**STEM CELL THERAPY FOR NEC**

1. Amniotic fluid (AF) is harvested at the time of amniocentesis or delivery.

2. AF cells are cultured in mesenchymal stem cell (MSC) induction medium to obtain MSC. AF-derived MSC are then frozen and stored for future use.

3. MSC or their secreted products are administered to protect the intestines from NEC.
Novel method of probiotic administration
PATHOGENESIS OF NEC

- Multifactorial
  - Prematurity
  - Low birth weight
  - Enteral feeds
  - Altered intestinal microflora
Infants that develop NEC have changes in microbial community:

- Increased Gammaproteobacteria (gram-neg facultative bacilli) and potentially pathogenic organisms including *E. coli, Enterobacter, Klebsiella*
- Decreased Negativicutes (gram-neg anaerobes) / Firmicutes (gram-pos anaerobic bacilli)


**Dysbiosis is associated with the development of NEC**
**TERMINOLOGY**

- **Prebiotic**
  - ✓ Compound that stimulates the growth of a probiotic
  - ✓ Non-digestible by host

- **Synbiotic**
  - ✓ Prebiotic + probiotic

- **Probiotic**
  - ✓ “live micro-organism which, when administered in adequate amounts, confers a health benefit on the host”
**CHALLENGES FOR PROBIOTICS**

- Not FDA approved
- **Formulations not regulated**
  - Typically only the # of live bacteria per dose provided
- **Formulations are different**
  - Foods (e.g. yogurt), pills, liquids
- Varying efficacy for each individual
- Difficulty colonizing in the gut
- **Probiotics face significant challenges within the host**
  - Low gastric pH
  - Host immune system
  - Commensal / pathogen competition
- Appropriate dose / frequency unknown
- Synbiotics rarely add improvement over probiotics alone
CHALLENGES FOR PREBIOTICS

- Orally consumed compounds don’t reach the target
- Significant dilution
- Unlikely to benefit probiotic bacteria if not in high concentration
PROBIOTIC ADMINISTRATION FOR NEC

- Decrease incidence and severity of NEC in infants (variable results)
- Animal models:
  - ✓ reduce intestinal permeability
  - ✓ inhibit inappropriate inflammation
  - ✓ reduce apoptosis
  - ✓ stimulate enterocyte proliferation
  - ✓ modulate T cell activation
  - ✓ inhibit TLR4 activation
LIMITATIONS OF PROBIOTIC ADMINISTRATION FOR NEC

- Must be given at least daily
- Do not significantly alter host microbiome
- Several case reports of bacteremia
Can we improve the way we deliver probiotics to protect the intestines from NEC?
PLANKTONIC vs. BIOFILM STATE

Planktonic state

Free-living *Lactobacillus reuteri*

Biofilm state

*Lactobacillus reuteri* surrounded by matrix of DNA, proteins, lipids, oligosaccharides

↑ BT stability

↑ BT activity
**Lactobacillus reuteri**

- Gram-positive, facultative anaerobic bacillus (microaerophilic, aerotolerant)
- Found in many food and milk products
- Convert sugars to lactic acid
- Common gut microbe in (most) mammals
- Antimicrobial properties
  - **Reuterin**, Reutericyclin, lactic acid
- Anti-inflammatory properties
  - **Histamine**
- Human feces-derived *Lr 23272* (clade II) from ATCC
REUTERIN (ANTIBACTERIAL)

- By-product of glycerol fermentation
- Induces oxidative stress and inhibits growth in a broad range of Gram +/- bacteria, yeast, mold, protozoa

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HO─CH─OH  ───────────>  HO─CH─COH
Glycerol      Reuterin
```
HISTAMINE (ANTI-INFLAMMATORY)

L. reuteri

BIOACTIVE HISTAMINE

Monocyte

↓ TNF

Macrophage

↓ TNF
NOVEL METHOD OF PROBIOTIC DELIVERY

Planktonic, free-living *Lactobacillus reuteri* + Biocompatible microspheres → Biofilm allowed to form

- Fluorescent Microscopy
- Scanning Electron Microscopy
SEPHADEX MICROSPHERES

- Spheres made of cross-linked dextran ("dextranomer")
- Separation Pharmacia Dextran
- Size exclusion chromatography
- Wide range of sizes (~15µm-600µm)
- ↑ available surface area for Lr growth
- Dextranomer composition similar to Lr native exopolysaccharide
SEPHADEX MICROSPHERES

- Precedence for medical use:
  ✓ Biostable
  ✓ GMP quality

- Medical uses:
  ✓ Debrisan® - dressing material
  ✓ Solesta® - fecal incontinence
  ✓ Deflux® - vesicoureteral reflux
**Spheres made of** dextran **cross-linked by** epichlorohydrin

- **Superfine:** 52 μm (hydrated)
Dextranomer microspheres provide a surface for *L. reuteri* to form a biofilm.
Microspheres can be filled with helpful compounds. Dextranomer microspheres provide a surface for *L. reuteri* to form a biofilm.
Dextranomer microspheres provide a surface for *L. reuteri* to form a biofilm.

Microspheres can be filled with helpful compounds.

Diffusion of helpful compounds directly to the biofilm community.
Microspheres can be loaded with beneficial cargo:

- **Glycerol** for reuterin production (antibacterial)
- **L-histidine** for histamine production (anti-inflammatory)
- **Maltose / Sucrose** for biofilm formation/attachment
**Lr Biofilm:**
- composed primarily of the polysaccharide **reuteran** (repeating glucose molecules)
- reuteran produced by the enzyme GtfW
BIOFILM FORMATION

Maltose

Lactobacillus reuteri

(substrate of GtfW)

Maltose

Lactobacillus reuteri

GtfW
Sucrose

Lactobacillus reuteri

(induces expression of GtfW)

BIOFILM FORMATION
BIOFILM FORMATION

L. reuteri on Empty Microspheres

L. reuteri on Sucrose-loaded Microspheres

Probtiotic

Microsphere Surface

Sugar
HYPOTHESIS

*Lactobacillus reuteri* grown on *sucrose-loaded or maltose-loaded microspheres* and administered in a biofilm state will produce superior and longer-lasting beneficial effects.
EFFECT OF *L*r ON INCIDENCE AND SEVERITY OF NEC

Single Dose Enteral Treatment!
EFFECT OF Lr ON GUT BARRIER FUNCTION IN NEC

![Graph showing the effect of Lr on gut barrier function in NEC](image)

- **Serum FITC-Dextran (ug/mL)**
- **Breast Fed**
- **NEC + Sterile Water**
- **NEC + Lr**
- **NEC + Lr + Sucrose-Loaded Microspheres**
- **NEC + Lr + Maltose-Loaded Microspheres**

* *p < .05*
CONCLUSIONS OF PROBIOTIC STUDIES

- A **single** dose of *Lr* administered in its biofilm state on sucrose- or maltose-loaded microspheres:
  - ✓ decreases the incidence of NEC
  - ✓ improves gut barrier function
  - ✓ has improved ability to colonize the intestine *in vivo*
  - ✓ positively affects the microbiome in NEC

- Optimizing delivery strategies may significantly improve probiotic efficacy

- Microspheres and prebiotics used are GRAS by FDA
We're not ALL BAD!!
- NIH STTR grant awarded April 2017
- Meeting with FDA planned for Q1 2018
LONG TERM GOALS

Clinical use of stem cells, novel probiotic delivery system in premature babies to prevent necrotizing enterocolitis in the future
THANK YOU