Pre-clinical tools for evaluating new components of TB regimens

Eric Nuermberger, M.D.
Center for TB Research
Johns Hopkins University
July 19, 2017
“All models are wrong, some are useful.”

George Box
**In vitro and in vivo models**

**Pros & cons**

**In vitro models**

**Pros**
- controlled microenvironment
- unlimited range of doses, schedules
- more precise measurement of drug conc. to which *Mtb* is exposed
- simple, serial sampling of *Mtb* products

**Cons**
- difficult to account for host effects on lesion microenvironment, microbial growth and susceptibility, and drug exposures at site of infection

**In vivo models**

**Pros**
- embody dynamic interaction b/w host, drug and microbe & represent impact of pathology
- enable simultaneous study of multiple sub-populations, perhaps in clinically relevant proportions

**Cons**
- limitations in dose size and schedules
- often difficult to mimic human PK
- may or may not represent diverse human disease states well
• HFS-TB qualified for use in drug development programs as an additional and complementary tool
• HFS-TB can be used in regulatory submissions, esp. for informed design and interpretation of clinical studies
• HFS-TB is recommended to be useful as follows:
  – To provide preliminary proof of concept for developing a specific drug or combination to treat tuberculosis
  – To select the pharmacodynamic target (e.g. T_{>MIC}, AUC/MIC)
  – To provide data to support PK/PD analyses leading to initial dose selection for non-clinical and clinical studies
  – To assist in confirming dose regimens for later clinical trials taking into account human PK data and exposure-response relationships
Some unanswered questions for HFS-TB

- Reproducibility?
- Obstacles to technology transfer and uptake?
- Reliability of estimates of drug exposures at site of infection (e.g., using free drug fraction, ELF penetration ratios)?
- Predictive accuracy for efficacy of regimens?
  - rank ordering existing and novel regimens
  - estimating absolute or relative treatment durations
- Optimal integration of log phase and sterilizing effect models to predict regimen efficacy?
“Correlations between drug concentration and pathogen survival that are based on in vitro models cannot be expected to reiterate all aspects of in vivo antimycobacterial treatment.”

Chilukuri et al, CID 2015; 61(S1):S32
Scheme for relapse-based experiments in mice

- **Day -14**: Initial treatment begins.
- **Day 0**: Inoculation of bacilli into mice.
- **M1** to **M5**: Sequential treatment phases.
- **Lung CFU counts**: Assessed on treatment.
- **Relapse-free cure** (absence of cultivable bacilli): Assessed after holding mice without treatment for 3-6 months.
Current uses of mouse models in the context of TB regimen development

- Derive (or confirm) PK/PD relationships for selecting optimal doses of component drugs
- Rank order drug combinations on the basis of efficacy
- Estimate treatment-shortening potential
- Assess impact of caseous pathology (eg, in Kramnik mice)
- Estimate potential for selection of drug-resistant mutants
Recapitulation of the short-course regimen in the mouse...as in humans

Log_{10} cfu in lungs

- INH + SM
- INH + RIF
- INH + RIF + PZA

AAC (2016); 60:1091
Performance of novel regimens in BALB/c mice (HDA model)

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Treatment-shortening effect (in months) relative to RHZ(E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMZ(E)</td>
<td>1 - 1.5</td>
</tr>
<tr>
<td>PaMZ</td>
<td>0 - 1</td>
</tr>
<tr>
<td>BPaMZ</td>
<td>3 - 3.5</td>
</tr>
<tr>
<td>BPaL</td>
<td>1-1.5 months</td>
</tr>
</tbody>
</table>

Pa = pretomanid; M = moxifloxacin; Z = pyrazinamide; B = bedaquiline; L = linezolid

• The PCS working group of CPTR has embarked on an effort to quantify the predictive accuracy of the “sterilizing” mouse model
• New regimens in, or advancing to, phase 3 trials will provide additional opportunities to evaluate the correspondence
Contribution of component drugs to the efficacy of the BPaMZ regimen

