PZA: A New Look Based on RNASeq, the Hollow Fiber System, and Patient Level Data

Tawanda Gumbo

Office of Global Health

University of Texas Southwestern Medical Center, Dallas, Texas

The team: this work has many fathers & mothers

- University of Texas Southwestern:

Shashikant Srivastava, Jotam Pasipanodya, Devyani Deshpande,

Sandirai Musuka, Carleton Sherman, Aurelia Schmalstieg

Chandima S. Wasana Siyambalapitiyage Dona, Nicolai van Oers

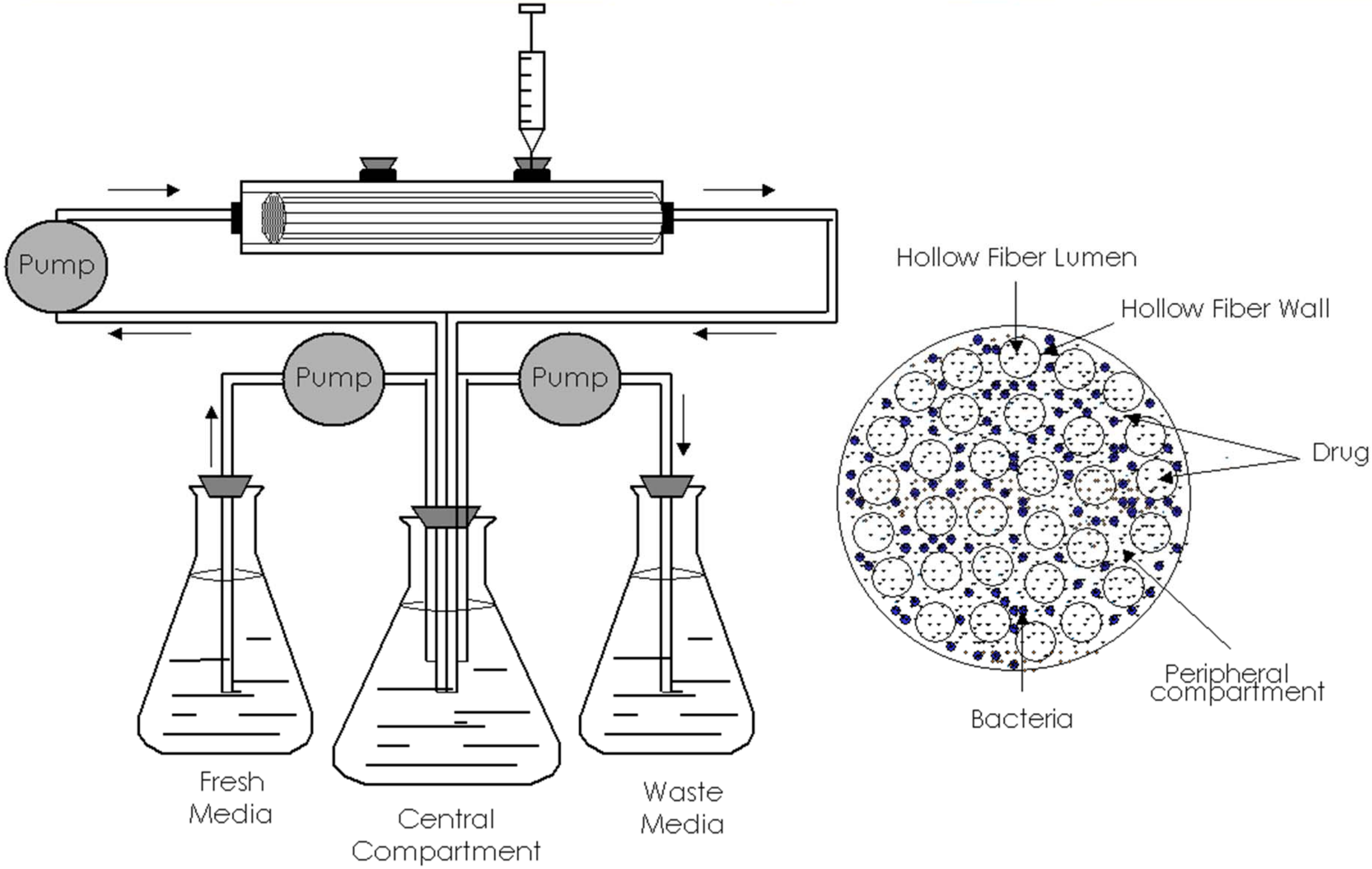
- 2. University of Cape Town:

Helen McIlleron, Peter Smith, Emmanuel Chigutsa

- 3. Brewelskloof hospital, Western Cape

Peter Wash, André Burger

M. tuberculosis in the hollow fiber system



Gumbo T, et al. (2006) ***J Infect Dis*** 2006;195:194-201

The Hollow Fiber Model of TB

Developed in 2001 for bactericidal effect

2004 for sterilizing effect (both semi-dormant bacteria at low pH and NRP [Wayne type II])

We also examine *M. tuberculosis* within macrophages

M. tuberculosis in the hollow fiber system

$$dX_1/dt = R(1) - (SCL/V_c) \times X_1 \; ; \qquad (1)$$

$$dN_S/dt = K_{gmax-S} \times (1 - L_S) \times N_S \times E - K_{kmax-S} \times M_S \times N_S \; ; \qquad (2)$$

$$dN_R/dt = K_{gmax-R} \times (1 - L_R) \times N_R \times E - K_{kmax-R} \times M_R \times N_R \; ; \qquad (3)$$

$$E = 1 - (N_R + N_S)/POPMAX \; ; \qquad (4)$$

$$L = (X_1/V_c)^H/[(X_1/V_c)^H + C_{50}g^H], \text{ where } H = H_{g-S} \text{ or } H_{g-R} \; , \qquad (5)$$

$$M = (X_2/V_c)^H/[(X_2/V_c)^H + C_{50}k^H], \text{ where } H = H_{k-S} \text{ or } H_{k-R} \; . \qquad (6)$$

Gumbo T et al. J Infect Dis. 2004;190:1642-1651

Population-median parameter estimates of pharmacodynamic model.

Parameter	Estimate	SD
Clearance rate, L/h	8.926	0.777
Volume of central compartment, L	179.349	27.041
K_{gmax-S}, $\log_{10}$ cfu/mL/h	0.415	0.411
$C_{50}g-S$, mg/L	5.103	3.909
H_{g-S}	4.759	5.276
K_{gmax-R}, $\log_{10}$ cfu/mL/h	0.023	0.014
$C_{50}g-R$, mg/L	1.090	1.178
H_{g-R}	8.857	8.179
K_{kmax-S}, $\log_{10}$ cfu/mL/h	8.699	4.047
$C_{50}k-S$, mg/L	14.009	12.710
H_{k-S}	5.122	3.693
K_{kmax-R}, $\log_{10}$ cfu/mL/h	10.459	12.187
$C_{50}k-R$, mg/L	16.867	11.864
H_{k-R}	3.316	1.126
POPMAX, cfu/mL	2.547×10^9	3.078×10^9
Total population, cfu/mL	1.962×10^6	5.878×10^5
Drug-resistant population, cfu/mL	1.123	0.059

Gumbo T et al. J Infect Dis. 2004;190:1642-1651

The Journal of Infectious Diseases

- *M. tuberculosis* in "**whatever**" metabolic state
- Pharmacokinetic system for 1-, 2-, 3-, or 4-drugs: control of dynamic drug concentrations to mimic exact PKs one wants
- Easy & repetitive sampling over up to 8 weeks (or up to 6 months) for several markers or assays to be used in quantitative & systems pharmacology:
  - cell counts, resistant *Mtb*, RNA Seq., molecular markers such as particular proteins (e.g., efflux pumps)

Pathways

HFS quantitative output on the relationship between changing concentration and microbial effect

