### Week 5: Neonatal Neurology

**Neonatal Neurology: Basic and Translational I**

**Tuesday, July 28   4:30-6:00 pm EDT**

**Moderators**
Kathryn Martinello  
Manon Benders

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Note: Schedule subject to change based on presenter availability.
CONTROL ID: 3379402

TITLE: Intranasal insulin-like growth factor 1 (IGF1) treatment to boost myelination in the injured preterm brain

PRESENTER: Josine E.G. Vaes

AUTHORS (LAST NAME, FIRST NAME): Vaes, Josine E.¹; van Kammen, Caren M.²; Baak, Lisanne M.²; Maas, Mirjam²; Groenendaal, Floris¹; Benders, Manon J.¹; Nijboer, Cora H.²

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CURRENT CATEGORY: Neonatology

CURRENT SUBCATEGORY: Neurology: Translational

KEYWORDS: Preterm white matter injury, Insulin-like growth factor 1, Regenerative medicine.

SESSION TITLE: Neonatal Neurology: Basic and Translational I | Neonatal Neurology: Basic and Translational I

SESSION TYPE: Webinar|Platform

ABSTRACT BODY:

Background: White matter injury (WMI) is a common cause of neurological morbidity in the preterm neonate. Impaired maturation of oligodendrocytes (OLs), followed by myelination failure, is a key underlying pathophysiological mechanism in preterm WMI. IGF1, an endogenous growth factor vital for normal white matter development, is shown to be downregulated following extreme preterm birth.

Objective: In this study we evaluated endogenous IGF1 changes in a mouse model of preterm WMI. Moreover, we explored the potential of intranasal IGF1 treatment to restore myelination in vivo. Furthermore, we assessed in vitro whether IGF1 could support OLs to overcome their maturational arrest.

Design/Methods: WMI was induced in C57Bl/6j mouse pups at postnatal day (P) 5 by combining a unilateral hypoxic-ischemic insult with systemic inflammation (1mg/kg LPS i.p.). Plasma and cerebral IGF1 levels were measured between P5-8. Mice were treated intranasally with IGF1 at different dosages during 6 consecutive days following WMI. At 3 weeks post-WMI, we assessed motor outcome using the cylinder rearing test. Brain sections were analyzed for myelination, cerebral inflammation, and axonal injury by immunohistochemistry. To explore the potential of IGF1 to support OLs in overcoming their maturational arrest, WMI was modelled in vitro by subjecting primary cultured immature OLs to inflammatory stimuli.

Results: Induction of preterm WMI led to a transient decrease in endogenous IGF1 levels compared to sham-operated control pups. Intranasal treatment with 25ug IGF1 restored myelination to control level (p<0.001) and improved motor and cognitive behavior of WMI mice by 80% when compared to vehicle (p<0.001). The experimental procedure did not lead to axonal/neuronal damage. IGF1 treatment dampened astrocyte activity compared to vehicle treatment while WMI-induced microglia activation remained unaffected. The cerebral distribution of hIGF1 was confirmed by ELISA at 30 min after treatment. Addition of IGF1 to OLs arrested in maturation in vitro directly boosted OL differentiation and increased myelin production.

Conclusion(s): Induction of preterm WMI is associated with a transient decrease in endogenous IGF1 levels, comparable to the human preterm neonate. Restoring IGF1 levels using intranasal administration of IGF1 is a potent new strategy to restore myelination in a mouse model of preterm WMI. IGF1 aids in white matter regeneration after preterm WMI by boosting OL differentiation following maturation arrest.

(No Image Selected)
Background: Melatonin is a promising neuroprotective agent with anti-oxidative properties. We have demonstrated augmentation of therapeutic hypothermia (HT) brain protection with an ethanol-free melatonin formulation (MEL) in a hypoxic-ischemic (HI) piglet model (1) of neonatal encephalopathy (NE). Erythropoietin (EPO) has anti-apoptotic and anti-inflammatory characteristics. Two multicentre phase III clinical trials of EPO with HT for newborns with NE are underway (HEAL and PAEAN). We hypothesized that the combination of HT+MEL+EPO augments neuroprotection more than MEL+HT and HT alone.

