

# Tissue Concentrations of *Pyrazinamide*

## PHARM-TB STUDY



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# *Pyrazinamide (PZA)*

## **First human use in 1952; remains essential drug**

- Shortening of drug-susceptible treatment
- Active versus slow growing/persisting organisms
- Synergistic with many newly introduced drugs



## **PZA Pharmacokinetics**

- Well absorbed; low protein binding; good CSF penetration
- Acidic pH important for activity
- Tissue penetration not well characterized

# PZA Penetration into Lung



$PZA_{\text{lung/lesion}} \rightarrow 0.7-0.8^1$

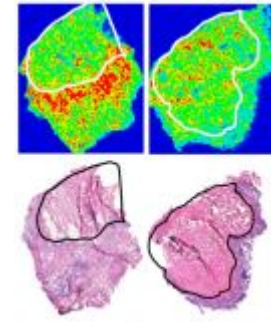


$PZA_{\text{lung/lesion}} \rightarrow 0.3-0.4^2$

Human



$PZA_{\text{lung/lesion}} \rightarrow 0.6-0.8^3$



<sup>1</sup>Lanoix et al. AAC 2016

<sup>2</sup>Via et al. ACS Infect Dis. 2015

<sup>3</sup>Prideaux et al. Nat Med 2015

# Study Question

*How well does PZA penetrate into  
Pulmonary TB Lesions?*



**AIM**

*Measure the intra lesional concentration  
of PZA utilizing microdialysis*

# Specific AIMS

- I. Determine serum and tissue pharmacokinetics of PZA among TB patients undergoing adjunctive surgery
- II. Determine radiological and pathological lesion characteristics associated with PZA tissue concentrations
- III. Investigate genotypic and phenotypic drug resistance profiles of *M. tuberculosis* isolates recovered from sputum and lung samples with DST and whole genome sequencing

# Methods I



## I. Setting

- National Center for TB and Lung Diseases in Tbilisi, Georgia
- High rates of M/XDR-TB; ~40-60 surgical resections per year

## II. Patients

- DR-TB cohort undergoing adjunctive surgical resection on PZA

## III. Pharmacokinetics

- Serum samples at 0, 1, 4 & 8 hours & time of resection
- $\mu$ D performed for intra lesional samples
- PZA concentrations done at U. of Florida (LC-MS/MS)

## IV. Laboratory

- LJ Cultures: preoperative sputum & 5 tissue cultures

# Methods II



## V. Pathology

- After  $\mu$ D, lesion bisected and pH tested
- Half of dissected lesion formalin-fixed/paraffin-embedded
- Lesions evaluated for cellularity, necrosis, granulomas, and AFB (0-3 scale)

## VI. Radiology

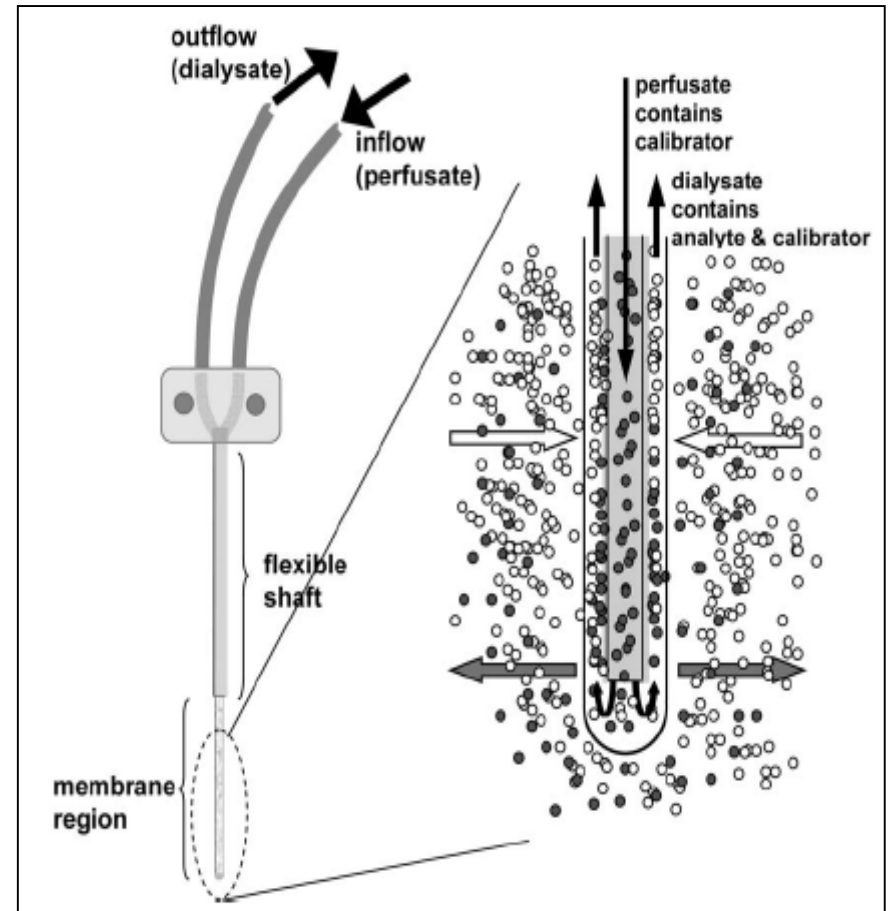
- When available, Chest CTs read by two Emory radiologists
- Characterized dominant resected lesion