Bactericidal effect

Sterilizing effect

<table>
<thead>
<tr>
<th>Regimen</th>
<th>M1.5 (+3)</th>
<th>M2 (+3)</th>
<th>M3 (+3)</th>
<th>M4 (+3)</th>
<th>M5 (+3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RHZ</td>
<td></td>
<td></td>
<td></td>
<td>10/15</td>
<td>2/15</td>
</tr>
<tr>
<td>PaMZ</td>
<td></td>
<td></td>
<td></td>
<td>10/14</td>
<td>3/15</td>
</tr>
<tr>
<td>JPaM</td>
<td></td>
<td></td>
<td></td>
<td>2/15</td>
<td>0/14</td>
</tr>
<tr>
<td>JPaZ</td>
<td>13/14</td>
<td>0/15</td>
<td>0/15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>JPaMZ</td>
<td>3/15</td>
<td>0/15</td>
<td>0/15</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Li et al, AAC 2017; in press
Challenges in translating mouse model results to the clinic

Differences in host response and lung pathology

Differences in:
- *Mtb* growth rate
- Intra/extracellular residence
- Drug distribution
- Lesion microenvironment
Examples of different outcomes in C3HeB/FeJ mice compared to BALB/c mice

• Lack of pyrazinamide bactericidal effect in large caseous lesions, where caseum has neutral pH\(^1\)
  – yet, addition of PZA to RIF+INH+EMB still shortens treatment duration\(^2\)

• Lack of clofazimine bactericidal effect in large caseous lesions, where CFZ diffuses poorly and caseum has neutral pH and is hypoxic\(^3\)

• Reduced bedaquiline effect in large caseous lesions, where BDQ diffuses poorly\(^4\)

1. Lanoix et al, AAC 2016; 60:735
2. Lanoix et al, AAC 2016; 60:1091
3. Irwin et al, AAC 2014; 58:4026
Limited experience comparing regimens in other animal models with caseous pathology

• Guinea pigs
  – Replacing RIF with RPT had no significant treatment-shortening effect\(^1\)
  – PaMZ had no significant treatment shortening effect compared to RHZ\(^2\)

• Rabbits
  – No regimen comparisons found

• Marmosets
  – RHZE reduced the extent of disease on PET-CT and lowered CFU counts in cavities compared to HS after 6 weeks of treatment\(^3\)

• Macaques
  – Metronidazole did not increase the bactericidal effect of RH

---

1. Ahmad et al, AAC 2012; 56:3726
2. Dutta et al, AAC 2013; 57:3910
3. Via et al, AAC 2014; 59:4181
Evaluating drug partitioning into TB lesions

MALDI-MSI

Laser-capture microdissection and LC/MS-MS

B Prideaux et al, Nature Medicine, 2015

M Zimmerman et al, AAC, 2017
Additional challenges in translating mouse model results to the clinic

- Inter-species differences in drug PK
- Experiments in inbred mice infected with 1 $Mtb$ strain and treated with identical drug doses cannot recapitulate the many sources of heterogeneity in human TB:
  - PK variability
  - Severity of disease (eg, presence of cavities, cavity size)
  - Immune status
  - Adherence to treatment
  - $Mtb$ drug susceptibility
Translational (mouse → human) PK/PD Model

Objectives

• Develop a translational PK/PD model that utilizes:
  – mouse PK data for RIF, RPT and MXF
  – mouse PD data (CFU counts) for RIF, RPT and MXF alone and in combinations including PZA + INH or EMB
  – human PK data, including rifamycin-MXF interaction
  – an immune effect on bacterial death derived from CFU differences between nude and BALB/c mice
  – inter-species differences in protein binding
  – effect of caseation and cavitation on lesion distribution of rifamycins

• Perform clinical trial simulations to predict trial outcomes
  – sputum culture status at 8 wks
  – relapse status at 1 yr
Translational (Mouse → Human) PK/PD Model

