

TITLE

**CELL THERAPIES AND METHODS OF TREATMENT FOR SMALL-VOLUME
STROKE**

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional Application No. 63/194,021 filed on May 27, 2021, the content of which is incorporated herein by reference in its entirety.

TECHNICAL FIELD

[0002] The present disclosure relates generally to the field of regenerative cell therapies, and, more specifically, to cell therapies and methods of treatment for small-volume stroke.

BACKGROUND

[0003] Worldwide, stroke is the second leading cause of death and the third leading cause of disability [1]. After an ischemic stroke, the acute period is generally defined from hours to several days after the stroke event or incident. Immediate post-stroke interventions for acute ischemic stroke focus on life support through respiratory and cardiac control of blood pressure, monitoring oxygen saturation and blood glucose levels, prevention of metabolic disturbances, maintenance of organ function, and management of elevated intracranial pressure.

[0004] One of the only approved therapies for acute ischemic stroke in the United States are thrombolytic agents, to be given to the patient within 3 hours of the onset of the stroke. Some studies have estimated that less than 5% of acute ischemic stroke subjects actually receive this therapy, likely due to the stringent criteria for thrombolytic intervention, the lack of adequate facilities, and the subject arriving beyond the 3-hour window [2]. Approximately 70% to 85% of patients that suffer their first stroke develop hemiplegia or at least some paralysis on one side of the patient's body. Six months following the stroke event or incident, only 60% of patients who develop hemiparesis and need inpatient rehabilitative care have achieved functional independence in performing simple daily activities [3].

[0005] Once a stroke patient has entered the chronic phase, generally defined as more than several months after the stroke event or incident, physical therapy is often the patient's only prescribed rehabilitative regimen. For these chronic-stroke patients, no proven

1 biological or pharmaceutical therapies have been shown to significantly reverse the damage
2 and improve the patients' motor functions. While a variety of cellular therapies have been
3 proposed and studied, clinical trials for these therapies have so far been limited with the
4 patients in such trials only showing modest improvements [4-6]. Moreover, no
5 investigation has been made as to whether certain therapies are more effective at treating
6 particular patient sub-populations such as stroke patients with differing ischemic core sizes
7 or volumes.

8 **[0006]** Therefore, there is a need for a safe and effective therapy for the treatment of
9 chronic stroke patients or those patients suffering from chronic stroke-induced motor
10 deficits.

11 SUMMARY

12
13 **[0007]** Disclosed are methods of treating a subject following a small-volume ischemic
14 stroke suffered by the subject and methods of treating a subject with a stroke-induced
15 motor deficit. Disclosed also is a composition for treating small-volume ischemic stroke.

16 **[0008]** In one aspect, a method of treating a subject following a small-volume ischemic
17 stroke comprises administering, to a brain region surrounding a small-volume ischemic
18 core of the subject, a therapeutically effective amount of cells. The cells can be descended
19 from mesenchymal stem cells transiently-transfected by a polynucleotide encoding a Notch
20 intracellular domain (NICD).

21 **[0009]** The cells can be made by a method comprising providing a culture of the
22 mesenchymal stem cells, contacting the culture of mesenchymal stem cells with a
23 polynucleotide encoding the NICD, selecting cells that comprise the polynucleotide, and
24 further culturing the selected cells in the absence of selection for the polynucleotide. The
25 mesenchymal stem cells can be human bone marrow-derived cells. Moreover, the
26 polynucleotide encoding the NICD does not encode a full-length Notch protein.

27 **[0010]** The small-volume ischemic core can be an ischemic core having an ischemic core
28 volume of less than 50 cubic centimeters (cc). For example, the small-volume ischemic
29 core can be an ischemic core having an ischemic core volume of between about 2 cc and 50
30 cc.

31 **[0011]** In some instances, the treatment is especially effective when the small-volume
32 ischemic core is located in a region of the brain of the subject other than a parietal region
33 such as a cortical frontal region, a cortical temporal region, a subcortical white matter, or a
34 subcortical grey matter of the brain of the subject.

1 **[0012]** In some instances, the treatment is effective even when the small-volume ischemic
2 stroke occurred more than six months prior to administering the cells. For example, the
3 treatment can be effective even when the small-volume ischemic stroke occurred between
4 six months and 90 months prior to administering the cells.

5 **[0013]** The step of administering the therapeutically effective amount of the cells can
6 comprise injecting a cell suspension comprising the cells at deposit sites within the brain of
7 the subject. The cell suspension can comprise the cells suspended in a sterile isotonic
8 crystalloid solution.

9 **[0014]** The step of administering the therapeutically effective amount of the cells can
10 further comprise injecting at least part of the cell suspension comprising the cells at one or
11 more deposit sites at an outer periphery of an ischemic penumbra or chronic ischemic
12 penumbra surrounding the small-volume ischemic core. For example, the step of
13 administering the therapeutically effective amount of the cells can comprise injecting at
14 least part of the cell suspension comprising the cells at one or more deposit sites proximal
15 to the ischemic penumbra or the chronic ischemic penumbra surrounding the small-volume
16 ischemic core.

17 **[0015]** The step of administering the therapeutically effective amount of the cells can
18 further comprise injecting at least part of the cell suspension comprising the cells at one or
19 more deposit sites within the ischemic penumbra or the chronic ischemic penumbra
20 surrounding the small-volume ischemic core. In some instances, the step of administering
21 the therapeutically effective amount of the cells can comprise injecting at least part of the
22 cell suspension comprising the cells at one or more deposit sites distal to the ischemic
23 penumbra/chronic ischemic penumbra or within the small-volume ischemic core.

24 **[0016]** The step of administering the therapeutically effective amount of the cells can
25 further comprise administering the therapeutically effective amount of the cells
26 stereotactically via a single burr-hole craniostomy.

27 **[0017]** The therapeutically effective amount of cells can be approximately 2.5 million cells
28 (or 2.5 million cells $\pm$ 0.1 million cells). The step of administering the approximately 2.5
29 million cells can comprise injecting a cell suspension comprising the cells at five deposit
30 sites along a first deposit track or needle track, five deposit sites along a second deposit
31 track or needle track, and five deposit sites along a third deposit track or needle track.
32 Approximately 20- μ L of the cell suspension can be injected at each deposit site. The cell
33 suspension can have a cell concentration of approximately 8.5×10^6 cells/mL.

1 **[0018]** The therapeutically effective amount of cells can also be approximately 5.0 million
2 cells (or 5.0 million cells $\pm$ 0.1 million cells). The step of administering the approximately
3 5.0 million cells can comprise injecting a cell suspension comprising the cells at five
4 deposit sites along a first deposit track or needle track, five deposit sites along a second
5 deposit track or needle track, and five deposit sites along a third deposit track or needle
6 track. Approximately 20- μ L of the cell suspension can be injected at each deposit site. The
7 cell suspension can have a cell concentration of approximately 17.0×10^6 cells/mL.

8 **[0019]** The method can further comprise subjecting a formulated dose of the cells to post-
9 release testing prior to administering the cells to the subject.

10 **[0020]** The method can further comprise determining a degree of disability of the subject
11 by determining an mRS score of the subject and administering the therapeutically effective
12 amount of cells only when the mRS score of the subject is between 2 and 4.

13 **[0021]** Also disclosed is a method of treating a subject with a stroke-induced motor deficit.
14 The method can comprise determining a volume of an ischemic core of the subject and
15 administering, to a brain region surrounding the ischemic core of the subject, a
16 therapeutically effective amount of cells only when the volume of the ischemic core is
17 determined to be less than 50 cubic centimeters (cc). For example, the method can
18 comprise administering, to the brain region surrounding the ischemic core of the subject,
19 the therapeutically effective amount of cells only when the volume of the ischemic core is
20 between about 2 cc and 50 cc.

21 **[0022]** The stroke-induced motor deficit can be the result of an ischemic stroke suffered by
22 the subject. In some instances, the ischemic stroke occurred more than six months prior to
23 administering the cells. For example, the small-volume ischemic stroke occurred between
24 six months and 90 months prior to administering the cells.

25 **[0023]** In certain instances, the method comprises administering, to the brain region
26 surrounding the ischemic core of the subject, the therapeutically effective amount of cells
27 only when the ischemic stroke occurred more than six months prior (e.g., between six
28 months and 90 months prior).

29 **[0024]** The cells can be descended from mesenchymal stem cells transiently-transfected by
30 a polynucleotide encoding a Notch intracellular domain (NICD).

31 **[0025]** The cells can be made by a method comprising providing a culture of the
32 mesenchymal stem cells, contacting the culture of mesenchymal stem cells with a
33 polynucleotide encoding the NICD, selecting cells that comprise the polynucleotide, and
34 further culturing the selected cells in the absence of selection for the polynucleotide. The

1 mesenchymal stem cells can be human bone marrow-derived cells. Moreover, the
2 polynucleotide encoding the NICD does not encode a full-length Notch protein.

3 **[0026]** In certain instances, the method comprises administering, to the brain region
4 surrounding the ischemic core of the subject, the therapeutically effective amount of cells
5 only when the ischemic core is located in a region of the brain of the subject other than a
6 parietal region. For example, the method can comprise administering, to the brain region
7 surrounding the ischemic core of the subject, the therapeutically effective amount of cells
8 only when part of the ischemic core is located in at least one of a cortical frontal region, a
9 cortical temporal region, a subcortical white matter, and a subcortical grey matter of the
10 brain of the subject.

11 **[0027]** The step of administering the therapeutically effective amount of the cells can
12 comprise injecting a cell suspension comprising the cells at deposit sites within the brain of
13 the subject. The cell suspension can comprise the cells suspended in a sterile isotonic
14 crystalloid solution.

15 **[0028]** The step of administering the therapeutically effective amount of the cells can
16 further comprise injecting at least part of the cell suspension comprising the cells at one or
17 more deposit sites at an outer periphery of an ischemic penumbra or chronic ischemic
18 penumbra surrounding the small-volume ischemic core. For example, the step of
19 administering the therapeutically effective amount of the cells can comprise injecting at
20 least part of the cell suspension comprising the cells at one or more deposit sites proximal
21 to the ischemic penumbra or chronic ischemic penumbra surrounding the small-volume
22 ischemic core. The step of administering the therapeutically effective amount of the cells
23 can also comprise injecting at least part of the cell suspension comprising the cells at one or
24 more deposit sites within the ischemic penumbra or chronic ischemic penumbra
25 surrounding the small-volume ischemic core. In some instances, the step of administering
26 the therapeutically effective amount of the cells can comprise injecting at least part of the
27 cell suspension comprising the cells at one or more deposit sites distal to the ischemic
28 penumbra/chronic ischemic penumbra or within the small-volume ischemic core.

29 **[0029]** The step of administering the therapeutically effective amount of the cells can
30 further comprise administering the therapeutically effective amount of the cells
31 stereotactically via a single burr-hole craniostomy.

32 **[0030]** The therapeutically effective amount of cells can be approximately 2.5 million cells
33 (or 2.5 million cells $\pm$ 0.1 million cells). The step of administering the approximately 2.5
34 million cells can comprise injecting a cell suspension comprising the cells at five deposit

1 sites along a first deposit track or needle track, five deposit sites along a second deposit
2 track or needle track, and five deposit sites along a third deposit track or needle track.
3 Approximately 20-μL of the cell suspension can be injected at each deposit site. The cell
4 suspension can have a cell concentration of approximately $8.5 * 10^6$ cells/mL.

5 **[0031]** The therapeutically effective amount of cells can also be approximately 5.0 million
6 cells (or 5.0 million cells $\pm$ 0.1 million cells). The step of administering the approximately
7 5.0 million cells can comprise injecting a cell suspension comprising the cells at five
8 deposit sites along a first deposit track or needle track, five deposit sites along a second
9 deposit track or needle track, and five deposit sites along a third deposit track or needle
10 track. Approximately 20-μL of the cell suspension can be injected at each deposit site. The
11 cell suspension can have a cell concentration of approximately $17.0 * 10^6$ cells/mL.

12 **[0032]** The method can further comprise subjecting a formulated dose of the cells to post-
13 release testing prior to administering the cells to the subject.

14 **[0033]** The method can further comprise determining a degree of disability of the subject
15 by determining an mRS score of the subject and administering the therapeutically effective
16 amount of cells only when the mRS score of the subject is between 2 and 4.

17 **[0034]** Also disclosed is a composition for treating small-volume ischemic stroke. The
18 composition can comprise a therapeutically effective amount of cells and a
19 pharmaceutically acceptable carrier or diluent. The cells can be descended from
20 mesenchymal stem cells transiently-transfected by a polynucleotide encoding a Notch
21 intracellular domain (NICD).

22 **[0035]** The cells can be made by a method comprising providing a culture of the
23 mesenchymal stem cells, contacting the culture of mesenchymal stem cells with a
24 polynucleotide encoding the NICD, selecting cells that comprise the polynucleotide, and
25 further culturing the selected cells in the absence of selection for the polynucleotide. The
26 mesenchymal stem cells can be transiently-transfected with a plasmid comprising the
27 polynucleotide encoding the NICD.

28 **[0036]** The mesenchymal stem cells can be human bone marrow-derived cells. Moreover,
29 the polynucleotide encoding the NICD does not encode a full-length Notch protein.

30 **[0037]** The pharmaceutically acceptable carrier or diluent can comprise a sterile isotonic
31 crystalloid solution (e.g., Plasma-Lyte A). The composition can be a cell suspension
32 packaged in a sealed vial prior to being administered.

33 **[0038]** The therapeutically effective amount of cells can be approximately 2.5 million
34 cells. When the therapeutically effective amount of cells is approximately 2.5 million cells,

1 the composition can be an approximately 0.3 mL cell suspension with a cell concentration
2 of approximately 8.5×10^6 cells/mL.

3 **[0039]** The therapeutically effective amount of cells can also be approximately 5.0 million
4 cells. When the therapeutically effective amount of cells is approximately 2.5 million cells,
5 the composition can be an approximately 0.3 mL cell suspension with a cell concentration
6 of approximately 17.0×10^6 cells/mL.

7 **[0040]** In some instances, the therapeutically effective amount of cells can be between
8 approximately 2.0 million cells and approximately 5.0 million cells.

9 **[0041]** Also disclosed is the use of cells in the manufacture of a medicament for treating a
10 subject following a small-volume ischemic stroke suffered by the subject, comprising:
11 administering to a brain region surrounding a small-volume ischemic core of the subject a
12 therapeutically effective amount of cells, wherein the cells are descended from
13 mesenchymal stem cells transiently-transfected by a polynucleotide encoding a Notch
14 intracellular domain (NICD).

