels of glutamate in the CM

1 were assessed. At two days of culture, the levels of glutamate in the CM increased 1.6-fold
2 suggesting that glutaminase indeed played a role in the glutamate released by
3 vandefitemcel.

4 **[0145]** Next, experiments were performed to determine if inhibition of glutaminase in
5 vandefitemcel (or SB623 cells) would affect the excitability observed in the coculture of
6 vandefitemcel and glutamatergic neurons (GluN). To this end, the activity of glutaminase
7 was inhibited in vandefitemcel with Telaglenastat (CB-839). Vandefitemcel (or SB623
8 cells) were incubated for two days with CB-839 and maintained in BrainPhys™ medium
9 for three days to assess the levels of glutamate in the CM.

10 **[0146]** Fig. 5C illustrates that after incubation with 5 μ M and 50 μ M of CB-839, the levels
11 of glutamate in the CM of vandefitemcel (shown as “SB (CB-839-5 μ M)” and “SB (CB-
12 839-50 μ M)” in Fig. 5C) were reduced 1.5-fold and 2.0-fold, respectively, compared to the
13 control of vandefitemcel incubated with dimethylsulfoxide (DMSO) (shown as “SB
14 (DMSO)” in Fig. 5C) (one-way ANOVA Holm-Sidak, ****p < 0.0001).

15 **[0147]** Glutamate can be involved in cell metabolism, where it is transformed into α -
16 ketoglutarate and then enters into the tricarboxylic acid cycle to generate ATP [25].
17 Therefore, to evaluate whether inhibition of glutaminase would affect cell metabolism and
18 viability, the viability of vandefitemcel (or SB623 cells) treated with CB-839 was assessed
19 using PrestoBlue™ high-sensitivity (HS) Cell Viability Reagent (from Thermo Fisher
20 Scientific, Waltham, MA). Fig. 5D illustrates that incubation with CB-839 did not alter the
21 viability of vandefitemcel (or SB623 cells) (one-way ANOVA Holm-Sidak, “ns” = no
22 difference in cell viability).

23 **[0148]** Next, glutamatergic neurons were cocultured with vandefitemcel that were pre-
24 incubated with both 5 μ M and 50 μ M of CB-839 to inhibit glutaminase. Figs. 5E and 5F
25 show that after one week of coculture, the inhibition of glutaminase in vandefitemcel
26 significantly reduced the spike activity (see Fig. 5E) and network bursts (see Fig. 5F) in the
27 cocultures of glutamatergic neurons and vandefitemcel (shown as “GluN+SB (CB-839-5
28 μ M)” and “GluN+SB (CB-839-50 μ M)” compared to the control culture of vandefitemcel
29 incubated with dimethylsulfoxide (DMSO) (shown as “SB (DMSO)” in Figs. 5E and 5F)
30 (one-way ANOVA Holm-Sidak, ***p < 0.0005; ****p < 0.0001).

31 **[0149]** Lastly, experiments were performed to investigate whether human astrocytes (hA)
32 could diminish the effect of vandefitemcel on glutamatergic neurons. Astrocytes have a key
33 role in brain homeostasis by the uptake of glutamate from the extracellular space [26].
34 Thus, three cocultures were prepared including 1) glutamatergic neurons with

1 vandefitemcel (shown in Figs. 5G and 5H as “GluN+SB”), 2) glutamatergic neurons with
2 vandefitemcel and human astrocytes (shown in Figs. 5G and 5H as “GluN+SB+hA”), and
3 3) glutamatergic neurons with human astrocytes (shown in Figs. 5G and 5H as
4 “GluN+hA”). Neuronal activity and synaptic plasticity were assessed in the form of the
5 number of spikes (i.e., action potentials that appear in the signal when neurons fire) and the
6 number of network bursts.

7 **[0150]** Figs. 5G and 5H illustrate that astrocytes did indeed reduce the number of spikes
8 and network bursts that would have been otherwise induced by vandefitemcel (or SB623
9 cells) on the glutamatergic neurons (one-way ANOVA Holm-Sidak, $*p<0.05$). This data
10 demonstrates that the increased neuronal activity and synaptic plasticity driven by
11 vandefitemcel was caused by tonic glutamate release.

12 **[0151]** Fig. 5I is a schematic diagram showing vandefitemcel (or SB623 cells) promoting
13 an increase in neuronal activity and synaptic plasticity via tonic glutamate release.