## VII. Whole Genome Sequencing

- DNA extracted for tissue cultures positive for *M. tb.*
- WGS performed using Illumina HiSeq2000; PZA mutations evaluated

# Microdialysis ( $\mu$ D)

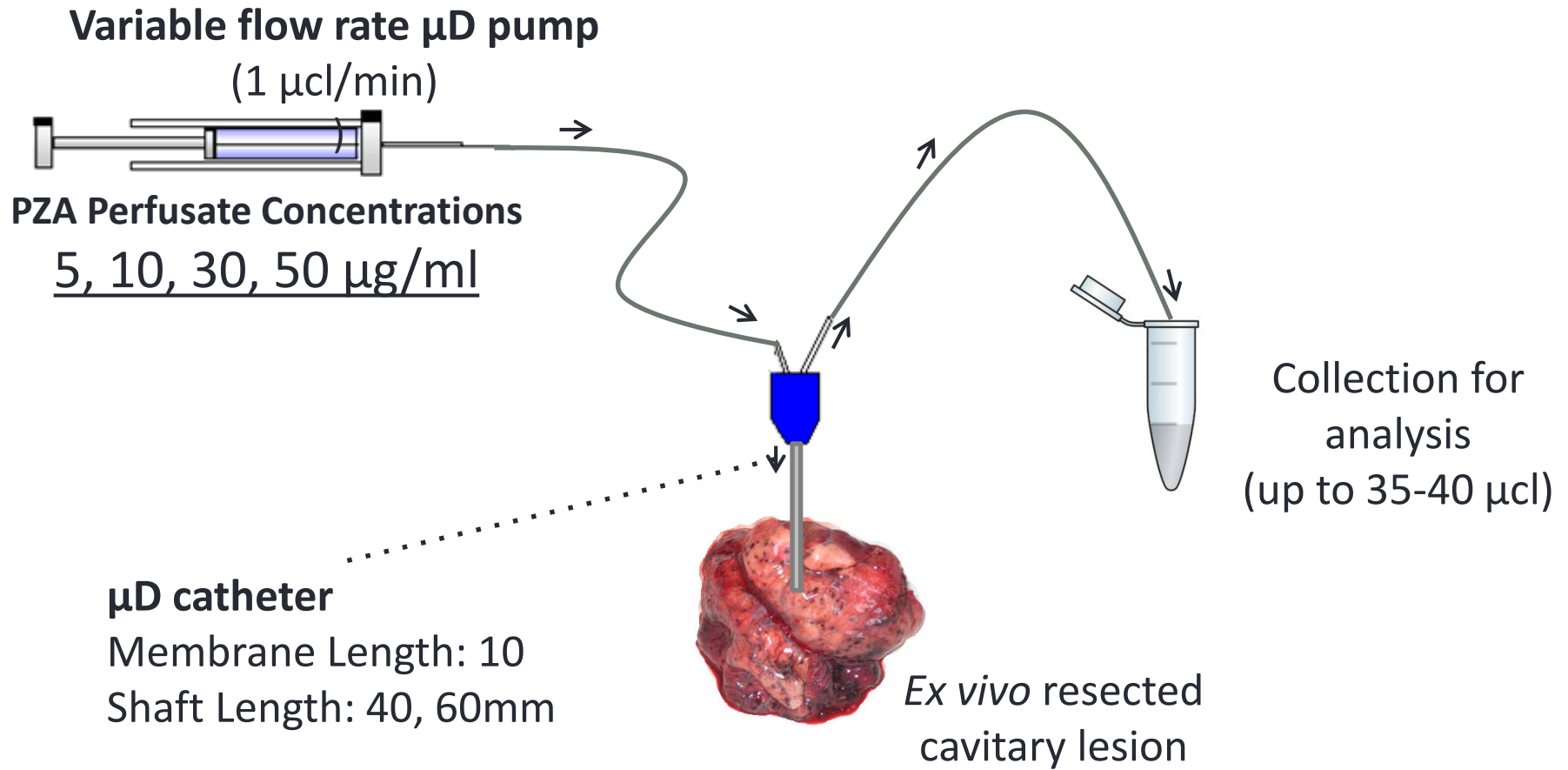
- Extracellular, unbound drug
- Must account for less than 100% recovery rate (calibration)
- No-net-flux method used