R = rifampin  
P = rifapentine  
M = moxifloxacin  

B_{\text{MAX}} = maximum number of bacteria  
K_{\text{growth}} = bacterial growth constant  
K_{\text{death}} = bacterial death constant  
\tau_{50} = time of 50\% of max. immune response  
\theta_{\text{KIND}} = max. immune kill rate (untreated mice)  
\gamma_{\text{immune response}} = sigmoidicity factor, defines shape of immune response effect  
\theta_{\text{KDOI.0}} = immune kill rate (treated mice) at average incubation time  
\theta_{\text{KDOI.t}} = increase in kill rate (treated mice) in expts w/above average incubation time  
E_{\text{drug}} = drug effect  
E_{\text{max}} = max. achievable drug effect  
E_{\text{C50}} = antibiotic conc. producing 50\% of E_{\text{max}}  
\gamma = sigmoidicity factor, defines the shape of drug effect

Bartelink et al, Clinical and Translational Science, In press
Using the final translational PK/PD model:
Predicted vs. observed trial results

pred/observed CFU free patients (solid culture)

RIFAXIN

data

Perprotocol
sim

pred/observed relapse free patients after treatment

rifampin?moxifloxacin MAMS trial

Bartelink et al, Clinical and Translational Science, In press
Translational PK/PD Model - conclusions

• The model performed reasonably well, especially in predicting higher relapse rates for 4-month arms

• Work is ongoing to:
  – incorporate individual PK/PD and dose-response for all drugs in regimens
  – simulate phase 3 trials with more novel regimens
  – incorporate drug-resistant sub-populations to predict rates of resistance emergence
  – merge PK/PD model with mechanistic within-host model to gain greater insight into factors driving regimen performance
Assessing the risk of resistance amplification

Low R exposures lead to acquired INH resistance in 2 of 10 mice

Impact of simulated RIF PK variability in C3HeB/FeJ mice

Impact of intermittency and immunodeficiency in nude mice

Recapitulating the arms of TBTC Study 22, selection of AHR and ARR were associated with:

- **immunosuppression**
  - nude mice more likely than BALB/c to have AHR (8.5% vs 0%, p= 0.001) and ARR (3.5% vs. 0%, p= 0.06)

- **intermittent vs daily initial phase therapy**
  - 30% vs 2.7% for AHR/ARR (p< 0.001)
  - 20% vs 2.7% for ARR only (p< 0.01)

- **once-weekly RPT vs RH in contin. phase**
  - 18% vs 3.3% for ARR (p< 0.05)

Park et al, submitted
Take-home points

• *In vitro* hollow fiber models are qualified as useful tools for exploring PK/PD relationships under controlled conditions

• Mouse models have an established track record in estimating the treatment-shortening potential of novel regimens

• The impact of certain variables that modify the effect of some drugs may require elucidation in caseous disease models:

• Emerging data from clinical trials with novel regimens will provide a great opportunity for further evaluating the predictive accuracy of these and other preclinical models

• Some factors are more difficult to account for in pre-clinical models and may be best address with more predictive PK/PD-based translational models:
  o inter-species PK differences in PK, protein binding, etc
  o human PK variability
  o heterogeneity in human host (eg, cavitation, immune response)
  o heterogeneity in bacterial pathogen (eg, MIC distribution)
Acknowledgements

• Members of the lab and the JHU Center for TB Research

• Collaborators
  – Veronique Dartois (Rutgers)
  – Tawanda Gumbo (Baylor)
  – Debra Hanna and CPTR PCS-WG
  – Anne Lenaerts, Scott Irwin (CSU)
  – Chuck Peloquin (UF)
  – Rada Savic, Imke Bartelink, Nan Zhang (UCSF)

• Funding
  – NIAID (R01-AI090820, R01-AI111992)
  – Global Alliance for TB Drug Development
  – FDA (U18-FD004004)
  – Gates Foundation (TBDA #42581, OPP1037174)
  – C-Path
  – CDC TB Trials Consortium
A 4-fold higher EC$_{50}$ for P in pts with cavitary disease.

