Umbilical Cord Blood Cell Therapy for Children with Cerebral Palsy

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Cerebral Palsy





- "Cerebral palsy (CP) describes a group of disorders of the development of movement and posture, causing activity limitation, that are attributed to non-progressive disturbances that occurred in the developing fetal or infant brain."

(Executive committee for the definition of cerebral palsy, 2005)

- Associated disabilities in cognition and activities of daily living
- Prevalence: 3 per 1000 *(Clark, 2003)*
- Lifelong functional deficits

From: **Prognosis for Gross Motor Function in Cerebral Palsy: Creation of Motor Development Curves**

JAMA. 2002;288(11):1357-1363. doi:10.1001/jama.288.11.1357

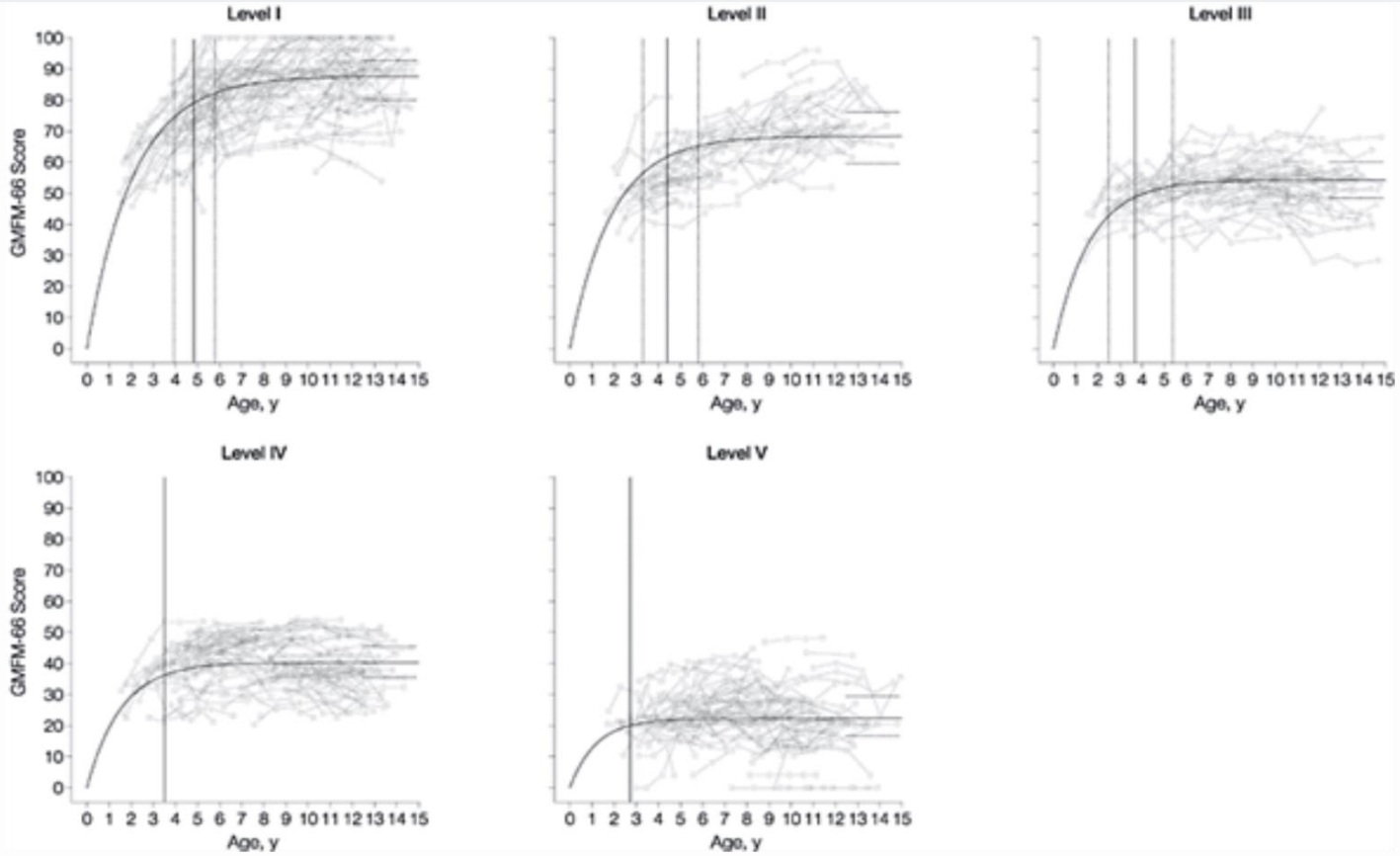


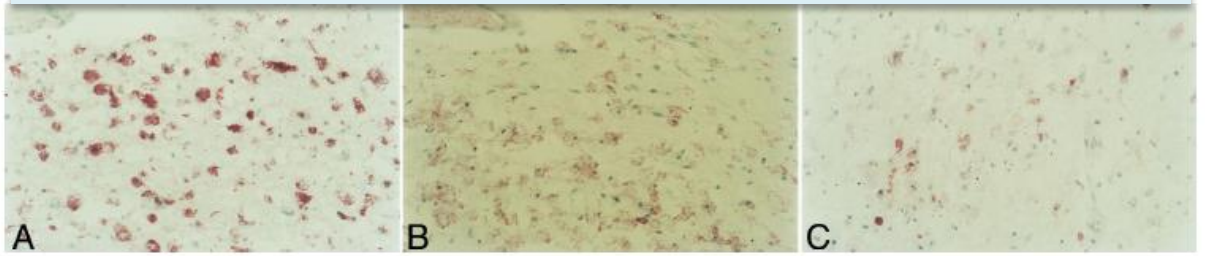
Figure Legend:

The curved solid lines indicate average performance. The horizontaldotted lines on the right of the figures indicate the band expected to encompass50% of children's limits of development. The solid vertical lines indicatethe average age-90. The dotted vertical lines indicate the bands expectedto encompass 50% of age-90 values around the average. The absence of 50% bandsin level IV and level V indicates low variation in age-90 values.



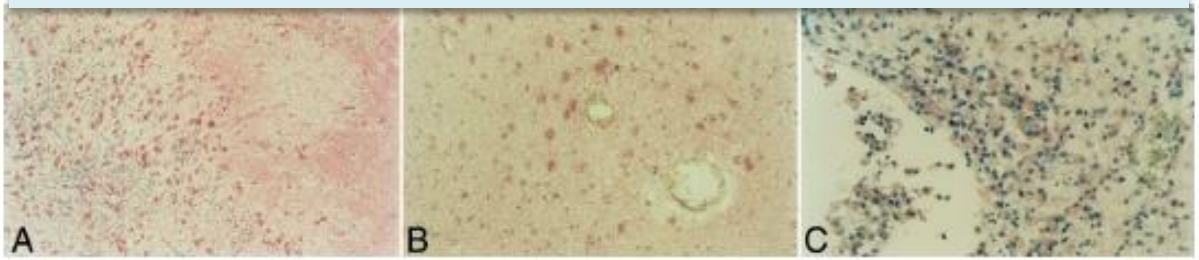
Periventricular leukomalacia (PVL)

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)

Kadhim, 2001 (Neurology)

Review

Developmental Neuroscience

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

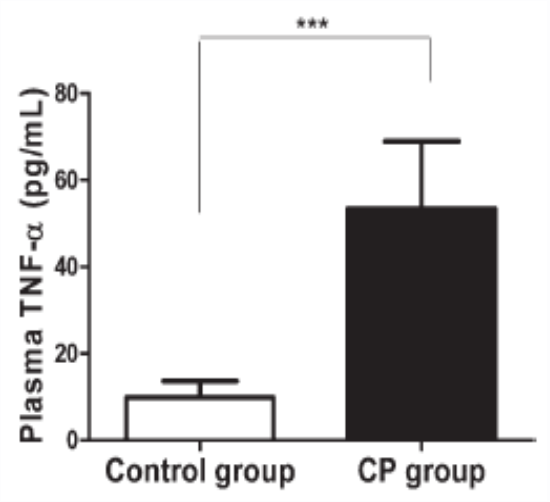
Received: December 3, 2008
Accepted after revision: March 3, 2009
Published online: August 11, 2009

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

Altered Inflammatory Responses in Preterm Children with Cerebral Palsy

Chang-Yi Lin, BS,[1] Ying-Chao Chang, MD, PhD,[2] Shan-Tair Wang, PhD,[3] Ting-Yang Lee, MS,[4] Chiou-Feng Lin, PhD,[5] and Chao-Ching Huang, MD[5,6]

ANN NEUROL 2010;68:204–212



- TNF-α ↑ in preterm children with PVL (average age: 7 years old)

▷ **Difficult to be recovered from accentuated inflammatory response in immature brain injury over existing brain lesion**

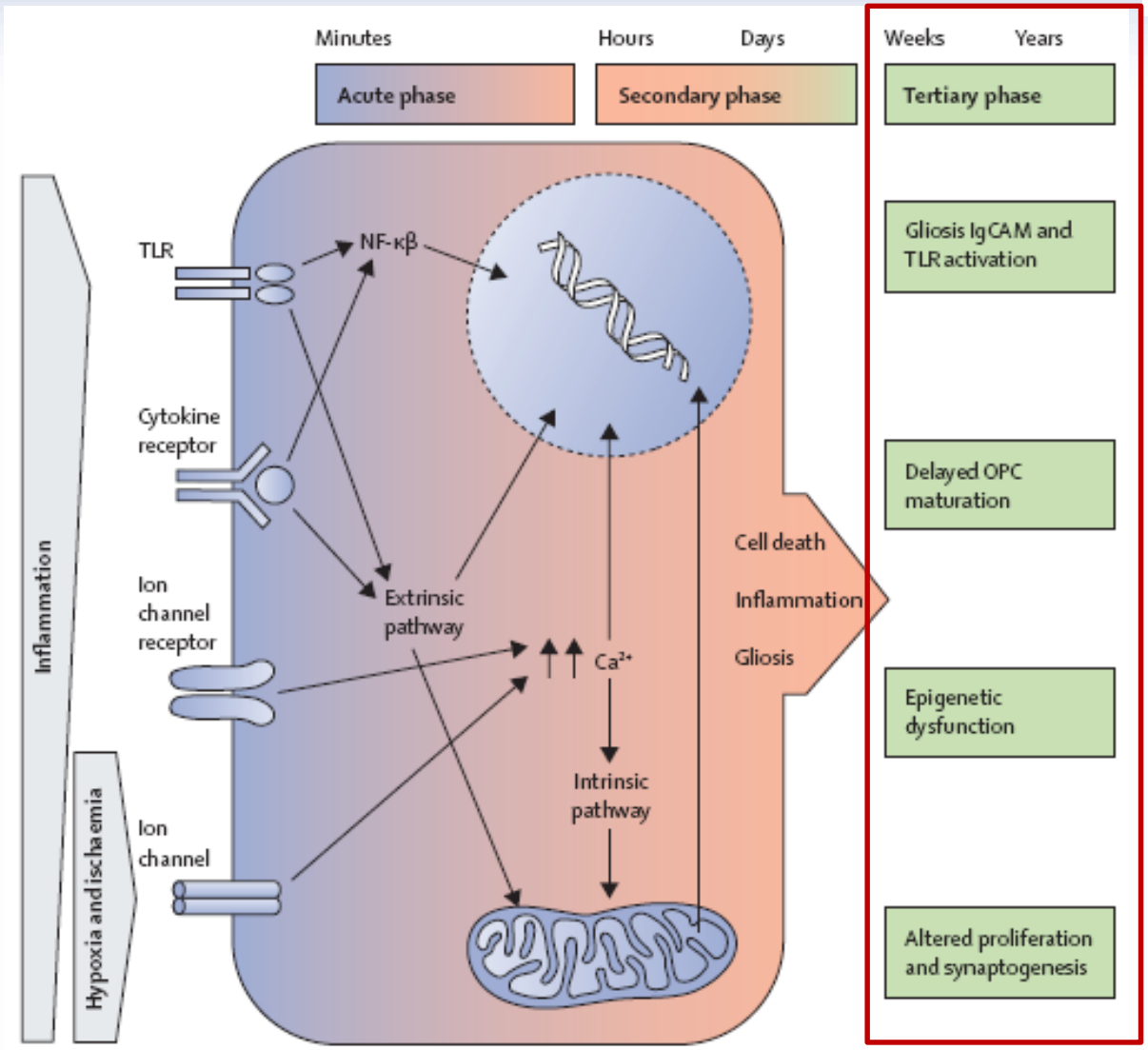
Persistent neuro-inflammation in cerebral palsy: a therapeutic window of opportunity?

Olaf Dammann[1,2,3] (dammann.olaf@mh-hannover.de)

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Tertiary Brain Damage in Cerebral Palsy

Fleiss, 2012 (Lancet Neurol)



Tertiary damage

- Results
  : Prone to further damage
  Prevent recovery
- Cause
  : Epigenetic change,
  Persistence of inflammation

Solution

Pharmacological drugs

- Erythropoietin
- G-CSF, GM-CSF
- Growth factors

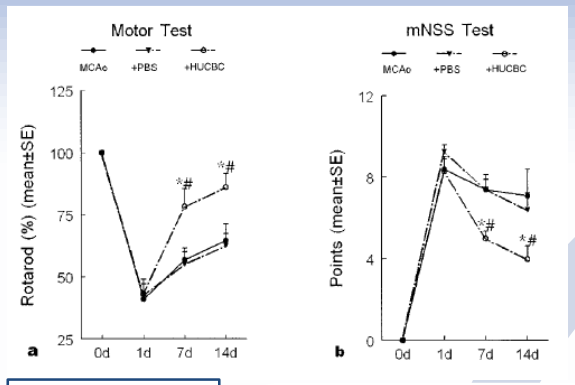
Cell therapy

- Umbilical cord blood
- Neural stem cell
- Mesenchymal stem cell

Duke University (USA; NCT00593242)

Medical College of Georgia (USA; NCT01072370)

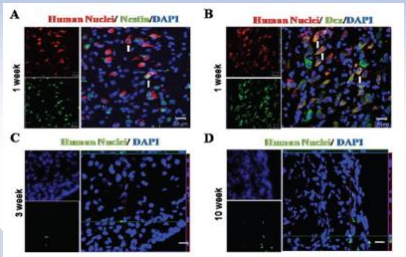
Umbilacal Cord Blood (UCB) in Brain Injury



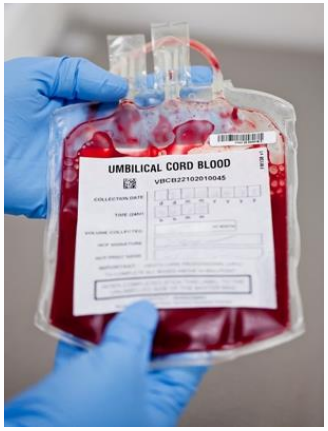
Functional improvement by UCB in brain infarct (Chen, 2001)

Functional recovery

Neurogenesis



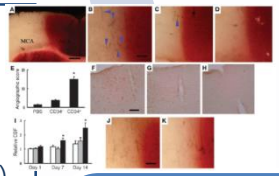
Survival of transplanted UCB cells in brain, anti-inflammatory cytokines (Bae, 2012)

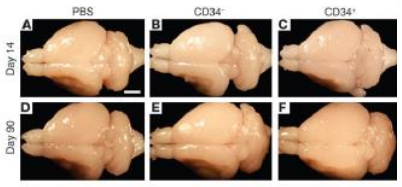


Paracrine effect

Cytokines and Chemokines	Control (pg/ml)	hUCBCs (pg/ml)
IL-1a	1	24
IL-1b	0	2
IL-1ra	0	4
IL-2	0	0
IL-3	1	0
IL-4	0	0
IL-5	1	1
IL-6(*)	0	2,921
IL-7	0	0
IL-8/CXCL8(*)	0	12,552
IL-9	3	0
IL-10	0	0
IL-12(p40)	9	0
IL-12(p70)	0	0
IL-13	2	2
IL-15	0	1
IL-17	0	0
IP-10(*)	4	550
IFNa2	0	5
IFNr	0	0
CCL2/MCP-1(*)	0	7,865
CCL3/MIP-1a	0	19
CCL4/MIP-1b	0	0
CCL5/RANTES(*)	0	196
CCL7/MCP-3(*)	0	906
CCL11/Eotaxin	5	6
CCL22/MDC	12	10
CXCL1/GROa(*)	0	>10,000
CX3CL1/Fractalkine	0	0
sCD40L	0	0
sIL-2Ra	0	0
EGF	12	12
FGF-2	0	0
Flt-3 ligand	3	3
G-CSF(*)	0	>10,000
GM-CSF	2	20
PDGF-AA	1	5
PDGF-AB/BB	0	0
TGFa	0	0

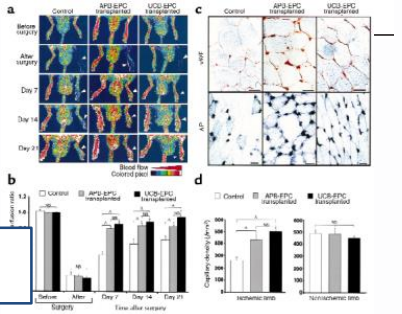
Decreased size of brain infarct area and vasculogenesis by administrating CD34+ UCB cells (Taguchi, 2004)





Decreased infarct size

Vasculogenesis



Neovascularization (Murohara, 2001)

The Efficacy of UCB in Animal Models





- **Source of stem cells**

Table 1 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

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

Cerebral hemorrhage

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

Spinal cord injury

- Cho et al, Neuroreport 2008

Traumatic brain injury

- Lu D at el, Cell Transplant 2002

Alzheimer's dementia

- Nikolic WV et al, Stem Cells Dev 2008

Cerebral palsy

- Meier C et al, Pediatr Res 2006

Advantages of UCB as a Source of Cell Therapy

Safety

Gluckman, 1989; Kurtzberg, 2005; Carter, 2006

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

Availability

Harris, 2008

- Off the shelf
- Cord blood banks

UCB

Lee, 2010

: suitable in regenerative medicine

Immune tolerance

- Immaturity

Tse, 2005; Vaziri, 1994; Lee, 2010

Neurogenicity

- Differentiable to neuronal, astrocytic, & oligo dendroglial cells

Neurhoff, 2007; Buzanska, 2006

Source of UCB & Immunosuppression



- **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 making up favorable environment for survival of allogeneic cells.