Objective: To assess the safety, pharmacokinetics and efficacy of the combination of EPO (3000U/kg given at 1h, 24, and 48h) + MEL (20mg/kg infusion over 2h at 1h, 24 and 48h) with HT compared with MEL+HT and HT alone. Primary efficacy outcome measures after HI were (i) aEEG recovery; (ii) Lactate+Threonine/total N-acetyl aspartate (Lac+Thr/tNAA) on 1H MRS; (iii) TUNEL positive cells across 8 brain regions.

Design/Methods: 49 piglets underwent HI followed by randomization to (i) HT+vehicle (n=12); (ii) HT+MEL (n=12); (iii) HT+EPO (n=13) or (iv) HT+MEL+EPO (n=12). aEEG was monitored continuously and MRS acquired at 30h and 66h followed by TUNEL cell death quantification at 72h.

Results: There was no difference in insult severity between the groups. Therapeutic levels of EPO and MEL were attained within 1h. Compared to HT, aEEG scores improved at 25-30h in HT+MEL (p=0.02) and HT+EPO (p=0.03) (Fig1A). At 66h, MRS basal ganglia and thalamic Lac+Thr/tNAA was significantly lower in HT+MEL (p=0.01) and HT+MEL+EPO (p=0.03) but not HT+EPO (p=0.07) compared to HT (Fig1B). There was a trend for reduced TUNEL positive cells in HT+MEL and HT+MEL+EPO (both p=0.08) compared to HT+EPO, with a reduced cell death in the sensory cortex in HT+MEL compared to HT (p=0.002) and HT+EPO (p=0.017) (Fig1C).

Conclusion(s): Intravenous melatonin started at 1h after HI augmented HT brain protection based on improved aEEG recovery, reduced Lac+Thr/tNAA and reduced cell death in the sensory cortex. Although HT+EPO improved aEEG recovery, there was no benefit on MRS and TUNEL and EPO did not confer additional neuroprotection over HT+MEL.

1. Robertson et al. Neurobiol Dis 2019
Fig1: aEEG (A), Magnetic Resonance Spectroscopy Lac+Thr/tNAA peak ratio (B) and cell death (TUNEL positive cells) (C) following HI treated with either hypothermia (HT), melatonin (MEL)+HT, erythropoietin (EPO)+HT or melatonin and erythropoietin combined+HT. aEEG scored from 0 (isoelectric) to 4 (continuous normal voltage). Lac/NAA at 30h and 66h in white matter and deep grey matter (BGT) voxels plotted against Log scale. Geometric mean values plotted +/- SEM, significance: *^+ denotes p<0.05, ** and ^^ p<0.01 compared to HT alone group.

IMAGE CAPTION:

Fig1: aEEG (A), Magnetic Resonance Spectroscopy Lac+Thr/tNAA peak ratio (B) and cell death (TUNEL positive cells) (C) following HI treated with either hypothermia (HT), melatonin (MEL)+HT, erythropoietin (EPO)+HT or melatonin and erythropoietin combined+HT. aEEG scored from 0 (isoelectric) to 4 (continuous normal voltage). Lac/NAA at 30h and 66h in white matter and deep grey matter (BGT) voxels plotted against Log scale. Geometric mean values plotted +/- SEM, significance: *^+ denotes p<0.05, ** and ^^ p<0.01 compared to HT alone group.

CONTROL ID: 3377926

TITLE: Neonatal Erythropoietin and Melatonin Mitigates Perineuronal Net Degradation and Chronic Pain Phenotype in Rats with Preterm Brain Injury

PRESENTER: Sarah Ahmed Hamimi Abdullah

AUTHORS (LAST NAME, FIRST NAME): Abdullah, Sarah A.¹; Burkhardt, Christopher²; Luhmann, Grant¹; Ainechi, Ana³; Muthukumar, Sankar⁴; Jantzie, Lauren L.⁵; Robinson, Shenandoah⁶

AUTHORS/INSTITUTIONS: S.A. Abdullah, G. Luhmann, Johns Hopkins University, Baltimore, Maryland, UNITED STATES; C. Burkhardt, Johns Hopkins University, Baltimore, Maryland, UNITED STATES; A. Ainechi, Johns Hopkins University, Baltimore, Maryland, UNITED STATES;
Background: Perinatal brain injury is a major cause of lifelong neurological deficits. The most common cause of cerebral palsy in the US is very preterm birth. As a sensorimotor disorder, cerebral palsy causes lifelong impairment. Chronic pain often emerges in adolescents and adults with cerebral palsy with both nociceptive and neuropathic qualities. The biological underpinnings of chronic pain in people with cerebral palsy remains elusive, limiting effective treatment advances. Perineuronal Nets (PNNs) are specialized extracellular matrix structures that ensheath interneurons and regulate synapses. PNNs have been shown to play a significant role in circuit refinement during adolescence and early adulthood.