Savic, R.M. et al. CPT 2017 Jan. 25

A 4-fold higher EC$_{50}$ for P in the cavity compartment compared to plasma was used in the simulation.

Bartelink, I. et al. CPT 2017
Contribution of each component to the efficacy of the BPaL regimen

**Bactericidal effect**

**Sterilizing effect**

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Proportion relapsing after treatment for:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 months</td>
</tr>
<tr>
<td>2RHZ/RH</td>
<td>8/14</td>
</tr>
<tr>
<td>JPa</td>
<td>3/14</td>
</tr>
<tr>
<td>JPaL</td>
<td>0/15</td>
</tr>
<tr>
<td>2JPaL/1JPa</td>
<td>6/15</td>
</tr>
<tr>
<td>1JPaL/2JPa</td>
<td>9/15</td>
</tr>
</tbody>
</table>
Recapitulation of the short-course regimen in the mouse...as in humans

![Graph showing Log10 cfu in lungs over months for INH + SM and INH + RIF regimens.]

- **INH + SM**:
  - 20% relapse (human: 7%)
  - 0% relapse (human: 1-3%)
  - 75% relapse (human 10%)

- **INH + RIF**:
  - 75% relapse (human 10%)

References:
Recapitulating the evolution of short-course therapy in mice and humans*

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Months</th>
<th>Proportion Relapsing after Treatment:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mice</td>
</tr>
<tr>
<td>INH+SM</td>
<td>6</td>
<td>100%</td>
</tr>
<tr>
<td>INH+SM</td>
<td>18</td>
<td>75%</td>
</tr>
<tr>
<td>INH+RIF</td>
<td>6</td>
<td>20%</td>
</tr>
<tr>
<td>INH+RIF</td>
<td>9</td>
<td>0-5%</td>
</tr>
<tr>
<td>INH+RIF+PZA</td>
<td>4</td>
<td>70-90%</td>
</tr>
<tr>
<td>INH+RIF+PZA</td>
<td>6</td>
<td>0-5%</td>
</tr>
</tbody>
</table>

*From Mitchison; and Grosset & Ji; in Gangadharam & Jenkins, Chapman & Hall, 1998
Moxifloxacin for treatment shortening: The REMoxTB phase 3 trial

Time to sputum conversion

- Pts in MXF arms converted sooner ($p < 0.01$)

Time to unfavorable outcome

- Pts in MXF arms relapsed more often and faster ($p < 0.01$)

Gillespie et al, NEJM 2014
Measuring the treatment-shortening effect of a test regimen relative to a control regimen in mice

Colored symbols represent the proportion of mice relapsing after receiving the indicated regimen for various durations (error bars represent the 95%CI).

Treatment-shortening effect of substituting moxifloxacin for isoniazid in the 1\textsuperscript{st}-line regimen in BALB/c mice

A 1 to 1.5-month treatment shortening effect is observed in our standard model.

Data from 5 experiments (4 JHU, 1 CSU)

Outcomes with REMox-TB regimens in mice

• In REMox-TB, substitution of M for H or E resulted in faster sputum conversion but did not permit shortening the duration of treatment by 2 months

• In mice, substitution of M for H resulted in:
  – treatment shortening of 1-1.5 months in high-dose infection models
  – treatment shortening of 0-1 month in low-dose infection models

• In mice, substitution of M for E resulted in:
  – treatment shortening of 0-1 month in low-dose infection models

• Results in mice are not inconsistent with those of REMox-TB
Treatment-shortening effect of substituting moxifloxacin for isoniazid in the 1st-line regimen

The treatment shortening effect is between 0-1 month in low-dose aerosol infection models in BALB/c and Kramnik mice.

Data from 2 experiments (1 JHU, 1 CSU) per mouse strain

Treatment-shortening effect of substituting moxifloxacin for ethambutol in the 1\textsuperscript{st}-line regimen

The treatment shortening effect is between 0-1 month in low-dose aerosol infection models in BALB/c and Kramnik mice.

