Clofazimine and its potential contribution to TB therapy

There and back again

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Began with a compound isolated from lichen
CFZ (B-663) was one of first riminophenazines synthesized & tested
MIC for *M. tuberculosis*: ≤ 0.25-1.0 µg/ml

Activity in mice was “prodigious”
Accumulation & crystallization in tissues recognized

<table>
<thead>
<tr>
<th>Drug</th>
<th>Screening</th>
<th>Daily dose x 14d</th>
<th>Increase in survival (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFZ</td>
<td>Protective</td>
<td>20 mg/kg</td>
<td>&gt;210</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8 mg/kg</td>
<td>&gt;210</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>Established</td>
<td>11 mg/kg</td>
<td>&gt;80</td>
</tr>
<tr>
<td></td>
<td>Protective (INH-R)</td>
<td>3.3 mg/kg</td>
<td>&gt;133</td>
</tr>
<tr>
<td>INH</td>
<td>Protective</td>
<td>25 mg/kg</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td>Established</td>
<td>39</td>
<td>67</td>
</tr>
</tbody>
</table>

Reddy et al, AAC 1996; 40:633
CFZ prompts intracellular xenobiotic sequestration response in mice

- CFZ accumulates inside acidic organelles by pH-dependent ion trapping & drug-induced autophagosome-like drug-membrane aggregates
- Initial accumulation in adipose tissue, then massive redistribution to reticulo-endothelial cells
- CFZ metabolically intact in CLDIs
- Causes general anti-inflammatory response (e.g., increased IL-1RA, decreased TNF & other chemokines)

Baik et al, AAC 2013; 57:1218
Redox cycling as a mechanism of action

- Redox cycling and toxic ROS production
  - enhanced activity against INH-resistant katG mutants
  - may be less active under hypoxic/anoxic conditions
- Redox cycling also shunts electrons from ETC and may poison respiration

Barry et al, Nature 1957
Yano et al, JBC 2011; 286:10276
Boshoff et al, JBC 2004; 279:40174
Cholo et al, JAC 2012; 67:290
The premature sunset of clofazimine
Boyle Medal Lecture

• In guinea pigs, high doses required to reverse established disease (but low plasma concentrations & sub-optimal endpoints = early mortality and pathology)
• In macaques, effective as prophylaxis but not established TB, despite adequate PK (but resistance emerged in most failures)
• In humans with chronic cavitary TB, 5-10 mg/kg/d was ineffective (later shown that bioavailability = ~15%)
• The spectacular activity of the 3-drug combination SM-INH-PAS totally overshadowed clofazimine in 1960!
Clofazimine in leprosy

• First studies in mice at NIH
  – (YT Chang, 1962)

• Accumulation of drug in macrophages & Schwann cells, long half-life were advantages

• Anti-inflammatory properties also useful for suppressing reversal reactions occurring with dapsone therapy

• 1\textsuperscript{st} approval (Switzerland): 1969

• Remains part of MDT
Tolerability

• Discontinuation is rare
• Skin discoloration
• Dry skin, ichthyosis
• GI symptoms (not uncommon, typically mild)
• Not known to be teratogenic or mutagenic
Skin discoloration

- Onset: 2 weeks - several mos. of Rx
- Prevalence - 37-100% for pts on 100mg/day for at least 6 months
- Color range: orange, pink, blue, red, purple, brown, black
- Color intensity: varies, skin-tone dependent
- Wash-out: several months to 2 years after >6 months Rx

Prevalence, intensity & duration, are duration-dependent

Counseling helps to prepare pts
The Bangladesh study
(van Deun et al., AJRCCM 2010; 182: 684-692)

• 206 patients with MDR-TB
• Daily treatment with 4(+)KCGEHZP followed by 5 GEZC
  K = kanamycin 1000mg (15mg/kg)
  C = clofazimine 100mg
  G = gatifloxacin 800mg
  E = ethambutol 1200mg
  H = isoniazid 600mg
  Z = pyrazinamide 2000mg
  P = prothionamide 750mg
• Results: 87.9% (95% CI, 82.7–91.6) successful outcomes after a 9-month treatment compared to the 48 % reported by WHO (MDR-TB FactSheet WHO update 2013).

