Efficacy of Allogeneic Cord Blood Cell Therapy Combined with Erythropoietin for Children with Cerebral Palsy

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Contents

- Introduction of cerebral palsy
- Reports of 3 RCTs of cord blood cell +/- erythropoietin therapy
- Efficacy factors in cord blood cell therapy
- Animal and in vitro study for efficacy evaluation of cord blood + erythropoietin combination therapy in stroke model
- IL-8 as an efficacy inducing molecule in cord blood therapy

Cerebral palsy (CP)

- A group of neurodevelopmental conditions with abnormal movement and posture resulted from non-progressive disturbances that occurred in developing brain
- The most common cause of motor disability in childhood, life-long functional deficits
- Prevalence: 3 per 1000

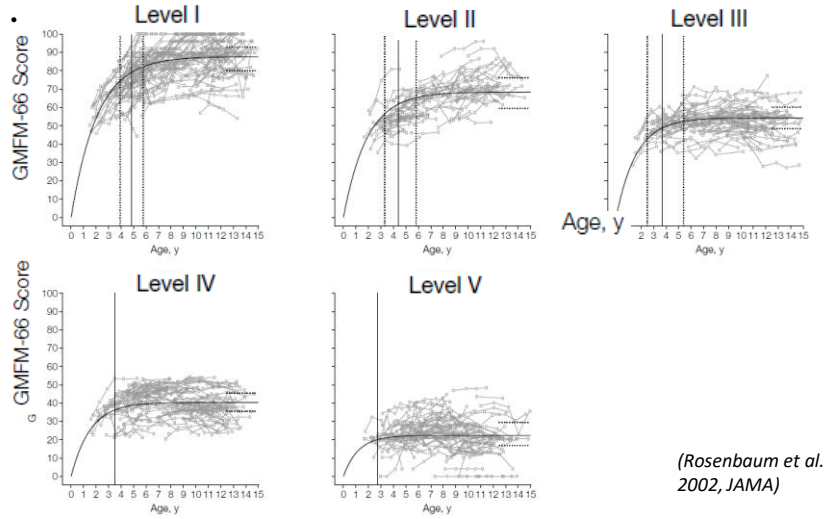
(Bax et al., 2005 Dev Med Child Neurol)

Regenerative medicine

- Most therapies: Palliative >> Restorative
- Cell therapy: Replacing or regenerating the affected neural tissues

(Harris et al. 2008, Stem Cell Dev)

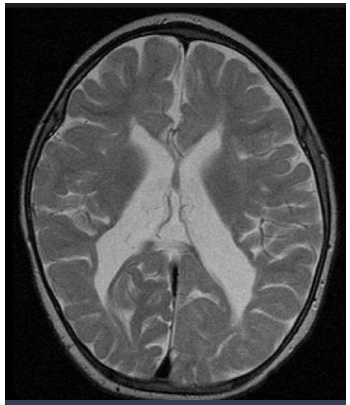
Observed and predicted GMFM scores in each level of Gross Motor Function Classification System (GMFCS)



(Rosenbaum et al. 2002, JAMA)

Cause of reluctance to recovery

- Inflammatory milieu in the brain of CP



Periventricular leukomalacia (PVL)

Developmental Neuroscience

Review

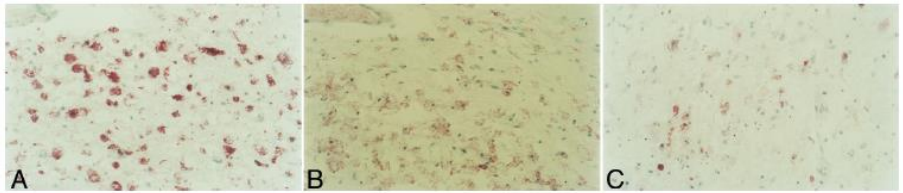
Dev Neurosci 2009;31:378–393
DOI: 10.1159/000232556

Received: December 3, 2008
Accepted after revision: March 3, 2009
Published online: August 11, 2009

Does Inflammation after Stroke Affect the Developing Brain Differently than Adult Brain?

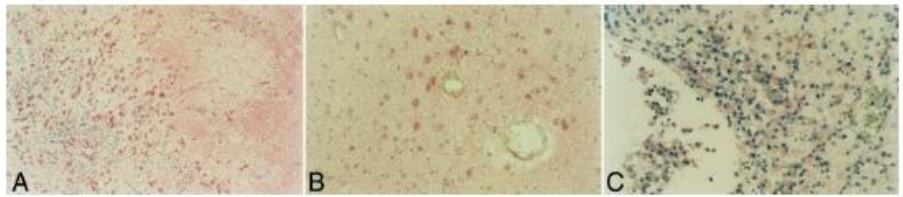
(Kadhim et al. 2001, Neurology)

Inflammatory cells in early PVL (3 to 5 days old)



A: CD68 B: Leukocyte common antigen C: Human leukocyte antigen II

in situ detection of cytokines in PVL

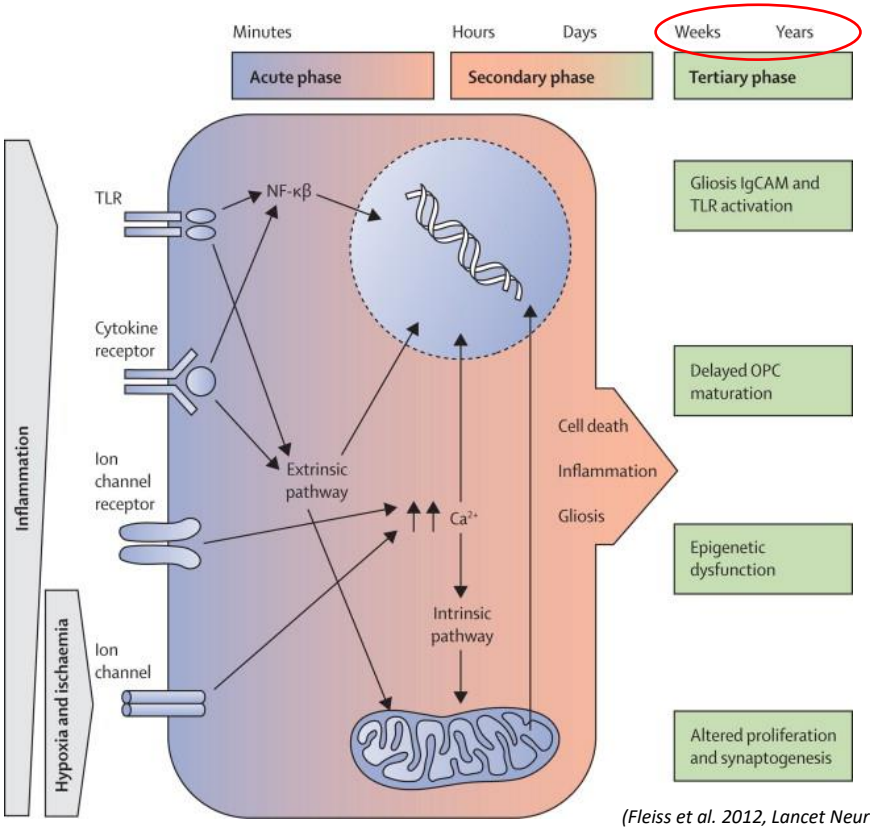


A: TNF α
Coagulative necrosis (early)

B: IL-6
Coagulative necrosis (early)

C: IL-1β
Cystic PVL (late)

Tertiary mechanisms of brain damage



(Fleiss et al. 2012, Lancet Neurol)

Tertiary brain damage

- Worsen outcome
- Predispose a patient to further injury
- Prevent repair or regeneration
  after an initial insult to the brain

Neuroprotection

Cell therapy

- Umbilical cord blood (UCB)
- Neural stem cell
- Mesenchymal stem cell

Pharmacological drugs

- Erythropoietin (EPO)
- Melatonin
- Growth factors

Duke University (USA; NCT00593242)

Medical College of Georgia (USA; NCT01072370), …

Advantages of UCB as a source of cell therapy

Umbilical Cord Blood

- Known as a stem cell source
  - Hematopoietic
  - Neurogenic
- Characteristics
  - Anti-inflammatory
  - Anti-apoptotic

Safety

- Used more over two decades
- Hematologic, immunologic, oncologic disorders, or inborn errors of metabolism

Gluckman, 1989; Kurtzberg, 2005; Carter 2006

Availability

- Off the shelf
- Cord blood banks

Harris, 2008

Lee, 2010

UCB

* suitable in regenerative medicine

Immune tolerance

- Immaturity

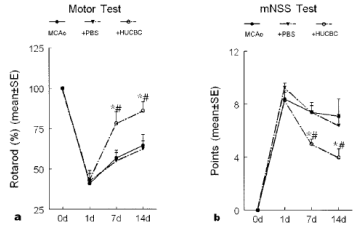
Tse, 2005; Vaziri, 1994; Lee, 2010

Differentiation

- Neuronal, astrocytic, oligo-dendroglial cell

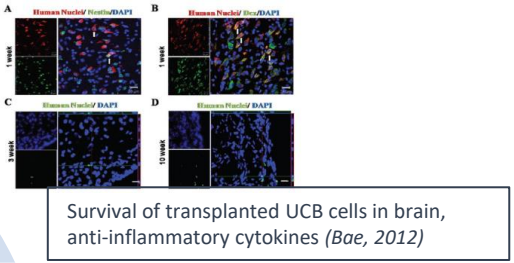
Neurhoff, 2007; Buzanska, 2006

Umbilical Cord Blood (UCB) in brain injury



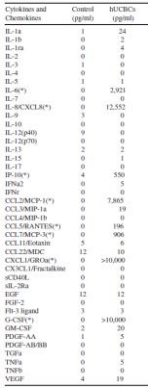
Functional improvement by UCB in brain infarct *(Chen, 2001)*

Neurogenesis

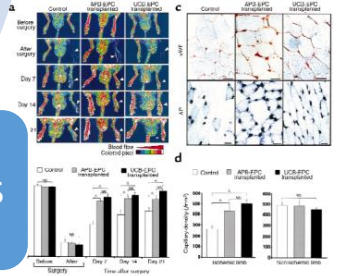


Survival of transplanted UCB cells in brain, anti-inflammatory cytokines *(Bae, 2012)*

Paracrine effect

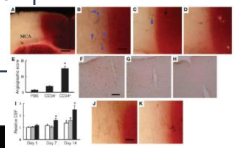


Vasculogenesis

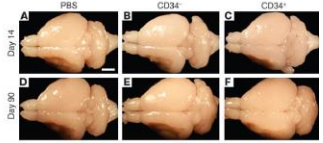


Neovascularization *(Murohara, 2001)*

Decreased infarct size



Decreased size of brain infarct area and vasculogenesis by administrating CD34+ UCB cells *(Taguchi, 2004)*



Functional recovery

The efficacy of UCB in animal models

- Source of stem cells

Immunophenotypic comparison of stem and progenitor cells derived from umbilical cord blood

Cell surface marker	HSC	MSC	USSC	CBE	MPC
CD34	+	−	−	+	−
CD133	+	−		+	−
CD14	−	−	−		+
CD45	+	−	−	−	+
CD44	+	+	+		+
CD54		+	+		+
CD73	−	+			−
CD90	+	+	+		−
CD105	−	+	+		+
CD166		+			+

Lee, 2010

Stroke (infarction)

- *Vendrame M et al. 2004, Stroke*
- *Willing AE et al. 2003, J Neurosci Res*

Stroke (hemorrhage)

- *Nan Z et al. 2005, Ann N Y Acd Sci*

Spinal cord injury

- *Cho et al. 2008, Neuroreport*

Traumatic brain injury

- *Lu D at el. 2002, Cell Transplant*

Alzheimer's dementia

- *Nikolic WV et al. 2008, Stem Cells Dev*

Cerebral palsy

- *Meier C et al. 2006, Pediatr Res*

Plan for clinical trials and immunosuppression

- Preliminary trial of (auto, allo) UCB infusion for small number of CP
- Rare preservation of autologous UCB in CP
- Plan to evaluate the efficacy of allogeneic UCB infusion for CP

Autologous UCB transplantation is the ideal approach in children with CP. However, most CP experience a difficult perinatal period that is unfavorable to harvest sufficient UCB.

Allogeneic UCB transplantation may thus represent a plausible alternative.

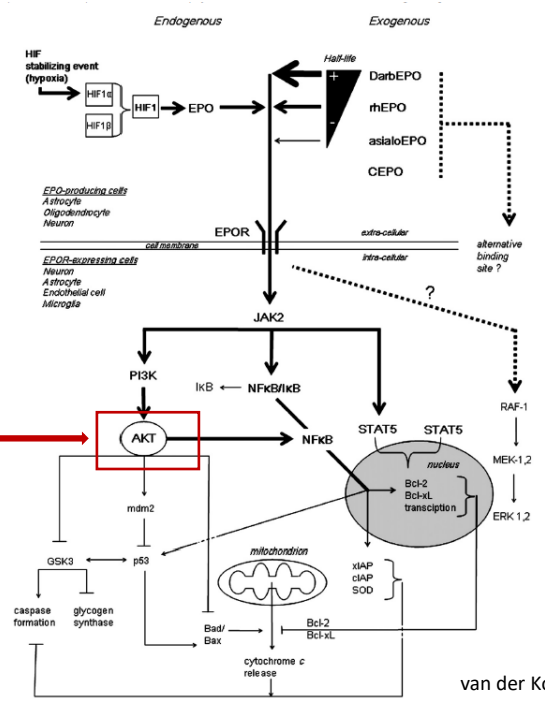
Immunosuppression is essential to prevent antibody generation and make up favorable environment for survival of allogeneic cells.

The 1st trial of UCB for children with CP

Title: Umbilical Cord Blood Therapy Potentiated with Erythropoietin for Children with Cerebral Palsy

- Potentiation with “Erythropoietin (EPO)”

UCB stem cells →



van der Kooij MA, 2008

Brain Res Rev Dasari VR, 2008 Neurobiol dis

1st Clinical Trial

UCB + Erythropoietin (EPO)

ClinicalTrials.gov
A service of the U.S. National Institutes of Health

Search for studies: Example: "Heart attack" AND "Los Angeles" Search
Advanced Search | Help | Studies by Topic | Glossary

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10 Completed Has Results — Allogenic Umbilical Cord Blood and Erythropoietin Combination Therapy for Cerebral Palsy
Condition: Cerebral Palsy
Interventions: Biological: Umbilical Cord Blood Infusion; Drug: Erythropoietin Injection; Other: Active Rehabilitation; Other: Placebo Umbilical Cord Blood; Other: Placebo Erythropoietin

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TRANSLATIONAL AND CLINICAL RESEARCH

Umbilical Cord Blood Therapy Potentiated with Erythropoietin for Children with Cerebral Palsy: A Double-blind, Randomized, Placebo-controlled Trial

Kyunghoon Min, MD,[a] Junyoung Song, MD[a], Jin Young Kang MD,[a] Jooyeon Ko PT, PhD,[a] Ju Seok Ryu Professor, MD, PhD,[a] Myung Seo Kang Professor, MD, PhD,[b] Su Jin Jang Professor, MD,[c] Sang Heum Kim Professor, MD,[d] Doyeun Oh Professor, MD, PhD,[e] Moon Kyu Kim Professor, MD, PhD,[f] Kim Sung Soo, Bio-statistician, PhD[g], MinYoung Kim, MD, PhD,[a]

(Min et al. 2013, Stem Cells)

Groups

Total 105 subjects
(Recruit: May 2010~ Nov 2010)

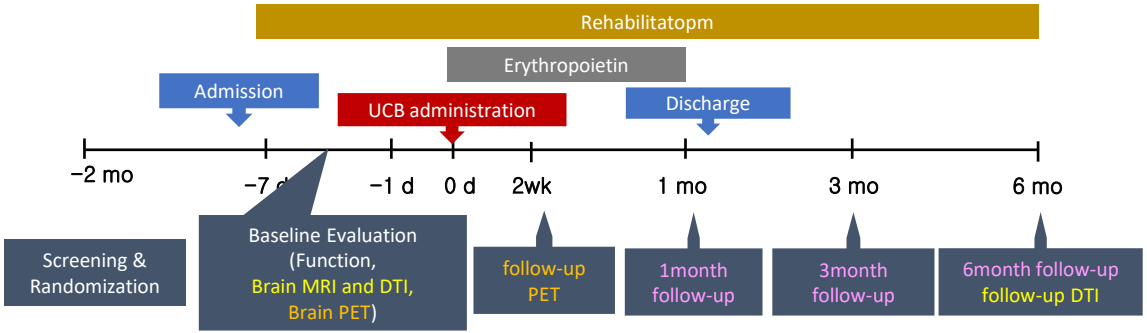
Randomized — Double-blind

pUCB (n=35)	EPO (n=36)	Control (n=34)
Allogeneic UCB + Erythropoietin + Rehabilitation	Placebo UCB + Erythropoietin + Rehabilitation	Placebo UCB + Placebo EPO + Rehabilitation

Intervention

- **IV administration**
- **Allogeneic UCB**
  - TNC>3×10^7/kg
  - HLA (A, B, DRB1) within 2 mismatch
- **EPO:** 500IU/kg × 2, 250IU/kg × 6
- **Cyclosporin**
  - Goal concentration: 100~200ng/mL
  - 4 weeks

Time line



Outcome measurements

- Gross Motor Function Measure (GMFM)
- Gross Motor Performance Measure (GMPM)
- Korean Bayley Scale 2nd version (BSID-II)
- Gross Motor Function Classification System (GMFCS)
- Alberta Infant Motor Scale (AIMS)
- Selective Control Assessment of the Lower Extremity (SCALE)
- Pediatric Evaluation of Disability Inventory (PEDI)
- Quality of Upper Extremity Skills Test (QUEST)
- Modified Ashworth Scale (MAS)
- Modified Tardiue Scale
- WeeFIM
- Range of Motion
- Manual Muscle Testing : 10 muscles in each side of upper and lower extremities
- Brain Diffusion Tensor Imaging (DTI) : FA value
- Brain ^{18}F-FDG-PET : analyzed using SPM3 implanted in MatLab R2011a, paired t-test statistics, voxels with an uncorrexted p-value <0.05

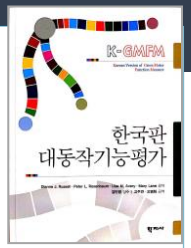
(Min et al. 2013, Stem Cells)

Reliability achievement for efficacy measures

GMFM-88

Reliability and Responsiveness of the Gross Motor Function Measure-88 in Children With Cerebral Palsy

Jooyeon Ko, MinYoung Kim *(Phys Ther, 2013)*



GMFM relative and absolute reliability

- 10 raters, 84 children with CP
- Relative reliability
  ICC (intraclass correlation coefficient): excellent (0.952-1.000)
- Absolute reliability
  SEM (standard error of measurement): 1.60 <10%
  SRD (smallest real difference): 3.14 <10% all acceptable

FA in DTI (Diffusion Tensor Imaging)

Reliability of fractional anisotropy measurement for children with cerebral palsy *(Neuropediatrics, 2013)*

Fractional anisotropy of corticospinal tract and ascending sensory pathway

- 4 raters, 78 children with CP
- Relative reliability
  Interrater ICC: excellent (≥ 0.91)
  Intrarater ICC: excellent (≥ 0.85)
- Absolute reliability
  Interrater SRD: acceptable <12%
  Intrarater SRD: acceptable <10%, All acceptable

GMPM

Original Article *(Ann Rehab Med, 2012)*

Ann Rehabil Med 2012; 36: 233-239
pISSN: 2234-0645 • eISSN: 2234-0653
http://dx.doi.org/10.5535/arm.2012.36.2.233

arm Annals of Rehabilitation Medicine

Inter-rater Reliability of the K-GMFM-88 and the GMPM for Children with Cerebral Palsy

Jooyeon Ko, P.T., Ph.D., Minyoung Kim, M.D.
Department of Rehabilitation Medicine, CHA Bundang Medical Center, CHA University, Seongnam 463-712, Korea

GMFM-88 and GMPM reliability and correlation

- 2 raters, 38 children with CP
- Reliability and correlation
  GMFM Inter & intrarater ICC: 0.916-0.997
  GMPM intrarater ICC: 0.863-0.929
  Correlation: r=0.859, p<0.01