Chaurasia CS, J Clin Pharm. 2007.



# $\mu$ D using No-Net Flux Method

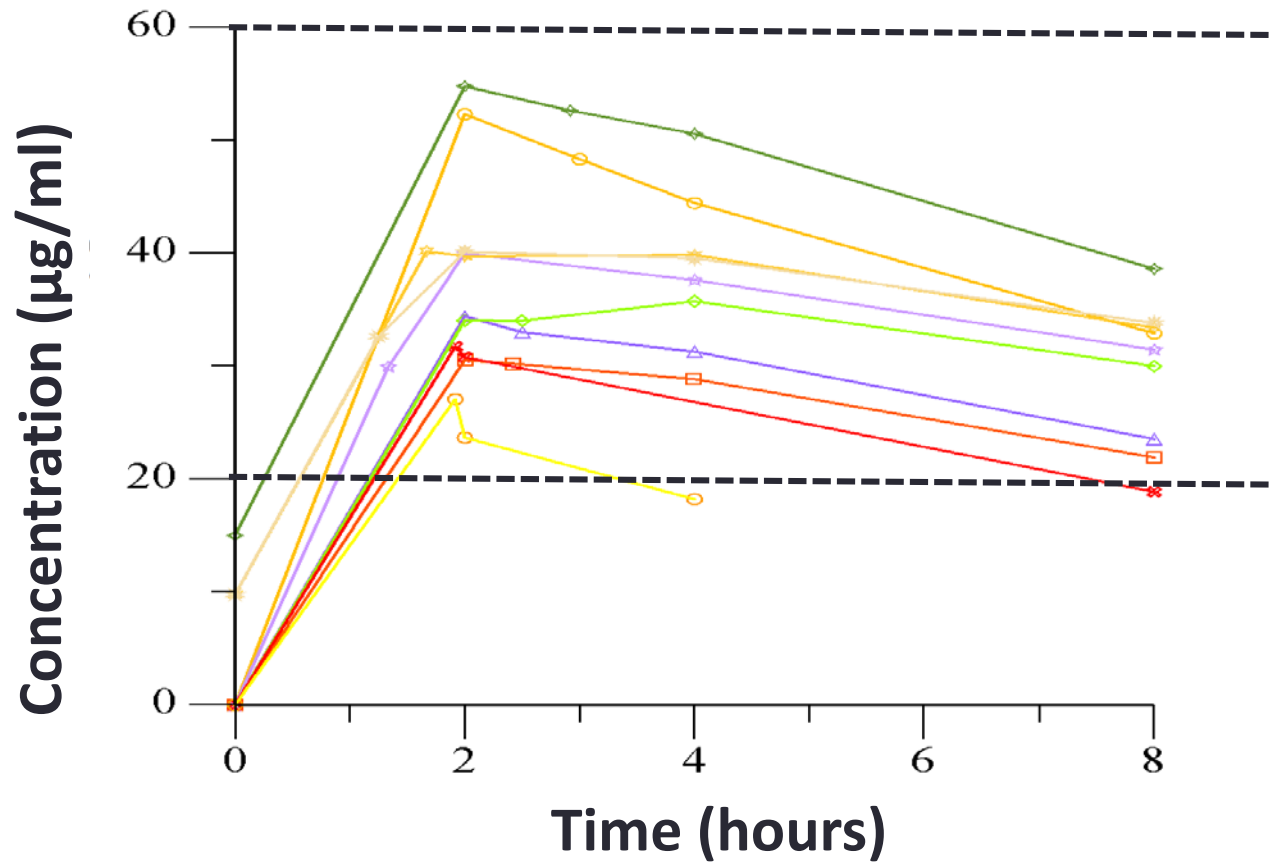


# Study Results: Table 1 (n=10)

| Characteristic            | Result       | Characteristic     | Result (N=12)    |
|---------------------------|--------------|--------------------|------------------|
| Male                      | 8 (80)       | CrCl (ml/min)*     | 91 (52-155)      |
| Georgian                  | 7 (70)       | Albumin (g/dl)*    | 4.2 (3.5-4.9)    |
| Age*                      | 30 (16-54)   | Hemoglobin (g/dl)* | 13.7 (12.4-15.5) |
| Diabetes                  | 1 (10)       | ALT*               | 18 (10-133)      |
| Hepatitis C               | 1 (10)       | PZA 1600 mg        | 8 (80)           |
| Weight (kg)*              | 53 (48-71)   | PZA (mg/kg)        | 24.7 (22.5-33.3) |
| BMI (kg/m <sup>2</sup> )* | 19.5 (15-22) | PZA Days           | 363 (120-504)    |
| New TB case               | 7 (70)       |                    |                  |
| Lobectomy                 | 5 (50)       |                    |                  |
