Outline

- Challenges in TB treatment
- Nanotechnology based drug delivery
- Status of TB nano drug delivery project
- Networking and HCD
- Market trends
Main Challenges facing South Africa

- TB leading cause of death in SA
  - TB co-infection with HIV/AIDS
    - 68% of TB patients are HIV+

- Patient non-compliance to treatment
  - High dose and dose frequency
## Anti-TB drugs

<table>
<thead>
<tr>
<th>First line drugs</th>
<th>Mode of action</th>
<th>Metabolism</th>
<th>Daily dose</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIF (MW = 822.9)</td>
<td>Inhibits assembly of bacterial DNA and protein into mature virus</td>
<td>deacetylation</td>
<td>10-12 mg/kg</td>
<td>0.2 ug/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inhibits initiation of RNA synthesis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>INH (MW = 137.1)</td>
<td>Inhibits synthesis of cell wall components</td>
<td>Acetylation and hydroxylation</td>
<td>5 mg/kg</td>
<td>0.3 ug/ml</td>
</tr>
<tr>
<td>PYR (MW = 123.1)</td>
<td>Disrupts membrane potential</td>
<td>hydrolysis</td>
<td>25 mg/kg</td>
<td>8 ug/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inhibits membrane transport functions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ETB (MW = 277.23)</td>
<td>Inhibits cell wall synthesis</td>
<td>Metabolised by hepatic enzymes</td>
<td>15-20 mg/kg</td>
<td>6 ug/ml</td>
</tr>
</tbody>
</table>
Main Challenges facing South Africa

- TB leading cause of death in SA
  - TB co-infection with HIV/AIDS
    - 68% of TB patients are HIV+

- Patient non-compliance to treatment
  - High dose and dose frequency
  - Length of treatment (6-9 months)
  - Poor bioavailability
    - SAEs

- Emergence of drug resistance
  - MDR and
  - XDR-TB
Main Challenges facing South Africa...

- DOT’s program
  - 53% cure rate
  - Logistics are impractical
  - Expensive program
  - Education

- Research to improve treatment is in progress
  - No new drug in the market in almost 50 years
### Current TB drug R&D portfolio (TB Alliance)

<table>
<thead>
<tr>
<th>Drug compound/analogue/derivative</th>
<th>Mechanism of action</th>
<th>Stage of development</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linezolid</td>
<td>Orally active anti-bacterial agents acting through inhibition of protein synthesis. Studied for treatment of MDR, it was the first antibiotic of its class to be approved.</td>
<td>FDA approved</td>
<td>[39,69]</td>
</tr>
<tr>
<td>TMC-207</td>
<td>Inhibits ATP synthase</td>
<td>Phase I human trials</td>
<td>[69]</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>Dual inhibition of ATP-dependant DNA-gyrase. Efficacy in pulmonary TB has already been reported.</td>
<td>Pivotal large scale phase III clinical trials in TB patients</td>
<td>[70-73]</td>
</tr>
<tr>
<td>PA-824</td>
<td>A prodrug that requires activation by a bacterial F420 dependent glucose-6-phosphate dehydrogenase and nitroreductase to activate components that then inhibit bacterial mycolic acid and protein synthesis. Active against MDR-TB.</td>
<td>Phase II clinical trials</td>
<td>[39,74]</td>
</tr>
<tr>
<td>OPC-67683</td>
<td>Chemically related to PA-824 with similar mechanism. Both drugs’ mechanism has not been fully elucidated.</td>
<td>Clinical trials</td>
<td>[69,75]</td>
</tr>
<tr>
<td>Rimaniphenazines</td>
<td>Thought to inhibit energy metabolism, which is needed even in latent <em>M. tb</em></td>
<td>Lead identification</td>
<td>[14]</td>
</tr>
<tr>
<td>Multifunctional molecules</td>
<td>Chemically linking multiple TB drugs to function as one, ensuring simultaneous optimal concentrations at the site of action</td>
<td>Lead optimization</td>
<td>[14]</td>
</tr>
<tr>
<td>Quinolone analogues</td>
<td>Inhibition of DNA-gyrase these 600 synthesized quinolones are being tested for an optimized quinolone</td>
<td>Preclinical</td>
<td>[14]</td>
</tr>
<tr>
<td>InhA inhibitors</td>
<td>Resistance to INH occurs as a result of KatG, the enzyme needed to activate INH, becoming nonfunctional. This new group of inhibitors will not need KatG, thus potentially eliminating INH-resistant strains</td>
<td>Lead optimization</td>
<td>[14]</td>
</tr>
<tr>
<td>Mycobacterial gyrase inhibitors</td>
<td>Novel inhibitor of DNA-gyrase. May be effective against fluoroquinolone-resistant strains.</td>
<td>Lead optimization</td>
<td>[14]</td>
</tr>
<tr>
<td>Pleuromutilins</td>
<td>New class of antibiotics able to selectively inhibit multiple steps in bacterial protein synthesis.</td>
<td>Lead optimization</td>
<td>[14]</td>
</tr>
<tr>
<td>Malate synthase inhibitors</td>
<td>Malate synthase thought to be a key enzyme used by <em>M. tb</em> to convert its food source to maintain its slow-growing, latent state. Hence inhibition may starve persisters and shorten therapy.</td>
<td>Lead identification</td>
<td>[14]</td>
</tr>
</tbody>
</table>
Main Challenges facing South Africa...