BSID-II

Original Article *(Ann Rehab Med, 2013)*

Ann Rehabil Med 2013;37(2):167-174
pISSN: 2234-0645 • eISSN: 2234-0653
http://dx.doi.org/10.5535/arm.2013.37.2.167

arm Annals of Rehabilitation Medicine

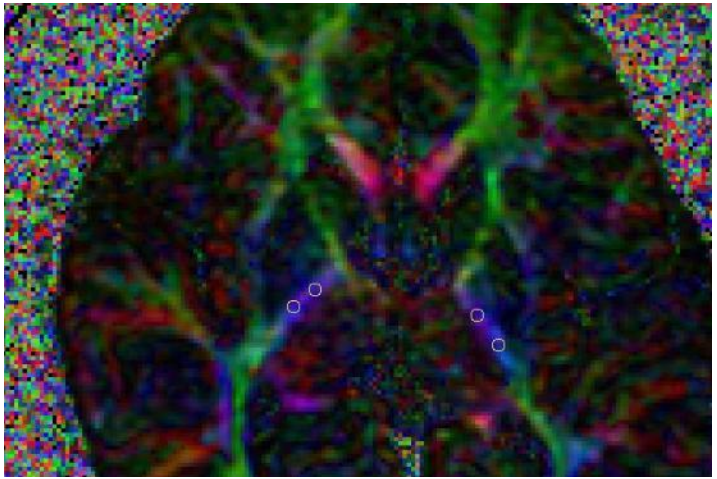
Reliability and Applicability of the Bayley Scale of Infant Development-II for Children With Cerebral Palsy

Ji Hyun Lee, MD, Hye Kyung Lim, EunYoung Park, Junyoung Song, MD, Hee Song Lee, MD, Jooyeon Ko, PhD, MinYoung Kim, MD

BSID-II reliability and validity

- 10 raters, 68 children with CP
- Interrater ICC: excellent (0.99)
- Correlation between Motor raw score and GMFM: r=0.84, p<0.001
- Correlation between Mental raw score and GMFM: r=0.65, p<0.001

DTI & fractional anisotropy (FA) measurements



- MRI : 3T GE Signa System
- DTI data were acquired using 2D axial spin echo echo-planar imaging with refocusing pulses
- Sequence parameters

  : TR/TE of 12000/108 msec
  1NEX, 48 slices
  24 cm FOV
  128 x 128 matrix
  3.0 mm slice thickness
  25 gradient directions
  B=900; with a non-diffusion weighted baseline image (B=0)

- Imaging data then was processed using DTI studio.
- FA value
  - Anterior & posterior portion at posterior limbs of internal capsule, bilaterally
  - Posterior lower pons, area of spinothalamic tract , bilaterally
- Rater : blind to subject information, 1 physiatrist
- ICC scores of test-retest reliability : 0.906 ~ 0.987 (1 rater, n=50)

Results

Demography and Typology

Group	pUCB (N=31)	EPO (N=33)	Control (N=32)
Male sex – no. (%)	23 (74.2%)	23 (69.7%)	23 (71.9%)
Age – months	36.84±19.4	43.9±24.7	38.3±18.4
Gestational day at birth (days)	237.6±34.6	230.3±35.0	246.4±28.7
Preterm – no. (%)	18 (58.1 %)	23 (69.7%)	17 (53.1%)
Birth weight – kg	2.2±0.9	2.0±0.9	2.4±0.7
NBW / LBW / VLBW / ELBW	13 / 9 / 8 / 1	11 / 8 / 10 / 4	16 / 13 / 2 / 1
GMFCS I / II / III / IV / V	4 / 3 / 5 / 10 / 9	5 / 4 / 11 / 7 / 6	2 / 1 / 12 / 9 / 8

Typology; SB: Spastic bilateral, SU: Spastic unilateral, D: Dystonia, C: Choreoathetosis, A: Ataxia (Bax, 2005)

(Min et al. 2013, Stem Cells)

Adverse events during study period of six months in three groups (N=105)

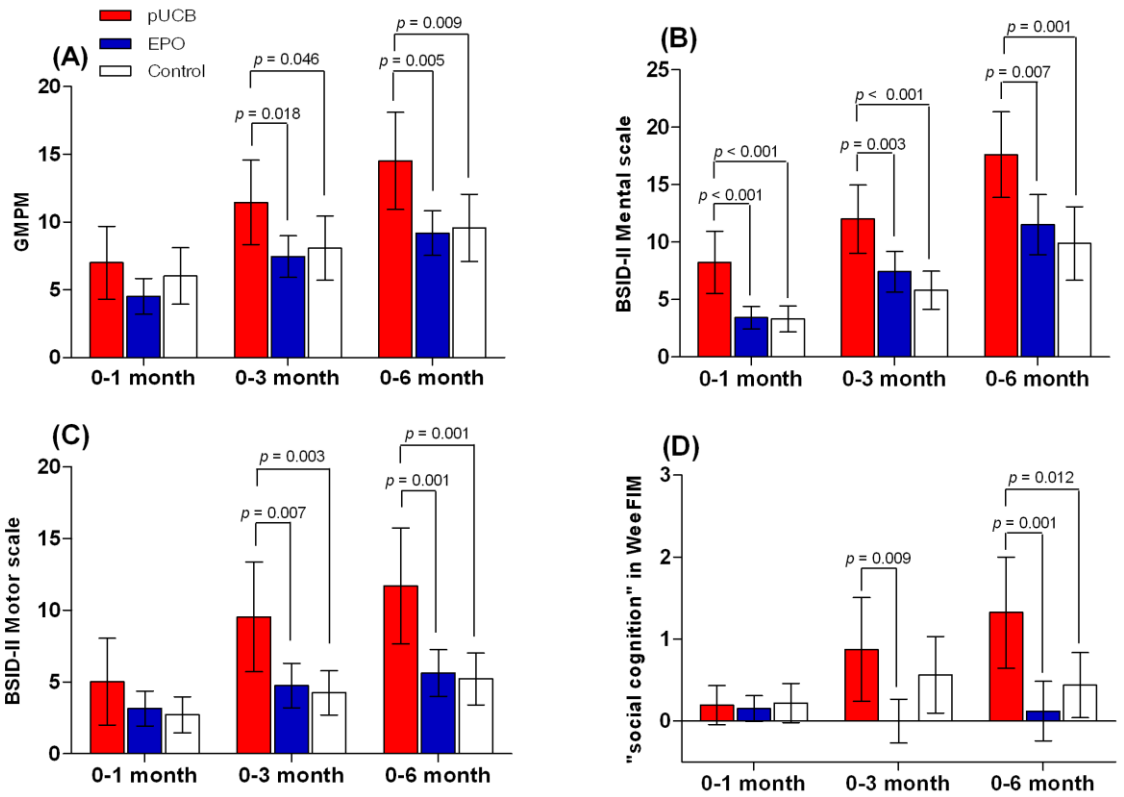
	pUCB (n=35)		EPO (n=36)		control (n=34)		p value§
	Number (percent)	Time of occurrences‡ (weeks post-treatment)	Number (percent)	Time of occurrences‡ (weeks post-treatment)	Number (percent)	Time of occurrences‡ (weeks post-treatment)	
Serious adverse events*							
Pneumonia	1 (2·9)	6~7	2 (5·6)	6~7, 18~19, 22~23	1 (2·9)	13~17	1·000
Seizure	0		1 (2·8)	16	0		1·000
Influenza	1 (2·9)	20	0		1 (2·9)	24~25	0·545
Urinary tract infection	0		0		1 (2·9)	12~13	0·324
Death	1 (2·9)	14	0		0		0·657
Other adverse events							
Upper respiratory tract infection	18(51·4)	0~5,10~13,23~24	19 (52·8)	0~5,9~13,19~25	21 (61·8)	0~4,8~17,23~25	0·666
Fever	12 (34·3)	0~6,17~18,21~22,24~25	4 (11·1)	1~4	8 (23·5)	0~3,18~19	0·067
Dyspepsia	5 (14·3)	0~4	2 (5·6)	1~3	2 (5·9)	0~2	0·459
Loose stool, diarrhea	6 (17·1)	0~3	2 (5·6)	0~2	2 (5·9)	0~2	0·246
Pneumonia	6 (17·1)	0~8, 20~22	0		0		0·002
Nausea, vomiting	6 (17·1)	0~4, 10~11	5 (13·9)	0~7	2 (5·9)	3~4	0·398
Anorexia	5 (14·3)	0~3	2 (5·6)	0~2	1 (2·9)	2~3	0·215
Bronchitis	4 (11·4)	0~8	4 (11·1)	0~6	3 (8·8)	1~5	1·000
Constipation	5 (14·3)	1~5	4 (11·1)	0~4, 15~16	5 (14·7)	0~4, 12~13	0·878
Irritability	4 (11·4)	0~2	0		0		0·021
Hypoxia†	3 (8·6)	0	1 (2·8)	3	1 (2·9)	3~4	0·527
Febrile convulsion	2 (5·7)	4,17,21	1 (2·8)	3	0		0·654
Herpangina	0		2 (5·6)	2~4	1 (2·9)	7~9	0·654
Urticaria	2 (5·7)	0~1, 3~4	1 (2·8)	3~4	4 (11·8)	0~3	0·254
Hirsuitism	2 (5·7)	3~26	0		0		0·212
Seizure	1 (2·9)	4	3 (8·3)	0, 8, 16, 18, 22, 23	3 (8·8)	2,3,4,6,13,24	0·625
Alopecia	1 (2·9)	1~3	0		0		0·657
Otitis media acute	1 (2·9)	4~5	1 (2·8)	2~3	0		1·000
Anemia	1 (2·9)	0~1	0		0		0·657
Colitis	0		1 (2·8)	6~7	2 (5·9)	1~4	0·317
Dermatitis	0		2 (5·6)	0~3	2 (5·9)	2~4	0·465
Insomnia	0		1 (2·8)	0	1 (2·9)	10~20	0·769
Conjunctival injection	0		1 (2·8)	3~4	1 (2·9)	1~4,22	0·769

(Min et al. 2013, Stem Cells)

Efficacy results (N=96)

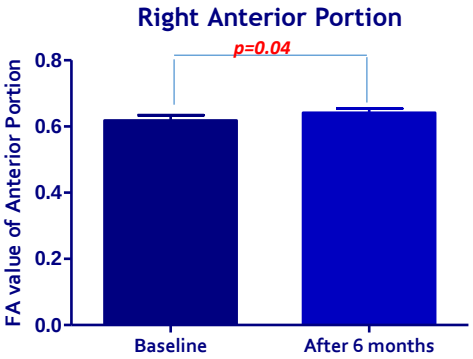
1st Clinical Trial

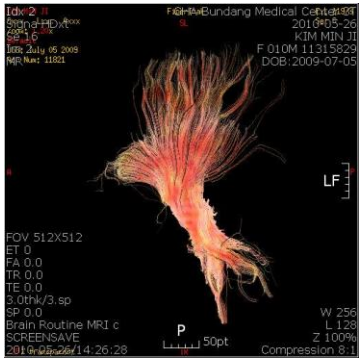
Changes in outcome scores from baseline to 1, 3, and 6 months post-treatment between pUCB, EPO and Control groups

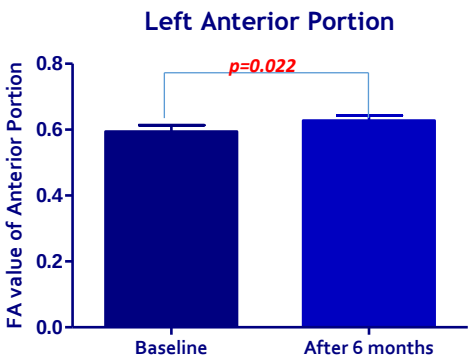


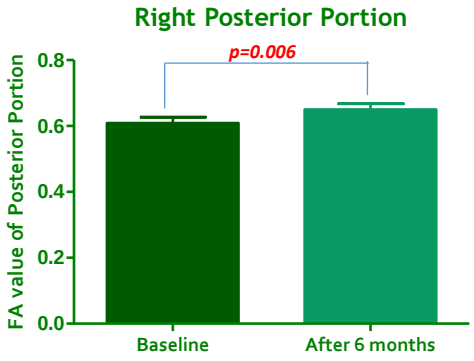
(Min et al. 2013, Stem Cells)

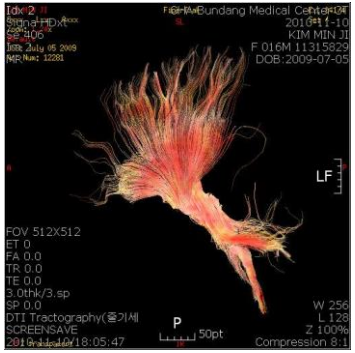
Changes of FA value in 4 portions of posterior internal capsule in potentiated UCB group (n=30)

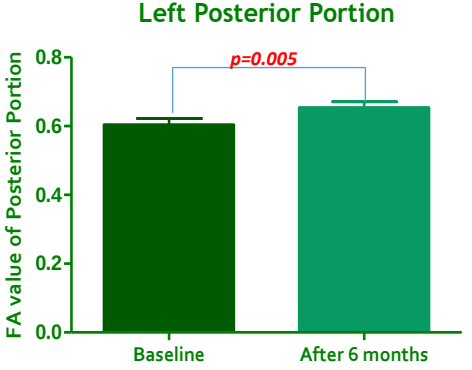








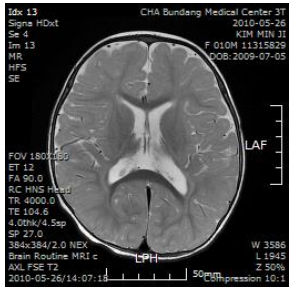




(Min et al. 2013, Stem Cells)

A case (F/11mo) who received UCB+EPO

1st Clinical Trial



CP due to periventricular leukomalacia and hypoxic brain damage

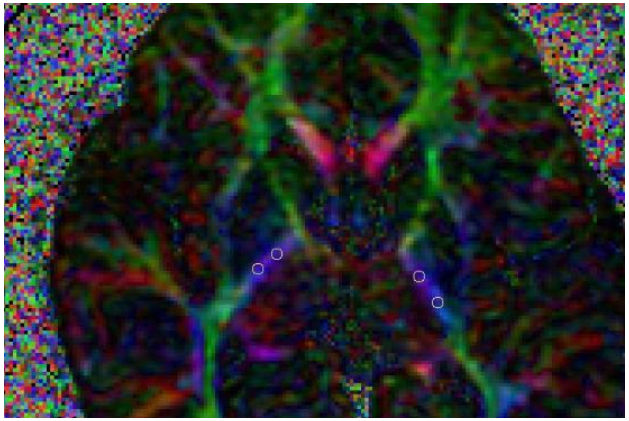
2 days before UCB administration

- She was unable to creep forward / severe irritability

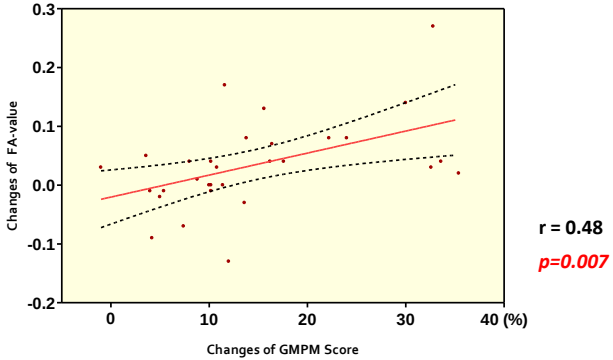
4 weeks after UCB administration

- She became able to creep forward / disappearance of irritability

Increment of FA correlated with increment of GMPM score

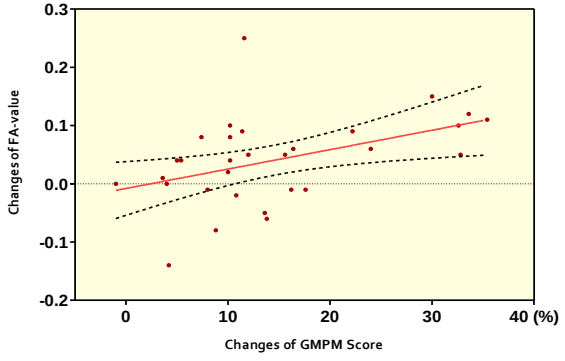


Correlation of Changes of GMPM % Score and Changes of FA value
in Ant. Portion of Left Posterior Internal Capsule in UCB Group (n=30)



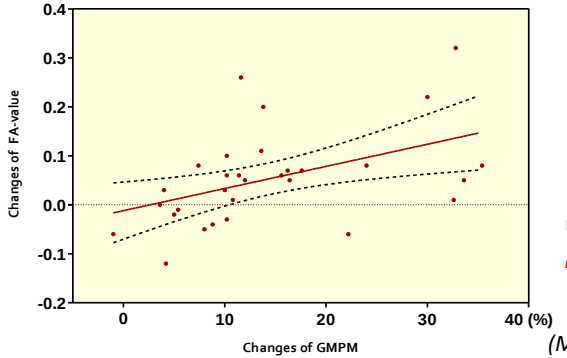
r = 0.48
p=0.007

Correlation of Changes of GMPM % Score and Changes of FA value
in Post. Portion of Right Posterior Internal Capsule in UCB Group (n=30)



r = 0.44
p=0.01

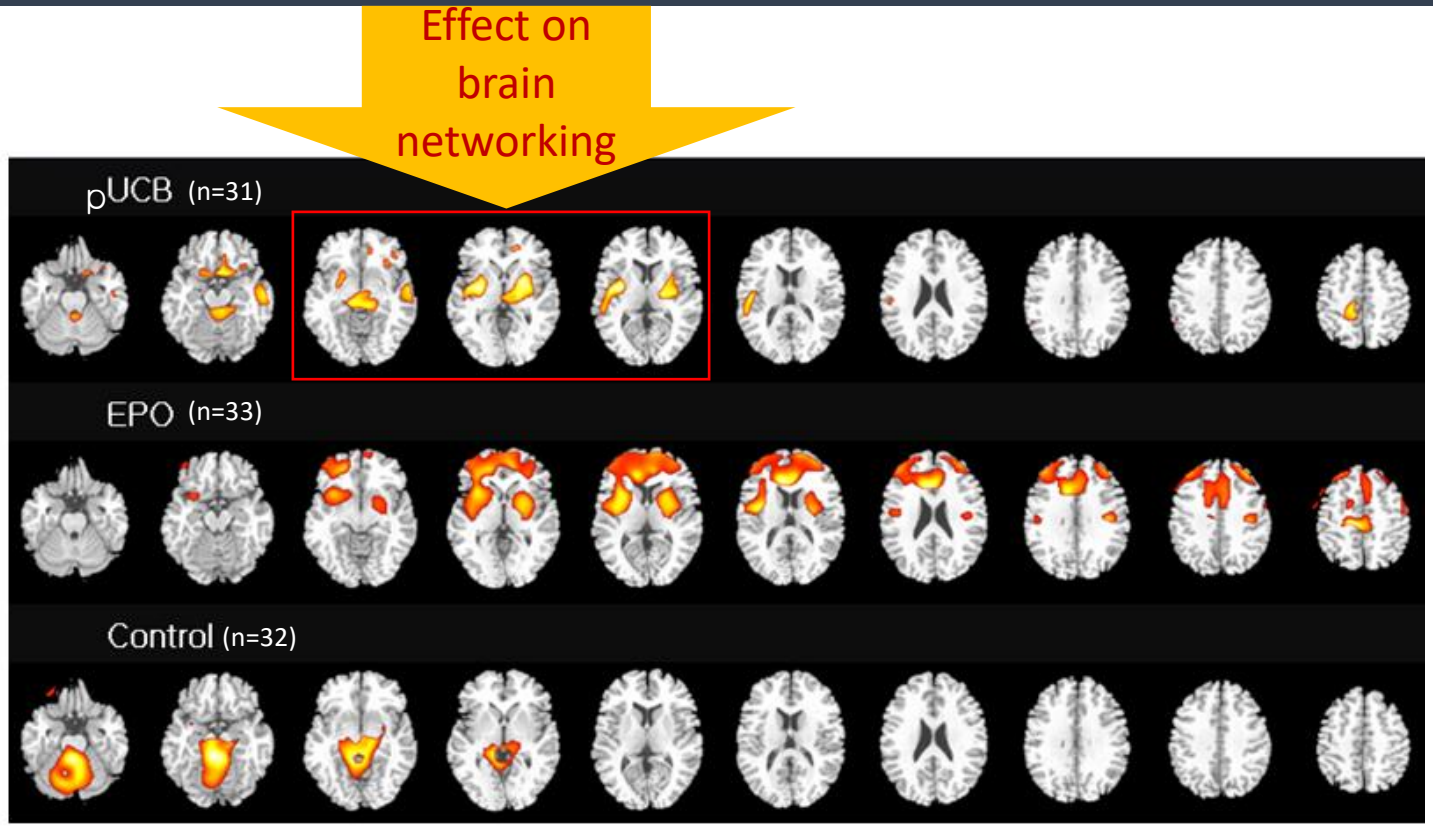
Correlation of Changes of GMPM % Score and Changes of FA value
in Post. Portion of Left Posterior Internal Capsule in UCB Group (n=30)



