


Complete Restoration of Motor Function in Acute Cerebral Stroke Treated with Allogeneic Human Umbilical Cord Blood Monocytes: Preliminary Results of a phase I Clinical Trial

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Abstract

Stem cell therapy has been explored for the treatment of cerebral stroke. Several types of stem cells have been investigated to ensure the safety and efficacy in clinical trials.

Cryopreserved umbilical cord blood (UCB) mononuclear cells (MNCs) obtained from healthy donors have a more stabilized quality, thereby ensuring a successful therapy. A phase I study was conducted on patients aged 45–80 years who sustained acute ischemic stroke. An UCB unit was obtained from a public cord blood bank based on ABO/Rh blood type, HLA matching score (6/6), and cell dose (total MNC count of $0.5\text{--}5 \times 10^7$ cells/kg). In addition, to facilitate blood brain barrier penetration of UCB, 4 doses of 100 mL mannitol was administered intravenously after 30 min after UCB transplantation and every 4 h thereafter. The primary outcomes were the number of disease (GVHD) within 100 days after transfusion. The secondary outcomes were changes in the National Institutes of Health Stroke Scale (NIHSS), Barthel index, and Berg Balance Scale scores. A 46-year-old male patient with identical ABO/Rh blood type, HLA matching score of 6/6, and MNC count of 2.63×10^8 cells/kg was enrolled. The patient did not present with serious AEs or GVHD during the 12-month study period. The patient's NIHSS score decreased from 9 to 1. Moreover, the Berg Balance Scale score increased from 0 to 48 and the Barthel index score from 0 to 90. This preliminary study showed that an adult patient with hemiplegia due to ischemic stroke completely recovered within 12 months after receiving allogeneic UCB therapy.

Keywords

acute cerebral stroke, allogeneic, clinical trial, stem cell, umbilical cord blood

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Abbreviations

BBB, blood-brain barrier; CB, cord blood; DWI, diffusion-weighted imaging; EGF, epidermal growth factor; G-CSF, granulocyte colony stimulating factor; GFs, growth factors; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; MNCs, mononuclear cells; PD, plasma depletion; TNF, tumor necrosis factor; UCB, umbilical cord blood; VEGF, vascular endothelial growth factor.

Introduction

Currently, acute stroke is the second and third leading cause of death and disability, respectively, worldwide. More than 15 million people present with stroke annually. Approximately 30%–35% die and nearly 75% of survivors sustain permanent disability¹. The current treatments in the acute phase include the use of thrombolytic, anticoagulant, and antiplatelet agents. Thrombolytic agents must be administered within 4.5 h of stroke onset. However, the use of this agent increases the incidence of hemorrhage by 15%–20%. After ischemic stroke, about 120 million neurons die every hour, which is equal to 3.6 years of aging brain function². Furthermore, the dead neurons cannot regenerate. Hence, treatment with new cells to facilitate regeneration is essential. The developing cell therapy is a breakthrough in the treatment of stroke. Our research team used granulocyte colony stimulating factor (G-CSF) injection combined with autologous hematopoietic stem cells (CD34+) brain transplantation for the treatment of patients with chronic ischemic stroke ($n=15$)³. Based on the current study, this treatment was found to be feasible and safe. However, results also showed that the patient's cell could significantly affect the degree of improvement. That is, autologous stem cell proliferation and differentiation in an elderly patient (aged over 60 years) is not as good as that of younger patients.

Compared with human adult peripheral blood, human umbilical cord blood contains richer hematopoietic primitive cells and numerous endothelial primitive cells, which have strong replication capacity in vitro and in vivo. Samples of cryopreserved plasma depletion (PD) cord blood products were assayed to determine cytokine profiles using a R&D Human XL Cytokine Discovery 14 Plex panel. The in-vitro analytic results listed in Table 1, which showed the level of anti-inflammatory cytokine, interleukin (IL)-10, is significantly higher than those of pro-inflammatory cytokine, IL-1-beta, IL-2, IL-6, interferon gamma (IFN)-gamma and tumor necrosis factor(TNF)-alpha. The relatively higher levels of growth factors (GFs), epidermal growth factor (EGF), FGF-basic, vascular endothelial growth factor (VEGF), G-CSF, and granulocyte-macrophage colony-stimulating factor (GM-CSF) were observed in comparison with those of cytokines, IL-1-beta, IL-2, IL-4, IL-5, IL-6, IFN-gamma and TNF-alpha. The high amounts of EGF, VEGF, G-CSF, and IL-10 in PD cord blood (CB) products allows us to hypothesize that the infusion of PD cytokine products may not only restore immune homeostasis but also enhance the repairing of the damaged brain nervous system in cerebral stroke patients⁴.