*If no culture conversion at 4 months, the initial phase is prolonged
Clinical studies evaluating the impact of CFZ

- **S. Africa (retrospective cohort study; new dx XDR-TB; 86% HIV+)**
  - CFZ 200-300mg/d (n=50) vs. no CFZ (n=35)
  - Sputum cx conversion: 40% vs. 28.6% (p=0.05)
  - Deaths: 36% vs 54.3% (p=0.12)
  - Only 3 CFZ discontinuations (1 skin, 1 GI, 1 CNS)

- **S. Africa (prospective observational study; XDR-TB; 45% HIV+)**
  - CFZ (n=22) vs. no CFZ (n=85)
  - Indep. predictor of sputum conversion (HR 0.14 [0.03-0.6]; p=0.007)
  - Indep. predictor of survival (HR 0.38 [0.16-0.87]; p=0.02); not in HIV+?

- **China (randomized trial; MDR-TB; no HIV+)**
  - CFZ 100mg/d (n=53) vs. no CFZ (n=52)
  - Treatment success: 73.6% vs. 53.8% (p=0.04)
  - Culture conversion & cavity closure faster in CFZ grp

N Padayatchi et al, JAC 2014; 69:3103
E Pietersen et al, Lancet 2014; 383:1230
S Tang et al, CID 2015; 60:1361
Additive effect of CFZ in 2nd-line regimen in mice

A, Amikacin 100mg/kg; M, Moxifloxacin, 100mg/kg; E, Ethambutol, 100mg/kg; Z, Pyrazinamide 150mg/kg, C, Clofazimine 25mg/kg

a 1 of 5 mice culture-positive 31 CFU (drug susceptible)
b 1 of 5 mice culture-positive 195 CFU (CFZ-resistant)
c 2 of 5 mice with ~1-10 colonies

J Grosset et al, AJRCCM 2013; 188:608
Incorporation of CFZ into the 1st-line regimen in mice

S Tyagi et al, PNAS 2015; 112:869
CFZ increases activity of bedaquiline (J) against an established mouse infection
### C adds sterilizing activity to JZ +/- rifapentine (P)

<table>
<thead>
<tr>
<th></th>
<th>Lung CFU cts</th>
<th>% (proportion) relapsing</th>
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<tr>
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<td>D-17</td>
<td>D0</td>
</tr>
<tr>
<td>Untreated</td>
<td>4.41 ± 0.08</td>
<td>8.32 ± 0.26</td>
</tr>
<tr>
<td>PZM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>JZ</td>
<td></td>
<td></td>
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<tr>
<td>JZP</td>
<td></td>
<td></td>
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<tr>
<td>JZC</td>
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<tr>
<td>JZPC</td>
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- **Unpublished data from Williams, et al.**

### Notes

- *p≤ 0.005 vs. JZ*
## C adds sterilizing activity to JZ +/- rifapentine (P)

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<td>JZ</td>
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<tr>
<td>JZPC</td>
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\(^1p \leq 0.005\) vs. JZ

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Williams et al, AAC (2012); 56:3114
Antagonism between CFZ and PA-824

<table>
<thead>
<tr>
<th></th>
<th>Mean lung CFU count</th>
<th>Proportion (%) relapsing after treatment for:</th>
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<tbody>
<tr>
<td></td>
<td>D-13</td>
<td>D0</td>
</tr>
<tr>
<td>None</td>
<td>4.44±0.10</td>
<td>7.95±0.19</td>
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<tr>
<td>JZC</td>
<td></td>
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<td></td>
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<tr>
<td>JZCPa</td>
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<tr>
<td>JZU</td>
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<td>JZUPa</td>
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</table>
Clofazimine has no EBA in TB patients
Serial sputum colony counts over 1st 14 days of treatment

A Diacon et al, AJRCCM 2015
More human-like pathology in C3HeB/FeJ ("Kramnik") mice

- Susceptibility allele at sst1
- Candidate gene is lpr1
- The cell death pathway in *Mtb*-infected lpr1-negative macrophages is necrosis rather than apoptosis
- Mice are not otherwise immunodeficient
- Necrotic lesions are hypoxic, but metronidazole is not active