- DOT’s program
  - 53% cure rate
  - Logistics are impractical
  - Expensive program
  - Education

- Research to improve treatment is in progress
  - No new drug in the market over 40 years

- Drug delivery system
  - address non compliance, toxicity, bioavailability and emergence of drug resistant strains

- Nanobased drug delivery system
  - Reduce dose, dose frequency and treatment time
How small?

- Water
- Glucose
- Antibody
- Virus
- Bacteria
- Cancer Cell
- A Period
- Tennis Ball

- $10^{-1}$
- 1
- 10
- $10^2$
- $10^3$
- $10^4$
- $10^5$
- $10^6$
- $10^7$
- $10^8$

Nanometers

- Liposome
- Dendrimer
- Gold Nanoshell
- Quantum Dot
- Fullerene
Promise of Nano-based drug delivery systems

- **Enhance drug properties**
  - Solubility
  - Rate of dissolution
  - Oral bioavailability
  - Targeting ability

- **Enhance dosing requirements**
  - Improved dose frequency
  - Minimal side effects
  - More convenient dosage forms
  - Shortened treatment time

- **Generic**
  - Anti-malarials
  - ARVs
  - Anti-cancer drugs
  - Long term pain killers
Nanoparticulate based drug delivery project

- **Natural polymers**
  - Chitosan
  - Alginate

- **Synthetic polymers**
  - PLGA
    - Spray-drying
    - Freeze drying

- **Micelle system**
  - PEG-PPS

- **Active targeted delivery**
  - Aptamer

TB drug delivery systems

- Oral delivery
- Pulmonary delivery

CSIR
our future through science
Objectives

➢ Improve the bioavailability of ATDs
  ▪ Minimise degradation of the drugs in the stomach
  ▪ Steady and controlled release

➢ Reduce the dosage and dose frequency
  ▪ Treatment 4 drugs/day – 4 drugs/weeks
  ▪ Improve patient compliance
  ▪ Minimise the toxicity of drugs
  ▪ Reduce the cost of TB treatment

➢ Targeting TB in infected macrophages
Synthesis/preparation SNP

Multiple emulsion solvent evaporation

- Aqueous drug solution
- Organic solvent, polymer
- Hardening
- Lyophilisation
- Dehydration
- Nanocapsules
- Harvest
- Spray drying
- Freeze drying
- Shear force
- Homogenisation
- Second aqueous phase, Emulsifier
- Sonication
Spray drying of a double emulsion \textit{W/O/W}
Solid Nanoparticles

- Successfully nano encapsulated 4 first line anti-TB drugs
  - Using double emulsion solvent evaporation - spray drying technique
  - PCT patent filed

Smallest human cell is ~ 2um

INH-loaded PLGA nanoparticles
Biocirculation

Nano particles uptake from the gut

Structure of the GIT

Internal structure of the intestine

- Para-cellular via M cell
- Intracellular via epithelial cell-intestine mucosa
- Peyer's patches
Routes and mechanisms of particle transport across epithelia

- **PARACELLULAR**
- **ENDOCYTOSIS** by ordinary enterocytes
- **UPTAKE via MALT**

**MUCOSAL SIDE**
- Cell junction
- Mucus
- Epithelial cell
- DC

**SEROSAL SIDE**
- Blood capillary
- Lymph capillary
- LN
- Blood circulation

- **Modified surface**
  - Increase circulation time: PEG
  - Enhance particle uptake: Chitosan
Uptake studies in CaCo-2 cells: PLGA

Z-stack 30 min incubation

60 min incubation
Particle uptake in THP-1 cells

a) Coumarin labelled

b) INH-PLGA

c) Rhodamine labelled
**In vitro efficacy: BACTEC 460**

The effect of FreeRIF, Freeze-dried RIF and Spray-dried RIF on THP-1 Cells Infected with M.tb (MOI=5)
In vivo assays (Prof Khuller: Laca mice)

- **Objectives:**
  - Determine plasma concentration of encapsulated RIF, INH and PZA
  - Compare three encapsulation techniques
    - Spray-drying
    - Freeze-drying