Moreover, cord blood stem cells proliferate into neural cells, and they were found to be effective in the treatment of

Table 1. Cytokine and Growth Factor Profiles in Cryopreserved PD Cord Blood Products vs. Adult Plasma/Serum.

Cytokines	Cryopreserved PD cord blood product		Adult blood Plasma/serum
	Concentration* pg/mL	Total AMT (~ 75 ml)* pg/unit	Concentration* pg/mL
IL-1 beta	14.96+4.45	1122+334	1.24+0.19
IL-2	56.03+33.12	4202+2484	2.29+0.27
IL-4	19.12+7.46	1434+559	6.05+0.23
IL-5	55.53+15.61	4165+1171	1.93+0.22
IL-6	60.57+16.73	4542+1228	1.20+0.17
IL-8	277.12+259.34	20784+19450	2.98+0.79
IL-10	144.02+69.66	10801+5224	1.91+0.14
IFN-gamma	38.98+8.42	2923+631	1.41+0.21
TNF-alpha	40.84+12.62	3063+946	2.77+0.25
GM-CSF	81.30+56.4	6097+4230	4.46+0.15
Growth Factors			
VEGF	277.79+84.01	20834+6300	2.94+0.11
G-CSF	134.09+20.45	10056+1534	46.22+0.13
EGF	191.13+23.98	14335+1798	4.64+0.13
FGF basic	87.80+33.86	6584+2539	2.35+0.12

*Levels of cytokines and growth factors presented as mean+ SD, IL: interleukine, IFN-gamma: interferon-gamma, TNF-alpha: tumor necrosis factor-alpha, GM-CSF: granulocyte-macrophage colony stimulating factor, G-CSF: granulocyte-colony stimulation factor, EGF: epithelial growth factor, FGF: basic fibroblast growth factor

Table 2. Inclusion criteria.

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1. Age between 45 and 80
 2. Acute ischemic stroke without t-PA therapy
 3. Brain MRI shows acute brain ischemic stroke in the MCA territory exclude hemorrhagic stroke. No mid line shift;no hemorrhagic transformation
 4. NIHSS Scale:8 to 16
 5. Check NIHSS 2 times, 2nd score can't be decrease 4 points compare with 1st
 6. HLA matched $\geq 4/6$ with ABO/Rh matched
 7. The cord blood will be administered once intravenously no later than 10 days after stroke
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several neurodegenerative diseases^{5,6}. In cerebral stroke, the intravenous injection of umbilical cord blood (UCB) mononuclear cells (MNCs) could restore exercise abilities, and it has neuroprotective effects⁷. After transplantation of human UCB, the expression of inflammatory markers such TNF- α , IL-1 β , and IL-2 decreased. Meanwhile, that of inflammatory markers including IL-6, IL-10, and transforming growth factor beta 1 increased. Consequently, after transplantation of human UCB, the anti-inflammatory processes can achieve the protective effect of neural cells^{7,8}. In addition to the anti-inflammatory effect, UCB MNCs can spontaneously transfer to the damaged central nervous system and the spleen^{7,9}. Therefore, UCB MNCs can participate in the biosynthesis of lymphocytes. Some studies have confirmed that lymphocytes are associated with neuroprotection in rats with acute stroke¹⁰⁻¹².

A phase I clinical trial on the intravenous administration of human UCB in acute ischemic stroke was conducted. To the best of knowledge, this study presented the first patient who completely recovered from right hemiplegia 12 months after receiving UCB therapy.

