CD271+ stem cell treatment of patients with chronic stroke: A retrospective case series report

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Abstract. Patients with chronic stroke have currently little hope for motor improvement towards regaining independent activities of daily living; stem cell treatments offer a new treatment option and needs to be developed. Patients with chronic stroke (more than 3 months prior to stem cell treatment, mean 21.2 months post-stroke) were treated with CD271+ stem cells, 7 patients received autologous and 1 allogeneic cells from first degree relative; administration was intravenous in 1 and intrathecal in 7 patients. Each patient received a single treatment consisting of 2-5x10^6 cells/kg and they were followed up for up to 12 months. There were significant improvements in expressive aphasia (2/3 patients) spasticity (5/5, of which 2 were transient), and small improvements in motor function (2/8 patients). Although motor improvements were minor in our chronic stroke patients, improvements in aphasia and spasticity were significant and in the context of good safety we are advocating further administration and clinical studies of CD271+ stem cells not only in chronic stroke patients, but also for spastic paresis/plegia; a different, yet unexplored application is pulmonary emphysema.

Introduction

Stem cell treatments have substantial therapeutic potential for many pathologies and together with cellular and gene therapies and biological agents represent an important change in the therapeutical paradigm, from the 'silver-bullet' single molecules acting at critical crossroads of cellular molecular pathways (which may be better suited for acute illness) to combination treatments acting on many more cellular molecules and pathways at levels closer to physiological functioning and thus with better overall results especially for chronic conditions.

Furthermore, having the potential to replace lost tissue and function, stem cells are uniquely positioned to offer truly regenerative treatments with rapid results and long-term advantages, and this is especially true when autologous implants are used. Towards these challenges, considering the scope and the level of detail of cell-level interactions, a multi-disciplinary effort including experimental models in various pathologies is necessary (1-5); advanced molecular imaging techniques with hybrid PET/CT systems enables assessment of brain tissue viability contributing to the *in vivo* imaging evaluation of response to stem cell-based therapies. Additionally, recent technological advances in instrumentation leading to the introduction into clinical practice of simultaneous PET/MRI systems, together with advances in radiochemical development of novel PET-tracers, hold promise for more accurate functional imaging of stroke-related molecular mechanisms, giving ground for designing more precise and individualized treatment strategies (6-8). At the same time much remains to be clarified about their optimal and individualized production, administration, actions, grafting and functional integration, and this work aims to bring a contribution in this regard.

Patients with cerebrovascular accidents (CVA, stroke) undergo multiple and simultaneous modifications at the cellular, molecular and genetic level which involve partially overlapping pathways, through activation or inhibition of specific molecules. Known so far are: dysfunction of hemostasis, vascular endothelium and blood-brain barrier; activation and increased levels of pro-inflammatory and adhesion molecules [cytokines, nuclear factor-κB (NF-κB), intercellular adhesion molecule (ICAM), vascular cell adhesion molecule (VCA), leukocyte infiltration, activation of microglia (1), functional neuronal impairment, oxidative stress, switching to anaerobic metabolism by activating hypoxia inducible factor-1α (HIF-1α)] (9) and apoptosis [B-cell lymphoma (Bcl), and Bcl-2-associated X protein (Bax)] (10,11).

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Post-stroke recovery is further influenced by excitotoxicity (activation of receptors for N-methyl-D-aspartate (NMDA), kainate, amino (hydroxy)-methyl-isoxazol-propanoic acid (AMPA), acidotoxicity and ionic imbalance (Ca, Na, K via modification of active transport systems or cell (12) lysis), neuroendocrine factors: insulin-like growth factor-1 (IGF-1) (13-15), hepatocyte growth factor (HGF) (16), transforming growth factor-β (TGF-β), nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF); and other factors (cross-talk between transcription, growth and neurotrophic factors, neurotransmitters, pathways including Wnt, notch and hedgehog, bone morphogenetic proteins (BMPs), and epigenetic modulators (11).