↓

Systems pharmacology equations

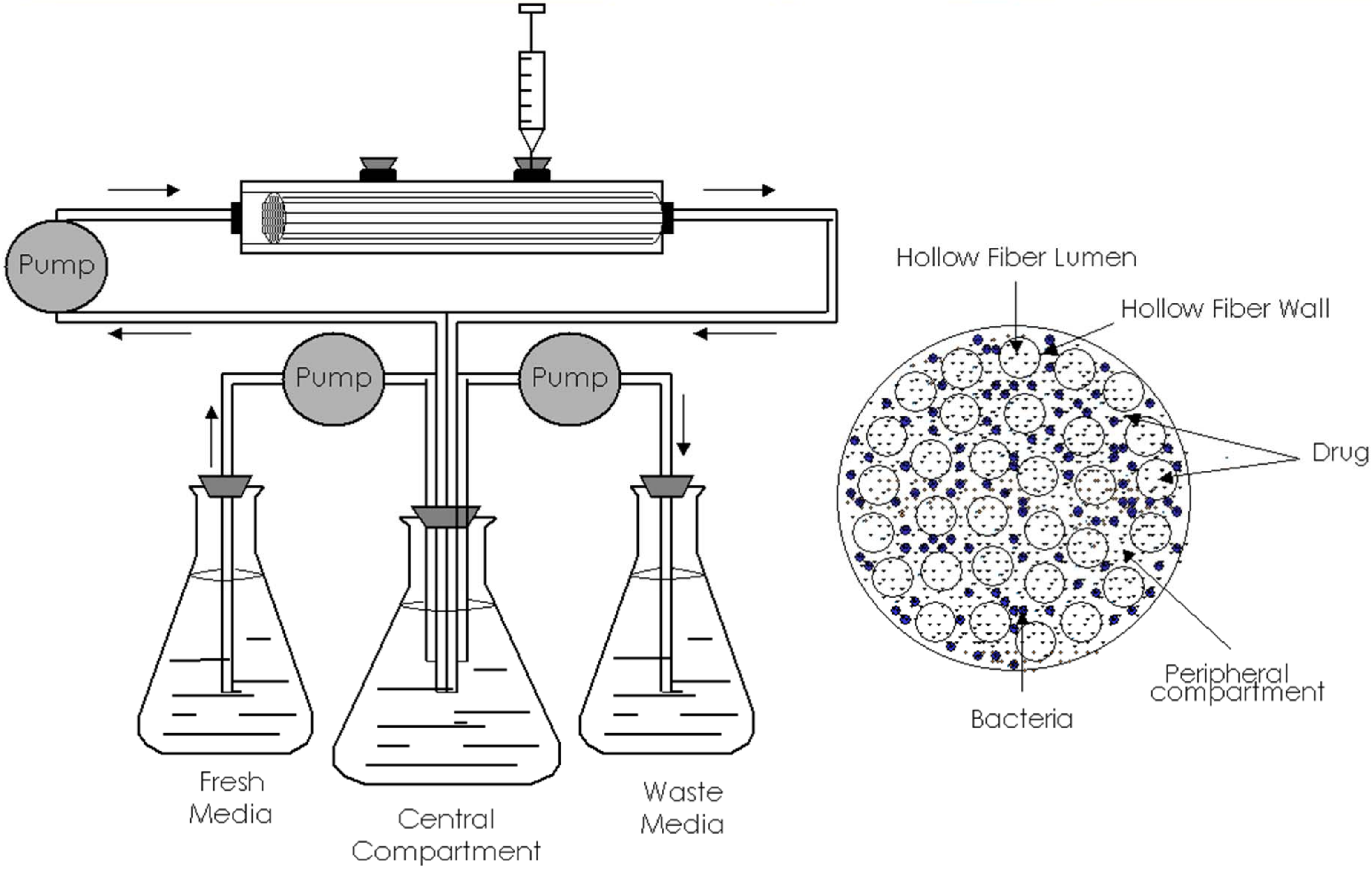
↓

Clinical prediction: Dose, Treatment & ADR rates

↓

Clinical validation: dose, treatment rates, ADR rates

M. tuberculosis in the hollow fiber system



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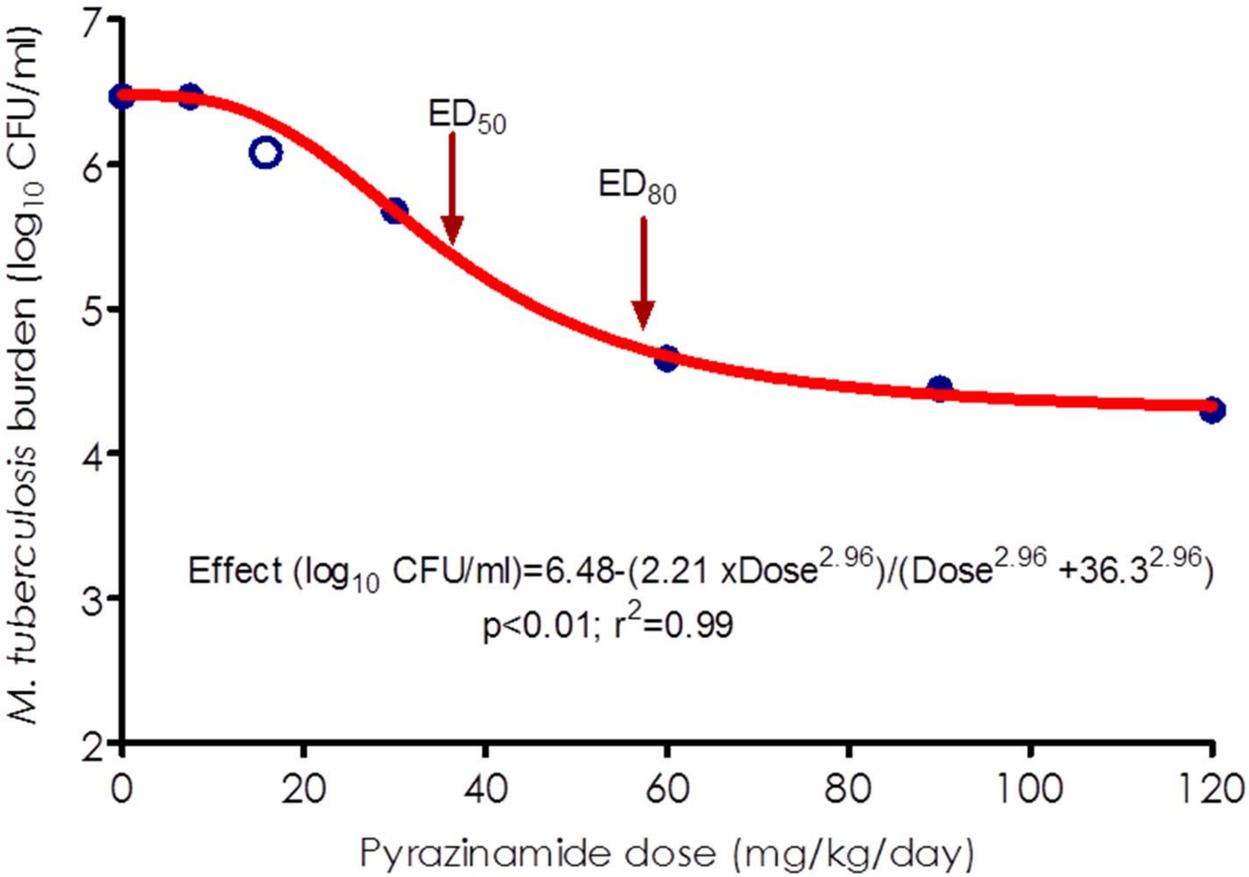


Gumbo et al. Antimicrob. Agents Chemother. 2009:53;3197-3204

PZA Standard Doses In HFS Vs. In patients

	Patients	Hollow fiber
Kill rates:		
Day 0-4 ($\log_{10}$ CFU/ml/day)	0.1±0.2	-0.1
Day 4-14 ($\log_{10}$ CFU/ml/day)	0.12±0.05	0.09-0.1
Time to resistance emergence:	2-3 wks	2-3 wks

Patient data sources: Jindani et al. Am Rev Respir Dis 1980; 121: 939-49
Yeager et al Trans Annu Meet Natl Tuberc Assoc 1952; 48: 178-201.

Gumbo et al. Antimicrob. Agents Chemother. 2009:53;3197-3204

Sterilizing effect Vs. PK/PD parameter

Time / PK/PD Index	Week 2	Week 3	Week 4
C_{max}/MIC	0.56	0.63	0.61
AUC/MIC	**0.90**	**0.89**	**0.80**
%T>MIC	0.76	0.78	0.75

EC_{90} AUC_{0-24}/MIC =210

Gumbo et al. Antimicrob. Agents Chemother. 2009:53;3197-3204

CLINICAL STUDY: PZA & Streptomycin

- 396 TB patients in Kenya, Tanzania and Uganda in 1965-7
- Each patient received 1g im streptomycin daily
- Groups of patients (dose is mean per patient day):
  - #1: PZA 184.8 mg/kg/week as 3 divided each day.
  - #2: PZA 182.4 mg/kg/week as a single daily dose.
  - #3:PZA 190.8 mg/kg/week as 3 doses per week
- Examined for sputum conversion each month.

East Africa/British Medical Research Council Pyrazinamide Investigation. Tubercle 1969;50:81-114.

Parameter (measurement)	Result for dosing regimen		
	500 mg three times a day	1,500 mg daily	3,000 mg alternate days
Serum AUC_{0-168} (mg · h/liter)	2,189	2,267	2,163
ELF AUC_{0-24}/MIC[a]	111	115	110
Serum C_{max} (mg/liter)	20.2	33.8	67.5
ELF C_{max}/MIC[a]	7.2	12.0	24
Sputum conversion (no. converted/total no. [%])			
2-month time point	30/65 (46)	34/70 (49)	39/71 (55)
End of 6 months	28/66 (42)	32/72 (44)	42/73 (58)
Any radiological improvement (no. showing improvement/total no. [%])	32/63 (51)	41/70 (59)	40/70 (57)
Pyrazinamide resistance (no. resistant/total no. [%])	21/63 (33)	20/65 (31)	17/68 (25)
Streptomycin resistance (no. resistant/total no. [%])	39/65 (60)	38/69 (55)	29/72 (40)

[a] A modal MIC of 50 mg/liter, a bioavailability of 1, and an ELF-to-plasma ratio of 17.8 were assumed based on published studies (13, 18, 82, 88, 106). Pyrazinamide resistance was as defined using methods that differ from current standards.

Pasipanodya & Gumbo. Antimicrob. Agents Chemother. 2011; 54: 24-34

Population PK data

- **PKs:** Wilkins J et al. *Eur. J Clin. Pharmacol.* 2006; 62:727-735.
  - Serum clearance= 3.4 L/h, Volume=30 L.
  - Serum clearance increases by 0.5 L/h for every 10 kg increase in weight above 48kg
  - Volume of distribution increases by4.3 L for every 10 kg increases in weight above 48kg.
  - Volume of distribution in men is 5L greater than in women
- **MICs at pH 5.8**: Salfinger M & Heifets LB. AAC 1988;32:1002-4.

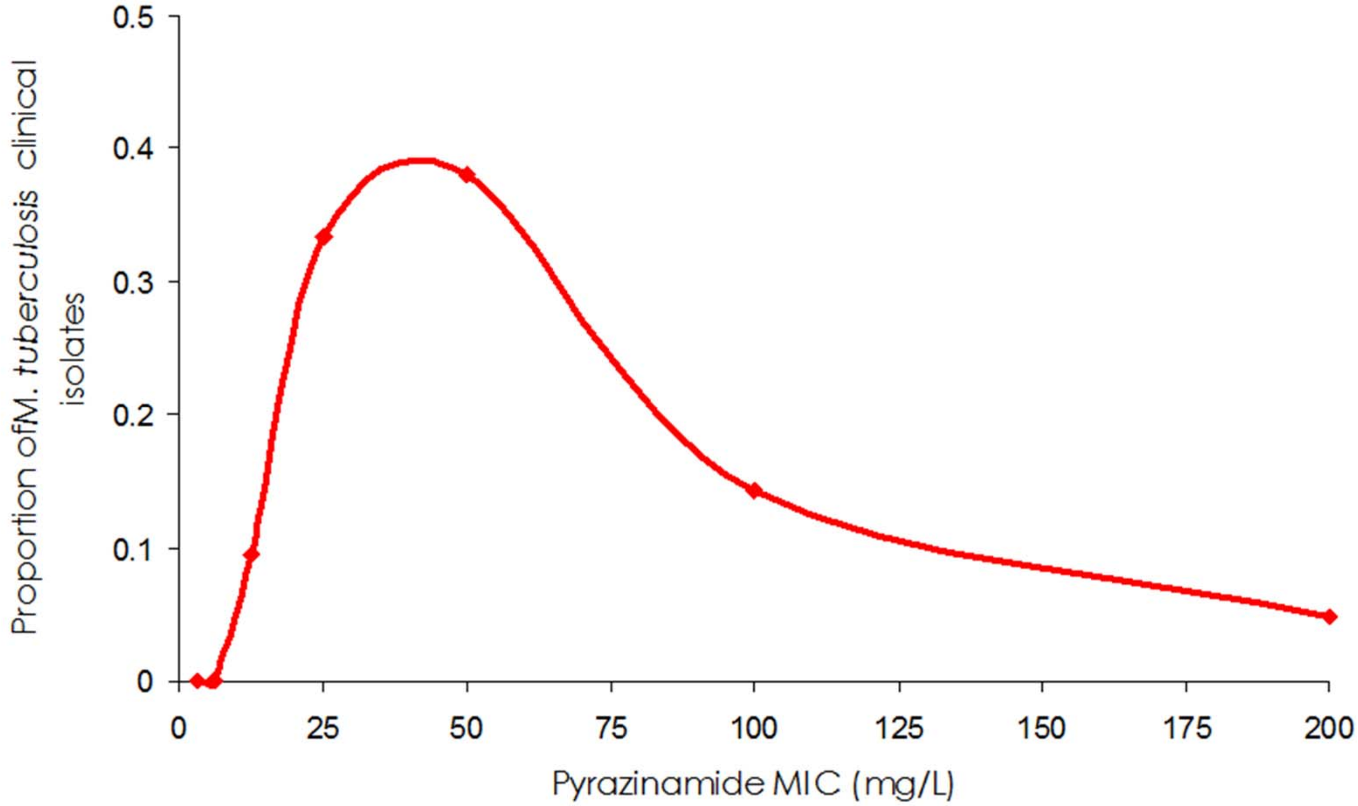
Gumbo et al. Antimicrob. Agents Chemother. 2009:53;3197-3204

Computer-aided clinical trial simulation

- 100,000 patients simulated
- Epithelial lining fluid conc. from Conte et al (AAC 1999; 43: 1329-1333)
- Man to woman ratio 68/32 (CDC)
- Weight distribution 2005 USA study
- How likely does 1.5g, 2g, 3g, 4g, 5g oral achieve the EC_{90} in these patients?
- Similar simulation for 10,000 children