| MDR/XDR                   | 6(60)/2(20)  |                    |                  |

\* Median value (range)

# PZA Concentration Time Graph (n=10)



# Non-Compartmental Analysis\*

| Parameter  | All<br>(n=10)    | Expected<br>Values^ |
|--|------------------|---------------------|
| $K_e$ ( $h^{-1}$ )                                     | 0.06 (.04-.13)   |                     |
| $T_{1/2}$ (h)  | 11.7 (5.3-17.6)  | 8-11                |
| $T_{max}$ (h)  | 2 (1.7-4)        | 1-2                 |
| $C_{max}$ ( $\mu g/ml$ )                               | 37.8 (27.1-54.7) | 20-60               |
| $AUC_{last}^*$ ( $h \cdot \mu g/ml$ )                  | 247 (70-353)     |                     |
| $AUC_{0-\infty}^*$ ( $h \cdot \mu g/ml$ ) <sup>+</sup> | 828 (209-1140)   |                     |
| CL/F ( $L/h$ ) <sup>+</sup>                            | 1.9 (1.4-7.7)    |                     |
| V/F (L) <sup>+</sup>                                   | 36.4 (29-59)     |                     |

\*Median values (range)

<sup>+</sup>Alsultan & Peloquin. Drugs 2014; Donald PR et al. Tuberculosis 2012.