Acting beyond modifications of gene transcription at epigenetic level, factors influencing stroke recovery at the genetic level are miRNAs (17) and genetic variations in molecules such as methyl tetrahidrofolate reductase (18,19), BDNF (20), apolipoprotein E (APO E), and others (21) most of these actions are summarized in Fig. 1.

The complexity of these pathological modifications which occur simultaneously may explain the fact that as of 2018 over 700 medications for stroke have failed clinical trials (22).

Current treatments for stroke (thrombolytics and endovascular thrombectomy) are limited in multiple ways: i) temporal, to first few hours after stroke; ii) resource-wise, to tertiary care centers; iii) focus-wise, by addressing mostly the vascular blockage but not the damaged and regenerating neurons; iv) scope-wise, by excluding hemorrhagic stroke; and v) patient-wise, due to co-morbid conditions and the risk for serious, sometimes fatal complications; these factors make their administration successful to less than 5% of stroke patients.

A new treatment for stroke which involves a combination of medications has shown good results in acute and subacute stroke and can be administered without the above limitations (23); however in chronic stroke patients with ample loss of brain tissue (>5 cubic centimeters or $>10^9$ neurons), cellular therapies can provide a better option, with the potential to improve neurological function years after stroke occurrence.

To achieve functional integration of stem cells in brain areas affected by stroke many aspects have to be successfully addressed: migration of administered cells to the injured areas; differentiation into functional neurons (mostly cholinergic) and supporting cells including capillaries; adhesion to existing neural networks and developing new synaptic connections, while at the same time avoiding new occlusions of capillaries by implanted cells, excessive multiplication (tumorigenesis), and errant stimulation (convulsions). On top of these, the procedure should not be overly complicated, expensive and practically out-of-reach for many patients.

Our approach relies on selecting and administering a larger population of stem cells ($>100x10^6$) with low propensity for multiplication (low $CD70^+$) and subsequent minimal risk for tumorigenesis, and also with more paracrine actions [cytokines and vascular endothelial growth factor (VEGF)-secretion] which favor formation of new synapses and capillaries. Specifically we are employing $CD271^+$ stem cells, growth factors (GFs) BDNF and IGF-1 added to culture media and a neurotrophic combination administered iv before and after stem cell administration; below we summarize the results of administering this treatment to chronic stroke patients.

Treatment

This is a case series report, resulting from non-standardized administration of treatments to individual patients on a case-by-case basis (under the EU rules governing compassionate care and hospital exemption), not under homogenous inclusion/exclusion criteria that would be applicable for a clinical study. The resultant patient data were analysed retrospectively, and the intention of this article was to provide insights into this type of treatment, which is currently undergoing further developments and refinements.

Each patient received a single treatment consisting of $2\text{-}5x10^6$ cells/kg administered via intrathecal injection (7 patients) or intravenously (1 patient). Cell suspensions containing adipose-derived stem cells (ASC) were prepared from patients' own adipose tissue (7 patients) or from a related donor; this was the case of a 77 year old stroke patient who received ASCs from a first degree relative.

Adipose tissue was harvested via standard lipoaspiration procedure under local anesthesia with lidocaine, with the resulting aspirate being a mixture of extracellular matrix (ECM), GFs and cells, a majority of which are adipocytes, and also vascular stromal cells and mesenchymal stem cells. A microscopic image (x200) of the raw lipoaspirate stained with Cresyl Violet revealing Nissl bodies in mesenchymal stem cells is shown in Fig. 2.

ECM constituents are type I-VII collagen, elastin, fibronectin, laminin and glycosaminoglycans, which contain the following GFs: TGF-β1, platelet-derived growth factor (PDGF), VEGF, NGF, IGF-1, basic fibroblast growth factor (bFGF), BMP4, epidermal growth factor (EGF), HGF; interspersed between adipocytes can be found in mesenchymal stem cells which have the potential to differentiate in multiple cellular lineage and express self-renewal genes and specific mesenchymal markers (24).