r = 0.46
p=0.009

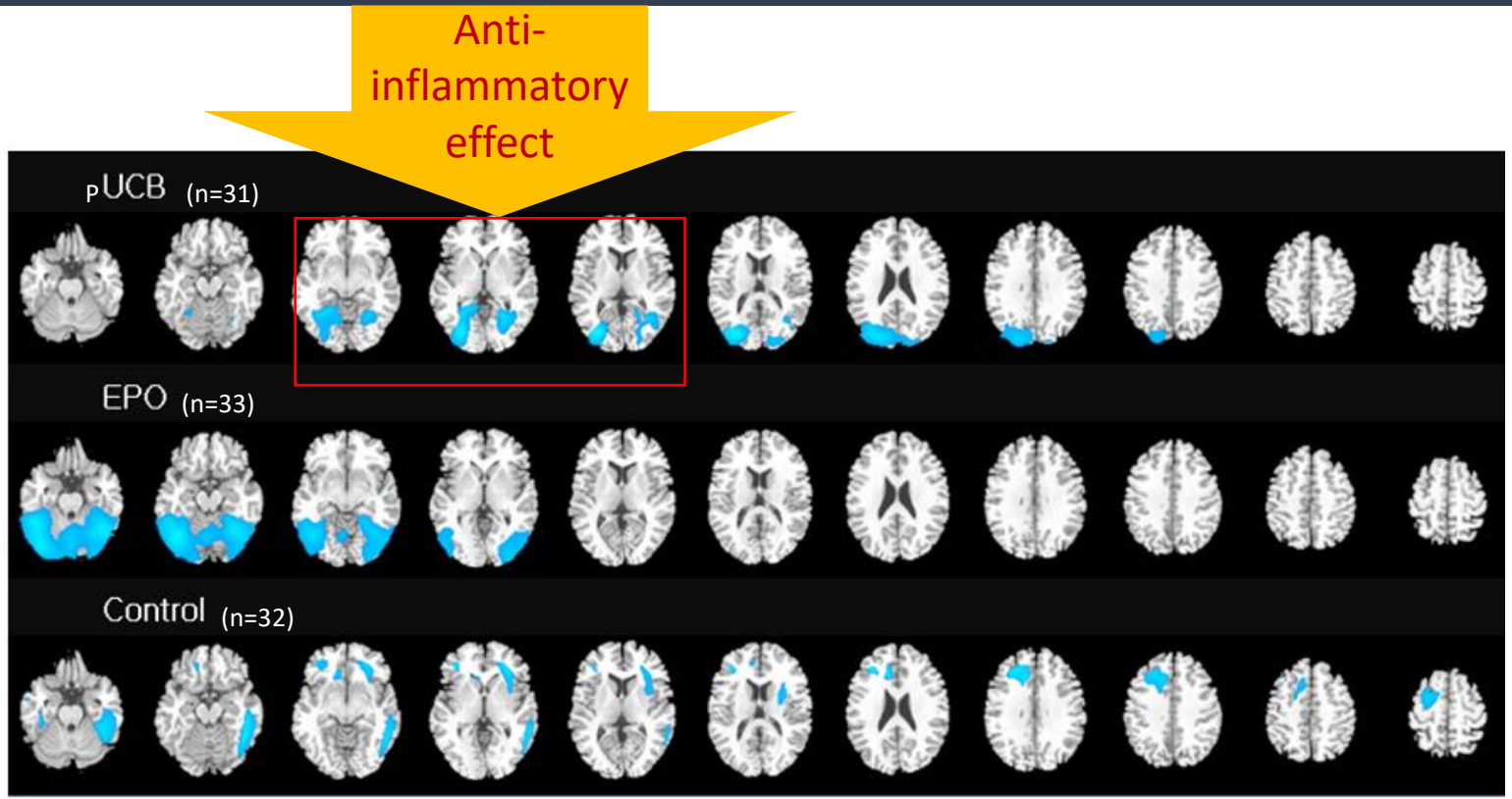
(Min et al. 2013, Stem Cells)

Activated areas in each group with brain FDG-PET



(Min et al. 2013, Stem Cells)

Deactivated areas in each group with brain FDG-PET



(Min et al. 2013, Stem Cells)

The 2nd trial with allogeneic UCB only

(Kang et al. 2015, Stem Cells Dev)

Purposes

The efficacy and safety of sole allogeneic UCB cell therapy

To investigate the therapy mechanism: assay of relevant cytokines and cell receptors

Design: double-blind RCT

2nd Clinical Trial

From February 2012 to July 2012, (NCT01528436 www.clinicaltrials.gov)

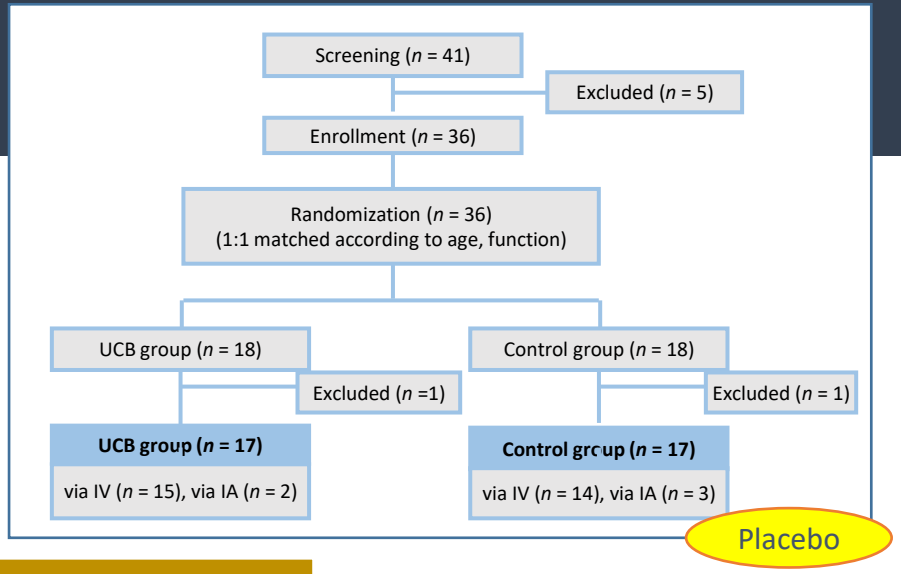
STEM CELLS AND DEVELOPMENT
Volume 00, Number 00, 2015

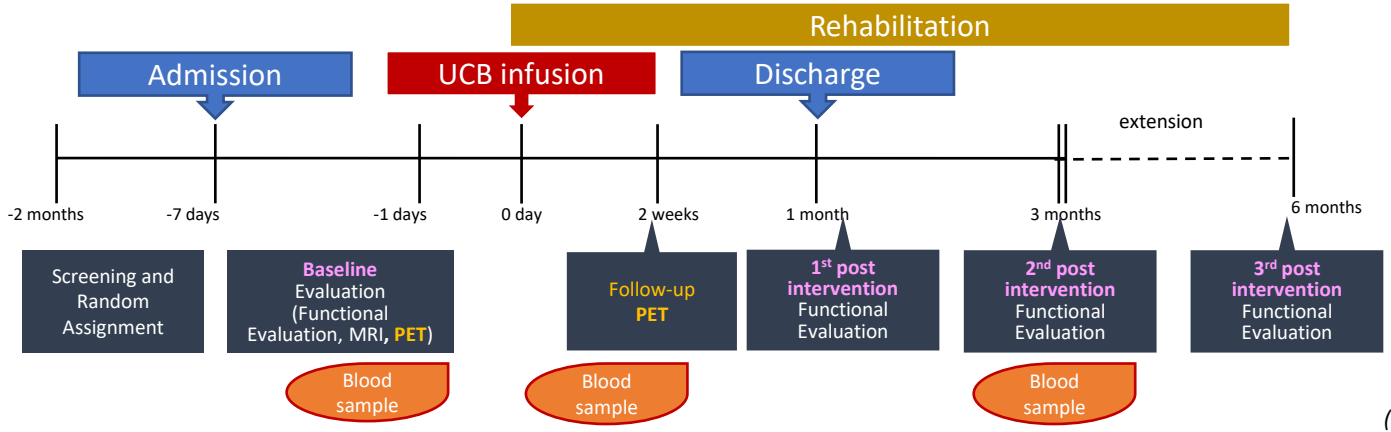
DOI: 10.1089/scd.2015.0074

ORIGINAL RESEARCH REPORT

Involvement of Immune Responses in the Efficacy of Cord Blood Cell Therapy for Cerebral Palsy

Mino Kang,[1,*] Kyunghoon Min,[2,*] Joonyoung Jang,[2] Seung Chan Kim,[1] Myung Seo Kang,[3] Su Jin Jang,[4] Ji Young Lee,[4] Sang Heum Kim,[5] Moon Kyu Kim,[6] SeongSoo A. An,[1] and MinYoung Kim[2]





Intervention

- **IV or IA (transfemoral) administration**
- **Allogeneic UCB**
  - TNC>2×10^7/kg
  - HLA (A, B, DRB1) within 2 mismatch
- **Cyclosporin**
  - Goal concentration: 100~200ng/mL
  - 2 weeks (3 d IV → oral)

(Kang et al. 2015, Stem Cells Dev)

Outcome measurements

- **Behaviors**
  - Manual Muscle Testing (MMT): 10 muscles in each side of upper and lower extremities; neck; and trunk muscles
  - Gross Motor Function Measure (GMFM)
  - Gross Motor Performance Measure (GMPM)
  - Bayley Scale 2nd version (BSID-II): Mental and Motor scales
  - Gross Motor Function Classification System (GMFCS)
  - WeeFIM

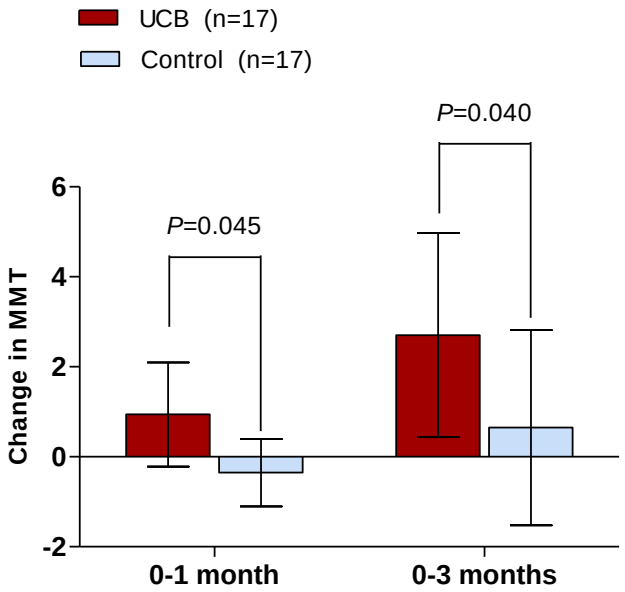
- **Cytokines** in peripheral blood: Innate immune and Inflammation related cytokines
- **Receptor assay** measured with Bradford assay
  - TLR-2, TLR-4
  - mTOR
- Brain ^{18}F-FDG-PET
  - analyzed using SPM3 implanted in MatLab R2011a
  - paired t-test statistics
  - voxels with an uncorrected p-value <0.05

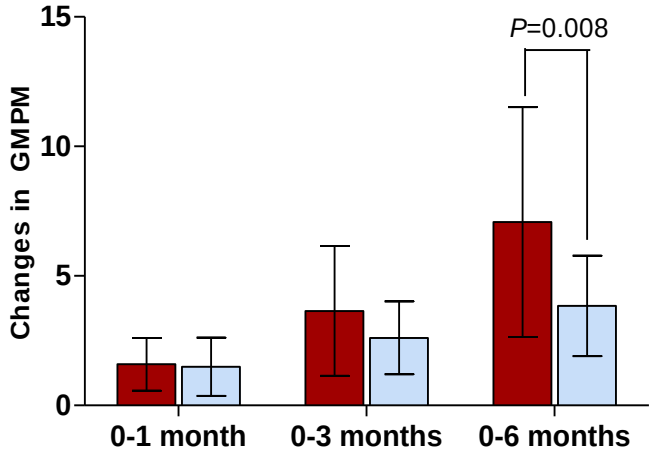
Demographic and baseline characteristics of patients (N = 34)

Group	UCB (n = 17)	Control (n = 17)
Demographics		
Sex, no. (% male)	10 (58.8)	8 (47.1)
Age, months; mean (SD; range); median	46.8 (60.1; 6–216);26.0	45.3 (41.7; 8–180); 35.5
Gestational age, weeks, mean (SD; range)	31.8 (4.7; 25–40)	33.4 (4.8; 27–40)
Preterm, no. (%)	14 (82.4)	12 (70.6)
Birth weight (SD; range), kg	1.9 (0.7; 1.0–3.1)	2.2 (0.8; 1.2–3.6)
NBW / LBW / VLBW / ELBW	6 / 2 / 8 / 1	6 / 5 / 6 / 0
GMFCS (I / II / III / IV / V)	3 / 0 / 1 / 5 / 8	2 / 2 / 1 / 2 / 10
MRI findings		
Periventricular leukomalacia	10 (58.8)	11 (64.7)
Diffuse encephalopathy	2 (11.8)	2 (11.8)
Focal ischemia/hemorrhage	5 (29.4)	4 (23.5)

Efficacy results (N=34)

Comparison of motor outcomes between UCB and control group





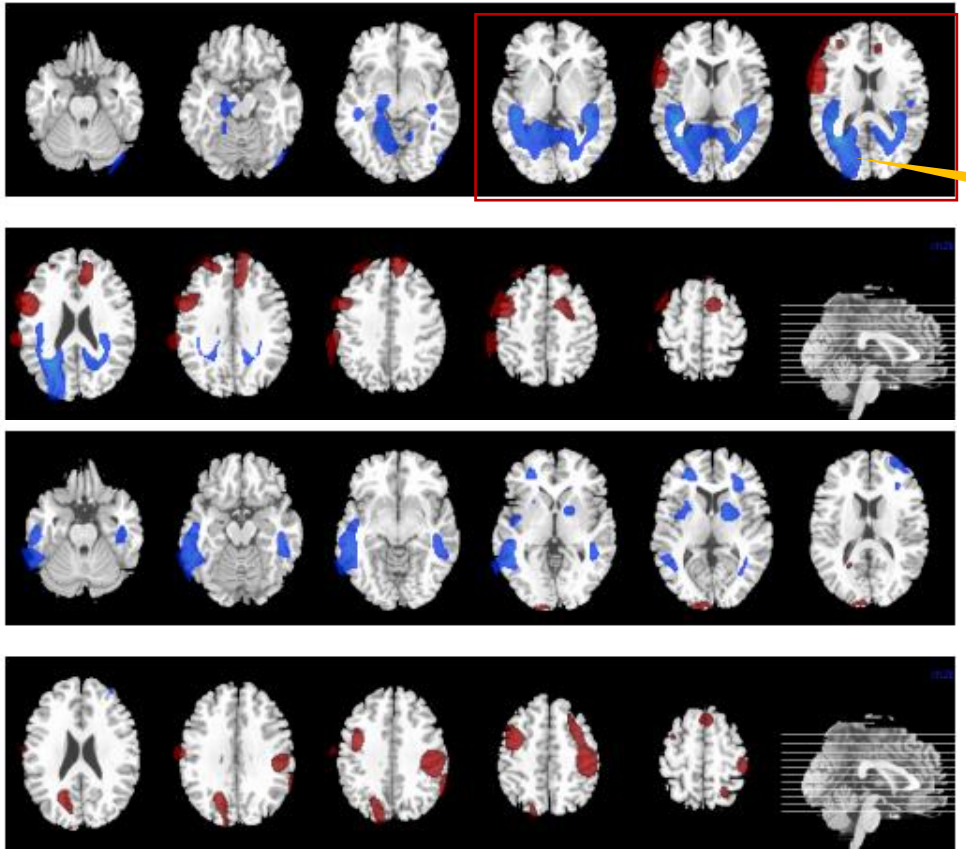
- Changes in **whole body muscle strength** with manual muscle test score showed **efficacy of UCB**
- Changes in **gross motor function** with gross motor performance measure score showed **efficacy of UCB**

(Kang et al. 2015, Stem Cells Dev)

Changes in ^{18}F-FDG PET/CT Glucose Metabolism during the period between baseline and 2 weeks post-treatment

UCB

Control

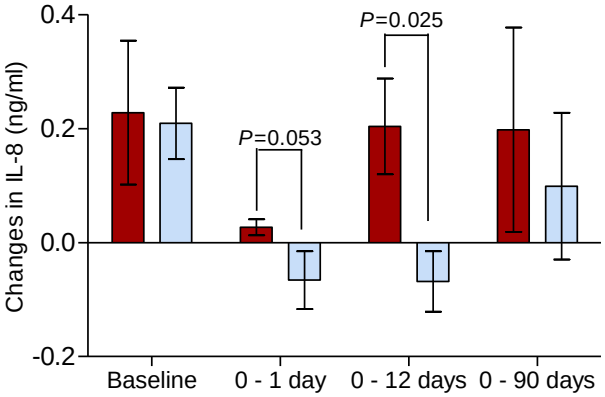


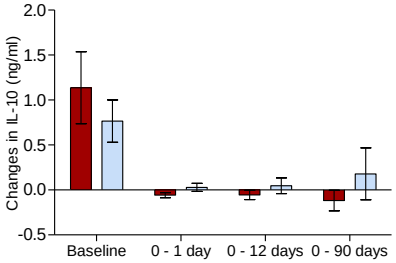
Again, **anti-inflammatory effect** in periventricular area, the most inflammatory region

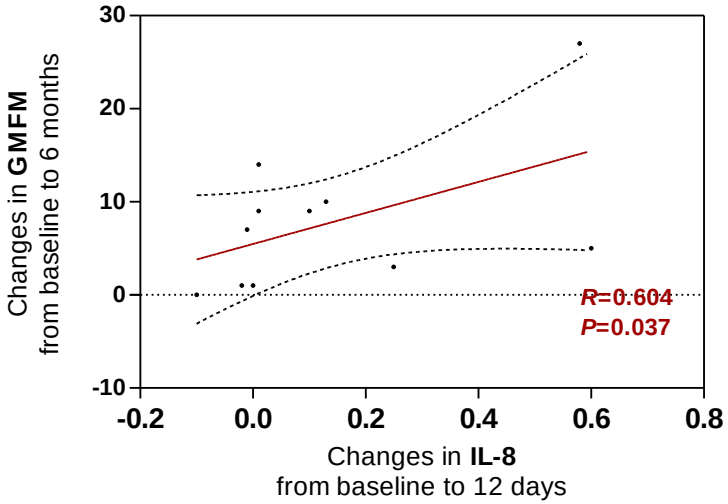
Glucose metabolism
Red color: increased activity
Blue color: decreased activity
(P-value < 0.05)

(Kang et al. 2015, Stem Cells Dev)

Changes in IL-8 from baseline to 1, 12, and 90 days and its correlation with motor outcome

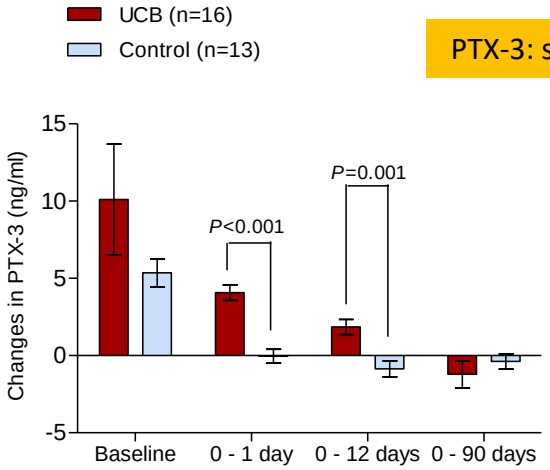




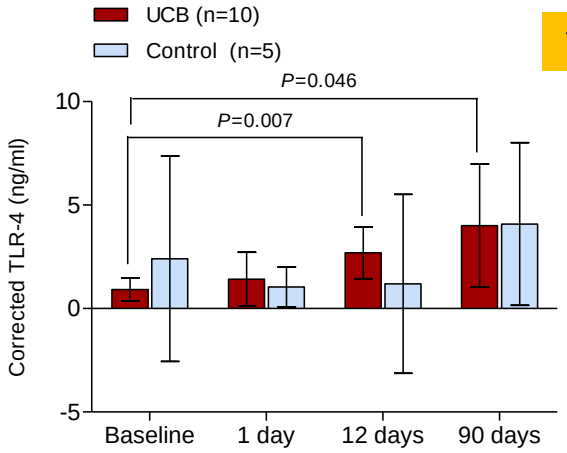


(Kang et al. 2015, Stem Cells Dev)