14 **[0152]** The results of the experiments disclosed herein show that vandefitemcel (or SB623
15 cells), which are genetically modified bone marrow-derived MSCs, increase neuronal
16 activity and synaptic plasticity in human neurons, and this effect is induced through tonic
17 glutamate release by vandefitemcel. As will be discussed in the following sections, the
18 findings from these experiments can be applied to MSC-based cell therapies involving the
19 transplantation of vandefitemcel (or SB623 cells) in human and animal subjects.

20 **[0153]** Moreover, functional magnetic resonance imaging (fMRI) [27] can be used prior to
21 cell transplantation to help determine optimal target brain regions for transplantation. For
22 example, fMRI can be used to select region(s) of the brain showing neuronal activity and
23 vandefitemcel can be administered to such region(s) of the brain selected based on the
24 fMRI scans. This can result in better efficacy of cell transplantation procedures and
25 reduction in clinical outcome variability and treatment-emergent adverse events.

26

27 **[0154] Methods of Treatment**

28 **[0155]** One unexpected result from the experiments described herein is that the
29 introduction of vandefitemcel to neurons induces, causes, or triggers tonic release of
30 glutamate to such neurons. Another unexpected result from the experiments described
31 herein is that the introduction of vandefitemcel to neurons increases synapse formation in
32 such neurons.

33 **[0156]** Based on the results of the experiments disclosed herein, a method of inducing,
34 causing, or triggering the tonic release of glutamate in a subject comprises administering

1 vandefitemcel to a region of the brain of the subject, wherein the vandefitemcel are cells
2 descended from mesenchymal stem cells (MSCs) transiently-transfected by a
3 polynucleotide encoding a Notch intracellular domain (NICD).

4 **[0157]** Administering the vandefitemcel can further comprise administering the
5 vandefitemcel by intracerebral implantation. The vandefitemcel can tonically release
6 glutamate to neurons of the brain when implanted via intracerebral implantation.

7 **[0158]** Administering the vandefitemcel can further comprise injecting the vandefitemcel
8 at multiple sites within the brain of the subject. In some cases, the vandefitemcel can be
9 administered stereotactically via a burr hole in the skull of a subject.

10 **[0159]** Additional details on stereotactic administration of the cells can be found in U.S.
11 Pat. No. 11,439,761, the content of which is incorporated herein by reference in its entirety
12 for the purposes of describing stereotactic administration of vandefitemcel and equipment
13 used for such purposes.

14 **[0160]** Administering the vandefitemcel can also comprise administering the vandefitemcel
15 to a site within a forebrain of the subject including at least one of the cerebrum, the
16 thalamus, and the hypothalamus of the subject.

17 **[0161]** Administering the vandefitemcel can further comprise administering the
18 vandefitemcel to a hippocampus of the subject.

19 **[0162]** Administering the vandefitemcel can further comprise administering the
20 vandefitemcel to a site of injury or disease in the brain region of the subject.

21 **[0163]** Administering the vandefitemcel can comprise administering the vandefitemcel by
22 parenteral administration.

23 **[0164]** The vandefitemcel can be suspended in a sterile isotonic crystalloid solution. For
24 example, the cell suspension can comprise the cells suspended in Plasma-Lyte™ A (Baxter
25 Healthcare Corporation). The cells can also be suspended in another physiologically
26 compatible carrier such as phosphate buffered saline.

27 **[0165]** The therapeutically effective amount of vandefitemcel can be between about 1.0
28 million cells and 10.0 million cells (e.g., about 1.0 million cells, 1.5 million cells, 2.0
29 million cells, 2.5 million cells, 3.0 million cells, 3.5 million cells, 4.0 million cells, 4.5
30 million cells, 5.0 million cells, 5.5 million cells, 6.0 million cells, 6.5 million cells, 7.0
31 million cells, 7.5 million cells, 8.0 million cells, 8.5 million cells, 9.0 million cells, 9.5
32 million cells, 10.0 million cells, and amounts therebetween).

33 **[0166]** As disclosed herein, the vandefitemcel can be made by a method comprising: (i)
34 providing a culture of mesenchymal stem cells; (ii) contacting the culture of mesenchymal

1 stem cells with a polynucleotide encoding a Notch intracellular domain (NICD) and where
2 the polynucleotide does not encode a full-length Notch protein; (iii) selecting cells that
3 comprise the polynucleotide; and (iv) further culturing the selected cells in the absence of
4 selection for the polynucleotide. The mesenchymal stem cells can be human bone marrow-
5 derived cells.