ASCs cultured with a medium containing 5% autologous serum express the CD29, CD44, CD73, CD90 and CD105 markers characteristic of stemness (17) and their properties are not affected after two freeze cycles; compared to other sources of stem cells, ASCs have similar phenotype maintenance as MSCs from bone marrow, also capacity for differentiation, and secretion of GFs-paracrine actions (25-31). Furthermore, ASCs had faster proliferation rate and also neural markers with significantly higher expression than MSCs derived from bone marrow, skin or umbilical cord (32-35).

Cultured ASCs can start to differentiate into neuronal precursors as early as 1-3 h (36) after adding insulin, hydrocortisone, valproic acid and butylated hydroxyanisole, with expression of nestin, GFAP and NeuN proteins, process augmented by addition of EGF and bFGF.

Regarding paracrine actions, it is widely agreed that ASC administration increases levels of neurotrophic factors such as BDNF irrespective of cellular engraftment (37); similarly a cell-free ASC extract was shown to modify substantially the expression of many genes involved in inflammation, immune response and apoptosis (38). Another important action of stem cells is the increase in VEGF and angiopoetin-1 production in brain endothelial cells and astrocytes, which increases angiogenesis (capillary tube formation) and vascular stabilization (39).

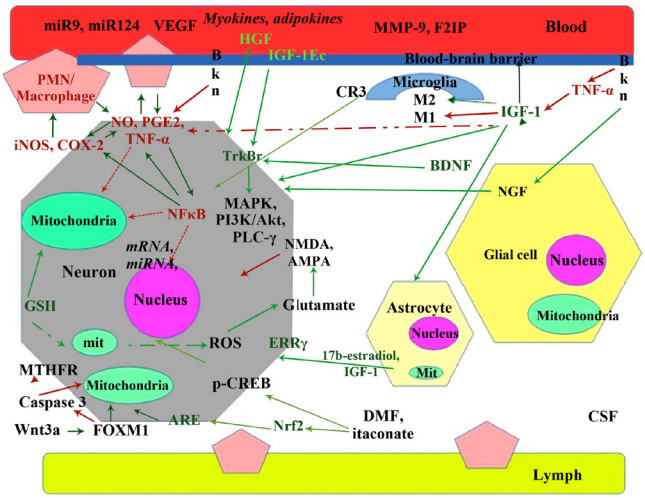

Figure 1. Pathological, molecular modifications in stroke and their respective actions. solid lines, direct action; dotted line, indirect action; green line, stimulating, red line, inhibitory actions.

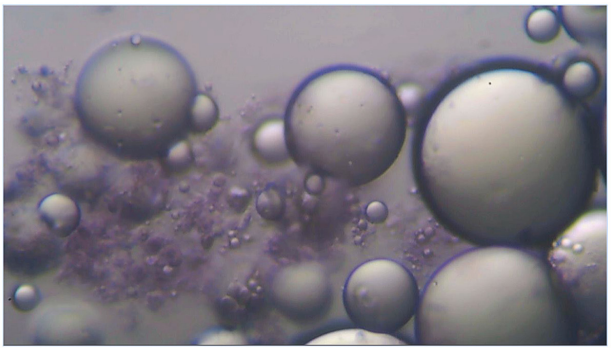

Figure 2. Raw lipoaspirate with adipose (clear) and stem cells (colored), Cresyl violet stain, x200.

Our method for preparing the cellular suspension focuses on the isolation of ASCs while preserving as much as possible the GF contained in the lipoaspirate; stem cells are isolated from the lipoaspirate after treatment with collagenase, filtration, centrifugation and subsequent incubation with CD271$^+$ monoclonal antibodies coated microspheres.

Fig. 3 shows flow cytometry from a freshly cultured ASC suspension, with cells showing no CD45 markers, 2% CD34$^+$ and 4% CD90$^+$.

The CD271 surface marker is a cell receptor belonging to the tumor necrosis factor (TNF) superfamily, also known as the low-affinity nerve growth factor receptor (LNGFR) or p75NTR (40) which was shown to be present on a specific population of stem cells, classically defined by cell markers CD73, CD90 and CD105 and plastic adherence (41).

