State of the Art: TB drug pharmacokinetics at the site of disease

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TB Steering Committee IMPAACT, June 2018
HYPOTHESIS

If TB drugs reach all bacterial populations at *sufficient* concentration in lesions, cure rates will increase and treatment duration will decrease.
What do we need to know to test this hypothesis?

1. Measure the concentrations of all TB drugs in cellular and necrotic lesion compartments
2. Measure the activity of all TB drugs against Mtb populations residing in each compartment
3. Use lesion-centric efficacy read-outs in human-like animal models to correlate lesion PK, lesion PD and efficacy

1. How much drug reaches each compartment?
2. How much drug does it take to kill the resident bugs?
3. How does that translate into lesion sterilization in an in vivo model?
Lesion PK-PD methods

- **Homogenize**
- **Extract**
- **LC/MS/MS**
- **Quantify**

**MALDI MS imaging**

**Laser capture microdissection**

**Drug activity in caseum**

**PK**

**PD**

100%
0%
MALDI Imaging of multiple drugs in a single section

Pyrazinamide

Moxifloxacin

Clofazimine

Cellular rim

Caseum

2mm

Limit of detection
Laser-capture microdissection / LCMS

Plasma or tissue concentration (ng/mL or ng/g)

PEAKS (2H)

- Compound A
- Compound B
- Compound C
- Compound D
- Compound E

- Uninvolved lung
- Cellular rim
- Outer caseum ring
- Inner caseum
An assay to measure the drug susceptibility of caseum Mtb

From frozen samples
(NIH & PHRI rabbits)

NO DRUG control
Growth kinetics in 10 caseum batches from D0 to D7

CFU / g of caseum

Batch # 1 2 3 4 5 6 7 8 9 10
Caseum Mtb is highly drug tolerant

MBC in broth

~ 100-fold shift

Antimicrob Agents Chemother. 2018 Jan 25;62(2)
Pyrazinamide (PZA): lesion PK and PD

PZA penetrates all lesion compartments and kills Mtb in caseum
The rabbit model of active Tuberculosis

Infection
Mtb HN878 (Beijing)

Cellular lesion
Necrotic (caseous) lesions

Scale bar: 500 μm
Pyrazinamide (PZA): in vivo efficacy in rabbits

PZA sterilizes cellular and necrotic lesions in rabbits
How much kill has happened in a lesion

**Cellular**

**Necrotic**

Log CEQ/CFU (bin center)
Moxifloxacin (MXF): lesion PK and PD

Laser-capture in lesions

Relative MXF concentration (% plasma)

Cidal activity in caseum

CFU/mL

MXF concentration (µM)
Moxifloxacin (MXF): in vivo efficacy

MXF sterilizes cellular and necrotic lesions in rabbits
Clofazimine (CFZ) and Bedaquiline (BDQ)

- CFZ
- BDQ

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Clofazimine (CFZ) and Bedaquiline (BDQ)

CFZ and BDQ concentrations in cellular rim, necrotic macrophages, neutrophil ring, and caseum.

Relative CFZ concentration (% cellular rim)

Relative BDQ concentration (% cellular rim)
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BDQ and CFZ: in vivo efficacy

- **BALB/c**
  - Lung Control
  - Lung CFZ
  - Spleen Control
  - Spleen CFZ

- **C3HeB/FeJ**
  - BDQ
  - CFZ

**Graphs:**
- Log CFU vs. Time (weeks)
- Log CFU vs. Treatment duration (weeks)
- Chemical structures of BDQ and CFZ
RIF accumulates in caseum... ... and kills the resident bugs
Clinical regimens: plug-and-play

But why does it take 6 to 24 months?

Zimmerman et al., AAC 61(9) (2017)
HYPOTHESIS

If TB drugs reach all bacterial populations at **sufficient** concentration in lesions, cure rates will increase and treatment duration will decrease.

1. How much drug reaches each compartment?
2. How much drug does it take to kill the resident bugs?
3. How does that translate in the clinic?
Even the best TB drugs come short of ideal target attainment in caseum.
Take Home

• MALDI mass spectrometry imaging combined with laser capture microdissection and LC-MS provides full drug quantitation at high spatial resolution

• Lesion-centric PK-PD helps understand the relative inefficiency of TB therapy

• Developing new PZA, MXF, RIF and BDQ derivatives that are more potent, less toxic, and/or higher in lesions is almost guaranteed to shorten therapy duration
Thank you!

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