Materials and Methods

Study Design

This is a multicenter phase I safety study on patients aged 45–80 years who sustained acute stroke but did not receive t-PA therapy. In total, six participants will be enrolled. The inclusion and exclusion criteria are presented in Tables 2 and 3, respectively. The participants will undergo a series of baseline neurological assessments, blood tests, and MRI. UCB units will be selected from a public CB bank based on the ABO/Rh blood type, HLA matching score (6/6), and cell dose (total nucleated cell count of $0.5\text{--}5 \times 10^7$ cells/kg). The UCB will be administered intravenously as a single infusion between 3 and 10 days after stroke onset. The participants will be monitored for 6 h after infusion, and follow-up will perform after 24 h. Subsequent follow-up phone calls will be conducted at 1, 6, and 12 months. The calls will include telephone surveys on post-stroke rehabilitation and functioning. The follow-up assessment after 90 days will include neurological examination, MRI, and blood tests. The

Research and Ethical Review Committees and Internal Review Board of Tzu Chi Hospital approved the protocol and procedures for the project (IRB105-71-A).

Selection of Umbilical Cord Blood Unit

An assessment was performed upon receipt of the participant's information, including HLA typing, from the transplant center. The report was used to create a Summary Search Report for Acute Stroke Clinical Trial, which included information on candidate CB units meeting the following criteria: at least a matching score of 4/6 based on low-resolution HLA typing, identical ABO/Rh blood type between the subject and donor, and total mononucleated cell (MNC) count of $2\text{--}5 \times 10^8$ cells/kg.

Case Presentation

A 40-year-old male patient, with a history of hypertension and hemodialysis for end-stage renal disease, presented with acute left hemiplegia at an emergency department. Emergent assessment conducted by a neurologist and MRI revealed infarction in the right ICA territory. Recombinant tissue plasminogen activator was not recommended owing to a time delay >3 h. Hence, the patient was enrolled in this study and the informed consent was written by patient himself. The baseline NIHSS score was 8, and it increased by 1 point after 24 h. A Summary Search Report for Acute Stroke Clinical Trial was created after assessing information from the CB bank. The report showed an identical ABO/Rh blood type, HLA matching score of 6/6, and total MNC count of 2.63×10^8 cells/kg. UCB transfusion was performed on the 8th day after stroke onset. Then, 4 doses of 100 mL mannitol were administered intravenously after 30 min after UCB transplantation and every 4 h thereafter. The patient was discharged 8 days after UCB transfusion, and he was followed-up at 1, 3, 6, and 12 months for the assessment of NIHSS score, neurological function, and MRI results.

Results

The patient's neurological function improved gradually after UCB transfusion. Moreover, the NIHSS score decreased from 9 at baseline to 1 at 12 months after UCB transfusion (Fig. 1A). Moreover, the Berg Balance Scale score increased from 0 to 48 (Fig. 1B), and the Barthel index score from 0 to 90 (Fig. 1C). The patient did not present with splenomegaly on abdominal ultrasonography during the 12-month observation period. However, he presented with insomnia and upper respiratory tract infection and was treated with medications, which led to satisfactory outcomes. No serious adverse event was observed. Diffusion-weighted imaging (DWI) performed 2 h and 8 days after stroke revealed infarction (increased white intensity) in the right corona radiata, which scattered after 3 months and disappeared after 6 months (Fig. 2A–D). T2-weighted images obtained 2 h,

Table 3. Exclusion Criteria.