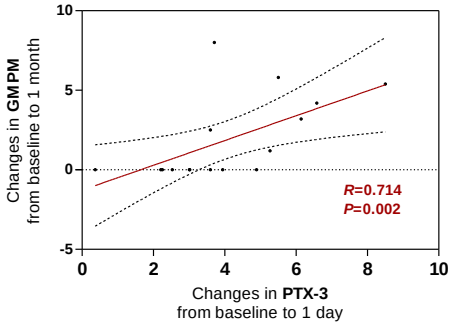
Changes in innate immune responses from baseline to 1, 12, and 90 days and the correlations with motor outcomes in UCB group

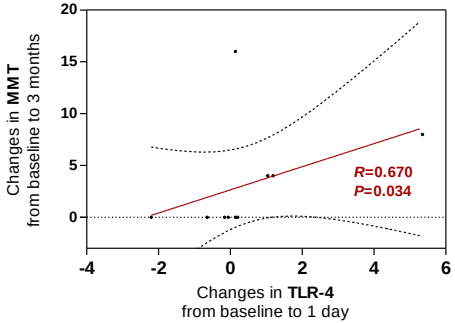


PTX-3: soluble factor



TLR-4: tissue bound receptor





(Kang et al. 2015, Stem Cells Dev)

The 3rd RCT for children with CP

Under permission of Korean FDA

NIH U.S. National Library of Medicine

ClinicalTrials.gov

Allogeneic UCB Therapy With EPO in Children With CP

ClinicalTrials.gov Identifier: NCT01991145

STUDY PROTOCOL

Safety and efficacy of allogeneic umbilical cord blood therapy combined with erythropoietin in children with cerebral palsy: study protocol for a double-blind, randomized, placebo-controlled trial

Kye Hee Cho, Kyunghoon Min, Sun Hee Lee, MinYoung Kim*

Department of Rehabilitation Medicine, CHA Bundang Medical Center, CHA University, Republic of Korea

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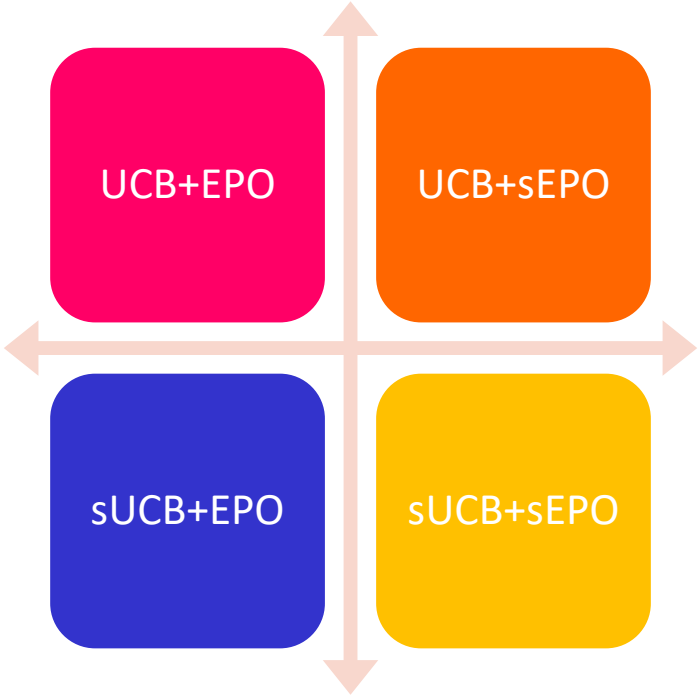
orcid: 0000-0001-5481-2985 (MinYoung Kim)

(Cho et al. 2017, Asia Pac J Clin Trials Nerv Syst Dis)

Allogeneic Umbilical Cord Blood Therapy combined with Erythropoietin for Children with Cerebral Palsy

: A 2x2 Factorial, Double-blind, Randomized, Placebo-controlled Trial



sUCB: sham UCB

sEPO: sham EPO

(Cho et al. 2017, Asia Pac J Clin Trials Nerv Syst Dis)

Protocol of the 3rd trial (approved by Korean FDA)

Study participants

Patients with cerebral palsy who visited rehabilitation clinic, in a University hospital from October 2013 to October 2015 and fulfill the following criteria are eligible.

Inclusion criteria

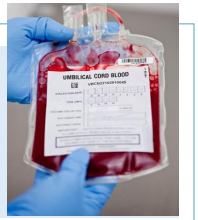
- Diagnosed with cerebral palsy
- Age of ≥ 10 months and ≤ 6 years
- Mismatch in HLA-A, -B, and -DR ≤ 2, and total nucleated cell count ≥ 3×10^7 /kg
- If the cell count is less than given values, more than 1 unit could be used
- Hemoglobin level < 13.6 mg/dL
- Written informed to participation in the study obtained from the subject's representative
- Willingness and ability to be hospitalized according to the schedule specified in the protocol and continue to participate for 12 months after study entry

(Cho et al. 2017, Asia Pac J Clin Trials Nerv Syst Dis)

Therapeutic regimen of the 3rd trial

UCB

- Allogeneic (Cryopreserved in CHA Medical Center)
- Number of total nucleated cell ≥ $3x10^7$/kg
- Matched for at least 4 out of 6 HLA-A, B, and DR



EPO

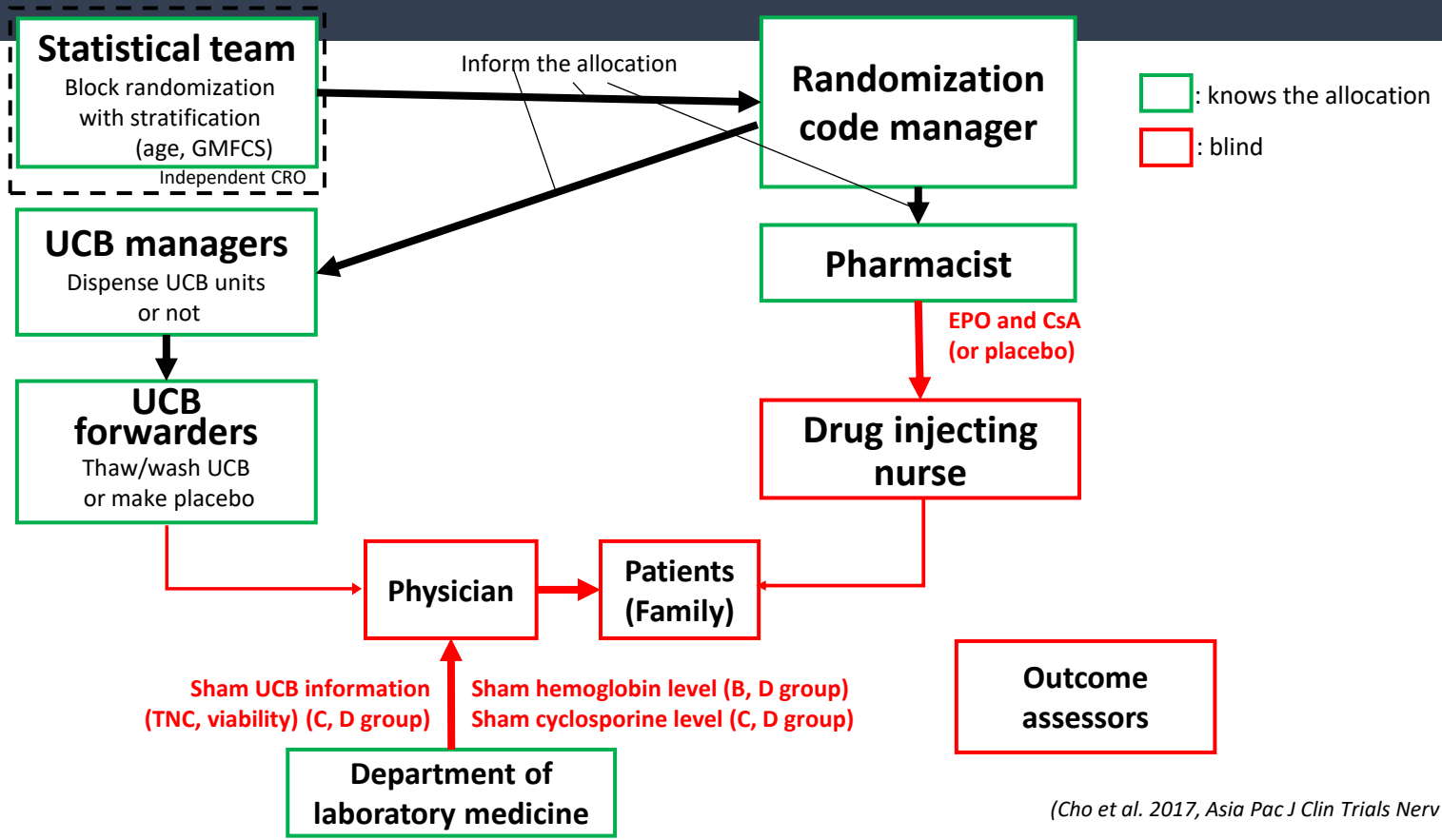
- Adjuvant to potentiate the UCB therapy
- IV 500 IU/kg at 2 hours before UCB (or placebo)
- From Day 3, SC 500 IU/kg EPO 5 times more every three days
- Placebo EPO was provided by the manufacturer (LG Chem.)

Cyclosporine

- Survival of infused UCB cells ↑, GVHD↓
- 7mg/kg bid for 16 days (A, B) or placebo (C, D)
- Placebo cyclosporine was provided by the manufacturer (ChongKunDang Pharm.)

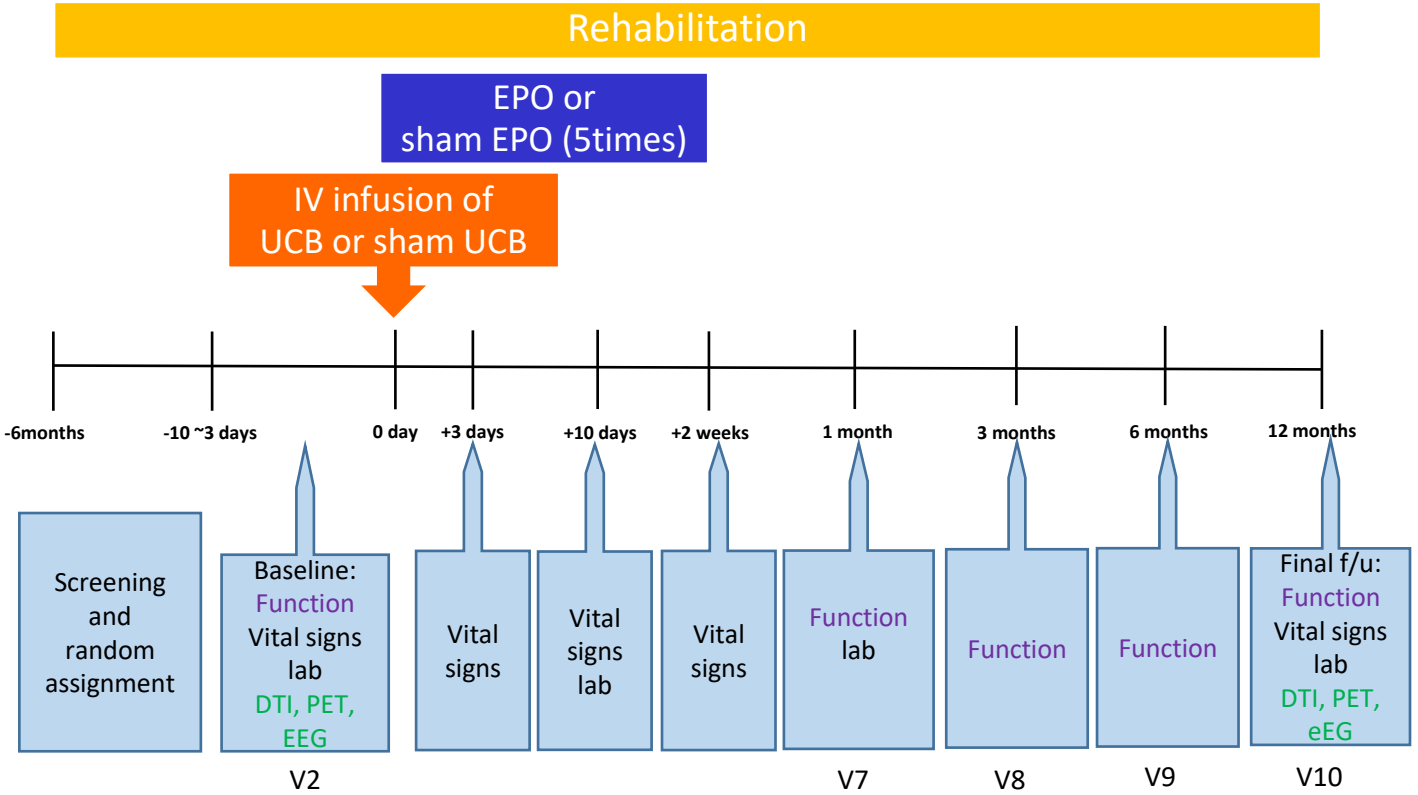
(Cho et al. 2017, Asia Pac J Clin Trials Nerv Syst Dis)

Randomization (1:1:1:1 block) & Double-blind Process



(Cho et al. 2017, Asia Pac J Clin Trials Nerv Syst Dis)

Timeline of the 3rd trial



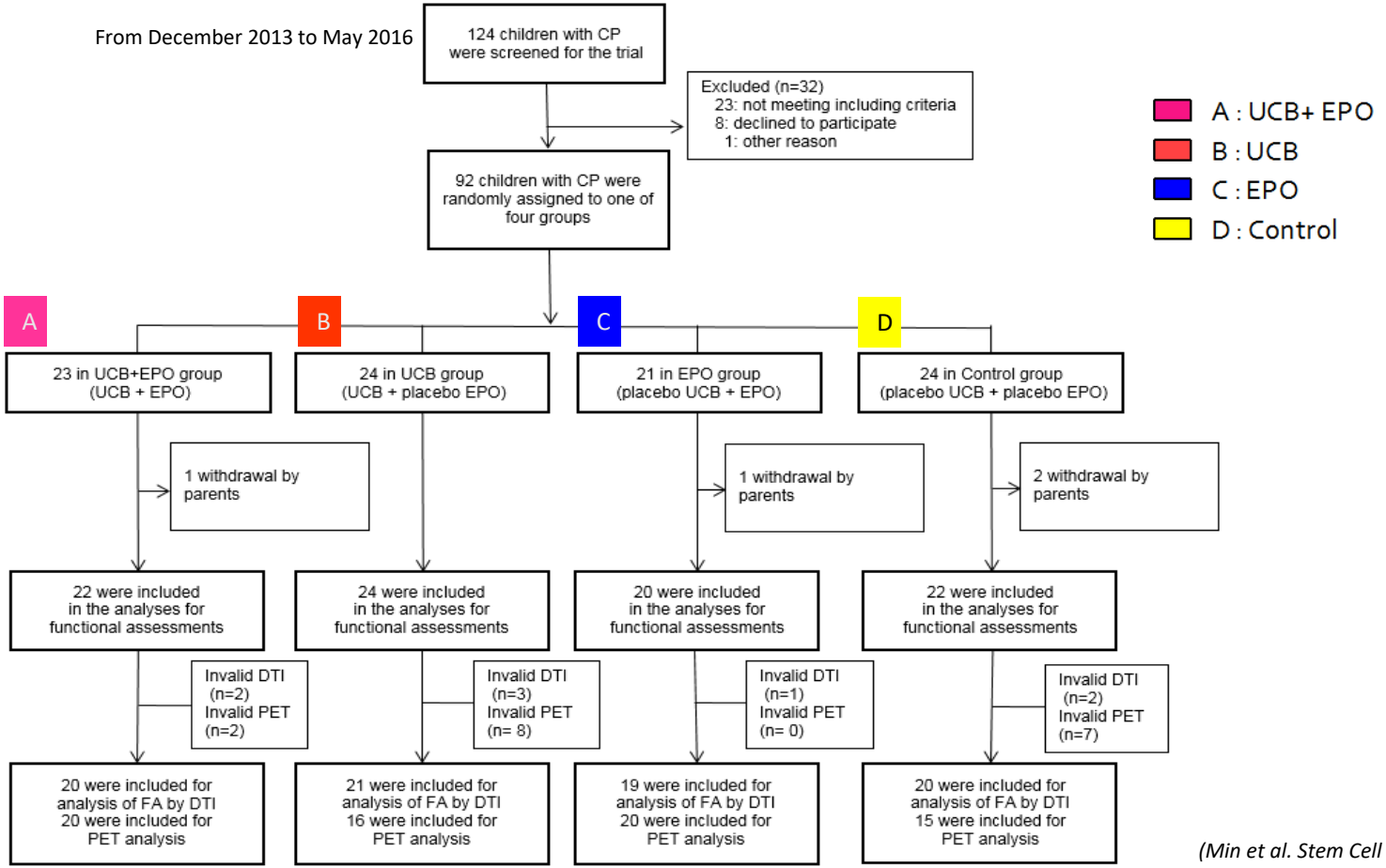
(Cho et al. 2017, Asia Pac J Clin Trials Nerv Syst Dis)

Outcome measures in the 3rd trial

- **Primary outcomes**
  - Gross Motor Performance Measure (GMPM)
  - Gross Motor Functional Measure (GMFM)
  - Bayley Scales for Infant Development-II (BSID-II)
- **Secondary outcomes**
  - Gross Motor Function Classification System (GMFCS)
  - Pediatric Evaluation of Disability Inventory (PEDI)
  - Functional Independence Measure for Children (WeeFIM)
  - Summed scores on manual muscle testing (MMT)
  - Visual motor integration (VMI)
  - Selective control assessment of lower extremity (SCALE)
  - modified Ashworth scale (MAS)
  - modified Tardieu scale for hamstring
  - Quality of Upper Extremity Skills Test (QUEST)
  - Fractional isotropy in DTI tractography
  - Findings in 18F-FDG-PET/CT

(Cho et al. 2017, Asia Pac J Clin Trials Nerv Syst Dis)

Study flow in the 3rd trial



(Min et al. Stem Cell Ther Res Accepted)

Demographic and baseline participant characteristics (N = 88)

Group	UCB+EPO[a] (n = 22)	UCB[b] (n = 24)	EPO[c] (n = 20)	Control[d] (n = 22)
Demographics				
Sex, no. % male	10 (45.5%)	11 (45.8%)	10 (50.0%)	15 (68.2%)
Age, year; mean (SD; range)	3.0 (1.2; 1.5-6.3)	2.9 (1.3; 1.0-5.0)	3.4 (1.3; 1.1-5.8)	3.0 (1.1; 1.2-6.0)
Gestational age, weeks; mean (SD; range)	32.3 (4.8; 26-41)	31.9 (3.9; 26-40)	31.9 (4.3; 26-40)	33.6 (5.4; 24-42)
Preterm, no. (%)	16 (72.7%)	16 (72.7%)	16 (72.7%)	13 (59.1%)
Birth weight (SD; range), kg	1.9 (0.8; 0.6 - 3.6)	1.9 (0.8; 0.8 - 3.4)	1.9 (0.8; 0.7 - 3.5)	2.2 (0.9; 0.7-4.2)
NBW / LBW / VLBW / ELBW[e]	6 / 7 / 8 / 1	5 / 10 / 7 / 2	5 / 8 / 5 / 2	10 / 7 / 3 / 2
GMFCS (I / II / III / IV / V)	1 / 2 / 5 / 6 / 8	2 / 2 / 5 / 3 / 12	1 / 6 / 3 / 7 / 3	0 / 1 / 5 / 10 / 6
Typology (SB / SU / D / C / A)[f]	18 / 0 / 3 / 0 / 1	20 / 0 / 4 / 0 / 0	15 / 0 / 4 / 0 / 1	17 / 0 / 4 / 0 / 1
MRI findings				
Acquired lesions (n = 87)				
Periventricular leukomalacia (n = 66)	17	20	14	15
Diffuse encephalopathy (n = 18)	4	4	5	5
Focal ischemia/hemorrhage (n = 1)	0	0	0	1
Multicystic encephalomalacia (n = 2)	1	0	0	1
Abnormality of white matter signal (n = 1)	0	0	1	0
Normal (n = 0)	0	0	0	0

Values represent number of patients unless otherwise noted. No baseline characteristics were significantly different among four groups (*p*-value > 0.05 for all comparisons).

[a]UCB+EPO group (n=22) received UCB and EPO. [b]UCB group (n=24) received UCB and placebo EPO. [c]EPO group (n=20) received placebo UCB and EPO. [d]Control group (n=22) received placebo UCB and placebo EPO.