Objective: To better understand cerebral cortical abnormalities that contribute to chronic pain in people with cerebral palsy, we studied pain and sensorimotor PNN in an established rat model with a spastic gait reminiscent of cerebral palsy. To test if PNN degradation and chronic pain were modifiable, we gave extended neonatal treatment of erythropoietin (EPO) plus Melatonin (MLT).

Design/Methods: Chorioamnionitis (CAM) is the leading cause of spontaneous preterm birth. To induce CAM, a laparotomy was performed on embryonic day 18, with uterine arteries transiently occluded for 60 min, and lipopolysaccharide (LPS) injected into each amniotic sac. Shams underwent anesthesia and laparotomy only. EPO (2500U/kg ip qd, postnatal day 1 (P1)-P5 and MLT (20mg/kg ip qd P1-P10), or vehicle, was given. Pain phenotype was quantified with tail immersion, von Frey filaments and formalin responses, by observers blinded to group. PNN were quantified with immunohistochemistry, confocal imaging and Imaris analysis. Two-way ANOVA with Bonferroni was used to compare differences.

Results: After preterm brain injury, vehicle-treated adult rats showed allodynia, thermal hypersensitivity, and excessive late-phase formalin response compared to shams or EPO+MLT-treated rats (all p<0.05). Sham PNN exhibited a typical mesh-like pattern, while PNNs in adult vehicle-treated CAM rats were severely degraded, with significantly decreased expression of PNN-specific aggrecan. Neonatal EPO+MLT prevented loss of aggrecan expression.

Conclusion(s): These data highlight a putative mechanism of nociception associated with cerebral palsy, while investigating the merit of a highly translatable neonatal treatment of EPO and MLT in modulating such deficits.
SESSION TYPE: Webinar|Platform

ABSTRACT BODY:

**Background:** Hypoxic-ischemic brain injury remains a significant cause of mortality and morbidity in infant and children. There is a critical need for additional therapies for hypoxic-ischemic (HI) encephalopathy (HIE) to improve survival and decrease morbidities. Intranasal insulin (InInsulin) administered immediately following HI exposure in P10 rats improves neurobehavioral outcomes and reduces ipsilateral brain damage at P11.

**Objective:** The objective of the project was to test the hypothesis that InInsulin provides long-term neuroprotection against HI brain injury in neonatal rats.

**Design/Methods:** At postnatal day 10 (P10), Sprague-Dawley rat pups were randomly divided into four groups: HI+Insulin (Ins); HI+Vehicle (Veh); Sham+Insulin; Sham+Veh, with an equal male/female ratio. Pups either had HI exposure by permanent ligation of right carotid artery followed by 90 min of hypoxia (8% oxygen) or sham surgery followed by room air exposure. Immediately after HI or Sham, pups received either intranasal recombinant human insulin (25 μg) or an equivalent volume of Veh (Phosphate buffer solution) in each naris, followed by 4 more doses every 24 h. The Sham+Veh served as control. A blinded observer performed neurobehavioral tests at P21-25. Statistical analysis was performed via two-way ANOVA followed by the Holm-Sidak method. The sample size was determined to find a difference of 22% between means with the power of 85% and significance of p < 0.05.

**Results:** Compared to the corresponding shams, pups in HI+Veh did, and HI+Ins did not have statistically significant poor weight gain, delayed right eye-opening, poor motor function during beam walking test, impaired motor & sensory integration during vibrissae forelimb test (P ≤ 0.001). For all outcomes, there was a statistically significant difference between pups from HI+Veh and HI+Ins. Only male pups in HI+veh had reduced working memory during the Y Maze test (P 0.001) compared to Sham+Veh and which was improved in pups from HI+Ins. (n = 4 pups/sex/group).