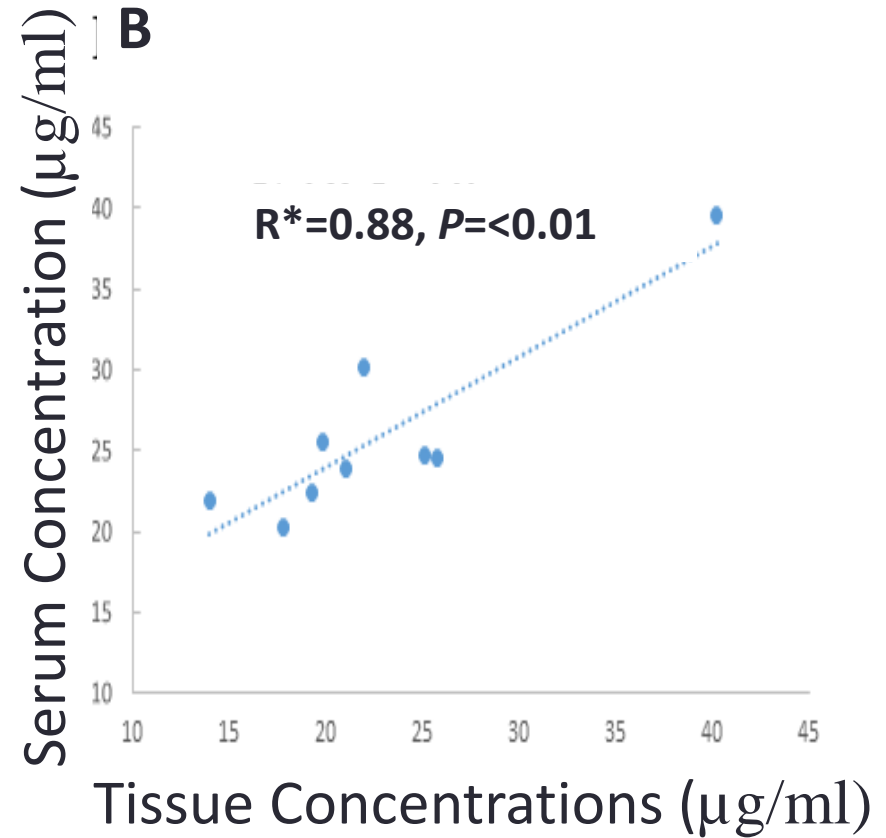
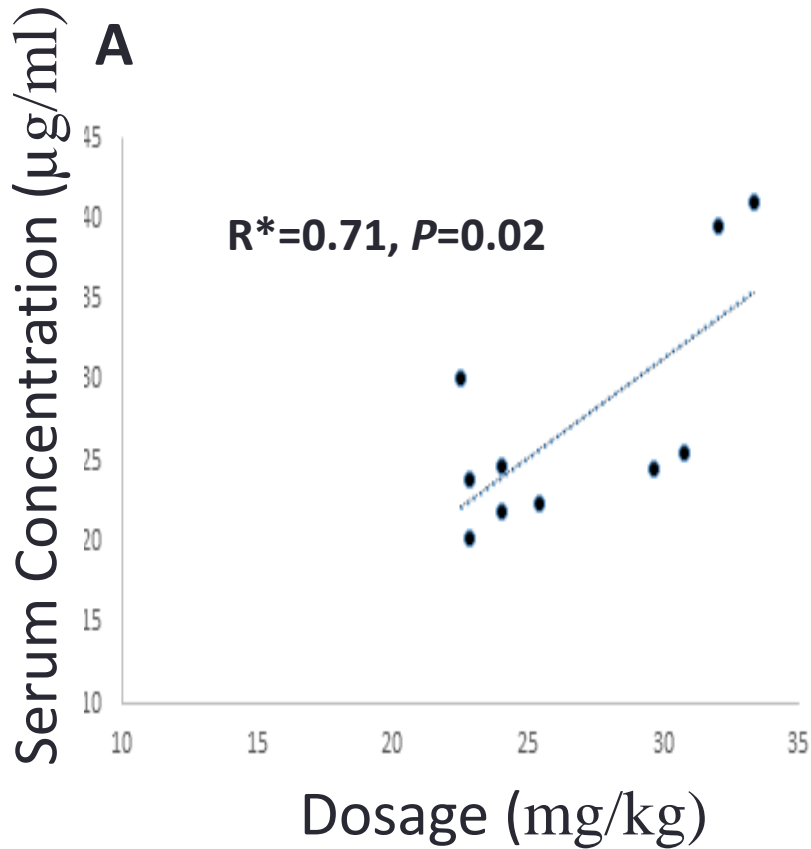
# PZA Serum & Tissue Concentrations

| ID                 | PZA<br>$C_{\text{serum}}^{\wedge}$ | PZA<br>$C_{\text{tissue}}$ | PZA<br>$C_{\text{tissue}}/C_{\text{serum}}$ |
|--------------------|------------------------------------|----------------------------|---|
| 1 <sup>&amp;</sup> | 41.04                              | NA                         | NA  |
| 2                  | 28.03                              | 25.06                      | 0.89  |
| 3                  | 25.67                              | 13.95                      | 0.54  |
| 4                  | 28.91                              | 19.78                      | 0.68  |
| 5                  | 25.44                              | 19.29                      | 0.76  |
| 6                  | 44.71                              | 40.17                      | 0.90  |
| 7                  | 34.13                              | 21.98                      | 0.64  |
| 8                  | 27.72                              | 25.76                      | 0.93  |
| 9                  | 26.95                              | 20.96                      | 0.78  |
| 10                 | 23.00                              | 17.75                      | 0.77  |
| <b>Median</b>      | <b>27.87</b>                       | <b>20.96 (13.95-40.17)</b> | <b>0.77 (0.54-0.93)</b>                     |