CD271$^+$ stem cells were successfully obtained from lipoaspirates (42) and it was shown to be one of the best markers (followed by CD146, CD106, CD13) for *in vitro* culturing and expansion of mesenchymal stem cells; CD271$^+$ stem cells also have the advantage of producing colony-forming unit-fibroblast (CFU-F), an important factor influencing grafting and development, activity which was shown to be absent in the CD271$^-$ cells (43).

CD271$^+$ cells were also shown to have superior paracrine actions than classic plastic-adherent mesenchymal stem cells (PA-MSCs) by secreting higher levels of molecules with chemoattractant actions [monocyte chemoattractant protein 1 (MCP-1), IL-8, IL-1β], pro-inflammatory [interferon-γ (IFN-γ), TNF-α], immunosuppressive (IL-10), and differentiation [granulocyte colony stimulating factor (G-CSF), granulocyte macrophage colony stimulating factor (GM-CSF)] (40).

To summarize, CD271$^+$ stem cells can be more easily and efficiently obtained from the respective tissue, their *in vitro* enriching potential is among the highest in the mesenchymal stem cells studied (43) and they have superior potential for engraftment as a consequence of their immunosuppresive and lymphohematopoietic properties (40).

For *in vitro* differentiation of stem cells into neuronal precursors we used either commercially available ready-made neuronal medium with no fetal bovine serum, containing neuronal-specific GFs and penicillin/streptomycin, or have prepared a culture medium from Dulbecco's Modified Eagle's medium (DMEM) to which we added neuronal GFs BDNF, IGF-1, ascorbic acid and aminophylline, which is in line with established neuronal differentiation protocols (44). Fig. 4 shows neuronal precursors with dendritic-like cellular extensions which stain positive with Cresyl violet, x400.

Patients

ASCs were administered to eight patients who were treated previously for stroke, which occurred more than 3 months prior to administration of the stem cell treatment; of these 7 received autologous ASCs and 1 patient of advanced age (77 years old) was treated with allogeneic cells from a younger first degree relative. This choice is supported by reports showing that aging has a deleterious effect on the potential of human MSCs to differentiate in neurons (45) to the point that neurogenesis from MSCs is virtually nonexistent in donors older than 60 years, irrespective of the use of different protocols which differentiate MSCs to neurons in single or multiple steps.

Patient demographics, co-morbid conditions and medication, time of administration of ASCs after stroke, presence of spasticity, and motor deficit are given in Table I. Of note is that 2 patients were treated with botulinum toxin for spasticity.

Regarding the route of administration, 7 patients received the cellular suspension via intrathecal injection (lumbar puncture) and 1 patient via intravenous administration; results are summarized in Table II.

No correlation was seen ($P>0.05$; Pearson correlation r) between the neurologic improvements and number of ASCs administered, size of ischemic/lacunar brain area, age of patients, co-morbid conditions, however, due to the small number of patients we cannot make inferrences on such correlations.

The differences noted were due to the timing of ASC administration relative to the stroke onset, so that earlier administration (3 months post-stroke) resulted in the best motor effects, manifested through sustained but involuntary isometric contraction of both upper and lower limbs. The other patients had either no motor improvement or transient improvement in spasticity (1 point on the modified Ashworth scale) and motor function (1 patient with trombophilia and 1 patient with aneurysmal disease, both with left hemiparesis) or minor

