WHAT  2nd Microbiome workshop
WHEN  17 & 18 November 2016
WHERE  Masur Auditorium at NIH Campus, Bethesda MD

Modulating the Vaginal Microbiome to Prevent HIV Infection

Laurel Lagenaur

For more information:  www.virology-education.com
Conflict of Interest

I work for Osel Inc., a microbiome company developing live biotherapeutic products to prevent diseases in women
The Vaginal Microbiome and HIV Infection

What’s normal /healthy/ optimal and what’s not?
- Ravel Community Groups
- Lactobacillus dominant vs. dysbiosis

Why are Lactobacilli important for HIV prevention?
- Dysbiosis = Inflammation = Increased risk of HIV acquisition
- Efficacy of Pre-Exposure Prophylaxis decreased

Modulation of the vaginal microbiome
- LACTIN-V, live biotherapeutic- *Lactobacillus crispatus*
- Ongoing clinical trials

How we can use Lactobacilli and go a step further
- Genetically modified Lactobacillus to prevent HIV acquisition
HIV is Transmitted Across Mucosal Surfaces

• HIV infection in women occurs in the mucosa of the vagina and cervix

• Infection of underlying target cells

All mucosal surfaces are continuously exposed to a community of microorganisms

Herrera and Shattock, *Curr Top Microbiol Immunol* 2013
Vaginal Microbiome: Ravel Community Groups

Vaginal microbiomes clustered into 5 groups:

4 were dominated by *Lactobacillus*, whereas the 5th had lower proportions of lactic acid bacteria and higher proportions of strictly anaerobic organisms.

- **Group V**: *L. jensenii*
- **Group II**: *L. gasseri*
- **Group I**: *L. crispatus*
- **Group III**: *L. iners*

**Group IV Diversity**:
- Prevotella
- Sneathia
- Megasphaera
- Atopobium

**Group 4 Diversity**:
- *Prevotella*
- *Sneathia*
- *Megasphaera*
- *Atopobium*

**Shannon Diversity**:

Ravel *et al.* PNAS 2011
Vaginal Microbiome

**Lactobacilli-dominated**
- Low diversity, low vaginal pH
- Antagonize pathogens
- Non-inflammatory

**Bacterial vaginosis**
- Decrease \( \text{H}_2\text{O}_2 \)-producing Lactobacilli
- Increased bacterial diversity
- High pH, odor, discharge
- Increased risk of STI and HIV

Adapted from Srinivasan 2012
Vaginal Microbiota vs. Host Inflammatory Responses

- Community types

Alpha diversity

Anahtar et al. Immunity 2015
Genital Inflammation is Linked to HIV infection

South African women in the CAPRISA study with *P. bivia* were **19 times** more likely to have genital inflammation and **13 times** more likely to acquire HIV.
Vaginal Microbiota May Influence Tenofovir Efficacy

• Tenofovir disoproxil - Nucleotide analog reverse-transcriptase inhibitor used in Pre-Exposure Prophylaxis

• Delivered as a 1% intravaginal gel (before and after sex)
Tenofovir gel effective against HIV with *Lactobacillus*-dominance

**Lactobacilli-dominated**
- 61% efficacy
- 95% CI, 11 to 84%

**Non-Lactobacillus dominant**
- 18% efficacy
- 95% CI, -77 to 63%

HR = 0.39 (95% CI: 0.20; 0.83)  
HR = 0.82 (95% CI: 0.40; 1.65)  
P = 0.644
Tenofovir was rapidly depleted by Gardnerella but not Lactobacillus

### Tenofovir (supernatant)

- **Abiotic**
- **L. iners**
- **G. vaginalis**

- **4 hours:**
  - G. vag vs. L. iners: $P=0.002$
  - G. vag vs Abiotic: $P=0.005$

- **24 hours:**
  - G. vag vs. L. iners: $P<0.001$
  - G. vag vs Abiotic: $P<0.001$

### Tenofovir (cell)

- **L. iners**
- **G. vaginalis**

- **4 hours:**
  - G. vag vs. L. iners: $P<0.001$

- **24 hours:**
  - G. vag vs. L. iners: $P<0.001$
What Can We Do to Decrease HIV Infection in Women?

