Novel preclinical animal models used in active drug development programs: the C3HeB/FeJ model

CPTR NIH Workshop 09-11-2017

Anne Lenaerts, Ph.D.
Topics

1) Brief description of the C3HeB/FeJ model
2) Measurements and tools used to evaluate treatment response and drug exposure
3) Current use in early drug development (single agents)
4) Current use in regimen development
5) Context-of-use for the C3HeB/FeJ mouse model – discussion points
Background: C3HeB/FeJ, aka the ‘Kramnik mouse model’

- Igor Kramnik et al. first to describe a highly susceptible mouse model developing necrotic lesions after *Mtb* infection (in < 4wks post i.v.)
- Mouse Strain **C3HeB/FeJ**; which is a sub-strain of C3H
- Necrotic lesions solely in the lung:
  - No other organ develops necrotic lesions
  - Macrophages in lungs die by necrosis rather than apoptosis (necrosis primarily due neutrophils)
- Due to **recessive allele in the Sst-1 locus**
  (SuperSusceptibility to Tuberculosis-1) at 54.0 cM

MGI:1888685, Informatics.jax.org

PNAS 2000, 97(15), 8560
Pulmonary Lesion Types in C3HeB/FeJ Mice

TYPE I – Caseous Necrotic Lesions
a. Highly organized
b. Central neutrophilic core
c. Caseous necrosis/ Hypoxic
d. Rim of foamy cells
e. Distinct fibrotic rim of collagen
f. High bacterial numbers

TYPE 2 – Uncontrolled, Neutrophilic Lesions
a. Disorganized, rapidly expanding
b. Neutrophil dominated
c. Few lymphocytes
d. No fibrosis
e. Cellular necrosis/ hypoxia
f. High bacterial numbers

TYPE 3 – Cellular Lesions
a. Lymphocyte dominated
b. Foamy Mf / functional Mf
c. Isolated pockets of neutrophils
d. No fibrosis/hypoxia/necrosis
e. Identical to Balb/c lesions
f. Few bacilli

Scott Irwin, et al. 2015. Dis Model Mech. 8(6); 591
Presence of Both Extracellular and Intracellular Bacteria in C3HeB/FeJ Mice

Inflammatory Cellular lesions (Balb/c and C3HeB/FeJ)

Caseous Necrotic lesions (C3HeB/FeJ only)

Bacterial location (by SYBR Gold staining – in green):

1. **Intracellular** bacteria in foamy macrophages around cuff
2. Numerous **extracellular** bacteria in caseum (hypoxic)
3. Few bacteria in activated mØ on the outside of the rim

C3HeB/FeJ mouse model

- Treatment starts 8 weeks after low dose aerosol
- Treatment for 4 weeks to 6 months, 5/7 days
- Larger standard deviations due to lesion heterogeneity
- Minimum 8 mice per group, (+20-25% extra mice due to early mortality)
**Chronic Balb/c**

Uniform pulmonary cellular lesions containing immune cell aggregates. Bacteria are ~99% intracellular in macrophages (mØ).

**Chronic C3HeB/FeJ**

Heterogeneity in pulmonary lesion pathology including caseous necrotic lesions. Bacteria are both intracellular (in mØ and neutrophils) and extracellular (in caseum). Caseum has a unique hypoxic environment, thought to contain more persistent bacterial phenotypes.

“Low Responders”

(Caseous necrotic lesions)

“Responders”