Data from 2 experiments (1 JHU, 1 CSU) per mouse strain

Substitution of M for H in the RHZ regimen in mice – data from 3 institutions, using low- & high-dose infection models

**A. High dose aerosol infection (BALB/c)**
- RHZ
- RMZ
- RHZ minus 1 month
- RHZ minus 2 months

**B. High dose IV infection**
- RMZ
- RHZ

**C. Low dose aerosol infection (BALB/c)**
- RMZ ± E
- RHZ ± E

**D. Low dose aerosol infection (C3HeB/FeJ)**
- RMZE
- RHZE
Substitution of M for E in RHZE – data from 2 institutions in chronic infections in 2 mouse strains

Low dose aerosol infection (BALB/c)

Low dose aerosol infection (C3HeB/FeJ)
Substitution of M for H in the RHZ regimen in mice – data from 3 institutions, using low- & high-dose infection models
Correspondence between results of REMox-TB trial and those in mice

• In REMox-TB, substitution of M for H or E resulted in faster sputum conversion but did not permit shortening the duration of treatment to 4 months

• In mice, substitution of M for H or E reduced CFU cts more rapidly, but relapse rates were inevitably higher when RMZ(E) or RHZM duration was reduced by 2 months relative to RHZ(E)

• Results in C3HeB/FeJ mice may be closer to those of REMox-TB
  – smaller difference for RMZE relative to RHZE
  – no difference between RHZM and RMZE

• The most severely affected C3HeB/FeJ mice may best represent pts most likely to relapse
Bacterial killing with $\text{Pa}_50\text{MZ}$ vs. RHZ: mouse vs. NC-002 results

Long = 6 wks from low-dose infxn to treatment onset
Short = 2 wks from high-dose infxn to treatment onset
PaMZ vs. RHZ, RMZ
HDA model in BALB/c mice – relapse data

**PaMZ vs. RHZ**

- RHZ
- Pa$_{100}$MZ

**PaMZ vs. RMZ**

- RMZ
- Pa$_{100}$MZ

Data from 5 experiments (3 with Pa 100mpk, 2 with Pa 50mpk)  
Data from 5 experiments (2 with head-to-head data)
PaMZ vs. RHZ, RMZ

HDA model in BALB/c mice – relapse data

The treatment shortening effect is between 0-1 month when compared to RHZ and depends on the Pa dose. PaMZ is no more effective than RMZ and appears less effective than RMZ when the Pa dose is 50 mpk.

Data from 5 experiments (3 with Pa 100mpk, 2 with Pa 50mpk)
PaMZ vs. RHZ
HDA model in BALB/c mice – relapse data

In one experiment in which a lower-dose aerosol infection was used in a 14-day incubation model to match Day 0 CFU counts with a low-dose chronic (42-day) infection model (right panel), the treatment shortening effect of PaMZ was at least 1 month.

Data from 5 experiments (3 with Pa 100mpk, 2 with Pa 50mpk)
PaMZ vs. RHZ
Chronic LDA model in BALB/c mice, guinea pigs

In the chronic low-dose aerosol model in BALB/c mice, Pa50MZ is equivalent to RHZ.
In the chronic guinea pig model, the treatment shortening effect of PaMZ is between 0 and 1 month.

Data from 3 experiments
Dutta et al, AAC (2013); 57:3910
PaMZ vs. RHZ
LDA model in C3HeB/FeJ mice – relapse data

In C3HeB/FeJ mice, Pa$_{50}$MZ is less effective than RHZ except in one expt in which Pa, M and Z dosing was divided twice daily.
Conclusions re: PaMZ

• Results of the abbreviated STAND trial comparing 2RHZE/4RH to 4PaMZ and 6PaMZ should be available soon & will provide a basis for comparison of mouse and human results

• In our standard high-dose aerosol model in BALB/c mice:
  – PaMZ requires 0-1 month less treatment to cure compared to RHZ, and this effect is somewhat dose-dependent
  – PaMZ requires 0-1 month more treatment to cure compared to RMZ
In the high-dose aerosol model in BALB/c mice, BPa_{100}MZ shortens the duration of treatment by 3-3.5 months compared to RHZ.
In the high-dose aerosol model in BALB/c mice, BPa\textsubscript{100}MZ shortens the duration of treatment by 3-3.5 months compared to RHZ.