• Treat their Bacterial vaginosis - Metronidazole

Lactobacillus dominant → Dysbiosis ↔ Abx → Post-Abx → Restoration

↓\( \text{H}_2\text{O}_2^+ \) *Lactobacillus*

Growth of diverse commensal and pathogenic bacteria

Live Biotherapeutic Product (LBP) • *Lactobacillus crispatus* CTV-05

• Give women exogenous *Lactobacillus*
Osel Product **LACTIN-V (Lactobacillus crispatus)** for Treatment of Bacterial Vaginosis

**LACTIN-V Clinical Studies**

**Phase 1**
- Safe and well-tolerated at all dose levels*  
  * Hemmerling et al., S Trans Dis 2009

**Phase 2a**
- Following Metronidazole treatment
- Colonization up to **78%** of women treated for BV**  
  ** Hemmerling et al., S Trans Dis 2010
- Colonization correlated with reduced BV recurrence

**Phase 2b (sponsor NIAID/DMID)**
- Prevention of recurrent BV
- 1st patient enrolled Q2 2016
- Locations: University of California, San Diego, San Francisco General Hospital, Cook County Health and Hospitals System, Chicago, Washington University School of Medicine in St. Louis
- Double Blind, Placebo Controlled, Randomized Clinical Trial
Microbiome Changes During Successful LACTIN-V Colonization

L. crispatus administered following ABX replenishes the vaginal microbiome

(Gnugi et al., 2011)
Increase in *Lactobacillus* and *L. crispatus* at follow-up


Two potential mechanisms:
1) colonizes directly (evidence) and 2) fosters growth of endogenous vaginal lactobacilli
Modulating the vaginal microbiome: Can we go further?

- Colonize the mucosa with live recombinant Lactobacillus
  - Natural defense, lactic acid,
  - “Anti-inflammatory”
  - Genetically modified to produce an HIV entry inhibitor
  - Protection at the site of HIV infection
Strain selection

• Screened >20 isolates of L. crispatus, L. jensenii and L. gasseri from healthy women

• Selected L. jensenii 1153
  - Growth rate-doubling time
  - D-Lactic acid production
  - Adherence to tissue
  - Activity against Gardnerella and Staphylococcus
  - Transform (no endogenous plasmids)

• Broad Institute/J. Craig Venter Institute -sequenced genome 1153
Inhibitor Selection

**Cyanovirin-N (NCI)**
- Identified by National Cancer Institute drug screen (Cyanobacterium)
- Activity against **all subtypes**
- Inhibited >85% HIV viruses in a representative panel of major subtypes, including Tier 2, CRF, T/F
- **CV-N** active pH 4-8 (vaginal pH range)

Constructing a recombinant *L. jensenii* 1153-1666

- Stably integrated mCV-N gene into the *Lactobacillus* chromosome by homologous recombination

- Biologically active level of inhibitor expression using a constitutive ribosomal promoter

\[ \text{Integrate into chromosome} \]

mCV-N *L. jensenii* 1153-1666 secreting mCVN

Liu *et al.* 2006, AAC
mCV-N Protein Seen on Coomassie Stain

- Parental *L. jensenii* 1153 is compared to recombinant *L. jensenii* 1153-1666 on protein gel
- Global protein expression unchanged
- Lactic acid production and growth unchanged
Intravaginal Inoculation of *L. jensenii* 1153-1666 in Macaques

In initial studies…..

Pellet + 3% hydroxyethyl cellulose

Freshly prepared overnight culture

Rhesus macaque

Brown and Hopps
Modified Gram stain
Recovery of *L. jensenii* 1153-1666 from Rhesus Macaques following 5-day dose

Followed

Vaginal colonization

*L. jensenii* 1153 expressing mCV-N
Proof of Principle

• Colonized rhesus macaques

• Cervical vaginal lavage taken weekly

• mCV-N protein detected by immunoblot for at least 6 weeks
Proof of Principle

- Colonized rhesus macaques
- Immunohistochemistry on vaginal biopsies for mCV-N protein
- mCV-N protein localized on mucosal surface at the site of virus entry
We selected a strain, and an HIV inhibitor. We made a recombinant L. jensenii.