15 **[0042]** Further disclosed is the use of cells in the manufacture of a medicament for treating
16 a subject with a stroke-induced motor deficit, comprising: determining a volume of an
17 ischemic core of the subject; and administering, to a brain region surrounding the ischemic
18 core of the subject, a therapeutically effective amount of cells only when the volume of the
19 ischemic core is determined to be less than 50 cubic centimeters (cc), wherein the cells are
20 descended from mesenchymal stem cells transiently-transfected by a polynucleotide
21 encoding a Notch intracellular domain (NICD).

22 **[0043]** Also disclosed are cells descended from mesenchymal stem cells transiently-
23 transfected by a polynucleotide encoding a Notch intracellular domain (NICD) for use in a
24 method of treating a subject following a small-volume ischemic stroke suffered by the
25 subject, wherein the method comprises administering, to a brain region surrounding a
26 small-volume ischemic core of the subject, a therapeutically effective amount of the cells.

27 **[0044]** Further disclosed are cells descended from mesenchymal stem cells transiently-
28 transfected by a polynucleotide encoding a Notch intracellular domain (NICD) for use in a
29 method of treating a subject with a stroke-induced motor deficit, wherein the method
30 comprises: determining a volume of an ischemic core of the subject; and administering, to
31 a brain region surrounding the ischemic core of the subject, a therapeutically effective
32 amount of cells only when the volume of the ischemic core is determined to be less than 50
33 cubic centimeters (cc), wherein the cells are descended from mesenchymal stem cells
34 transiently-transfected by a polynucleotide encoding a Notch intracellular domain (NICD).

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BRIEF DESCRIPTION OF THE DRAWINGS

3 [0045] Fig. 1 illustrates a method of treating a subject with a stroke-induced motor deficit.

4 [0046] Fig. 2 illustrates an ischemic core of a stroke patient surrounded by an ischemic
5 penumbra.

6 [0047] Fig. 3A illustrates the relationship between baseline FMMS and volume of stroke
7 for various treatment and control groups.

8 [0048] Fig. 3B illustrates the relationship between baseline mRS and baseline FMMS for
9 various treatment and control groups.

10 [0049] Fig. 3C illustrates the relationship between baseline mRS and volume of stroke for
11 various treatment and control groups.

12 [0050] Fig. 4 illustrates the delta composite rate as a function of stroke volume for
13 different dosage groups.

14 [0051] Fig. 5 illustrates various regions or areas of the brain including, among others, the
15 subcortical white matter, the subcortical grey matter, the cortical frontal region, the cortical
16 parietal region, and the cortical temporal region.

17 [0052] Fig. 6 is a table comprising information concerning the stroke location, baseline
18 characteristics, and delta response rates of the study population broken down by population
19 percentage

20 [0053] Fig. 7 is a plot showing the delta composite response rates of Fig. 6 plotted against
21 the population percentages.

22

DETAILED DESCRIPTION

23 [0054] The below terms are defined as follows for purposes of this disclosure.

24 [0055] **Definitions**

25 [0056] “Angiogenesis” or “angiogenic” refers to the formation of new vasculature (e.g.,
26 blood vessels; e.g., veins, arteries, venules, arterioles, capillaries). Angiogenesis can occur
27 by sprouting of new vessels from an existing vessel, and/or by in situ coalescence of
28 endothelial cells to form new blood vessels. Angiogenesis also includes the attendant
29 processes of matrix remodeling and cell recruitment (e.g., recruitment of smooth muscle
30 cells, monocytes and/or pericytes). Angiogenesis further includes proliferation and/or
31 migration of endothelial cells.

32 [0057] The terms “DNTT-MSCs” (“descendants of NICD transiently-transfected MSCs”)
33 and “SB623 cells” refer to populations of cells obtained following transient expression of
34 an exogenous Notch intracellular domain (NICD) in MSCs. For example, a population of

1 DNTT-MSCs can be obtained by transient transfection of MSCs with a vector comprising
2 sequences encoding a NICD (e.g., from the human Notch 1 protein) but not encoding a
3 full-length Notch protein followed by selection (e.g., with G418). The selected cells can be
4 further cultured in a standard culture medium, optionally supplemented with a serum, in the
5 absence of any added growth factors or differentiation factors (other than those which may
6 be present in the serum, if serum is present in the culture medium). DNTT-MSCs can be
7 derived from human bone marrow MSCs by transient transfection of human bone marrow
8 MSCs with NICD (e.g., the human Notch1 intracellular domain (NICD1)), followed by
9 selection, and subsequent expansion. This process produces a cell population that
10 demonstrates superior angiogenic and neuropoietic properties in vitro, compared to the
11 parental MSCs [7-9]. The neuropoietic effects of DNTT-MSCs have been attributed to the
12 increased expression, and correspondingly, increased secretion, of FGF1, FGF2, and BMPs
13 [7, 9].

14 **[0058]** “Fugl-Meyer Assessment” or “FMA” is an index or scale used to assess the
15 sensorimotor impairment in individuals who have had a stroke. The FMA index or scale is
16 applied clinically to determine impairment severity, assess motor recovery, and plan
17 treatment. The FMA index or scale was first proposed by Axel Fugl-Meyer and his
18 colleagues in 1975 as a standardized assessment for post-stroke recovery [10]. The
19 assessment is made in five domains, including: (1) motor function (upper extremity and
20 lower extremity motor function); (2) sensory function; (3) balance; (4) joint range of
21 motion; and (5) joint pain. A total of 155 items are assessed with a maximum score of 226
22 points across all five domains.

23 **[0059]** “Fugl-Meyer Motor Scale or Score” or “FMMS” is a subscale used to assess the
24 motor functioning or impairment of individuals who have had a stroke including range of
25 motion in both the upper and lower limbs. The FMMS ranges from 0 points (hemiplegia) to
26 100 points (normal motor performance). The FMMS maximum score of 100 points
27 accounts for almost half of the maximum total FMA score (226 points) across all five
28 domains. The FMMS maximum score is divided into 66 points for the upper extremity
29 (based on an evaluation of 33 items) and 34 points for the lower extremity (based on an
30 evaluation of 17 items).

31 **[0060]** “FMMS Upper Extremity” or “FMMS UE” is a more specific subscale used to
32 assess the motor functioning of the upper extremity or upper limbs of individuals who have
33 had a stroke.

1 **[0061]** “FMMS Lower Extremity” or “FMMS LE” is a more specific subscale used to
2 assess the motor functioning of the lower extremity or lower limbs of individuals who have
3 had a stroke.

4 **[0062]** The terms “implantation” and “transplantation” are used to denote the introduction
5 of exogenous cells into a subject or patient. Exogenous cells can be autologous (i.e.
6 obtained from the subject) or allogeneic (i.e., obtained from an individual other than the
7 subject).

8 **[0063]** “Mesenchymal cells” refer to cells of mesenchymal tissue (e.g., chondroblasts,
9 chondrocytes, osteoblasts, osteocytes, adipocytes) and their precursors and include, for
10 example, fibroblasts (e.g., human foreskin fibroblasts), MSCs (as defined herein) and cells
11 derived from MSCs such as, for example, DNTT-MSCs, as defined herein.

12 **[0064]** “Minimally Clinically Important Difference” or “MCID” is the smallest change in a
13 treatment outcome that a patient or individual would perceive or identify as meaningful or
14 important. For example, an MCID as it relates to FMA can be the smallest change in a
15 score used to evaluate a stroke patient in one or more of the five FMA domains. As a more
16 specific example, an MCID threshold for evaluating a stroke treatment can require that a
17 stroke patient exhibit an improvement of at least 6 points in their FMMS UE score from a
18 baseline measurement.

19 **[0065]** “Modified Rankin Scale” or “mRS” is a clinician-reported ordinal scale used to
20 measure the degree of disability or disability level of patients who have had a stroke. The
21 scale ranges from a grade or score of 0 (no symptoms at all) to 6 (death). Those with a
22 grade or score of 1 have no significant disability and are able to carry out all usual duties
23 and activities. Those with a grade or score of 2 have a slight disability and are unable to
24 perform all previous activities but are able to look after their own affairs without
25 assistance. Those with a grade or score of 3 are moderately disabled, require some help, but
26 are able to walk without assistance. Those with a grade or score of 4 exhibit moderately
27 severe disability and are unable to walk and unable to attend to their own bodily needs
28 without assistance. Those with a grade or score of 5 are severely disabled, bedridden, and
29 incontinent and require constant nursing care and attention.

30 **[0066]** “MSCs” (“mesenchymal stem cells”) refer to adherent, non-hematopoietic
31 pluripotent cells obtained from bone marrow. These cells are variously known as
32 mesenchymal stem cells, mesenchymal stromal cells, marrow adherent stromal cells,
33 marrow adherent stem cells and bone marrow stromal cells. MSCs can also be obtained
34 from, e.g., umbilical cord blood, adipose tissue, dental pulp, Wharton's jelly, and various

1 types of connective tissue. MSCs can be obtained by selecting (e.g., by growth in culture)
2 adherent cells (i.e., cells that adhere to tissue culture plastic) from bone marrow. To obtain
3 MSC populations having a sufficient number of cells for use in therapy, populations of
4 adherent cells are expanded in culture after selecting for adherence. Expansion in culture
5 also enriches for MSCs, since contaminating cells (such as monocytes) do not proliferate
6 under the culture conditions.

7 **[0067]** Exemplary disclosures of MSCs are provided in U.S. Patent Publication No.
8 2003/0003090; Prockop (1997) *Science* 276:71-74 and Jiang (2002) *Nature* 418:41-49.
9 Methods for isolating and purifying MSCs can be found, for example, in U.S. Pat. No.
10 5,486,359; Pittenger et al. (1999) *Science* 284:143-147 and Dezawa et al. (2001) *Eur. J.*
11 *Neurosci.* 14:1771-1776. Human MSCs are commercially available (e.g., BioWhittaker,
12 Walkersville, Md.) or can be obtained from donors by, e.g., bone marrow aspiration,
13 followed by culture and selection for adherent bone marrow cells. See, e.g., WO
14 2005/100552.

15 **[0068]** MSCs can also be isolated from umbilical cord blood. See, for example,
16 Campagnoli et al. (2001) *Blood* 98:2396-2402; Erices et al. (2000) *Br. J.*
17 *Haematol.* 109:235-242 and Hou et al. (2003) *Int. J. Hematol.* 78:256-261. Additional
18 sources of MSCs include, for example, adipose tissue, dental pulp and Wharton's jelly.

19 **[0069]** "Neuropoiesis" or "neuropoietic" refers or relates to the growth and/or
20 differentiation of neural precursor cells (NPCs) into neurons and/or glial cells (e.g.,
21 astrocytes, oligodendrocytes). Examples of neuropoietic processes include, but are not
22 limited to, NPC proliferation, neurogenesis (e.g., formation of new neurons) and
23 gliogenesis (e.g., formation of astrocytes and/or oligodendrocytes). Other processes related
24 to neuronal development include, for example, neurite outgrowth, outgrowth of axon(s),
25 and outgrowth of dendrite(s).

26 **[0070]** The "Notch protein" (e.g., Notch 1 protein) is a transmembrane receptor, found in
27 all metazoans, that influences cell differentiation through intracellular signaling. Contact of
28 the Notch extracellular domain (e.g., the extracellular domain of the Notch 1 protein) with
29 a Notch ligand (e.g., Delta, Serrate, Jagged) results in two proteolytic cleavages of the
30 Notch protein, the second of which is catalyzed by a γ -secretase and releases the Notch
31 intracellular domain (NICD) into the cytoplasm. In the mouse Notch protein, this cleavage
32 occurs between amino acids gly1743 and val1744. The NICD translocates to the nucleus,
33 where it acts as a transcription factor, recruiting additional transcriptional regulatory
34 proteins (e.g., MAM, histone acetylases) to relieve transcriptional repression of various

1 target genes (e.g., Hes 1). Additional details and information regarding Notch signaling are
2 found, for example in Artavanis-Tsakonas et al. (1995) Science 268:225-232; Mumm
3 and Kopan (2000) Develop. Biol. 228:151-165 and Ehebauer et al. (2006) Sci. STKE 2006
4 (364), cm7. [DOI: 10.1126/stke.3642006cm7].

5 **[0071]** “Stroke” is the name given to conditions resulting from impairment of blood flow
6 in the brain. Such cerebrovascular impairment can result, for example, from intracranial
7 hemorrhage, or from reduction or blockage of blood flow in the brain (i.e., cerebral
8 ischemia). Ischemic blockages can result from thrombosis (i.e., formation of a clot in situ
9 in a cranial vessel or a vessel supplying the brain) or from a cerebral embolism migration
10 of a clot to a site in the brain). The damage resulting from ischemic or hemorrhagic stroke
11 usually results in impairment of certain neurological and physiological functions.
12 Additional information relating to different types of stroke, and their characteristics, is
13 found in co-owned U.S. Pat. Nos. 8,092,792 and 10,245,286; the disclosures of which are
14 incorporated by reference in their entireties herein for the purpose of describing different
15 types of stroke and their characteristics.

16 **[0072] Treatments**

17 **[0073]** Fig. 1 illustrates a method 100 of treating a subject with a stroke-induced motor
18 deficit. The stroke-induced motor deficit can be the result of an ischemic stroke suffered by
19 the subject. The method 100 can also be considered a method of treating a subject
20 following an ischemic stroke or a method of treating a subject following a small-volume
21 ischemic stroke.

22 **[0074]** The method 100 can comprise determining a volume of an ischemic core of the
23 subject with the stroke-induced motor deficit in operation 102. In some instances, operation
24 102 can comprise receiving data or information concerning the volume of the ischemic
25 core of the subject.

26 **[0075]** For purposes of the present disclosure, the ischemic core or infarct core can refer to
27 brain tissue that has already infarcted (i.e., suffered necrosis or tissue death) or is
28 irreversibly destined to infarct as a result of the ischemic stroke suffered by the subject.

29 **[0076]** The volume of the ischemic core can be determined using computed tomography
30 (CT) perfusion (CTP), diffusion-weighted magnetic resonance imaging (DWI), or, on some
31 occasions, baseline non-contrast CT (NCCT). When measured using CTP, the ischemic
32 core can be defined as brain tissue with a relative cerebral blood flow (CBF) level of less
33 than 30% of normal brain blood flow. When measured using DWI, the ischemic core can

1 be defined as brain tissue with an apparent diffusion coefficient of less than $620 \mu\text{m}^2/\text{s}$
2 [11].