[d] Age corrected for preterm birth.

[e]NBW was defined as birth body weight ≥ 2500 g, LBW < 2500 g, VLBW < 1500 g, and ELBW < 1000 g.

[f]Typology was divided as follows: SB, SU, D, C, and A.

Abbreviations: NBW, normal birth weight; LBW, low birth weight; VLBW, very low birth weight; ELBW, extremely low birth weight; SB, spastic bilateral; SU, spastic unilateral; D, dystonic; C, choreoathetoid; A, ataxic

(Min et al. Stem Cell Ther Res Accepted)

Adverse events during study period of 12 months in four groups (N=88)

Number of patients	UCB+EPO[a] (n=22)	UCB[b] (n=24)	EPO[c] (n=20)	Control[d] (n=22)	*p value*[e]
Serious adverse events[f]					
Pneumonia	1			1	0.724
Seizure	1			2	0.138
Otitis media acute	1				0.727
Pyrexia		1			1
Entropion			1		0.227
Hepatitis viral			1		0.227
Nasopharyngitis				1	0.727
Labial frenectomy			1		0.227
Other adverse events					
Upper respiratory infection	13	16	10	17	0.305
Pyrexia	3	2	3		0.287
Constipation	5	3	7	6	0.353
Urticaria	2				0.17
Seizure				2	0.17
Mucocutaneous rash	4	2	2		0.217
Eczema	1		1		0.472
Pruritus		2			0.242
Cellulitis		1		1	1
Dehydration			1	1	0.472
Tachycardia	2				0.17
Tachypnoea	1				0.727
Fatigue	1				0.727
Otitis media acute		1		1	1
Swelling of eyelid	1				0.727

[a]UCB+EPO group (n=22) received UCB and EPO. [b]UCB group (n=24) received UCB and placebo EPO. [c]EPO group (n=20) received placebo UCB and EPO [c]Control group (n =22) received placebo UCB and placebo EPO. [e]*p* values were calculated for difference among four groups of the number of patients with reported adverse events using Fisher's exact analysis.

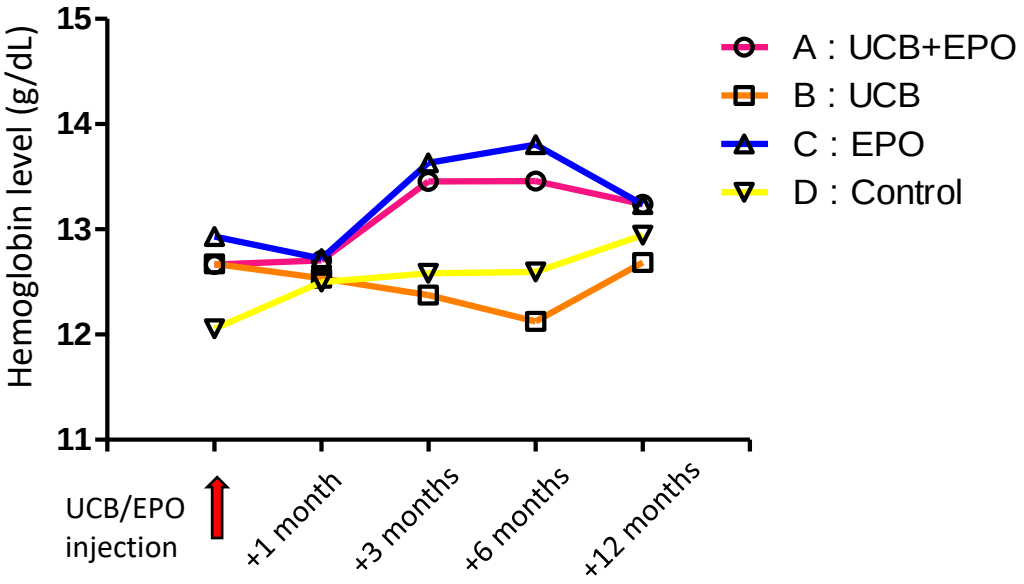
[f]Serious adverse events were defined as any event, resulting in death, life-threatening, requiring hospitalization or prolongation of hospital stay. The source of terminology was Medical Dictionary for Regulatory Activities (MedDRA) 21.1.

(Min et al. Stem Cell Ther Res Accepted)

Safety of UCB + EPO combination treatment

- At least 10 years (since 2009), from previous trials of UCB only or UCB + EPO therapy, no long-term safety related issues were raised, including
  - Tumorigenesis: whole body
  - Aggravation or occurrence of newly-developed seizure

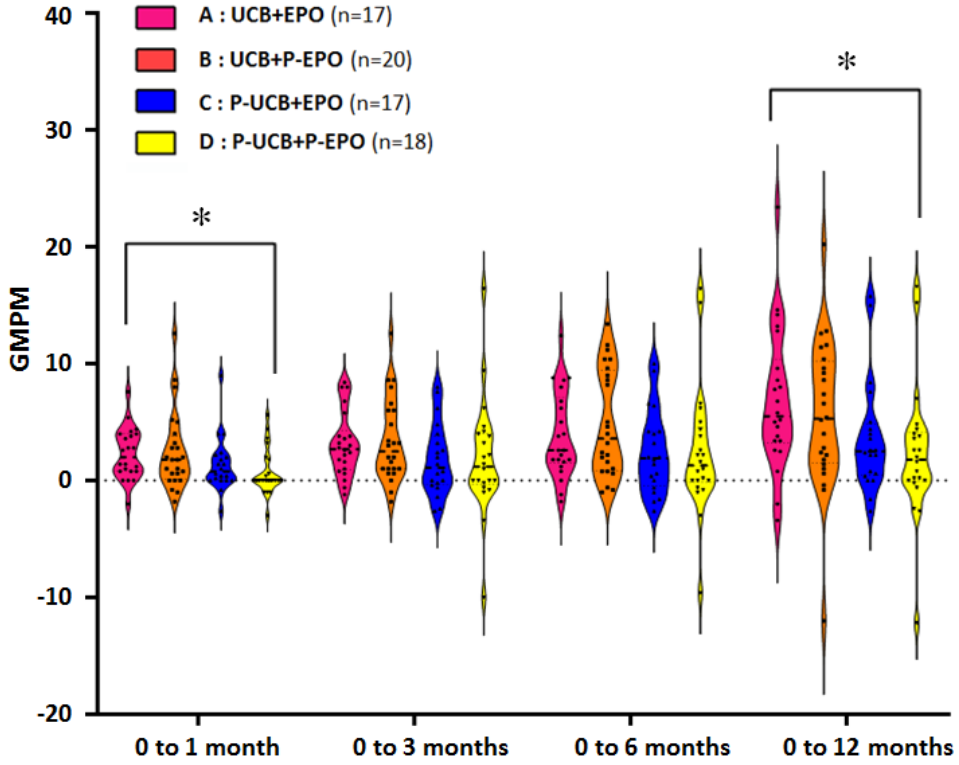
Erythropoiesis caused by EPO administration



(Min et al. Stem Cell Ther Res Accepted)

Efficacy results (N=88)

3rd Clinical Trial



(Min et al. Stem Cell Ther Res Accepted)

Efficacy results (N=88)

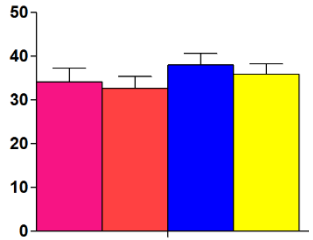
3rd Clinical Trial

GMPM change ratio

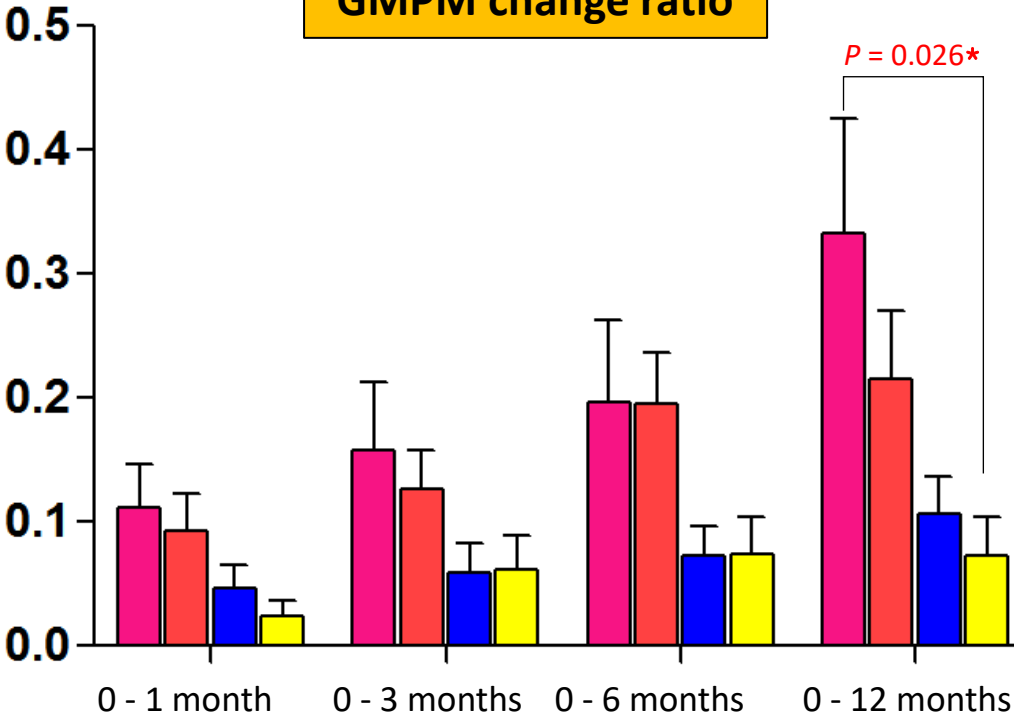
$$= \frac{\Delta \text{GMPM}}{\text{GMPM}}$$

$$= \frac{\text{GMPM}_{follow-up} - \text{GMPM}_{initial}}{\text{GMPM}_{initial(=baseline)}}$$

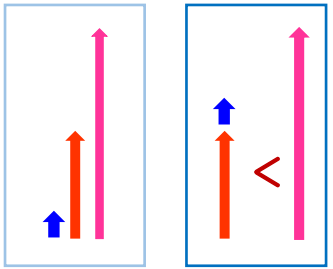
Baseline GMPM



GMPM change ratio



A : UCB+ EPO
B : UCB
C : EPO
D : Control



(Min et al. Stem Cell Ther Res Accepted)

Efficacy results (N=88)

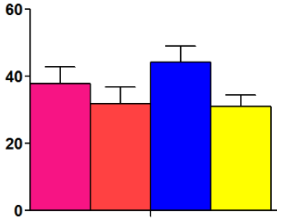
3rd Clinical Trial

GMPM change ratio

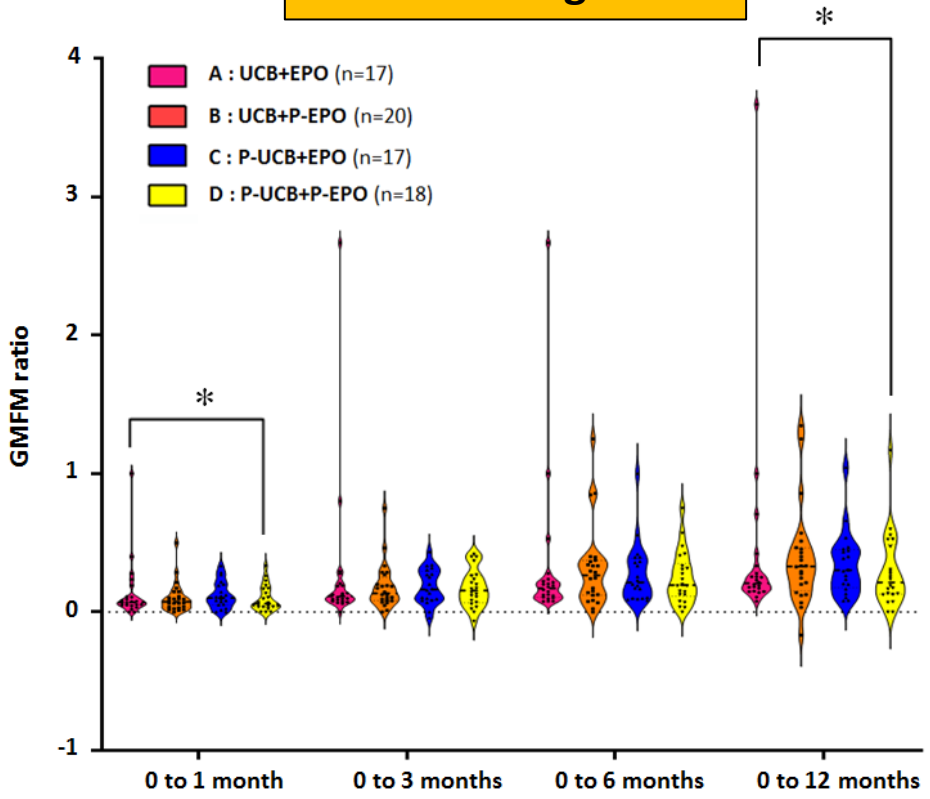
$$= \frac{\Delta \text{GMPM}}{\text{GMPM}}$$

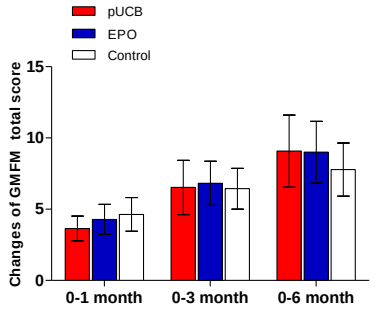
$$= \frac{\text{GMPM}_{follow-up} - \text{GMPM}_{initial}}{\text{GMPM}_{initial(=baseline)}}$$

Baseline GMFM

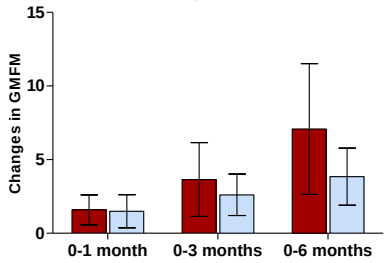


GMPM change ratio





(Min et al. 2013, Stem Cells)



(Kang et al. 2015, Stem Cells Dev)

Similar responses without statistical significance (same to previous trials)

(Min et al. Stem Cell Ther Res Accepted)

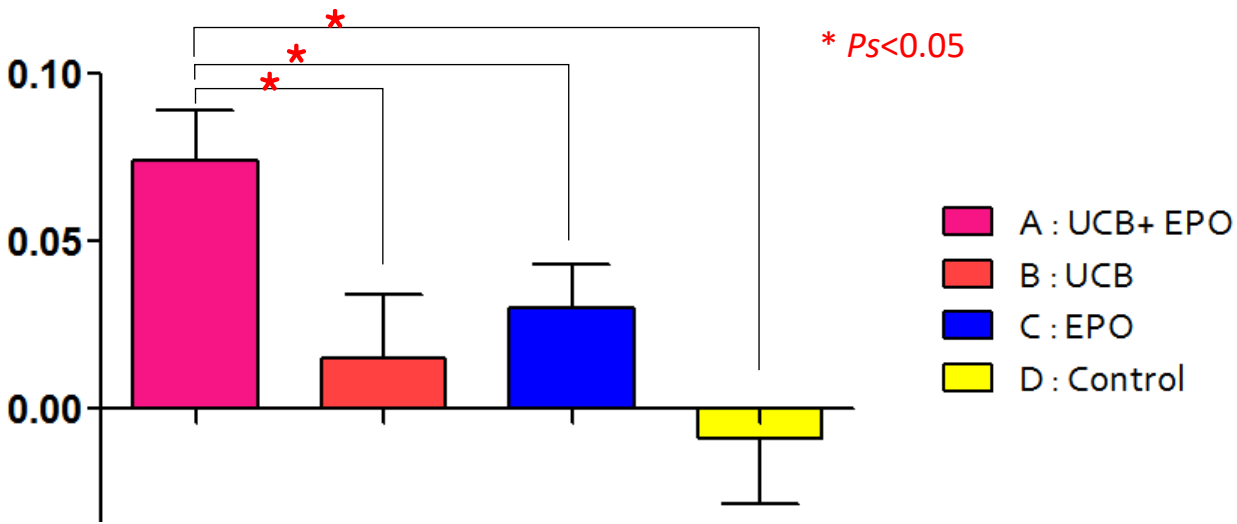
FA value of anterior thalamic radiation, Rt. (ATRR) in Age ≥ 3

FA change ratio

$$= \frac{\Delta FA}{FA}$$

$$= \frac{FA_{follow-up} - FA_{initial}}{FA_{initial}}$$

FA value change ratio in ATRR between baseline and post-UCB 12 months

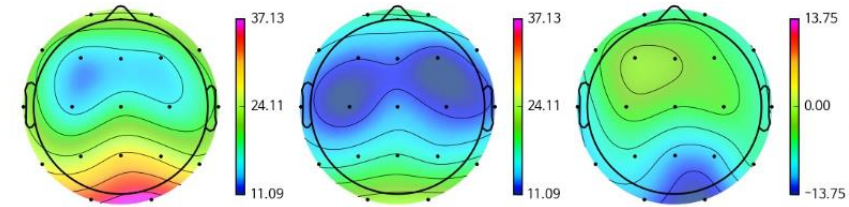


(Min et al. Stem Cell Ther Res Accepted)

EEG mapping before and after therapy

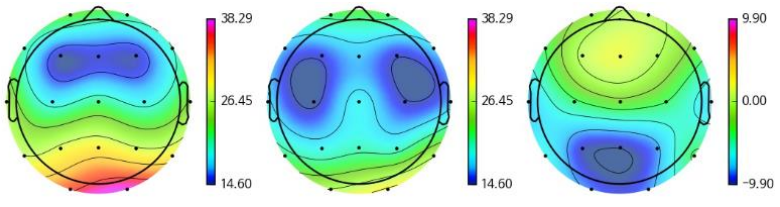
* Delta/alpha ratio : DAR

Group A (UCB + EPO)



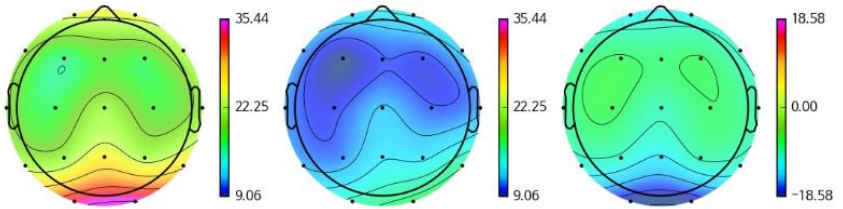
DAR (baseline) | DAR (1yr follow-up) | DAR (Δ)

Group B (UCB + sEPO)



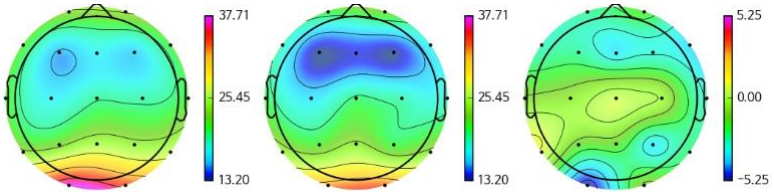
DAR (baseline) | DAR (1yr follow-up) | DAR (Δ)

Group C (sUCB + EPO)



DAR (baseline) | DAR (1yr follow-up) | DAR (Δ)

Group D (sUCB + sEPO)



DAR (baseline) | DAR (1yr follow-up) | DAR (Δ)

(Min et al. Stem Cell Ther Res Accepted)

Allogeneic Umbilical Cord Blood Therapy Combined with Erythropoietin in Children with Cerebral Palsy: a Two Year Follow Up

Protocol

IRB No.2015-06-093

ClinicalTrials.gov NCT03130816

Study participants

Among the patients with cerebral palsy who were enrolled in the first trial, those who agreed to take the second trial were included

Inclusion criteria of the first trial

- Diagnosed with cerebral palsy
- Age of ≥ 10 months and ≤ 6 years
- Mismatch in HLA-A, -B, and -DR ≤ 2, and total nucleated cell count ≥ 3×10^7 /kg
- If the cell count is less than given values, more than 1 unit could be used
- Written informed to participation in the study obtained from the subject's representative
- Willingness and ability to be hospitalized according to the schedule specified in the protocol and continue to participate for 12 months after study entry

(Unpublished Data)