Purposes of the Trials

- To evaluate the efficacy of allogeneic UCB infusion for cerebral palsy

Umbilical Cord Blood

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



1st Trial of UCB for Children with CP

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

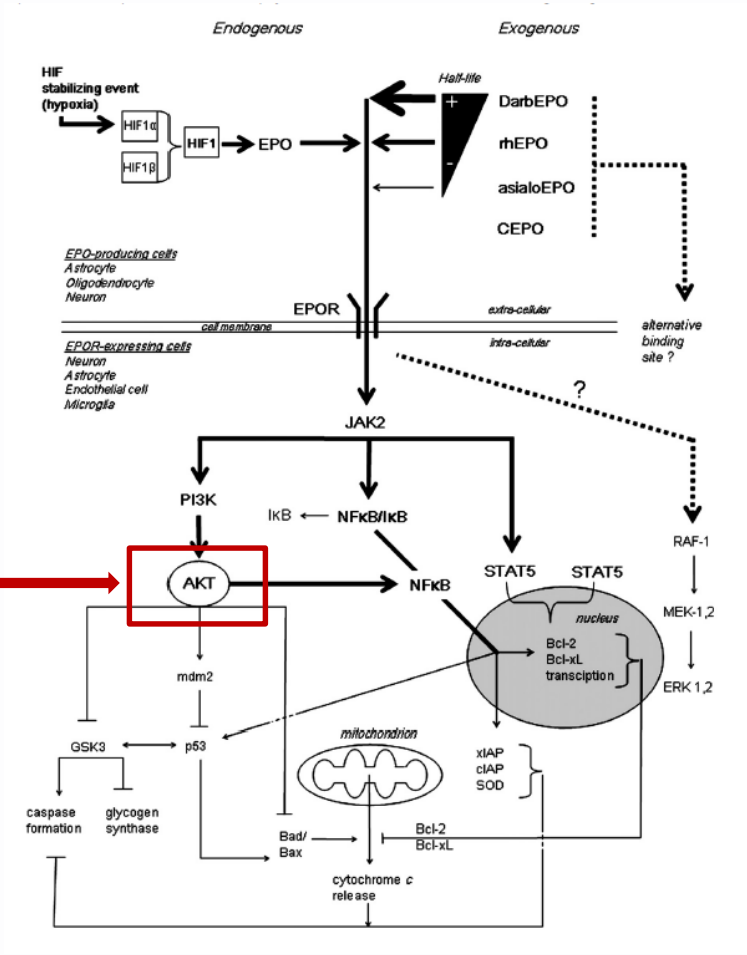
- Potentiation with "Erythropoietin"

van der Kooij MA, Brain Res Rev 2008

Dasari VR, Neurobiol dis 2008



UCB stem cells



1st Clinical Trial

Umbilical Cord Blood (UCB) + Erythropoietin

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

Search for studies: Example: "Heart attack" AND "Los Angeles" Search
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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]

Groups

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

Randomized — Double-blind

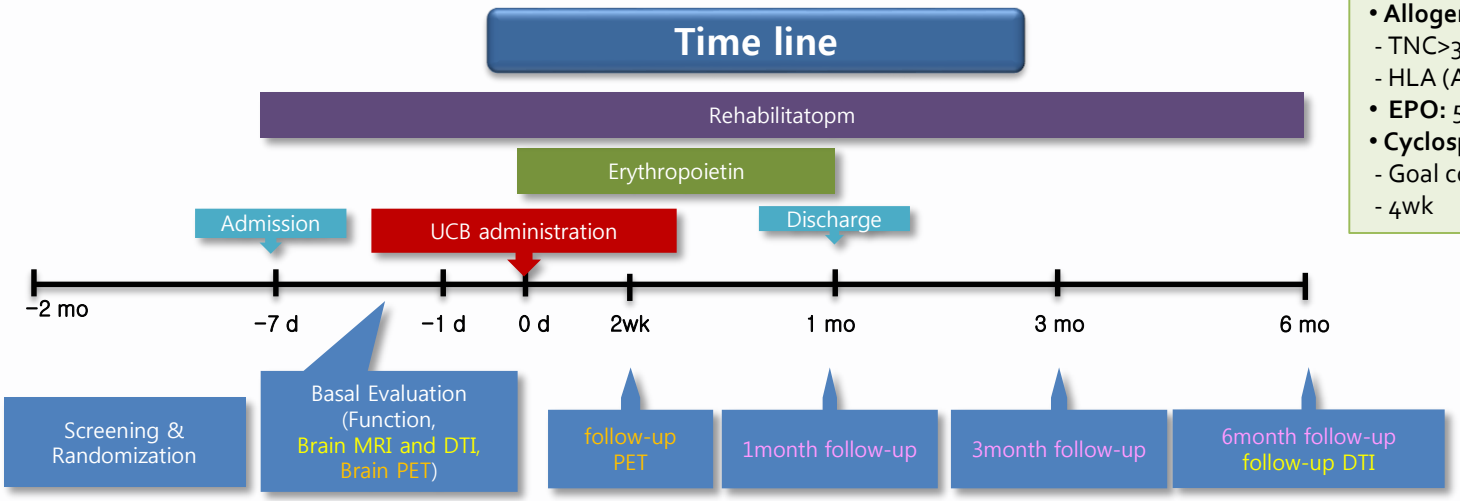
pUCB (n=35)	EPO (n=36)	Control (n=34)
Allogeneic UCB + Erythropoietin + Rehabilitation	Placebo UCB + Erythropoietin + Rehabilitation	Placebo UCB + Placebo EPO + Rehabilitation

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
  - 4wk



Time line

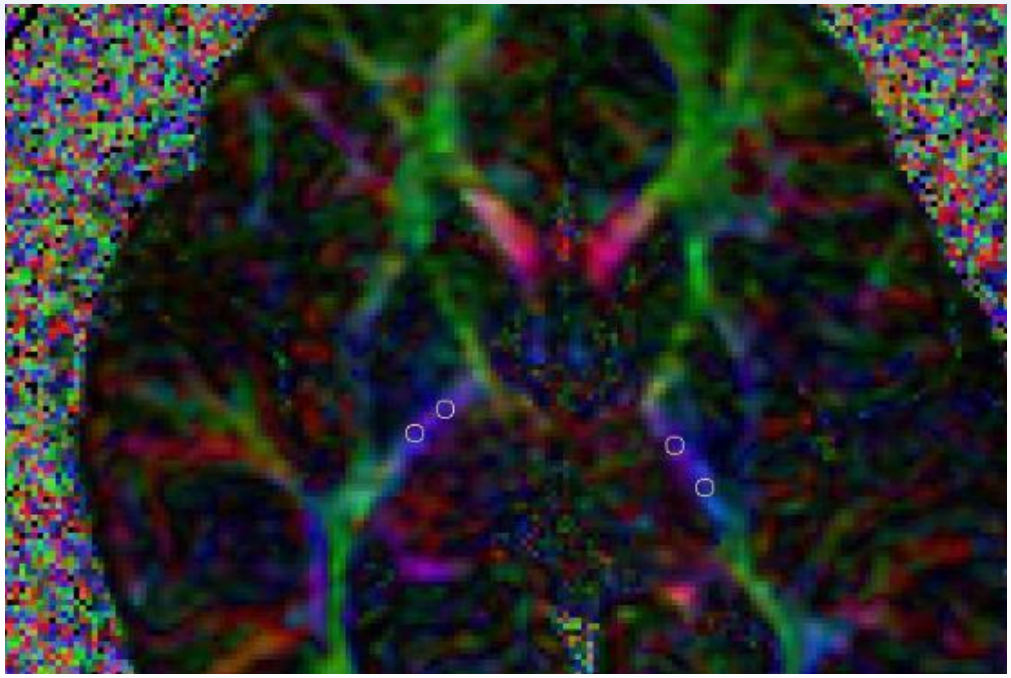




Outcome measurements

- 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

DTI & Frational 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)

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

(Ann Rehab Med, 2012)

Original Article

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.

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

(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

Results

Demography and Typology

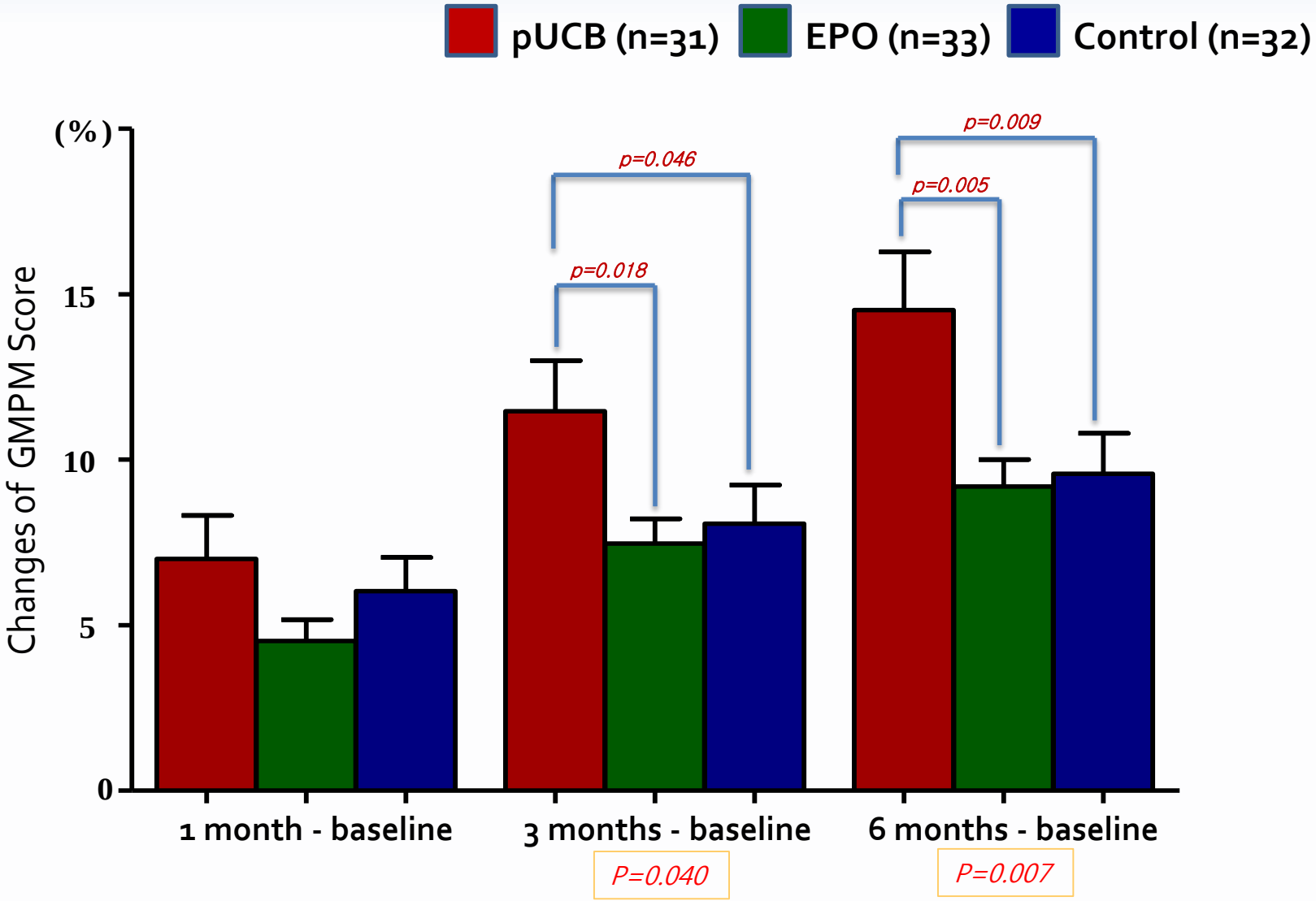
Group	pUCB (N=31)	EPO (N=33)	Control (N=32)
Male sex — no. (%)	23 (74.2%)	23 (69.7%)	23 (71.9%)
Age — months	36.84±19.4	43.9±24.7	38.3±18.4
Gestational day at birth (days)	237.6±34.6	230.3±35.0	246.4±28.7
Preterm — no. (%)	18 (58.1 %)	23 (69.7%)	17 (53.1%)
Birth weight — kg	2.2±0.9	2.0±0.9	2.4±0.7
NBW / 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)