BPamZ data from 2 experiments
BPaL vs. RHZ

HDA model in BALB/c mice

BPaL has a 1-1.5 month treatment-shortening effect in BALB/c mice.
"Dichotomous" activity of PZA in C3HeB/FeJ mice

C3HeB/FeJ

BALB/c

Treatment group

Lung log_{10} CFU

D0 None PZA

Lung log_{10} CFU

D0 None PZA

4.60 7.68
Z adds sterilizing activity to RHE in BALB/c and C3HeB/FeJ mice

**BALB/c**

- **RHE/RH**: 100
- **RHEZ/RH**: 70
- **RHE/RH**: 40
- **RHE/RH**: 10

**Relapse proportion**

- 3 months
- 4.5 months
- 6 months

**C3HeB/FeJ**

- **RHE/RH**: 100
- **RHEZ/RH**: 80
- **RHE/RH**: 40
- **RHEZ/RH**: 20

**Relapse proportion**

- 3 months
- 4.5 months
- 6 months

* indicates significance levels.
Sterilizing activity of Z in 1st-line regimen in 2 mouse strains

**A**

- **BALB/c mice**
  - Relapse proportion
  - M3+3: 100%
  - M4.5+3: 67%
  - M6+3: 0%

**B**

- **C3HeB/FeJ mice**
  - Relapse proportion
  - M3+3: 95%
  - M4.5+3: 53%
  - M6+3: 20%

**C**

- **BALB/c mice**
  - CFU count (log10/lung)
  - M3+3: 100%
  - M4.5+3: 67%
  - M6+3: 7%

**D**

- **C3HeB/FeJ mice**
  - CFU count (log10/lung)
  - M3+3: 95%
  - M4.5+3: 53%
  - M6+3: 20%
Increasing duration of PZA increases sterilizing effect in C3HeB/FeJ mice

M3+3  M4.5+3  M6+3

Lanoix et al, AAC (2016); 60:1091
Z Adds Sterilizing Activity to RHE in BALB/c and C3HeB/FeJ Mice

<table>
<thead>
<tr>
<th>Study</th>
<th>Regimen*</th>
<th>Follow-up (months)</th>
<th>Patients assessed</th>
<th>Bacteriological relapses</th>
</tr>
</thead>
<tbody>
<tr>
<td>East African/BMRC (current)</td>
<td>2SHRZ/HRZ</td>
<td>24</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2SHRZ/HR</td>
<td>24</td>
<td>40</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Singapore/BMRC (1981)</td>
<td>2SHRZ/HRZ</td>
<td>24</td>
<td>78</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2SHRZ/HR</td>
<td>24</td>
<td>80</td>
<td>2 (2%)</td>
</tr>
</tbody>
</table>

Can we really conclude that continuing Z beyond 2 months would not benefit the most difficult-to-cure patients?

*Z was associated with lower mean CFU count among relapsing mice
†2RHEZ/2.5RHZ was associated with fewer relapses than 2RHEZ/2.5RH

Lanoix et al, AAC (2016); 60:1091
Fox, Br J Dis Chest (1981); 75:331
Activity of clofazimine in C3HeB/FeJ mice

BALB/c

CFZ effect size in lungs

4 log

C3HeB/FeJ

1 log

SM Irwin et al, AAC 2014; 58:4026
Activity of clofazimine in C3HeB/FeJ mice

CFZ effect size in spleens

SM Irwin et al, AAC 2014; 58:4026
Activity of clofazimine in C3HeB/FeJ mice

3-wk incubation

CFZ effect size in lungs

7-wk incubation

5 log

1.5 log
Compartmentalized activity of CFZ – PK/distribution

• Slow, steady accumulation in adipose tissue & macrophages
  – pH-dependent ion trapping in lysosomes
• Poor distribution into caseum relative to cavity wall
Data inventory