Pan et al, Nature 2005
Apt & Kramnik, Tuberculosis 2009

Davis et al, AAC 2010
Harper et al, JID 2012
Driver et al, AAC, 2012
Activity of clofazimine in C3HeB/FeJ mice

CFZ effect size in lungs

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

BALB/c

C3HeB/FeJ

CFZ effect size in spleens

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

CFZ effect size in lungs

3-wk incubation

7-wk incubation

In vitro activity
CFZ: slow onset and compartmentalized activity – PK/distribution

- Long time to reach steady state
- Slow & steady accumulation in adipose tissue & macrophages
- pH-dependent ion trapping in lysosomes
- Formation of intracellular crystal-like inclusions of intact drug
- Poor distribution into caseum
CFZ: slow onset and compartmentalized activity – mechanism(s) of action

- Redox cycling and toxic ROS production
  - may be less active under hypoxic/anoxic conditions
- Redox cycling also shunts electrons from ETC and may poison respiration
  - slower onset of action, like BDQ
- Other proposed mechanisms have included membrane disruption by:
  - increased lysophospholipid production
  - inhibition of K⁺ transporters

Barry et al, Nature 1957
Yano et al, JBC 2011; 286:10276
Boshoff et al,
Cholo et al, JAC 2012; 67:290
CFZ: slow onset and compartmentalized activity – host-directed effects?

- Dose-dependent inhibition of neutrophil motility
- Enhancement of neutrophil and macrophage phagocytic activity and superoxide production
- Inhibition of mitogen-induced lymphocyte transformation
- inhibits Kv1.3 K+ channel
- Induction of apoptosis via caspase activation
CFZ: back to the future

It was widely accepted that the tendency of a tuberculostatic compound to concentrate in macrophages where many of the bacilli had already been phagocytosed was a favourable feature. By 1959, however, we had began to suspect that this was much too simple a view and to suspect that while it might well be a favourable property in the treatment of an infection where the bacilli are for the most part intracellular, it could well be unfavourable in an infection where the bacilli were, to any considerable extent, extracellular. If our suspicions were well founded it would fit in with the excellent therapeutic results in murine disease where the bacilli are for the most part believed to be intracellular, except in a terminal condition, and with the unpromising (generally presumed) results in clinical tuberculosis where large numbers of bacilli are extracellular and must be dealt with by a drug circulating in body fluids. The more efficiently and rapidly a drug is removed from the blood stream by the macrophages the lower will be its blood level and the less effective therapeutically it may be under certain pathologic conditions.
However, the phenazines/rimino compounds, specifically B.663, were disappointing in human tuberculosis, probably due to the highly selective distribution of the drug in the tissues of the body (adipose, RES) not coinciding with the distribution of *Mycobacterium tuberculosis* in human disease (pulmonary cavity).

Barry et al. *Bull. Int. Union against Tuberculosis* 29 (1959), 582-593
How might we succeed with CFZ?

- Accept that its utility may be limited to bacteria in the intracellular compartment
- Identify the dose (and duration) that most effectively targets this compartment
- Combine it carefully with agents that complement its PK and MOA while being mindful of potential additive toxicity and cross-resistance
- Study it in a way that specifically tests its contribution to the regimen
- Be prepared for long trials
Dose-ranging activity of CFZ in BALB/c mice

**4 wks**

- $R^2 = 0.69$
- $E_{\text{max}} = -1.1 \log$
- $EC_{50} = 1.6 \text{mpk (95\% CI: 0.9-3.0)}$
- $EC_{90} = 3.7 \text{mpk}$

**Ormond & Nuermberger, unpublished**

**8 wks**

- Log$_{10}$ dose (mg/kg)
- Lung $\log_{10}$ CFU count

**Swanson et al, AAC 2015;**

- CFZ conc. $\mu g/ml$
- log$_{10}$ CFU per Lung
- Days on treatment
Predicted Plasma Exposure after Multiple Oral Doses of Clofazimine

3x300 mg/d followed by 100 mg/d expected to match 100 mg/d at steady state: $C_{max}$ on day 14 = 895 nM
In NC-003, JPaC regimen, D14 $C_{min} = 142$; $C_{max} = 270$

Identifying derivatives that improve upon CFZ’s PK and safety properties

Attributes:

