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
The curved solid lines indicate average performance. The horizontal dotted lines on the right of the figures indicate the band expected to encompass 50% of children's limits of development. The solid vertical lines indicate the average age-90. The dotted vertical lines indicate the bands expected to encompass 50% of age-90 values around the average. The absence of 50% bands in 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  
B: IL-6  
C: IL-1β

Kadhim, 2001 (Neurology)

Coagulative necrosis (early)  
Coagulative necrosis (early)  
Cystic PVL (late)
Difficult to be recovered from accentuated inflammatory response in immature brain injury over existing brain lesion

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

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

Olaf Dammann¹,²,³ (dammann.olaf@mh-hannover.de)
Tertiary Brain Damage in Cerebral Palsy

**Pharmacological drugs**
- Erythropoietin
- G-CSF, GM-CSF
- Growth factors

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

**Solution**
- **Cell therapy**
  - Umbilical cord blood
  - Neural stem cell
  - Mesenchymal stem cell

**Others**
- Duke University (USA; NCT00593242)
- Medical College of Georgia (USA; NCT01072370)

**Fleiss, 2012 (Lancet Neurol)**
Umbilical Cord Blood (UCB) in Brain Injury

Background

- Neurogenesis
  - Decreased infarct size
  - Functional recovery
  - Paracrine effect

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

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

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

Neovascularization (Murohara, 2001)

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

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

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

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

Functional improvement by UCB in brain infarct (Chen, 2001)
The Efficacy of UCB in Animal Models

- **Source of stem cells**

<table>
<thead>
<tr>
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<th>USSC</th>
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<td>CD166</td>
<td>+</td>
<td>+</td>
<td>+</td>
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</tbody>
</table>

Lee, 2010

**Stroke**
- Vendrame M et al, Stroke 2004

**Cerebral hemorrhage**
- Nan Z et al, Ann N Y Acad 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**
### Advantages of UCB as a Source of Cell Therapy

<table>
<thead>
<tr>
<th>Safety</th>
<th>Availability</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Used more over two decades</td>
<td>• Off the shelf</td>
</tr>
<tr>
<td>• Hematologic, immunologic, oncologic disorders, or inborn errors of metabolism</td>
<td>• Cord blood banks</td>
</tr>
</tbody>
</table>

#### Immune tolerance
- Immaturity

- Tse, 2005; Vaziri, 1994; Lee, 2010

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#### Neurogenicity
- Differentiable to neuronal, astrocytic, & oligodendroglial 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.
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
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
**1st Clinical Trial**

**Umbilical Cord Blood (UCB) + Erythropoietin**

**Total 105 subjects**
(Recruit: May 2010 - Nov 2010)

**Groups**

- **Randomized**
- **Double-blind**

**Intervention**

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

**Time line**

- Admission
- UCB administration
- Discharge
- Basal Evaluation (Function, Brain MRI and DTI, Brain PET)
- follow-up PET
- 1month follow-up
- 3month follow-up
- 6month follow-up follow-up DTI

**UCB**

- Allogeneic UCB + Erythropoietin + Rehabilitation
- Placebo UCB + Erythropoietin + Rehabilitation
- Placebo UCB + Placebo EPO + Rehabilitation

**Control (n=34)**

**pUCB (n=35)**

**EPO (n=36)**

**STEM CELLS**

**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, Junyoung Song, MD, Jin Young Kang MD, Jooyeon Ko PT, PhD, Ju Seok Ryu Professor, MD, PhD, Myung Soo Kang Professor, MD, PhD, Su Jin Jang Professor, MD, Sang Heun Kim Professor, MD, Doyeun Oh Professor, MD, PhD, Moon Kyu Kim Professor, MD, PhD, Kim Sung Soo, Bio-statistician, PhD, Min Young Kim, MD, PhD,
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 Tardieu 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 uncorrected 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 (intra-class 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

BSID-II

Reliability and Applicability of the Bayley Scale of Infant Development-II for Children With Cerebral Palsy
Il Hyun Lee, MD, Hye Kyung Lim, EunYoung Park, Junyoung Song, MD, Hoo Song Lee, MD, Jooyeon Ko, PhD, MinYoung Kim, MD

(Ann Rehab Med, 2013)

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

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

Typology; SB: Spastic bilateral, SU: Spastic unilateral, D: Dystonia, C: Choreoathetosis, A: Ataxia (Bax, 2005)
# 1st Clinical Trial