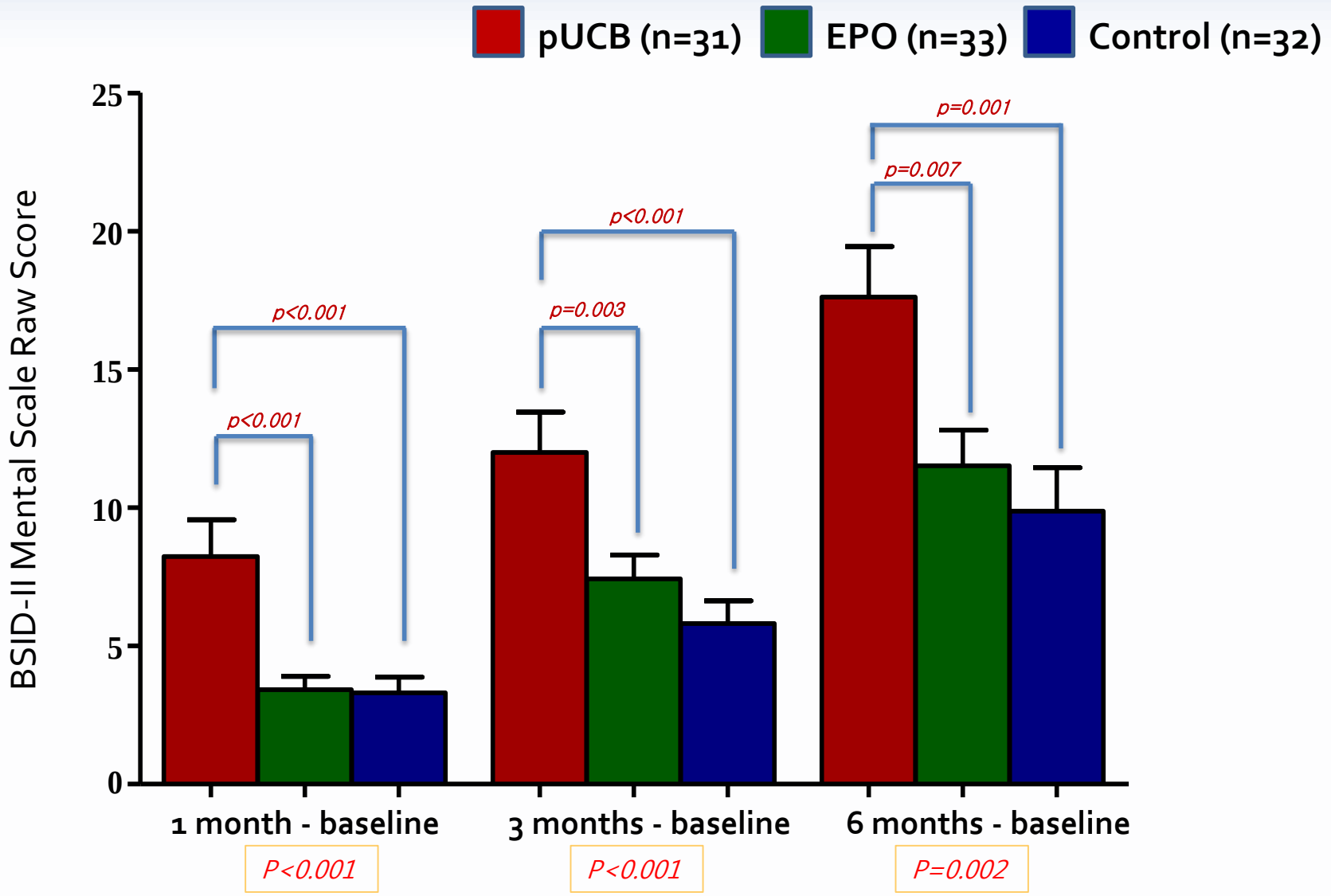
Adverse events during study period of six months in three groups (N=105)

	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

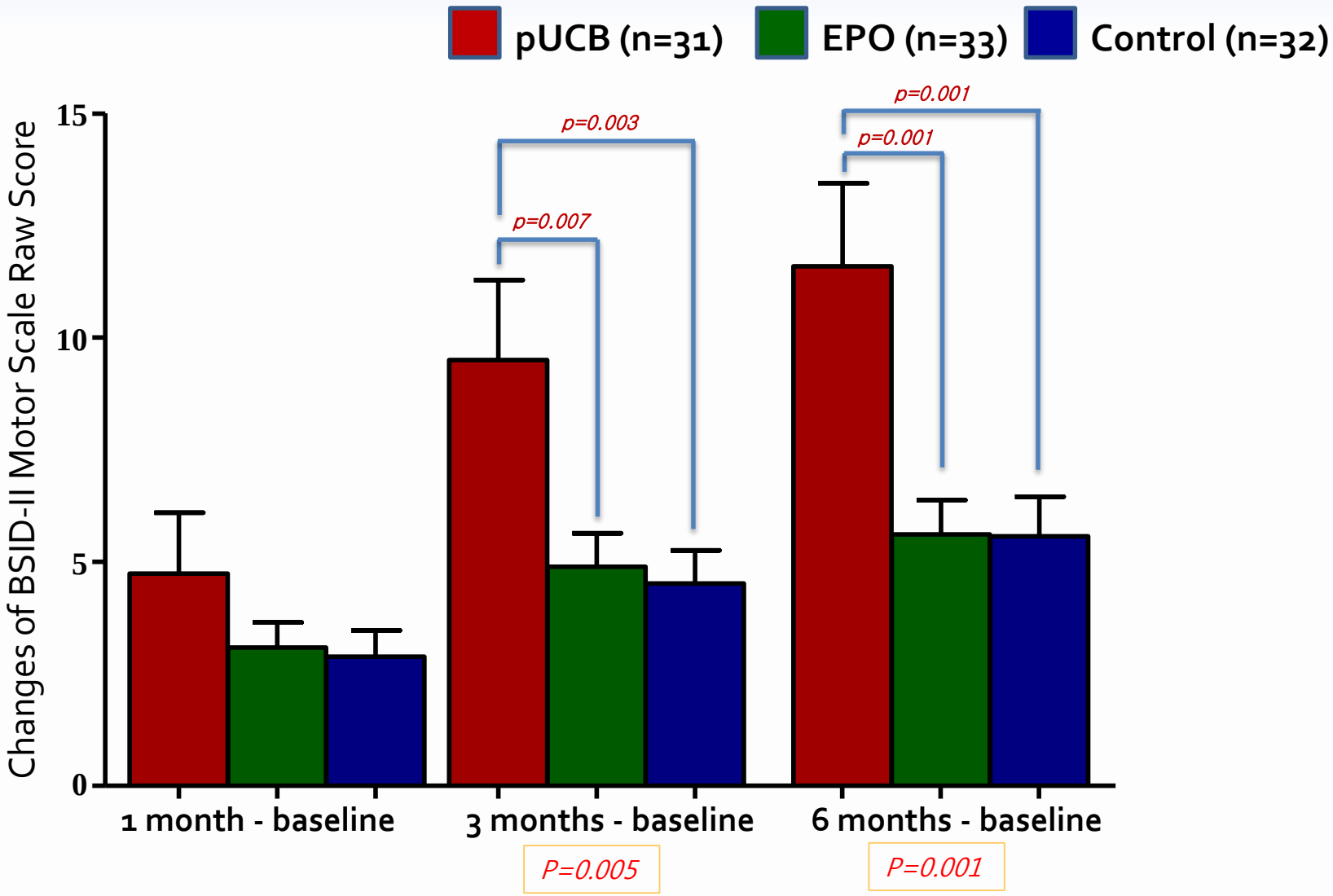
Changes of GMPM % Score from Baseline to Each Visit



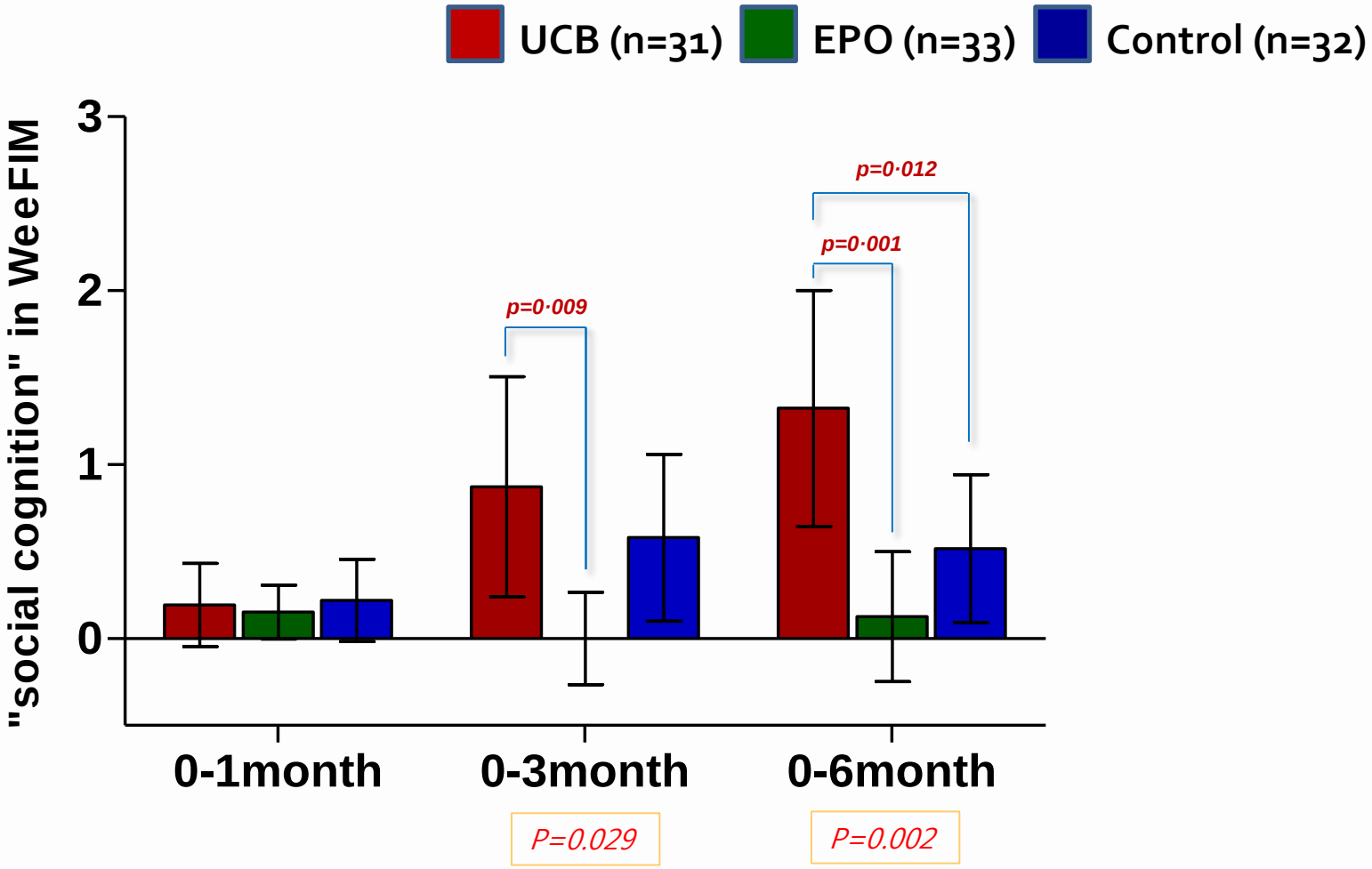
Changes of BSID-II Mental Scale Raw Score from Baseline to Each Visit



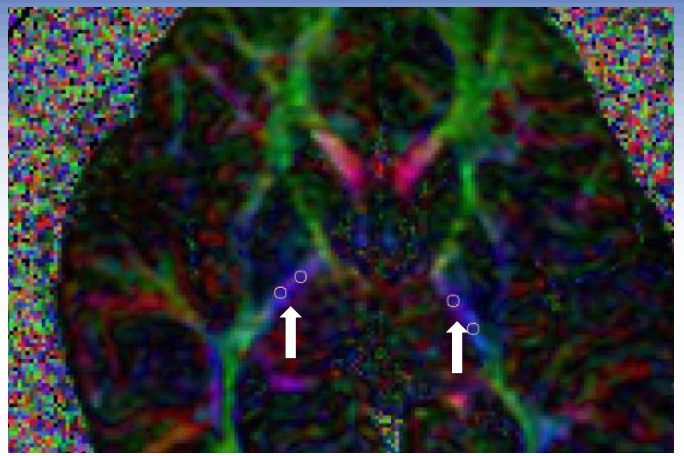
Changes of BSID-II Motor Scale Raw Score from Baseline to Each Visit

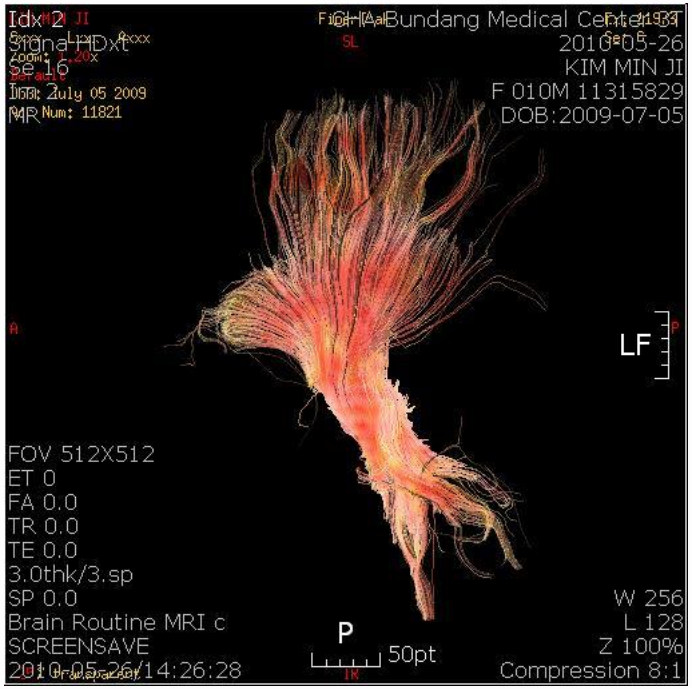


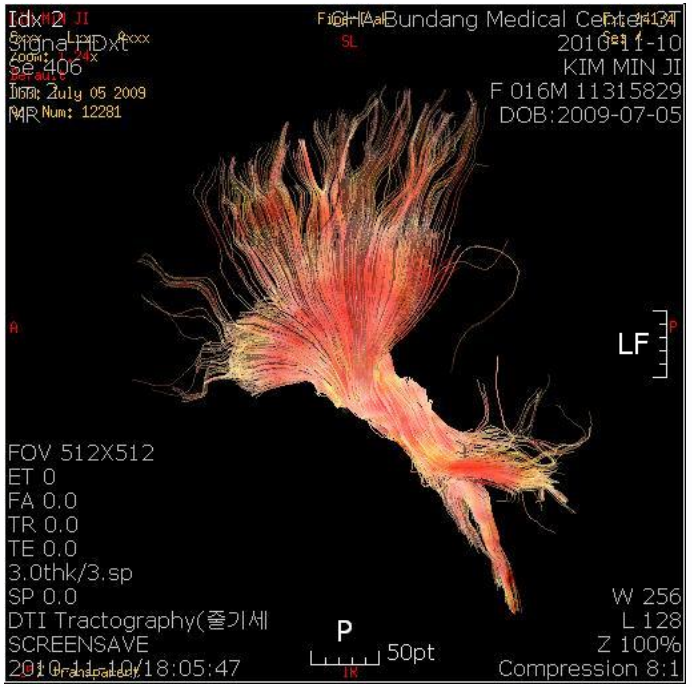
Changes of "social cognition" score in WeeFIM (Functional Independence Measure)



Changes in DTI
– a case in pUCB group

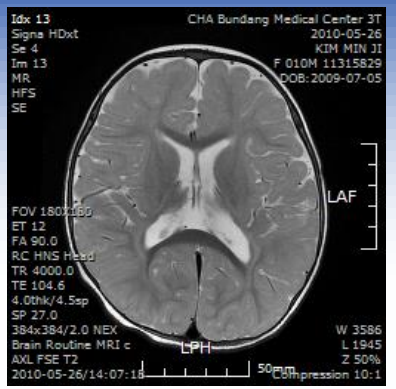






A case (F/11mo)

: Cerebral palsy due to periventricular leukomalacia



2 days before UCB administration



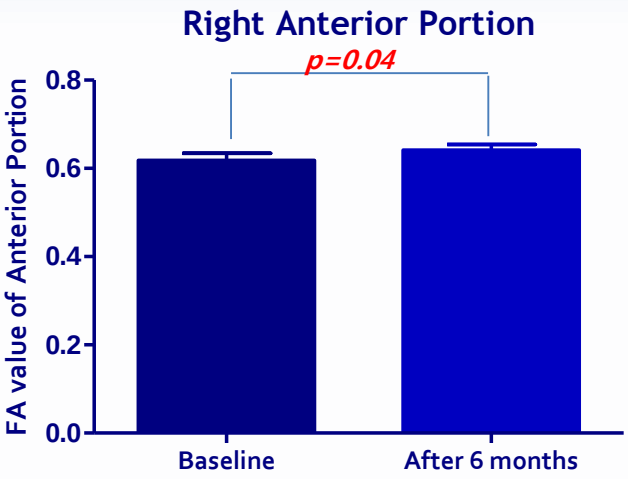
- She was unable to creep forward / severe irritability

