Placental Stem Cell Transplant Improves PKU Symptoms in Mice

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Current Treatments

- Physiological / non-physiological amino acid therapy
- Phe-restricted diet
  - Lifelong strict compliance
  - Expensive
  - Taste?
- BH4 (Kuvan™) supplementation
  - Expensive
  - Does not work for everyone
- PEG-PAL
  - Phase 2 clinical trials
- Gene Therapy
- Cell Transplant
  - Liver cells
  - Placental cells

A Mouse Model of PKU

Hi, I have PKU!
A mouse model of PKU

- No enzyme activity
- ↑ Phe in the blood, organs, and brain
- Disruptions in brain amino acids / neurotransmitters
- Hypopigmented (fur changes from black to light brown)
A mouse model of PKU

- No enzyme activity
- ↑ Phe in the blood, organs, and brain
- Disruptions in brain amino acids / neurotransmitters
- Hypopigmented (fur changes from black to light brown)
- Smaller than healthy siblings
- Cognitive (memory, learning) problems
- Offspring of PKU moms suffer defects similar to human maternal PKU

PKUenu² mouse is a great model for human PKU.
Previous Studies – Liver Cell Transplant
Benefits of Liver Cell Transplant

1. **Less** expensive (5-10% the cost of liver transplant)

2. **Less** invasive, fast recovery (several infusions over 24-48h)

3. **Fewer** incidents of serious complications

4. Transplanted cells are from "recycled" livers - Still relying on donor livers (limited) - lifelong(?) immunosuppression

5. Transplanted cells only have **ONE JOB** - If cells fail, the patient would only go back to the condition he/she was in before undergoing cell transplant.
Liver Cell Transplant and Disease

• **Animal models of disease**
  – Crigler-Najjar
  – Maple Syrup Urine Disease (K. Skvorak)
  – **PKU** (C. Harding, K. Skvorak)
  – Glycogen storage disease
  – Wilson’s Disease

• **Patients – bridge therapy and beyond**
  – **PKU** (ongoing clinical trial, Children’s Hospital of Pittsburgh)
  – Crigler-Najjar (1998)
  – Glycogen storage disease
  – Ornithine Transcarbamylase (OTC) deficiency
  – Factor VII deficiency
  – Biliary Atresia
  – Additional Urea Cycle disorders
  – Liver failure (1997)
Placental Stem Cells: Amnion Epithelial (AE) Cells
**Amnion** – thin membrane surrounding the fetus during pregnancy

**Epithelium** – cells that line the inner and outer body surfaces

- Acquired after **full term birth**
  - **Plentiful** – C-sections account for a third of all births in the USA
- **Easy** to isolate, easy to grow in the lab
- Non-controversial source of stem cells
- **Not** cord blood cells
- Anti-fibrotic, anti-inflammatory, and anti-microbial characteristics
- Can “hide” from the immune system
- Freeze/thaw well
  - Cell banking potential
Procedure and Rationale

- Healthy donor cells have normal enzyme activity
  - <10% activity: more manageable disease; increased protein tolerance
  - 10-20% activity: potential cure (Harding & Gibson, 2010)

- Treatment at birth – clinically relevant to treat this way

- No surgery required (mice)
  - Clearly see the liver through the skin

- Newborn mouse livers are rapidly growing
Previous Results – MSUD mouse

After AE cell transplant:

- Growth rate was normalized
- Survival was significantly lengthened
- Enzyme activity was doubled (6% to 13%)
- Leucine was normalized, neurotransmitters were significantly improved
Isolate human amnion epithelial cells (hAE)

1 million cells/mouse transplanted directly to liver (birth)

hAEC Tx

7 days  14 days  21 days  28 days

PAHenu^2 (birth)

On normal diet

No immunosuppression

1 month post-tx (young adult)

100 days Post-tx

AA Analysis (blood/brain)
Neurotransmitters
PAH activity
Human DNA in mouse liver
Results – Blood Phe Improvement

- Male vs Female
- ~25% reduction
- ~60% reduction

Statistics
* = p<0.05
** = p<0.01
*** = p<0.001
Results – Brain Phe Improvement

- Male + Female
- ~60% reduction
- NORMALIZED!

Statistics
* = p<0.05
** = p<0.01
*** = p<0.001
Results - Neurotransmitter Improvement

Phe → Tyrosine → Dopamine → DOPAC → HVA → 3-MT

Also Normalized: Taurine, Glycine, Aspartate

PAH

50% 20% NS = not statistically different from control mice
Summary

1. Cell therapy was tested in a mouse model of PKU
   – Mouse liver cells and human placental stem cells

2. Blood and Brain Phe with cell transplant
   – Brain Phe was normalized with placental stem cells

3. Additional corrections in brain (Neurotransmitters!)

4. Viable alternative therapy for PKU (and other liver-based metabolic diseases)
   – Placental stem cells are a non-controversial source of cells, which has immunomodulatory properties
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Questions?