Gumbo et al. Antimicrob. Agents Chemother. 2009:53;3197-3204

Clinical *Mtb* isolates: MICs at pH 5.8



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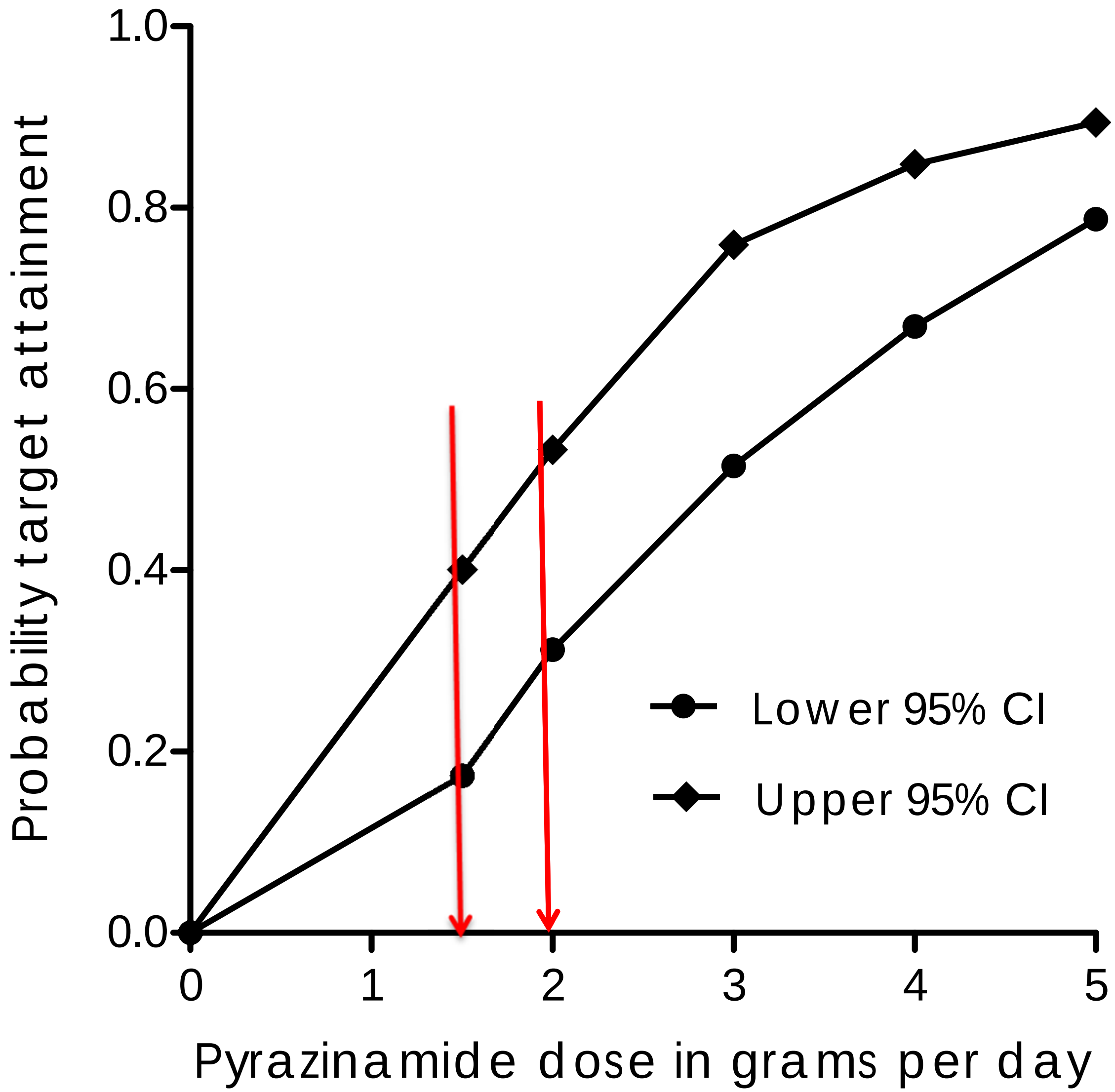

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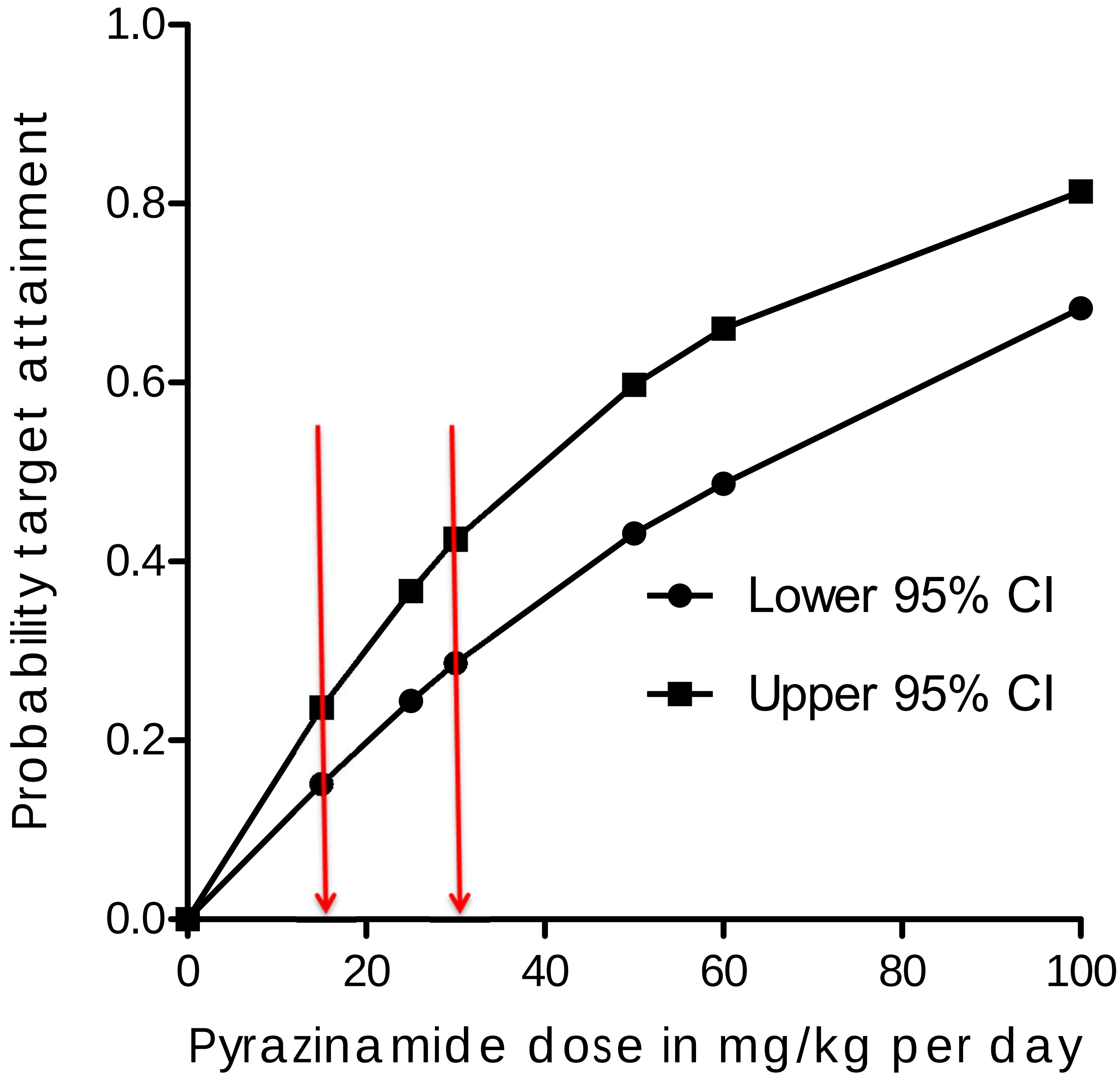


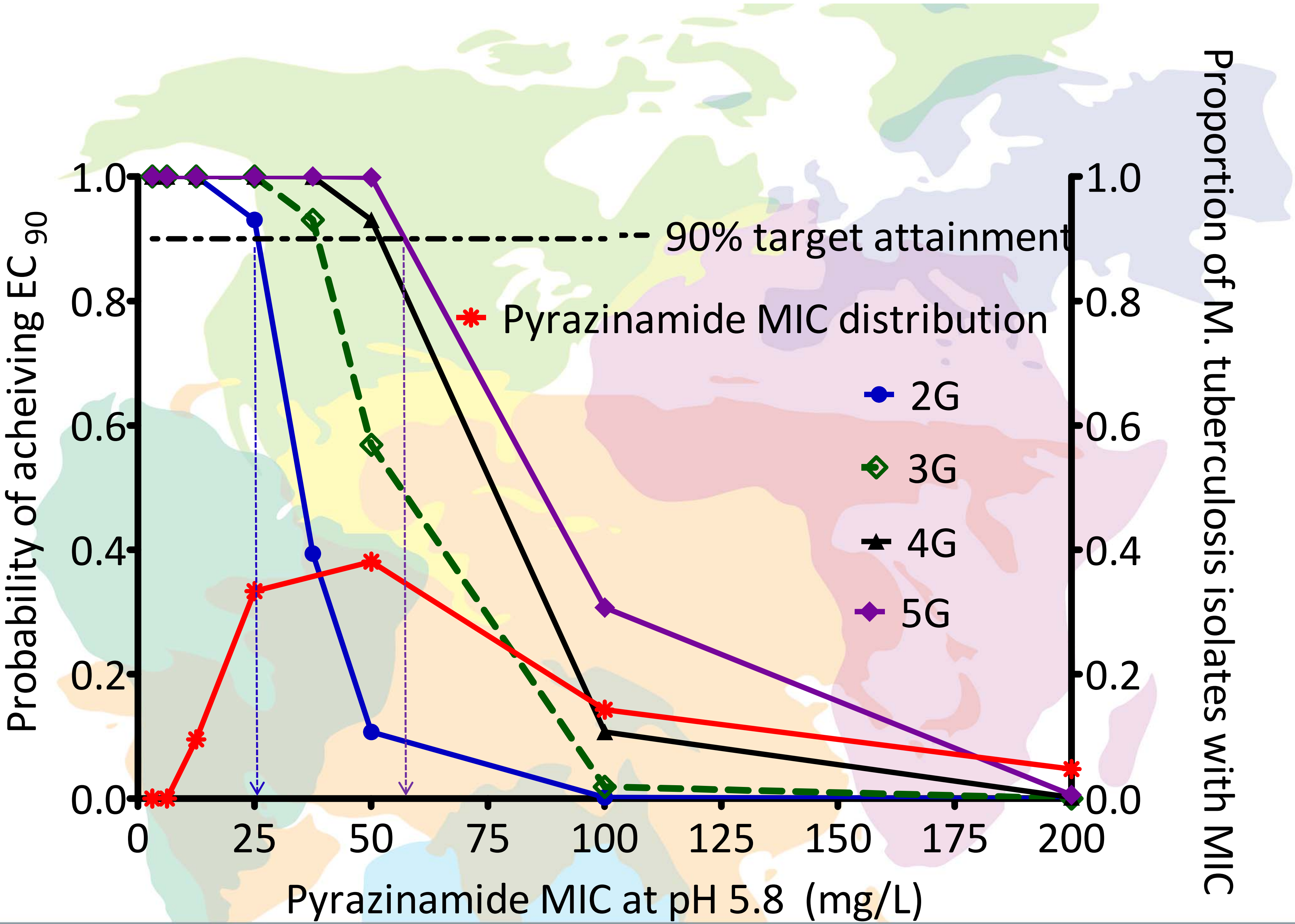
Gumbo et al. Antimicrob. Agents Chemother. 2009:53;3197-3204

Use of HFS-derived PK/PD to identify resistance breakpoints

- Use of population PK parameters
- Variability of PZA clearance with weight
- Variability of INH clearance with NAT2*4 alleles
- Rifampin pre-and post auto-induction
- Ethambutol & Moxifloxacin

- ELF concentrations utilized
- Monte Carlo simulations for ability to achieve AUC/MIC associated with 90% effect (EC_{90})
- Resistance= inability to achieve EC_{90} in >90% of TB patients

Gumbo T. Antimicrob. Agents Chemother., 2010: 53: 3197-3204



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Gumbo et al. Antimicrob. Agents Chemother. 2010:54;1484-1491

HFS/pop PK/MIC breakpoint

Drug	Old breakpoint (mg/L)	Proposed (mg/L)
Moxifloxacin	1	1
Ofloxacin	2	0.5
Isoniazid	0.2/1.0	0.03/0.125
Rifampin	1	0.0625
Ethambutol	5/7.5	4
Pyrazinamide	100	50

Gumbo T. Antimicrob. Agents Chemother., 2010: 53: 3197-3204

PZA PK/PD: Mice & Guinea pig

- Examined in BALB/c mice, a typical example of intracellular bacteria

- Examined in Guinea pigs, a typical example of extracellular bacteria

- Effect was AUC/MIC linked

- Also found that higher doses than current needed

Ahmad et al, Antimicrob. Agents. Chemother. 2011 55:1527-1532

Examination of PZA transcriptome as a single agents and in combination

- PZA, INH, RIF and the combination of the 3 effect against *Mtb* at AUCs similar to those achieved in patients; “NRP”, semi-dormant bacilli
- *Mtb* examined using RNAseq at several time points
- Analyzed using formal algebraic models and systems pharmacology differential equations
- Also simple STRING predicted protein-protein interactions for ease of visualization

Srivastava S, et al. Submitted.

	Up-regulated	Down-regulated
• INH	403	427
• RIF	363	646
• PZA	253	240
• Combo	355	732
• Day 42 NRP	419	928
• SDB pH 5.8	985	923

Srivastava S, et al. Submitted.