Medical Conditions

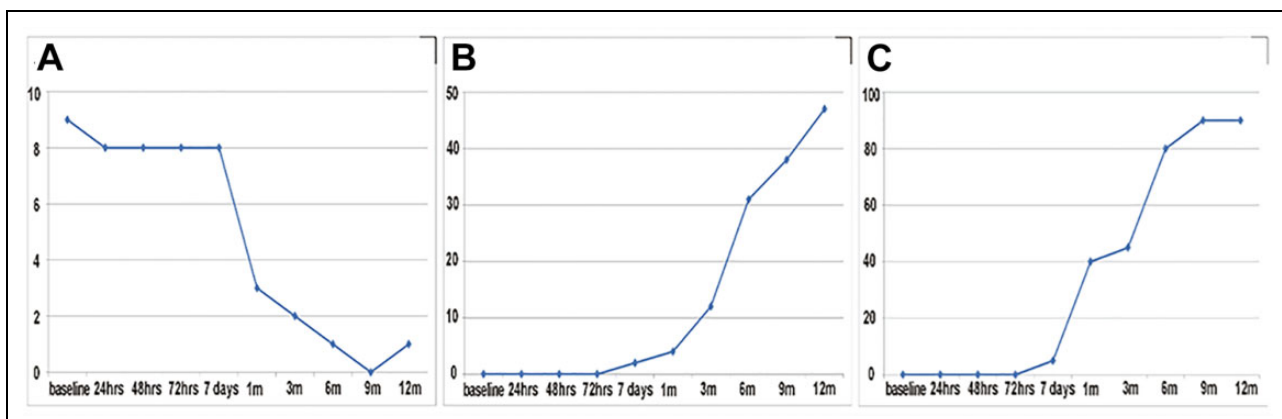
1. Has a medical history of neurological or orthopedic pathology with a deficit as a consequence that results in a modified Rankin Scale >1 before stroke or has a pre-existing cognitive deficit
2. Has clinically significant and/or symptomatic hemorrhage associated with stroke
3. Has new intracranial hemorrhage, edema, or mass effect that may place patient at increased risk for secondary deterioration when assessed prior to infusion
4. Has hypotension as defined as the need for IV pressor support of systolic blood pressure <90
5. Has isolated brain stem stroke
6. Has pure lacunar stroke
7. Requires mechanical ventilation
8. Requires a craniotomy
9. Has a serious psychiatric or neurological disease which could alter evaluation on functional or cognitive scales
10. Has an active systemic infection or is HIV positive
11. Has had an active malignancy within 3 years prior to the start of screening excluding skin cancers other than melanoma
12. Has known coagulopathy such as Factor V Leyden, AntiPhospholipid Syndrome (APC), Protein C, Protein S deficiency, sickle cell, anticardiolipin antibody, or phospholipid syndrome
13. Has any concurrent illness or condition that in the opinion of the investigator might interfere with treatment or evaluation of safety
14. Has current or recent history of alcohol or drug abuse, or stroke associated with drug abuse
15. Pregnant as documented by urine or blood test

Concomitant or Prior Therapies

1. Subjects currently receiving immunosuppressant drugs
2. History of prior transfusion reaction
3. Currently on dialysis
4. Recipient of bone marrow or organ transplant
5. Renal insufficiency with serum creatinine >2.0 mg/dL
6. Hepatic insufficiency (bilirubin >2.5mg/dL or transaminases >5x the upper limit of normal). Patients with Gilberts syndrome are eligible for study enrollment if other liver function tests are normal, regardless of bilirubin level.
7. Any previous or current treatment with angiogenic growth factors, cytokines, gene or stem cell therapy
8. Subjects participating in another interventional clinical trial of an investigational therapy within 30 days of screening

Other

1. Pregnant or lactating women
2. Unable to be evaluated for follow up visits

**Figure 1.** Neurological function from day 1 prior to UCB transfusion (baseline) to 12 months after UCB transfusion. (A) National Institutes of Health Stroke Scale score, (B) Berg Balance Scale score, and (C) Barthel index score

8 days, 3 months, and 6 months after the onset of infarction revealed increased white intensity in the right corona radiata (Fig. 3A–D).

Discussion

The efficacy of stem cell therapy for cerebral stroke in terms of cell type, cell origin, cell number, timing of cell