6 **[0167]** As disclosed herein, the method can further comprise capturing functional magnetic
7 resonance imaging (fMRI) scans of regions of the brain of the subject, selecting one region
8 of the brain showing neuronal activity based on the fMRI scans, and administering the
9 vandefitemcel to the one region of the brain.

10 **[0168]** As disclosed herein, the vandefitemcel can be made by a method comprising: (i)
11 providing a culture of mesenchymal stem cells; (ii) contacting the culture of mesenchymal
12 stem cells with a polynucleotide encoding a Notch intracellular domain (NICD) and where
13 the polynucleotide does not encode a full-length Notch protein; (iii) selecting cells that
14 comprise the polynucleotide; and (iv) further culturing the selected cells in the absence of
15 selection for the polynucleotide. The mesenchymal stem cells can be human bone marrow-
16 derived cells.

17 **[0169]** As disclosed herein, the method can further comprise capturing functional magnetic
18 resonance imaging (fMRI) scans of regions of the brain of the subject, selecting one region
19 of the brain showing neuronal activity based on the fMRI scans; and administering the
20 vandefitemcel to the one region of the brain.

21 **[0170]** Also disclosed is a method of increasing synapse formation in neurons, comprising
22 administering vandefitemcel to a region of the brain of the subject comprising the neurons,
23 wherein the vandefitemcel are cells descended from mesenchymal stem cells (MSCs)
24 transiently-transfected by a polynucleotide encoding a Notch intracellular domain (NICD).

25 **[0171]** Administering the vandefitemcel can further comprise administering the
26 vandefitemcel by intracerebral implantation. The vandefitemcel can tonically release
27 glutamate to neurons of the brain when implanted via intracerebral implantation.

28 **[0172]** Administering the vandefitemcel further comprises injecting the vandefitemcel at
29 multiple sites within the brain of the subject. In some cases, the vandefitemcel can be
30 administered stereotactically via a burr hole in the skull of a subject.

31 **[0173]** Administering the vandefitemcel can further comprise administering the
32 vandefitemcel to a hippocampus of the subject.

33 **[0174]** Administering the vandefitemcel can further comprise administering the
34 vandefitemcel to a site of injury or disease in the brain region of the subject.

1 [0175] Administering the vandefitemcel can comprise administering the vandefitemcel by
2 parenteral administration.

3 [0176] The vandefitemcel can be suspended in a sterile isotonic crystalloid solution. For
4 example, the cell suspension can comprise the cells suspended in Plasma-Lyte™ A (Baxter
5 Healthcare Corporation). The cells can also be suspended in another physiologically
6 compatible carrier such as phosphate buffered saline.

7 [0177] The therapeutically effective amount of vandefitemcel can be between about 1.0
8 million cells and 10.0 million cells (e.g., about 1.0 million cells, 1.5 million cells, 2.0
9 million cells, 2.5 million cells, 3.0 million cells, 3.5 million cells, 4.0 million cells, 4.5
10 million cells, 5.0 million cells, 5.5 million cells, 6.0 million cells, 6.5 million cells, 7.0
11 million cells, 7.5 million cells, 8.0 million cells, 8.5 million cells, 9.0 million cells, 9.5
12 million cells, 10.0 million cells, and amounts therebetween).

13 [0178] As disclosed herein, the vandefitemcel can be made by a method comprising: (i)
14 providing a culture of mesenchymal stem cells; (ii) contacting the culture of mesenchymal
15 stem cells with a polynucleotide encoding a Notch intracellular domain (NICD) and where
16 the polynucleotide does not encode a full-length Notch protein; (iii) selecting cells that
17 comprise the polynucleotide; and (iv) further culturing the selected cells in the absence of
18 selection for the polynucleotide. The mesenchymal stem cells can be human bone marrow-
19 derived cells.

20 [0179] As disclosed herein, the method can further comprise capturing functional magnetic
21 resonance imaging (fMRI) scans of regions of the brain of the subject, selecting one region
22 of the brain showing neuronal activity based on the fMRI scans; and administering the
23 vandefitemcel to the one region of the brain.

24 [0180] Also disclosed is a method of inducing, causing, or triggering the release of
25 glutamate in a subject. The method can comprise capturing functional magnetic resonance
26 imaging (fMRI) scans of regions of the brain of the subject, selecting a region of the brain
27 showing neuronal activity based on the fMRI scans, and administering vandefitemcel to the
28 region of the brain selected. The vandefitemcel are cells descended from mesenchymal
29 stem cells (MSCs) transiently-transfected by a polynucleotide encoding a Notch
30 intracellular domain (NICD). The vandefitemcel can release glutamate after being
31 administered via intracerebral implantation.