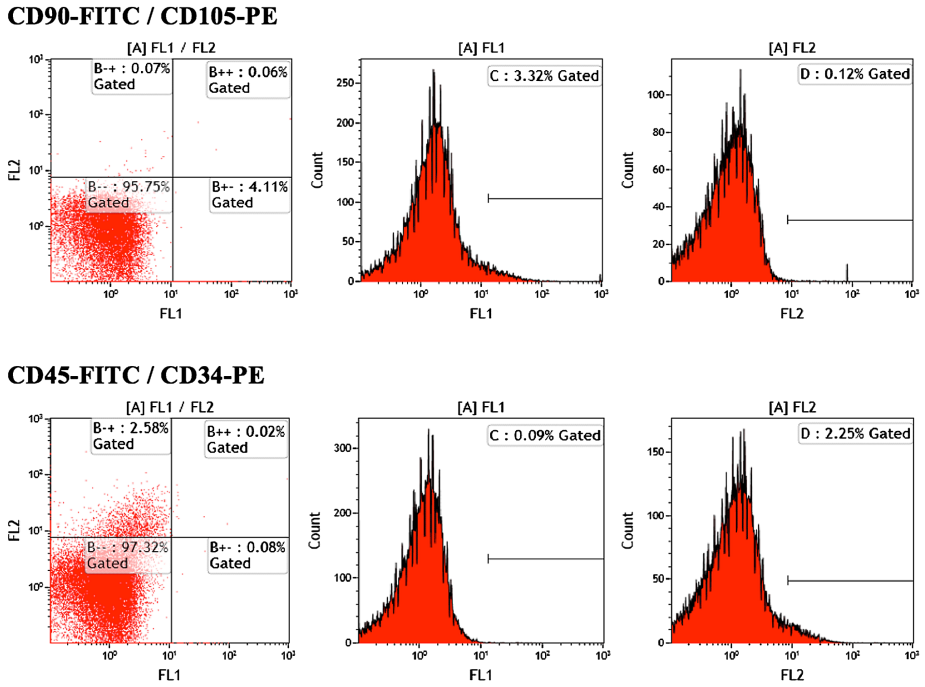

Figure 3. Flow cytometry of fresh ASC suspension, with relative percentage of CD90+ and CD34+. ASC, adipose-derived stem cells.

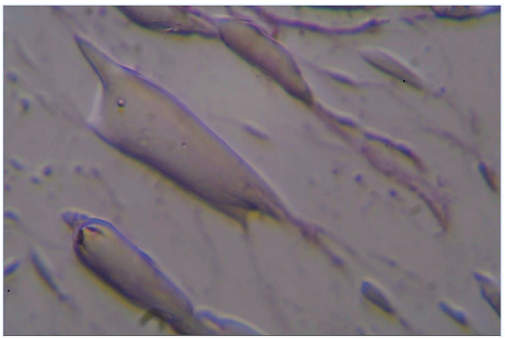

Figure 4. Neuronal precursors with Nissl bodies on Cresyl violet stain, 3 days after stem cell culture in neuronal medium, optical microscope, x400.

improvements; one patient with hemorrhagic stroke was able to initiate dorsiflexion of paretic foot with improved ambulation and stair climbing/descending, which was helped by a decrease in spasticity, and another patient with trombophilia and left hemiparesis was able to ambulate better (2 points on Fugl-Meyer on upper extremity).

Aphasia improved in the 2 patients with right hemiparesis who received ASCs via intrathecal injection, with the most important improvement observed in a patient progression from monosyllabic answers to full sentences (6 points increase on Quick Aphasia Battery); this patient had also improved motor ability of affected leg and better ambulation (increased balance and unassisted walking distance).

There was also a difference between the intrathecal and intravenous administration, with the treatment effects installed later (approximately 24 h compared to 3-4 h after intrathecal) and manifested as vertigo, alongside a decrease in spasticity.

It is worth mentioning that from the two patients with spasticity who were previously treated with botulinum toxin and hyperbaric oxygen therapy (HBOT), one had sustained improvement in spasticity (with iv administration) and another had transient, episodic amelioration, during which motor ability was significantly improved (1 point Ashworth Scale and 1 point NIHSS).

Also 2 patients with spasticity had been treated with HBOT prior to stem cell treatment with minor and transient improvement in spasticity, but no motor improvement; these two patients had sustained improvement in spasticity after ASC treatment.

Side effects were pain after administration starting 3-4 h post intrathecal injection, initially in the lumbar area, then ascending to cervical area. This was experienced with different intensity by all patients, and it responded well to administration of paracetamol, ketoprofen, and/or metamizole.

This pain diminished quickly so that after 24-36 h there was no need for analgesics.

Three patients experienced transient rise of body temperature (max 38.5˚C) which also responded well to paracetamol.