(Small necrotic & cellular lesions)
<table>
<thead>
<tr>
<th></th>
<th><strong>Balb/c</strong></th>
<th><strong>C3HeB/FeJ (‘Kramnik’)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mouse Strain</strong></td>
<td>Inbred</td>
<td>Inbred</td>
</tr>
<tr>
<td><strong>Mtb strain</strong></td>
<td>Mtb Erdman (CSU), H37Rv (JHU)</td>
<td>Mtb Erdman (CSU), H37Rv (JHU)</td>
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<tr>
<td><strong>Pulmonary Mtb lesions</strong></td>
<td>Uniform lesions, consisting of immune cell aggregates</td>
<td>Heterogeneous pathology showing 3 lesion types, including hypoxic caseous necrotic lesions</td>
</tr>
<tr>
<td>(Mtb lesions in spleens)</td>
<td>Uniform: immune cell aggregates</td>
<td>Uniform: immune cell aggregates (similar as Balb/c, same pathology and containing similar bacterial numbers)</td>
</tr>
<tr>
<td><strong>Bacterial location in lungs</strong></td>
<td>~99% intracellular in macrophages (mØ)</td>
<td>High ratio of extracellular bacteria (in caseum) as well as intracellular Mtb (in mØ and neutrophils)</td>
</tr>
<tr>
<td><strong>Bacterial phenotype</strong></td>
<td>Mostly uniform (intracellular in mØ)</td>
<td>Heterogeneity in bacterial phenotypes in various micro-environments, including caseum</td>
</tr>
<tr>
<td><strong>Treatment response</strong></td>
<td>Uniform between mice in a treatment group</td>
<td>Drugs often times show heterogeneous treatment response: reduced activity vs Balb/c, or bimodal activity</td>
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<tr>
<td><strong>Context-of-Use</strong></td>
<td>Efficacy testing of early compounds against a chronic Mtb infection as single agent or in drug regimens; to assess dose response, drug combinations, and assess relapse</td>
<td>Efficacy testing against advanced disease (stringent), for lead compounds, or compounds with unique PK properties, or a drug target (potentially) present in C3HeB/FeJ only. Plus confirmatory for Balb/c results</td>
</tr>
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<td><strong>Data Output (CFU)</strong></td>
<td>Typically tight</td>
<td>Larger standard deviations, which is pathology related (more representative of human response)</td>
</tr>
<tr>
<td><strong>Cost (efficacy test only, 5 cmpds + ctrl drug)</strong></td>
<td>~ $22,500</td>
<td>~ $32,000</td>
</tr>
<tr>
<td><strong>Other benefits:</strong></td>
<td>Robust, historical data available</td>
<td>Model can be used for determination of resistance and lesion PK studies (correlate local drug exposure-efficacy)</td>
</tr>
</tbody>
</table>
**Linking Treatment Response to Pathology and Local Drug Exposure**

Understanding PK/PD of single drugs in mouse model with advanced pathology ....
... to increase our understanding of the *in vivo* efficacy of an individual cmpd,
... thereby identifying a potential role/contribution of a drug in a regimen

**Drug distribution across lung lesions**
(at locations where the bacteria are)

+ **Potency against bacterial phenotypes, in diverse micro-environments (e.g. caseum)**

- Unique micro-environment of caseum:
  - Neutral to elevated ph (ph 7.4-7.6)
  - Hypoxia
  - Elevated RNI (Measured Carl Nathan)
  - High glycerol, lipid content
  - High protein and DNA content (affects PPB)
  - ???

_Modified from figure in V. Dartois, Nat Rev Microbiol. 2014. 12(3):159_
Local Lesion Pharmacokinetics and Drug Distribution

Laser Capture Microdissection

MALDI-MRM-MS imaging

Caseum

Reduced clofazimine levels in caseum

Clofazimine (green – cellular lesion), Cholesterol (blue – in caseum), PC 32:0 (red – uninvolved lung)

In collaboration with Dr. V. Dartois and Dr. B. Prideaux (Rutgers University, New Jersey, NY)
Tools and measurements

**Treatment Response:**
- CFU
- PETscan (JHU)
- Resistance frequency

New tool development:
- RNA-based methods (live/dead)
- *In vitro* assays (MBC in caseum – Rutgers, NR assays + serum, ...)

**Pathology:**
- Gross necropsy results
- Histopathology (classical)
- PETscan (JHU)

New tool development:
- Histopathology (new software devel.)

**Drug distribution in lung:**
- Plasma/lung/Lesion PK (using dissection)
- Local Lesion PK (laser microdissection)
- MALDI imaging (not quantitative, yet!)

New tool development:
- MALDI imaging (quantitative, Rutgers)
- *In vitro* assays (intrabact. and mØ conc, caseum binding, etc.)
When Treatment Response is Different in C3HeB/FeJ versus Balb/c mice ...