32 [0181] Also disclosed is a method for increasing synapse formation in neurons. The
33 method can comprise capturing functional magnetic resonance imaging (fMRI) scans of
34 regions of the brain of the subject, selecting a region of the brain showing neuronal activity

1 based on the fMRI scans, and administering vandefitemcel to the region of the brain
2 selected comprising the neurons. The vandefitemcel are cells descended from
3 mesenchymal stem cells (MSCs) transiently-transfected by a polynucleotide encoding a
4 Notch intracellular domain (NICD). The vandefitemcel can release glutamate to the
5 neurons after being administered via intracerebral implantation.

6 **[0182] Composition, Formulations, and Kits**

7 **[0183]** The composition can increase synapse formation in neurons and can comprise
8 vandefitemcel in an amount between about 1.0 million cells and 10.0 million cells. The
9 vandefitemcel can be produced by modifying mesenchymal stem cells derived from bone
10 marrow. The composition can also comprise one or more pharmaceutically acceptable
11 excipients.

12 **[0184]** As previously discussed, the vandefitemcel can be descended from mesenchymal
13 stem cells transiently-transfected by a polynucleotide encoding a Notch intracellular
14 domain (NICD). The vandefitemcel can be made by a process comprising providing a
15 culture of the mesenchymal stem cells (i.e., human bone marrow-derived cells) and
16 contacting the culture of mesenchymal stem cells with the polynucleotide encoding the
17 NICD. In certain instances, the polynucleotide does not encode a full-length Notch protein.
18 The vandefitemcel can further be made by selecting cells that comprise the polynucleotide;
19 and further culturing the selected cells in the absence of selection for the polynucleotide.

20 **[0185]** The composition can further comprise additional mesenchymal stem cells not
21 transiently-transfected by the polynucleotide.

22 **[0186]** The mesenchymal stem cells can be transiently-transfected with a plasmid vector
23 comprising the polynucleotide encoding the NICD.

24 **[0187]** The vandefitemcel can be suspended in a sterile isotonic crystalloid solution.

25 **[0188]** The one or more pharmaceutically acceptable excipients can comprise at least one
26 of buffers, proteins, stabilizers, and preservatives. The one or more pharmaceutically
27 acceptable excipients can also comprise carriers or diluents to form the cell suspension.

28 **[0189]** The pharmaceutically acceptable carrier can be a physiologically compatible carrier
29 for implantation. As used herein, the term “physiologically compatible carrier” can refer to
30 a carrier that is compatible with the other ingredients of the formulation and not deleterious
31 to the recipient thereof. Examples of suitable carriers or diluents include cell culture
32 medium (e.g., Eagle’s minimal essential medium), phosphate buffered saline, Hank’s
33 balanced salt solution+/-glucose (HBSS), and multiple electrolyte solutions. The

1 pharmaceutically acceptable carrier or diluent can also be or comprise a sterile isotonic
2 crystalloid solution such as Plasma-Lyte™ A (Baxter Healthcare Corporation).

3 **[0190]** The composition can comprise the vandefitemcel packaged in a sealed vial. In some
4 instances, the sealed vial can comprise 0.3 mL of the cell suspension with a cell
5 concentration of approximately 8.5×10^6 cells/mL. Alternatively, the sealed vial can
6 comprise 0.3 mL of the cell suspension with a cell concentration of approximately $17.0 \times$
7 10^6 cells/mL.

8 **[0191]** Also disclosed are other examples of materials which can serve as
9 pharmaceutically-acceptable carriers or excipients including: sugars, such as lactose,
10 glucose and sucrose; starches, such as corn starch and potato starch; cellulose and its
11 derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate;
12 powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository
13 waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil
14 and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol,
15 mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar;
16 buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid;
17 pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; phosphate buffer
18 solutions; and other non-toxic compatible substances. Wetting agents, emulsifiers and
19 lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring
20 agents, release agents, coating agents, sweetening, flavoring and perfuming agents,
21 preservatives, and antioxidants can also be present in the composition.