3 **[0077]** In some instances, the method 100 can comprise proceeding with the cell therapy or
4 treatment only when the ischemic core is determined to be less than 50 cubic centimeters
5 (cc). For example, the method 100 can comprise only proceeding with the cell therapy or
6 treatment when the ischemic core is determined to be between about 2 cc and 50 cc.

7 **[0078]** The method 100 can further comprise determining a degree of disability of the
8 subject by determining an mRS score of the subject in operation 104. In some instances,
9 operation 104 can comprise receiving data or information concerning the mRS score of the
10 subject.

11 **[0079]** Although Fig. 1 illustrates operation 104 as following operation 102, it should be
12 understood by one of ordinary skill in the art that the order of such operations can be
13 switched or the operations can occur concurrently.

14 **[0080]** In some instances, the method 100 can comprise proceeding with the cell therapy or
15 treatment only when the mRS score of the subject is between 2 and 4. For example, those
16 with an mRS score of less than 2 can be considered not sufficiently disabled enough to
17 warrant the cell therapy disclosed herein and those with an mRS score of greater than 4 can
18 be considered too severely disabled for the treatment.

19 **[0081]** The method 100 can further comprise administering, to a brain region surrounding
20 the ischemic core of the subject, a therapeutically effective amount of cells in operation
21 106. In some instances, the brain region surrounding the ischemic core can be an ischemic
22 penumbra of the subject. The method 100 can comprise administering a therapeutically
23 effective amount of cells to the ischemic penumbra without injecting directly into the
24 ischemic core.

25 **[0082]** For purposes of the present disclosure, the ischemic penumbra can refer to hypo-
26 perfused brain tissue or brain tissue which is at risk for irreversible damage but is still
27 salvageable. Some studies have defined the ischemic penumbra as a region of reduced CBF
28 where CBF levels are reduced to between approximately 10 and 15 ml/100 g/min and
29 approximately 25 mL/100 g/min [12].

30 **[0083]** The method 100 can comprise administering, to the ischemic penumbra of the
31 subject, a therapeutically effective amount of cells in operation 106. In some instances, the
32 method 100 can comprise administering, to the ischemic penumbra of the subject, the
33 therapeutically effective amount of cells only when the volume of the ischemic core is
34 determined to be less than 50 cc. For example, the method 100 can comprise administering,

1 to the ischemic penumbra of the subject, the therapeutically effective amount of cells only
2 when the volume of the ischemic core is determined to be between about 2 cc and 50 cc.

3 **[0084]** In these and other instances, the method 100 can comprise administering, to the
4 ischemic penumbra of the subject, the therapeutically effective amount of cells only when
5 the mRS score of the subject is between 2 and 4. The method 100 can also comprise
6 administering, to the ischemic penumbra of the subject, the therapeutically effective
7 amount of cells only when the volume of the ischemic core is determined to be less than 50
8 cc and the mRS score of the subject is between 2 and 4.

9 **[0085]** As will be discussed in more detail in the following sections, the Applicant
10 discovered that in subjects with an mRS score of between 2 and 4 who had suffered a
11 small-volume stroke (e.g., ischemic core volume < 50 cc), a statistically significant
12 difference (p-value = 0.02) was observed in the proportion of such subjects in the treatment
13 group (i.e., those who were administered the cells) that exhibited clinically important
14 improvements in their motor functioning than those same subjects in the sham/control
15 group.

16 **[0086]** In certain instances, the method 100 can be considered a method of treating a
17 subject with a chronic stroke-induced motor deficit or a method of treating chronic stroke.
18 For example, the method 100 can comprise administering, to the brain region surrounding
19 the ischemic core of the subject, the therapeutically effective amount of cells only when the
20 ischemic stroke occurred more than six months prior (e.g., between six months and 90
21 months prior) to administering the cells.

22 **[0087]** The method 100 can also be considered a method of treating small-volume chronic
23 stroke (as opposed to acute stroke). For example, the method 100 can comprise
24 administering, to the brain region surrounding the ischemic core of the subject, the
25 therapeutically effective amount of cells only when the ischemic stroke occurred more than
26 six months prior (e.g., between six months and 90 months prior) to administering the cells
27 and only when the volume of the ischemic core is determined to be less than 50 cc.

28 **[0088]** When the stroke incident occurred more than six months prior to treatment (e.g.,
29 administering the cells), the penumbra surrounding the chronic ischemic core of the subject
30 can be considered a chronic penumbra (as opposed to an acute penumbra). The chronic
31 penumbra can exhibit CBF levels even less than those exhibited by the acute penumbra of
32 subjects who had recently suffered a stroke. In these instances, the method 100 of treating
33 such patients can comprise administering, to the chronic penumbra surrounding the chronic
34 ischemic core of the subject, the therapeutically effective amount of cells. In some

1 instances, the method 100 can comprise administering, to the chronic penumbra
2 surrounding the chronic ischemic core of the subject, the therapeutically effective amount
3 of cells only when the volume of the chronic ischemic core is determined to be less than 50
4 cc.

5 **[0089] DNTT-MSCs**

6 **[0090]** The cells administered can be allogeneic cells descended from mesenchymal stem
7 cells transiently-transfected by a polynucleotide encoding a Notch intracellular domain
8 (NICD). The cells can be made by a method comprising providing a culture of the
9 mesenchymal stem cells, contacting the culture of mesenchymal stem cells with the
10 polynucleotide encoding a NICD (where the polynucleotide does not encode a full-length
11 Notch protein), selecting cells that comprise the polynucleotide, and further culturing the
12 selected cells in the absence of selection for the polynucleotide. The mesenchymal stem
13 cells can be human bone marrow-derived cells. For purposes of this disclosure, the cells
14 administered can be referred to as DNTT-MSCs.

15 **[0091]** As previously discussed, DNTT-MSCs can be obtained from marrow adherent
16 stromal cells, also known as MSCs, by transiently expressing the intracellular domain of
17 the Notch protein in the MSCs. Transient expression of the Notch intracellular domain
18 (e.g., the NICD from the human Notch 1 protein)) in a MSC can be sufficient to convert a
19 population of MSCs into a population of DNTT-MSCs. Additional treatment with growth
20 and/or differentiation factors is not required. Thus, a population of MSCs can be converted
21 to a population of DNTT-MSCs by transient transfection of MSCs with a vector
22 comprising sequences encoding a NICD (but not encoding full-length Notch protein),
23 followed by selection for cells comprising the vector and further culture of the selected
24 cells in serum-containing medium, in the absence of exposure to additional growth and/or
25 differentiation factors. See, for example, U.S. Pat. No. 7,682,825 (Mar. 23, 2010); U.S.
26 Patent Application Publication No. 2010/0266554 (Oct. 21, 2010); and WO 2009/023251
27 (Feb. 19, 2009); the disclosures of which are incorporated herein by reference in their
28 entireties for the purposes of describing isolation of mesenchymal stem cells and
29 conversion of mesenchymal stem cells to DNTT-MSCs (denoted “neural precursor cells”
30 and “neural regenerating cells” in those documents).

31 **[0092]** In these methods, any polynucleotide encoding a Notch intracellular domain (e.g.,
32 vector) can be used, and any method for the selection and enrichment of transfected cells
33 can be used. For example, MSCs can be transfected with a vector containing sequences
34 encoding a Notch intracellular domain (e.g., the human Notch 1 intracellular domain) and

1 also containing sequences encoding a selection marker (e.g., drug resistance; e.g.,
2 resistance to G418). In some instances, two vectors, one containing sequences encoding a
3 Notch intracellular domain and the other containing sequences encoding a drug resistance
4 marker, can be used for transfection of MSCs. In these instances, selection is achieved,
5 after transfection of a cell culture with the vector or vectors, by adding a selective agent
6 (e.g., G418) to the cell culture in an amount sufficient to kill cells that do not comprise the
7 vector but spare cells that do. Absence of selection entails removal of said selective agent
8 or reduction of its concentration to a level that does not kill cells that do not comprise the
9 vector. Following selection (e.g., for seven days) the selective agent can be removed and
10 the cells can be further cultured (e.g., for two passages) in serum-containing culture
11 medium.

12 **[0093]** It is also possible, depending on the nature of the selection marker and/or the
13 concentration of the selective agent used, that not every cell that lacks a vector encoding a
14 selection marker will be killed during the selection process. For example, a selective agent
15 may inhibit growth of a cell not comprising the selection marker and, after removal of the
16 selective agent, that cell may recover and resume growth.

17 **[0094]** Preparation of DNTT-MSCs thus involves transient expression of an exogenous
18 Notch intracellular domain in a MSC. To this end, MSCs can be transfected with a vector
19 comprising sequences encoding a Notch intracellular domain (e.g., the human Notch 1
20 intracellular domain) wherein said sequences do not encode a full-length Notch protein. All
21 such sequences are known and readily available to those of skill in the art. For example,
22 Del Amo et al. (1993) Genomics 15:259-264 present the complete amino acid sequences of
23 the mouse Notch protein; while Mumm and Kopan (2000) Devel. Biol. 228:151-165
24 provide the amino acid sequence, from mouse Notch protein, surrounding the so-called S3
25 cleavage site which releases the intracellular domain. Taken together, these references
26 provide the skilled artisan with each and every peptide containing a Notch intracellular
27 domain that is not the full-length Notch protein; thereby also providing the skilled artisan
28 with every polynucleotide comprising sequences encoding a Notch intracellular domain
29 that does not encode a full-length Notch protein. The foregoing documents (Del Amo and
30 Mumm) are incorporated by reference in their entireties for the purpose of disclosing the
31 amino acid sequence of the full-length Notch protein and the amino acid sequence of the
32 Notch intracellular domain, respectively.

33 **[0095]** Similar information is available for Notch proteins and nucleic acids from
34 additional species, including rat, Xenopus, Drosophila and human. See, for example,

1 Weinmaster et al. (1991) Development 113:199-205; Schroeter et al.
2 (1998) Nature 393:382-386; NCBI Reference Sequence No. NM_017167 (and references
3 cited therein); SwissProt P46531 (and references cited therein); SwissProt Q01705 (and
4 references cited therein); and GenBank CAB40733 (and references cited therein). The
5 foregoing references are incorporated by reference in their entireties for the purpose of
6 disclosing the amino acid sequence of the full-length Notch protein and the amino acid
7 sequence of the Notch intracellular domain in a number of different species.

8 **[0096]** In some instances, DNTT-MSCs can be prepared by introducing, into MSCs, a
9 nucleic acid comprising sequences encoding a Notch intracellular domain such that the
10 MSCs do not express exogenous Notch extracellular domain. Such can be accomplished,
11 for example, by transfecting MSCs with a vector comprising sequences encoding a Notch
12 intracellular domain wherein said sequences do not encode a full-length Notch protein.

13 **[0097]** Additional details on the preparation of DNTT-MSCs, and methods for making
14 cells with properties similar to those of DNTT-MSCs which can be used in the methods
15 disclosed herein, can be found in U.S. Pat. No. 7,682,825 (Mar. 23, 2010); and U.S. Patent
16 Application Publication Nos. 2010/0266554 (Oct. 21, 2010) and 2011/0229442 (Sep. 22,
17 2011); the disclosures of which are incorporated herein by reference in their entireties for
18 the purposes of describing alternative methods for the preparation of, DNTT-MSCs, and
19 for providing methods for making cells with properties similar to those of DNTT-MSCs.
20 See also Dezawa et al. (2004) J. Clin. Invest. 113:1701-1710.

21 **[0098] Cell Culture**

22 **[0099]** Standard methods for cell culture are known in the art. See, for example, R. I.
23 Freshney "Culture of Animal Cells: A Manual of Basic Technique," Fifth Edition, Wiley,
24 New York, 2005.

25 **[0100] Transfection**

26 **[0101]** Methods for introduction of exogenous DNA into cells (i.e., transfection), and
27 selection of transfected cells, are also known in the art. See, for example, Sambrook et al.
28 "Molecular Cloning: A Laboratory Manual," Third Edition, Cold Spring Harbor
29 Laboratory Press, 2001; Ausubel et al., "Current Protocols in Molecular Biology," John
30 Wiley & Sons, New York, 1987 and periodic updates.

31 **[0102]** Fig. 2 depicts an ischemic core 200 of a subject having suffered an ischemic stroke.
32 As shown in Fig. 2, the ischemic core 200 can be surrounded by an ischemic penumbra
33 202. When the ischemic stroke occurred more than six-months prior to the commencement

1 of treatment, the ischemic core 200 can be considered a chronic ischemic core and the
2 ischemic penumbra 202 can be considered a chronic ischemic penumbra.

3 **[0103]** As previously discussed, the ischemic core 200 can have a core volume or ischemic
4 core volume. The core volume can be determined using, among other imaging techniques,
5 computed tomography perfusion or diffusion-weighted magnetic resonance imaging. When
6 the core volume is determined to be less than 50 cc (for example, between about 2 cc and
7 50 cc), the ischemic stroke can be considered a small-volume ischemic stroke.

8 **[0104]** Also, as previously discussed, a method of treating a small-volume ischemic stroke
9 can comprise administering a therapeutically effective amount of allogeneic cells to an
10 ischemic penumbra surrounding a small-volume ischemic core 200 of a subject having
11 suffered the small-volume ischemic stroke. The cells can be descended from mesenchymal
12 stem cells transiently-transfected by a polynucleotide encoding a NICD. For purposes of
13 this disclosure, the cells can be referred to as DNTT-MSCs. Moreover, when the ischemic
14 stroke occurred more than six-months prior to the commencement of the treatment, the
15 method can be considered a method of treating a small-volume chronic ischemic stroke.

16 **[0105]** As shown in Fig. 2, the method can comprise administering the therapeutically
17 effective amount of the cells by injecting a cell suspension comprising the cells at one or
18 more deposit sites 204 within the brain of the subject. The step of administering the
19 therapeutically effective amount of the cells can further comprise injecting at least part of
20 the cell suspension comprising the cells at one or more deposit sites 204 at an outer
21 periphery of the ischemic penumbra or chronic ischemic penumbra surrounding the small-
22 volume ischemic core. For example, the step of administering the therapeutically effective
23 amount of the cells can comprise injecting at least part of the cell suspension comprising
24 the cells at one or more deposit sites 204 proximal to the ischemic penumbra or chronic
25 ischemic penumbra surrounding the small-volume ischemic core.

26 **[0106]** The step of administering the therapeutically effective amount of the cells can also
27 comprise injecting at least part of the cell suspension comprising the cells at one or more
28 deposit sites 204 within the ischemic penumbra surrounding the small-volume ischemic
29 core. In some instances, the step of administering the therapeutically effective amount of
30 the cells can comprise injecting at least part of the cell suspension comprising the cells at
31 one or more deposit sites 204 distal to the ischemic penumbra or the ischemic chronic
32 penumbra. In these and other instances, the step of administering the therapeutically
33 effective amount of the cells can comprise injecting at least part of the cell suspension

1 comprising the cells at one or more deposit sites 204 within the small-volume ischemic
2 core.

