TB-Epidemiology

- 2 billion people are infected with latent TB
- 1 in 10 people infected with TB bacilli will become sick with active TB in their lifetime
- Nearly 2 million deaths are caused by TB annually (5000 people/day)
- ~10 million new cases/year
- TB is a leading killer among HIV-infected people with weakened immune systems
- Resistant TB
  - 500,000 cases of MDR-TB in all 109 WHO countries
  - 55 countries: XDR-TB
Anti-TB Drugs

First-line drugs
- Rifampin
- Isoniazid
- Pyrazinamide
- Ethambutol

Second-line drugs
- Fluoroquinolones (moxifloxacin, gatifloxacin, levofloxacin)
- Injectable agents: Aminoglycosides (streptomycin, amikacin, kanamycin)
- Polyamides (capreomycin)
- Oral bacteriostatic agents: ethionamides, prothionamides, cycloserine, terizidone, p-aminosalicylic acid, thioacetazone
- Agents with unclear efficacy: clofazimine, amoxicillin plus clavulanate, clarithromycin, linezolid

Lee 562: Pharmacokinetics

<table>
<thead>
<tr>
<th>Route</th>
<th>Half-life (hr)</th>
<th>AUCinf (µg-hr/L)</th>
<th>Volume of Distribution (L/kg)</th>
<th>Clearance (L/hr/kg)</th>
<th>Bioavailability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV</td>
<td>1.3</td>
<td>956</td>
<td>28.9</td>
<td>12.9</td>
<td></td>
</tr>
<tr>
<td>ORAL</td>
<td>3.69</td>
<td>1519</td>
<td>386</td>
<td>71.6</td>
<td>15.9</td>
</tr>
</tbody>
</table>

- % Excreted unchanged Lee 562 into urine and feces was less than 1% after both oral and IV administration

MIC 0.006 mg/L

ORAL, 100 mg/kg

IV, 10 mg/kg
Lee 562: Metabolic Stability

Pooled rat liver microsomes

% Remaining after 60 min: 0.46%

Second Generation: Structure

1st generation

2nd generation

Lee 562

1st generation

Lee 878

- MIC 0.006 mg/L
- Microsomal stability: 31.4% remaining after 90 min

Lee 952

- MIC 0.006 mg/L
- Microsomal stability: 4.3% remaining after 90 min
Second Generation: In Vivo PK

Lee 878

IV, 10 mg/kg

ORAL, 100mg/kg

F = 27.4%

Lee 952

IV, 10 mg/kg

ORAL, 100mg/kg

F = 15.9%

Third Generation

Metabolic Stability

% Remaining

OBC-67683
Lee 980
Lee 1119
Lee 1180
Lee 1131
Lee 1120
Lee 992
Lee 1053
Lee 879
Lee 1106
Lee 1119
Lee 880
Third Generation: Structure

1st generation

2nd generation

3rd generation

Third Generation: In Vivo PK

Lee 1106

Good in vitro activity: MIC 0.025 mg/L
Good metabolic stability: >70% @ 90 min
Long elimination half-life: 10.3 hr
Poor Oral Bioavailability (4.6%) secondary to limited aqueous solubility overcome by formulation

Hurdle et al., J Antimicrob Chemother 2008; 62: 1037-45
In vitro PK/PD Model to Determine Time-Kill Curves of Antibiotics against Slow-Growing Microorganisms

✓ *Mycobacterium bovis BCG* as surrogate for virulent *M. tuberculosis*

### Time-Kill Curves: Experimental

- Sample to be counted
- 100 µL
- 100 µL
- 100 µL
- 100 µL
- 100 µL
- 100 µL
- 100 µL
- 100 µL
- 900 µL medium
- 1/100 (10⁻²)
- 1/1000 (10⁻³)
- 1/10000 (10⁻⁴)
- 1/100000 (10⁻⁵)
- 1/1000000 (10⁻⁶)
- 1/10000000 (10⁻⁷)
- Incubation at 37°C for 4 weeks
- Plate 100 µL samples
- Too many colonies to count
- 159 colonies
- 17 colonies
- 2 colonies
- 0 colonies
- Plate count
- 159
- Dilution factor
- 10⁴
- CFU/mL of Original Sample
- 1.59 x 10⁴
Time-Kill Curves: Isoniazid