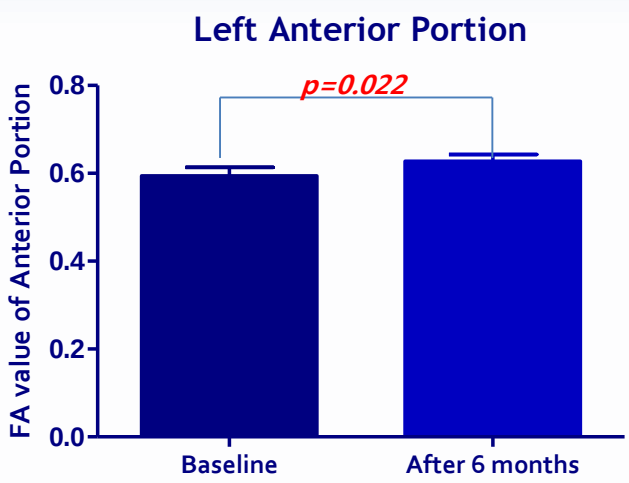
4 weeks after UCB administraion

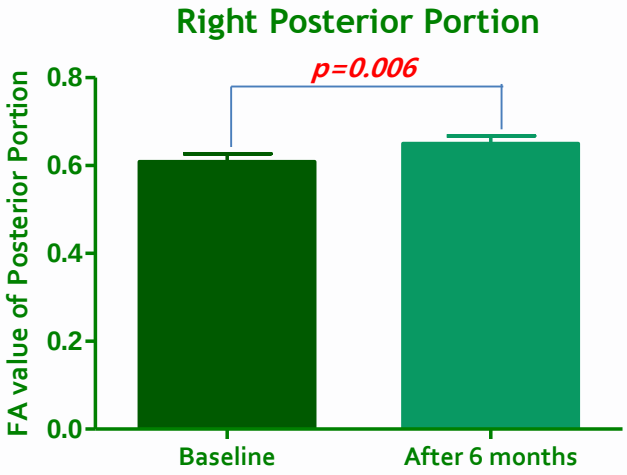


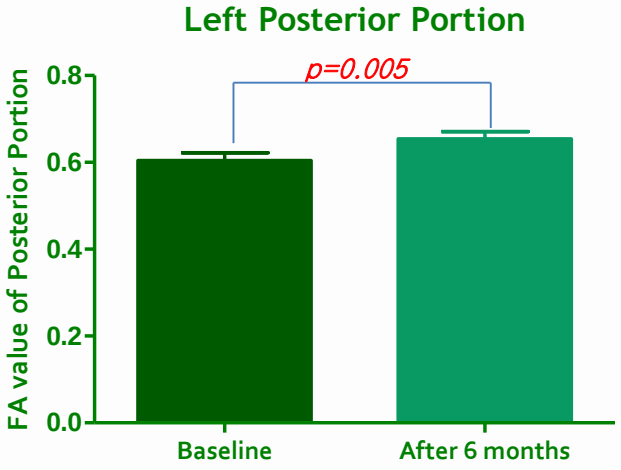
- She became able to creep forward / disappearance of irritability

Changes of FA value in 4 Portions of Posterior Internal Capsule in UCB Group (n=30)

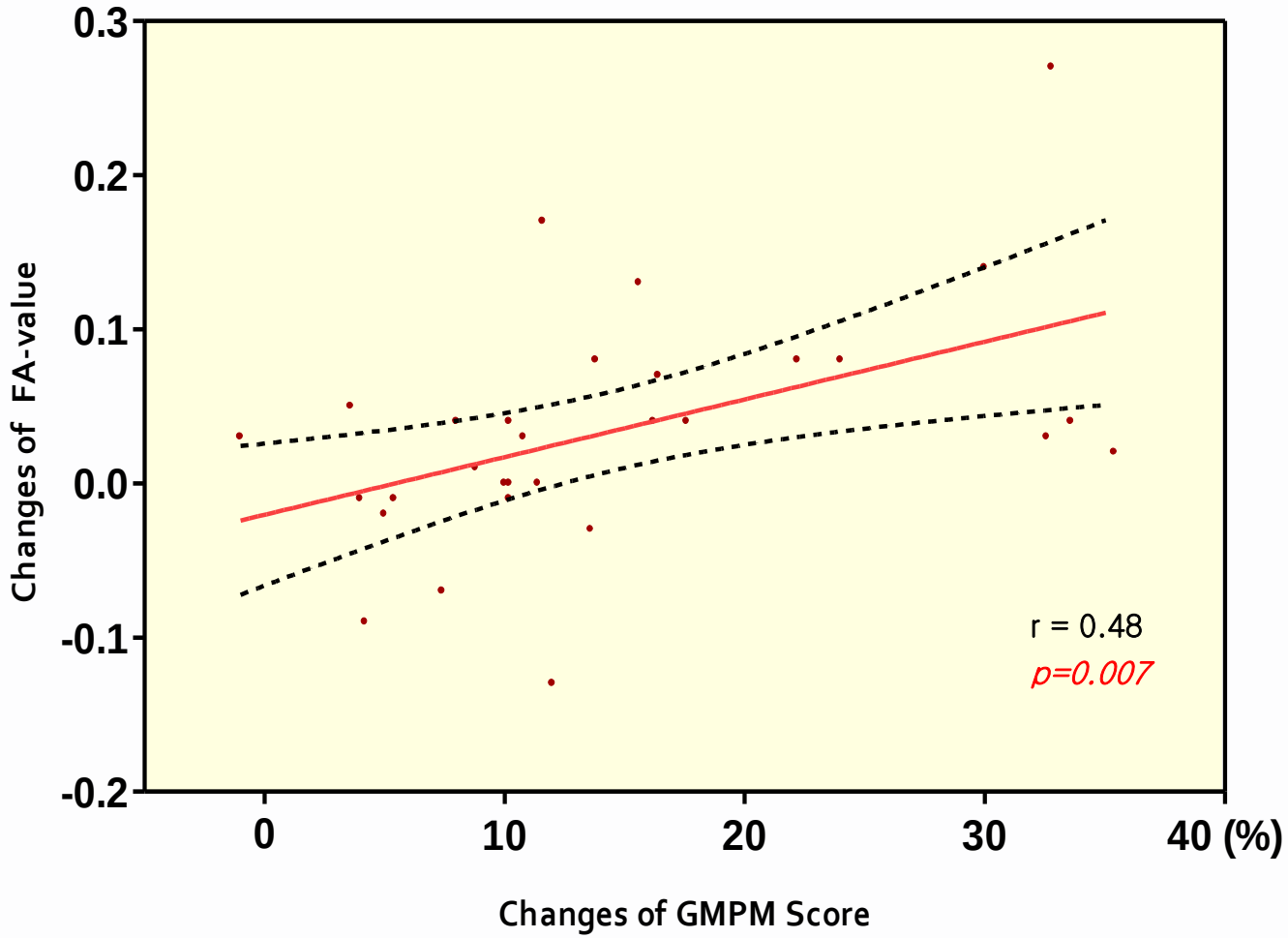




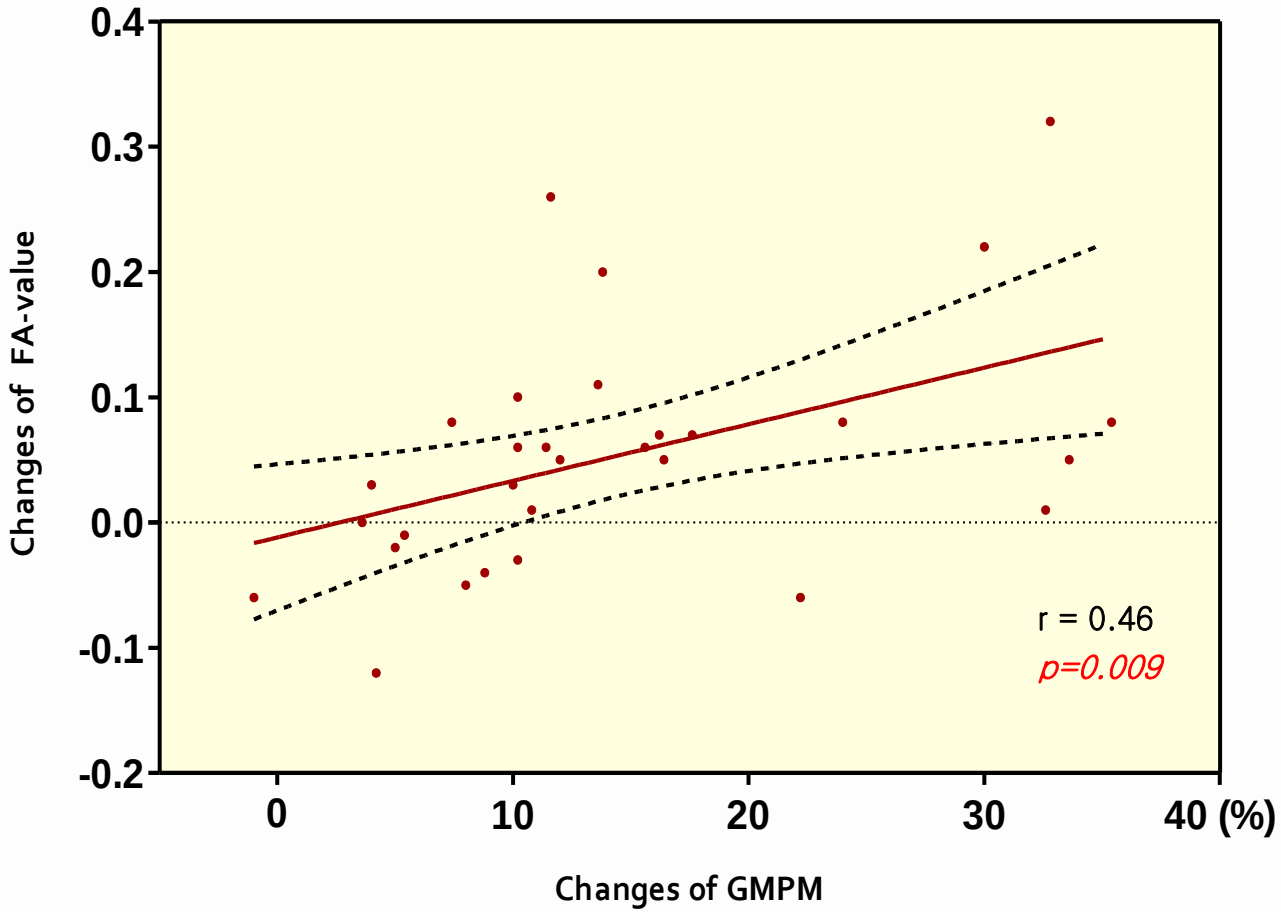




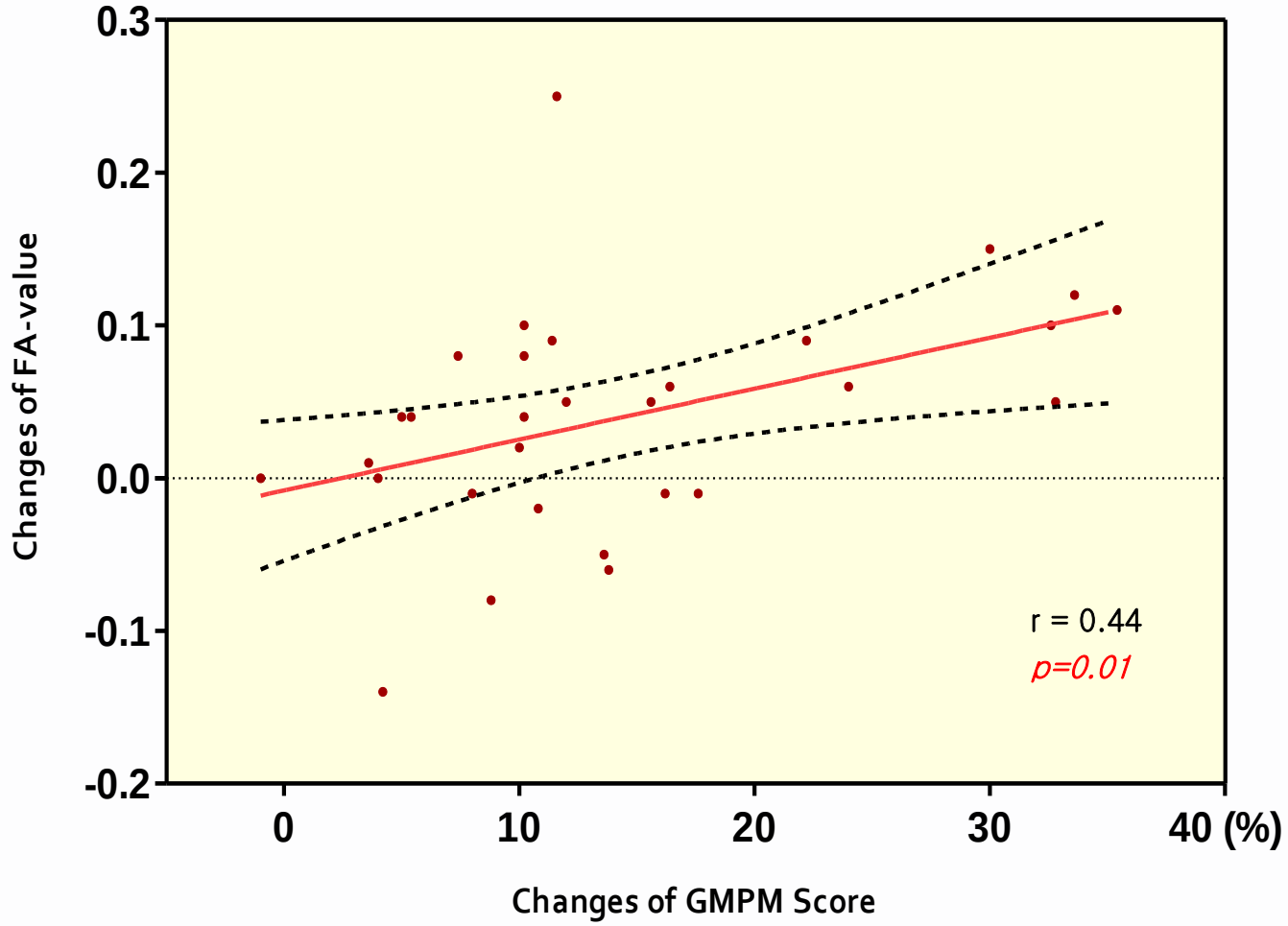
Correlation of Changes of GMPM % Score and Changes of FA value in Ant. Portion of Left Posterior Internal Capsule in UCB Group (n=30)