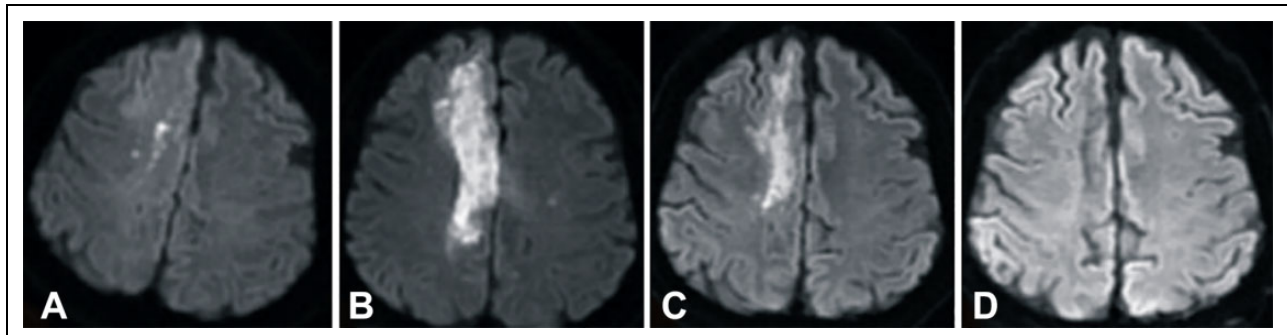


Figure 2. Diffusion-weighted images obtained 2 h after stroke onset and 6 months after mononucleated cell UCB transfusion. (A) 2 h after stroke, (B) 1 day after umbilical cord blood transfusion, (C) 3 months after UCB transfusion, and (D) 6 months after MNC transfusion

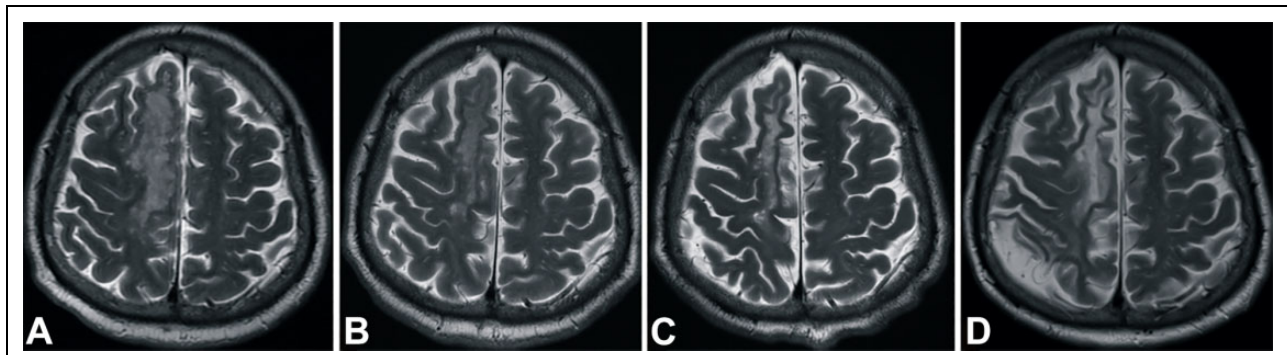


Figure 3. T2-weighted images obtained 2 h after stroke and 6 months after UCB transfusion. (A) 2 h after stroke, (B) 1 day after UCB transfusion, (C) 3 months after UCB transfusion, and (D) 6 months after UCB transfusion

treatment, and route of cell delivery has been subjected to optimization evaluations. The optimal cell transplant strategy has been examined in several preclinical and clinical studies. Although a conclusion has not been established, translational guidance on the safe and effective transplant regimen has been recognized. The time window for neural cell repair or renewal after the initiation of stroke is within 72 h based on an animal experiment¹³. Early cell therapy in the acute or subacute stage of stroke might result in better outcomes. This patient presented with subclinical second stroke on MRI 3 months after stroke. The T2-weighted images revealed loss of brain parenchyma and CSF accumulation only in the second stroke region, but not in the first stroke region, which has been treated with cell therapy in the acute stage (Fig. 3c, 3d).

Human UCB MNCs have been shown to exert robust therapeutic effects in experimental models of acute and subacute stage of stroke. These cells have immunomodulatory and anti-inflammatory effects in addition to regenerative effects. Hence, they can protect the penumbral tissue from further injury caused by inflammation after stroke¹⁴. MNCs have immunomodulatory effects by changing the phenotype of splenocytes⁹. In the acute or subacute stages, intravenous MNC therapy may achieve neural protection via the modification of systemic immunomodulation.

MNCs, and other adult tissue-derived stem cells can be easily isolated and expanded in the laboratory. The safety

profile of MNCs has been consistently demonstrated clinical trials. However, their efficacy is unclear^{15,16}. The quality, activity, and stemness of donor stem cells are the fundamental requirement for a successful stem cell therapy. The cell conditions are significantly correlated to donor origin. In autologous transplantation, the donor cells are collected from sick patients thus the stem cells are possibly unhealthy. Cryopreserved MNCs in allogeneic transplantation, unlike autologous transplantation, are collected from a healthy donor, and they have a stabilizing quality.

