MALDI-IMAGING AS A TOOL FOR DRUG DISTRIBUTION STUDIES OF SELECTED ANTI-TUBERCULAR DRUGS

Adeola Shobo (PhD)
CPRU-www.cpru.ac.za
School of Pharmacy, UKZN
Principal Investigators

Thavi Govender  Gert Kruger  Glenn Maguire  Tricia Naicker

Fernando Albericio  Beatriz de la Torre  Raveen Parboosing  Per Arvidsson  Bahareh Honarparsvar
Principal Investigators

Prof Fernando Albericio – Peptide Chemistry, CPRU and ex- Executive Director of the Barcelona Science Park (Spain).

Prof Per I. Arvidsson – Organocatalysis and Drug Development, CPRU, Director of Medicinal Research of SciLifeLab at Karolinska Institute (Sweden).

Prof Gert Kruger – Computational Chemistry, Cage chemistry, CPRU (SA).

Prof Thavi Govender – Asymmetric Catalysis and Analytical Chemistry, Director of CPRU at UKZN (SA).

Prof Beatriz de la Torre – Peptide Chemistry, CPRU, (SA)

Dr Glenn Maguire – Physical Organic and Metallo Chemistry, CPRU (SA).

Dr Tricia Naicker – Organocatalysis and NMR, CPRU (SA).

Dr Raveen Parboosing – Virologist, CPRU, Albert Luthuli Hospital (SA).

Dr Bahareh Honarparvar – Computational Chemistry, CPRU, (SA)
Background

- Tuberculosis (TB) is a major health problem and one of the leading cause of death globally¹

- MDR and XDR TB pose substantial public health problems all around the world²

Tuberculosis meningitis (TBM): most common form of extra-pulmonary TB within HIV co-infected patients

Morbidity and mortality remains high

Possibly due to reduced penetration of orally administered TB drugs in the central nervous system

### Background

**Estimated epidemiological burden of TB, 2014**

<table>
<thead>
<tr>
<th>Country</th>
<th>Population</th>
<th>Mortality</th>
<th>HIV-Positive TB Mortality</th>
<th>Prevalence</th>
<th>Incidence</th>
<th>HIV-Positive Incident TB cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>China</td>
<td>1,369,436</td>
<td>38</td>
<td>0.7</td>
<td>1,200</td>
<td>930</td>
<td>13</td>
</tr>
<tr>
<td>Ethiopia</td>
<td>96,959</td>
<td>32</td>
<td>5.5</td>
<td>190</td>
<td>200</td>
<td>19</td>
</tr>
<tr>
<td>India</td>
<td>1,295,292</td>
<td>220</td>
<td>31</td>
<td>2,500</td>
<td>2,200</td>
<td>110</td>
</tr>
<tr>
<td>Nigeria</td>
<td>177,476</td>
<td>170</td>
<td>78</td>
<td>590</td>
<td>570</td>
<td>100</td>
</tr>
<tr>
<td><strong>South Africa</strong></td>
<td>53,969</td>
<td>24</td>
<td>72</td>
<td>380</td>
<td>450</td>
<td>270</td>
</tr>
<tr>
<td>Thailand</td>
<td>67,726</td>
<td>7.4</td>
<td>4.5</td>
<td>160</td>
<td>120</td>
<td>15</td>
</tr>
</tbody>
</table>

*Numbers in thousands

Background

- Blood brain barrier permeability is a major source of concern for potential drug candidates

- Previous studies have incomplete data on drug penetration into intracranial compartments
The Blood-Brain Barrier (BBB)
Problem Statement

* Quantity of administered anti-TB drugs in the cerebrospinal fluid erroneously viewed as true reflection of brain concentrations

* Failure to utilize a label free approach for drug visualization
Aims of the study

* To evaluate the concentrations and distribution profiles of all the classes of TB drugs in the pipeline
Global TB Drug Pipeline

### Preclinical Development

<table>
<thead>
<tr>
<th>Early Stage</th>
<th>GLP Tox.</th>
<th>Phase 1</th>
<th>Phase 2</th>
<th>Phase 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Riminophenazine</td>
<td>BTZ-043*</td>
<td>Q203*</td>
<td>Sutezolid (PNU-100480)</td>
<td>Rifapentine - Moxifloxacin for Drug Sensitive TB</td>
</tr>
<tr>
<td>TBI-166</td>
<td></td>
<td></td>
<td>Linezolid EBA</td>
<td>Delamanid (OPC-67683) with OBR for MDR-TB</td>
</tr>
<tr>
<td>Caprazene nucleoside</td>
<td>PBTZ169*</td>
<td></td>
<td>High Dose Rifampicin for DS-TB</td>
<td>Pretomanid-Moxifloxacin-Pyrazinamide Regimen (STAND)</td>
</tr>
<tr>
<td>CPZEN-45*</td>
<td></td>
<td></td>
<td>Bedaquiline (TMC207)-Pretomanid (PA-824) - Pyrazinamide Regimen</td>
<td>Bedaquiline-Pretomanid-Linezolid (NiX-TB Regimen)</td>
</tr>
<tr>
<td>Capuramycin</td>
<td>TBA-7371*</td>
<td></td>
<td>Levofloxacin with OBR for MDR-TB</td>
<td>Bedaquiline-STREAM MDR-TB Trial Stage 2 with oral OBR (9 mo) or OBR with injectables (6 mo)</td>
</tr>
<tr>
<td>SQ609*</td>
<td></td>
<td></td>
<td></td>
<td>Bedaquiline-Linezolid with OBR for MDR-TB (NEXT Trial)</td>
</tr>
<tr>
<td>Spectinamide 1599*</td>
<td>GSK-070*</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Chemical classes: **fluoroquinolone**, rifamycin, oxazolidinone, nitroimidazole, diarylquinoline, benzothiazinone, imidazopyridine amide. New chemical class*