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

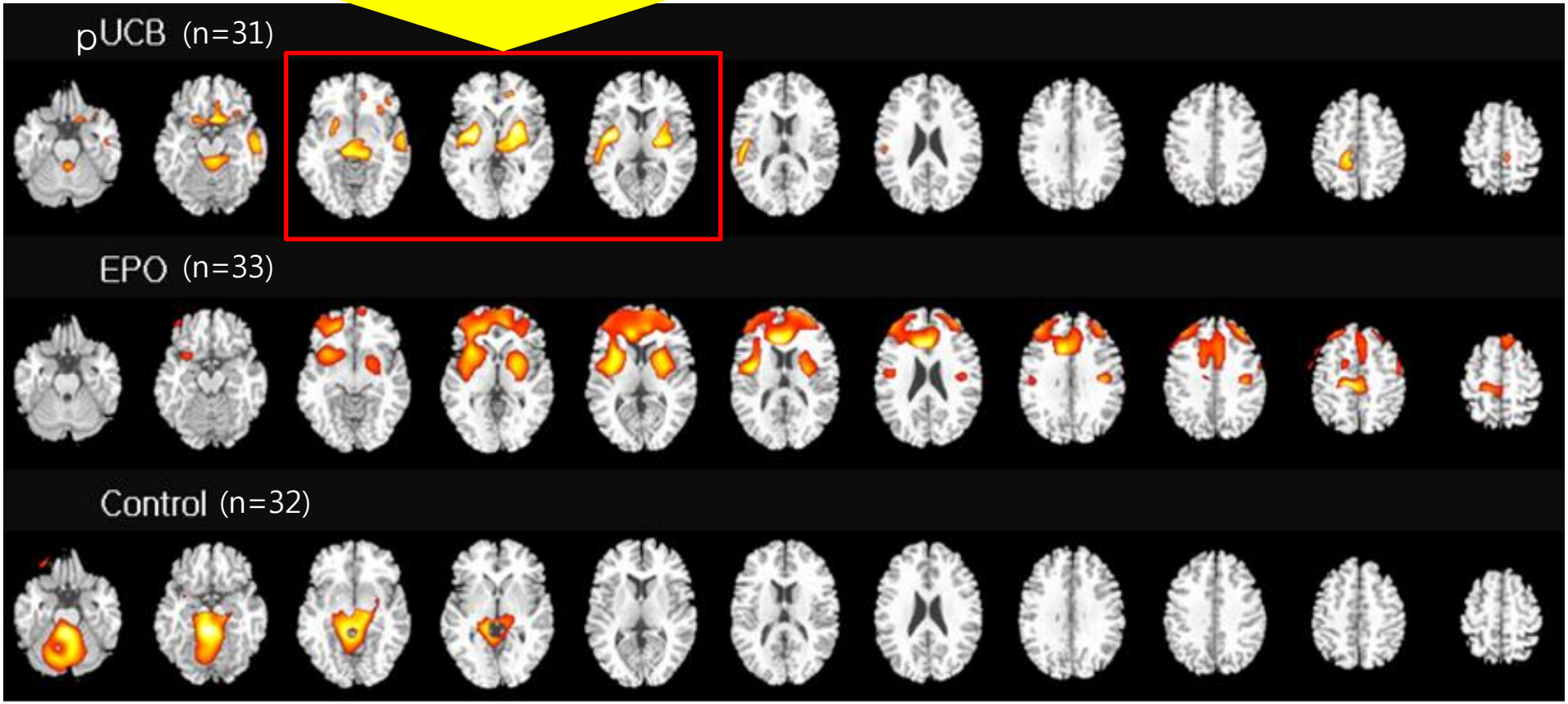


Correlation of Changes of GMPM % Score and Changes of FA value in Post. Portion of Right Posterior Internal Capsule in UCB Group (n=30)



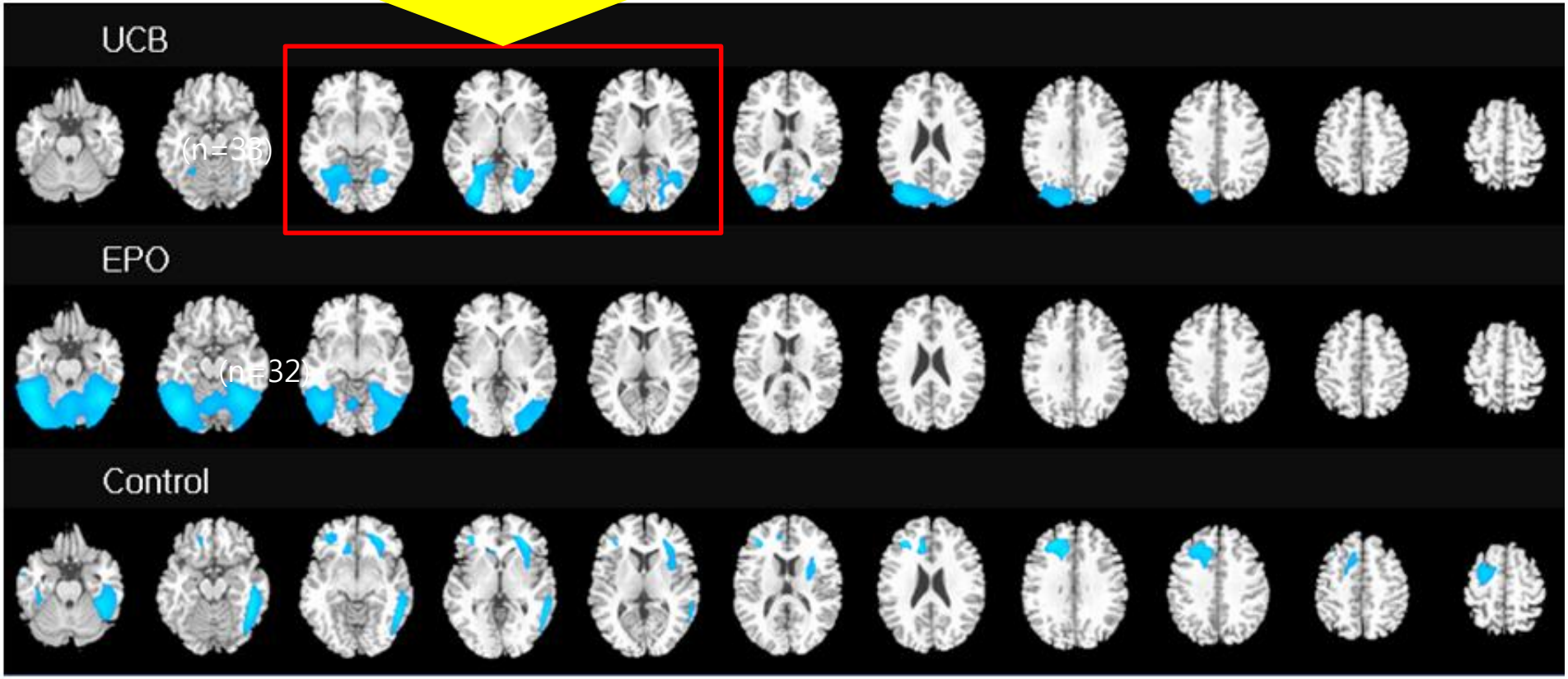
Difference of Activated Areas in Each Group with Brain FDG-PET

Effect on brain networking



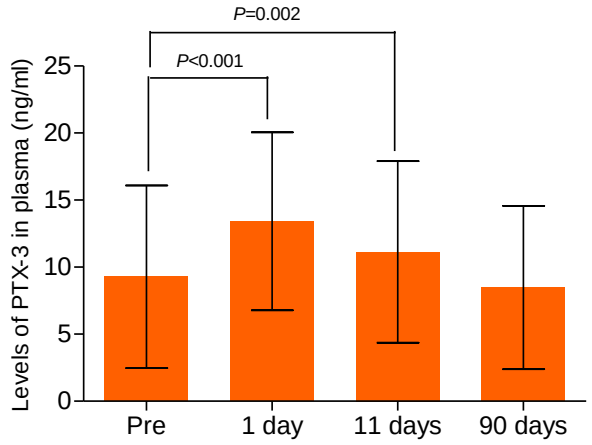
Difference of Deactivated Areas in Each Group with Brain FDG-PET

Anti-inflammatory effect



Need of Mechanism Study

- Selection of interesting molecules
  - Results from our preliminary studies
  - Review of previous researches
- Cytokines
  - Pentraxin 3 (PTX3)
  - Interleukin (IL)-8, IL-10
- Receptors
  - Toll-like receptor (TLR) 4, TLR 2
  - Mammalian target of rapamycin (mTOR)
- Changes in inflammatory status of brain tissue using ^{18}F-FDG-PET



Pentraxin-3 (PTX-3) level was elevated at 1 and 11 days post-administration

Second 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 therapeutic mechanism: assay of relevant cytokines and cell receptors

Design: double-blind RCT

Subject Enrollement Criteria

Indications

- Cerebral palsy
- Age: 6 months to 20 years (mostly under 4)
- Gross Motor Function Classification System: I, II, III, IV, V



Exclusion

- Medically unstable due to pneumonia or renal dysfunction at enrollment
- Known genetic syndrome
- Hypersensitivity reaction to study drugs
- Clinically uncontrolled epilepsy
- Lack of family support

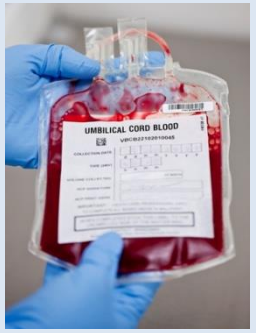
2nd Clinical Trial

UCB

Allogeneic UCB

- Matched for at least 4 out of 6 HLA-A, B, and DR
- Total nucleated cell (TNC) ≥ $2x10^7$/kg
- Multiple units can be used

  if single unit is not sufficient to the criteria of TNC.



Route: intravenous (IV) or intra-arterial (IA)

- IA was considered: age ≥ 4 years old and TNC < $6x10^7$/kg

Placebo

Cyclosporine

- For allogeneic UCB, survival of infused UCB cells ↑, Graft versus Host Disease ↓
- 2 mg/kg twice IV from 12 hours pre UCB therapy
- 1 ~ 2 mg/kg twice per day for the next 3 days post UCB therapy
- Oral solution was used for the next 9 days
- Target range of drug level: 100 ~ 200 ng/ml

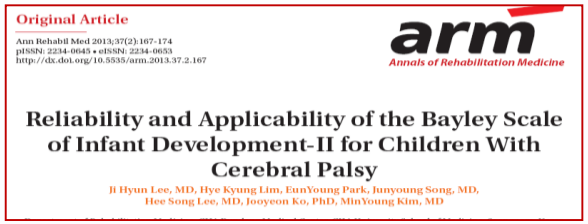
Outcome Measurements

- 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



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

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

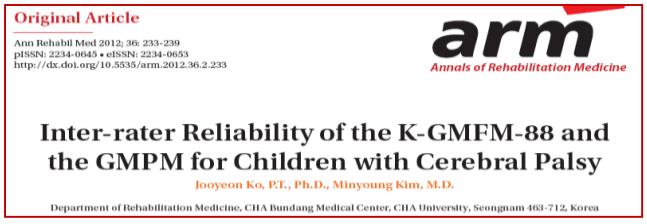

Original Article

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


Original Article

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

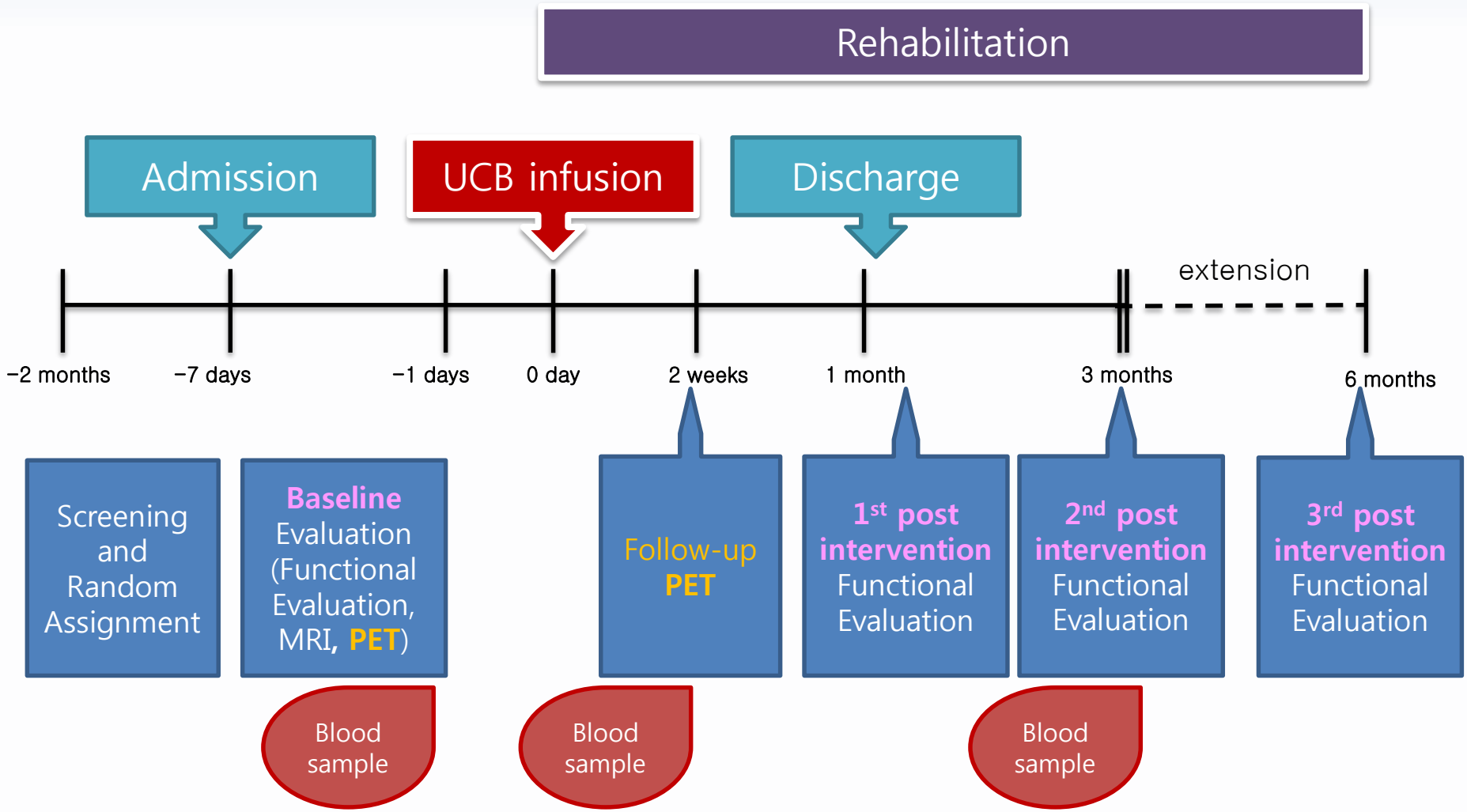
Jooyeon Ko, P.T., Ph.D., Minyoung Kim, M.D.

Department of Rehabilitation Medicine, CHA Bundang Medical Center, CHA University, Seongnam 463-712, Korea

Outcome Measurements

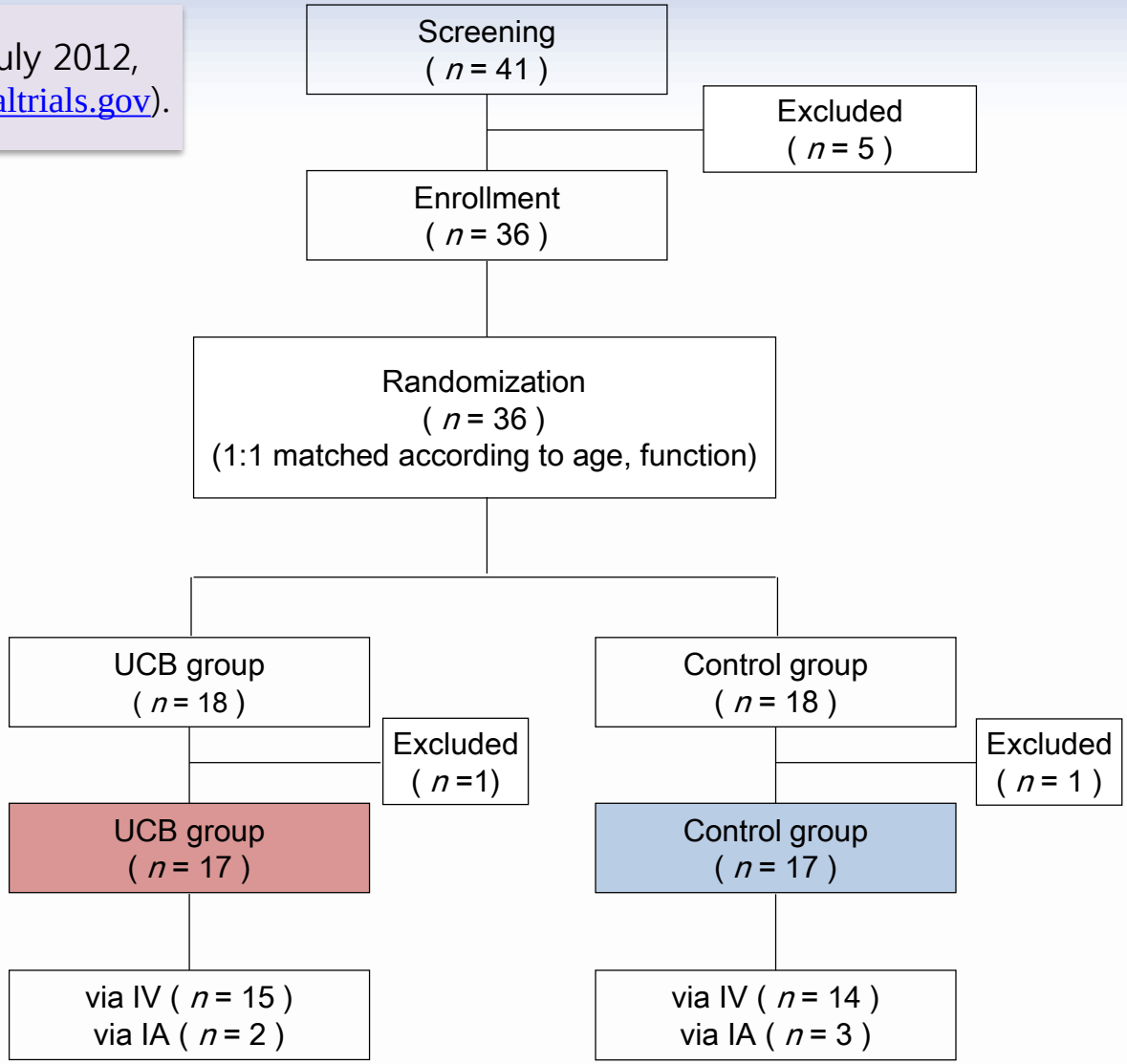
- **Cytokines** in peripheral blood: Inflammation related ones
- **Receptor assay** measured with Bradford assay
  - TLR-2, TLR-4
  - mTOR
- Brain ^{18}F-FDG-PET
  - analyzed using SPM3 implanted in MatLab R2011a
  - paired t-test statistics
  - voxels with an uncorrected p-value <0.05