<sup>^</sup> Free serum concentration  $\rightarrow$  PZA concentration  $\times$  0.85

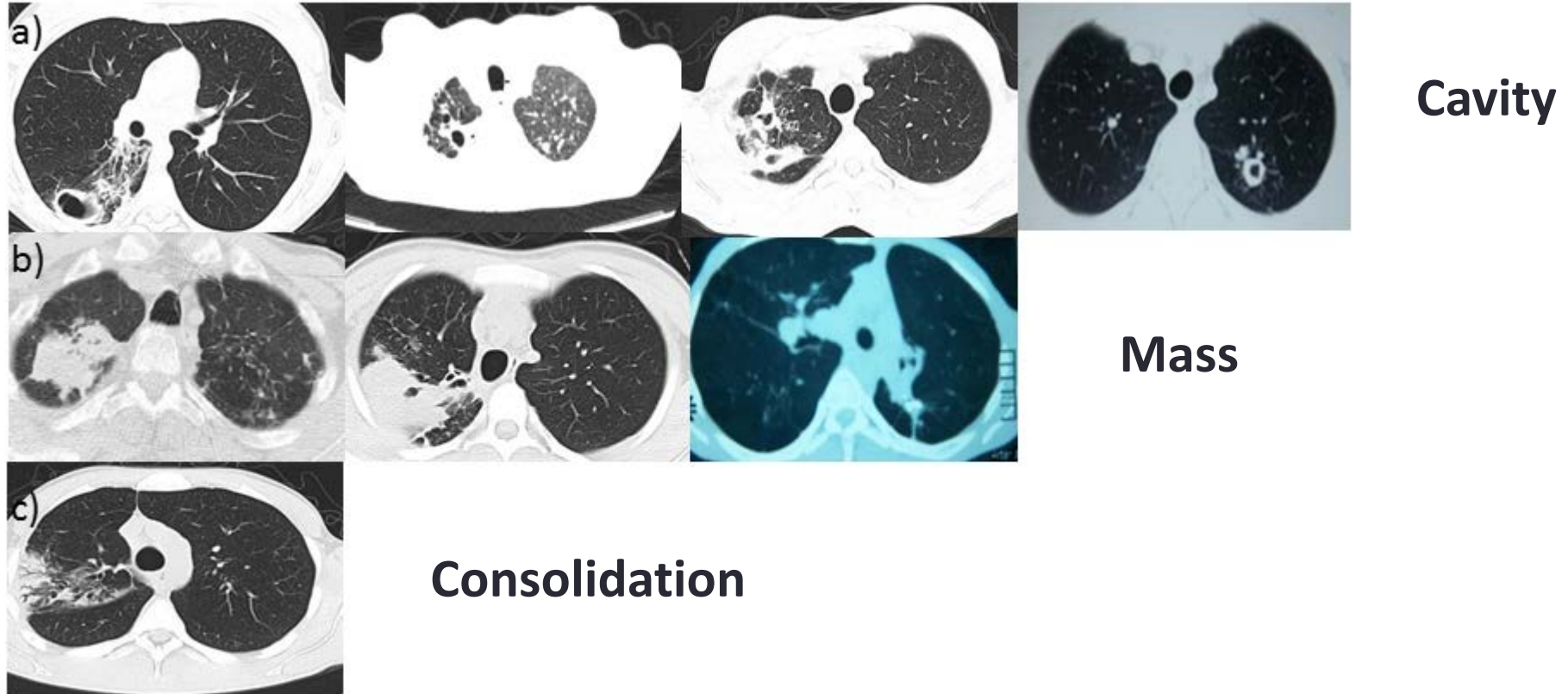
<sup>&</sup> No cavity concentration was available for subject 2 due to low dialysate volume

# Correlations



\* Pearson Correlation Coefficient

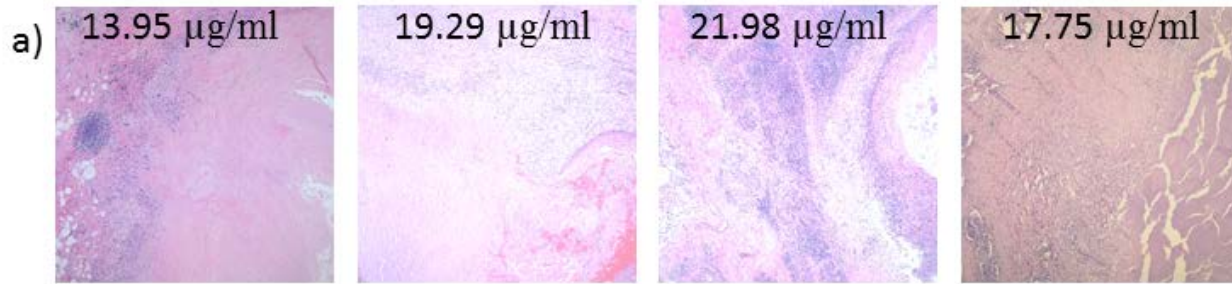
# Chest CT Findings (n=8)