Therapeutic Regimen

UCB

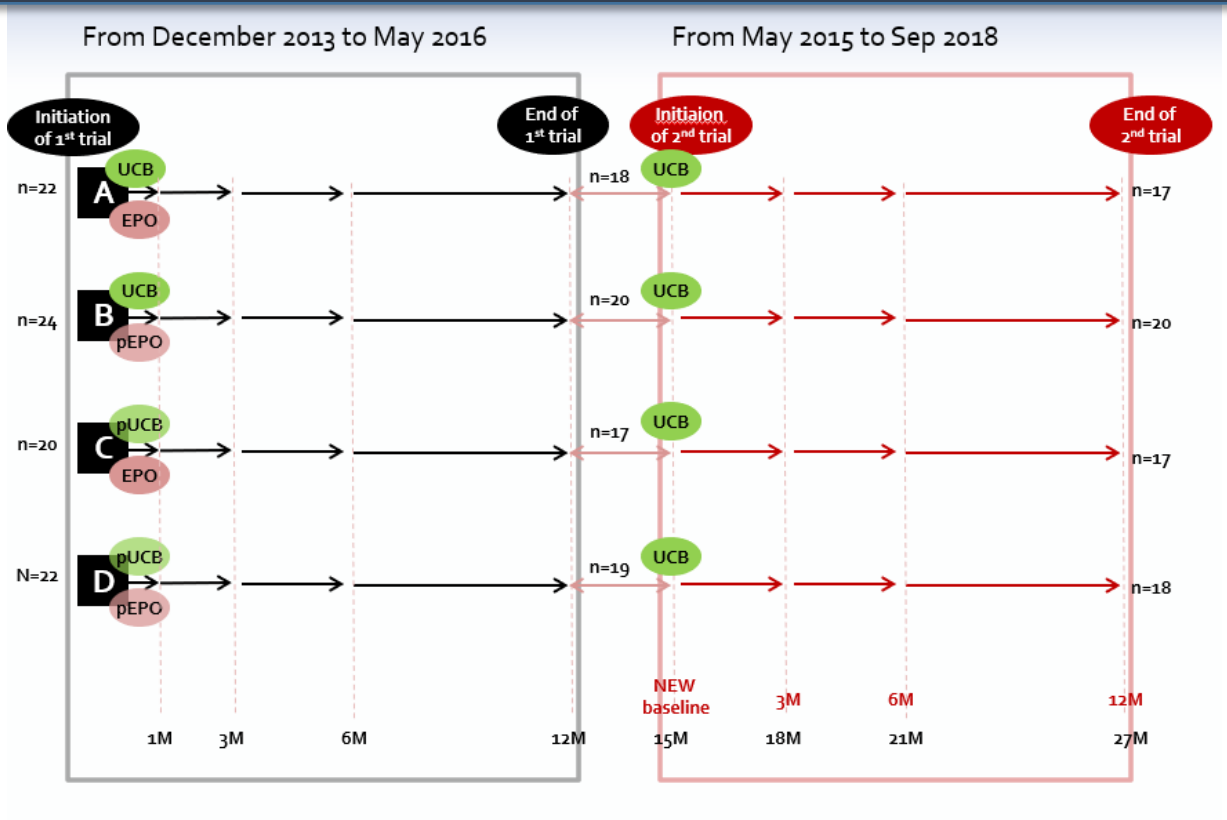
- Allogeneic, total nucleated cell ≥ $2x10^7$/kg
- Matched for at least 3 out of 6 HLA-A, B, and DR
- Intravenous infusion

Cyclosporine

- Survival of infused UCB cells ↑, GVHD↓
- 7mg/kg bid oral administration for 9 days (from 2 d before UCB infusion)

And, essentially intensive rehabilitation.

Study Flow



(Unpublished Data)

Functional Assessment

- **Primary outcomes**
  - Gross Motor Performance Measure (GMPM)
  - Gross Motor Functional Measure (GMFM)
  - Bayley Scales for Infant Development-II (BSID-II)

  At baseline, and 3, 6, and 12 months after the second intervention

- **Subgroup Analysis**
  - Performed among those with severe impairments, whose gross motor functional classification system (GMFCS) levels were either IV or V

Baseline Characteristics

Demographic and baseline participant characteristics (n = 72)				
Group	**Group A[a] (n = 17)**	**Group B[b] (n = 20)**	**Group C[c] (n = 17)**	**Group D[d] (n = 18)**
Demographics				
Sex, no. % male	6 (35.3%)	9 (45.0%)	7 (41.2%)	12 (66.7%)
Age, year (mean± SD)	4.3 ± 1.1	4.0 ± 1.4	4.6 ± 1.4	4.5 ± 1.2
Gestational age, weeks (mean± SD)	32.3 ± 4.7	31.9 ± 4.0	31.7 ± 4.3	33.6 ± 5.0
Preterm, no. (%)	13 (76.5%)	16 (80.0%)	14 (82.4%)	11 (61.1%)
Birth weight (mean± SD) , kg	2.0± 0.8	1.9± 0.8	1.9± 0.8	2.2± 0.8
NBW / LBW / VLBW / ELBW[e]	4 / 7 / 5 / 1	4 / 8 / 6 / 2	4 / 8 / 3 / 2	8 / 6 / 3 / 1
GMFCS (I / II / III / IV / V)	2 / 2 / 4 / 3 / 6	0 / 5 / 4 / 3 / 8	2 / 5 / 2 / 5 / 3	0 / 2 / 5 / 10 / 6

Values represent number of patients unless otherwise noted. No baseline characteristics were significantly different among four groups (*p*-value > 0.05 for all comparisons). [a]Group A (n=17) received UCB and EPO, [b]group B (n=20) received UCB and placebo EPO, [c]group C (n=17) received placebo UCB and EPO, and [d]group D (n=18) received placebo UCB and placebo EPO at the beginning of the 1st trial. [d] Age corrected for preterm birth. [e]NBW was defined as birth body weight ≥ 2500 g, LBW < 2500 g, VLBW < 1500 g, and ELBW < 1000 g.

Abbreviations: NBW, normal birth weight; LBW, low birth weight; VLBW, very low birth weight; ELBW, extremely low birth weight

(Unpublished Data)

Functional Improvements

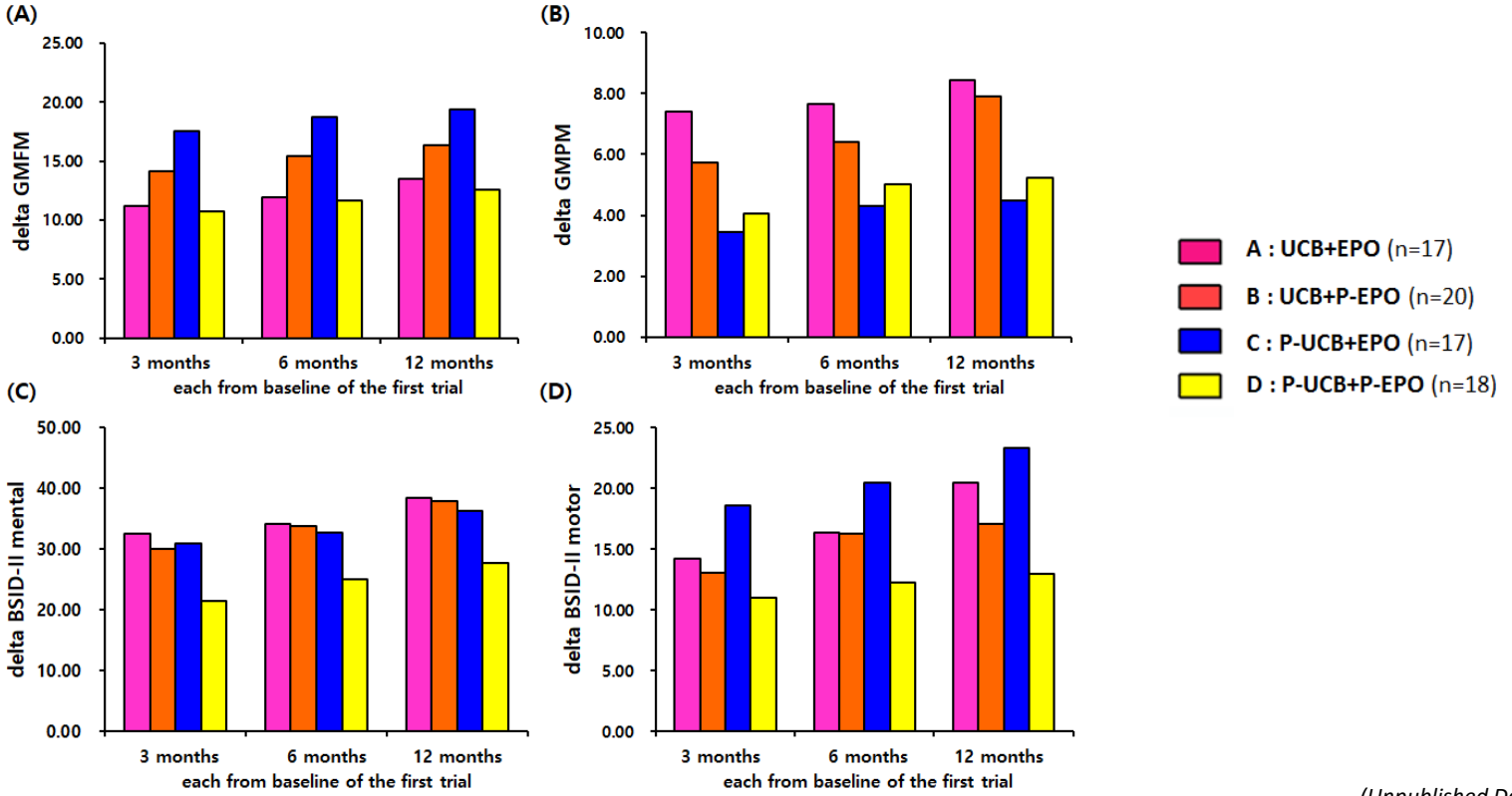
Primary Outcome Measures at the Second Trial

Outcome measures	Group[a]	baseline	3 months	6 months	12 months
GMFM	A	48.18±6.53	49.06±6.62*	49.76±6.66*	51.29±6.86*
	B	44.65±6.09	45.85±6.26*	47.15±6.42*	48.10±6.61*
	C	60.59±5.93	61.35±6.11*	62.53±6.09*	63.12±6.21*
	D	40.28±4.50	40.72±4.95	41.61±4.99	42.56±5.09*
GMPM	A	41.48±2.92	42.02±2.91	42.26±3.00	43.07±3.02*
	B	38.32±2.76	38.95±2.87*	39.64±2.72*	41.13±2.48*
	C	42.21±2.84	42.34±2.75*	43.20±2.60*	43.39±2.98*
	D	37.84±3.09	38.42±3.06	39.38±3.18*	39.59±3.25*
BSID-II mental score	A	132.12±10.16	137.18±10.05*	138.82±10.19*	143.06±10.19*
	B	121.05±10.60	125.50±10.74*	129.30±10.59*	133.40±10.79*
	C	148.00±7.21	151.47±7.41*	153.18±7.54*	156.71±7.60*
	D	117.56±10.09	120.83±10.05*	124.50±10.24*	127.11±10.36*
BSID-II motor score	A	61.18±6.34	62.76±6.42*	64.88±6.45*	69.00±7.01*
	B	59.80±5.80	60.50±5.87*	63.75±6.28*	64.55±6.32*
	C	75.88±5.44	78.59±5.64*	80.41±5.54*	83.29±5.57*
	D	57.22±5.31	59.06±5.40*	59.78±5.38*	57.78±5.25*

Data are shown as mean ± standard error. Each are GMFM, GMPM, BSID-II mental and motor scores at baseline of second trial, and at 3, 6, and 12 months after 2nd intervention. [a]Group A (n=17) received UCB and EPO, group B (n=20) received UCB and placebo EPO, group C (n=17) received placebo UCB and EPO, and group D (n=18) received placebo UCB and placebo EPO at the beginning of the 1st trial. * shows significant changes($p<0.05$) of each value compared to the baseline value of the second trial based on paired t-test. Abbreviation: BSID-II, Bayley Scales of Infant Development II; GMPM, gross motor performance measure; GMFM, gross motor function measure.

(Unpublished Data)

Changes in Primary Outcome Measures Compared to the Baseline of the 1st Trial



(Unpublished Data)

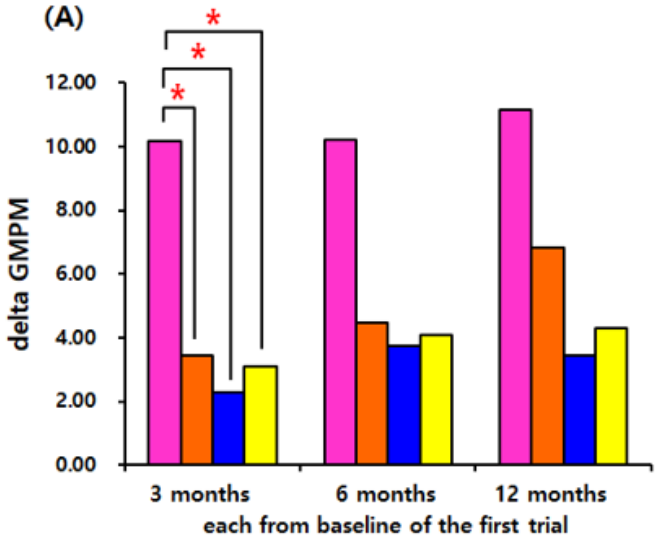
Changes in Motor Function among Participants with Severe Impairment (GMFCS levels IV-V) Compared to the Baseline of the 1st Trial (n=44)

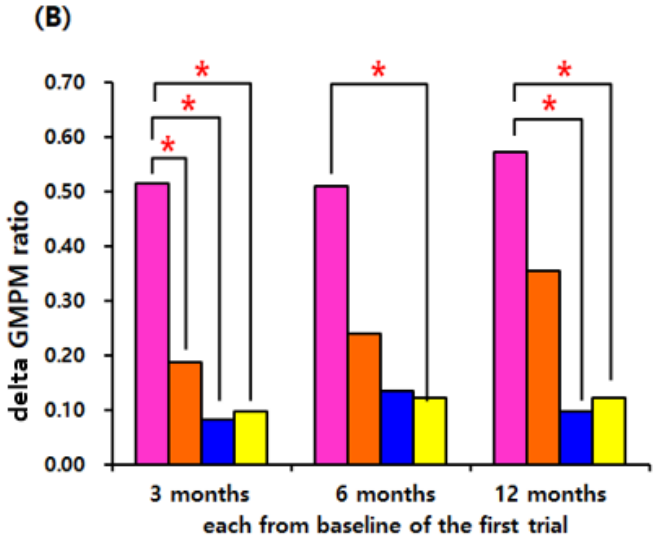
Delta GMPM ratio

$$= \frac{\Delta GMPM}{GMPM}$$

$$= \frac{GMPM_{follow-up} - GMPM_{initial}}{GMPM_{initial(=baseline)}}$$

A : UCB+EPO (n=9)
B : UCB+P-EPO (n=11)
C : P-UCB+EPO (n=8)
D : P-UCB+P-EPO (n=16)

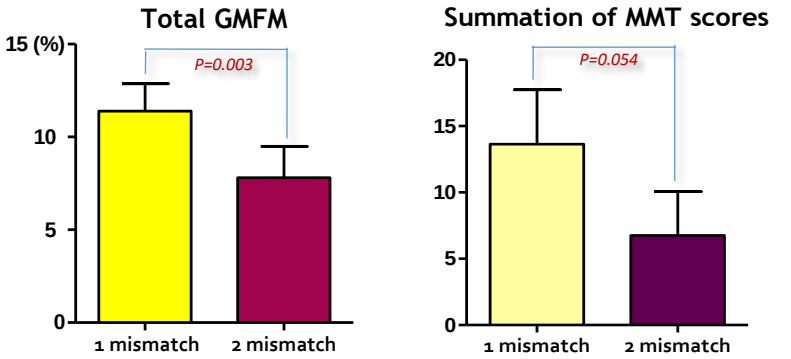




(Unpublished Data)

Efficacy related factor: UCB immune compatibility

Differences in score change from baseline to 6 mo in UCB group by HLA mismatching (1: n=11; 2: n=20)



(Min et al., 2013, Stem Cells)

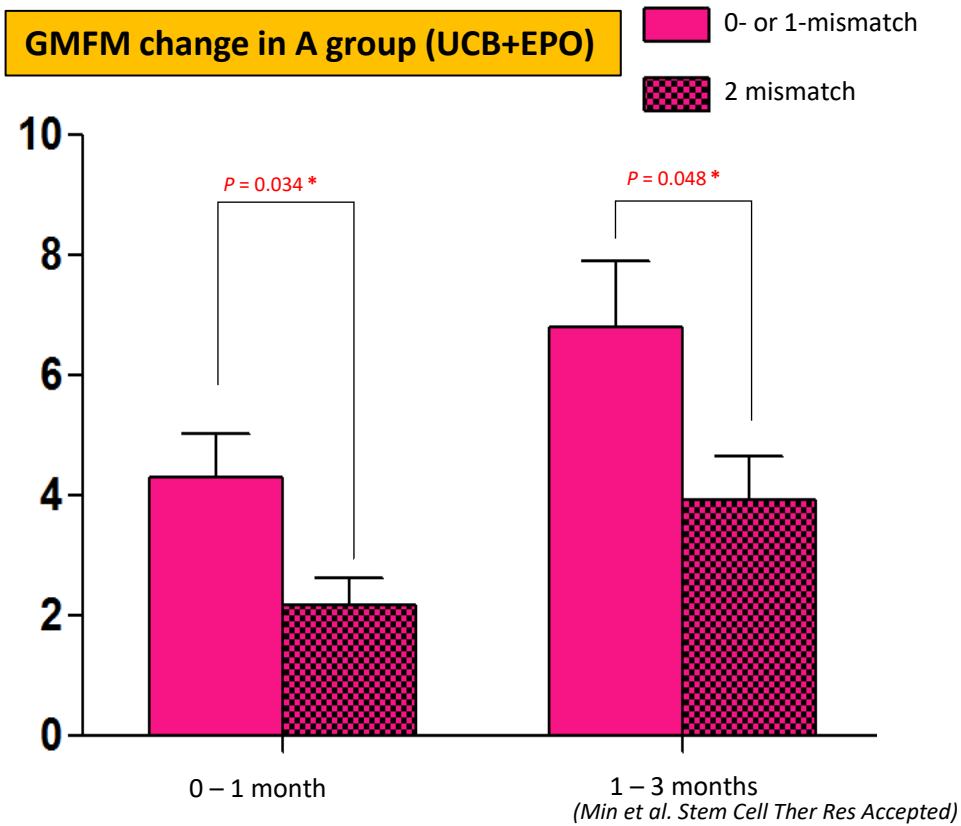
- More improvements (*Ps*<0.05) in

0- or 1-mismatched (*n*=5) > 2-mismatched (*n*=12)

- MMT score at 3 months
- BSID-II Motor scale at 1 month
- WeeFIM total score at 3 months

(Kang et al. 2015, Stem Cells Dev)

GMFM change in A group (UCB+EPO)



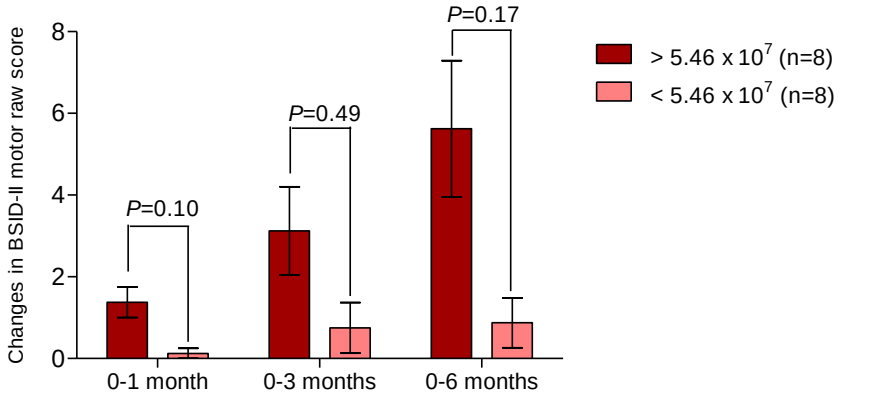
(Min et al. Stem Cell Ther Res Accepted)

Efficacy related factor: Number of total nucleated cells

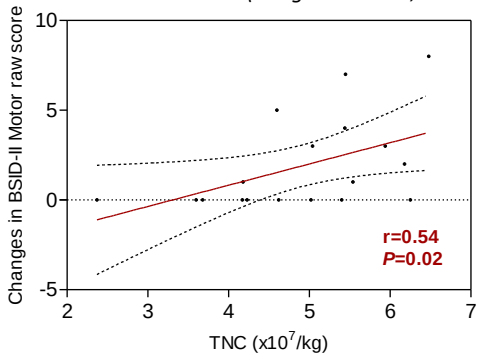
Clinical outcomes and Total Nucleated Cells (TNC) and CD34+ cells/kg of body weight in pUCB group (*n* = 31)

