Animal models for pre-clinical testing of antibiotics against gonorrhea: Established and new models under development

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- Chiron Corporation
- Glaxo-Smith-Kline
- Microbiotx, Inc.
- The Population Council
- Reoxcyn Discoveries Group
- Tetraphase, Inc.
- Topcaid
- VenatoRx
- Yaso Therapeutics

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Animal Modeling of *Neisseria gonorrhoeae* Infections

*Neisseria gonorrhoeae* (Ng)

- Human-specific pathogen, no outside animal or environmental reservoir
- Well-adapted to human mucosae
- Numerous host-restricted factors
  - Colonization receptors ( pilus receptor, CEACAMs, C3b/C3R receptor)
  - Iron-binding glycoproteins ( transferrin, lactoferrin)
  - Calprotectin
  - Soluble regulators of the complement cascade
    (factor H, C4b-binding protein)  
    *(Reviewed in Jerse 2011; Jean 2016)*

Published animal models of *Ng* urogenital infection

- Chimpanzees
  - Males: urethritis
  - Females: cervicitis
  - Natural transmission from male to female documented
    *Lucas 1971, Brown 1972*

- Estradiol-treated female mice
  - Lower reproductive tract (LRT) model
    - cervico-vaginal infection
    *Taylor-Robinson 1990, Jerse 1999, 2011*
Murine model of lower reproductive tract (LRT) infection model

- Treat with estradiol and antibiotics to promote long-term susceptibility; vaginal inoculation
- Localization of infection: Vaginal lumen, associated with nucleated and squamous epithelial cells in cervical and vaginal tissue; seen within PMNs and in the lamina propria
- Recovery: $10^2$ – $10^5$ CFU/100 µl of a single vaginal swab suspension
- Cyclical recovery pattern (hormonally driven): mimics human cervical infections in women of reproductive age

Characteristic Recovery Pattern (vaginal swabs)

- Innate responses (BALB/c mice): Vaginal PMN influx and localized proinflammatory cytokine and chemokine response; induction of cationic antimicrobial peptides (CAMPs)
- Adaptive response is suppressed and not protective

(Reviewed in Jerse 2011)

- BALB/c mice are treated with estradiol and antibiotics (STR, TMP).
- Mice are vaginally inoculated with Ng; pre-tx cultures taken on days +1 and +2 post-inoculation.
- Test compound(s), vehicle control, and positive control [ceftriaxone (CRO) or gentamicin (GEN) are administered on day 2 of infection.
- Clearance rate and bioburden are measured over 8 consecutive days by quantitative vaginal culture.

**Current test strains**
Laboratory Strains: FA1090, MS11, FA19, F62
MDR Clinical Isolate: H041
Published pre-clinical efficacy trials using murine models of *Ng* lower reproductive tract infection

<table>
<thead>
<tr>
<th>PRODUCT TYPE</th>
<th>MODEL</th>
<th>STUDY OUTCOME</th>
<th>CITATION</th>
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<tbody>
<tr>
<td>Antibiotics</td>
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<tr>
<td>Aminomethyl spectinomycins and spectinomycin (Microbiotix)</td>
<td>BALB/c mice</td>
<td>All compounds (5QD) significantly cleared infection by a MDR <em>Ng</em> strain compared to vehicle; results comparable to GEN.</td>
<td>Butler 2018</td>
</tr>
<tr>
<td>Resorufin pentyl ether (RPE)</td>
<td>BALB/c mice</td>
<td>5QD significantly reduced the number of <em>Ng</em> recovered over time compared to vehicle; a trend for faster clearance of infection was observed.</td>
<td>Schmitt 2016</td>
</tr>
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<td>Acylaminooxadiazole (trans-translation inhibitor) (Microbiotix)</td>
<td>BALB/c mice</td>
<td>A single oral dose of MBX-4132 significantly cleared infection by a MDR <em>Ng</em> strain compared to vehicle; results comparable to GEN.</td>
<td>Aron 2021</td>
</tr>
<tr>
<td>Tri-cyclic topoisomerase inhibitor REDX05931</td>
<td>Ovariectomized BALB/c mice</td>
<td>Dose-dependent decrease in <em>Ng</em> bioburden at 1 and 24 hr post-tx compared to vehicle. Highest dose resulted in no recovery of <em>Ng</em> after 24 hr, and no or low numbers of <em>Ng</em> after 7 days.</td>
<td>Savage 2016</td>
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</table>
A single 5 mg/kg dose of CRO showed 100% efficacy against a CRO strain (FA1090) and produced a therapeutic time of 23.6 hours.
A multiple CRO dosing regimen with a therapeutic time of 22.9 h cleared a CRO\textsuperscript{R} strain (H041) in 90% of mice at 48 hr
CIP: The lowest dose that cleared infection by a CIP^S strain (FA1090) in 100% of mice within 48 h has a fAUC/MIC of 264
Need for *Ng* upper reproductive tract infection models

- Limited number assessments of treatments for clearance of endometrium and fallopian tube infection or for prevention of tubal infertility and ectopic pregnancy (Workowski and Bolan, 2015)
  (Walker 1991)
- Efficacy study for cefotetan with doxycycline and cefoxitin with doxycycline in 108 women with acute salpingitis due to *Ct* or *Ng*, with or without anaerobes
  - Clinical cure in 51 of 54 (94%) patients in each group.
  - All six patients whose treatment failed had positive cultures for *Ng* and facultative/anaerobic bacteria; none had *Ct*.
  - Only 92% effective against gonorrhea (66/72 cured)
  - Pre-dates emergence of *Ng* strains with reduced cephalosporin susceptibility
Are PK/PD modeling predictions for clearance of cervical infections the same for endometrial and fallopian tube infections?