3 **[0107]** The cell suspension can comprise the cells suspended in a pharmaceutically
4 acceptable carrier or diluent. In some instances, the pharmaceutically acceptable carrier or
5 diluent can be a sterile isotonic crystalloid solution. For example, the cell suspension can
6 comprise the cells suspended in Plasma-Lyte™ A (Baxter Healthcare Corporation). The
7 cells can also be suspended in another physiologically compatible carrier such as phosphate
8 buffered saline.

9 **[0108]** The cells can be administered stereotactically via a single burr-hole craniostomy.
10 Additional details on stereotactic administration of the cells can be found in U.S. Patent
11 Publication No. US 2019/0290846 (Sep. 26, 2019), the content of which is incorporated
12 herein by reference in its entirety for the purposes of describing stereotactic administration
13 of DNTT-MSCs and equipment used for such purposes.

14 **[0109]** The method can further comprise subjecting a formulated dose of the cells to post-
15 release testing prior to administering the cells to the subject.

16 **[0110]** The therapeutically effective amount of cells (or DNTT-MSCs) can be
17 approximately 2.5 million cells (or 2.5 million cells $\pm$ 0.1 million cells). When the amount
18 of cells administered is approximately 2.5 million cells, the treatment method can comprise
19 injecting a cell suspension comprising the cells at five deposit sites 204 (see Fig. 2) along a
20 first deposit track 206A or needle track, five deposit sites 204 along a second deposit track
21 206B or needle track, and five deposit sites along a third deposit track 206C or needle
22 track. Approximately 20- μ L of the cell suspension can be injected at each deposit site.
23 Moreover, the cell suspension can have a cell concentration of approximately $8.5 * 10^6$
24 cells/mL.

25 **[0111]** The therapeutically effective amount of cells (or DNTT-MSCs) can be
26 approximately 5.0 million cells (or 5.0 million cells $\pm$ 0.1 million cells). When the amount
27 of cells administered is approximately 5.0 million cells, the treatment method can comprise
28 injecting a cell suspension comprising the cells at five deposit sites 204 (see Fig. 2) along a
29 first deposit track 206A or needle track, five deposit sites 204 along a second deposit track
30 206B or needle track, and five deposit sites along a third deposit track 206C or needle
31 track. Approximately 20- μ L of the cell suspension can be injected at each deposit site.
32 Moreover, the cell suspension can have a cell concentration of approximately $17.0 * 10^6$
33 cells/mL.

1 **[0112]** In some cases, the therapeutically effective amount of cells (or DNTT-MSCs) can
2 be between approximately 2.5 million cells and 5.0 million cells. For example, the
3 therapeutically effective amount of cells (or DNTT-MSCs) can be approximately 3.0
4 million cells (or 3.0 million cells $\pm$ 0.1 million cells), 3.5 million cells (or 3.5 million cells
5 $\pm$ 0.1 million cells), 4.0 million cells (or 4.0 million cells $\pm$ 0.1 million cells), or 4.5 million
6 cells (or 4.5 million cells $\pm$ 0.1 million cells).

7 **[0113]** As shown in Fig. 2, at least some of the deposit sites 204 along each of the deposit
8 tracks or needle tracks can be within the ischemic penumbra or chronic ischemic
9 penumbra. In some instances, at least one or more of the deposit sites 204 along each of the
10 deposit tracks or needle tracks can be proximal to or at the outer periphery of the ischemic
11 penumbra or chronic ischemic penumbra. In other instances, all of the deposit tracks or
12 needle tracks can be within the ischemic penumbra or chronic ischemic penumbra. In
13 additional instances, at least one or more of the deposit sites 204 along each of the deposit
14 tracks or needle tracks can be within or at an outer periphery of the ischemic core or
15 chronic ischemic core.

16 **[0114]** In certain instances, the treatment is especially effective when the ischemic core
17 200 is located in a region, lobe, or area of the brain of the subject other than a parietal
18 region or lobe. For example, the method of treating the small-volume ischemic stroke can
19 comprise administering, the therapeutically effective amount of cells, to the brain region
20 (e.g., the ischemic penumbra 202) surrounding the ischemic core 200 of the subject only
21 when part of the ischemic core 200 is located in at least one of a cortical frontal region, a
22 cortical temporal region, a subcortical white matter, and a subcortical grey matter of the
23 brain of the subject (see, e.g., Fig. 5). In some instances, the method can comprise
24 administering, to the brain region (e.g., the ischemic penumbra 202) surrounding the
25 ischemic core 200 of the subject, the therapeutically effective amount of cells only when
26 the ischemic core 200 is located in a region of the brain of the subject other than a parietal
27 region.

28 **[0115] Composition, Formulations, and Kits**

29 **[0116]** Also disclosed are compositions, formulations, and kits for treating small-volume
30 ischemic stroke or small-volume chronic ischemic stroke. The composition can comprise a
31 therapeutically effective amount of cells and a pharmaceutically acceptable carrier or
32 diluent. As previously discussed, the cells, also referred to as DNTT-MSCs, can be
33 descended from mesenchymal stem cells transiently-transfected by a polynucleotide
34 encoding a Notch intracellular domain (NICD). The cells (DNTT-MSCs) can be made by a

1 process comprising providing a culture of the mesenchymal stem cells (e.g., human bone
2 marrow-derived cells) and contacting the culture of mesenchymal stem cells with the
3 polynucleotide encoding the NICD. For example, the mesenchymal stem cells can be
4 transiently-transfected with a plasmid comprising the polynucleotide encoding the NICD.
5 The process can further comprise selecting cells that comprise the polynucleotide, and
6 further culturing the selected cells in the absence of selection for the polynucleotide. In
7 certain instances, the polynucleotide does not encode a full-length Notch protein. The
8 compositions disclosed herein can be useful for, inter alia, stimulating proliferation and
9 differentiation of neural precursor cells and/or endothelial cells.

10 **[0117]** The “therapeutically effective amount” of the composition can comprise cells of an
11 amount suitable for treatment of small-volume ischemic stroke or small-volume chronic
12 ischemic stroke by, inter alia, stimulating proliferation and differentiation of neural
13 precursor cells and/or endothelial cells. In some instances, the therapeutically effective
14 amount of the composition can comprise approximately 2.5 million cells (or 2.5 million
15 cells $\pm$ 0.1 million cells). The therapeutically effective amount of the composition can also
16 comprise approximately 5.0 million cells (or 5.0 million cells $\pm$ 0.1 million cells). The
17 therapeutically effective amount of the composition can also comprise between
18 approximately 2.0 million cells and approximately 5.0 million cells.

19 **[0118]** In other instances, the therapeutically effective amount of the composition can vary
20 based on the nature and severity of the injury, the weight and general health of the subject
21 and other criteria that are known to those of skill in the art. For example, dosage amounts
22 can vary from about 100; 500; 1,000; 2,500; 5,000; 10,000; 20,000; 50,000; 100,000;
23 500,000; 1,000,000; 2,500,000; 5,000,000 to 10,000,000 cells or more (or any integral
24 value therebetween); with a frequency of administration of, e.g., a single dose, once per
25 day, twice per week, once per week, twice per month, once per month, depending upon,
26 e.g., body weight, route of administration, severity of disease, etc.

27 **[0119]** The cells described herein can be suspended in a pharmaceutically acceptable
28 carrier or diluent to form the cell suspension. The pharmaceutically acceptable carrier can
29 be a physiologically compatible carrier for implantation. As used herein, the term
30 “physiologically compatible carrier” can refer to a carrier that is compatible with the other
31 ingredients of the formulation and not deleterious to the recipient thereof. Examples of
32 suitable carriers or diluents include cell culture medium (e.g., Eagle’s minimal essential
33 medium), phosphate buffered saline, Hank’s balanced salt solution+/-glucose (HBSS), and
34 multiple electrolyte solutions. The pharmaceutically acceptable carrier or diluent can also

1 be or comprise a sterile isotonic crystalloid solution such as Plasma-Lyte™ A (Baxter
2 Healthcare Corporation).

3 **[0120]** Various pharmaceutical compositions and techniques for their preparation and use
4 are known to those of skill in the art in light of the present disclosure. For a detailed listing
5 of suitable pharmacological compositions and techniques for their administration one may
6 refer to texts such as Remington's Pharmaceutical Sciences, 17th ed. 1985; Brunton et al.,
7 "Goodman and Gilman's The Pharmacological Basis of Therapeutics," McGraw-Hill,
8 2005; University of the Sciences in Philadelphia (eds.), "Remington: The Science and
9 Practice of Pharmacy," Lippincott Williams & Wilkins, 2005; and University of the
10 Sciences in Philadelphia (eds.), "Remington: The Principles of Pharmacy Practice,"
11 Lippincott Williams & Wilkins, 2008.

12 **[0121]** The composition can comprise the cell suspension packaged in a sealed vial. In
13 some instances, the sealed vial can comprise 0.3 mL of the cell suspension with a cell
14 concentration of approximately 8.5×10^6 cells/mL. Alternatively, the sealed vial can
15 comprise 0.3 mL of the cell suspension with a cell concentration of approximately $17.0 \times$
16 10^6 cells/mL.

17 **[0122]** Also disclosed are other examples of materials which can serve as
18 pharmaceutically-acceptable carriers including: sugars, such as lactose, glucose and
19 sucrose; starches, such as corn starch and potato starch; cellulose and its derivatives, such
20 as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered
21 tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils,
22 such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean
23 oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and
24 polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents,
25 such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water;
26 isotonic saline; Ringer's solution; ethyl alcohol; phosphate buffer solutions; and other non-
27 toxic compatible substances employed in pharmaceutical formulations. Wetting agents,
28 emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as
29 coloring agents, release agents, coating agents, sweetening, flavoring and perfuming
30 agents, preservatives and antioxidants can also be present in the compositions.

31 **[0123]** Exemplary formulations include, but are not limited to, those suitable for parenteral
32 administration, e.g., intrapulmonary, intravenous, intra-arterial, intra-ocular, intra-cranial,
33 sub-meningeal, or subcutaneous administration, including formulations encapsulated in
34 micelles, liposomes or drug-release capsules (active agents incorporated within a

1 biocompatible coating designed for slow-release); ingestible formulations; formulations for
2 topical use, such as eye drops, creams, ointments and gels; and other formulations such as
3 inhalants, aerosols and sprays. The dosage of the compositions of the disclosure can vary
4 according to the extent and severity of the need for treatment, the activity of the
5 administered composition, the general health of the subject, and other considerations well
6 known to the skilled artisan.

7 **[0124]** In additional embodiments, the compositions described herein can also be delivered
8 locally. Localized delivery allows for the delivery of the composition non-systemically,
9 thereby reducing the body burden of the composition as compared to systemic delivery.
10 Such local delivery can be achieved, for example, through the use of various medically
11 implanted devices including, but not limited to, stents and catheters, or can be achieved by
12 inhalation, injection or surgery. Methods for coating, implanting, embedding, and
13 otherwise attaching desired agents to medical devices such as stents and catheters are
14 established in the art and contemplated herein.

15 **[0125]** Another aspect of the present disclosure relates to kits for carrying out the
16 administration of the cells to a subject. For example, the kit can comprise the composition
17 of cells, formulated as appropriate (e.g., in a pharmaceutical carrier), in one or more
18 separate pharmaceutical preparations.

19 **[0126]** Compositions comprising DNTT-MSCs can be used in combination with other
20 compositions comprising substances that stimulate angiogenesis (“pro-angiogenic agents”).
21 The compositions can be administered sequentially in any order or concurrently.
22 Accordingly, therapeutic compositions as disclosed herein can contain both DNTT-MSCs
23 and a pro-angiogenic agent. In additional embodiments, separate therapeutic compositions,
24 one comprising DNTT-MSCs and the other comprising a pro-angiogenic agent, can be
25 administered to the subject, either separately or together.

26 **[0127]** In some instances, the pro-angiogenic agent can be a protein (e.g., fibroblast growth
27 factor, platelet-derived growth factor, transforming growth factor alpha, hepatocyte growth
28 factor, vascular endothelial growth factor, sonic hedgehog, MAGP-2, HIF-1, PR-39,
29 RTEF-1, c-Myc, TFII, Egr-1, ETS-1) or a nucleic acid encoding such a protein. See, for
30 example, Vincent et al. (2007) Gene Therapy 14:781-789. In other instances, the pro-
31 angiogenic agent can be a small RNA molecule (e.g., siRNA, shRNA, microRNA) or a
32 ribozyme that targets a nucleic acid encoding an inhibitor of angiogenesis. Moreover, the
33 pro-angiogenic agent can be a triplex-forming nucleic acid that binds to DNA sequences

1 regulating the expression of a protein that inhibits angiogenesis, such as to block
2 transcription of the gene encoding the protein.

3 **[0128]** The pro-angiogenic agent can be a transcription factor that activates expression of a
4 pro-angiogenic molecule (e.g., protein). Naturally-occurring transcription factors (such as,
5 for example, HIF-1alpha) that regulate the expression of pro-angiogenic proteins, are
6 known. In addition, synthetic transcriptional regulatory proteins can be constructed by
7 genetic engineering. For example, methods for the design of zinc finger DNA-binding
8 domains that bind to a sequence of interest, and methods for the fusion of such zinc finger
9 DNA-binding domains to transcriptional activation and repression domains, have been
10 described. See, for example, U.S. Pat. Nos. 6,534,261; 6,607,882; 6,785,613; 6,794,136;
11 6,824,978; 6,933,169; 6,979,539; 7,013,219; 7,177,766; 7,220,719; and 7,788,044. These
12 methods can be used to synthesize non-naturally-occurring proteins that activate
13 transcription of any gene encoding a pro-angiogenic protein. In addition, synthetic zinc
14 finger transcriptional activators of the vascular endothelial growth factor (VEGF) gene
15 have been described. See, e.g., U.S. Pat. Nos. 7,026,462; 7,067,317; 7,560,440; 7,605,140;
16 and 8,071,564. Accordingly, a non-naturally-occurring (i.e., synthetic) zinc finger protein
17 that activates transcription of the VEGF gene can be used, in combination with SB623
18 cells, for augmenting angiogenesis, e.g., in the treatment of stroke. Furthermore, a natural
19 or synthetic transcriptional regulatory protein (e.g., a synthetic zinc finger transcriptional
20 regulatory protein) that inhibits transcription of an anti-angiogenic molecule can also be
21 used as a pro-angiogenic agent.