One patient (with allogeneic implant) had a period of confusion after stem cell administration, which gradually improved with complete remission after 2 weeks; subsequently this patient had significant improvement in speech (from monosyllabic answers to full sentences) and ambulation.

There were no signs of infection either local or systemic (erythema, edema, fever, leukocytosis, or neurological symptoms).

Discussion

Administration of MSCs in stroke patients via intravenous, intra-arterial, intracerebral and intrathecal routes was performed during the past two decades by many teams in many countries, mostly with cells from bone marrow, more recently from adipose tissue and umbilical cord blood (46,47).

Table I. Initial patient data.

	Age @ CVA, sex	Tx admin after CVA	Co-morbid conditions	Spasticity	Motor deficit	Medication
P1	54, F	10 mo	AF, UTI, hepatic cytolysis, right hemiplegia, reactive depression, anarthria, expressive aphasia	No	R, aphasia	Concor, tianeptine, valproic acid
P2	65, F	3 mo	Anemia, pressure ulcers, right femoral fracture, hepatic cytolysis, HBP, malnutrition	No	L	Indapamide, amlodipine, piracetam, clopidogrel
P3	40, F	31 mo	AF, HBP, septal hypertrophic cardiomyopathy, severe mitral valve regurgitation; MTHFR C677T^{+}, factor XIII, PAI 4G/5G	Yes	L	Acenocumarol, metoprolol, eutirox, baclofen
P4	35, M	26 mo	NIDDM, HBP, nistagmus, dyslipidemia, Thrombophilia MTHFR C677T^{+}, A1298C^{+}; prothrombin	Yes	L	Acenocumarol, metoprolol, metformin, atorvastatin
P5	51, F	36 mo	Subarachnoid hemorrhage, left arachnoid cyst 4x9 cm; urinary frequency, vertigo, spasticity	Yes	L	Baclofen, betahistine, Aspirin, Feminost
P6	75, M	23 mo	Atrial fibrillation, UTIs, neurological bladder, reactive depression	No	R, aphasia	Dabigatran, carvedilol, atorvastatin, tianeptin omeprazol
P7	36, F	20 mo	Multiple aneurisms on right middle cerebral, ophtalmic, left cerebellar, supraclinoid, with surgery and stenting; subarachnoid hemorrhage	Yes	L	HBOT, baclofen, botulinum toxin
P8	50, F	21 mo	Thrombophilia with homozygous mutations for MTHFR, PAI1, EPCR; dyslipidemia	Yes	R, aphasia	Acenocumarol, baclofen, Levetiracetam, Fluoxetine botulinum toxin

F/M, female/male; CVA, cerebrovascular accident; mo, months; R, L, right, left hemiplegia/paresis; HBP, high blood pressure; UTI, urinary tract infection; AF, atrial fibrillation; NIDDM, non-insulin dependent diabetes mellitus; HBOT, hyperbaric oxygen therapy.

Table II. Treatment and results.

	No. of patients	Associated condition	Route of admin	Cell no.	Allogen	Spasticity improved	Motor improved	Other
Right hemiplegia	(3)	Atrial fibrillation (2); thrombophilia (1)	IT (2) IV (1)	2.2x10^{6}/kg	(1)	Sustained (2)	Minor (1)	Aphasia improved (2/3)
Left hemiplegia	(5)	Thrombophilia (2), aneurismal disease (1), brain hemorrhage (1), right femoral fracture (1)	IT (5)	3.1x10^{6}/kg		Transient (2) Sustained (1)	Transient (2) Minor (1)	Myoclonus (1)

IT, intrathecal; IV, intravenous. Number of respective patients is given in paranthesis.

Our search in the clinicaltrials.gov database for 'stem cells' and 'stroke' in January 2020 listed 86 ongoing or completed clinical studies, but a majority of them although completed had not posted results; fewer clinical trials to date were performed with ASCs, and one such example is AMASCIS-01 trial (48) which involved iv administration of ASCs in up to 19 patients with subacute stroke, and for which also no results were posted a few years after completion.