**Conclusion(s):** InInsulin prevents HI induced poor weight gain and long-term neurobehavioral disturbances in rat pups with HI brain damage and thus has the potential to be a promising non-invasive therapy to improve outcomes of newborns with HIE.
Right eye opening

Beam walking test

Vibrissa test - Rt Forelimb-placement

**IMAGE CAPTION:**

Body weight

Right eye opening

Beam walking test
CONTROL ID: 3382166
TITLE: PPARγ activation enhances myelination and improves neurological recovery in preterm rabbits with intraventricular hemorrhage
PRESENTER: Sunil Krishna

AUTHORS (LAST NAME, FIRST NAME): Krishna, Sunil; BALLABH, PRAVEEN

AUTHORS/INSTITUTIONS: S. Krishna, P. BALLABH, Children's Hospital at Montefiore, Bronx, New York, UNITED STATES;

CURRENT CATEGORY: Neonatology
CURRENT SUBCATEGORY: Neurology: Basic

KEYWORDS:

SESSION TITLE: Neonatal Neurology: Basic and Translational I | Neonatal Neurology: Basic and Translational I
SESSION TYPE: Webinar|Platform

ABSTRACT BODY:

**Background:** Intraventricular hemorrhage (IVH) leads to white matter injury causing neurological deficits. The occurrence of IVH results in inflammation, maturational arrest of oligodendrocyte progenitor cells (OPCs) and hypomyelination. The peroxisome proliferator-activated receptor γ (PPARγ) promotes maturation of OPCs in both culture and animal models of demyelinating diseases.

**Objective:** We evaluated the effect of IVH on PPARγ expression in the periventricular neural cells in rabbit kit model of IVH and human autopsy samples from preterm infants. We next assessed the effect of genetic and pharmacological activation of PPARγ on myelination, gliosis, and neurobehavior in preterm kits with IVH.

**Design/Methods:** Premature rabbit kits, delivered by C-section were treated with I.P glycerol to induce IVH. Head ultrasound was done at 24 h age to detect IVH. Kits with IVH were treated by intracerebroventricular (ICV) Ad-PPARγ and Ad-GFP (adenovirus) and compared for myelination and gliosis. We also evaluated rosiglitazone versus vehicle treated pups with IVH for myelination and astroglisis. Motor behavior was studied by open field test. Autopsy samples from preterm infants with and without IVH were also studied.

**Results:** In autopsy samples from preterm infants, PPARγ was expressed on a few OPC and microglial cells of the periventricular region; and the occurrence of IVH increased the number of PPARγ expressing Iba1+ cells (P<0.05). Accordingly in rabbits, IVH increased gene transcription of PPARγ and the density of PPARγIba1+ microglia (P<0.05). ICV Ad-PPARγ further increased PPARγ expression on Iba1+ microglia. More importantly, Ad-PPARγ treatment in kits with IVH elevated volume fractions of myelin basic protein relative to Ad-GFP treated controls (P<0.05), which was confirmed by western blot analyses (P<0.04). Ad-PPARγ treatment in kits with IVH also reduced GFAP+ astrocytic arborization compared to controls (P<0.01). Ad-PPARγ treatment enhanced OPC maturation, and reduced proinflammatory cytokines. Similarly, rosiglitazone treatment enhanced myelination and reduced gliosis. The motor function was better for Ad-PPARγ treated kits compared to Ad-GFP controls (P<0.05).

**Conclusion(s):** Both pharmacological and genetic activation of PPARγ promotes myelination and minimizes gliosis in preterm rabbits with IVH, which we attribute to reduced inflammation and increased differentiation of OPCs. PPARγ activation might improve the neurological outcome of premature infants with IVH.

Supported by R21 NS102897-01A1

(No Image Selected)
**neonatal HI injury**

PRESENTER: Raul Chavez-Valdez

AUTHORS (LAST NAME, FIRST NAME): Chavez-Valdez, Raul1; Lechner, Charles R.1; Emerson, Paul C.1; Northington, Frances J.1; Martin, Lee J.1

AUTHORS/INSTITUTIONS: R. Chavez-Valdez, C.R. Lechner, P.C. Emerson, F.J. Northington, L.J. Martin, Johns Hopkins University, Baltimore, Maryland, UNITED STATES;

CURRENT CATEGORY: Neurology

CURRENT SUBCATEGORY: Neonatal Neurology: Basic

KEYWORDS: Neonatal hypoxia-ischemia, GABA, Neurodegeneration.