22

23 **Clinical Trial**

24 **[0129]** A randomized double-blind sham surgery-controlled clinical trial was conducted
25 evaluating the safety and efficacy of stereotactic intracranial injection of DNTT-MSCs
26 (also known as SB623 cells) in subjects suffering from chronic motor deficits as a result of
27 ischemic stroke. The subjects were randomized as part of the clinical trial at a ratio of
28 roughly 1:1:1 to receive: (1) approximately 2.5 million DNTT-MSCs, (2) approximately
29 5.0 million DNTT-MSCs, or (3) the sham surgery (the control group). A total of 158
30 subjects were randomized with 51 subjects receiving the sham surgery, 52 subjects
31 receiving the approximately 2.5 million DNTT-MSCs, and 55 subjects receiving the
32 approximately 5.0 million DNTT-MSCs.

33

34 **Study Population and Administration Procedure**

1 **[0130]** The study population included adult subjects with chronic motor deficits caused by
2 an ischemic stroke event occurring between 6 months and 90 months (7.5 years) prior. The
3 post-stroke interval of 6 months to 90 months was selected based on studies that had shown
4 that more than 90% of ischemic stroke subjects were stable by 90 days post-stroke. All
5 subjects also had an mRS score of between 2 and 4 at screening. Subjects with an mRS
6 score of less than 2 were deemed not sufficiently disabled to justify the risk of such a
7 procedure and subjects with an MRS score of greater than 4 were considered at increased
8 risk due to severe disability).

9 **[0131]** As previously discussed, a total of 158 subjects were randomized as part of the
10 clinical trial with 51 subjects receiving the sham surgery and 107 subjects receiving the
11 treatment (either the approximately 2.5 million or the approximately 5.0 million DNTT-
12 MSCs). Of the 158 subjects, 77 subjects had suffered a small-volume ischemic stroke or a
13 stroke with an ischemic core volume of less than 50 cc (based on CT or MRI scans
14 included as part of the subjects' medical history).

15 **[0132]** The subjects received either the sham surgery or an intracranial administration of
16 approximately 2.5 million DNTT-MSCs or approximately 5.0 million DNTT-MSCs. The
17 DNTT-MSCs were administered stereotactically through a single burr-hole craniostomy.

18 **[0133]** The one burr-hole craniostomy (about 1 cm to about 1.5 cm) was fashioned under
19 local anesthesia and sedation. The subject's dura was opened and an implantation cannula
20 was inserted. Five 20- μ L volumes of a cell suspension comprising the DNTT-MSCs were
21 injected slowly into five implantation sites selected by stereotactic targeting (so a total of
22 100- μ L of the cell suspension was injected among the five implantation sites along a single
23 deposit track). This procedure was repeated with two other deposit tracks with different
24 trajectories inserted through the same burr-hole craniostomy (see, e.g., Fig. 2). Those who
25 received the approximately 2.5 million DNTT-MSCs were injected with a cell suspension
26 having a target concentration of approximately 8.5×10^6 cells/mL and those who received
27 the approximately 5.0 million DNTT-MSCs were injected with a cell suspension having a
28 target concentration of approximately 17×10^6 cells/mL.

29 **[0134]** The sham surgical group received a sham surgery under local anesthesia and
30 sedation with the same stereotactic planning procedure, partial-thickness skull outer table
31 burr hole, and scalp sutures but no penetration of the dura matter or cell implantation. The
32 sham surgery procedure was scripted to mimic as closely as possible the procedure
33 undertaken by the treatment group.

[0135] The DNTT-MSCs or SB623 cells were provided as sterile cell suspensions in a unit volume of 1 mL containing $\geq 8 \times 10^6$ cells/mL and cryopreserved in CryoStore™ freezing media in a 2 mL vial. The cryopreserved cells were thawed, washed, centrifuged, and re-suspended in Plasma-Lyte A to achieve the aforementioned target concentrations. Prior to administration, the formulated dose for injection underwent post release testing and the formulated dose for injection was administered to the subject within three hours post-release testing.

Results of Clinical Trial

[0136] With respect to safety, DNTT-MSCs administered to subjects intracranially at doses of approximately 2.5 million cells or approximately 5.0 million cells were well tolerated in all subjects with stroke-induced chronic motor deficits.

[0137] With respect to efficacy, the study did not meet its primary efficacy endpoint of a statistically significant difference in the proportion of subjects in the “combined treatment group” (those who were administered either the ~2.5 million cells or the ~5.0 million cells) that exhibited an improvement of at least 10-points in their total FMMS over the control/sham group at week 24 (~month 6). The response rate was similar in the sham surgery group (~15.6%) and the combined treatment group (~15.0%) and there was no statistically significant difference between the combined treatment group response rate and the sham surgery response rate based on statistical analysis using generalized linear mixed model (GLMM) analysis or logistic regression analysis.

Retrospective Analysis of Clinical Trial Data

[0138] A retrospective analysis was conducted of data collected from the aforementioned clinical trial.

[0139] With respect to efficacy, one unexpected result discovered by the Applicant from the retrospective analysis was that in subjects who had suffered a small-volume stroke (e.g., ischemic core volume < 50 cc), a statistically significant difference (p-value = 0.02) was observed in the proportion of subjects in the combined treatment group (i.e., those who were administered either the ~2.5 million cells or the ~5.0 million cells) that exhibited clinically important improvements in their motor functioning compared to the sham/control group. In view of these unexpected findings, administration of DNTT-MSCs may be considered an effective treatment for subjects suffering from chronic motor deficits caused by a small-volume ischemic stroke.

[0140] In addition, another unexpected result discovered by the Applicant was that in subjects who had suffered a stroke in a region/lobe of the brain not in the parietal region/lobe (i.e., the ischemic of the stroke was located in a region/lobe of the brain not in the parietal region/lobe), a statistically significant difference (p-value = 0.05) was observed in the proportion of subjects in the combined treatment group that exhibited clinically important improvements in their motor functioning compared to the sham/control group.

[0141] Moreover, yet another unexpected result discovered by the Applicant was that in subjects who had suffered a small-volume stroke (e.g., ischemic core volume < 50 cc) in a region of the brain not in the parietal region/lobe, an even greater statistically significant difference (p-value < 0.01) was observed in the proportion of subjects in the combined treatment group that exhibited clinically important improvements in their motor functioning compared to the sham/control group.

[0142] As part of the retrospective analysis, clinical trial subjects were considered “Composite Responders” if they exhibited clinically meaningful improvements in their motor functioning by achieving at least one of the following MCID thresholds (the “MCID Thresholds”):

1. Change from baseline (“CFB”) improvement in their total FMMS at week 24 (“W24”) of at least 9 points (W24 CFB Total FMMS Improvement $\geq$ 9 points)
2. CFB improvement in their total FMMS UE at week 24 of at least 6 points (W24 CFB FMMS UE Improvement $\geq$ 6 points)
3. CFB improvement in their total FMMS LE at week 24 of at least 4 points (W24 CFB FMMS LE Improvement $\geq$ 4 points)

[0143] Tables 1 and 2 below show the composite response rates, average baseline scores, and average stroke volumes for the overall population and the small-volume stroke subpopulation, respectively.

Table 1: Treatment and control group values at 24 weeks for overall population

Group	Count	Composite Responders	Response Rate	Average Baseline FMMS	Average Baseline mRS	Average Volume of Stroke
Combined Treatment Group	107	42	39%	44.87	2.74	53.19

Control Group	51	16	31%	47.35	2.49	56.32
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1

2 Table 2: Treatment and control group values at 24 weeks for small-volume stroke

3 (ischemic core volume < 50 cc) population

4

Group	Count	Composite Responders	Response Rate	Average Baseline FMMS	Average Baseline mRS	Average Volume of Stroke
Combined Treatment Group	51	25	49%	48.55	2.69	15.26
Control Group	26	5	19%	49.42	2.54	14.4

5

6 [0144] Table 3 below shows the delta composite response rates and their accompanying p-values for the overall population and the small-volume stroke subpopulation. For purposes of this disclosure, a “delta composite response rate” refers to a difference in response rates between the treatment group and the control group.

10

11 Table 3: Delta composite response values and accompanying p-values for overall population and small-volume stroke population at 24 weeks

13

Group	Treatment Count	Treatment Composite Responders	Control Count	Control Composite Responders	Delta Composite Response Rate	p-value of Delta Composite Response Rate
Overall Population	107	42	51	16	8%	0.42
Small-Volume Stroke Population	51	25	26	5	30%	0.02

14

1 [0145] As shown by Tables 1-3 above, the population subsegment with small-volume
2 stroke (e.g., patients who suffered an ischemic stroke with an ischemic core volume < 50
3 cc) had a significantly higher response rate (~49%) in the treatment arm in comparison to
4 the control arm (~19%). The delta composite response rate of ~30% for this population
5 subsegment was determined to be statistically significant (p-value = 0.02) based on GLMM
6 and logistic regression analysis. This is in stark contrast to the delta composite response
7 rate of 8% for the overall population, which was determined to be not statistically
8 significant (p-value = 0.42).

9 [0146] The retrospective analysis also determined that this statistically significant delta
10 composite response rate is driven by the subjects' volume of stroke rather than the subjects'
11 baseline mRS. More specifically, the subjects' volume of stroke did not have any
12 considerable effect (and did not materially impact) the subjects' baseline mRS.

13 [0147] Figs. 3A-3C are plots illustrating the relationship between baseline FMMS, baseline
14 mRS, and volume of stroke (e.g., the volume of the stroke patients' ischemic core) for the
15 2.5 million (2.5 M) dosage group, the 5.0 million (5.0 M) dosage group, and the control
16 group. Fig. 3A illustrates the relationship between baseline FMMS and volume of stroke
17 for the 2.5 M dosage group, the 5.0 M dosage group, and the control group. A Spearman
18 correlation was calculated between these two factors yielding a correlation of -0.23 with a
19 p-value of 0.002. This indicates that the subjects' baseline FMMS is significantly
20 correlated with the subjects' volume of stroke meaning that those with smaller stroke
21 volumes generally exhibited greater motor function than those with larger stroke volumes.

22 [0148] Fig. 3B illustrates the relationship between baseline mRS and baseline FMMS for
23 the 2.5 M dosage group, the 5.0 M dosage group, and the control group. A Spearman
24 correlation was calculated between these two factors yielding a correlation of -0.34 with a
25 p-value of <0.001. This indicates that the subjects' baseline mRS is significantly correlated
26 with the subjects' baseline FMMS meaning that those subjects with less baseline motor
27 function generally were more disabled than subjects with greater baseline motor function.

28 [0149] Fig. 3C illustrates the relationship between baseline mRS and volume of stroke for
29 the 2.5 M dosage group, the 5.0 M dosage group, and the control group. A Spearman
30 correlation was calculated between these two factors yielding a correlation of 0.06 with a
31 p-value of 0.44. This indicates that the subjects' baseline mRS is not significantly
32 correlated with the subjects' volume of stroke.

33

34 Dosage Comparison

1 **[0150]** Table 4 below shows composite response rates, average baseline scores, and
 2 average stroke volumes of all subjects broken down by treatment dosage, the control
 3 population, and the overall population.

4

5 Table 4: Composite response rates, average baseline scores, and average stroke volumes for
 6 various populations at 24 weeks

Population	Count	Composite Responders	Response Rate	Average Baseline FMMS	Average Baseline mRS	Average Volume of Stroke
2.5 M Cell Dose	52	24	46.15%	44.38	2.65	48.82
5.0 M Cell Dose	55	18	32.73%	45.33	2.82	57.33
Control	51	16	31.37%	47.35	2.49	56.32
Overall (2.5 M + 5.0 M + Control)	158	58	36.71%	45.67	2.66	54.20

7

8 **[0151]** Table 5 below shows composite response rates, average baseline scores, and
 9 average stroke volumes of subjects with small-volume strokes (e.g., patients who suffered
 10 an ischemic stroke with an ischemic core volume < 50 cc) broken down by treatment
 11 dosage, the control population, and the overall population.

12

13 Table 5: Composite response rates, average baseline scores, and average stroke volumes for
 14 various small-volume stroke populations at 24 weeks

15

Population	Count	Composite Responders	Response Rate	Average Baseline FMMS	Average Baseline mRS	Average Volume of Stroke
2.5 M Cell Dose	29	16	55.17%	48.17	2.66	15.02
5.0 M Cell Dose	22	9	40.91%	49.05	2.73	15.58
Control	26	5	19.23%	49.42	2.54	14.40
Overall (2.5 M + 5.0 M + Control)	77	30	38.96%	48.84	2.64	14.97

16

1 **[0152]** Table 6 below shows composite response rates, average baseline scores, and
 2 average stroke volumes of subjects with stroke volumes equal to or greater than 50 cc (e.g.,
 3 patients who suffered an ischemic stroke with an ischemic core volume ≥ 50 cc) broken
 4 down by treatment dosage, the control population, and the overall population.

5
 6 Table 6: Composite response rates, average baseline scores, and average stroke volumes for
 7 various stroke subject populations with stroke volumes equal to or greater than 50 cc at 24
 8 weeks

Population	Count	Composite Responders	Response Rate	Average Baseline FMMS	Average Baseline mRS	Average Volume of Stroke
2.5 M Cell Dose	23	8	34.78%	39.61	2.65	91.43
5.0 M Cell Dose	33	9	27.27%	42.85	2.88	85.17
Control	25	11	44.00%	45.20	2.44	99.91
Overall (2.5 M + 5.0 M + Control)	81	28	34.57%	42.65	2.68	91.50

10

11 **[0153]** Table 7 below shows composite response rates, average baseline scores, and
 12 average stroke volumes of subjects with stroke volumes equal to or greater than 100 cc
 13 (e.g., patients who suffered an ischemic stroke with an ischemic core volume ≥ 100 cc)
 14 broken down by treatment dosage, the control population, and the overall population.

15

16 Table 7: Composite response rates, average baseline scores, and average stroke volumes for
 17 various stroke subject populations with stroke volumes equal to or greater than 100 cc at 24
 18 weeks

19

Population	Count	Composite Responders	Response Rate	Average Baseline FMMS	Average Baseline mRS	Average Volume of Stroke
2.5 M Cell Dose	9	3	33.33%	33.00	3.00	114.08
5.0 M Cell Dose	7	2	28.57%	37.14	3.14	132.83
Control	13	5	38.46%	46.46	2.23	124.82

Overall (2.5 M + 5.0 M + Control)	29	10	34.48%	40.03	2.69	123.42
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1

2 **[0154]** Tables 4-7 above shows that when treatment populations are subdivided by dosage
3 (e.g., 2.5 M cell dosage or the 5.0 M cell dosage) and compared with the control
4 population, those subjects who were administered approximately 2.5 million DNTT-MSCs
5 responded better than those subjects who were administered approximately 5.0 million
6 DNTT-MSCs. This was maintained across all stroke-volume subpopulations as shown in
7 Tables 5-7.