22 **[0192]** Other examples of pharmaceutically-acceptable carriers or excipients include
23 substances that stimulate angiogenesis ("pro-angiogenic agents"). In some instances, the
24 pro-angiogenic agent can be a protein (e.g., fibroblast growth factor, platelet-derived
25 growth factor, transforming growth factor alpha, hepatocyte growth factor, vascular
26 endothelial growth factor, sonic hedgehog, MAGP-2, HIF-1, PR-39, RTEF-1, c-Myc, TFII,
27 Egr-1, ETS-1) or a nucleic acid encoding such a protein. See, for example, Vincent et al.
28 (2007) Gene Therapy 14:781-789. In other instances, the pro-angiogenic agent can be a
29 small RNA molecule (e.g., siRNA, shRNA, microRNA) or a ribozyme that targets a
30 nucleic acid encoding an inhibitor of angiogenesis. Moreover, the pro-angiogenic agent can
31 be a triplex-forming nucleic acid that binds to DNA sequences regulating the expression of
32 a protein that inhibits angiogenesis, so as to block transcription of the gene encoding the
33 protein.

1 [0193] Exemplary formulations include, but are not limited to, those suitable for parenteral
2 administration, e.g., intrapulmonary, intra-arterial, intra-ocular, intra-cranial, sub-
3 meningeal, or subcutaneous administration, including formulations encapsulated in
4 micelles, liposomes or drug-release capsules (active agents incorporated within a
5 biocompatible coating designed for slow-release); ingestible formulations; formulations for
6 topical use, such as eye drops, creams, ointments and gels; and other formulations such as
7 inhalants, aerosols and sprays. The dosage of the compositions of the disclosure can vary
8 according to the extent and severity of the need for treatment, the activity of the
9 administered composition, the general health of the subject, and other considerations well
10 known to the skilled artisan.

11 [0194] In additional embodiments, the compositions described herein can also be delivered
12 locally. Localized delivery allows for the delivery of the composition non-systemically,
13 thereby reducing the body burden of the composition as compared to systemic delivery.
14 Such local delivery can be achieved, for example, through the use of various medically
15 implanted devices including, but not limited to, stents and catheters, or can be achieved by
16 inhalation, injection or surgery. Methods for coating, implanting, embedding, and
17 otherwise attaching desired agents to medical devices such as stents and catheters are
18 established in the art and contemplated herein.

19 [0195] Another aspect of the present disclosure relates to kits for carrying out the
20 administration of the cells to a subject. For example, the kit can comprise the composition
21 of cells, formulated as appropriate (e.g., in a pharmaceutical carrier), in one or more
22 separate pharmaceutical preparations.

23 [0196] Compositions comprising vandefitemcel can be used in combination with other
24 compositions comprising substances that stimulate angiogenesis ("pro-angiogenic agents").
25 The compositions can be administered sequentially in any order or concurrently.
26 Accordingly, therapeutic compositions as disclosed herein can contain both vandefitemcel
27 and a pro-angiogenic agent. In additional embodiments, separate therapeutic compositions,
28 one comprising vandefitemcel and the other comprising a pro-angiogenic agent, can be
29 administered to the subject, either separately or together.

30 [0197] The pro-angiogenic agent can be a transcription factor that activates expression of a
31 pro-angiogenic molecule (e.g., protein). Naturally-occurring transcription factors (such as,
32 for example, HIF-1alpha) that regulate the expression of pro-angiogenic proteins, are
33 known. In addition, synthetic transcriptional regulatory proteins can be constructed by
34 genetic engineering. For example, methods for the design of zinc finger DNA-binding

1 domains that bind to a sequence of interest, and methods for the fusion of such zinc finger
2 DNA-binding domains to transcriptional activation and repression domains, have been
3 described. See, for example, U.S. Pat. Nos. 6,534,261; 6,607,882; 6,785,613; 6,794,136;
4 6,824,978; 6,979,539; 7,013,219; 7,177,766; 7,220,719; and 7,788,044. These methods can
5 be used to synthesize non-naturally-occurring proteins that activate transcription of any
6 gene encoding a pro-angiogenic protein. In addition, synthetic zinc finger transcriptional
7 activators of the vascular endothelial growth factor (VEGF) gene have been described. See,
8 e.g., U.S. Pat. Nos. 7,026,462; 7,067,317; 7,560,440; 7,605,140; and 8,071,564.
9 Accordingly, a non-naturally-occurring (i.e., synthetic) zinc finger protein that activates
10 transcription of the VEGF gene can be used, in combination with vandefitemcel, for
11 augmenting angiogenesis, e.g., in the treatment of stroke. Furthermore, a natural or
12 synthetic transcriptional regulatory protein (e.g., a synthetic zinc finger transcriptional
13 regulatory protein) that inhibits transcription of an anti-angiogenic molecule can also be
14 used as a pro-angiogenic agent.