---


2. OBR = Optimized Background Regimen
Quantitative Analysis of Rifampicin in Brain Tissue Homogenates and the MALDI Images


MALDI Images of Pretomanid Distribution in the Brain


MALDI Images of Doxycycline Distribution in the Brain

Evidence for the presence of clofazimine in the brain

MALDI brain images

Neuro-protective properties of Linezolid in the brain

Global TB Drug Pipeline

Preclinical Development

Early Stage
- Riminophenazine TBI-166
- Caprazene nucleoside CPZEN-45*
- Capuramycin SQ609*
- Spectinamide 1599*

GLP Tox.
- BTZ-043*
- PBTZ169*
- TBA-7371*
- GSK-070*

Clinical Development

Phase 1
- Q203*
- Sutezolid (PNU-100480)
- Linezolid EBA
- High Dose Rifampicin for DS-TB
- Bedaquiline (TMC207)- Pretomanid (PA-824) - Pyrazinamide Regimen
- Levofloxacin with OBR for MDR-TB

Phase 2

Rifapentine - Moxifloxacin for Drug Sensitive TB
Delamanid (OPC-67683) with OBR for MDR-TB
Pretomanid-Moxifloxacin – Pyrazinamide Regimen (STAND)
Bedaquiline-Pretomanid - Linezolid (NiX-TB Regimen)
Bedaquiline-STREAM MDR-TB Trial Stage 2 with oral OBR (9 mo) or OBR with injectables (6 mo)
Bedaquiline-Linezolid with OBR for MDR-TB (NExT Trial)

Chemical classes: fluoroquinolone, rifamycin, oxazolidinone, nitroimidazole, diarylquinoline, benzothiazinone, imidazopyridine amide. New chemical class*

1 Details for projects listed can be found at http://www.newtbdrugs.org/pipeline.php and ongoing projects without a lead compound series identified can be viewed at http://www.newtbdrugs.org/pipeline-discovery.php.

2 OBR = Optimized Background Regimen

www.newtbdrugs.org

Updated: May 2016
Preclinical neuro-toxicological evaluations of fluoroquinolones

MALDI MSI and LCMS/MS: Towards preclinical determination of the neurotoxic potential of fluoroquinolones Drug Test Anal.
DOI: 10.1002/dta.1862
<table>
<thead>
<tr>
<th>Name of TB drug</th>
<th>Minimum inhibitory concentration $\mu$g/mL</th>
<th>Maximum concentration in the brain ($C_{max}$) $\mu$g/mL</th>
<th>Brain compartments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rifampicin</td>
<td>0.20-0.40</td>
<td>0.089</td>
<td>Cortex, striatum</td>
</tr>
<tr>
<td>Gatifloxacin</td>
<td>0.007-0.12</td>
<td>0.228</td>
<td>Cortex</td>
</tr>
<tr>
<td>Pretomanid</td>
<td>0.015-0.25</td>
<td>0.237</td>
<td>Corpus callosum</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>16.00</td>
<td>1.034</td>
<td>Wide spread</td>
</tr>
<tr>
<td>Linezolid</td>
<td>0.12-0.50</td>
<td>30.31*</td>
<td>Brain stem</td>
</tr>
<tr>
<td>Clofazimine</td>
<td>0.12-0.24</td>
<td>0.156*</td>
<td>Wide spread, concentrated at the lateral ventricles</td>
</tr>
</tbody>
</table>

*Multiple oral doses
Complementary efforts of LC-MS/MS and MALDI MSI are sensitive and powerful tools for evaluating time dependent drug distribution in the brain even at relatively low concentrations.
**Conclusion**

- MALDI MSI technique has great potential for examining drug distribution in tissue samples.
- Does not need molecular labeling, thus strengthening the need of this technique in pharmacological applications.
Acknowledgements

* National Research Foundation, South Africa
* Medical Research Council, South Africa
* Aspen Pharmacare, South Africa
* College of Health Science, University of KwaZulu-Natal
* Prof. Thavendran Govender, Prof. Gert Kruger, Dr. Glenn Maguire and Dr. Tricia Naicker
* Analytical team members
Thank you for listening