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

<table>
<thead>
<tr>
<th>Event</th>
<th>pUCB (n=35)</th>
<th>EPO (n=36)</th>
<th>control (n=34)</th>
<th>p value†</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Number (percent)</td>
<td>Time of occurrences‡ (weeks post-treatment)</td>
<td>Number (percent)</td>
<td>Time of occurrences‡ (weeks post-treatment)</td>
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<tr>
<td><strong>Serious adverse events</strong></td>
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<td></td>
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<tr>
<td>Pneumonia</td>
<td>1 (2·9)</td>
<td>6~7</td>
<td>2 (5·6)</td>
<td>6<del>7, 18</del>19, 22~23</td>
</tr>
<tr>
<td>Seizure</td>
<td>0</td>
<td></td>
<td>1 (2·8)</td>
<td>16</td>
</tr>
<tr>
<td>Influenza</td>
<td>1 (2·9)</td>
<td>20</td>
<td>0</td>
<td>1 (2·9)</td>
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<tr>
<td>Urinary tract infection</td>
<td>0</td>
<td></td>
<td>0</td>
<td>1 (2·9)</td>
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<tr>
<td>Death</td>
<td>1 (2·9)</td>
<td>14</td>
<td>0</td>
<td>0</td>
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<tr>
<td><strong>Other adverse events</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Upper respiratory tract infection</td>
<td>18 (51·4)</td>
<td>0<del>5, 10</del>13, 23~24</td>
<td>19 (52·8)</td>
<td>0<del>5, 9</del>13, 19~25</td>
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<tr>
<td>Fever</td>
<td>12 (34·3)</td>
<td>0<del>6, 17</del>18, 21<del>22, 24</del>25</td>
<td>4 (11·1)</td>
<td>1~4</td>
</tr>
<tr>
<td>Dyspepsia</td>
<td>5 (14·3)</td>
<td>0~4</td>
<td>2 (5·6)</td>
<td>1~3</td>
</tr>
<tr>
<td>Loose stool, diarrhea</td>
<td>6 (17·1)</td>
<td>0~3</td>
<td>2 (5·6)</td>
<td>0~2</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>6 (17·1)</td>
<td>0<del>8, 20</del>22</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nausea, vomiting</td>
<td>6 (17·1)</td>
<td>0<del>4, 10</del>11</td>
<td>5 (13·9)</td>
<td>0~7</td>
</tr>
<tr>
<td>Anorexia</td>
<td>5 (14·3)</td>
<td>0~3</td>
<td>2 (5·6)</td>
<td>0~2</td>
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<td>Bronchitis</td>
<td>4 (11·4)</td>
<td>0~8</td>
<td>4 (11·1)</td>
<td>0~6</td>
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<td>Constipation</td>
<td>5 (14·3)</td>
<td>1~5</td>
<td>4 (11·1)</td>
<td>0<del>4, 15</del>16</td>
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<td>Irritability</td>
<td>4 (11·4)</td>
<td>0~2</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Hypoxia†</td>
<td>3 (8·6)</td>
<td>0</td>
<td>1 (2·8)</td>
<td>3</td>
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<td>Febrile convulsion</td>
<td>2 (5·7)</td>
<td>4, 17, 21</td>
<td>1 (2·8)</td>
<td>3</td>
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<td>Herpangina</td>
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<td>2 (5·6)</td>
<td>2~4</td>
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<td>Urticaria</td>
<td>2 (5·7)</td>
<td>0<del>1, 3</del>4</td>
<td>1 (2·8)</td>
<td>3~4</td>
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<td>Hirsuitism</td>
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<td>3~26</td>
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<td>0</td>
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<tr>
<td>Seizure</td>
<td>1 (2·9)</td>
<td>4</td>
<td>3 (8·3)</td>
<td>0, 8, 16, 18, 22, 23</td>
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<tr>
<td>Alopecia</td>
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<td>1~3</td>
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<td>Otitis media acute</td>
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<td>1 (2·8)</td>
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<td>0~1</td>
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<td>Colitis</td>
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<td>1 (2·8)</td>
<td>6~7</td>
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<tr>
<td>Dermatitis</td>
<td>0</td>
<td></td>
<td>2 (5·6)</td>
<td>0~3</td>
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<tr>
<td>Insomnia</td>
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<td>Conjunctival injection</td>
<td>0</td>
<td></td>
<td>1 (2·8)</td>
<td>3~4</td>
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</tbody>
</table>
Changes of GMPM % Score from Baseline to Each Visit

- pUCB (n=31)
- EPO (n=33)
- Control (n=32)

1 month - baseline: p=0.018
3 months - baseline: p=0.046
6 months - baseline: p=0.005