We showed that our L. jensenii recombinant could colonize the macaque and express mCV-N protein in situ on the mucosa.

Now we wanted to challenge macaques with (S)HIV to test whether infection could be prevented.
SHIV Repeated Dose Challenge

- Two arm study
  - 12 macaques received *L. jensenii* expressing mCV-N
  - 12 control (8 macaques received HEC placebo gel/4 no gel
  - Macaques were challenged each week for 6 weeks with (S)HIV
  - Monitored for viral infection each week

- Repeated dose challenges measure the rate of infection per exposure

- Infection rate about 33% macaques/exposure (100-1000X higher than amount found in human)

- Monitor time to infection
Tested recombinant *L. jensenii* 1153-1666 SHIV Repeated Dose Vaginal Challenge

- 300 TCID\(_{50}\) SHIV\(_{SF163P3}\)
- **Controls**: 35% infection per exposure
- **L. jensenii** 1153-1666: 13% infection per exposure
- 63% reduction in acquisition \(p\lt0.004\)
- 6X reduction in viral load

Studies performed at BIOQUAL Inc.

Lagenaur *et al*. 2011 Mucosal Immunology

A second smaller experiment confirmed these results
Based on the results

- Moved forward with formulation of the product that could be used in the real life
  - Developed a vaginal tablet (VT)
  - Tablets are potent, stable, colonized macaques

Lagenaur et al. 2011 PLoSOne

New formulation less potent, but more stable at 37°C
Next Steps in Assay and Product Development

• Developed ELISA to analyze mCV-N protein-limit of detection 1ng
• Developed qPCR to detect *L. jensenii* 1153-1666
• Submitted documents for pre-IND meeting with FDA (initial comments received)
• Formulation needs to be sufficient for first in human studies (our stability of sufficient)
• Transfer strain and process to an industrial partner
• IND-submission

IND=Investigation New Drug Application
Summary MucoCept Product Development

- Identified a **need**: to develop novel HIV prevention strategies for women
- Shown that formulated *L. crispatus* can be delivered and can colonize women
- Selected a *Lactobacillus jensenii* (vaginal colonizer) and an **HIV inhibitor**
- Constructed a **recombinant L. jensenii** that secretes mCV-N, **tested fitness**
- Tested colonization, protein expression, efficacy, safety of the recombinant *L. jensenii*
- **Formulated** the concept into a product (**Vaginal Tablet**)
- Tested colonization and efficacy of the tablet
- Developed assays to detect mCV-N protein and recombinant *L. jensenii*
- With submission of an IND, we are moving toward phase 1 safety trial in women
Acknowledgments

Peter Lee, MD
Thomas Parks, PhD
Trine Nilsen, PhD
KT Moortgat, PhD
Iwona Swedek
Angela Marcobal, PhD
Caitlyn Dela Cruz

UCSF
Craig Cohen, MD, MPH
Anke Hemmerling, MD, PhD, MPH

Bioqual, Inc.
Hanne Andersen, PhD
Brigitte Sanders, PhD

University of California, Davis
Christopher Miller, DVM, PhD
Tim Carroll, PhD,
Linda Fritts

NIH
Dean Hamer, PhD
Jay Berzofsky, MD, PhD
Nancy Miller, PhD
Carole Bewley, PhD

Former Osel colleagues
Chia-Hwa Chang, PhD
Teresa Chang, PhD
Andrew Cheng, PhD
Kirsten Essenmacher, PhD
Courtney Frasier, PhD
Letong Jia
Wenjun Huang
Xiaowen Liu, PhD
Yang Liu, PhD
Pat Martin, PhD
David Simpson, PhD
Kimberly Smith
Qing Xia
Qiang Xu, PhD
Rosa Yu, PhD
Yonghong Zhu, PhD

UCSF
Craig Cohen, MD, MPH
Anke Hemmerling, MD, PhD, MPH