Timeline


Rehabilitation
Admission
UCB infusion
Discharge
extension
−2 months
−7 days
−1 days
0 day
2 weeks
1 month
3 months
6 months
Screening and Random Assignment
Baseline Evaluation (Functional Evaluation, MRI, PET)
Follow-up PET
1st post intervention Functional Evaluation
2nd post intervention Functional Evaluation
3rd post intervention Functional Evaluation
Blood sample
Blood sample
Blood sample

Results

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



Safety

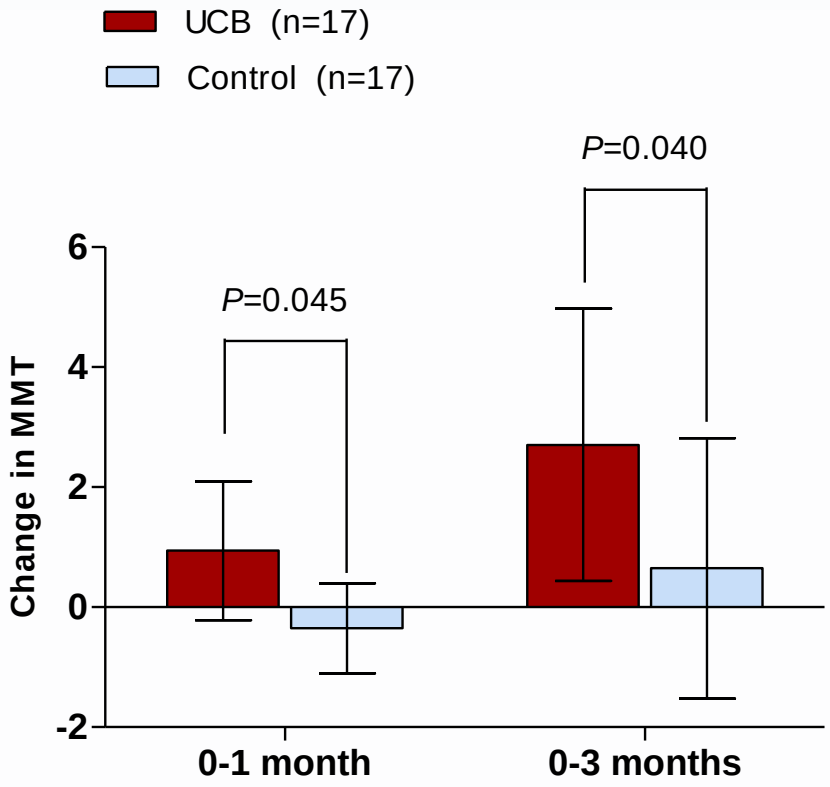
Group (n=36)	UCB (n=18)		Control (n=18)	
Number	Patient	Event	Patient	Event
Serious adverse events	0	0	0	0
Other adverse events				
Upper respiratory tract infection	6	7	4	6
Pyrexia	6	6	4	7
Vomiting	4	5	1	1
Nausea	2	2	4	6
Bronchitis	4	4	1	1
Pneumonia	2	2	2	3
Urticaria	4	4	2	2

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)

Comparison of Motor Outcome between UCB and Control group

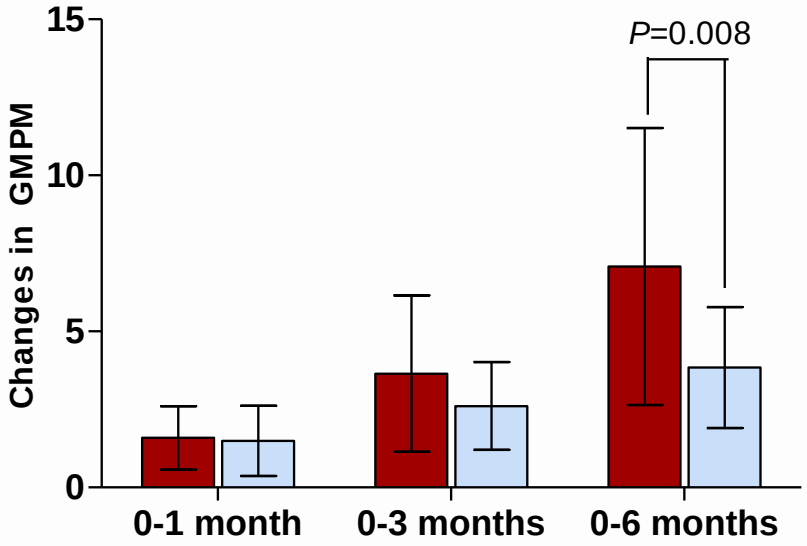
(Kang et al. 2015, Stem Cells Dev)

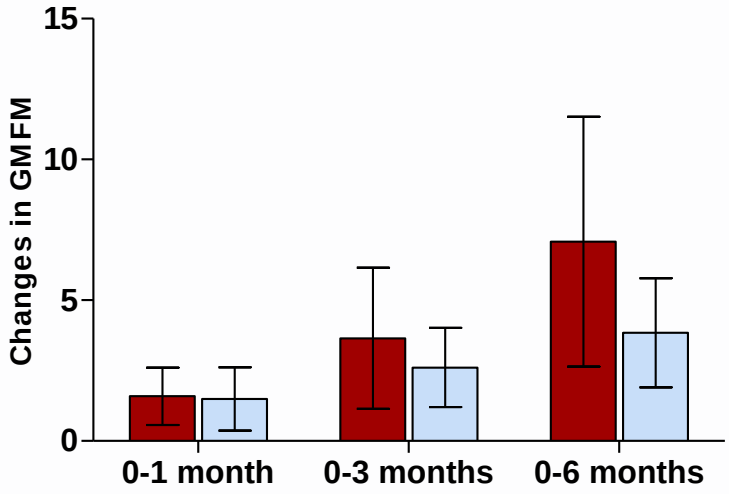


- Changes in **muscle strength** with manual muscle test score showed **efficacy of UCB**

Comparison of Motor Outcome between UCB and Control group

(Kang et al. 2015, Stem Cells Dev)





• Changes in **gross motor function** with gross motor performance measure score showed **efficacy of UCB**

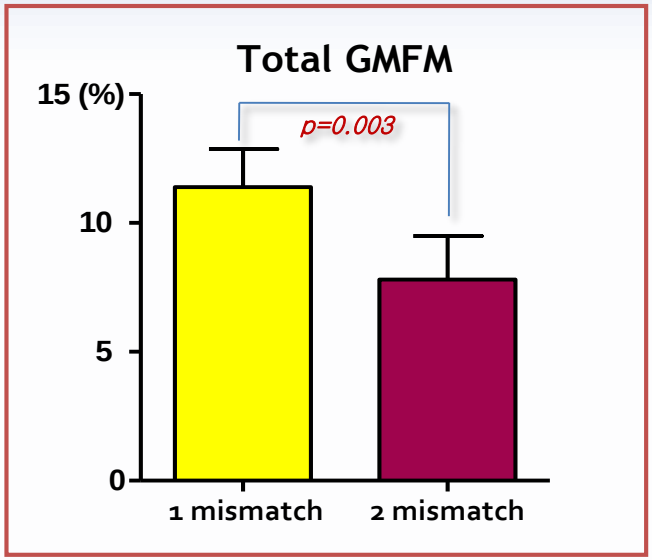
EFFICACY RELATED FACTORS

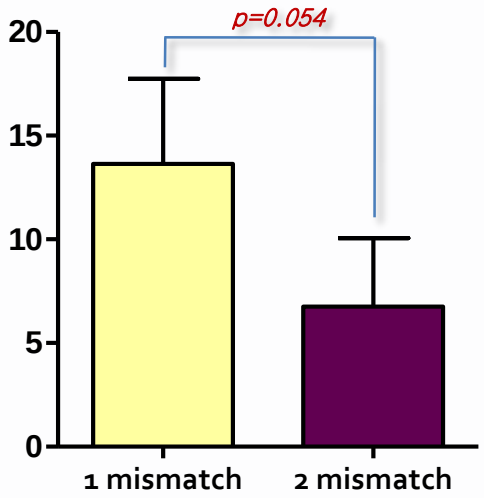
- HLA matching
- Total cell number
- CD 34+ cell number
- Innate immune response

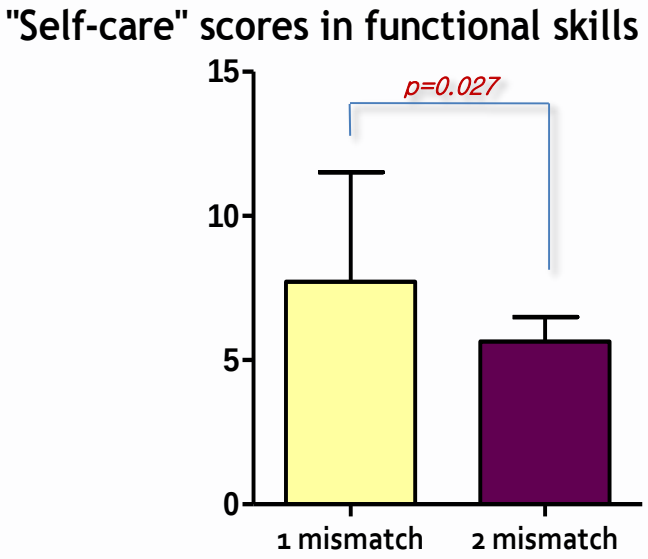
1st Clinical Trial

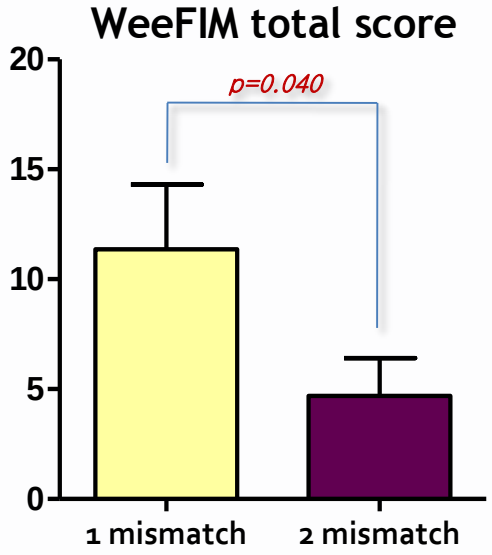
(Min et al., 2013, Stem Cells)

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









Correlation of Clinical Outcomes and HLA Matching in UCB Group

- 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

1st Clinical Trial

(Min et al., 2013, Stem Cells)

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

TNC number/kg of body weight		> 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 of body weight		**> 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

Values are mean (SE).

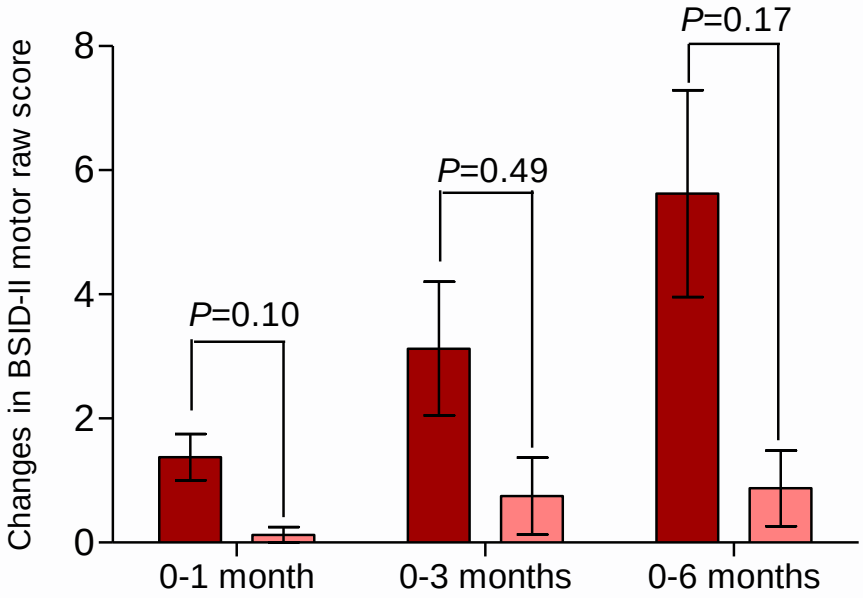
GMFM denotes Gross Motor Performance Measure; GMPM, Gross Motor Performance Measure; BSID-II, Bayley scales of infant development, 2nd edition

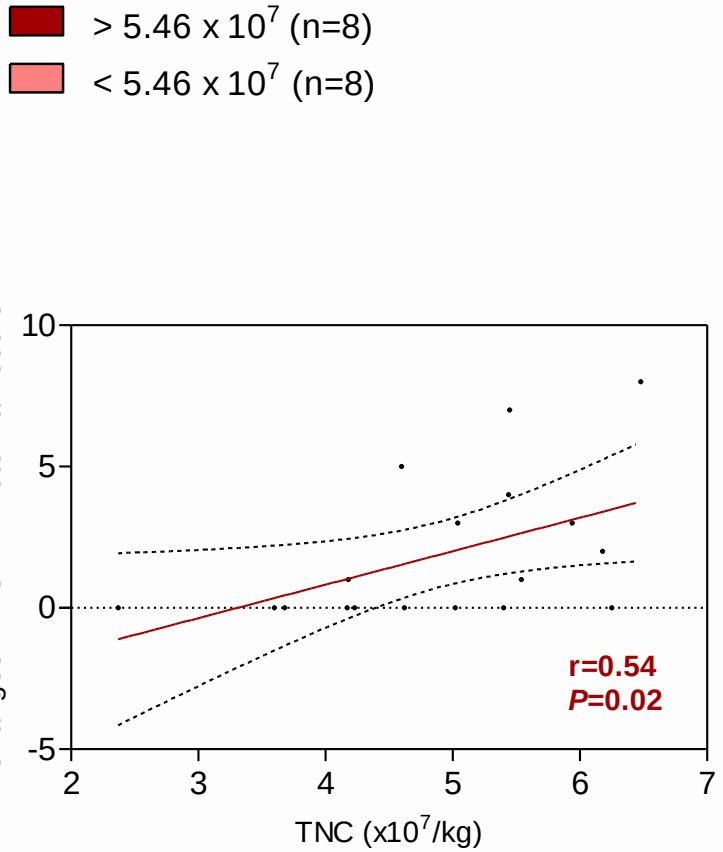
For TNC/kg, 6.69 x 10^7 was median value and for CD34+ cell numbers/kg, 1.46 x 10^5 was median value.

pUCB group received umbilical cord blood potentiated with recombinant human erythropoietin and rehabilitation.

* *p*-values are reported for difference between two groups, based on Mann-Whitney analysis.