SESSION TITLE: Neonatal Neurology: Basic and Translational I | Neonatal Neurology: Basic and Translational I

SESSION TYPE: Webinar|Platform

**ABSTRACT BODY:**

**Background:** In hippocampus, the number of parvalbumin (PV)+ interneurons (INs), presynaptic GAD65/67 boutons, and dendritic arborization of INs are decreased by 8 days after neonatal hypoxia-ischemia (HI), indicating GABAergic disruption. The post-transcriptional glycosylation of neural cell adhesion molecule (NCAM) with polysialic acid (PSA-NCAM) is an essential process shaping synaptic plasticity, because a decline in expression from early life levels is needed to trigger IN maturation.

**Objective:** To test the hypothesis that neonatal HI perturbs the developmental decline in PSA-NCAM immunoreactivity (IR) and its cellular localization contributing to GABAergic disruption, and early stages of neurodegeneration within the dorsal CA1.

**Design/Methods:** Cerebral HI was induced in postnatal day (P)10 in C57BL6 mice by right carotid artery ligation and 45 min of hypoxia (FiO₂=0.08), followed by normothermia (36°C, NT) or therapeutic hypothermia (TH, 31°C) for 4h with anesthesia-exposed shams as controls. At P11, P18 and P40, we assessed histologically in the dorsal CA1 cell degeneration, atrophy, and IR to PSA-NCAM in relation to: i) PV, GFAP, and Iba-1, cell subtype markers; ii) GAD65/67 and synaptophysin (SYP), pre-synaptic markers; iii) pSer396 Tau, neuronal cytoskeletal marker; and iv) GAP43, axonal regeneration marker.

**Results:** PSA-NCAM IR was low in sham and hypoxia-alone dorsal CA1 at P11. After HI, cell death (nuclear pyknosis) and atrophy was extensive in the CA1 pyramidal cell layer. PSA-NCAM IR was intense in injured pyramidal cells (PCs), and minimally in PV+ INs, but not in activated microglia or astroglia. Although lower than at P11, PSA-NCAM IR was persistently increased at P18 and P40, and became more prominent in the perikaryal cytoplasm of injured PCs. PSA-NCAM IR directly correlated with the degree of HI injury (pyknosis and CA1 atrophy). HI injury decreased GAD 65/67 and SYP IR in the CA1 at P18 and P40, but the relationship with PSA-NCAM IR was weak. HI injury increased the total number of PCs and PV+ INs expressing pSer396 Tau, which variably co-localized with PSA-NCAM at P40. While those PCs with marginalized PSA-NCAM had increased perisomatic GAP43, those demonstrating perikaryal cytoplasmic PSA-NCAM had minimal GAP43 8 and 30d after HI.

**Conclusion(s):** Increased PSA-NCAM level is likely a generic response of CA1 PCs to HI injury. Impaired intracellular trafficking in injured surviving neurons, may lead to PSA-NCAM aberrant localizations and aggravate neuronal degeneration.

![Image](image_url)
Impact of delayed hypothermia plus rEpo on white matter damage after cerebral ischemia in near-term fetal sheep

Alistair J. Gunn

Wassink, Guido1; Crisostomo, Alyssa1; Davidson, Joanne O.1; Galinsky, Robert2; Bennet, Laura1; Gunn, Alistair J.1

G. Wassink, A. Crisostomo, J.O. Davidson, L. Bennet, A.J. Gunn, Physiology, The University of Auckland, Auckland, NEW ZEALAND; R. Galinsky, Monash University, Melbourne, Victoria, AUSTRALIA;

Neurology
Neonatal Neurology: Basic
therapeutic hypothermia, erythropoietin, neuroprotection.

Neonatal Neurology: Basic and Translational I | Neonatal Neurology: Basic and Translational I
Webinar|Platform

Background: Current hypothermia protocols for term infants with hypoxic-ischemic encephalopathy provide partial white matter protection. In experimental models, recombinant erythropoietin (rEpo) can also improve white matter recovery after hypoxia-ischemia, but it is unclear whether adding rEpo therapy to hypothermia can further improve white matter outcomes.

Objective: To test whether delayed, prolonged infusion of rEpo plus hypothermia can further improve white matter recovery compared with hypothermia alone.