8 **[0155]** Fig. 4 illustrates that the delta composite response rate (or the difference in the
9 composite response rate between the treatment group and the control group) for the 2.5 M
10 dosage population exceeded the delta composite response rate for the 5.0 M dosage
11 population across almost all subject stroke-volumes. As can be seen Fig. 4, the difference
12 in the delta composite response rates is especially pronounced in subjects with small-
13 volume strokes (e.g., patients who suffered an ischemic stroke with an ischemic core
14 volume < 50 cc). These results were unexpected and suggest that a dosage of 2.5 million
15 DNTT-MSCs may be an even more therapeutically effective dose for treatment of chronic
16 ischemic stroke than a dosage of 5.0 million DNTT-MSCs, despite the latter having more
17 cells. This may be especially true for subjects with small-volume strokes (e.g., patients who
18 suffered an ischemic stroke with an ischemic core volume < 50 cc).

19 **[0156]** The graph in Fig. 4 excluded results for subjects with stroke-volumes below 2 cc
20 (e.g., patients who suffered an ischemic stroke with an ischemic core volume < 2 cc) as the
21 number of subjects with stroke-volumes less than 2 cc in the control group greatly
22 exceeded the number of such subjects in the 5.0 M dosage group from a relative standpoint.

23 **[0157]** Tables 5-7 also shows that with the exception of the small-volume stroke
24 subpopulation (e.g., patients who suffered an ischemic stroke with an ischemic core
25 volume < 50 cc) (see Table 5), the control group actually responded better than either of the
26 dosage groups for those subjects with stroke volumes equal to or greater than 50 cc (see
27 Table 6) or equal to or greater than 100 cc (see Table 7). These results indicate that DNTT-
28 MSCs may be less effective for treating chronic stroke patients with ischemic core volumes
29 greater than 50 cc.

30

1 Location of Stroke

2 **[0158]** The retrospective analysis also examined the efficacy of the treatment based on the
 3 stroke location. Among the subject population, strokes (or, more aptly, the ischemic cores
 4 of such strokes) were recorded in the following regions/areas of the brain: (1) subcortical
 5 white matter, (2) subcortical grey matter, (3) cortical frontal region, (4) cortical parietal
 6 region, and (5) cortical temporal region. Fig. 5 illustrates these regions/areas of the brain
 7 along with other regions/areas of the brain.

8 **[0159]** As previously mentioned, one unexpected result discovered by the Applicant was
 9 that in subjects who had suffered a stroke in a region of the brain not in the cortical parietal
 10 region/lobe, a statistically significant difference (p-value = 0.05) was observed in the
 11 proportion of subjects in the combined treatment group that exhibited clinically important
 12 improvements in their motor functioning compared to the sham/control group.

13 **[0160]** Moreover, yet another unexpected result discovered by the Applicant was that in
 14 subjects who had suffered a small-volume stroke (e.g., ischemic core volume < 50 cc) in a
 15 region of the brain not in the cortical parietal region/lobe, an even greater statistically
 16 significant difference (p-value < 0.01) was observed in the proportion of subjects in the
 17 combined treatment group that exhibited clinically important improvements in their motor
 18 functioning compared to the sham/control group.

19 **[0161]** Table 8 below shows the delta composite response rates and their accompanying p-
 20 values for the overall population and the subject population that did not suffer a stroke in
 21 their cortical parietal region.

22

23 Table 8: Delta composite response values and accompanying p-values for overall
 24 population and subjects who did not suffer a stroke in their cortical-parietal region at 24
 25 weeks

26

Group	Treatment Count	Treatment Composite Responders	Control Count	Control Composite Responders	Delta Composite Response Rate	p-value of Delta Composite Response Rate
Overall Population	107	42	51	16	8%	0.42

No Stroke in Cortical Parietal Region	62	26	21	4	23%	0.05
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1

2 **[0162]** Table 9 below shows various delta response rates and scores at 24 weeks for those
3 in the small-volume stroke population (ischemic core volume < 50 cc) who either suffered
4 or did not suffer a stroke in their cortical parietal regions.

5

6 Table 9: Response rates and scores at 24 weeks for small-volume stroke population broken
7 down by presence or absence of stroke in cortical parietal region.

8

Stroke in Cortical Parietal	Treatment Count	Treatment Composite Response Rate	Control Count	Control Composite Response Rate	Delta Composite Response Rate	Avg. Baseline FMMS	Avg. Baseline mRS	Av. Vol. of Stroke
No	40	52.5%	15	6.7%	45.8% (p-value < 0.01)	47.95	2.71	11.59
Yes	11	36.4%	11	36.4%	0%	51.09	2.45	23.42

9

10 **[0163]** As shown by Table 8 above, the subjects in the study population that did not suffer
11 a stroke in their cortical parietal region had a significantly higher response rate (~42%) in
12 the treatment arm in comparison to the control arm (~19%). The delta composite response
13 rate of ~23% for this population subsegment was determined to be statistically significant
14 (p-value = 0.05) based on GLMM or logistic regression analysis.

15 **[0164]** Table 9 above shows that the best delta composite response rate (45.8%) was
16 observed in the segment of the population with small-volume strokes (ischemic core
17 volume < 50 cc) who did not suffer a stroke in their cortical parietal region.

18 **[0165]** In view of these unexpected findings, administration of DNTT-MSCs may be
19 considered an effective treatment for subjects suffering from chronic motor deficits caused
20 by a ischemic stroke where the ischemic core was not located in the subjects' cortical
21 parietal region. Moreover, administration of DNTT-MSCs may be an even more effective
22 treatment for subjects suffering from chronic motor deficits caused by a small-volume
23 ischemic stroke where the small-volume (core volume < 50 cc) ischemic core was not
24 located in the subjects' cortical parietal region.

1 **[0166]** Fig. 6 is a reference table generated as part of the retrospective analysis comprising
2 information concerning the stroke location, baseline characteristics, and delta response
3 rates of the study population broken down by population percentage.

4 **[0167]** Fig. 7 is a plot showing the delta composite response rates of Fig. 6 plotted against
5 the population percentages. As shown in Fig. 6, point P1 on the graph represents
6 approximately 9% of the population and includes subjects with low stroke-volume
7 (ischemic core volume < 50 cc) and where the stroke did not occur in the subjects'
8 subcortical grey matter or the cortical frontal region, point P2 on the graph represents
9 approximately 11% of the population and includes subjects with low stroke-volume and
10 where the stroke did not occur in the subjects' cortical parietal region, point P3 on the
11 graph represents approximately 25% of the population and includes subjects with low
12 stroke-volume and where the stroke did not occur in the subjects' cortical temporal region,
13 point P4 on the graph represents approximately 31% of the population and includes
14 subjects with low stroke-volume and where the stroke did not occur in either the cortical
15 parietal region or the cortical temporal region, point P5 on the graph represents
16 approximately 47% of the population and includes all study subjects with low stroke-
17 volume (regardless of stroke location), point P6 on the graph represents approximately
18 51% of the population and includes all study subjects where the stroke did not occur in the
19 subjects' cortical parietal region, point P7 on the graph represents approximately 63% of
20 the population and includes all study subjects where the stroke occurred at least partly in
21 the subjects' subcortical white matter, point P8 on the graph represents approximately 79%
22 of the population and includes all study subjects who received cells processed in less than
23 180 minutes (considered a low cell processing time), and point P9 on the graph represents
24 approximately 97% of the population (essentially, the entire study population).

25 **[0168]** Figs. 6 and 7 illustrate that the population segments represented by points P5 (low
26 stroke-volume) and P6 (no stroke in cortical parietal region) were the only two population
27 segments where the response rates, including the delta composite response rate, were
28 calculated from close to 50% of the total study population and both delta composite
29 response rates were statistically significant.

30 **[0169]** It should also be noted that there is noticeable overlap between the P5 (low-stroke
31 volume) and P6 (no stroke in cortical parietal region) populations as 55 out of the 83
32 subjects with no stroke in their cortical parietal regions also had low-volume strokes.

33

34 Progress of Composite Responders at 48 Weeks

[0170] In order to establish whether subjects who were W24 composite responders also showed improvement in their disability scores 24 weeks later (i.e., at 48 weeks or W48), clinical trial data at W48 was also analyzed for mRS CFB for the overall population and the subpopulation with low-stroke volume (ischemic core volume < 50 cc).

[0171] Tables 10 and 11 below show W48 mRS changes for the overall population and the low-stroke volume subpopulation, respectively. It is important to note that a reduction in a subject's mRS is an improvement in the subject's degree of disability or disability level.

Table 10: mRS change of composite responders and non-responders in overall population at 48 weeks

	Combined Treatment Group Count (n)	Control Group Count (n)	Combined Treatment Group W48 mRS CFB	Control Group W48 mRS CFB	Delta W48 mRS CFB
Composite Responders	42	16	-0.14	0.13	-0.27
Composite Non-Responders	65	35	-0.11	-0.09	-0.02

Table 11: mRS change of composite responders and non-responders in low-stroke volume subpopulation at 48 weeks

	Combined Treatment Group Count (n)	Control Group Count (n)	Combined Treatment Group W48 mRS CFB	Control Group W48 mRS CFB	Delta W48 mRS CFB
Composite Responders	25	5	-0.20	0.20	-0.40
Composite Non-Responders	26	21	-0.12	-0.10	-0.02

[0172] As shown in Tables 10 and 11 above, composite responders in the low-stroke volume (ischemic core volume < 50 cc) subpopulation exhibited better delta mRS CFB at 48 weeks than the overall population. Since improvement in a subject's motor response is a

1 prerequisite to improvement in the subject's disability (this is known as the "lead lag
2 effect"), the results show that the lead lag effect is present in composite responders in both
3 the overall population and the low-stroke volume subpopulation at week 48 and the effect
4 is more prominent in composite responders in the low-stroke volume subpopulation.

5 **[0173]** A number of embodiments have been described. Nevertheless, it will be understood
6 by one of ordinary skill in the art that various changes and modifications can be made to
7 this disclosure without departing from the spirit and scope of the embodiments. Elements
8 of systems, devices, apparatus, and methods shown with any embodiment are exemplary
9 for the specific embodiment and can be used in combination or otherwise on other
10 embodiments within this disclosure. For example, the steps of any methods depicted in the
11 figures or described in this disclosure do not require the particular order or sequential order
12 shown or described to achieve the desired results. In addition, other steps operations may
13 be provided, or steps or operations may be eliminated or omitted from the described
14 methods or processes to achieve the desired results. Moreover, any components or parts of
15 any apparatus or systems described in this disclosure or depicted in the figures may be
16 removed, eliminated, or omitted to achieve the desired results. In addition, certain
17 components or parts of the systems, devices, or apparatus shown or described herein have
18 been omitted for the sake of succinctness and clarity.

19 **[0174]** Accordingly, other embodiments are within the scope of the following claims and
20 the specification and/or drawings may be regarded in an illustrative rather than a restrictive
21 sense.

22 **[0175]** Each of the individual variations or embodiments described and illustrated herein
23 has discrete components and features which may be readily separated from or combined
24 with the features of any of the other variations or embodiments. Modifications may be
25 made to adapt a particular situation, material, composition of matter, process, process act(s)
26 or step(s) to the objective(s), spirit or scope of the present invention.

27 **[0176]** Methods recited herein may be carried out in any order of the recited events that is
28 logically possible, as well as the recited order of events. Moreover, additional steps or
29 operations may be provided or steps or operations may be eliminated to achieve the desired
30 result.

31 **[0177]** Furthermore, where a range of values is provided, every intervening value between
32 the upper and lower limit of that range and any other stated or intervening value in that
33 stated range is encompassed within the invention. Also, any optional feature of the
34 inventive variations described may be set forth and claimed independently, or in

1 combination with any one or more of the features described herein. For example, a
2 description of a range from 1 to 5 should be considered to have disclosed subranges such as
3 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
4 numbers within that range, for example 1.5, 2.5, etc. and any whole or partial increments
5 therebetween.

6 **[0178]** All existing subject matter mentioned herein (e.g., publications, patents, patent
7 applications) is incorporated by reference herein in its entirety except insofar as the subject
8 matter may conflict with that of the present invention (in which case what is present herein
9 shall prevail). The referenced items are provided solely for their disclosure prior to the
10 filing date of the present application. Nothing herein is to be construed as an admission that
11 the present invention is not entitled to antedate such material by virtue of prior invention.

12 **[0179]** Reference to a singular item, includes the possibility that there are plural of the
13 same items present. More specifically, as used herein and in the appended claims, the
14 singular forms “a,” “an,” “said” and “the” include plural referents unless the context clearly
15 dictates otherwise. It is further noted that the claims may be drafted to exclude any optional
16 element. As such, this statement is intended to serve as antecedent basis for use of such
17 exclusive terminology as “solely,” “only” and the like in connection with the recitation of
18 claim elements, or use of a “negative” limitation. Unless defined otherwise, all technical
19 and scientific terms used herein have the same meaning as commonly understood by one of
20 ordinary skill in the art to which this invention belongs.

21 **[0180]** Reference to the phrase “at least one of”, when such phrase modifies a plurality of
22 items or components (or an enumerated list of items or components) means any
23 combination of one or more of those items or components. For example, the phrase “at
24 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
25 and C; or (vii) A and C.

26 **[0181]** In understanding the scope of the present disclosure, the term “comprising” and its
27 derivatives, as used herein, are intended to be open-ended terms that specify the presence
28 of the stated features, elements, components, groups, integers, and/or steps, but do not
29 exclude the presence of other unstated features, elements, components, groups, integers
30 and/or steps. The foregoing also applies to words having similar meanings such as the
31 terms, “including”, “having” and their derivatives. Also, the terms “part,” “section,”
32 “portion,” “member” “element,” or “component” when used in the singular can have the
33 dual meaning of a single part or a plurality of parts. As used herein, the following
34 directional terms “forward, rearward, above, downward, vertical, horizontal, below,

1 transverse, laterally, and vertically” as well as any other similar directional terms refer to
2 those positions of a device or piece of equipment or those directions of the device or piece
3 of equipment being translated or moved.