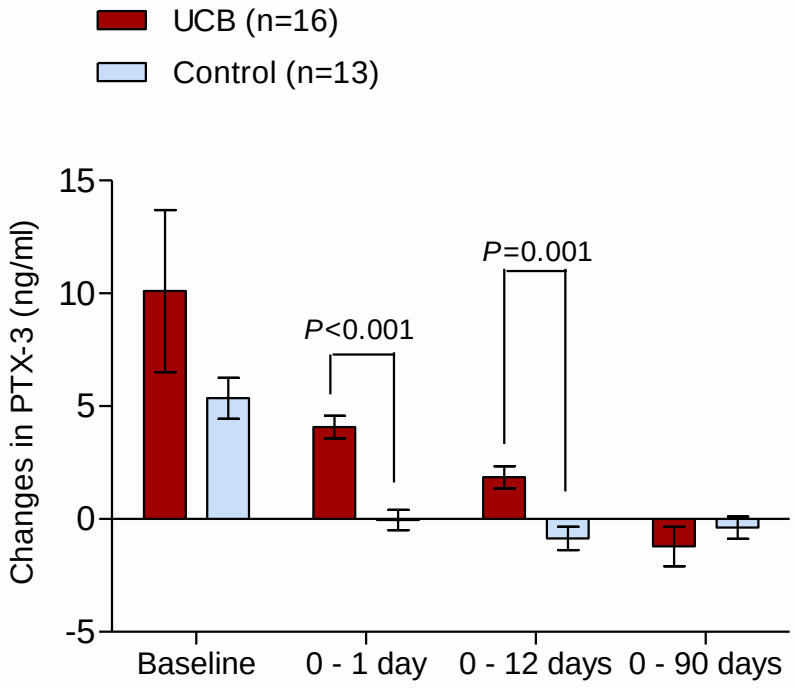
Correlation of Clinical Outcomes and Cell Number (TNC) in UCB Group

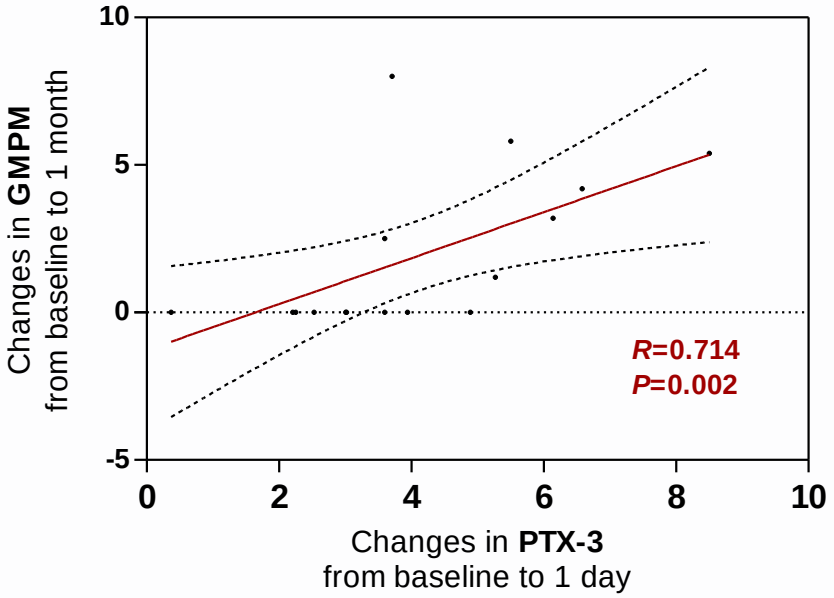




Changes in PTX-3 from baseline to 1, 12, and 90 days and its Correlation with Motor Outcome in UCB group

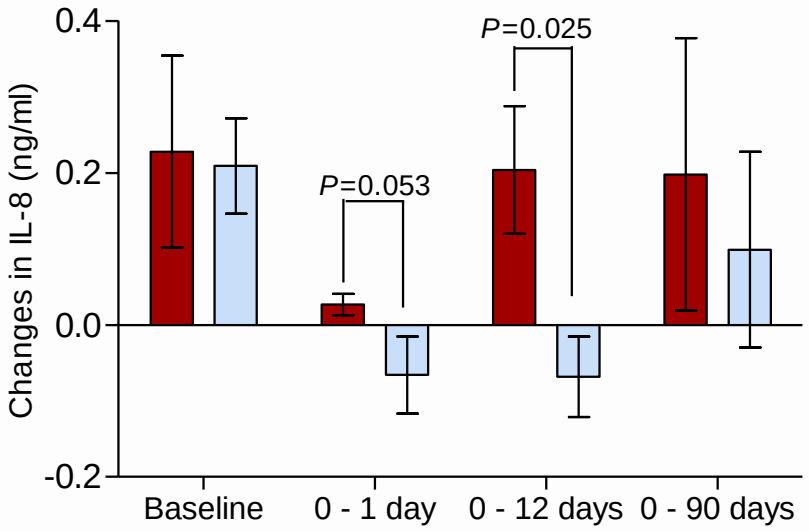
(Kang et al. 2015, Stem Cells Dev)

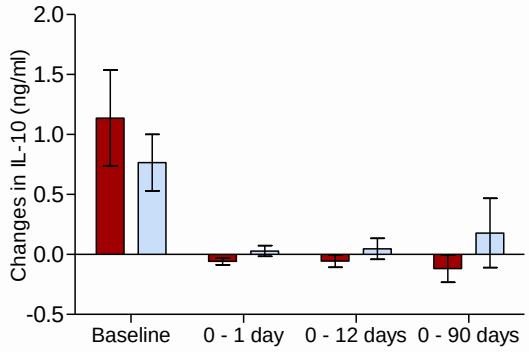


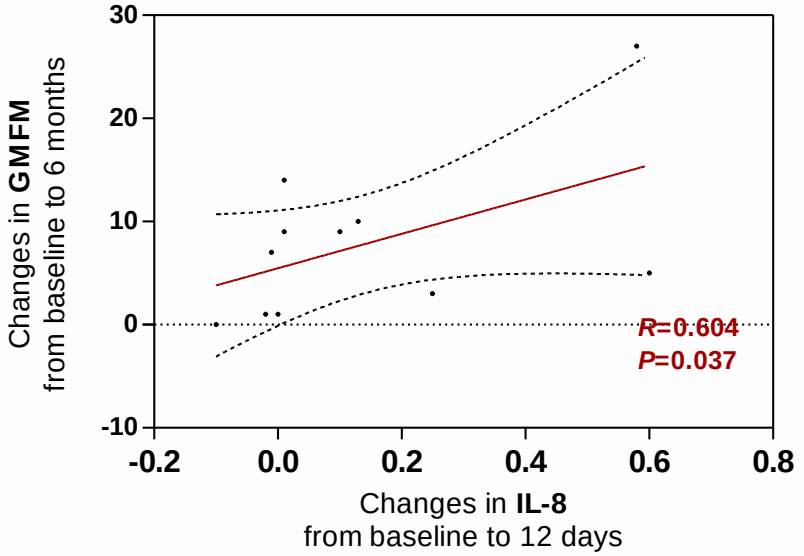


Changes in IL-8 from baseline to 1, 12, and 90 days and its Correlation with Motor Outcome

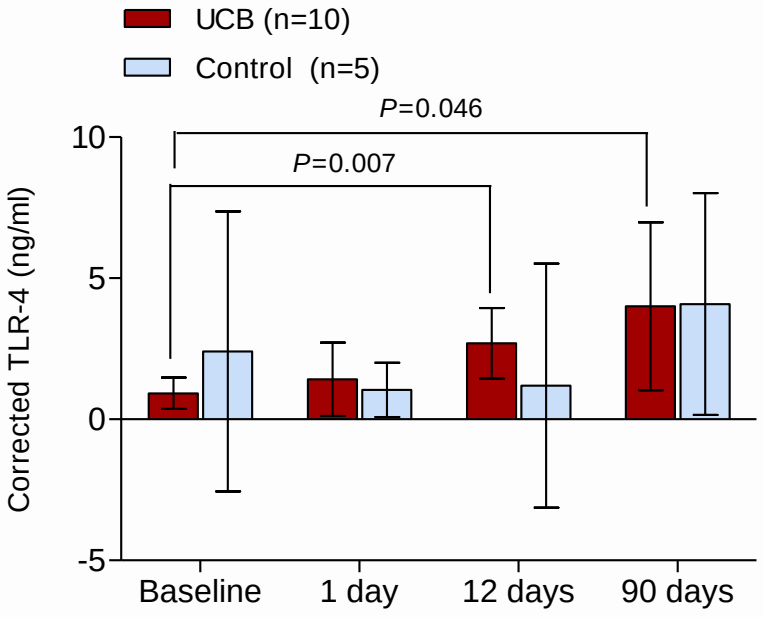
(Kang et al. 2015, Stem Cells Dev)

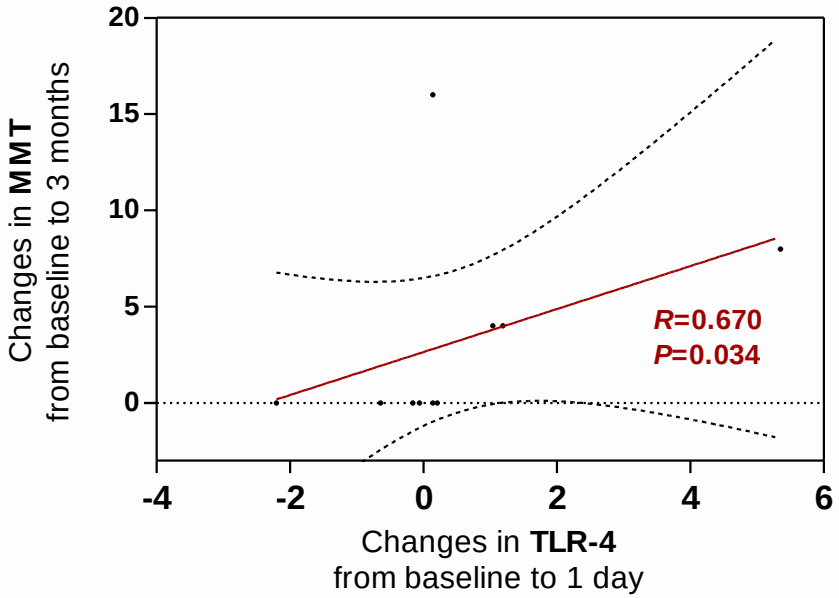






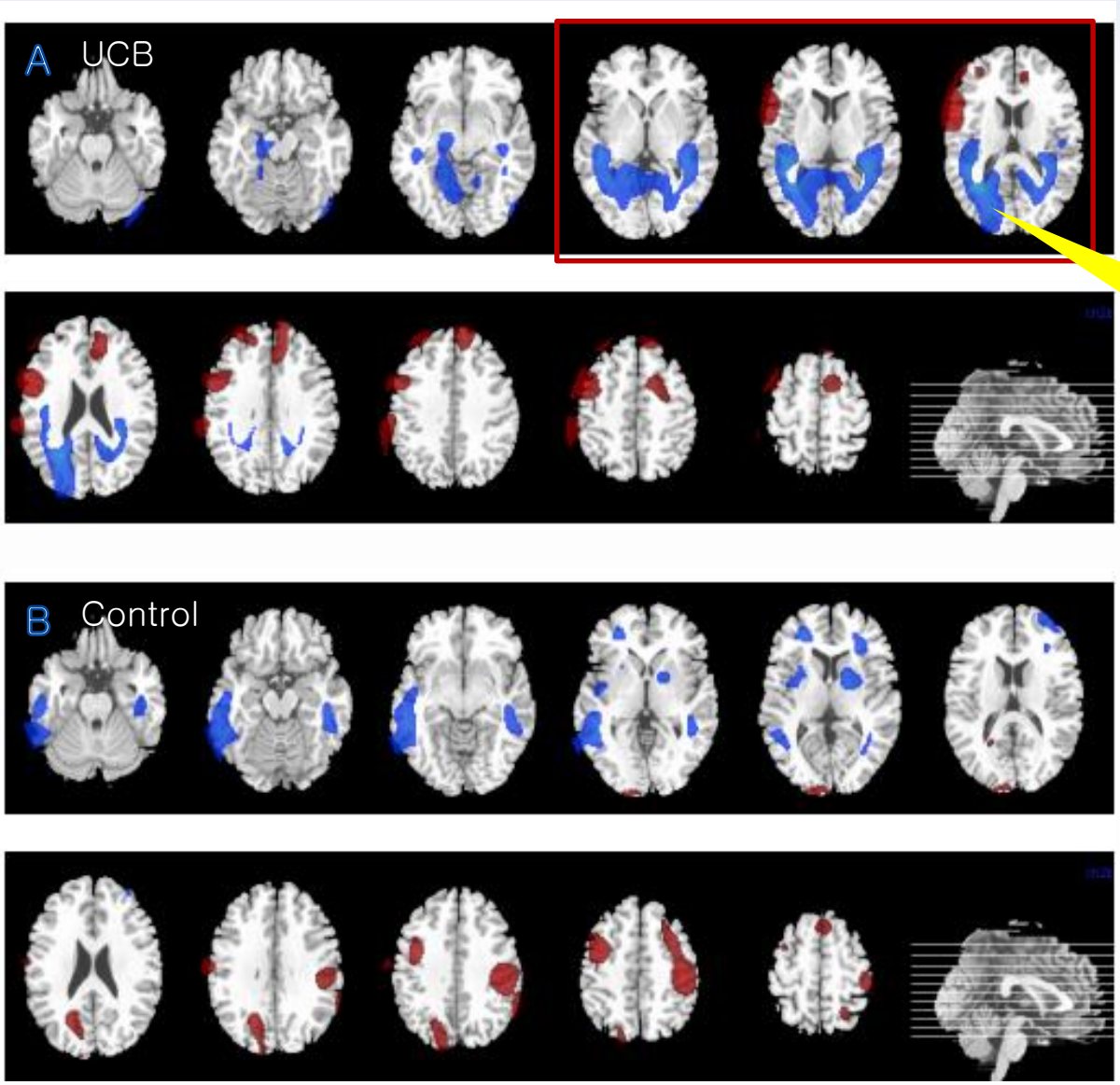
Increment in TLR-4 Expression in UCB Group





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

(Kang et al. 2015, Stem Cells Dev)



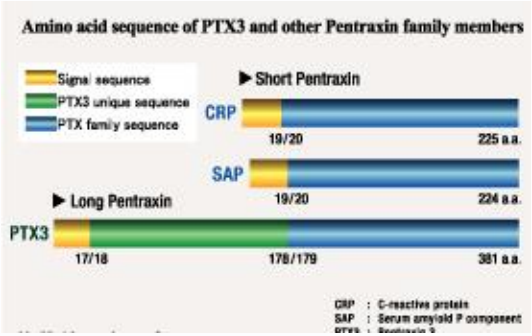
Again, **anti-inflammatory effect** in periventricular area, the most inflammatory region

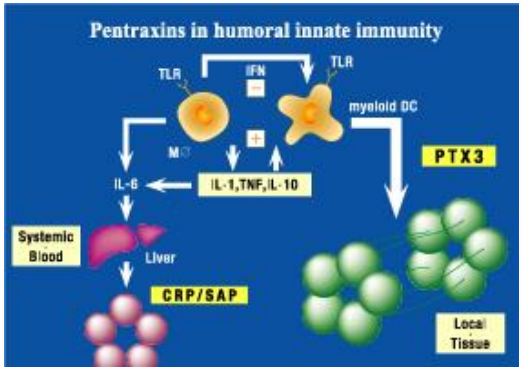
Glucose metabolism
Red color: increased activity
Blue color: decreased activity
(P-value < 0.05)

Discussion for mechanism

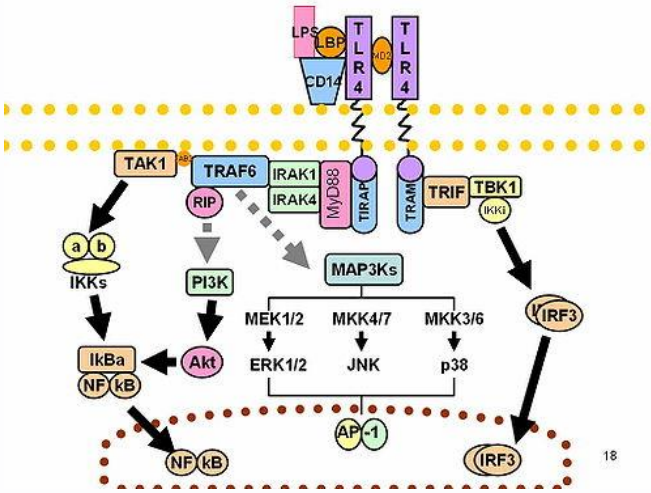
- Innate immunity

- Brief stimulation of innate immunity is related to the therapeutic efficacy
- PTX-3 (fluid-phase soluble)





- Toll-like Receptors (TLRs: cell associated receptors)



- mTOR orchestrates innate immune cells

- Cytoprotection, wound healing, and modulation of inflammation

Discussion for mechanism

- Anti-inflmmation

- Resultant responses in the brain tissue was anti-inflammatory effect as shown in brain PET study
- Nevertheless, future trials are needed to confirm the long-term efficacy and more direct therapeutic mechanism of UCB therapy for CP.