15 **[0198]** A number of embodiments have been described. Nevertheless, it will be understood
16 by one of ordinary skill in the art that various changes and modifications can be made to
17 this disclosure without departing from the spirit and scope of the embodiments. Elements
18 of systems, devices, apparatus, and methods shown with any embodiment are exemplary
19 for the specific embodiment and can be used in combination or otherwise on other
20 embodiments within this disclosure. For example, the steps of any methods depicted in the
21 figures or described in this disclosure do not require the particular order or sequential order
22 shown or described to achieve the desired results. In addition, other steps or operations may
23 be provided, or steps or operations may be eliminated or omitted from the described
24 methods or processes to achieve the desired results. Moreover, any components or parts of
25 any apparatus or systems described in this disclosure or depicted in the figures may be
26 removed, eliminated, or omitted to achieve the desired results. In addition, certain
27 components or parts of the systems, devices, or apparatus shown or described herein have
28 been omitted for the sake of succinctness and clarity.

29 **[0199]** Accordingly, other embodiments are within the scope of the following claims and
30 the specification and/or drawings may be regarded in an illustrative rather than a restrictive
31 sense.

32 **[0200]** Each of the individual variations or embodiments described and illustrated herein
33 has discrete components and features which may be readily separated from or combined
34 with the features of any of the other variations or embodiments. Modifications may be

1 made to adapt a particular situation, material, composition of matter, process, process act(s)
2 or step(s) to the objective(s), spirit or scope of the present invention.

3 **[0201]** Methods recited herein may be carried out in any order of the recited events that is
4 logically possible, as well as the recited order of events. Moreover, additional steps or
5 operations may be provided or steps or operations may be eliminated to achieve the desired
6 result.

7 **[0202]** Furthermore, where a range of values is provided, every intervening value between
8 the upper and lower limit of that range and any other stated or intervening value in that
9 stated range is encompassed within the invention. Also, any optional feature of the
10 inventive variations described may be set forth and claimed independently, or in
11 combination with any one or more of the features described herein. For example, a
12 description of a range from 1 to 5 should be considered to have disclosed subranges such as
13 from 1 to 3, from 1 to 4, from 2 to 4, from 2 to 5, from 3 to 5, etc. as well as individual
14 numbers within that range, for example 1.5, 2.5, etc. and any whole or partial increments
15 therebetween.

16 **[0203]** All existing subject matter mentioned herein (e.g., publications, patents, patent
17 applications) is incorporated by reference herein in its entirety except insofar as the subject
18 matter may conflict with that of the present invention (in which case what is present herein
19 shall prevail). The referenced items are provided solely for their disclosure prior to the
20 filing date of the present application. Nothing herein is to be construed as an admission that
21 the present invention is not entitled to antedate such material by virtue of prior invention.

22 **[0204]** Reference to a singular item, includes the possibility that there are plural of the
23 same items present. More specifically, as used herein and in the appended claims, the
24 singular forms “a,” “an,” “said” and “the” include plural referents unless the context clearly
25 dictates otherwise. It is further noted that the claims may be drafted to exclude any optional
26 element. As such, this statement is intended to serve as antecedent basis for use of such
27 exclusive terminology as “solely,” “only” and the like in connection with the recitation of
28 claim elements, or use of a “negative” limitation. Unless defined otherwise, all technical
29 and scientific terms used herein have the same meaning as commonly understood by one of
30 ordinary skill in the art to which this invention belongs.

31 **[0205]** Reference to the phrase “at least one of,” when such phrase modifies a plurality of
32 items or components (or an enumerated list of items or components) means any
33 combination of one or more of those items or components. For example, the phrase “at

1 least one of A, B, and C” means: (i) A; (ii) B; (iii) C; (iv) A, B, and C; (v) A and B; (vi) B
2 and C; or (vii) A and C.

3 **[0206]** In understanding the scope of the present disclosure, the term “comprising” and its
4 derivatives, as used herein, are intended to be open-ended terms that specify the presence
5 of the stated features, elements, components, groups, integers, and/or steps, but do not
6 exclude the presence of other unstated features, elements, components, groups, integers
7 and/or steps. The foregoing also applies to words having similar meanings such as the
8 terms, “including,” “having,” and their derivatives. Also, the terms “part,” “section,”
9 “portion,” “member” “element,” or “component” when used in the singular can have the
10 dual meaning of a single part or a plurality of parts. As used herein, the following
11 directional terms “forward, rearward, above, downward, vertical, horizontal, below,
12 transverse, laterally, and vertically” as well as any other similar directional terms refer to
13 those positions of a device or piece of equipment or those directions of the device or piece
14 of equipment being translated or moved.