The appropriate stem cell number for stroke therapy is not fully elucidated. However, a minimum single dose of 840 million is required for the intravenous injection of mesenchymal stem cells¹⁷. A benefit-to-risk compromise between cell number and toxicity should be based on the maximal benefit and the lowest cell dosage¹⁸. Enhancing the permeability of stem cells when crossing the blood–brain barrier (BBB) may reduce the required amount of stem cells. Mannitol can break the BBB to facilitate the peripheral delivery of stem cells¹⁹. Although the cell count in this case was 263 million, mannitol was used to open the BBB 30 min after MNC infusion. In addition to increasing the permeability of stem cells, the expression of neurotrophic and neural growth factors across the BBB can be enhanced by this drug^{20,21}. Therefore, aside from MNC cell regeneration, the effects of by-products derived from transplanted MNCs can

contribute to synaptogenesis, immature neuron proliferation, and neuronal cell migration.

Conclusion

This preliminary result showed that an adult patient with ischemic stroke significantly recovered within a short period after receiving allogeneic umbilical cord blood MNCs with an HLA matching score of 6/6.

Author Contributions

T.K.L., C.Y.L., S.T.T. and P.H.T. designed the research, performed the experiments, and wrote the manuscript; Y.C.L., and S.Z.L. analyzed the data and interpreted data; J.C.W., and C.Y.H. contributed analytical tool. All authors were involved in editing the manuscript and gave final approval of the submitted final published format.

Tian-Kuo Lee and Sheng-Tzung Tsai shares equal contribution.

Declaration of Conflicting Interests

PRS and CVB hold patents with the University of South Florida for the use of human umbilical cord blood-derived cells, including MNCs, as a cell therapy for stroke and several disorders. PRS and CVB provided the intellectual and technological expertise in the use of mannitol and human umbilical cord blood-derived cells. The authors declare that they have no conflict of interest.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Ethical approval to report this case series was obtained from *The Research and Ethical Review Committees and Internal Review Board of Tzu Chi Hospital (IRB105-71-A)*.

Statement of Human and animal Rights

All procedures in this study were conducted in accordance with the *The Research and Ethical Review Committees and Internal Review Board of Tzu Chi Hospital (IRB105-71-A)* approved protocols.




Statement of Informed Consent

Written informed consent was obtained from a legally authorized representative for anonymized patient information to be published in this article.

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References

- Singh M, Pandey PK, Bhasin A, Padma MV, Mohanty S. Application of stem cells in stroke: a multifactorial approach. *Front Neurosci.* 2020;14:473.
- Lakhan SE, Kirchgessner A, Hofer M. Inflammatory mechanisms in ischemic stroke: therapeutic approaches. *J Transl Med.* 2009;7:97.
- Chen DC, Lin SZ, Fan JR, Lin CH, Lee W, Lin CC, Liu YJ, Tsai CH, Chen JC, Cho DY, Lee CC, et al. Intracerebral implantation of autologous peripheral blood stem cells in stroke patients: a randomized phase II study. *Cell Transplant.* 2014;23(12):1599–1612.
- Ehrhart J, Sanberg PR, Garbuzova-Davis S. Plasma derived from human umbilical cord blood: Potential cell-additive or cell-substitute therapeutic for neurodegenerative diseases. *J Cell Mol Med.* 2018;22(12):6157–6166.
- Garbuzova-Davis S, Willing AE, Zigova T, Saporta S, Justen EB, Lane JC, Hudson JE, Chen N, Davis CD, Sanberg PR. Intravenous administration of human umbilical cord blood cells in a mouse model of amyotrophic lateral sclerosis: distribution, migration, and differentiation. *J Hematother Stem Cell Res.* 2003;12(3):255–270.
- Nikolic WV, Hou H, Town T, Zhu Y, Giunta B, Sanberg CD, Zeng J, Luo D, Ehrhart J, Mori T, Sanberg PR, et al. Peripherally administered human umbilical cord blood cells reduce parenchymal and vascular beta-amyloid deposits in Alzheimer mice. *Stem Cells Dev.* 2008;17(3):423–439.
- Vendrame M, Cassady J, Newcomb J, Butler T, Pennypacker KR, Zigova T, Sanberg CD, Sanberg PR, Willing AE. Infusion of human umbilical cord blood cells in a rat model of stroke dose-dependently rescues behavioral deficits and reduces infarct volume. *Stroke.* 2004;35(10):2390–2395.
- Vendrame M, Gemma C, de Mesquita D, Collier L, Bickford PC, Sanberg CD, Sanberg PR, Pennypacker KR, Willing AE. Anti-inflammatory effects of human cord blood cells in a rat model of stroke. *Stem Cells Dev.* 2005;14(5):595–604.
- Vendrame M, Gemma C, Pennypacker KR, Bickford PC, Davis Sanberg C, Sanberg PR, Willing AE. Cord blood rescues stroke-induced changes in splenocyte phenotype and function. *Exp Neurol.* 2006;199(1):191–200.
- Traggi E, Chicha L, Mazzucchelli L, Bronz L, Piffaretti JC, Lanzavecchia A, Manz MG. Development of a human adaptive immune system in cord blood cell-transplanted mice. *Science.* 2004;304(5667):104–107.
- Wolf SA, Fisher J, Bechmann I, Steiner B, Kwidzinski E, Nitsch R. Neuroprotection by T-cells depends on their subtype and activation state. *J Neuroimmunol.* 2002;133(1-2): 72–80.
- Rosenkranz K, Tenbusch M, May C, Marcus K, Meier C. Changes in Interleukin-1 alpha serum levels after transplantation of umbilical cord blood cells in a model of perinatal hypoxic-ischemic brain damage. *Ann Anat.* 2013;195(2): 122–127.
- Boltze J, Schmidt UR, Reich DM, Kranz A, Reymann KG, Strassburger M, Lobsien D, Wagner DC, Forschler A, Schabitz