PZA major gene networks

- 240 down-regulated genes (~6%)
- Genes involved in **fatty acid synthesis**
- Genes involved in **cysteine metabolism**
- Genes involved in **NAD biosynthesis**
- Genes involved in metal/cation transport
- Efflux pump genes & their regulators (at least 12 efflux pumps within 24hrs)

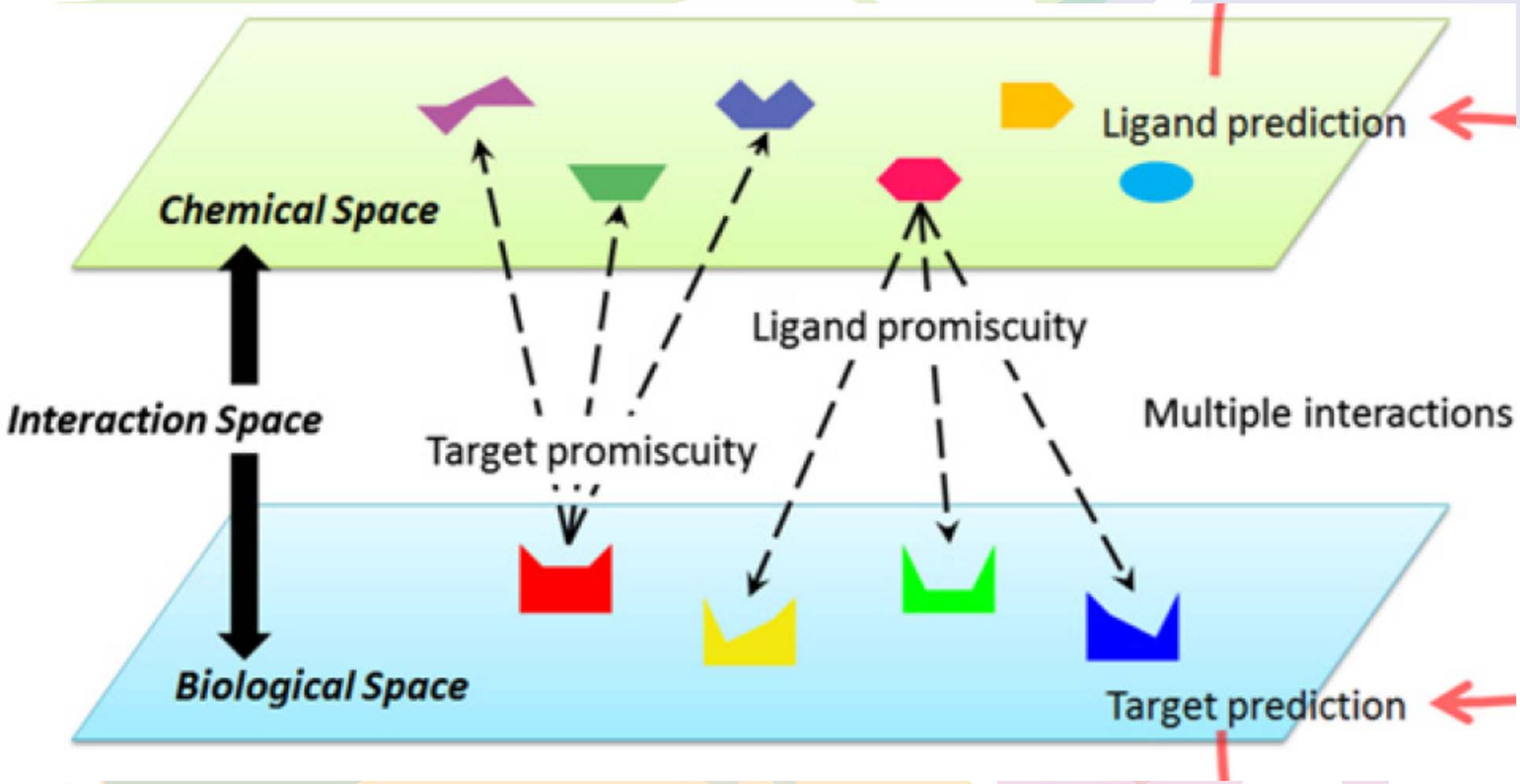
Srivastava S, et al. In prep.

Combination

- Direction and pathways most closely resembles combination of PZA and RIF
- Down-regulation of ~18% of all *Mtb* genes by this combination
- Up-regulation consistent with starvation signaling-stringent response

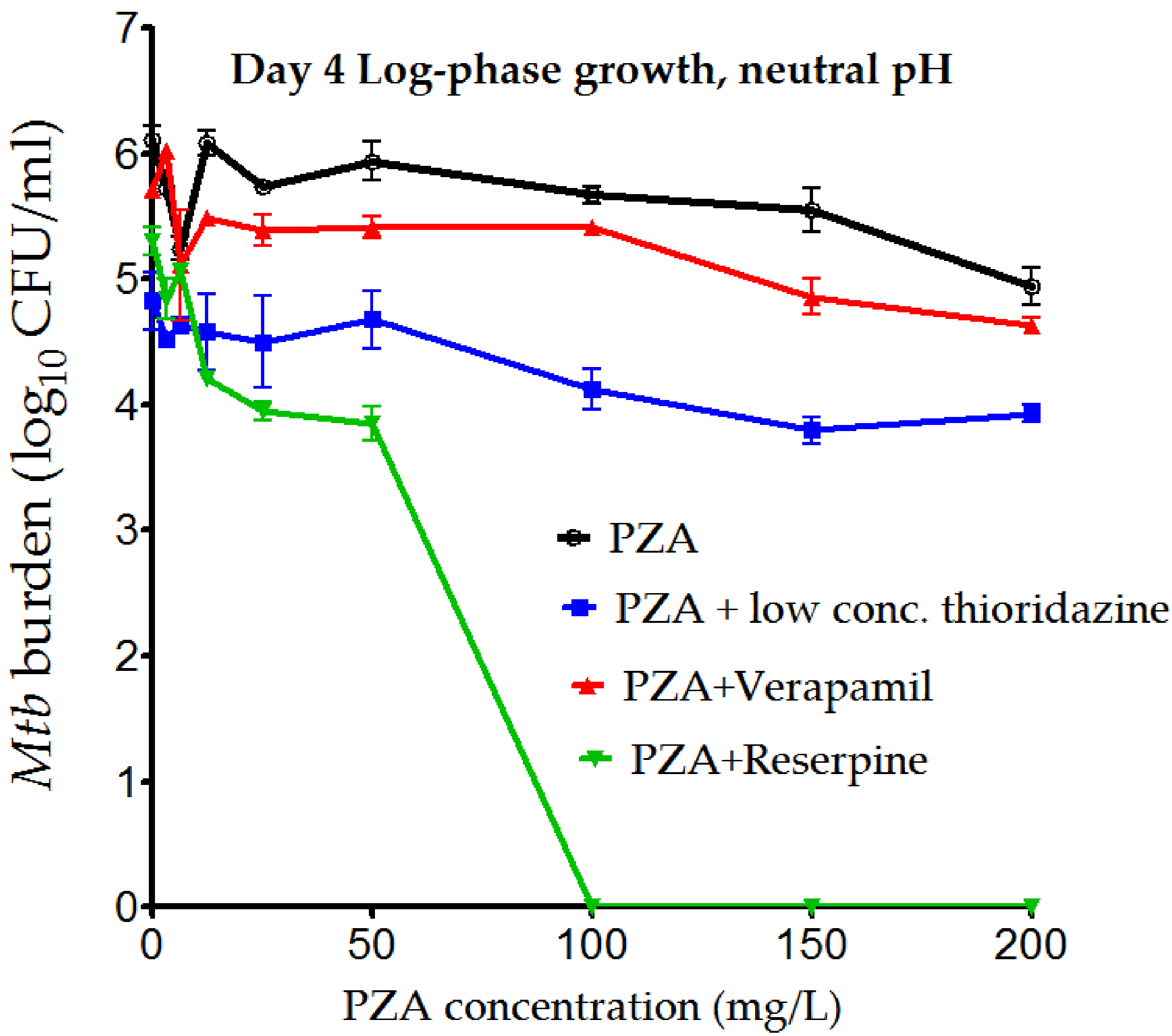
Srivastava S, et al. In Prep.

Many-to-many interactions versus “one-ligand one receptor” or “one on one”



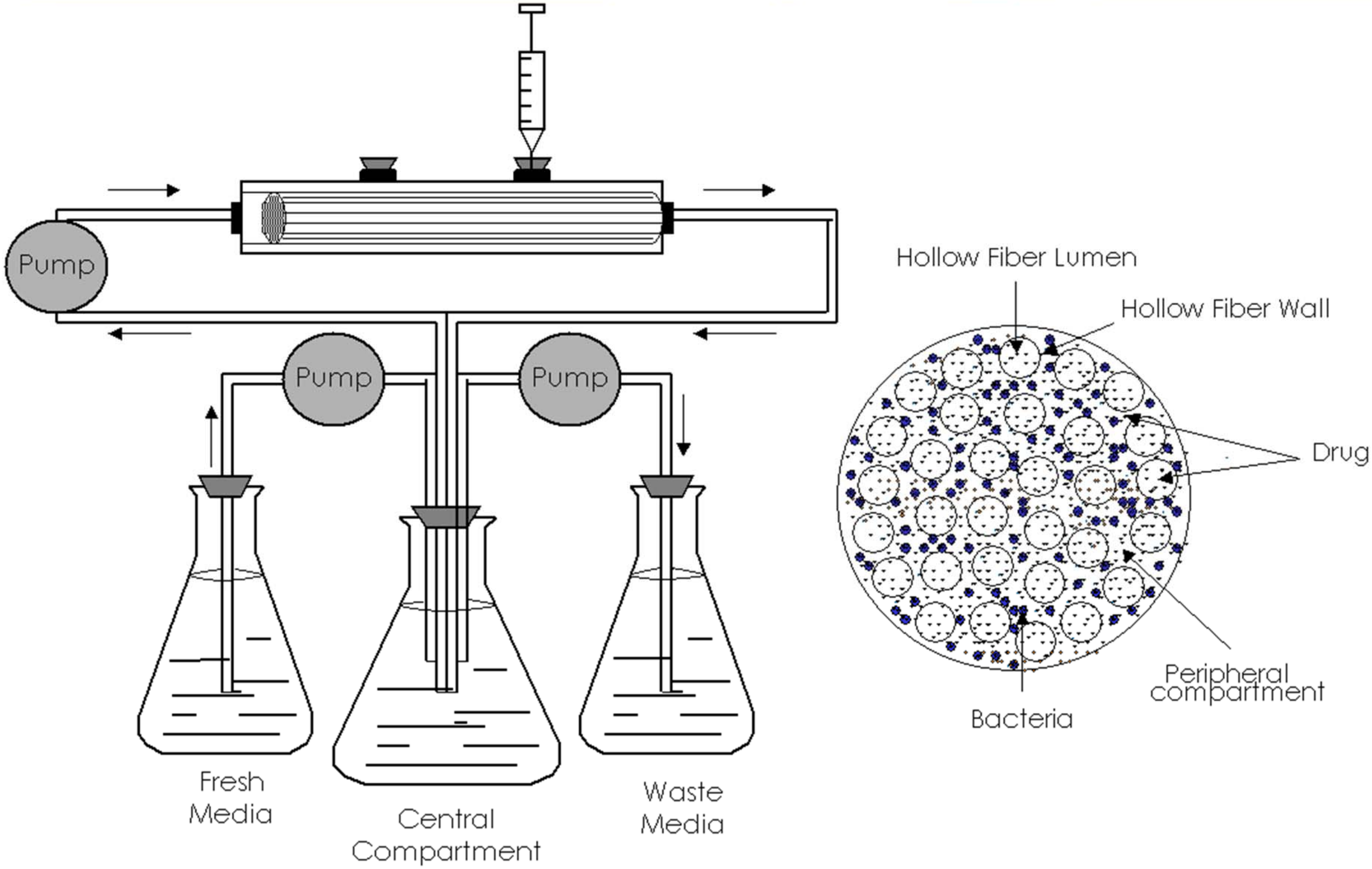
Excellent machine learning based analysis of net-work wide interaction and drug design

Brown & Okuno, Chemistry & Biology 2012; 19: 23-28.



Srivastava, In prep.