15 **[0207]** Finally, terms of degree such as “substantially”, “about” and “approximately” as
16 used herein mean the specified value or the specified value and a reasonable amount of
17 deviation from the specified value (e.g., a deviation of up to $\pm 0.1\%$, $\pm 1\%$, $\pm 5\%$, or $\pm 10\%$,
18 as such variations are appropriate) such that the end result is not significantly or materially
19 changed. For example, “about 1.0 cm” can be interpreted to mean “1.0 cm” or between
20 “0.9 cm and 1.1 cm.” When terms of degree such as “about” or “approximately” are used
21 to refer to numbers or values that are part of a range, the term can be used to modify both
22 the minimum and maximum numbers or values.

23 **[0208]** This disclosure is not intended to be limited to the scope of the particular forms set
24 forth, but is intended to cover alternatives, modifications, and equivalents of the variations
25 or embodiments described herein. Further, the scope of the disclosure fully encompasses
26 other variations or embodiments that may become obvious to those skilled in the art in
27 view of this disclosure.

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19

CLAIMS

1 We claim:

- 2 1. A method of inducing tonic release of glutamate in a subject, the method
3 comprising:
4 administering vandefitemcel to a region of the brain of the subject, wherein the
5 vandefitemcel are cells descended from mesenchymal stem cells (MSCs)
6 transiently-transfected by a polynucleotide encoding a Notch intracellular
7 domain (NICD).
8
- 9 2. The method of claim 1, wherein administering the vandefitemcel further comprises
10 administering the vandefitemcel by intracerebral implantation.
- 11 3. The method of claim 2, wherein the vandefitemcel tonically release glutamate to
12 neurons of the brain when implanted via intracerebral implantation.
- 13 4. The method of claim 2, wherein administering the vandefitemcel further comprises
14 injecting the vandefitemcel at multiple sites within the region of the brain.
- 15 5. The method of claim 2, wherein administering the vandefitemcel further comprises
16 administering the vandefitemcel stereotactically via a burr hole in the skull of the
17 subject.
- 18 6. The method of claim 1, wherein the region of the brain is a forebrain of the subject.
- 19 7. The method of claim 1, wherein the region of the brain is a site of injury or disease.
- 20 8. The method of claim 1, wherein the region of the brain is a hippocampus of the
21 subject.
- 22 9. The method of claim 1, wherein administering the vandefitemcel further comprises
23 administering the vandefitemcel by parenteral administration.
- 24 10. The method of claim 1, wherein the vandefitemcel is suspended in a sterile isotonic
25 crystalloid solution.
- 26 11. The method of claim 1, wherein administering the vandefitemcel further comprises
27 administering between about 1.0 million cells and 10.0 million cells.
- 28 12. The method of claim 1, wherein the vandefitemcel is made by a method
29 comprising:
30 providing a culture of MSCs;
31 contacting the culture of MSCs with the polynucleotide encoding the NICD,
32 wherein the polynucleotide does not encode a full-length Notch protein,
33 selecting cells that comprise the polynucleotide; and

- 1 further culturing the selected cells in the absence of selection for the
2 polynucleotide.
- 3 13. The method of claim 1, wherein the MSCs are human bone marrow-derived cells.
- 4 14. The method of claim 1, further comprising:
5 capturing functional magnetic resonance imaging (fMRI) scans of regions of the
6 brain of the subject;
7 selecting one region of the brain showing neuronal activity based on the fMRI
8 scans; and
9 administering the vandefitemcel to the one region of the brain.
- 10 15. A method of increasing synapse formation in neurons, the method comprising:
11 administering vandefitemcel to a region of the brain of the subject comprising the
12 neurons, wherein the vandefitemcel are cells descended from mesenchymal
13 stem cells (MSCs) transiently-transfected by a polynucleotide encoding a Notch
14 intracellular domain (NICD).
- 15 16. The method of claim 15, wherein administering the vandefitemcel further
16 comprises administering the vandefitemcel by intracerebral implantation.
- 17 17. The method of claim 16, wherein the vandefitemcel tonically release glutamate to
18 the neurons when implanted via intracerebral implantation.
- 19 18. The method of claim 16, wherein administering the vandefitemcel further
20 comprises injecting the vandefitemcel at multiple sites within the region of the
21 brain.
- 22 19. The method of claim 16, wherein administering the vandefitemcel further
23 comprises administering the vandefitemcel stereotactically via a burr hole in the
24 skull of the subject.
- 25 20. The method of claim 15, wherein the region of the brain is a forebrain of the
26 subject.
- 27 21. The method of claim 15, wherein the region of the brain is a site of injury or
28 disease.
- 29 22. The method of claim 16, wherein the region of the brain is a hippocampus of the
30 subject.
- 31 23. The method of claim 15, wherein administering the vandefitemcel further
32 comprises administering the vandefitemcel by parenteral administration.
- 33 24. The method of claim 15, wherein the vandefitemcel is suspended in a sterile
34 isotonic crystalloid solution.