- WR. Determination of the therapeutic time window for human umbilical cord blood mononuclear cell transplantation following experimental stroke in rats. *Cell Transplant*. 2012;21(6):1199–1211.
14. Sanberg PR, Ehrhart J. A Hallmark clinical study of cord blood therapy in adults with ischemic stroke. *Cell Transplant*. 2019;28(9-10):1329–1332.
 15. Lalu MM, Montroy J, Dowlatshahi D, Hutton B, Juneau P, Wesch N, Zhang SY, McGinn R, Corbett D, Stewart DJ, Fergusson DA. From the lab to patients: a systematic review and meta-analysis of mesenchymal stem cell therapy for stroke. *Transl Stroke Res*. 2020;11(3):345–364.
 16. Zheng H, Zhang B, Chhatbar PY, Dong Y, Alawieh A, Lowe F, Hu X, Feng W. Mesenchymal stem cell therapy in stroke: a systematic review of literature in pre-clinical and clinical research. *Cell Transplant*. 2018;27(12):1723–1730.
 17. Borlongan CV. Concise review: stem cell therapy for stroke patients: are we there yet? *Stem Cells Transl Med*. 2019;8(9):983–988.
 18. Sarmah D, Kaur H, Saraf J, Pravalika K, Goswami A, Kalia K, Borah A, Wang X, Dave KR, Yavagal DR, Bhattacharya P. Getting closer to an effective intervention of ischemic stroke: the big promise of stem cell. *Transl Stroke Res*. 2018;9(4):356–374.
 19. Tajiri N, Lee JY, Acosta S, Sanberg PR, Borlongan CV. Breaking the blood-brain barrier with mannitol to aid stem cell therapeutics in the chronic stroke brain. *Cell Transplant*. 2016;25(8):1453–1460.
 20. Yasuhara T, Hara K, Maki M, Xu L, Yu G, Ali MM, Masuda T, Yu SJ, Bae EK, Hayashi T, Matsukawa N, et al. Mannitol facilitates neurotrophic factor up-regulation and behavioural recovery in neonatal hypoxic-ischaemic rats with human umbilical cord blood grafts. *J Cell Mol Med*. 2010;14(4):914–921.
 21. Gonzales-Portillo GS, Sanberg PR, Franzblau M, Gonzales-Portillo C, Diamandis T, Staples M, Sanberg CD, Borlongan CV. Mannitol-enhanced delivery of stem cells and their growth factors across the blood-brain barrier. *Cell Transplant*. 2014;23(4-5):531–539.