Design/Methods: Term-equivalent (0.85 gestation) fetal sheep received 30 min of global cerebral ischemia, then from 3 to 72 hours after ischemia, either normothermia plus vehicle (ischemia-control, n=8), cerebral hypothermia (ischemia-hypo, n=8), rEpo infusion (ischemia-rEpo, 5000 IU/kg loading dose, then 5000 IU/kg every 6 hours, n=8), or co-treatment with hypothermia plus rEpo (ischemia-rEpo-hypo, n=8). Sheep were killed 7 days after ischemia.

Results: Cerebral ischemia was associated with loss of total (Olig2-positive) oligodendrocytes in the intragyral and periventricular white matter (p<0.05), with reduced area fraction of myelin basic protein (MBP, p<0.05), immature-to-
mature (CNPase) oligodendrocytes (p<0.05) and loss of linearity of myelin and SMI-312-positive axons while Ki67-positive cell proliferation was increased in both regions (p<0.05). Hypothermia attenuated loss of Olig2 (p<0.05) in the intragryal and periventricular white matter tracts and improved MBP- and SMI-312 area fraction (p<0.05) in the intragryal white matter after hypothermia. rEpo did not improve Olig2 survival and had an intermediate effect on MBP and SMI-positive axons. Hypothermia+rEpo improved CNPase-area fraction in the intragyral white matter (p<0.05) compared with each intervention alone, but did not improve Olig2 or MBP or axonal integrity, compared to hypothermia alone. Ki67-positive cell proliferation was suppressed after both hypothermia and hypothermia+rEpo, but not rEpo(p<0.05).

Conclusion(s): These data suggest that both therapeutic hypothermia and a high dose infusion of rEpo started 3 hours after cerebral ischemia provide independent white matter protection in near-term fetal sheep. However, although combined treatment increased survival of immature and mature oligodendrocytes compared to either intervention alone, it did not further improve overall protection of myelination or axonal integrity, suggesting overlapping mechanisms of action.

(No Image Selected)

CONTROL ID: 3382051

TITLE: Regenerative Medicine’s 1st biomarker for predicting responsiveness to stem cell therapy-based on mechanism-of-action (MOA): evidence from perinatal cerebral hypoxic-ischemic injury (HII) & proposed patient stratification for a clinical trial

PRESENTER: Evan Yale Snyder

AUTHORS (LAST NAME, FIRST NAME): Snyder, Evan Y.1; Hartman, Richard E.2; Nathan, Neal H.1; Ghosh, Nirmalya2; Pernia, Cameron D.1; Law, Janessa3; Nuryyev, Ruslan1; Tone, Beatriz4; Obenaus, Andre5; Ashwal, Stephen2

AUTHORS/INSTITUTIONS: E.Y. Snyder, N.H. Nathan, C.D. Pernia, R. Nuryyev, Center for Stem Cells & Regen Med Pediatrics, Sanford Burnham Prebys Medical Discovery Institute & UCSD, La Jolla, California, UNITED STATES; R.E. Hartman, N. Ghosh, S. Ashwal, Loma Linda University, Loma Linda, California, UNITED STATES; J. Law, University of Washington, Seattle, Washington, UNITED STATES; B. Tone, Pediatrics, Loma Linda Medical Scholl, Loma Linda, California, UNITED STATES; A. Obenaus, University of California Irvine, Irvine, California, UNITED STATES;

CURRENT CATEGORY: Neonatology

CURRENT SUBCATEGORY: Neurology: Translational

KEYWORDS: biomarkers, hypoxic-ischemic cerebral injury, stem cells.

SESSION TITLE: Neonatal Neurology: Basic and Translational I |Neonatal Neurology: Basic and Translational I

SESSION TYPE: Webinar|Platform

ABSTRACT BODY:

Background: In Regenerative Medicine (RM), no stem cell therapy for any condition has been directed to specific recipients & withheld from others based on the expression of a clinical or molecular profile congruent with the cell’s therapeutic MOA. Ultimately, such an MOA-based selection of patients will be required to optimize safety, efficacy, & resource allocation. We addressed this issue by approaching a condition for which stem cell transplantation is being advanced – perinatal cerebral HII.

Objective: We offer an MRI algorithm for the selection of newborns with lesions responsive to a stem cell's neuroprotective MOA for HII.

