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

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**The team: this work has many fathers & mothers**

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- **2. University of Cape Town:**
  Helen McIlIeron, Peter Smith, Emmanuel Chigutsa

- **3. Brewelskloof hospital, Western Cape**
  Peter Wash, André Burger
M. tuberculosis in the hollow fiber system

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

\[
\frac{dX_t}{dt} = R(1) - (SCL/V_c) \times X_t ;
\]

\[
\frac{dN_s}{dt} = K_{g_{\max-s}} \times (1 - L_s) \times N_s \\
\times E - K_{k_{\max-s}} \times M_s \times N_s ;
\]

\[
\frac{dN_r}{dt} = K_{g_{\max-r}} \times (1 - L_r) \times N_r \\
\times E - K_{k_{\max-r}} \times M_r \times N_r ;
\]

\[
E = 1 - (N_r + N_s)/\text{POPMAX} ;
\]

\[
L = (X_t/V_c)^H/[(X_t/V_c)^H + C_{50}g^H],
\]

where \( H = H_{g-s} \) or \( H_{g-r} \),

\[
M = (X_s/V_r)^H/[(X_s/V_r)^H + C_{50}k^H],
\]

where \( H = H_{k-s} \) or \( H_{k-r} \).
**Population-median parameter estimates of pharmacodynamic model.**

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

\[ \Downarrow \]

Systems pharmacology equations

\[ \Downarrow \]

Clinical prediction: Dose, Treatment & ADR rates

\[ \Downarrow \]

Clinical validation: dose, treatment rates, ADR rates
M. tuberculosis in the hollow fiber system

M. tuberculosis burden (log₁₀ CFU/ml) = 6.48 - (2.21 \times \text{Dose}^{2.96})/(\text{Dose}^{2.96} + 36.3^{2.96})

p < 0.01; r² = 0.99

ED₅₀

ED₈₀

Effect (log₁₀ CFU/ml)
## PZA Standard Doses In HFS Vs. In patients

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Hollow fiber</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Kill rates:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0-4 (log$_{10}$ CFU/ml/day)</td>
<td>0.1±0.2</td>
<td>-0.1</td>
</tr>
<tr>
<td>Day 4-14 (log$_{10}$ CFU/ml/day)</td>
<td>0.12±0.05</td>
<td>0.09-0.1</td>
</tr>
<tr>
<td><strong>Time to resistance emergence:</strong></td>
<td>2-3 wks</td>
<td>2-3 wks</td>
</tr>
</tbody>
</table>

**Patient data sources:**
### Sterilizing effect Vs. PK/PD parameter

<table>
<thead>
<tr>
<th>PK/PD Index</th>
<th>Time</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$/MIC</td>
<td></td>
<td>0.56</td>
<td>0.63</td>
<td>0.61</td>
</tr>
<tr>
<td>AUC/MIC</td>
<td></td>
<td>0.90</td>
<td>0.89</td>
<td>0.80</td>
</tr>
<tr>
<td>%T$&gt;$MIC</td>
<td></td>
<td>0.76</td>
<td>0.78</td>
<td>0.75</td>
</tr>
</tbody>
</table>

EC$_{90}$ AUC$_{0-24}$/MIC = 210

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.
<table>
<thead>
<tr>
<th>Parameter (measurement)</th>
<th>Result for dosing regimen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>500 mg three times a day</td>
</tr>
<tr>
<td>Serum AUC$_{0-168}$ (mg \cdot h/liter)</td>
<td>2,189</td>
</tr>
<tr>
<td>ELF AUC$_{0-24}$/MIC$^a$</td>
<td>111</td>
</tr>
<tr>
<td>Serum $C_{\text{max}}$ (mg/liter)</td>
<td>20.2</td>
</tr>
<tr>
<td>ELF $C_{\text{max}}$/MIC$^a$</td>
<td>7.2</td>
</tr>
<tr>
<td>Sputum conversion (no. converted/total no. [%])</td>
<td>30/65 (46)</td>
</tr>
<tr>
<td>2-month time point</td>
<td></td>
</tr>
<tr>
<td>End of 6 months</td>
<td>28/66 (42)</td>
</tr>
<tr>
<td>Any radiological improvement (no. showing improvement/total no. [%])</td>
<td>32/63 (51)</td>
</tr>
<tr>
<td>Pyrazinamide resistance (no. resistant/total no. [%])</td>
<td>21/63 (33)</td>
</tr>
<tr>
<td>Streptomycin resistance (no. resistant/total no. [%])</td>
<td>39/65 (60)</td>
</tr>
</tbody>
</table>

$^a$ 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.
Population PK data

  - 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 48 kg.
  - Volume of distribution increases by 4.3 L for every 10 kg increase in weight above 48 kg.
  - Volume of distribution in men is 5 L greater than in women.