M. tuberculosis in the hollow fiber system



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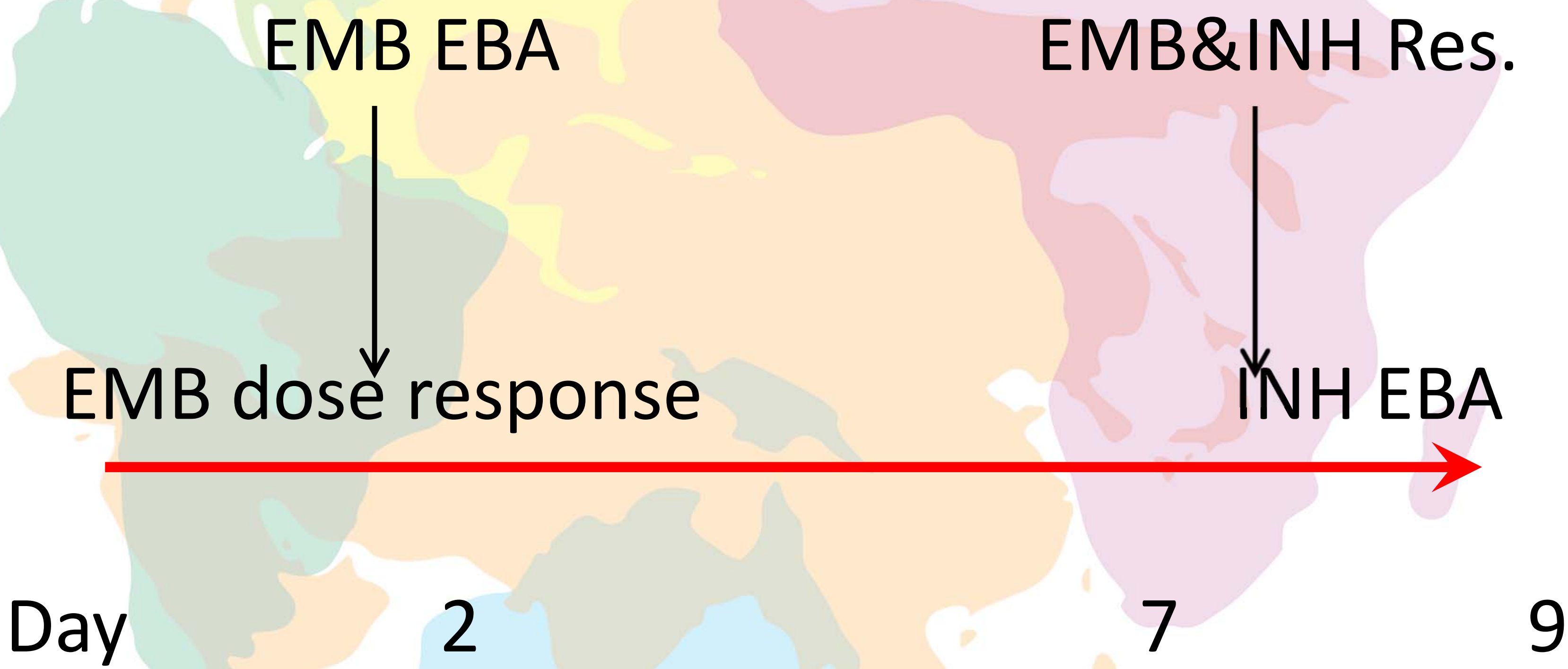
Scheme of hollow fiber study



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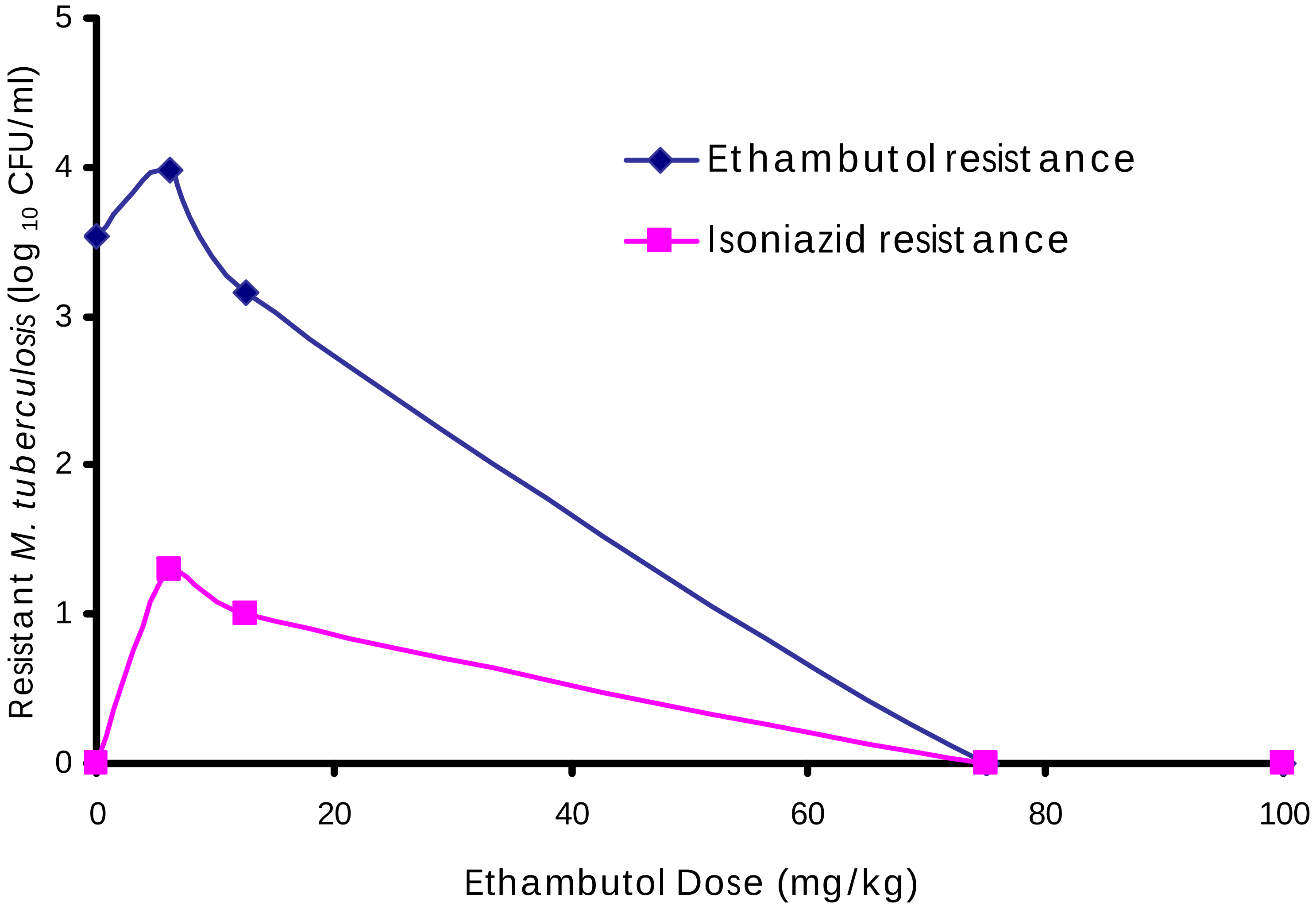
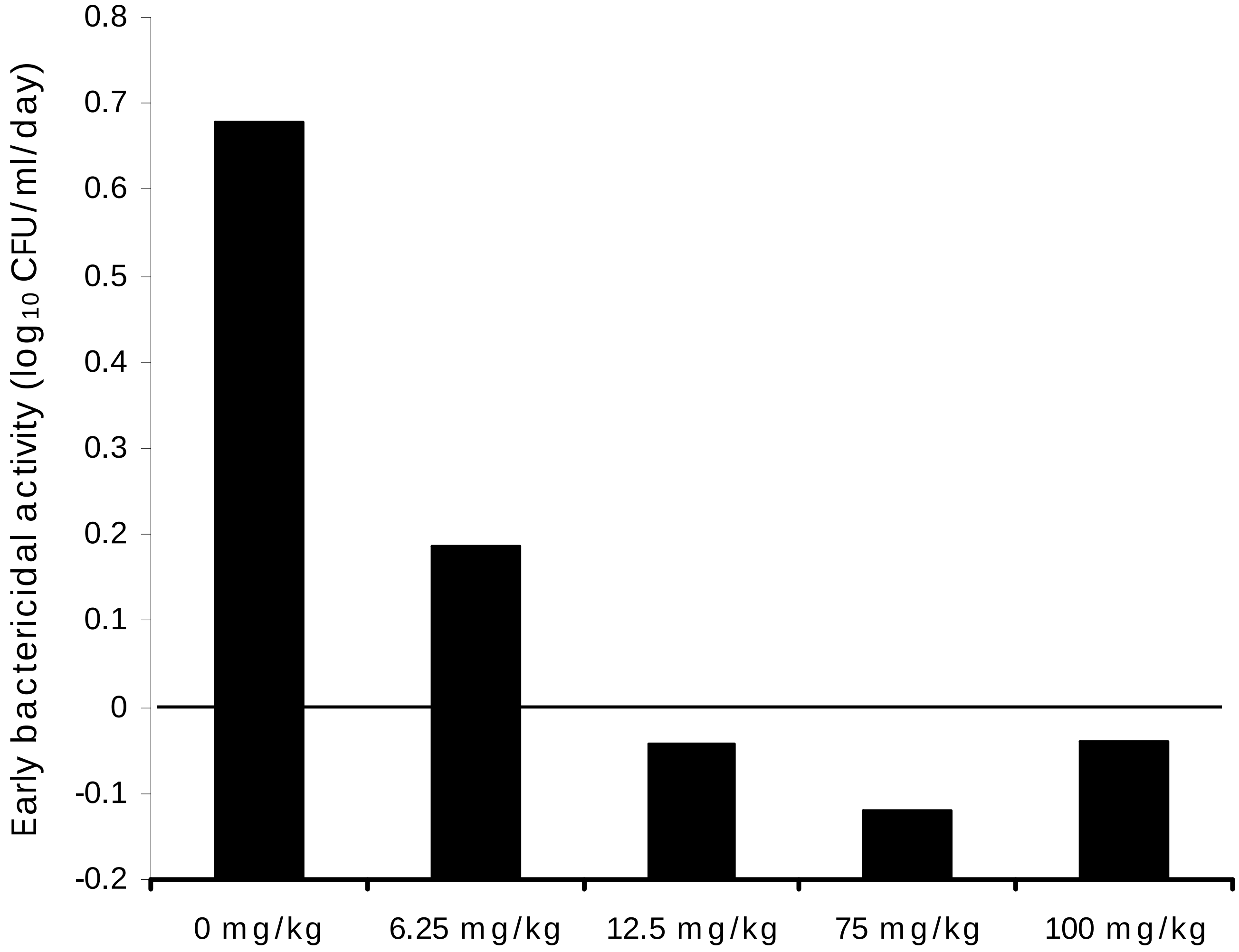
Srivastava et al. *J Infect. Dis*. 2010: 201: 1225