Despite the high interest in the field, published results of MSC treatment in stroke patients are not as numerous and most

have a low number of patients: 5 MSC-treated patients and 25 controls (49) had a significant difference in Barthel index at 3 and 6 months, but no significant difference at 12 months or modified Rankin; 16 MSC-treated iv and 36 controls (50) showed that clinical improvement in the MSC group was associated with serum levels of stromal cell-derived factor-1 (Sdf-1) and the degree of involvement of the subventricular region of the lateral ventricle; in another study 12 stroke patients safely received 4 intrathecal injections each with modest improvement (51).

A meta-analysis of 7 clinical trials performed until August 2018 on MSC treatments for stroke patients (52) showed that most studies had less than 32 participants; result-wise there was long-term (at least 6 months) improvement in motor function assessed with the National Institute of Health Stroke Scale, but not Barthel Index nor modified Rankin.

While the iv/intra-arterial/intra-thecal administration of MSCs was mostly done in acute/subacute stroke patients with modest improvements, small studies showed better results via neurosurgery (53) so that 16 of 18 patients had motor improvement at 12 months after intracerebral implant of MSCs in chronic stroke patients. To our knowledge there is no known report of administration of $CD271^+$ cells to stroke patients to which we could compare our results.

A crucial aspect of stem cell treatment is the homing of stem cells in the brain after their administration; it was shown that Sdf-1 levels (46) elevated in subacute phase of stroke favors homing of stem cells in ischemic areas and a similar finding was published by a different team (54), however, we do not know if measuring the level of Sdf-1 has predictive value for successful administration of stem cells in specific patients and ASC homing remains an aspect in need of clarification (55).

A multitude of intertwined factors are acting in concert and influencing stem cell multiplication and differentiation; they can be grouped in signaling pathways based on respective sequences and roles: pro-multiplication-Jak/Stat, modulated by cytokines such as G-CSF interferons, granulocyte colony-stimulating factor (G-CSF), and interleukins (IL-6 and IL-10); Sonic Hedgehog (SHh) and Notch Signaling Pathways, important in proliferation and differentiation of neural stem cells; Wnt/β-catenin, important in embryonic development and tumorigenesis in adult tissues, and PI3K/Akt which is intertwined with the MAPK/ERK path via protein kinase B (Akt-1) which blocks apoptosis; and pro-differentiation: MAPK/ERK; activated through tyrosine kinase and G-protein receptors, and extracellular effectors such as EGF and FGF; and the TGF-β signaling pathway which activates apoptosis; finally at epigenetic levels important roles are held by demethylases (ex ascorbic acid) and histone deacetylases (ex valproic acid) (56).

Testing limitations make objective verification of homing and grafting of stem cells in the human brain difficult; using Technetium-99m-labeled autologous bone-marrow mononuclear cells (BMMNCs) in 6 patients (55) with chronic ischemic stroke (days 59-82 post-stroke) of the middle cerebral artery (MCA); around $2x10^7$ cells were labeled with ^{99m}Tc from a total of $1.25\text{-}5x10^8$ and intraarterial administration into MCA. There were no complications at the 120-day follow-up; whole body scintigraphy with Single-Photon Emission Computed Tomography (SPECT) showed at 2 h post-administration the presence of labeled stem cells in the brain of all patients, with preference for the infarcted hemisphere.