- **MICs at pH 5.8:** Salfinger M & Heifets LB. *AAC* 1988;32:1002-4.
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
Clinical *Mtb* isolates: MICs at pH 5.8

Pyrazinamide dose in grams per day

Probability target attainment

Lower 95% CI

Upper 95% CI

The graph shows the probability of target attainment as a function of the pyrazinamide dose in mg/kg per day. The x-axis represents the pyrazinamide dose, while the y-axis represents the probability of target attainment. The graph includes two curves: one for the lower 95% confidence interval (Lower 95% CI) and another for the upper 95% confidence interval (Upper 95% CI). The arrows indicate specific dose points that are within the confidence intervals.
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


Pyrazinamide MIC distribution at pH 5.8 (mg/L)

- 2G
- 3G
- 4G
- 5G

Probability of achieving EC$_{90}$

Proportion of M. tuberculosis isolates with MIC

90% target attainment

UT SOUTHWESTERN
Office of Global Health
HFS/pop PK/MIC breakpoint

<table>
<thead>
<tr>
<th>Drug</th>
<th>Old breakpoint (mg/L)</th>
<th>Proposed (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moxifloxacin</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td>Isoniazid</td>
<td>0.2/1.0</td>
<td>0.03/0.125</td>
</tr>
<tr>
<td>Rifampin</td>
<td>1</td>
<td>0.0625</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>5/7.5</td>
<td>4</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>100</td>
<td>50</td>
</tr>
</tbody>
</table>

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
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

<table>
<thead>
<tr>
<th></th>
<th>Up-regulated</th>
<th>Down-regulated</th>
</tr>
</thead>
<tbody>
<tr>
<td>INH</td>
<td>403</td>
<td>427</td>
</tr>
<tr>
<td>RIF</td>
<td>363</td>
<td>646</td>
</tr>
<tr>
<td>PZA</td>
<td>253</td>
<td>240</td>
</tr>
<tr>
<td>Combo</td>
<td>355</td>
<td>732</td>
</tr>
<tr>
<td>Day 42 NRP</td>
<td>419</td>
<td>928</td>
</tr>
<tr>
<td>SDB pH 5.8</td>
<td>985</td>
<td>923</td>
</tr>
</tbody>
</table>

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)

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

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
Day 4 Log-phase growth, neutral pH

- **PZA**
- **PZA + low conc. thioridazine**
- **PZA + Verapamil**
- **PZA + Reserpine**

**Mtb burden (log_{10} CFU/ml)**

**PZA concentration (mg/L)**

Srivastava, In prep.
M. tuberculosis in the hollow fiber system

Scheme of hollow fiber study

EMB EBA

EMB dose response

EMB&INH Res.

INH EBA

Day 2 7 9

DAY 7 EMB AND INH RESISTANCE

Ethambutol Dose (mg/kg)

Isoniazid EBA

Early bactericidal activity (log_{10} CFU/ml/day)

Mycobacterium tuberculosis exposure to dynamic concentrations of drug A

Efflux pump induction

Low level resistance to chemical A

Continued replication in presence of dynamic concentrations of chemical A

Chromosomal mutations and high level resistance to chemical A

Low level resistance to chemical B

Simultaneous/Multiple drug resistance

Low level resistance to chemical B
M. tuberculosis in the hollow fiber system

Resistance suppression

- The higher the $\%T_{\text{MIC}}$, the less the smaller the resistant population for the same AUC/MIC
- $\%T_{\text{MIC}}$ of 66.7% minimal exposure associated with best suppression of resistance
\[ Y = aX^2 + bX + k \]

- \( Y \): 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”
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)

M. tuberculosis in the hollow fiber system

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

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

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 \( \geq 80\% \) non-compliance failed.