DAY 7 EMB AND INH RESISTANCE

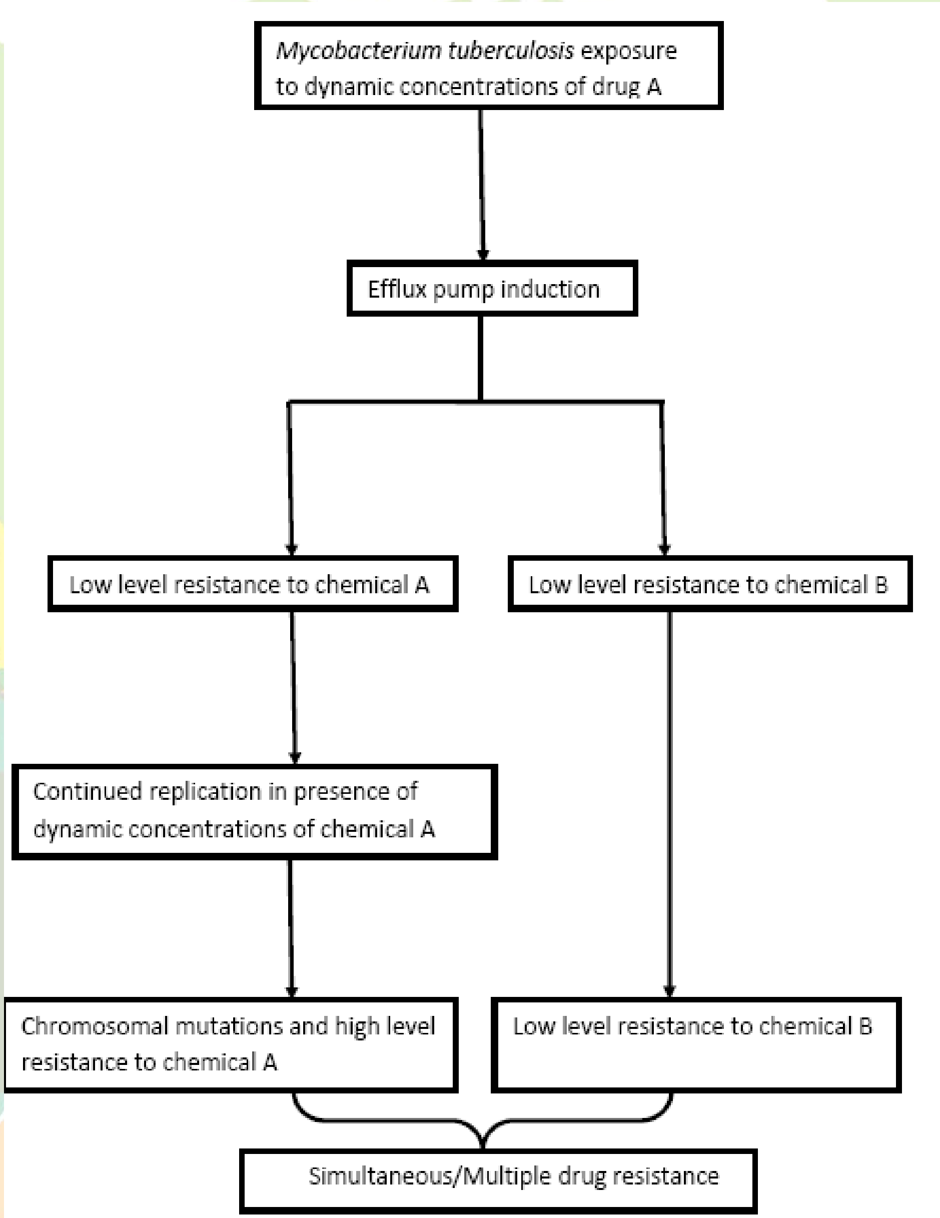


Srivastava et al. *J Infect. Dis*. 2010: 201: 1225

Isoniazid EBA



Srivastava et al. ***J Infect. Dis***. **2010: 201: 1225**



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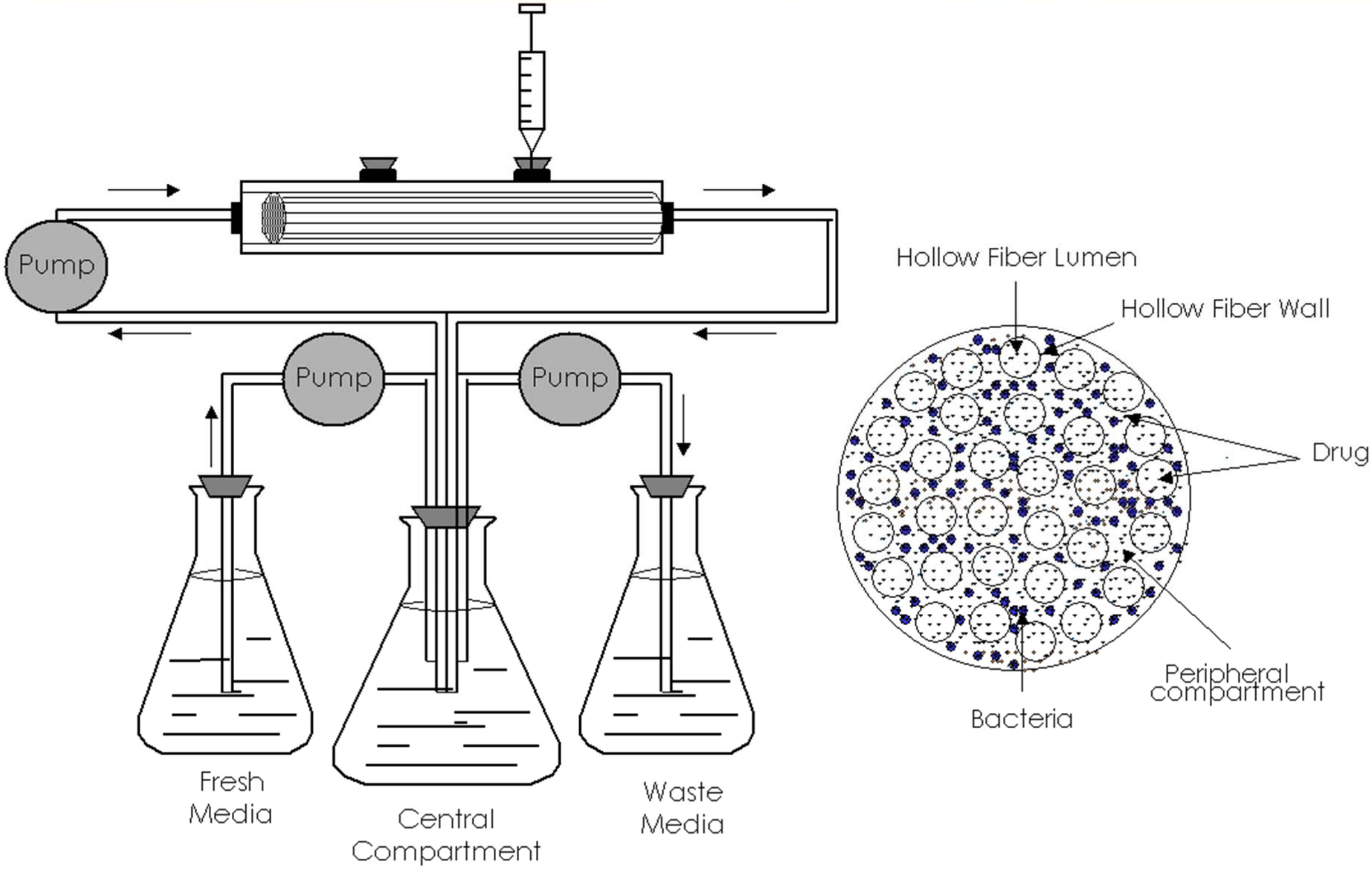

Gumbo T, et al. (2006) ***J Infect Dis*** 2006;195:194-201

Resistance suppression

- The higher the $\%T_{MIC}$, the less the smaller the resistant population for the same AUC/MIC
- $\%T_{MIC}$ of 66.7% minimal exposure associated with best suppression of resistance

Gumbo et al. Antimicrob. Agents Chemother. 2009:53;3197-3204

$$Y = aX^2 + bX + k$$

- Y : size of the resistant sub-population
- X: chemotherapeutic drug exposure value
- k: drug resistant subpopulation in non-treated controls.
- a: leading coefficient (starts as a positive value in the upright “U” curve, but changes to a negative value with increased duration of therapy
- -b/2a: drug exposure associated with the highest drug-resistant sub-population in the inverted “U”, or the lowest resistant subpopulation with an upright “U”

 Gumbo et al. Antimicrob. Agents Chemother. 2009:53;3197-3204

PZA major gene networks

- 240 down-regulated genes (~6%)
- Genes involved in **fatty acid synthesis**
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Srivastava S, et al. In prep.

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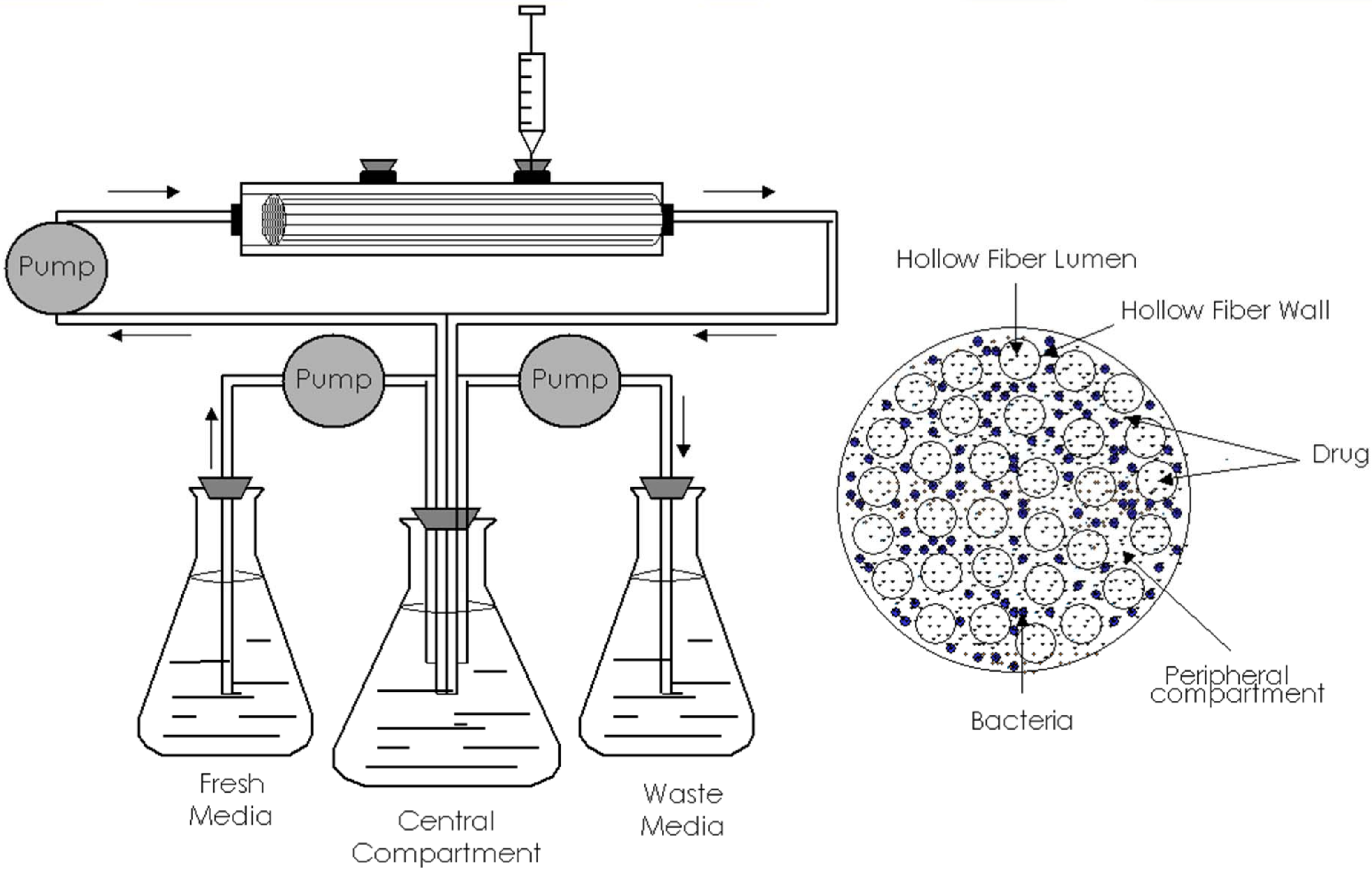
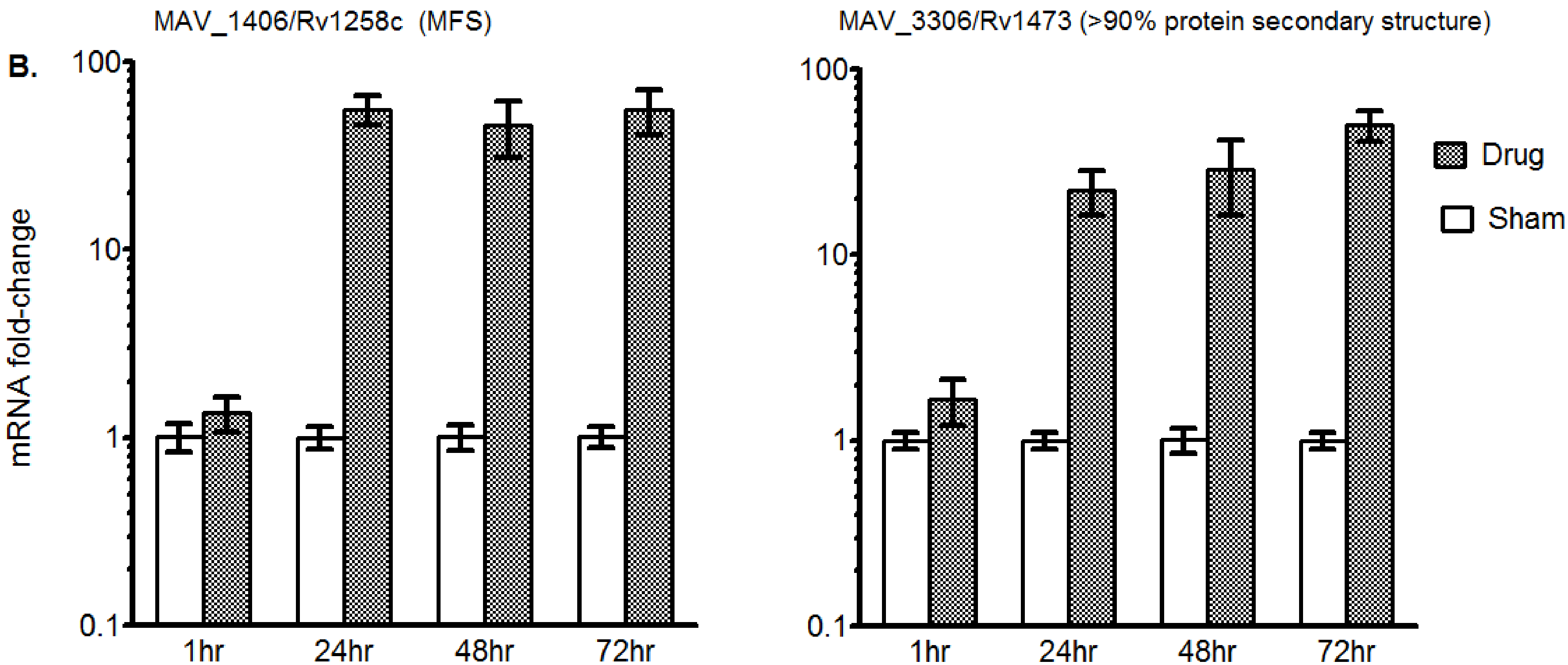