1st Clinical Trial
Changes of BSID-II Mental Scale Raw Score from Baseline to Each Visit

- **pUCB (n=31)**
- **EPO (n=33)**
- **Control (n=32)**

**1 month**
- p<0.001

**3 months**
- p<0.001
- p<0.001

**6 months**
- p=0.001
- p<0.001
- p=0.007

**1st Clinical Trial**
Changes of BSID-II Motor Scale Raw Score from Baseline to Each Visit

- pUCB (n=31)
- EPO (n=33)
- Control (n=32)

1 month - baseline
3 months - baseline
6 months - baseline

P=0.005
P=0.001
Changes of “social cognition” score in WeeFIM (Functional Independence Measure)

- **UCB (n=31)**
- **EPO (n=33)**
- **Control (n=32)**

<table>
<thead>
<tr>
<th>Time</th>
<th>UCB</th>
<th>EPO</th>
<th>Control</th>
<th>Significance</th>
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<td>0-1month</td>
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<td>0-3month</td>
<td><img src="graph_0-3month.png" alt="Graph" /></td>
<td><img src="graph_0-3month.png" alt="Graph" /></td>
<td><img src="graph_0-3month.png" alt="Graph" /></td>
<td>P = 0.009</td>
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<tr>
<td>0-6month</td>
<td><img src="graph_0-6month.png" alt="Graph" /></td>
<td><img src="graph_0-6month.png" alt="Graph" /></td>
<td><img src="graph_0-6month.png" alt="Graph" /></td>
<td>P = 0.012</td>
</tr>
</tbody>
</table>

*P = 0.002*
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 administration

- 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)

Right Anterior Portion

Baseline: 0.6
After 6 months: 0.6

Left Anterior Portion

Baseline: 0.6
After 6 months: 0.6

Right Posterior Portion

Baseline: 0.6
After 6 months: 0.6

Left Posterior Portion

Baseline: 0.6
After 6 months: 0.6

1st Clinical Trial
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 Left Posterior Internal Capsule in UCB Group (n=30)

$r = 0.46$
$p = 0.009$
Correlation of Changes of GMPM % Score and Changes of FA value in Post. Portion of Right Posterior Internal Capsule in UCB Group (n=30)

$r = 0.44$
$p = 0.01$
Difference of Activated Areas in Each Group with Brain FDG-PET

Effect on brain networking

pUCB (n=31)
EPO (n=33)
Control (n=32)
Difference of Deactivated Areas in Each Group with Brain FDG-PET

Anti-inflammatory effect

UCB (n=33)

EPO (n=32)

Control
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
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

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
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

2nd Clinical Trial

Admission
-2 months

Baseline Evaluation (Functional Evaluation, MRI, PET)
-7 days

UCB infusion
-1 days

Follow-up PET
0 day

1st post intervention Functional Evaluation
2 weeks

Blood sample

Discharge
1 month

2nd post intervention Functional Evaluation
3 months

Blood sample

Rehabilitation
extension

3rd post intervention Functional Evaluation
6 months

Blood sample

Follow-up PET

Blood sample

Blood sample

Blood sample
Results


2nd Clinical Trial

Enrollment (n = 36)

Randomization (n = 36)
(1:1 matched according to age, function)

UCB group (n = 18)

Excluded (n = 1)

UCB group (n = 17)

via IV (n = 15)
via IA (n = 2)

Control group (n = 18)

Excluded (n = 1)

Control group (n = 17)

via IV (n = 14)
via IA (n = 3)
## Safety

<table>
<thead>
<tr>
<th>Group (n=36)</th>
<th>UCB (n=18)</th>
<th>Control (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patient</td>
<td>Event</td>
</tr>
<tr>
<td>Serious adverse events</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other adverse events</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper respiratory tract infection</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Pyrexia</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Vomiting</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Nausea</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Bronchitis</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Urticaria</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>
## Demographic and baseline characteristics of patients (n = 34)