• Breakpoint non-compliance associated with failure of therapy is therefore just below 60\% for daily therapy

Drug Resistance

- There was no emergence of MDR-TB

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., $\log_{10} \text{CFU/ml/day}$)
- Known clinical kill rates, sterilizing effect rates (i.e., $\log_{10} \text{CFU/ml/day}$)
- Performed MCS in 10,000 Western Cape Patients on the FULL REGIMEN

Further assumptions

- PK and PK variability from Western Cape, South Africa, patients (studies by Wilkins & McIlheron)
- 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?
Sputum conversion rate predicted = 56% of patients

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

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 **0.68% of all pts** on therapy in first 2 months despite 100% adherence.

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

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

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, $C_{\text{max}}$)
- Examined both 2 month and 2 year outcomes
## PK/PD in HFS models

<table>
<thead>
<tr>
<th>Drug</th>
<th>Microbial kill</th>
<th>Resistance suppression</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rifampin</td>
<td>AUC/MIC</td>
<td>$C_{\text{max}}$/MIC</td>
<td>Gumbo et al. AAC 2007</td>
</tr>
<tr>
<td>Isoniazid</td>
<td>AUC/MIC</td>
<td>AUC/MIC; $C_{\text{max}}$/MIC</td>
<td>Gumbo et al. AAC 2007</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>AUC/MIC</td>
<td>%T&gt;MIC</td>
<td>Gumbo et al. AAC 2009</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>AUC/MIC</td>
<td>%T&gt;MIC</td>
<td>Srivastava et al. JID 2010</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>AUC/MIC</td>
<td>AUC/MIC</td>
<td>Gumbo et al. JID 2004</td>
</tr>
</tbody>
</table>
Top 3 predictors: Long term outcomes

B.

**Study sample=142 patients**

Long-term outcome

<table>
<thead>
<tr>
<th></th>
<th>Good</th>
<th>Poor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good</td>
<td>107 (75%)</td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>35 (25%)</td>
<td></td>
</tr>
</tbody>
</table>

**Pyrazinamide AUC<=363: 29 patients**

Long-term outcome

<table>
<thead>
<tr>
<th></th>
<th>Good</th>
<th>Poor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good</td>
<td>16 (55%)</td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>13 (45%)</td>
<td></td>
</tr>
</tbody>
</table>

**Pyrazinamide AUC>363: 113 patients**

Long-term outcome

<table>
<thead>
<tr>
<th></th>
<th>Good</th>
<th>Poor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good</td>
<td>91 (80%)</td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>22 (20%)</td>
<td></td>
</tr>
</tbody>
</table>

**Rifampin AUC>13: 73 patients**

Long-term outcome

<table>
<thead>
<tr>
<th></th>
<th>Good</th>
<th>Poor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good</td>
<td>64 (88%)</td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>9 (12%)</td>
<td></td>
</tr>
</tbody>
</table>

**Rifampin AUC<=13: 40 patients**

Long-term outcome

<table>
<thead>
<tr>
<th></th>
<th>Good</th>
<th>Poor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good</td>
<td>27 (67%)</td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>13 (33%)</td>
<td></td>
</tr>
</tbody>
</table>

**Isoniazid AUC< 62: 10 patients**

Long-term outcome

<table>
<thead>
<tr>
<th></th>
<th>Good</th>
<th>Poor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good</td>
<td>3 (30%)</td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>7 (70%)</td>
<td></td>
</tr>
</tbody>
</table>

**Isoniazid AUC> 62: 30 patients**

Long-term outcome

<table>
<thead>
<tr>
<th></th>
<th>Good</th>
<th>Poor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good</td>
<td>24 (80%)</td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>6 (20%)</td>
<td></td>
</tr>
</tbody>
</table>

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 $\text{PZA}_{\text{AUC}}$ accounted for 83% of all poor long-term outcomes

Summary

• 2 Month Outcomes (sputum culture conversion)
  - PZA $C_{\text{max}}$
  - RIF $C_{\text{max}}$
  - INH $C_{\text{max}}$

• Long-term Outcomes (failure, relapse & death)
  - PZA AUC
  - RIF AUC
  - INH AUC

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
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
FUNDING & Acknowledgements

- National Institutes of Health:
  - DP2 OD001886
  - RO1 A1079497
- UT Southwestern:
  - Office of Global Health & Leadership
  - Department of Medicine $ Leadership
- UT Southwestern leadership

I Thank You