Schmalstieg R, et al. Antimicrob. Agents Chemother. 2012:56:4806-1553



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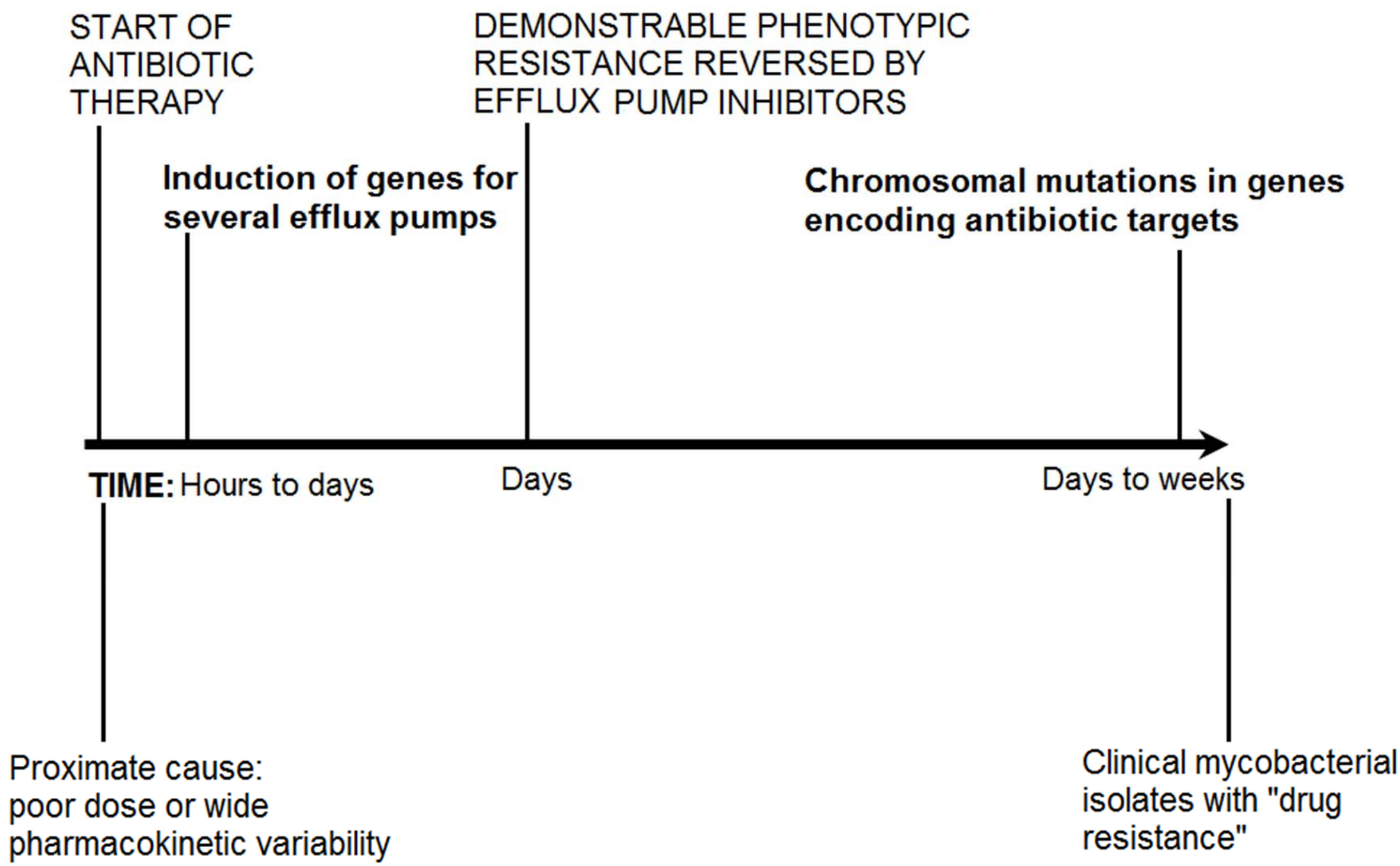


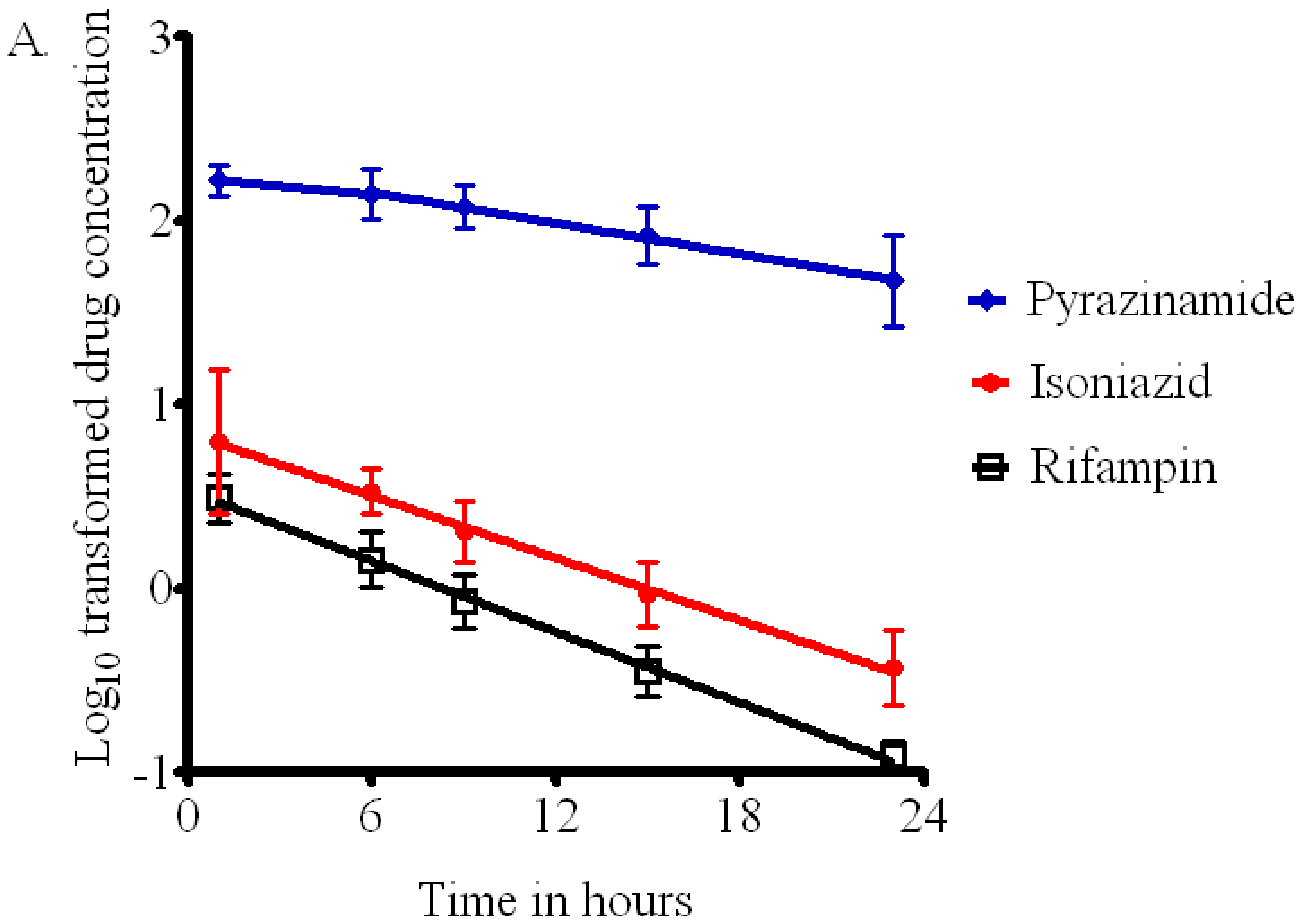
Schmalstieg R, et al. Antimicrob. Agents Chemother. 2012:56:4806-1553

What degree of non-compliance leads to emergence of acquired drug resistance?

Study design

- Three drug therapy in HFS (INH, RIF, PZA), with 3 different $t_{1/2}$ in HFS
- Bactericidal effect: drug susceptible
- Sterilizing effect: drug susceptible
- Bactericidal effect: pre-seeded with INH resistant (katG 315) and RIF resistant (rpoB S531L) of 0.5% total proportion each
- Different patterns of non adherence examined: random forgetting, start-stop, start-stop-start-stop
- Duration of therapy: 28 & 56 days

Srivastava S, et al. J. Infect. Dis. 2011; 204:1951-9.



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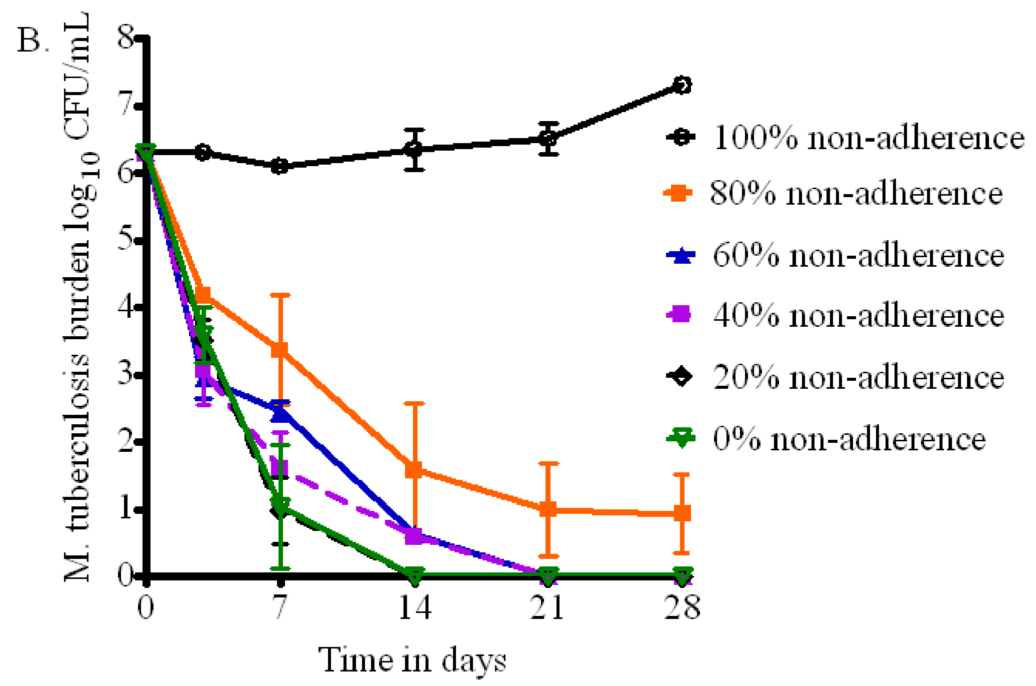
Srivastava S, et al. J. Infect. Dis. 2011; 204:1951-9.

Failure of therapy vs proportion of non-adherence

- Degree of non-adherence = proportion of doses missed
- Reference regimen = daily therapy for 56 doses
- 5/7 regimen = de facto 29% non-adherence
- Three times a week regimen = de facto 57% non-adherence

Srivastava S, et al. J. Infect. Dis. 2011; 204:1951-9.



Srivastava S, et al. J. Infect. Dis. 2011; 204:1951-9.