4 **[0182]** Finally, terms of degree such as “substantially”, “about” and “approximately” as
5 used herein mean the specified value or the specified value and a reasonable amount of
6 deviation from the specified value (e.g., a deviation of up to $\pm 0.1\%$, $\pm 1\%$, $\pm 5\%$, or $\pm 10\%$,
7 as such variations are appropriate) such that the end result is not significantly or materially
8 changed. For example, “about 1.0 cm” can be interpreted to mean “1.0 cm” or between
9 “0.9 cm and 1.1 cm.” When terms of degree such as “about” or “approximately” are used
10 to refer to numbers or values that are part of a range, the term can be used to modify both
11 the minimum and maximum numbers or values.

12 **[0183]** This disclosure is not intended to be limited to the scope of the particular forms set
13 forth, but is intended to cover alternatives, modifications, and equivalents of the variations
14 or embodiments described herein. Further, the scope of the disclosure fully encompasses
15 other variations or embodiments that may become obvious to those skilled in the art in
16 view of this disclosure.

17

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CLAIMS

1 We claim:

- 2 1. A method of treating a subject following a small-volume ischemic stroke suffered
3 by the subject, comprising:
4 administering, to a brain region surrounding a small-volume ischemic core of the
5 subject, a therapeutically effective amount of cells, wherein the cells are
6 descended from mesenchymal stem cells transiently-transfected by a
7 polynucleotide encoding a Notch intracellular domain (NICD).
8
- 9 2. The method of claim 1, wherein the small-volume ischemic core is an ischemic core
10 having an ischemic core volume of less than 50 cubic centimeters (cc).
- 11 3. The method of claim 1, wherein the small-volume ischemic core is an ischemic core
12 having an ischemic core volume of between about 2 cc and 50 cc.
- 13 4. The method of claim 1, wherein administering the therapeutically effective amount
14 of the cells further comprises injecting at least part of a cell suspension comprising
15 the cells at one or more deposit sites at an outer periphery of a chronic penumbra
16 surrounding the small-volume ischemic core.
- 17 5. The method of claim 1, wherein administering the therapeutically effective amount
18 of the cells further comprises injecting at least part of a cell suspension comprising
19 the cells at one or more deposit sites proximal to or within a chronic penumbra
20 surrounding the small-volume ischemic core.
- 21 6. The method of claim 1, wherein administering the therapeutically effective amount
22 of the cells further comprises injecting at least part of a cell suspension comprising
23 the cells at one or more deposit sites distal to a chronic penumbra surrounding the
24 small-volume ischemic core.
- 25 7. The method of claim 1, wherein administering the therapeutically effective amount
26 of the cells further comprises administering the therapeutically effective amount of
27 the cells stereotactically via a single burr-hole craniostomy.
- 28 8. The method of claim 1, wherein the small-volume ischemic core is located in a
29 region of the brain of the subject other than a parietal region.
- 30 9. The method of claim 1, wherein at least part of the small-volume ischemic core is
31 located in at least one of a cortical frontal region, a cortical temporal region, a
32 subcortical white matter, and a subcortical grey matter of the brain of the subject.
- 33 10. The method of claim 1, wherein the cells are made by a method comprising:
34 providing a culture of the mesenchymal stem cells;

- 1 contacting the culture of mesenchymal stem cells with the polynucleotide encoding
2 the NICD, wherein the polynucleotide does not encode a full-length Notch
3 protein,
4 selecting cells that comprise the polynucleotide; and
5 further culturing the selected cells in the absence of selection for the
6 polynucleotide.
- 7 11. The method of claim 1, wherein the therapeutically effective amount of cells is
8 approximately 2.5 million cells.
- 9 12. The method of claim 11, wherein administering the approximately 2.5 million cells
10 comprises injecting a cell suspension comprising the cells at five deposit sites along
11 a first deposit track, five deposit sites along a second deposit track, and five deposit
12 sites along a third deposit track, wherein approximately 20-μL of the cell
13 suspension is injected at each deposit site and wherein the cell suspension has a cell
14 concentration of approximately 8.5×10^6 cells/mL.
- 15 13. The method of claim 1, wherein the therapeutically effective amount of cells is
16 approximately 5.0 million cells.
- 17 14. The method of claim 13, wherein administering the approximately 5.0 million cells
18 comprises injecting a cell suspension comprising the cells at five deposit sites along
19 a first deposit track, five deposit sites along a second deposit track, and five deposit
20 sites along a third deposit track, wherein approximately 20-μL of the cell
21 suspension is injected at each deposit site and wherein the cell suspension has a cell
22 concentration of approximately 17.0×10^6 cells/mL.
- 23 15. The method of claim 1, wherein the small-volume ischemic stroke occurred more
24 than six months prior to administering the cells.
- 25 16. The method of claim 15, wherein the small-volume ischemic stroke occurred
26 between six months and 90 months prior to administering the cells.
- 27 17. The method of claim 1, further comprising evaluating a degree of disability of the
28 subject by determining an mRS score of the subject and administering the
29 therapeutically effective amount of cells only when the mRS score of the subject is
30 between 2 and 4.
- 31 18. The method of claim 1, wherein the mesenchymal stem cells are human bone
32 marrow-derived cells.
- 33 19. The method of claim 1, wherein the therapeutically effective amount of cells are
34 suspended in a sterile isotonic crystalloid solution.

- 1 20. The method of claim 1, further comprising subjecting a formulated dose of the cells
2 to post-release testing prior to administering the cells to the subject.
- 3 21. A method of treating a subject with a stroke-induced motor deficit, comprising:
4 determining a volume of an ischemic core of the subject; and
5 administering, to a brain region surrounding the ischemic core of the subject, a
6 therapeutically effective amount of cells only when the volume of the ischemic
7 core is determined to be less than 50 cubic centimeters (cc), wherein the cells
8 are descended from mesenchymal stem cells transiently-transfected by a
9 polynucleotide encoding a Notch intracellular domain (NICD).
- 10 22. The method of claim 21, further comprising administering, to the brain region
11 surrounding the ischemic core of the subject, the therapeutically effective amount of
12 cells only when the volume of the ischemic core is between about 2 cc and 50 cc.
- 13 23. The method of claim 21, wherein administering the therapeutically effective
14 amount of the cells further comprises injecting at least part of a cell suspension
15 comprising the cells at one or more deposit sites at an outer periphery of a chronic
16 penumbra surrounding the ischemic core.
- 17 24. The method of claim 21, wherein administering the therapeutically effective
18 amount of the cells further comprises injecting at least part of a cell suspension
19 comprising the cells at one or more deposit sites proximal to or within a chronic
20 penumbra surrounding the ischemic core.
- 21 25. The method of claim 21, wherein administering the therapeutically effective
22 amount of the cells further comprises injecting at least part of a cell suspension
23 comprising the cells at one or more deposit sites distal to a chronic penumbra
24 surrounding the ischemic core.
- 25 26. The method of claim 21, wherein administering the therapeutically effective
26 amount of the cells further comprises administering the therapeutically effective
27 amount of the cells stereotactically via a single burr-hole craniostomy.
- 28 27. The method of claim 21, wherein the ischemic core is located in a region of the
29 brain of the subject other than a parietal region.
- 30 28. The method of claim 21, wherein at least part of the ischemic core is located in at
31 least one of a cortical frontal region, a cortical temporal region, a subcortical white
32 matter, and a subcortical grey matter of the subject.
- 33 29. The method of claim 21, wherein the cells are made by a method comprising:
34 providing a culture of the mesenchymal stem cells;

- 1 contacting the culture of mesenchymal stem cells with the polynucleotide encoding
2 the NICD, wherein the polynucleotide does not encode a full-length Notch
3 protein,
4 selecting cells that comprise the polynucleotide; and
5 further culturing the selected cells in the absence of selection for the
6 polynucleotide.
- 7 30. The method of claim 21, wherein the therapeutically effective amount of cells is
8 approximately 2.5 million cells.
- 9 31. The method of claim 30, wherein administering the approximately 2.5 million cells
10 comprises injecting a cell suspension comprising the cells at five deposit sites along
11 a first deposit track, five deposit sites along a second deposit track, and five deposit
12 sites along a third deposit track, wherein approximately 20- μ L of the cell
13 suspension is injected at each deposit site and wherein the cell suspension has a cell
14 concentration of approximately $8.5 * 10^6$ cells/mL.
- 15 32. The method of claim 21, wherein the therapeutically effective amount of cells is
16 approximately 5.0 million cells.
- 17 33. The method of claim 32, wherein administering the approximately 5.0 million cells
18 comprises injecting a cell suspension comprising the cells at five deposit sites along
19 a first deposit track, five deposit sites along a second deposit track, and five deposit
20 sites along a third deposit track, wherein approximately 20- μ L of the cell
21 suspension is injected at each deposit site and wherein the cell suspension has a cell
22 concentration of approximately $17.0 * 10^6$ cells/mL.
- 23 34. The method of claim 21, wherein the stroke-induced motor deficit is a result of an
24 ischemic stroke suffered by the subject.
- 25 35. The method of claim 34, wherein the ischemic stroke occurred more than six
26 months prior to administering the cells.
- 27 36. The method of claim 35, wherein the ischemic stroke occurred between six months
28 and 90 months prior to administering the cells.
- 29 37. The method of claim 21, further comprising evaluating a degree of disability of the
30 subject by determining an mRS score of the subject and administering the
31 therapeutically effective amount of cells only when the mRS score of the subject is
32 between 2 and 4.
- 33 38. The method of claim 21, wherein the mesenchymal stem cells are human bone
34 marrow-derived cells.

- 1 39. The method of claim 21, wherein the therapeutically effective amount of cells are
2 suspended in a sterile isotonic crystalloid solution.
- 3 40. The method of claim 21, further comprising subjecting a formulated dose of the
4 cells to post-release testing prior to administering the cells to the subject.
- 5 41. A composition for treating small-volume ischemic stroke, comprising:
6 a therapeutically effective amount of cells, wherein the cells are descended from
7 mesenchymal stem cells transiently-transfected by a polynucleotide encoding a
8 Notch intracellular domain (NICD); and
9 a pharmaceutically acceptable carrier or diluent.
- 10 42. The composition of claim 41, wherein the cells are made by a process comprising:
11 providing a culture of the mesenchymal stem cells;
12 contacting the culture of mesenchymal stem cells with the polynucleotide encoding
13 the NICD, wherein the polynucleotide does not encode a full-length Notch
14 protein,
15 selecting cells that comprise the polynucleotide; and
16 further culturing the selected cells in the absence of selection for the
17 polynucleotide.
- 18 43. The composition of claim 41, wherein the mesenchymal stem cells are human bone
19 marrow-derived cells.
- 20 44. The composition of claim 41, wherein the mesenchymal stem cells are transiently-
21 transfected with a plasmid comprising the polynucleotide encoding the NICD.
- 22 45. The composition of claim 41, wherein the therapeutically effective amount of cells
23 is approximately 2.5 million cells.
- 24 46. The composition of claim 41, wherein the therapeutically effective amount of cells
25 is approximately 5.0 million cells.
- 26 47. The composition of claim 41, wherein the therapeutically effective amount of cells
27 is between approximately 2.0 million cells and approximately 5.0 million cells.
- 28 48. The composition of claim 41, wherein the pharmaceutically acceptable carrier or
29 diluent comprises a sterile isotonic crystalloid solution.
- 30 49. The composition of claim 45, wherein the composition is a cell suspension
31 packaged in a sealed vial, wherein the cell suspension is approximately 0.3 mL in
32 volume and has a cell concentration of approximately 8.5×10^6 cells/mL.

- 1 50. The composition of claim 46, wherein the composition is a cell suspension
2 packaged in a sealed vial, wherein the cell suspension is approximately 0.3 mL in
3 volume and has a cell concentration of approximately $17.0 * 10^6$ cells/mL.
- 4 51. Use of cells in the manufacture of a medicament for treating a subject following a
5 small-volume ischemic stroke suffered by the subject, comprising:
6 administering to a brain region surrounding a small-volume ischemic core of the
7 subject a therapeutically effective amount of cells, wherein the cells are
8 descended from mesenchymal stem cells transiently-transfected by a
9 polynucleotide encoding a Notch intracellular domain (NICD).
- 10 52. The use of claim 51, wherein the small-volume ischemic core is an ischemic core
11 having an ischemic core volume of less than 50 cubic centimeters (cc).
- 12 53. The use of claim 51, wherein the small-volume ischemic core is an ischemic core
13 having an ischemic core volume of between about 2 cc and 50 cc.
- 14 54. The use of claim 51, wherein administering the therapeutically effective amount of
15 the cells further comprises injecting at least part of a cell suspension comprising the
16 cells at one or more deposit sites at an outer periphery of a chronic penumbra
17 surrounding the small-volume ischemic core.
- 18 55. The use of claim 51, wherein administering the therapeutically effective amount of
19 the cells further comprises injecting at least part of a cell suspension comprising the
20 cells at one or more deposit sites proximal to or within a chronic penumbra
21 surrounding the small-volume ischemic core.
- 22 56. The use of claim 51, wherein administering the therapeutically effective amount of
23 the cells further comprises injecting at least part of a cell suspension comprising the
24 cells at one or more deposit sites distal to a chronic penumbra surrounding the
25 small-volume ischemic core.
- 26 57. The use of claim 51, wherein administering the therapeutically effective amount of
27 the cells further comprises administering the therapeutically effective amount of the
28 cells stereotactically via a single burr-hole craniostomy.
- 29 58. The use of claim 51, wherein the small-volume ischemic core is located in a region
30 of the brain of the subject other than a parietal region.
- 31 59. The use of claim 51, wherein at least part of the small-volume ischemic core is
32 located in at least one of a cortical frontal region, a cortical temporal region, a
33 subcortical white matter, and a subcortical grey matter of the brain of the subject.
- 34 60. The use of claim 51, wherein the cells are made by a method comprising:

- 1 providing a culture of the mesenchymal stem cells;
2 contacting the culture of mesenchymal stem cells with the polynucleotide encoding
3 the NICD, wherein the polynucleotide does not encode a full-length Notch
4 protein,
5 selecting cells that comprise the polynucleotide; and
6 further culturing the selected cells in the absence of selection for the
7 polynucleotide.
- 8 61. The use of claim 51, wherein the therapeutically effective amount of cells is
9 approximately 2.5 million cells.
- 10 62. The use of claim 61, wherein administering the approximately 2.5 million cells
11 comprises injecting a cell suspension comprising the cells at five deposit sites along
12 a first deposit track, five deposit sites along a second deposit track, and five deposit
13 sites along a third deposit track, wherein approximately 20-μL of the cell
14 suspension is injected at each deposit site and wherein the cell suspension has a cell
15 concentration of approximately $8.5 * 10^6$ cells/mL.
- 16 63. The use of claim 51, wherein the therapeutically effective amount of cells is
17 approximately 5.0 million cells.
- 18 64. The use of claim 63, wherein administering the approximately 5.0 million cells
19 comprises injecting a cell suspension comprising the cells at five deposit sites along
20 a first deposit track, five deposit sites along a second deposit track, and five deposit
21 sites along a third deposit track, wherein approximately 20-μL of the cell
22 suspension is injected at each deposit site and wherein the cell suspension has a cell
23 concentration of approximately $17.0 * 10^6$ cells/mL.
- 24 65. The use of claim 51, wherein the small-volume ischemic stroke occurred more than
25 six months prior to administering the cells.
- 26 66. The use of claim 51, wherein the small-volume ischemic stroke occurred between
27 six months and 90 months prior to administering the cells.
- 28 67. The use of claim 51, further comprising evaluating a degree of disability of the
29 subject by determining an mRS score of the subject and administering the
30 therapeutically effective amount of cells only when the mRS score of the subject is
31 between 2 and 4.
- 32 68. The use of claim 51, wherein the mesenchymal stem cells are human bone marrow-
33 derived cells.