Time-Kill Curves: Lee 1106

Data Analysis

\[ \frac{dN}{dt} = \left[ k_o \cdot \left( 1 - \frac{N}{N_{\text{max}}} \right) - \left( \frac{I_{\text{max}} \cdot C}{IC50A + C} \right) \right] \cdot N \]

Growth function \hspace{1cm} \text{Kill function (drug effect)}

\[ IC50_A = IC50 \cdot e^{\left( \text{Adaptation Constant} \cdot \frac{N}{AUC_{0-24}} \right)} \]

- \( N \): Number of bacteria (CFU/mL)
- \( N_0 \): Initial cell numbers in the flask
- \( N_{\text{max}} \): Maximum cell numbers in the flask
- \( k_o \): Maximum growth rate constant in the absence of drug
- \( I_{\text{max}} \): Maximum bacterial kill rate
- \( IC50 \): Free concentration required to produce half-maximal effect
- \( IC50A \): Adaptive \( IC50 \)
- \( AUC_{0-24} \): Area under free concentration-time profile of Lee 1106
Time-Kill Curves: Lee 1106

**Observed & Predicted Data vs. Time**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Estimate (% CV)</th>
<th>Between Experiment Variability (%CV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_0$</td>
<td>hr$^{-1}$</td>
<td>0.0568 (15.7)</td>
<td>na</td>
</tr>
<tr>
<td>IC50</td>
<td>mg/L</td>
<td>0.55 (35.3)</td>
<td>57.1</td>
</tr>
<tr>
<td>Adaptation constant</td>
<td>L/mg.hr</td>
<td>0.00016 (91.2)</td>
<td>154.2</td>
</tr>
<tr>
<td>$I_{max}$</td>
<td>hr$^{-1}$</td>
<td>0.338 (13.1)</td>
<td>na</td>
</tr>
<tr>
<td>Residual Variability (%)</td>
<td>(%)</td>
<td>60.9 (33.4)</td>
<td></td>
</tr>
</tbody>
</table>

**Numerical Simulations**

- For dose optimization of efficacy studies:
  - Efficacy studies in mouse models of TB infection
  - Extrapolation of PK from rat to mouse using allometric scaling

- To predict the bactericidal effect of Lee 1106 on *M. bovis BCG* at untested dosing regimens in mice
  - 1000 datasets simulated with stochastic variability in PD parameters for once daily, twice daily, once weekly and twice weekly dosing regimens
Lee 1106: Simulated Efficacy

Once Daily Dosing Regimens

0.1 mg/kg

0.3 mg/kg

1 mg/kg

3 mg/kg

10 mg/kg

Lee 1106: Simulated Efficacy

Twice Daily Dosing Regimens

0.1 mg/kg

0.3 mg/kg

1 mg/kg

3 mg/kg

10 mg/kg
Lee 1106: Simulated Efficacy

Once Weekly Dosing Regimens

- 0.1 mg/kg
- 0.3 mg/kg
- 1 mg/kg
- 3 mg/kg
- 10 mg/kg

Lee 1106: Simulated Efficacy

Twice Weekly Dosing Regimens

- 0.1 mg/kg
- 0.3 mg/kg
- 1 mg/kg
- 3 mg/kg
- 10 mg/kg
PK/PD Guided Lead Optimization

- Design of Lead Compounds
- In silico Predictions
- Chemical Synthesis
- Screening for in vitro Activity
- In vitro Screen of Biopharmaceutic/Pharmacokinetic Properties (Solubility, Metabolic Stability and Protein Binding)
- In vivo PK in Rats
- In vitro Time-Kill Studies
- In vivo Efficacy Models (e.g., M.tb mouse infection model)

Feedback

Budha et al., AAPS J 2008; 10: 157-65

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