Microbial Kill

- Non-compliance accounted for 70.5% of all the variance in bacterial load ($p<0.0001$)
- 60% non-compliance was associated with a slower rate of kill up to day 14 ($p=0.001$), after which it stopped kill
- All regimens with ≥80% non-compliance failed.
- Breakpoint non-compliance associated with failure of therapy is therefore just below 60% for daily therapy

Srivastava S, et al. J. Infect. Dis. 2011; 204:1951-9.

Drug Resistance

- **There was no emergence of MDR-TB**

Srivastava S, et al. J. Infect. Dis. 2011; 204:1951-9.

If MDR-TB does not arise from poor compliance, why does it?

- Hypothesis: Perhaps the PK system (i.e., patient's xenobiotic metabolism) is to blame
- HFS output: kill rates, sterilizing effect rates (i.e., $\log_{10}$ CFU/ml/day)
- Known clinical kill rates, sterilizing effect rates (i.e., $\log_{10}$ CFU/ml/day)
- Performed MCS in 10,000 Western Cape Patients on the FULL REGIMEN

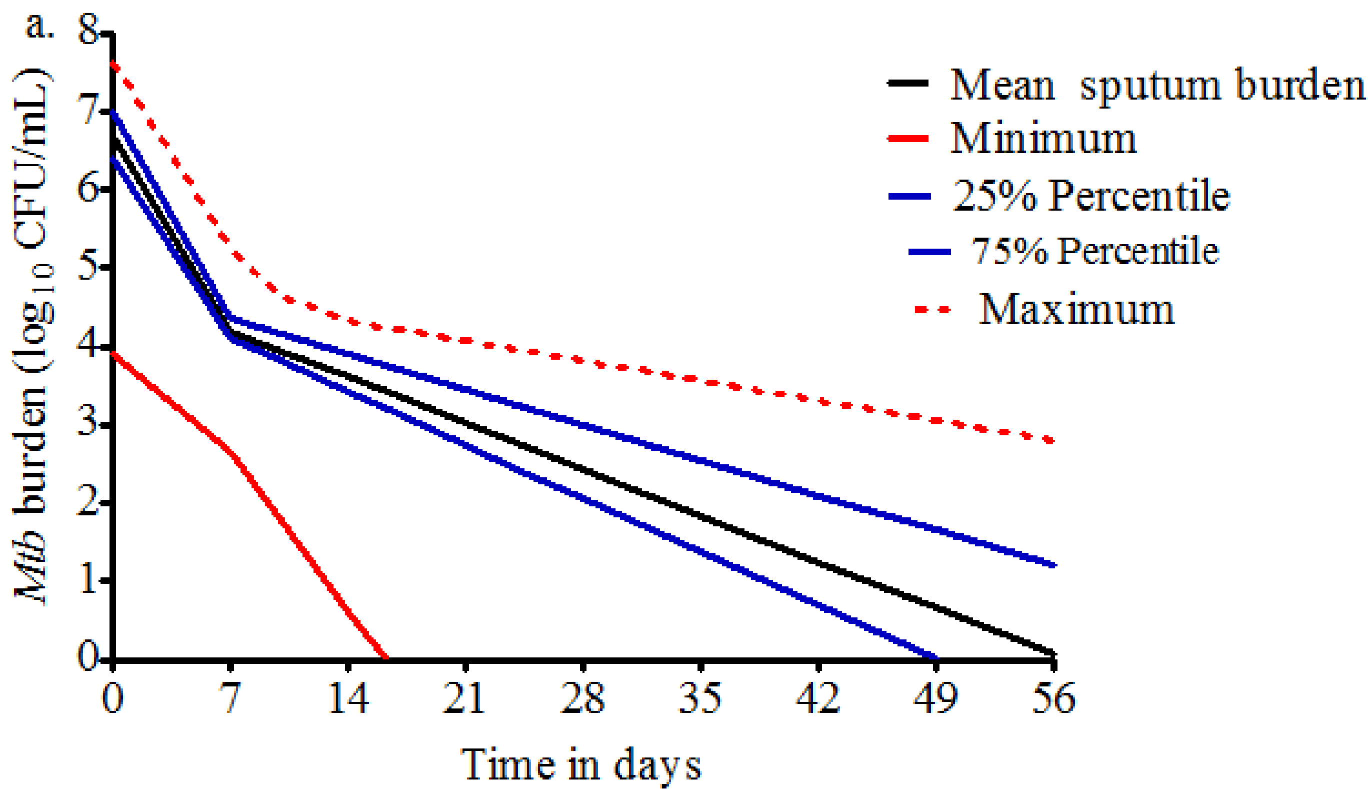
Srivastava S, et al. J. Infect. Dis. 2011; 204:1951-9.

Further assumptions

- PK and PK variability from Western Cape, South Africa, patients (studies by Wilkins & McIlleron)
- Resistance to INH and RIF arises as Poisson type event: 100% biofitness
- Patients 100% adherent to INH, RIF and PZA
- Resistance rates constrained to those observed with monotherapy in clinical studies in the 1960s and 1970s
- **How many patients on the REGIMEN will be effectively on monotherapy due to PK variability and in what proportion will MDR-TB arise?**

Srivastava S, et al. J. Infect. Dis. 2011; 204:1951-9.

External validation of model: sputum conversion rates in 10,000 patients



Sputum conversion rate predicted = 56% of patients

Sputum conversion rate from prospective clinical studies in Western Cape= 51-63%

Srivastava S, et al. J. Infect. Dis. 2011; 204:1951-9.

Many (simulated) patients had 1-2 of the 3 drugs at very low concentration throughout, leading to monotherapy of the remaining drug

Drug resistance predicted to arise in <u>0.68% of all pts</u> on therapy in first 2 months despite 100% adherence

Srivastava S, et al. J. Infect. Dis. 2011; 204:1951-9.

Prospective study of 142 patients in the Western Cape province of South Africa

Jotam Pasipanodya, Helen McIlleron*, André Burger, Peter A. Wash, Peter Smith, Tawanda Gumbo

Pasipanodya J, et al. Submitted.

What was done

- All patients hospitalized first 2 months
- All had 100% adherence first 2 months
- Drug concentrations measured at 8 time points over 24hrs in month 2
- Followed for 2 years, 6% non-adherence

Pasipanodya J, et al. Submitted.

Classification and Regression Tree analysis

- Non-parametric machine learning technique
- **Main objective is predictive accuracy**
- Ranks important predictors of outcome
- Also calculate thresholds for continuous variables
- Clinical factors in the model were age, gender, weight, cavities, HIV status, streptomycin, non-adherence, PK factors (AUC, trough, C_{max})
- Examined both 2 month and 2 year outcomes

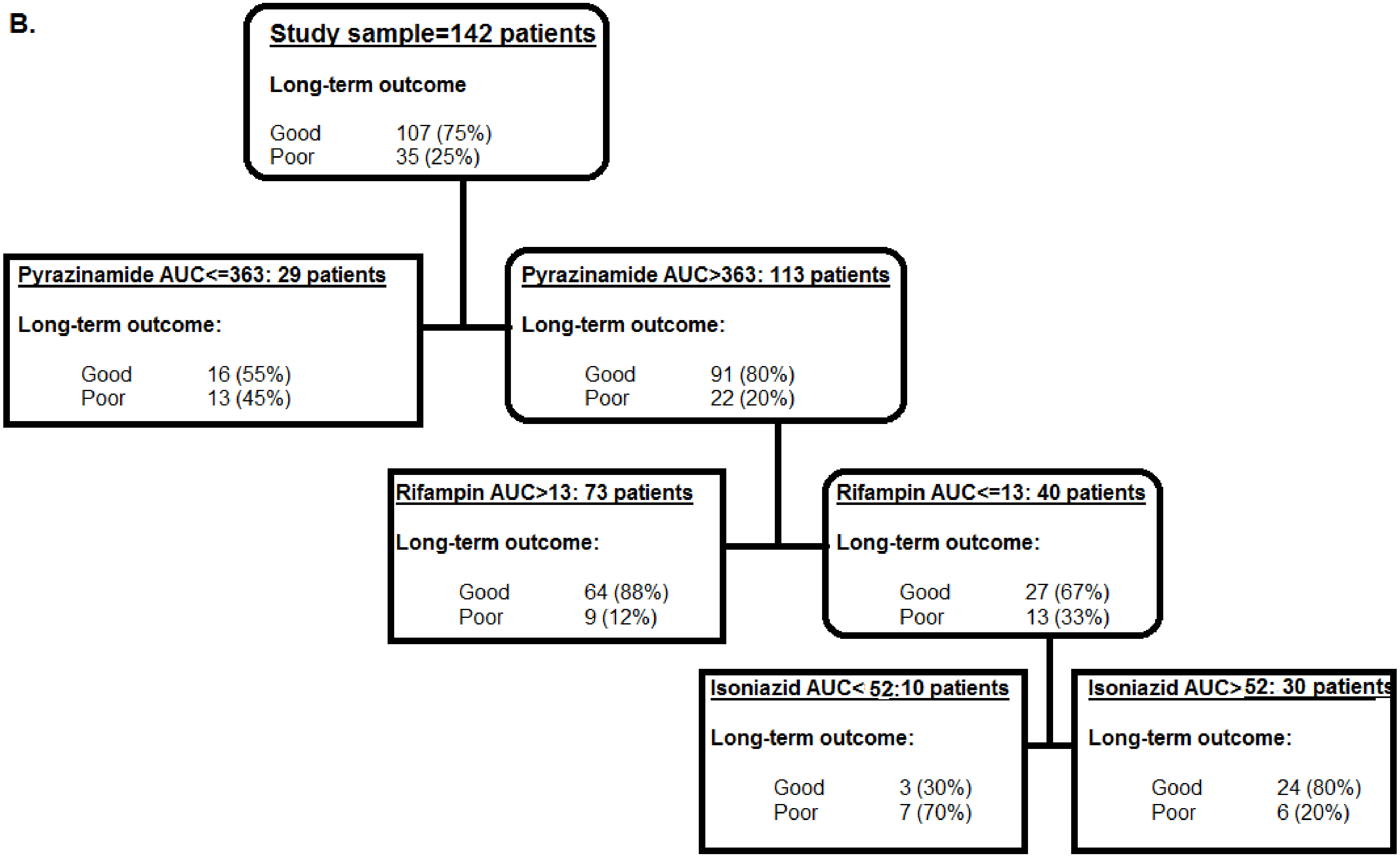
Pasipanodya J, et al. Submitted.

PK/PD in HFS models

Drug	Microbial kill	Resistance suppression	Reference
Rifampin	AUC/MIC	**C_{max}/MIC**	Gumbo et al. AAC 2007
Isoniazid	AUC/MIC	**AUC/MIC; C_{max}/MIC**	Gumbo et al. AAC 2007
Pyrazinamide	AUC/MIC	**%T>MIC**	Gumbo et al. AAC 2009
Ethambutol	AUC/MIC	**%T>MIC**	Srivastava et al. JID 2010
Moxifloxacin	AUC/MIC	**AUC/MIC**	Gumbo et al. JID 2004

Top 3 predictors: Long term outcomes

B.



Pasipanodya J, et al. Submitted.

Long-term outcomes

- 91% of patients with poor outcomes had at least one drug with low AUC
- All ADR had low concentrations of at least one drug
- Low PZA_{AUC} accounted for 83% of ***all poor long-term outcomes***

Pasipanodya J, et al. Submitted.

Summary

- 2 Month Outcomes
  (sputum culture conversion)

- Long-term Outcomes
  (failure, relapse & death)

- PZA C_{max}
- RIF C_{max}
- INH C_{max}

↓

- PZA AUC
- RIF AUC
- INH AUC

↓

Pasipanodya J, et al. Submitted.

Summary

- Pyrazinamide is likely the dominant drug in current combinations
- PK/PD studies, and patient data, suggest that higher doses may be necessary to achieve optimal AUCs and AUC/MIC associated with efficacy
- Suggest that new drug regimens include a PZA backbone, with optimized dose

Pasipanodya J, et al. Submitted.

Drug resistance: Proposal

- Mtb isolates with different MICs from say 12.5-200 with M. bovis as negative control
- Set up parallel HFS, mouse, Guinea pig studies
- Treatment with full regimens, see when failure occurs (when it looks like *M. bovis*)
- That MIC when poor response occurs, is breakpoint

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- UT Southwestern:
- Office of Global Health & Leadership
- Department of Medicine $ Leadership
- UT Southwestern leadership

Pasipanodya J, et al. Submitted.

I Thank You