<table>
<thead>
<tr>
<th>Group</th>
<th>UCB (n = 17)</th>
<th>Control (n = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex, no. (% male)</td>
<td>10 (58.8)</td>
<td>8 (47.1)</td>
</tr>
<tr>
<td>Age, months; mean (SD; range); median</td>
<td>46.8 (60.1; 6–216); 26.0</td>
<td>45.3 (41.7; 8–180); 35.5</td>
</tr>
<tr>
<td>Gestational age, weeks, mean (SD; range)</td>
<td>31.8 (4.7; 25–40)</td>
<td>33.4 (4.8; 27–40)</td>
</tr>
<tr>
<td>Preterm, no. (%)</td>
<td>14 (82.4)</td>
<td>12 (70.6)</td>
</tr>
<tr>
<td>Birth weight (SD; range), kg</td>
<td>1.9 (0.7; 1.0–3.1)</td>
<td>2.2 (0.8; 1.2–3.6)</td>
</tr>
<tr>
<td>NBW / LBW / VLBW / ELBW</td>
<td>6 / 2 / 8 / 1</td>
<td>6 / 5 / 6 / 0</td>
</tr>
<tr>
<td>GMFCS (I / II / III / IV / V)</td>
<td>3 / 0 / 1 / 5 / 8</td>
<td>2 / 2 / 1 / 2 / 10</td>
</tr>
<tr>
<td><strong>MRI findings</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Periventricular leukomalacia</td>
<td>10 (58.8)</td>
<td>11 (64.7)</td>
</tr>
<tr>
<td>Diffuse encephalopathy</td>
<td>2 (11.8)</td>
<td>2 (11.8)</td>
</tr>
<tr>
<td>Focal ischemia/hemorrhage</td>
<td>5 (29.4)</td>
<td>4 (23.5)</td>
</tr>
</tbody>
</table>
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
Differences in score change from baseline to 6 mo in UCB group by **HLA mismatching** (1: n=11; 2: n=20)

**Total GMFM**

- 1 mismatch: 10
- 2 mismatch: 5

\[ p=0.003 \]

**Summation of MMT scores**

- 1 mismatch: 15
- 2 mismatch: 10

\[ p=0.054 \]

"Self-care" scores in functional skills scales of PEDI

- 1 mismatch: 10
- 2 mismatch: 5

\[ p=0.027 \]

**WeeFIM total score**

- 1 mismatch: 15
- 2 mismatch: 10

\[ p=0.040 \]
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
Clinical outcomes and **Total Nucleated Cells (TNC) and CD34+ cells per kg** of body weight in pUCB group \((n = 31)\)

<table>
<thead>
<tr>
<th></th>
<th>TNC number/kg of body weight</th>
<th>&gt; 6.69 (\times) 10^7 ((n = 15))</th>
<th>&lt; 6.69 (\times) 10^7 ((n = 16))</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GMFM</strong></td>
<td>3–6month</td>
<td>3.7 (0.8)</td>
<td>1.4 (0.3)</td>
<td>0.024</td>
</tr>
<tr>
<td></td>
<td>1–6month</td>
<td>7.9 (2.2)</td>
<td>3.1 (1.78)</td>
<td>0.021</td>
</tr>
<tr>
<td><strong>GMPM</strong></td>
<td>1–3month</td>
<td>5.3 (0.7)</td>
<td>3.73 (1.2)</td>
<td>0.030</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>CD 34+ cell number/kg of body weight</th>
<th>&gt; 1.46 (\times) 10^5 ((n = 16))</th>
<th>&lt; 1.46 (\times) 10^5 ((n = 15))</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSID-II</td>
<td>3–6month</td>
<td>8.3 (1.9)</td>
<td>2.7 (0.9)</td>
<td>0.030</td>
</tr>
<tr>
<td>Mental scale raw score</td>
<td>1–6month</td>
<td>13.2 (2.2)</td>
<td>5.3 (0.9)</td>
<td>0.008</td>
</tr>
</tbody>
</table>

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 \(\times\) 10^7 was median value and for CD34+ cell numbers/kg, 1.46 \(\times\) 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 BSID-II Motor raw score

- 0-1 month
- 0-3 months
- 0-6 months

- > 5.46 x 10^7 (n=8)
- < 5.46 x 10^7 (n=8)

P=0.10
P=0.49
P=0.17

TNC (x10^7/kg)

Changes in BSID-II Motor raw score

- 0
- 2
- 4
- 6
- 8

r=0.54
P=0.02
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-inflammation

- 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

(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

(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

Ischemic brain injury
Hypoxia-ischemia

Transplantation of hUCB cells

Neurogenesis
Neuronal survival

Neovascularization

Chemokine released by host cells

Angiogenesis

IL-8

p38 phosphorylation

IκB degradation
NFκB activation

Nuclear translocation
Gene regulation

Angiogenesis (VEGF, bFGF, PDGF)
ACKNOWLEDGEMENT

Acknowledgement: These studies were supported by grants of the Korean Health Industry Development Institute (HI3C1204) and CHA University.

Supporter/Collaborators

• Prof. Dong-Wook Kim, Yonsei University
• Prof. SeongSoo An, Gacheon University
Thank you for listening!