TNC number/kg		**> 6.69 × 10^7 (*n* = 15)**	**< 6.69 × 10^7 (*n* = 16)**	***P*-value***
GMFM	3–6month	3.7 (0.8)	1.4 (0.3)	0.024
	1–6month	7.9 (2.2)	3.1 (1.78)	0.021
GMPM	1–3month	5.3 (0.7)	3.73 (1.2)	0.030
CD 34+ cell number/kg		**> 1.46 × 10^5 (*n* = 16)**	**< 1.46 × 10^5 (*n* = 15)**	***P*-value***
BSID-II Mental scale raw score	3–6month	8.3 (1.9)	2.7 (0.9)	0.030
	1–6month	13.2 (2.2)	5.3 (0.9)	0.008

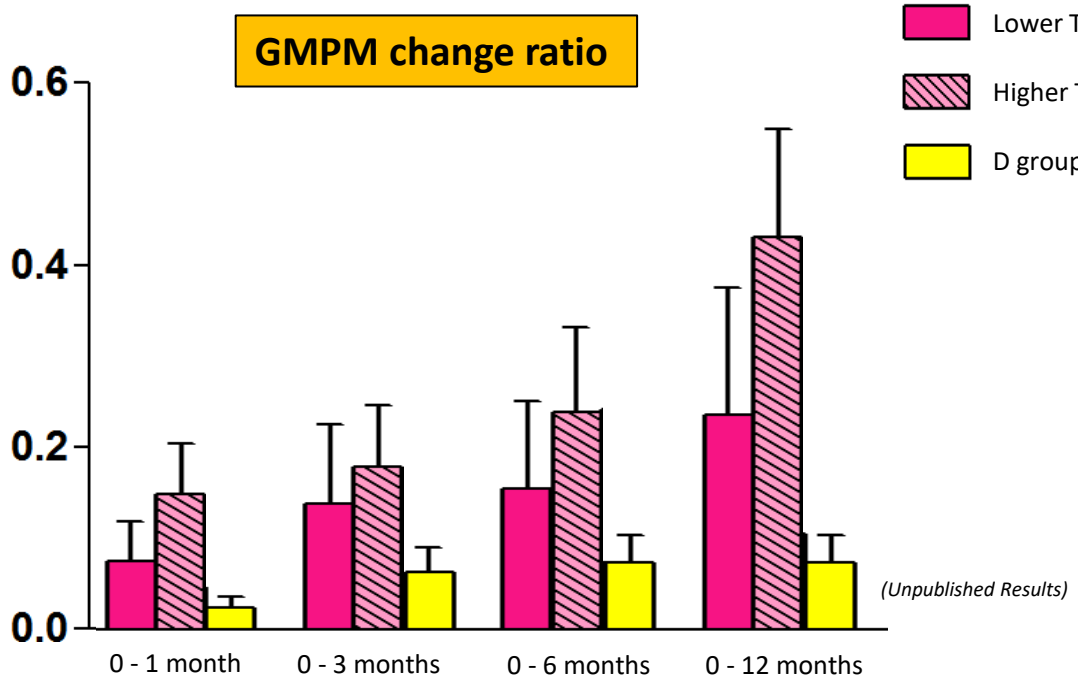
(Min et al., 2013, Stem Cells)

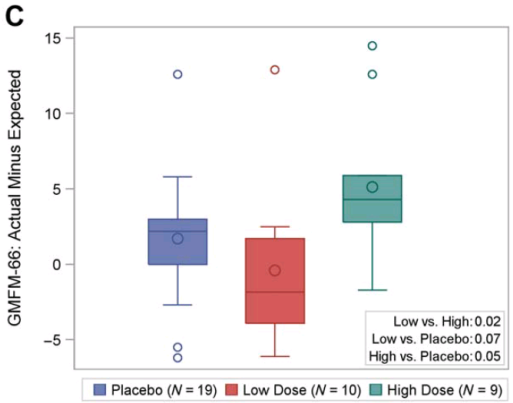


(Kang et al. 2015, Stem Cells Dev)



Efficacy related factor: Number of total nucleated cells





- Higher TNC group showed better improvement in the comparison of GMPM ratio.

(Sun et al. Stem Cells Transl Med 2017)

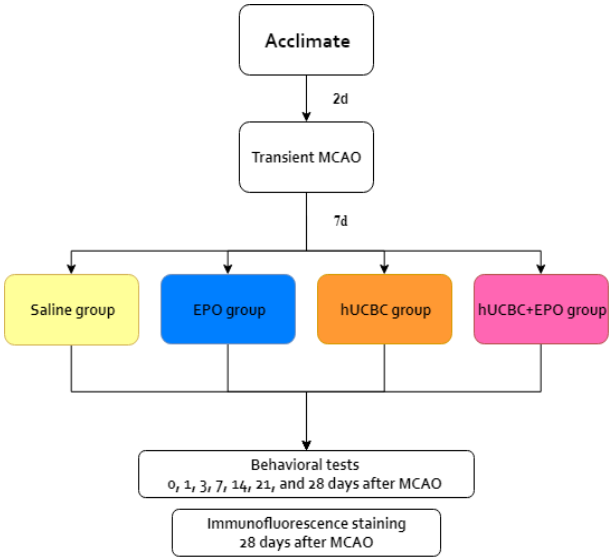
Mechanism research revealing synergistic effect of Combination treatment with UCB Cells (UCBC) and EPO

frontiers in Neurology

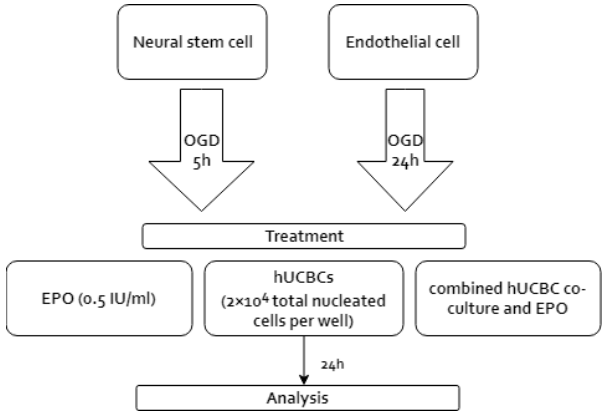
Combining Human Umbilical Cord Blood Cells with Erythropoietin Enhances Angiogenesis/Neurogenesis and Behavioral Recovery after Stroke

- Saline, intraperitoneal injection for five consecutive days from 7 d post-MCAO
- EPO, 500 IU/kg, intraperitoneal injection for 5 consecutive days from 7 d post-MCAO
- hUCBC, 1.2×10^7 total nucleated cells, tail vein injection once at 7 d post-MCAO
- hUCBC+EPO treatment at the same dose and schedule as the other groups

in vivo ischemia model



in vitro assay



(Hwang et al. 2019, Front Neurol)

Reliability of behavior tests in MCA occlusion model

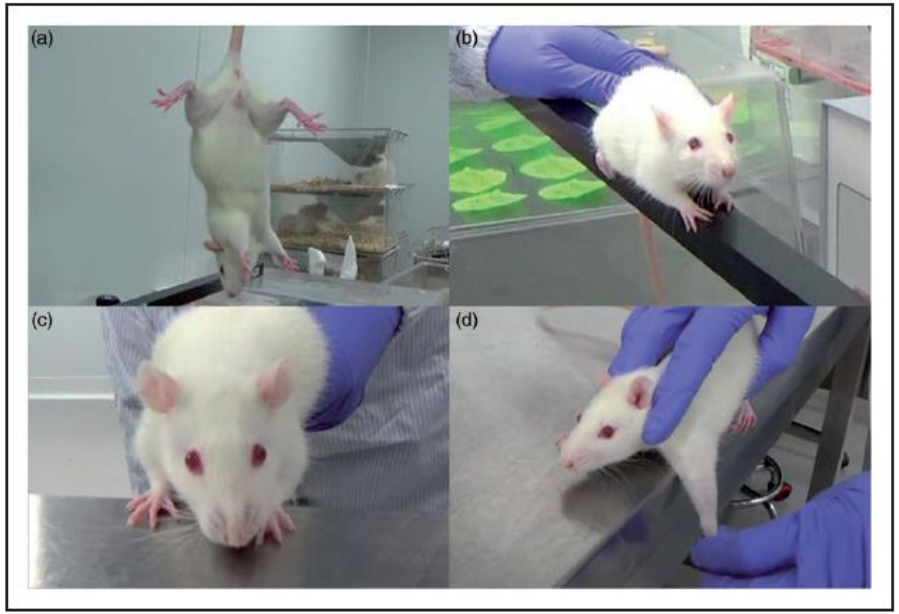


Figure 1. Modified Neurological Severity Score (mNSS). (a) Raising rat by tail; (b) Beam balance test; (c) Placing test; and (d) Proprioceptive test.

Laboratory Animals Limited

Original Article

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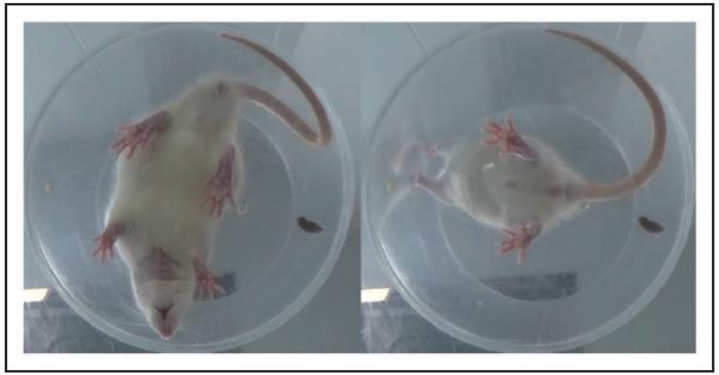
SAGE

Reliability of behavioral tests in the middle cerebral artery occlusion model of rat

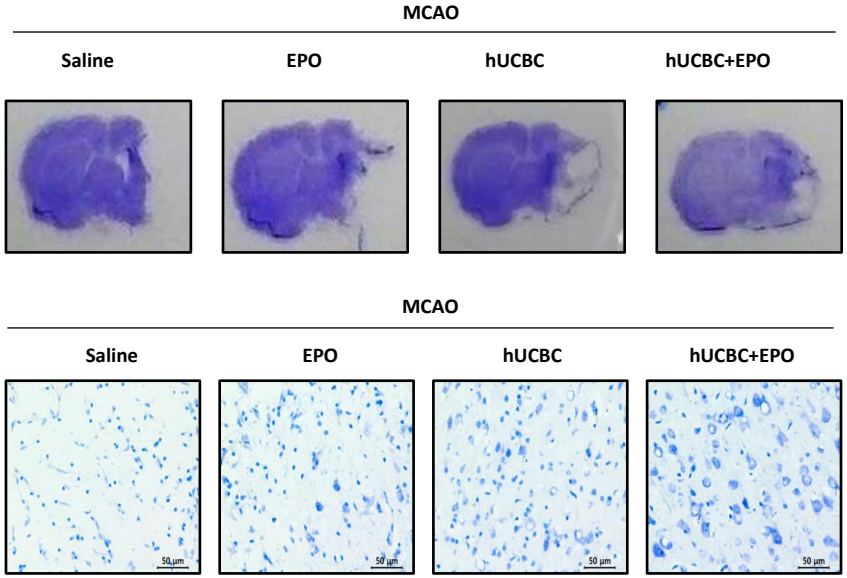
Junghoon Yu[1], Jinkyoo Moon[1], Joonyoung Jang[1], Jee In Choi[2], Jooeun Jung[3], Sunyoung Hwang[2] and MinYoung Kim[1,2]

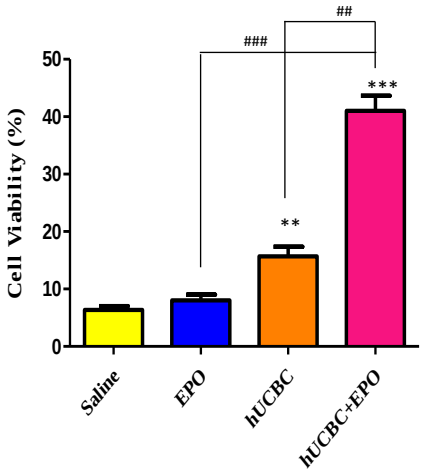


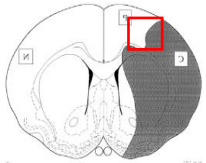
Cylinder Test. Spontaneous forelimb use of rat being assessed.

Attenuation of ischemic brain damage by EPO, hUCBC and hUCBC+EPO

Lesion volume were determined 28d after MCAO







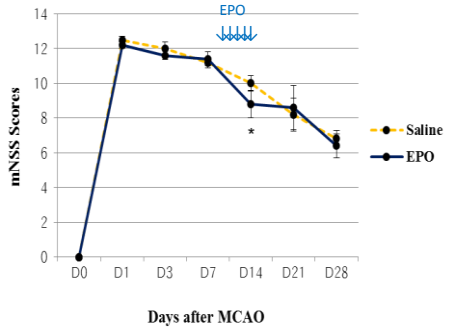
(Representative images of cresyl violet staining in the cortex)

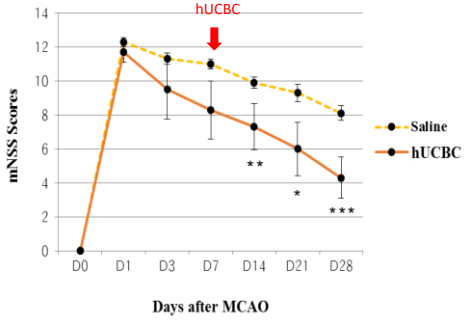
- **Treatment with hUCBC+EPO achieved the most significant alleviation of cell damage in rat brains**

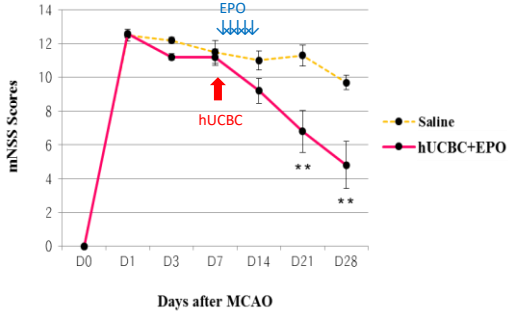
(Hwang et al. 2019, Front Neurol)

Comparison of behavioral tests by administration of EPO, hUCBC and hUCBC+EPO

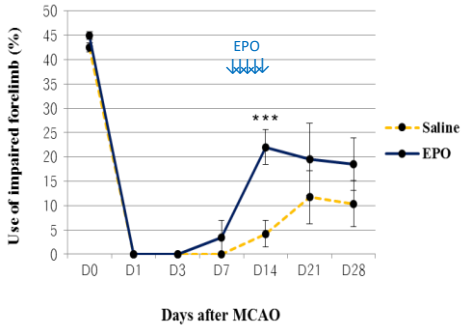
Modified nueurological severity score (mNSS)

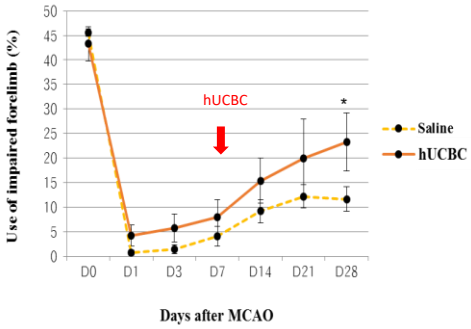


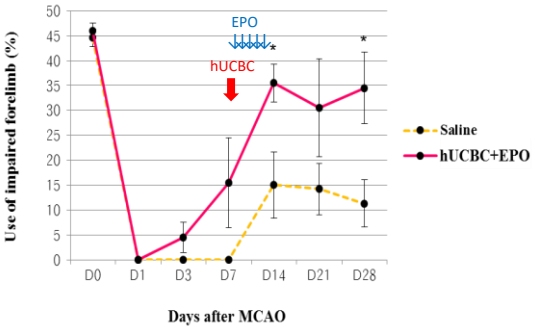




Cylinder test

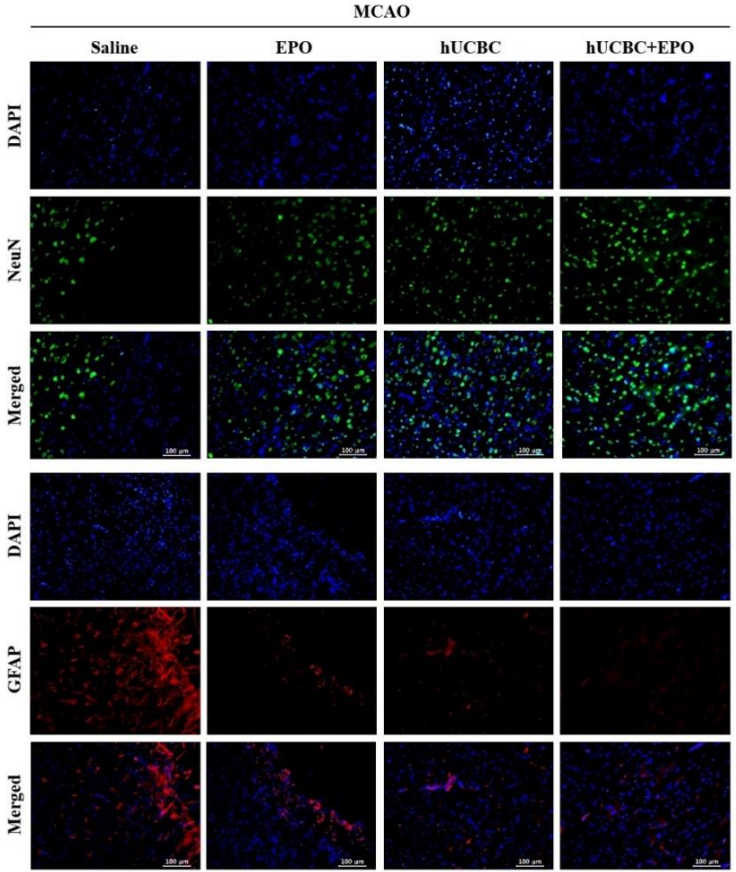


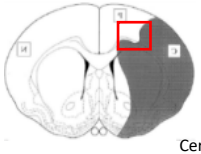




(Hwang et al. 2019, Front Neurol)

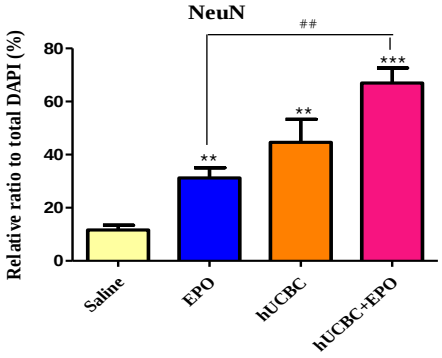
Survival of Neuronal Cells and Decreased astrogliosis by EPO, hUCBC and hUCBC+EPO

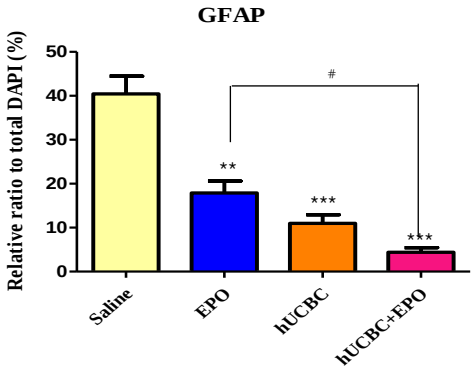




Cerebral cortex

in vivo ischemia model





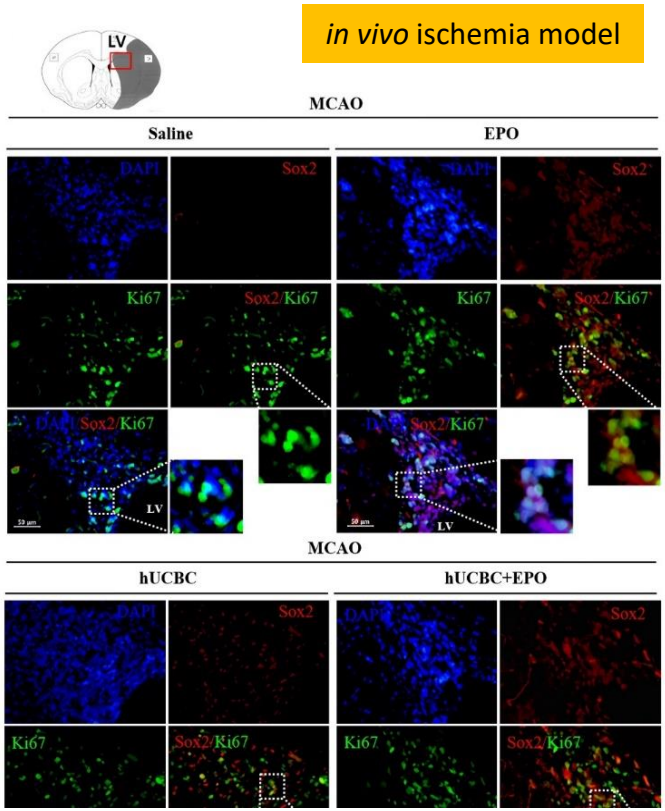
$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$ *vs* saline-treated group.