Interestingly, studies performed in rodents following iv administration of human ASCs marked with green fluorescent protein (40,42) showed that at 75 days post infusion with cultured ASCs after 4 passages, most implanted ASCs were found in lungs and spleen (approximately 10-30 ASCs per 10.000 resident cells) followed by brain with 2-10 ASCs/10,000 resident cells, and there were significant differences in ASC tissue distribution between animals as well as between the left and right brain hemispheres in some animals (42). There was also a different cellular marker expression between cells at passage 0 (freshly harvested) and passage 4, so that initially there was a higher proportion of $CD34^+$ and $CD45^+$ cells (50 and 38%, respectively) vs. passage 4 (2 and 7%, respectively), while the opposite trend was observed for $CD90^+$ and $CD271^+$ markers, which initially were present in 52 and 5% of cells, and at passage 4 were identified in 98 and 62% of cultured ASCs (37). The proportion of hematopoietic vs neuronal precursor cell markers is important because it seem to greatly influence the site of ASC grafting, as it was shown that when $CD271^+$ and $CD34^+$ are co-transplanted in a 1:1 ratio, they migrate preferentially to the brain, while when administered in a ratio of 8:1 the stem cells migrated preferentially to the lungs and to a much lesser extent to the liver, brain and heart (40). The difference in stem cell homing may be due to differences in tissue oxygenation levels in combination with local chemokines; proportion of $CD34^+$ in the transplanted stem cells can be a crucial factor when administering stem cell treatments for neurological vs pulmonary pathologies, especially emphysema, for which as yet, there is no regenerative therapy available.

In conclusion, we have safely administered $CD271^+$ mesenchymal stem cells to eight patients with stroke beginning April 2018; those patients had improvements especially in areas of spasticity, aphasia, and to a lesser degree in motor strength and coordination. Improvements varied from patient to patient; some of these were transient (24-36 h) and some were long-lasting (one year and continuing). Earliest administration (3 months post stroke) was followed by best motor improvement, but other factors were not correlated with patient improvement.

Side effects were mild to moderate (pain responding well to non-opiate analgesics) and transient; pain was mostly absent after 48 h; two patients experienced confusion which lasted approximately 2 weeks but was followed by significant improvements in aphasia and spasticity.

Based on above data and the cited literature on mesenchymal stem cells - of which $CD271^+$ represent a subpopulation - it can be said that treating chronic stroke patients with $CD271^+$ stem cells administered intrathecally and/or intravenously is a safe therapeutic option with good results especially for spasticity and aphasia; at the same time it needs to be further studied and improved (e.g., repeated or combined intrathecal/iv administration, control of stem cell homing by administering different proportions of $CD271^+/CD34^+$) with the goal of obtaining ample motor improvements and subsequent functional independence of the patient.

Note added in proof (added October 6, 2023)

Subsequently to the publication of this article, the following changes have been made, which were not included or reflected in the article as it was originally published. The title of the article has been changed to '$CD271^+$ stem cell treatment of patients with

chronic stroke: A retrospective case series report', to reflect that this study should be regarded as a case series report. Secondly, the following text (first paragraph) has been added at the start of the 'Treatment' subsection: 'This is a case series report, resulting from non-standardized administration of treatments to individual patients on a case-by-case basis (under the EU rules governing compassionate care and hospital exemption), not under homogenous inclusion/exclusion criteria that would be applicable for a clinical study. The resultant patient data were analysed retrospectively, and the intention of this article was to provide insights into this type of treatment, which is currently undergoing further developments and refinements.' Thirdly, on p. 2061, the left-hand column, the first sentence in the final paragraph of the Discussion has been changed to read as follows: 'Based on above data and the cited literature on mesenchymal stem cells - of which $CD271^+$ represent a subpopulation - it can be said that treating chronic stroke patients with $CD271^+$ stem cells administered intrathecally and/or intravenously is a safe therapeutic option with good results especially for spasticity and aphasia; at the same time it needs to be further studied and improved (e.g., repeated or combined intrathecal/iv administration, control of stem cell homing by administering different proportions of $CD271^+/CD34^+$) with the goal of obtaining ample motor improvements and subsequent functional independence of the patient.'

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Availability of data and materials

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Authors' contributions

FS: manuscript writing, data collection and analysis; GP: manuscript planning and data evaluation; GL: manuscript planning and data evaluation; DAS: manuscript planning and data evaluation; AT: manuscript planning and data evaluation; MF: data collection; CB: manuscript planning and evaluation; CB: manuscript review and data evaluation.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Informed consent was obtained from each patient prior to procedure.

Competing interests

DAS is the Editor-in-Chief for the journal, but had no personal involvement in the reviewing process, or any influence in terms of adjudicating on the final decision, for this article. The other authors declare that they have no competing interests.

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