**PZA (300 mg/kg)**

**BDQ at 25 mg/kg**

**CFZ at 20 mg/kg**

**C3HeB/FeJ**

**Balb/c**

**PK analysis (LC/MS):**

- BDQ drug level (lung): 22 ug/ml
- BDQ drug level (caseum): 0.97
- MIC BDQ: 0.002-0.06, 99% PPB

**Irwin et al. 2015 ACS Inf Dis**
Use in Lead Optimization Programs

Efficacy Data

<table>
<thead>
<tr>
<th>Activity</th>
<th>MIC</th>
<th>Fu (unbound fraction)</th>
<th>MIC + 4% HSA</th>
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<tbody>
<tr>
<td>Standard PK</td>
<td></td>
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<tr>
<td>Plasma (Cmax, Cmin)</td>
<td></td>
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<tr>
<td>Whole Lung (Cmax, Cmin)</td>
<td></td>
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<tr>
<td>Lesion (Cmax, Cmin)</td>
<td></td>
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<tr>
<td>Laser capture PK</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Uninvolved Lung</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Rim (collag rim, foamy macs)</td>
<td></td>
<td></td>
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<tr>
<td>Outer caseum (neutrophils)</td>
<td></td>
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<td></td>
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<tr>
<td>Inner caseum</td>
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<thead>
<tr>
<th>Efficacy</th>
<th>Lung (Log10CFU reduction)</th>
<th>Spleen (Log10CFU reduction)</th>
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Cmax mean levels

Trough mean levels

“Productive protein binding”

Laser Capture Microdissection (LCM) & LC/MS by V. Dartois, B. Prideaux (Rutgers Univ., NJ)
Confirmation of Sterilizing Regimens from Balb/c Relapse studies in C3HeB/FeJ mice (NiX Study)

Balb/c Lungs

C3HeB/FeJ Lungs

C3HeB/FeJ Lung CFU and Relapse

<table>
<thead>
<tr>
<th>Drug Regimen</th>
<th>PreRx</th>
<th>M1</th>
<th>M2</th>
</tr>
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<tbody>
<tr>
<td>Untreated</td>
<td>7.47 ± 0.14 (6/6)</td>
<td></td>
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<tr>
<td>2HRZE/HR</td>
<td>4.42 ± 0.45 (6/6)</td>
<td>1.25 ± 0.31 (5/6)</td>
<td></td>
</tr>
<tr>
<td>BPa</td>
<td>4.58 ± 0.31 (6/6)</td>
<td>2.39 ± 0.22 (6/6)</td>
<td>15/15 (100)</td>
</tr>
<tr>
<td>BPaL</td>
<td>3.53 ± 0.59 (6/6)</td>
<td>2.42 ± 0.35 (5/6)</td>
<td>14/18 (78)</td>
</tr>
<tr>
<td>BPaLZ</td>
<td>1.22 ± 0.27 (4/6)</td>
<td>0.70 ± 0.00 (1/6)</td>
<td>1/15 (7)</td>
</tr>
<tr>
<td>1BPaL/BPa</td>
<td>2.06 ± 0.53 (5/6)</td>
<td></td>
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Proportion (%) relapsing after treatment for:

<table>
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<tr>
<th>2mo+3obs</th>
<th>3mo+3obs</th>
<th>4mo+3obs</th>
</tr>
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<tbody>
<tr>
<td>15/15 (100)</td>
<td>12/14 (86)</td>
<td>1/15 (7)</td>
</tr>
<tr>
<td>9/16 (56)</td>
<td>4/15 (27)</td>
<td>0/20 (0)</td>
</tr>
<tr>
<td>11/21 (52)</td>
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</table>

BPaLZ > 1BPaL/BPa = BPaL > BPa > 2HRZE/HR
Proposed Context-of-Use of the C3HeB/FeJ mouse model

- More stringent mouse model: most drugs show reduced efficacy in C3HeB/FeJ versus Balb/c, or show bimodal activity
- Efficacy testing against advanced disease with lesion heterogeneity - practical implications: larger standard dev (high mouse numbers, more labor-intensive).
- C3HeB/FeJ mouse model is not likely to replace the Balb/c mouse model
- Evaluation of Single Drugs:
  - For leads and drug candidates, to confirm efficacy as a single drug
  - Use the C3HeB/FeJ model as a tool to understand PK/PD against advanced disease
  - For compounds with a unique drug target or metabolic pathway in *Mtb* which is only or more highly expressed in C3HeB/FeJ, due to unique local microenvironmental conditions
  - For compounds with unique PK properties, e.g. with high protein binding, or accumulation in mØ (potentially overestimated in Balb/c with mostly intracellular bacilli)
  - Resistance evaluation *in vivo* (facilitated due to high bacterial numbers in lungs)
- Evaluation of Regimens:
  - To confirm sterilizing potential of a novel regimen identified in Balb/c relapse mouse model versus the standard regimen, confirm rank order
  - Resistance evaluation *in vivo* (facilitated due to high bacterial numbers in lungs)
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