- 1 25. The method of claim 15, wherein administering the vandefitemcel further
2 comprises administering between about 1.0 million cells and 10.0 million cells.
- 3 26. The method of claim 15, wherein the vandefitemcel is made by a method
4 comprising:
5 providing a culture of mesenchymal stem cells;
6 contacting the culture of mesenchymal stem cells with the polynucleotide encoding
7 the NICD, wherein the polynucleotide does not encode a full-length Notch
8 protein,
9 selecting cells that comprise the polynucleotide; and
10 further culturing the selected cells in the absence of selection for the
11 polynucleotide.
- 12 27. The method of claim 15, wherein the MSCs are human bone marrow-derived cells.
- 13 28. The method of claim 15, further comprising:
14 capturing functional magnetic resonance imaging (fMRI) scans of regions of the
15 brain of the subject;
16 selecting one region of the brain showing neuronal activity based on the fMRI
17 scans; and
18 administering the vandefitemcel to the one region of the brain.
- 19 29. A composition for increasing synapse formation in neurons, the composition
20 comprising:
21 vandefitemcel in an amount between about 1.0 million cells and 10.0 million cells,
22 wherein the vandefitemcel is produced by modifying mesenchymal stem cells
23 derived from bone marrow; and
24 one or more pharmaceutically acceptable excipients.
- 25 30. The composition of claim 29, wherein the vandefitemcel is made by a process
26 comprising:
27 providing a culture of mesenchymal stem cells;
28 contacting the culture of mesenchymal stem cells with a polynucleotide encoding a
29 Notch intracellular domain (NICD), wherein the polynucleotide does not encode
30 a full-length Notch protein,
31 selecting cells that comprise the polynucleotide; and
32 further culturing the selected cells in the absence of selection for the
33 polynucleotide.

- 1 31. The composition of claim 30, wherein the mesenchymal stem cells are human bone
2 marrow-derived cells.
- 3 32. The composition of claim 29, wherein the mesenchymal stem cells are transiently-
4 transfected with a plasmid vector comprising the polynucleotide encoding the
5 NICD.
- 6 33. The composition of claim 29, wherein the one or more pharmaceutically acceptable
7 excipients comprises at least one of buffers, proteins, stabilizers, and preservatives.
- 8 34. The composition of claim 29, wherein the vandefitemcel is suspended in a sterile
9 isotonic crystalloid solution.
- 10 35. The composition of claim 29, wherein the one or more pharmaceutically acceptable
11 excipients comprises carriers or diluents.
- 12 36. A method of inducing release of glutamate in a subject, the method comprising:
13 capturing functional magnetic resonance imaging (fMRI) scans of regions of the
14 brain of the subject;
15 selecting a region of the brain showing neuronal activity based on the fMRI scans;
16 and
17 administering vandefitemcel to the region of the brain selected, wherein the
18 vandefitemcel are cells descended from mesenchymal stem cells (MSCs)
19 transiently-transfected by a polynucleotide encoding a Notch intracellular
20 domain (NICD), and wherein the vandefitemcel release glutamate after being
21 administered via intracerebral implantation.
- 22 37. A method of increasing synapse formation in neurons, the method comprising:
23 capturing functional magnetic resonance imaging (fMRI) scans of regions of the
24 brain of the subject;
25 selecting a region of the brain showing neuronal activity based on the fMRI scans;
26 and
27 administering vandefitemcel to the region of the brain selected comprising the
28 neurons, wherein the vandefitemcel are cells descended from mesenchymal
29 stem cells (MSCs) transiently-transfected by a polynucleotide encoding a Notch
30 intracellular domain (NICD), and wherein the vandefitemcel release glutamate
31 to the neurons after being administered via intracerebral implantation.