Studies on women undergoing prophylactic antibiotic treatment prior to hysterectomy

- Cephalosporins and cephamycin antibiotics may have similar plasma concentrations, but can differ in uterine and fallopian tube tissues and not be high enough for all potential PID pathogens.
- Comparisons of ceforanide and cefazolin:
  - Ceforanide levels in endometrial samples exceeded the MIC90 for *E. coli*
  - Cefazolin levels were below the MIC90 in 50% of myometrial and 67% of endometrial samples
  (Elder 1977; Souney 1988)

Pregnancy

- PK of antibiotics may also differ during pregnancy and for several weeks after pregnancy due to:
  - Changes in renal function
  - Increased uterine weight
  - Physiological changes that may cause poor antibiotic perfusion into the uterus
  (i.e. changes in blood volume, extracellular fluid and endometrial blood flow)
  (Fortunato and Dodson, 1988)

Antibiotic bioavailability with respect to the menstrual cycle – not investigated
**Ng upper reproductive tract (URT) infection model**

- Supplement mice with human transferrin (hTF) to relieve the host-restriction in the URT for a usable iron source
- Inoculate vaginally or transcervically with Ng

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**Vaginal swabs**

- Left graph: Comparison of CFUs/mL between hTF-supplemented (red) and untreated (blue) groups.
- X-axis: Days post-inoculation (0-12)
- Y-axis: Log10 CFUs/mL

**PMN influx**

- Right graph: PMN influx (out of 100 cells) over days post-inoculation (0-11).
- X-axis: Days post-inoculation
- Y-axis: % PMNs

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Need for *Ng*/*Chlamydia* coinfection models

- Gonorrhea/chlamydial coinfections are very common
- Question: For dually active new drugs, what is the most effective treatment regimen for both pathogens? - also for co-packaged or fixed-dose combination therapies

*Ng*/*Chlamydia* coinfections are very common

<table>
<thead>
<tr>
<th>Activity against coinfecting STI pathogens</th>
<th>Short-term (up to 5 years)</th>
<th>Long-term (up to 10 years)</th>
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<tr>
<td></td>
<td>Ideal</td>
<td>Acceptable</td>
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<tr>
<td>Indication</td>
<td>(First-line) treatment of uncomplicated, urogenital gonorrhea (susceptible and MDR)</td>
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<td>First-line treatment of extra-genital gonorrhea (ano-rectal and oropharyngeal)</td>
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*Alirol 2017* Target Product Profile for gonorrhea
Ng/Chlamydia muridarum (Cm) lower reproductive tract coinfection model

- Inoculate with Cm first, followed by Ng 2-5 days later
- Higher numbers of Ng are isolated from Cm-infected mice compared to mice infected with Ng alone.

Results are consistent with a human study in which higher numbers of Ng were isolated from adolescent girls with concurrent Chlamydia trachomatis infection compared to Ng infection alone.

*The Natural History of Incident Gonococcal Infection in Adolescent Women*  Stupiansky et al.  Sex Trans Dis 2011
**Ng/Chlamydia muridarum** endometrial coinfection model

- An upper reproductive tract co-infection model also has been established
  - *Ng* and *Cm* are recovered from vaginal swabs and endometrial tissue for as long as 10 days post transcervical inoculation of both organisms.
  - Have identified time points and a positive control two-drug regimen for drug efficacy studies

*Costenoble-Caherty, in preparation*
Extragenital tract infection models

- Pharyngeal infections are more refractory to treatment than lower urogenital tract infections
  - hTF is not sufficient; appears to be a more host-restricted body site (Connolly and Jerse, unpublished)
  - A combination of host-restricted factors is likely needed

- Rectal infections
  - Not yet successful in female mice
  - Attempt to infect cotton rats unsuccessful (Spencer and Jerse, unpublished)

Disseminated gonococcal infection (DGI) model

- No models yet that replicate dissemination from a mucosal site to the bloodstream
  - Older literature reports bacteremia in mice following intraperitoneal (IP) injection of Ng (Kita, 1985)
  - C1q infant rat model: IP injection of Ng +C1q resulted in bacteremia for 6 days (Nowicki 1995)

- Arthritis models
  - Synovial injection of rats, rabbits (Goldenberg 1983; Flemming 1986)
Animal modeling of Ng infections is indeed a work in progress.

- Identification of in vivo breakpoints for different antibiotics is ongoing and will facilitate testing combination therapies.

- Alleviating the host restriction for iron has allowed establishment of models of Ng endometrial and oviduct infection.
  - PK/PD studies are needed to explore difference in bioavailability in the upper reproductive tract.
  - Characterization of the Ng URT model with respect to host immune responses and pathology is ongoing.

- Ng/C. muridarum lower and upper reproductive tract coinfection models have been established.
  - The higher bioburden of Ng seen in Chlamydia-infected mice and adolescent girls supports the need to test antibiotic efficacy in the context of coinfection.
  - We have established a positive control for testing dual therapies in the Ng/Cm endometrial coinfection model.
  - Attempts to establish a Ng/C. trachomatis coinfection model are underway.

- Extra-genital tract infection models and a DGI model are challenged by more host restrictions.
  - Supplementation of mice with host factors or transgenic mice may solve this problem.
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