- Focus first on mouse strains other than C3HeB/FeJ ("Kramnik")
- Inventory identified a variety of relapse-based pre-clinical studies with corresponding clinical trial outcomes data

<table>
<thead>
<tr>
<th>Test regimen intervention</th>
<th>Regimen comparison</th>
<th># of expts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combining INH+STR</td>
<td>HS vs. H or S monotherapy</td>
<td>1</td>
</tr>
<tr>
<td>Shortening duration of INH+STR</td>
<td>6HS vs. 18HS</td>
<td>1</td>
</tr>
<tr>
<td>Adding RIF to INH+STR or INH+EMB+PZA</td>
<td>HR (or HRS or HREZ) vs. HS (or HEZ)</td>
<td>4</td>
</tr>
<tr>
<td>Adding STR to INH+RIF</td>
<td>HRS vs. HR</td>
<td>1</td>
</tr>
<tr>
<td>Adding PZA to INH+RIF (±STR/EMB)</td>
<td>HRZ (or HRSZ or HREZ) vs. HR (or HRS or HRE)</td>
<td>4</td>
</tr>
<tr>
<td>Shortening duration of PZA</td>
<td>2HREZ/4RH vs. 6HREZ</td>
<td>1</td>
</tr>
<tr>
<td>Increasing dose of RIF</td>
<td>High-dose R plus HEZ vs. HREZ</td>
<td>2</td>
</tr>
<tr>
<td>Extending dosing interval of 1st-line Rx</td>
<td>HREZ (2/7) vs. HREZ (daily)</td>
<td>1</td>
</tr>
<tr>
<td>Replacing EMB with MXF</td>
<td>HRMZ vs. HRZ(E)</td>
<td>3</td>
</tr>
<tr>
<td>Replacing INH with MXF</td>
<td>MRZ(E) vs. HRZ(E)</td>
<td>10</td>
</tr>
<tr>
<td>Replacing RIF with RPT</td>
<td>HPZ(E) vs. HRZ(E)</td>
<td>7</td>
</tr>
<tr>
<td>Replacing RIF+EMB with RPT+MXF</td>
<td>HPMZ vs. HRZ</td>
<td>3</td>
</tr>
<tr>
<td>Replacing RIF with RPT and extending dosing interval</td>
<td>HP(1/7) cont phase vs. HR(2/7)</td>
<td>2</td>
</tr>
<tr>
<td>(in continuation phase)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comparing INH+RIF+PZA+EMB with PMD+MXF+PZA</td>
<td>PaMZ vs. HRZ(E)</td>
<td>8</td>
</tr>
</tbody>
</table>
Summary points

• An initial step to address the “translational gap” is to learn what data from what models analyzed in what way best inform key trial design decisions.

• Evidence-based validation of pre-clinical models is important:
  – to confidently place preclinical models on the critical development path,
  – to increase the efficiency of regulatory interactions,
  – to set a precedent for objective, data-driven processes to apply to other models (e.g., C3HeB/FeJ mouse, marmoset), and
  – to identify gaps in knowledge & in existing tools to drive future research.

• Evaluation of sterilizing mouse models is the appropriate first step for *in vivo* models, with other models to follow
Clofazimine has no EBA in TB patients
Serial sputum colony counts over 1st 14 days of treatment

A Diacon et al, AJRCCM 2015
Compartmentalized activity of CFZ and slow onset of effect – PK/distribution

- >1 month to reach steady state
- Slow, steady accumulation in adipose tissue & macrophages
  - pH-dependent ion trapping in lysosomes

D Everitt et al, derived from Schaad-Lanyi et al

![Graph showing simulated clofazimine plasma concentration over time.](image.png)
*In vitro* EBA of INH and CFZ at similar multiples of their MICs