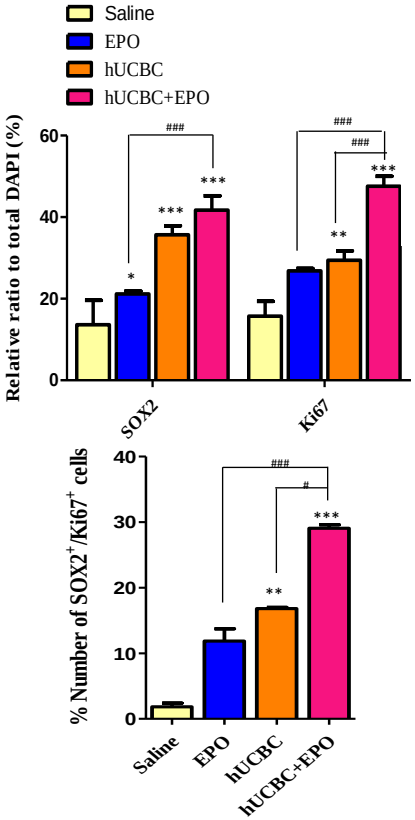
$^{\#}P < 0.05$, $^{\#\#}P < 0.01$ for inter-treatment group comparison

(Hwang et al. 2019, Front Neurol)

Neurogenic effect by EPO, hUCBC and hUCBC+EPO

in vivo ischemia model

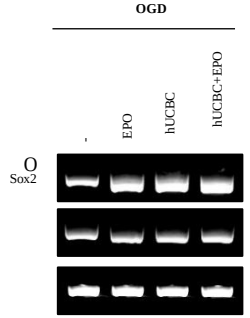


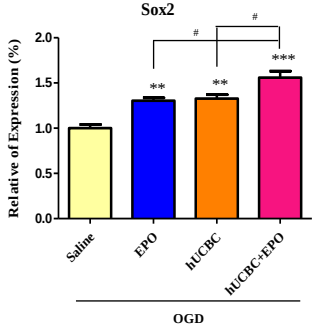


- Proliferation of neuronal cells in the lateral subventricular zone by treatment with EPO, hUCBC, and hUCBC+EPO (Stained with Ki67 or Sox2 antibodies at 28d after MCAO)

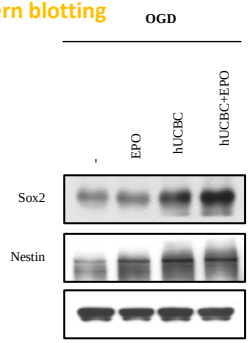
in vitro assay using OGD-injured neural stem cells

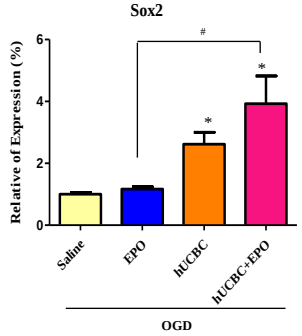
RT PCR





Western blotting



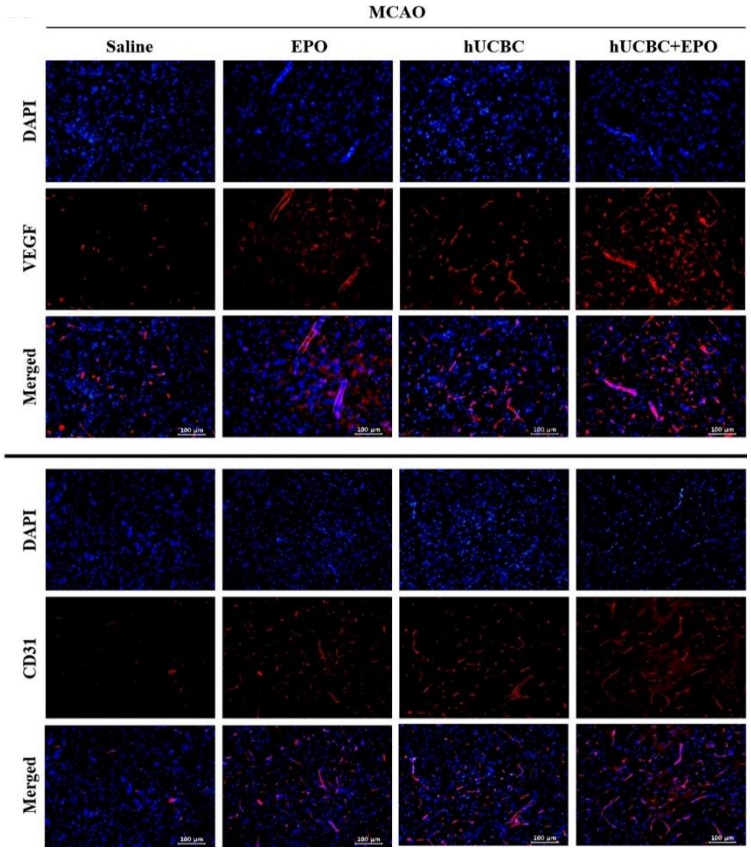


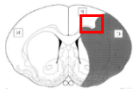
- Sox2 gene expression was significantly increased by EPO or hUCBC treatment, and it was most increased by hUCBC+EPO
- Sox2 was upregulated by hUCBC and hUCBC+EPO treatment

(Hwang et al. 2019, Front Neurol)

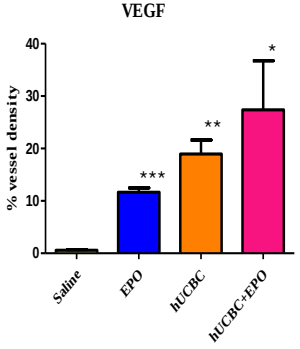
Angiogenic effect by EPO, hUCBC and hUCBC+EPO

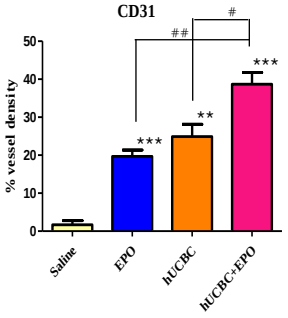
in vivo ischemia model





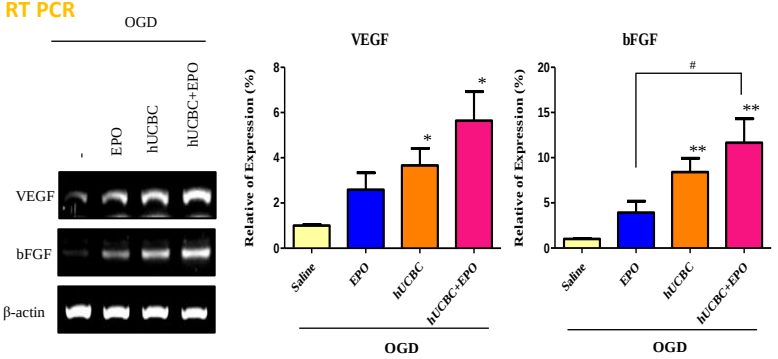
Cerebral cortex



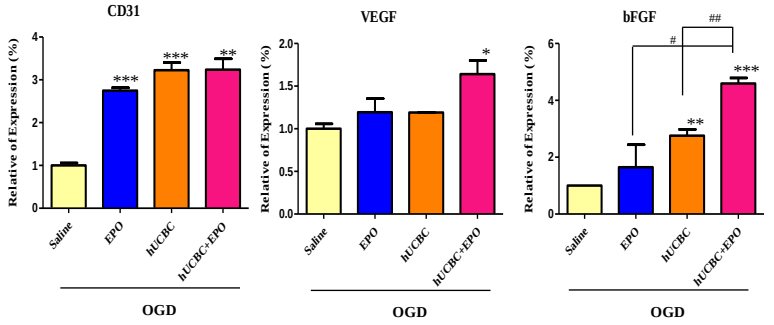


in vitro assay using OGD-injured endothelial cells

RT PCR



Western blotting



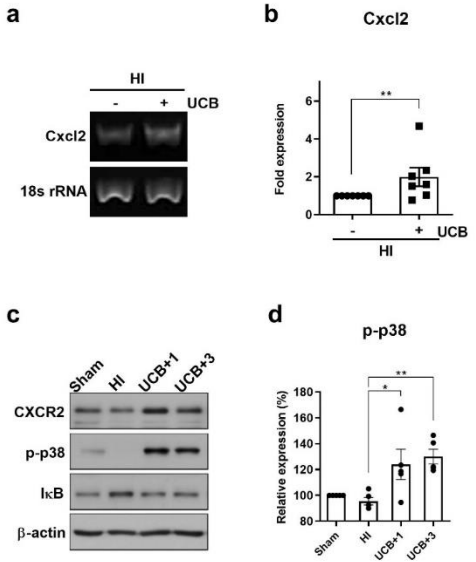
(Hwang et al. 2019, Front Neurol)

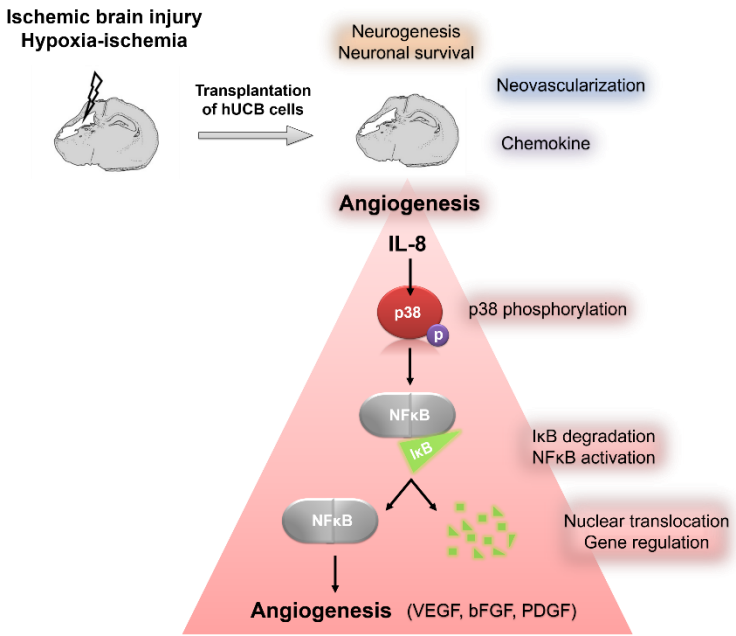
Therapeutic mechanism of cord blood mononuclear cells via the IL-8-mediated angiogenic pathway in neonatal hypoxic-ischaemic brain injury

Cho et al, Scientific Reports | (2020) 10:4446

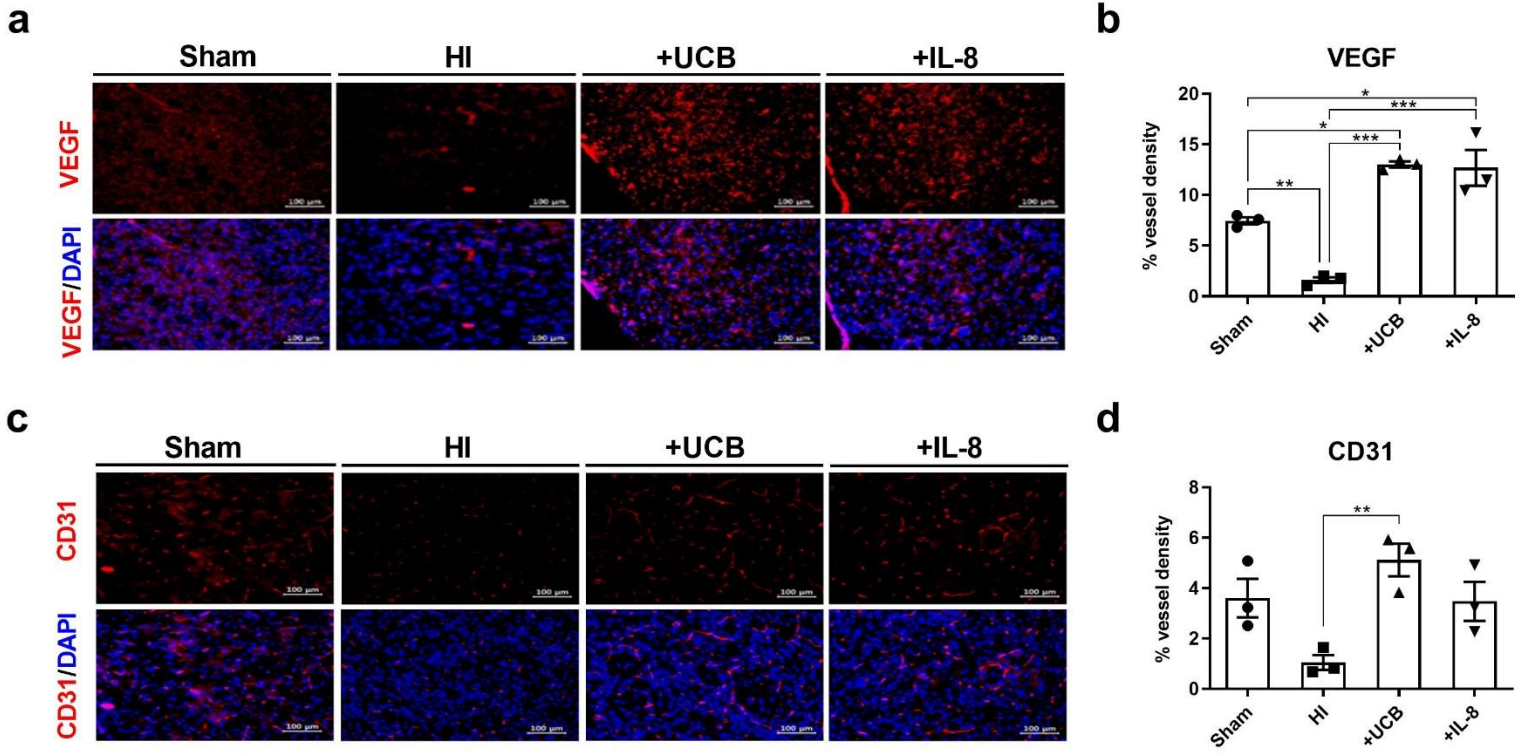
- IL-8 elevation by UCBC in neonatal HIE model
- In mice, deletion of IL-8 gene coding → Cxcl2 is a functional homologue
- CXCR2 mediates signal transduction (human IL-8)

Brain tissue RT- PCR





Angiogenic effect by UCB or IL-8 in vivo



Summary

- Three double-blind RCT showed therapeutic efficacy of allogeneic UCB +/- EPO without harmful side effects
  - 1st RCT (allogeneic UCB+EPO *vs* EPO *vs* control)
    - UCB+EPO therapy showed superior outcomes in motor function at 3rd and 6th months post-intervention compared to EPO-received and control groups.
    - White matter integrity was increased at 6 month post-intervention by UCB+EPO therapy.
    - Amelioration of inflammation in the brain at 2 weeks after the intervention were observed.
  - 2nd RCT (IV and IA allogeneic UCB *vs* control)
    - Infusion without EPO showed superior outcomes in motor function at 3rd and 6th months post-intervention
    - Involvement of innate immunity was observed by elevation of related molecules and correlation with outcomes
    - Amelioration of inflammation in the brain at 2 weeks after the intervention were observed.
  - 3rd RCT (allogeneic UCB+EPO *vs* UCB *vs* EPO *vs* control) → 4th clinical study
    - UCB+EPO therapy showed superior outcome in motor function at 1 year & 2 years post-intervention.
    - White matter integrity was increased at 1 year post-intervention by UCB+EPO therapy in a thalamic radiation.
    - Electroencephalographic findings showed maturation of brain wave by any of the interventions.

Summary

- Efficacy factors were consistently found to be significant in allogeneic UCB therapy
  - Immune histocompatibility
    - More matched units of HLA-A, B, DRB1 showed better outcomes than less matched units (all 3 RCTs)
  - Numbers of total nuclear cells
    - More number of TNCs showed better outcomes than less number of TNCs (all 3 RCTs)
- Mechanism of UCB +/- EPO therapy in brain damage
  - Decrement of neuroinflammation
    - appeared in FDG-PET in 2 RCTs with 2-weeks follow-up
  - Involvement of innate immunity
  - Increment of neuronal survival, neurogenesis, and angiogenesis
  - Decrement of astrogliogenesis
- Therapeutic efficacy of allogeneic UCB can be potentiated with concomitant EPO use
  - Functional outcomes in clinical results, and *in vivo* and *in vitro* findings also supported the findings

Conclusion

- UCB has efficacy without harm for neural recovery in brain damage
  - Cerebral palsy, Stroke,..
- Efficacy of UCB is enhanced by combined treatment of erythropoietin
  - No harmful effect, under monitoring of hemoglobin level
- Suggested therapeutic mechanism
  - Neurogenesis, improvement in white matter integrity
  - Anti-inflammation in the brain tissue
  - Stimulation of innate immunity that may be related with healing
  - Angiogenesis

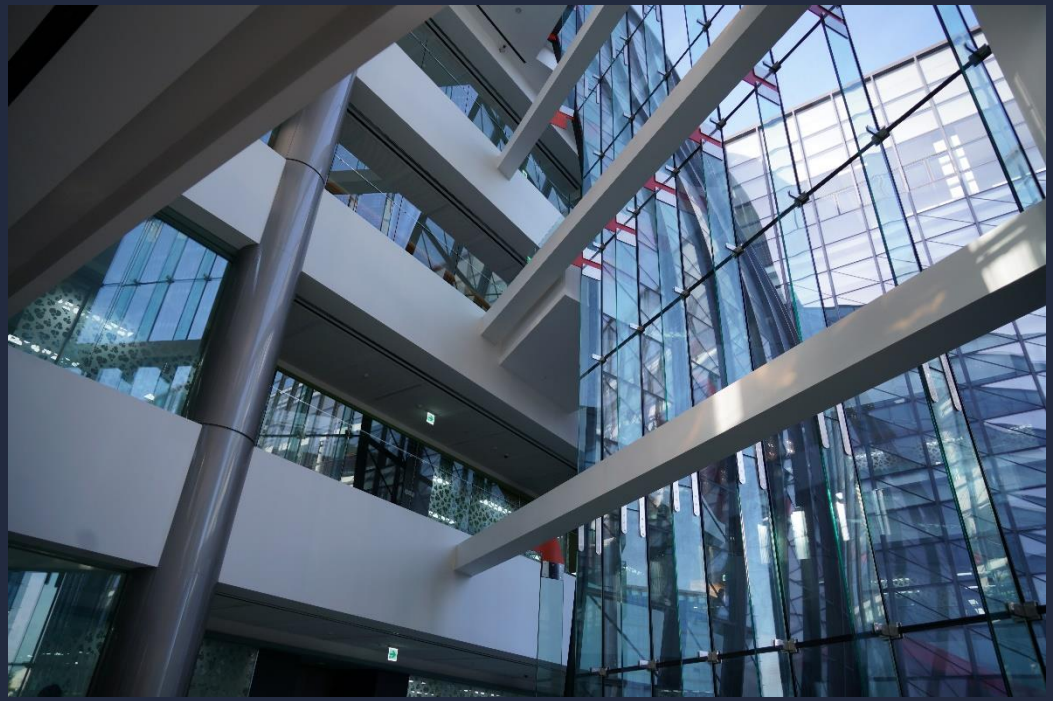
Supporter, Study team, & Collaborators

Rehabilitation & Regeneration Research Center, CHA University

- CHA Medical Center Cord Blood Bank
- CHA Global Clinical Trial Center
- Korean Network for Organ Sharing in Korea Centers for Disease Control & Prevention, Ministry of Health & Welfare
- Prof. Dong-Wook Kim, Yonsei University
- Prof. Seongsoo An, Gacheon University
- iSyncBrain®: analysis of EEG
- Seoul CRO

Rehabilitation Medicine Clinical Research Team

Thank you for your attention!



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