- 1 69. The use of claim 51, wherein the therapeutically effective amount of cells are
2 suspended in a sterile isotonic crystalloid solution.
- 3 70. The use of claim 51, further comprising subjecting a formulated dose of the cells to
4 post-release testing prior to administering the cells to the subject.
- 5 71. Use of cells in the manufacture of a medicament for treating a subject with a stroke-
6 induced motor deficit, comprising:
7 determining a volume of an ischemic core of the subject; and
8 administering, to a brain region surrounding the ischemic core of the subject, a
9 therapeutically effective amount of cells only when the volume of the ischemic
10 core is determined to be less than 50 cubic centimeters (cc), wherein the cells
11 are descended from mesenchymal stem cells transiently-transfected by a
12 polynucleotide encoding a Notch intracellular domain (NICD).
- 13 72. The use of claim 71, further comprising administering, to the brain region
14 surrounding the ischemic core of the subject, the therapeutically effective amount of
15 cells only when the volume of the ischemic core is between about 2 cc and 50 cc.
- 16 73. The use of claim 71, wherein administering the therapeutically effective amount of
17 the cells further comprises injecting at least part of a cell suspension comprising the
18 cells at one or more deposit sites at an outer periphery of a chronic penumbra
19 surrounding the ischemic core.
- 20 74. The use of claim 71, wherein administering the therapeutically effective amount of
21 the cells further comprises injecting at least part of a cell suspension comprising the
22 cells at one or more deposit sites proximal to or within a chronic penumbra
23 surrounding the ischemic core.
- 24 75. The use of claim 71, wherein administering the therapeutically effective amount of
25 the cells further comprises injecting at least part of a cell suspension comprising the
26 cells at one or more deposit sites distal to a chronic penumbra surrounding the
27 ischemic core.
- 28 76. The use of claim 71, wherein administering the therapeutically effective amount of
29 the cells further comprises administering the therapeutically effective amount of the
30 cells stereotactically via a single burr-hole craniostomy.
- 31 77. The use of claim 71, wherein the ischemic core is located in a region of the brain of
32 the subject other than a parietal region.

- 1 78. The use of claim 71, wherein at least part of the ischemic core is located in at least
2 one of a cortical frontal region, a cortical temporal region, a subcortical white
3 matter, and a subcortical grey matter of the subject.
- 4 79. The use of claim 71, wherein the cells are made by a method comprising:
5 providing a culture of the 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 80. The use of claim 71, wherein the therapeutically effective amount of cells is
13 approximately 2.5 million cells.
- 14 81. The use of claim 80, wherein administering the approximately 2.5 million cells
15 comprises injecting a cell suspension comprising the cells at five deposit sites along
16 a first deposit track, five deposit sites along a second deposit track, and five deposit
17 sites along a third deposit track, wherein approximately 20- μ L of the cell
18 suspension is injected at each deposit site and wherein the cell suspension has a cell
19 concentration of approximately $8.5 * 10^6$ cells/mL.
- 20 82. The use of claim 81, wherein the therapeutically effective amount of cells is
21 approximately 5.0 million cells.
- 22 83. The use of claim 82, wherein administering the approximately 5.0 million cells
23 comprises injecting a cell suspension comprising the cells at five deposit sites along
24 a first deposit track, five deposit sites along a second deposit track, and five deposit
25 sites along a third deposit track, wherein approximately 20- μ L of the cell
26 suspension is injected at each deposit site and wherein the cell suspension has a cell
27 concentration of approximately $17.0 * 10^6$ cells/mL.
- 28 84. The use of claim 71, wherein the stroke-induced motor deficit is a result of an
29 ischemic stroke suffered by the subject.
- 30 85. The use of claim 84, wherein the ischemic stroke occurred more than six months
31 prior to administering the cells.
- 32 86. The use of claim 85, wherein the ischemic stroke occurred between six months and
33 90 months prior to administering the cells.

- 1 87. The use of claim 71, further comprising evaluating a degree of disability of the
2 subject by determining an mRS score of the subject and administering the
3 therapeutically effective amount of cells only when the mRS score of the subject is
4 between 2 and 4.
- 5 88. The use of claim 71, wherein the mesenchymal stem cells are human bone marrow-
6 derived cells.
- 7 89. The use of claim 71, wherein the therapeutically effective amount of cells are
8 suspended in a sterile isotonic crystalloid solution.
- 9 90. The use of claim 71, further comprising subjecting a formulated dose of the cells to
10 post-release testing prior to administering the cells to the subject.
- 11 91. Cells descended from mesenchymal stem cells transiently-transfected by a
12 polynucleotide encoding a Notch intracellular domain (NICD) for use in a method
13 of treating a subject following a small-volume ischemic stroke suffered by the
14 subject, wherein the method comprises administering, to a brain region surrounding
15 a small-volume ischemic core of the subject, a therapeutically effective amount of
16 the cells.
- 17 92. The cells of claim 91, wherein the small-volume ischemic core is an ischemic core
18 having an ischemic core volume of less than 50 cubic centimeters (cc).
- 19 93. The cells of claim 91, wherein the small-volume ischemic core is an ischemic core
20 having an ischemic core volume of between about 2 cc and 50 cc.
- 21 94. The cells of claim 91, wherein administering the therapeutically effective amount of
22 the cells further comprises injecting at least part of a cell suspension comprising the
23 cells at one or more deposit sites at an outer periphery of a chronic penumbra
24 surrounding the small-volume ischemic core.
- 25 95. The cells of claim 91, wherein administering the therapeutically effective amount of
26 the cells further comprises injecting at least part of a cell suspension comprising the
27 cells at one or more deposit sites proximal to or within a chronic penumbra
28 surrounding the small-volume ischemic core.
- 29 96. The cells of claim 91, wherein administering the therapeutically effective amount of
30 the cells further comprises injecting at least part of a cell suspension comprising the
31 cells at one or more deposit sites distal to a chronic penumbra surrounding the
32 small-volume ischemic core.

- 1 97. The cells of claim 91, wherein administering the therapeutically effective amount of
2 the cells further comprises administering the therapeutically effective amount of the
3 cells stereotactically via a single burr-hole craniostomy.
- 4 98. The cells of claim 91, wherein the small-volume ischemic core is located in a
5 region of the brain of the subject other than a parietal region.
- 6 99. The cells of claim 91, wherein at least part of the small-volume ischemic core is
7 located in at least one of a cortical frontal region, a cortical temporal region, a
8 subcortical white matter, and a subcortical grey matter of the brain of the subject.
- 9 100. The cells of claim 91, wherein the cells are made by a method comprising:
10 providing a culture of the mesenchymal stem cells;
11 contacting the culture of mesenchymal stem cells with the polynucleotide encoding
12 the NICD, wherein the polynucleotide does not encode a full-length Notch
13 protein,
14 selecting cells that comprise the polynucleotide; and
15 further culturing the selected cells in the absence of selection for the
16 polynucleotide.
- 17 101. The cells of claim 91, wherein the therapeutically effective amount of cells is
18 approximately 2.5 million cells.
- 19 102. The cells of claim 101, wherein administering the approximately 2.5 million cells
20 comprises injecting a cell suspension comprising the cells at five deposit sites along
21 a first deposit track, five deposit sites along a second deposit track, and five deposit
22 sites along a third deposit track, wherein approximately 20- μ L of the cell
23 suspension is injected at each deposit site and wherein the cell suspension has a cell
24 concentration of approximately $8.5 * 10^6$ cells/mL.
- 25 103. The cells of claim 91, wherein the therapeutically effective amount of cells is
26 approximately 5.0 million cells.
- 27 104. The cells of claim 103, wherein administering the approximately 5.0 million cells
28 comprises injecting a cell suspension comprising the cells at five deposit sites along
29 a first deposit track, five deposit sites along a second deposit track, and five deposit
30 sites along a third deposit track, wherein approximately 20- μ L of the cell
31 suspension is injected at each deposit site and wherein the cell suspension has a cell
32 concentration of approximately $17.0 * 10^6$ cells/mL.
- 33 105. The cells of claim 91, wherein the small-volume ischemic stroke occurred more
34 than six months prior to administering the cells.

- 1 106. The cells of claim 91, wherein the small-volume ischemic stroke occurred
2 between six months and 90 months prior to administering the cells.
- 3 107. The cells of claim 91, further comprising evaluating a degree of disability of the
4 subject by determining an mRS score of the subject and administering the
5 therapeutically effective amount of cells only when the mRS score of the subject is
6 between 2 and 4.
- 7 108. The cells of claim 91, wherein the mesenchymal stem cells are human bone
8 marrow-derived cells.
- 9 109. The cells of claim 91, wherein the therapeutically effective amount of cells are
10 suspended in a sterile isotonic crystalloid solution.
- 11 110. The cells of claim 91, further comprising subjecting a formulated dose of the cells
12 to post-release testing prior to administering the cells to the subject.
- 13 111. Cells descended from mesenchymal stem cells transiently-transfected by a
14 polynucleotide encoding a Notch intracellular domain (NICD) for use in a method
15 of treating a subject with a stroke-induced motor deficit, wherein the method
16 comprises:
17 determining a volume of an ischemic core of the subject; and
18 administering, to a brain region surrounding the ischemic core of the subject, a
19 therapeutically effective amount of cells only when the volume of the ischemic core
20 is determined to be less than 50 cubic centimeters (cc), wherein the cells are
21 descended from mesenchymal stem cells transiently-transfected by a polynucleotide
22 encoding a Notch intracellular domain (NICD).
- 23 112. The cells of claim 111, further comprising administering, to the brain region
24 surrounding the ischemic core of the subject, the therapeutically effective amount of
25 cells only when the volume of the ischemic core is between about 2 cc and 50 cc.
- 26 113. The cells of claim 111, wherein administering the therapeutically effective
27 amount of the cells further comprises injecting at least part of a cell suspension
28 comprising the cells at one or more deposit sites at an outer periphery of a chronic
29 penumbra surrounding the ischemic core.
- 30 114. The cells of claim 111, wherein administering the therapeutically effective
31 amount of the cells further comprises injecting at least part of a cell suspension
32 comprising the cells at one or more deposit sites proximal to or within a chronic
33 penumbra surrounding the ischemic core.

- 1 115. The cells of claim 111, wherein administering the therapeutically effective
2 amount of the cells further comprises injecting at least part of a cell suspension
3 comprising the cells at one or more deposit sites distal to a chronic penumbra
4 surrounding the ischemic core.
- 5 116. The cells of claim 111, wherein administering the therapeutically effective
6 amount of the cells further comprises administering the therapeutically effective
7 amount of the cells stereotactically via a single burr-hole craniostomy.
- 8 117. The cells of claim 111, wherein the ischemic core is located in a region of the
9 brain of the subject other than a parietal region.
- 10 118. The cells of claim 111, wherein at least part of the ischemic core is located in at
11 least one of a cortical frontal region, a cortical temporal region, a subcortical white
12 matter, and a subcortical grey matter of the subject.
- 13 119. The cells of claim 111, wherein the cells are made by a method comprising:
14 providing a culture of the mesenchymal stem cells;
15 contacting the culture of mesenchymal stem cells with the polynucleotide encoding
16 the NICD, wherein the polynucleotide does not encode a full-length Notch
17 protein,
18 selecting cells that comprise the polynucleotide; and
19 further culturing the selected cells in the absence of selection for the
20 polynucleotide.
- 21 120. The cells of claim 111, wherein the therapeutically effective amount of cells is
22 approximately 2.5 million cells.
- 23 121. The cells of claim 120, wherein administering the approximately 2.5 million
24 cells comprises injecting a cell suspension comprising the cells at five deposit sites
25 along a first deposit track, five deposit sites along a second deposit track, and five
26 deposit sites along a third deposit track, wherein approximately 20-μL of the cell
27 suspension is injected at each deposit site and wherein the cell suspension has a cell
28 concentration of approximately 8.5×10^6 cells/mL.
- 29 122. The cells of claim 111, wherein the therapeutically effective amount of cells is
30 approximately 5.0 million cells.
- 31 123. The cells of claim 122, wherein administering the approximately 5.0 million
32 cells comprises injecting a cell suspension comprising the cells at five deposit sites
33 along a first deposit track, five deposit sites along a second deposit track, and five
34 deposit sites along a third deposit track, wherein approximately 20-μL of the cell

- 1 suspension is injected at each deposit site and wherein the cell suspension has a cell
2 concentration of approximately $17.0 * 10^6$ cells/mL.
- 3 124. The cells of claim 111, wherein the stroke-induced motor deficit is a result of an
4 ischemic stroke suffered by the subject.
- 5 125. The cells of claim 124, wherein the ischemic stroke occurred more than six
6 months prior to administering the cells.
- 7 126. The cells of claim 125, wherein the ischemic stroke occurred between six
8 months and 90 months prior to administering the cells.
- 9 127. The cells of claim 111, further comprising evaluating a degree of disability of
10 the subject by determining an mRS score of the subject and administering the
11 therapeutically effective amount of cells only when the mRS score of the subject is
12 between 2 and 4.
- 13 128. The cells of claim 111, wherein the mesenchymal stem cells are human bone
14 marrow-derived cells.
- 15 129. The cells of claim 111, wherein the therapeutically effective amount of cells are
16 suspended in a sterile isotonic crystalloid solution.
- 17 130. The cells of claim 111, further comprising subjecting a formulated dose of the
18 cells to post-release testing prior to administering the cells to the subject.
19