- MICs: INH = 0.05 µg/mL, CFZ = 0.25 µg/mL

N. Ammerman et al, accepted for publication
EBA of INH and CFZ in BALB/c mice

N. Ammerman et al, accepted for publication
Incorporation of CFZ into the 1\textsuperscript{st}-line regimen in mice

\begin{table}
\centering
\begin{tabular}{|c|c|c|c|c|c|}
\hline
Regimen & 2 mo & 3 mo & 4 mo & 5 mo & 6 mo \\
\hline
2RHZE/4RH & -- & -- & 5/15 & 3/15 & 1/10 \\
2RHZC/2RHC & 8/15 & 0/15 & 0/15 & -- & -- \\
\hline
\end{tabular}
\end{table}
Comparative activity of RIF and RPT in BALB/c and C3HeB/FeJ mice over 4 wks of treatment
## RHZE vs. PHZE in 2 mouse strains

<table>
<thead>
<tr>
<th>Drug regimen</th>
<th>Mouse strain</th>
<th>% (proportion) of mice with relapsing after treatment for:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 month</td>
</tr>
<tr>
<td>R_{10}HZE</td>
<td>BALB/c</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>C3HeB/FeJ</td>
<td>ND</td>
</tr>
<tr>
<td>P_{10}HZE</td>
<td>BALB/c</td>
<td>100% (14/14)</td>
</tr>
<tr>
<td></td>
<td>C3HeB/FeJ</td>
<td>100% (13/13)</td>
</tr>
</tbody>
</table>

2 month shortening with P in BALB/c

Rosenthal et al, AAC 2012; 56:4331
**RHZE vs. PHZE in 2 mouse strains**

<table>
<thead>
<tr>
<th>Drug regimen</th>
<th>Mouse strain</th>
<th>% (proportion) of mice with relapsing after treatment for:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 month</td>
</tr>
<tr>
<td><strong>Relapse</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R\textsubscript{10}HZE</td>
<td>BALB/c</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>C3HeB/FeJ</td>
<td>ND</td>
</tr>
<tr>
<td>P\textsubscript{10}HZE</td>
<td>BALB/c</td>
<td>100% (14/14)</td>
</tr>
<tr>
<td></td>
<td>C3HeB/FeJ</td>
<td>100% (13/13)</td>
</tr>
</tbody>
</table>

< 1 month shortening with P in C3HeB/FeJ

Rosenthal et al, AAC 2012; 56:4331
PZA PK/PD in non-clinical models

• AUC/MIC correlates best with activity\(^1\)

• AUC associated with a -0.11 CFU/day reduction:
  – Hollow fiber system = 1500 µg-h/ml\(^1\)
  – BALB/c mouse = 323 µg-h/ml\(^2\)

• More potent effect of PZA in mice is likely due to lower pH (≤ 5) inside mature phagosomes of activated macrophages\(^3\) vs. that in the HFS-TB (5.8) which effectively reduces the PZA MIC by ~10x

• Increasing current dose by 2-4x increases kill rate\(^1,2\)

---

\(^1\) Gumbo et al, AAC (2009); 53:3197
\(^2\) Lanoix et al, AAC (2016); 60:735
\(^3\) Vandal et al, Nat Med 2008; 14:849
PZA (Z) is relatively ineffective in immunocompromised mice

As in IFN-γ-KO mice (above), phagosomes containing M.tb do not mature & develop sufficiently low pH for cidal effect of PZA

Almeida et al, Mycobact Dis 2014; 4:145

Clofazimine PK: mouse vs. human dose equivalence

**Mouse**
- 20 mg/kg = ~200 mg
- 10 mg/kg = ~100 mg

**Human**

---

Swanson et al, AAC 2015; 59:3042

In vitro EBA of INH and CFZ at similar multiples of their MICs

MICs: INH = 0.05 µg/mL, CFZ = 0.25 µg/mL

N. Ammerman, R. Swanson, J. Grosset, JHU