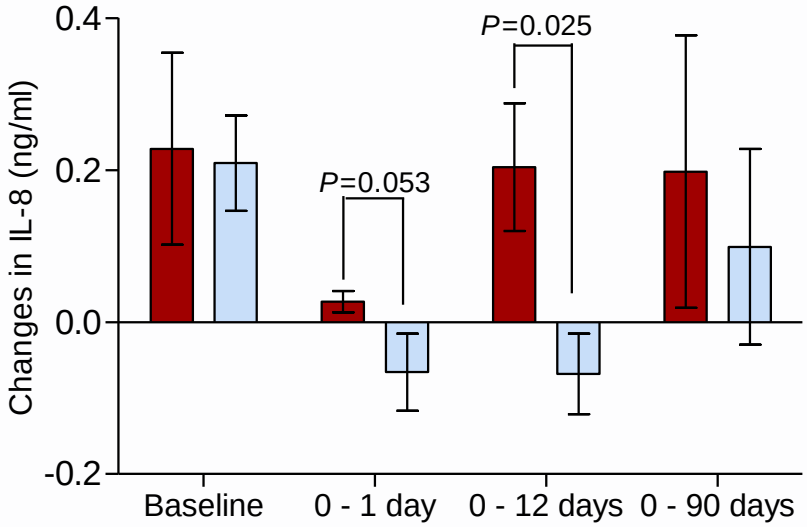
This study was published in Stem Cells Dev, 2015 (Kang et al.)

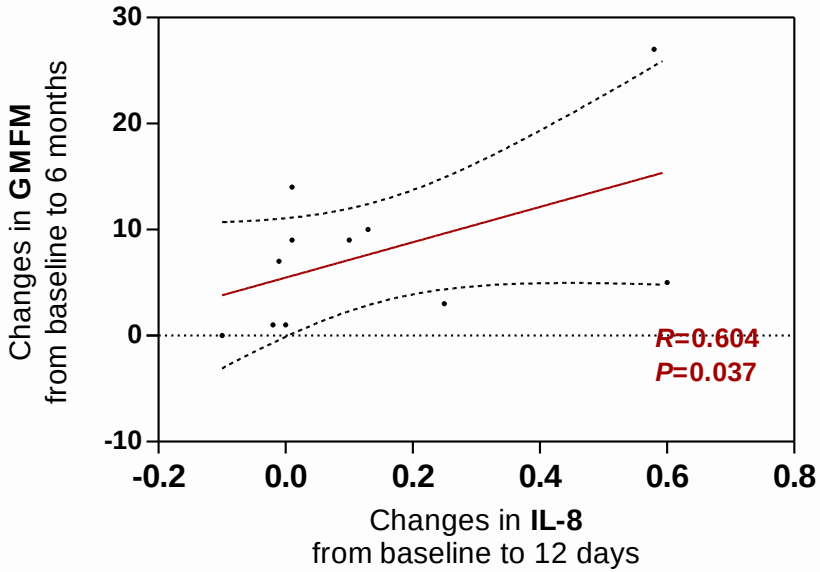
Acknowledgement: This study was supported by a grant of the Korean Health Industry Development Institute (HI3C1204).

Discussion for mechanism

- Association with clinical outcome and IL-8 elevation

(Kang et al. 2015, Stem Cells Dev)



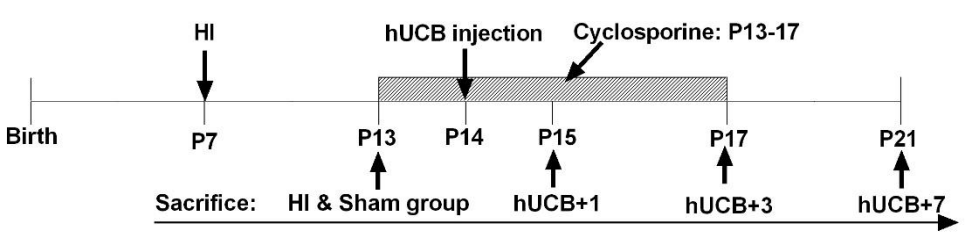


- IL-8 is known to be related with angiogenesis.
- In mouse, IL-8 encoding gene is deleted; instead of IL-8, Cxcl1 and Cxcl2 are homologues.

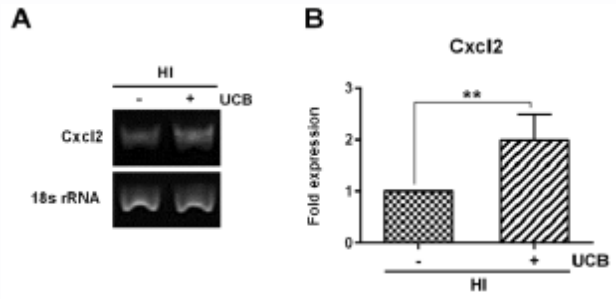
Further mechanism study

- Angiogenesis through IL-8 elevation as host response

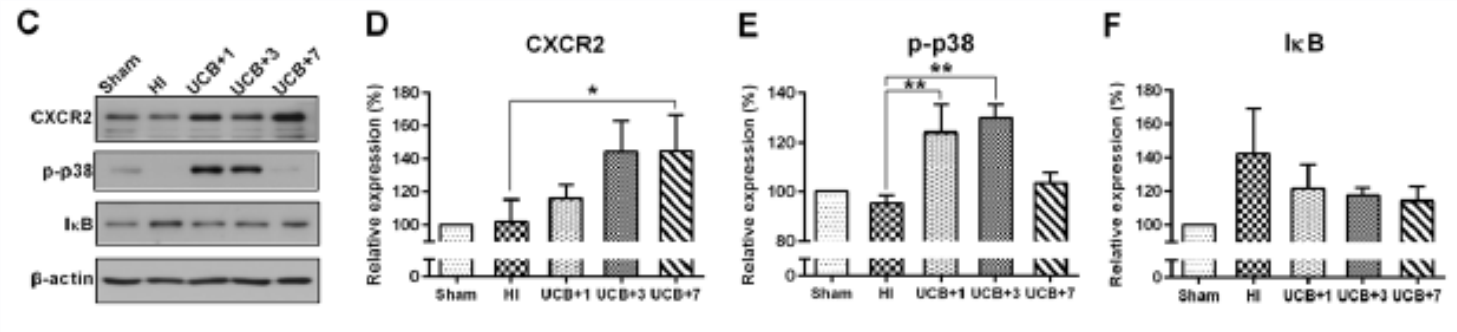
- CP model mouse: Hypoxic-ischemic brain injury
- Intraperitoneal UCB injection



- Upregulation of mouse Cxcl2 gene and CXCR2 in mouse CP brain tissue



RT-PCR



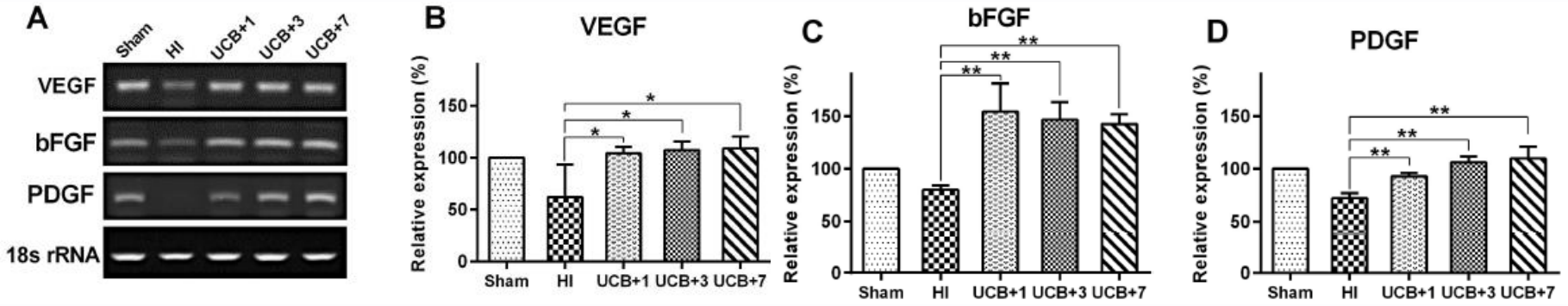
Western blotting

(Unpublished, in submission)

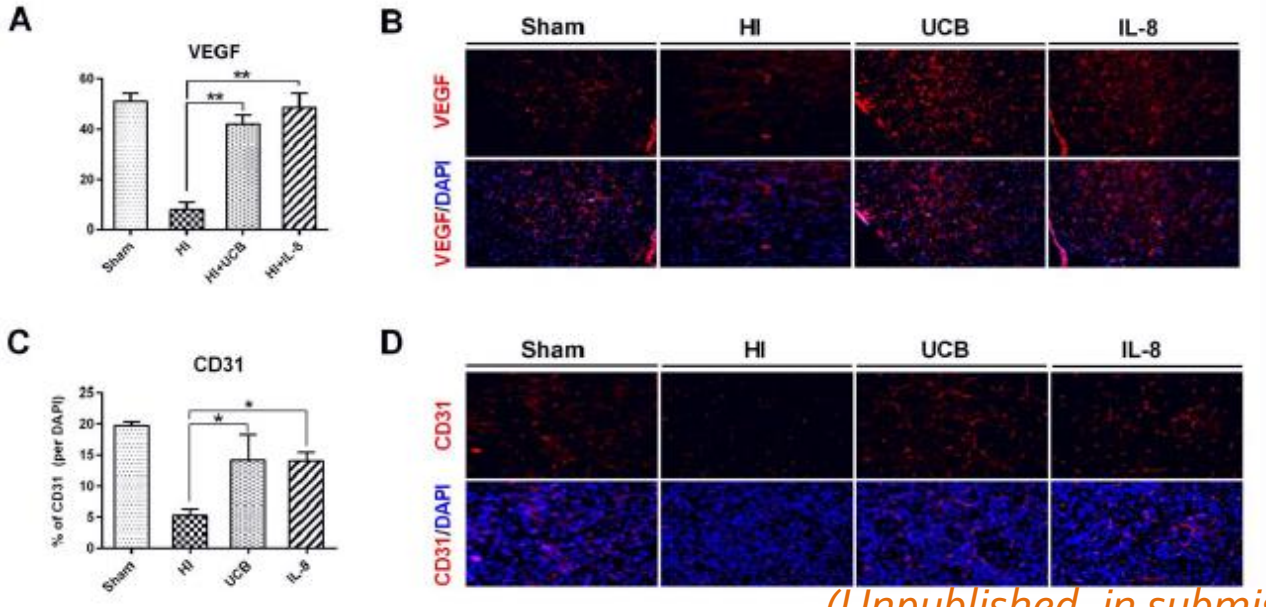
Further mechanism study

- Angiogenesis through IL-8 elevation as host response

- Upregulation of angiogenic genes



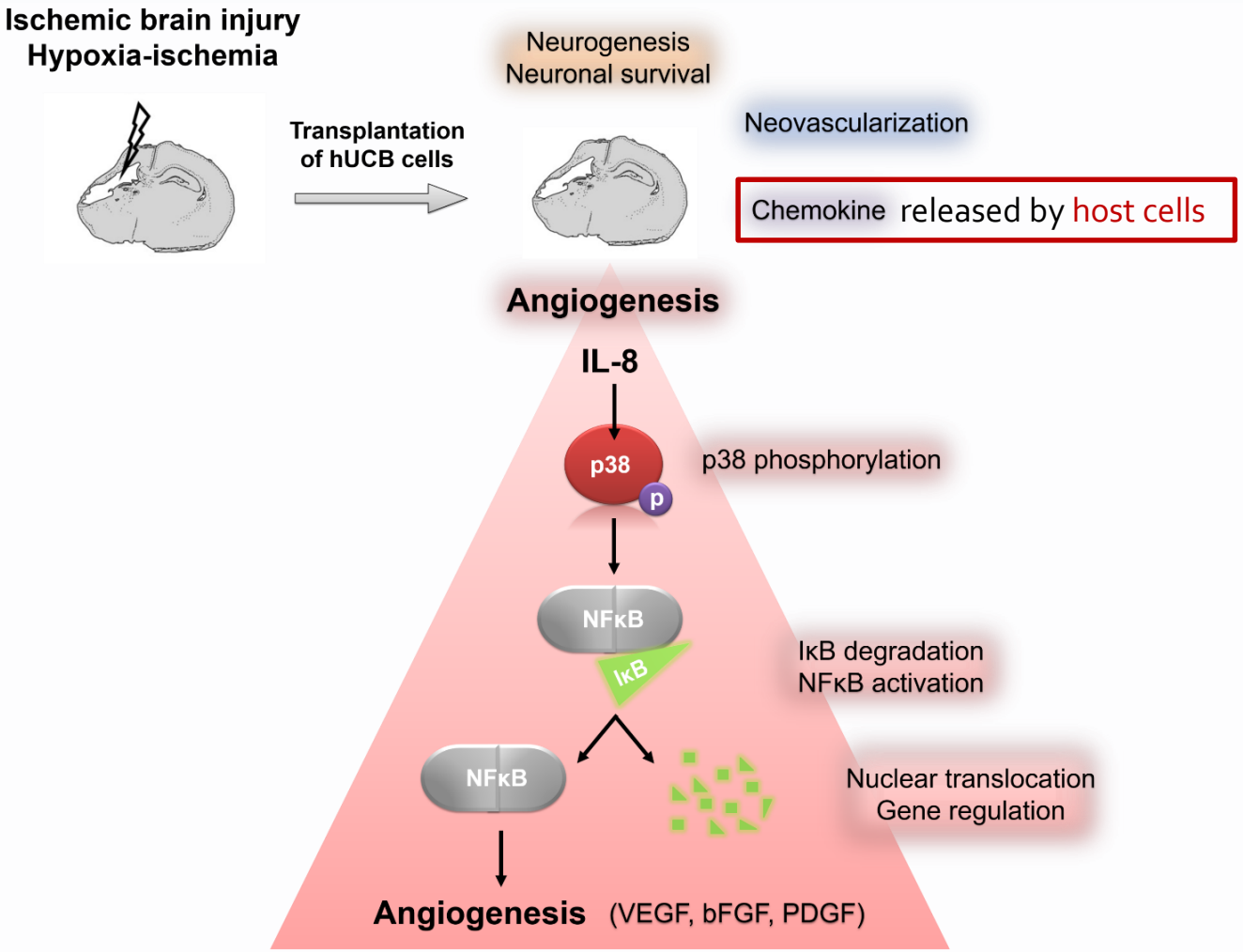
- Angiogenesis



(Unpublished, in submission)

Further mechanism study

- Angiogenesis through IL-8 elevation as host response



(Unpublished, in submission)

ACKNOWLEDGEMENT

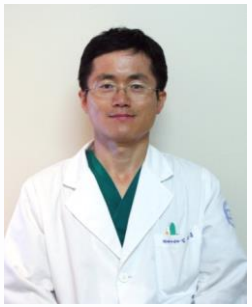
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Supporter/Collaborators

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- Prof. SeongSoo An, Gacheon University











Thank you for listening!