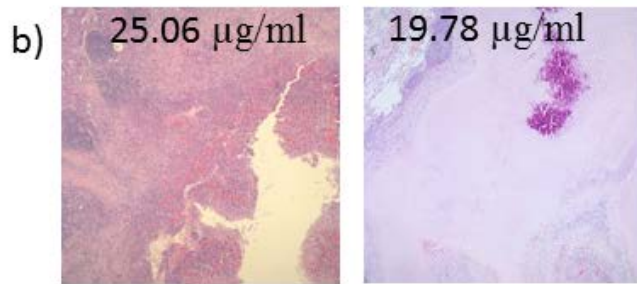
PZA tissue concentrations similar in Cavitory & Mass Lesions  
(22.1 vs. 24.5  $\mu\text{g/ml}$ ) & (0.78 and 0.73)

# Pathological Examination (n=9)

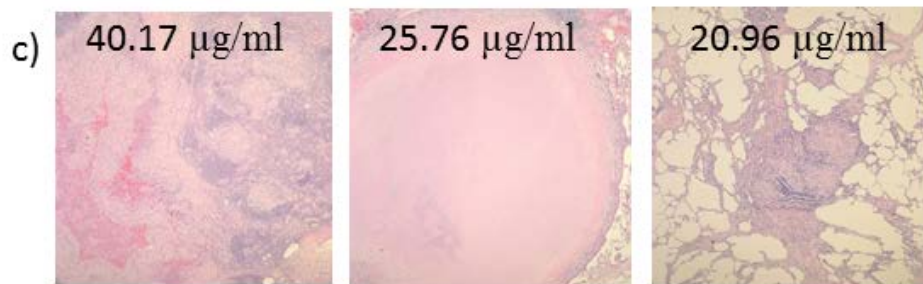
NECROSIS/Mean PZA<sub>tissue</sub>



Severe (18.28  $\mu\text{g/ml}$ )



Moderate (22.5  $\mu\text{g/ml}$ )



Rare (29.0  $\mu\text{g/ml}$ )

Negative Correlation between

PZA<sub>tissue</sub> and Necrosis  $\rightarrow R=-0.66, P=0.04$

PZA<sub>tissue</sub> and AFB grade  $\rightarrow R=-0.75, P=0.01$



# Culture & pH Results

- Two patients with (+) resected tissue culture
- Median pH 5.5; 3 with > median pH (7.0, 7.2, 7.2)



## Patient 1 (XDR)

$C_{\max}$  = 41.04  $\mu\text{g/ml}$

Pre Op Smear (-)/Cx(+)

**pH=7.2**

2 sputum & 5 tissue *M.tb*  
isolates



## Patient 2 (pre XDR)

$C_{\text{tissue}}$  = 25.06  $\mu\text{g/ml}$

Pre Op Smear/Cx (-)

**pH=7.2**

1 sputum & 3 tissue *M.tb*  
isolates



WGS: Same Beijing strain and DST patterns for all intra patient *M. tb* isolates; no *pncA* or *rpsA* mutations

# Conclusions

## Limitations

- POA concentrations not measured
- pH measured ~3 hours after resection; in future will compare immediate probe measurements to pH test strips

## Summary

- Good penetration of PZA into pulmonary TB lesions (0.54-0.93)
- PZA  $C_{\text{serum}}$  correlates with PZA  $C_{\text{issue}}$
- High grade necrosis & AFBs assoc. with lower PZA tissue concentrations
- Most lesions with  $\text{pH} \leq 5.5$ ; two patients with positive tissue cultures had high pH (1<sup>st</sup> human lung pH measurements > 50 yrs)\*

\*Weiser O, et al. Transactions Conference Chemo of TB. 1953

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