- Potent anaerobic and intracellular activity
- Efficacious against acute and chronic murine TB models
- Synergistic effect in combo studies in mouse
- Low frequency of resistance development
- Novel MOA (ox. phos.) – active against M(X)DR-TB
- Low COG

Challenges:

1. CV liability
   - hERG IC50 <1 uM
   - Clinical QTc finding: BDQ+CFZ 40.5 ms > BDQ alone 12.9 ms

2. PK and tissue distribution/accumulation
   - Human T\(_{1/2}\) > 10 days – accumulation > 1.5 g
   - Crystal formation in tissue, intracellular precipitation
   - Intrinsic color (skin discoloration)

3. Limited “modern” safety/tox/DMPK data available

Khisi Mdluli, Anna Upton (TB Alliance)
Example of an advanced lead compound

![Chemical structures of TBI-1077 and Clofazimine]

<table>
<thead>
<tr>
<th>Cmpd</th>
<th>MABA MIC</th>
<th>LORA MIC</th>
<th>Vero CC50</th>
<th>ClogP</th>
<th>Mouse PK (20 mpk, agar formulation)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ug/mL</td>
<td>ug/mL</td>
<td>ug/mL</td>
<td></td>
<td>t ½ (h)</td>
</tr>
<tr>
<td>CLF</td>
<td>0.24</td>
<td>0.573</td>
<td>&gt;64</td>
<td>7.70</td>
<td>46.34</td>
</tr>
<tr>
<td>TBI-1077</td>
<td>&lt;0.0075</td>
<td>0.216</td>
<td>&gt;64</td>
<td>4.88</td>
<td>19.54</td>
</tr>
</tbody>
</table>
Results after 8 weeks of treatment in combination with bedaquiline & sutezolid

Lung CFU counts before and after treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>D0</th>
<th>W8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>7.52+0.15</td>
<td>N/A</td>
</tr>
<tr>
<td>BDQ+SZD</td>
<td>3.42+0.19</td>
<td></td>
</tr>
<tr>
<td>BDQ+SZD+CFZ</td>
<td>1.43+0.38</td>
<td></td>
</tr>
<tr>
<td>BDQ+SZD+TBI-1077</td>
<td>0.54+0.34</td>
<td></td>
</tr>
</tbody>
</table>
TBI-1077 has sterilizing activity similar to CFZ when combined with BDQ+PZA

<table>
<thead>
<tr>
<th>Treatment</th>
<th>6 weeks</th>
<th>8 weeks</th>
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<tbody>
<tr>
<td>BDQ+PZA</td>
<td>N/A</td>
<td>9/15 (60%)</td>
</tr>
<tr>
<td>BDQ+PZA+CFZ</td>
<td>4/15 (27%)</td>
<td>0/15 (0%)</td>
</tr>
<tr>
<td>BDQ+PZA+TBI-1077</td>
<td>5/15 (33%)</td>
<td>0/14 (0%)</td>
</tr>
</tbody>
</table>

TBI-1077 had improved solubility, PK and accumulation profile and at least similar efficacy compared to CFZ, but could not match its safety.
Conclusions

• CFZ has substantial potential in new TB regimens
  – potently targets intracellular bacilli via novel (sterilizing) mechanism(s)
  – active against most strains on the planet, especially INH-resistant *Mtb*
  – Pre-clinically, combines well w/rifamycins, PZA, BDQ, oxazolidinones

• But proving it is challenging
  – more work needed to characterize exposure-response profiles *in vivo*, including impact of immunodeficiency on intracellular accumulation and efficacy
  – more work needed to characterize its contribution to combination therapy in models with more human-like pathology
  – clinical trials will need to focus on sputum sterilization and relapse rather than early microbiological markers
Acknowledgements

• Colleagues
  – Faculty and staff of the JHU Center for TB Research
  – Anna Upton, Khisi Mdluli, Tian Yang (TB Alliance)
  – David McNeeley (Novartis)
  – Charles Peloquin (Univ. of Florida)
  – Anne Lenaerts and colleagues (Colorado St.)
  – Veronique Dartois (Rutgers)
  – Omar Vandal (Gates Foundation)

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  – TB Alliance
  – NIAID (R01-AI111992, R01-AI090820)