Design/Methods: We transplanted human neural stem cells (hNSCs) into experimentally-asphyxiated rat pups & examined them under MRI at intervals post-HII, subjecting the image to “hierarchical region splitting” (HRS) to visualize & quantify (i) total lesion, (ii) necrotic core, & (iii) salvageable penumbral volumes which, in turn, were correlated with histology & behavior 90 d post-HII.

Results: hNSCs migrated to the penumbra & improved histologic, motor, & cognitive outcomes only when there was an MRI-detectable penumbra that could be forestalled from evolving into necrotic core (the core never improved) [Fig 1; Table]. The penumbra was characterized by a molecular profile associated with salvageability & induction of neuroprotective mechanisms, the surrogate marker for which is heat shock protein 27 (HSP27) [Fig. 2]. The penumbra

Table.
was reduced only when hNSCs migrated there, hNSCs were plentiful only in regions where HSP27 was upregulated, & HSP27 was induced only in the penumbra. “Penumbral>Core Volume” defined HII which was responsive to hNSCs (“moderate”); “Core>Penumbral” Volume defined “severe” HII which was unresponsive. “Mild” HII had no core & little penumbra & was unaltered by hNSCs. Not only did host cells upregulate HSP27, but so did engrafted hNSCs, concomitant with reduced penumbral volume & better function.

Conclusion(s): This work represents RM’s 1st selection “biomarker”. Given that HRS of actual babies can yield the same classifications & molecularly responsive regions, this approach should enable the expeditious, non-invasive, prospective selection of HII neonates most responsive to stem cell-based therapy while sparing others an intervention. This method of HII newborn stratification will be used in a prospective clinical trial using hNSCs.

### Table 1: Impact of hNSCs on animals with “moderate” HII, as defined by MRI & HRS

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<th>hNSCsöt + saline-treated</th>
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<tbody>
<tr>
<td>Body weight (g)</td>
<td>22.0</td>
<td>21.9</td>
<td>21.8</td>
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<tr>
<td>Brain volume (mm³)</td>
<td>94.2</td>
<td>94.0</td>
<td>94.1</td>
<td>94.0</td>
<td>94.2</td>
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<tr>
<td>Total lesion area (mm²)</td>
<td>10.2</td>
<td>10.2</td>
<td>10.3</td>
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<td>10.2</td>
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<tr>
<td>Penumbral area (mm²)</td>
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<td>6.8</td>
<td>6.9</td>
<td>6.9</td>
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<tr>
<td>Core area (mm²)</td>
<td>3.4</td>
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*Lower panels: [ii]MRI slices subjected to HRS, most rostral to most caudal.

**Fig. 1:** MRI of the “necrotic core” & “salvageable penumbra” of HII lesion following hNSC transplantation: Although the core does not diminish the penumbra does & becomes “normal” by MRI; penumbra not rescued progresses to “core”.

[A] Upper Panels [i] show T2 coronal images at 2d after HII (prior to hNSC implantation) where hyperintensity (white) indicates injured tissue. At 90 d post-HII (after hNSC implantation), the remaining injured tissue has become cystic (bright) (green arrows). **Lower Panels [ii]** demonstrate the “core” (red) & “penumbra” (blue) after HRS. Note transition from “penumbra” (blue) (blue arrows) at 2d to “core” (red) (red arrows) at 90d post-HII in saline-treated brains.

[B] Quantification of phenomena.

[C] MRI slices subjected to HRS, most rostral to most caudal.
FIG 2. HSP27 expression is positively related to the Rat Pup Severity Score (RPSS) & injury site.

[A] RPSS & HSP27 immunoreactivity in ischemic areas, particularly the penumbra, have a positive correlation ($R^2 = 0.403$) with a peak RPSS of 2.5 – consistent with presence of salvageable penumbra & identical to peak density of hNSC engraftment.

[B] HSP27 expression by host cells (greatest in penumbra of moderate HII rats (a = 0.05).

[C] Endogenous cells in penumbra of representative moderate HII rat.

[D] HSP27 (red) & donor hNSCs (Stem 101+, nuclear green) co-localize in the penumbra. HII induces upregulation of HSP27 in donor in addition to host cell. Bar=100 µm.

*HNSCs migrate to areas of moderate & severe (though not mild) HII (influenced by upregulation of repair-associated genes which is most robust in regions where cells are salvageable.*

**IMAGE CAPTION:**

TABLE 1: Impact of hNSCs on animals with “moderate” HII, as defined by MRI & HRS

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