- **Method**
  - 6 unchallenged Laca mice per group (20-25g)
  - Resuspended in UHQ
  - Administered orally to mice via gavage
  - Collect blood daily for 5 days
Results: Spray-dried formulation

Release profile Free Drugs vs Spray dried Nanocarriers

![Graph showing release profile of various drugs over time.](image)

- INH-PLGA
- RIF-PLGA
- PZA-PLGA
- Free INH
- Free RIF
- Free PZA
- MIC INH
- MIC RIF
- MIC PZA
Results: Freeze-dried formulation

![Graph showing plasma concentration over days for different drugs in a freeze-dried formulation. The graph includes lines for INH, MIC-INH, RIF, MIC-RIF, PZA, and MIC-PZA. The x-axis represents days, and the y-axis represents plasma concentration in ug/ml.]
FACS: Peritoneal lavage cells: anti-MOMA-2 and CD11c

Control: Saline

IP administration of PLGA particles

Oral administration of PLGA particles

IP administration of Rhodamine labelled particles
FACS: Peritoneal lavage…

Determine macrophage activation or phagocytosis

- Low TNF production
  - indicates no infection/inflammation
- Low IL-12p70 production
  - Generally produced in response to antigen stimulation
- Higher levels of IL-4 and IFN gamma
  - Activation of mononuclear phagocytes
In vivo toxicity assays

- **Histopathology**
  - Various doses
    - Therapeutic
    - above therapeutic and
    - overdosing
  - Formalin preservation method and H/E staining
  - Liquid nitrogen preservation and H/E staining
    - best of the two methods
  - Heart, brain, kidney, liver, lung and spleen
    - Four doses: 4, 8, 16 and 60 mg over 24 hrs
    - No abnormalities
Tissue sections @ 60 mg of PLGA particles

- Brain
- Kidney
- Heart muscle
- Liver
- Lung
- Spleen
Summary

- **Encapsulated first line ATDs**
  - PCT patent filed
  - 2 further invention disclosers
  - Publications

- **In vitro assays**
  - *In vitro* efficacy
  - *In vitro* stability and slow release profile
  - Particle uptake

- **In vivo assays**
  - Macrophage uptake
  - No abnormalities in tissues
  - No inflammatory response
  - Sustained release profile over 5 days
Pre-Clinical Trial (MRC PTA and UCT)
- Characterise particle uptake
- Determine safety
- Determine PK/PD and bioavailability
- Determine efficacy

Pre-Clinical trial in Non-human Primates
- Characterise particle uptake in higher primates
- Determine dose tolerance
- Determine PK/PD and bioavailability
- Tissue distribution

Proof concept clinical trial (Early Bacterial Activity)
- Establish the safety of the delivery system
- Establish the PK and PD of the encapsulated drugs
- Illustrate the efficacy of the delivery system

Clinical trials
- Phase I, II, III

Current phase

Stakeholder engagement/funding
- TB Alliance
- EDCTP
- Dr Swai (DCCC)
- DoH
Human Capacity Development

Post doctoral training
- Post doc (EPFL, Switzerland and UK, Nottingham University, 2006)
- Post doc (UK, Nottingham University, 2007)

PhD exchange programme
- PhD student (UK, University of London, 2005 and 2008)
- PhD student (UK, Cardiff University and University of Liverpool, 2008)

Students/Researchers
- 3 Post doc fellows
- 4 PhD Students (UP, MWU, and TUT)
- 2 MSc (UNISA)
- 1 Hons (UP)
- 4 Internship students (TUT)

Further training planned for 2009/10
- Dosage form design and PK/PD studies
- GMP production
- Pulmonary drug delivery systems
- Microdialysis
Current applications of nanoparticle delivery systems

Many cosmetics, sunscreens and parenterals have been formulated using nanotechnology.
Nanotechnology based drug delivery

Value of Nanotechnology Based DDS 2006-2015

Source: The Nanoparticle Drug Delivery Report
The nano-enabled drug discovery market will generate revenues of $1.3 billion by 2009 and $2.5 billion by 2012, predicts a new report, "The Impact of Nanotechnology in Drug Discovery: Global Developments, Market Analysis and Future Prospects", by the US consultancy, NanoMarkets, Mark Phillips, 2005
Future Trends in drug delivery systems

Closed loop systems:
- External
- Internal

Combined impact
- Microtechnology impact, mainly delivery
- Nanotechnology impact, mainly formulation

- Closed loop systems
- Implantable release reservoirs
- Implantable infusion pumps
- External infusion pumps
- Transdermal microneedles
- Transdermal iontophoresis
- Transdermal ultrasound
- Needle-less injectors
- Inhaler delivery devices:
  - Chlorofluorocarbon-free
  - Ease of use
  - Deep lung
  - Variable dose
- Oral/nasal sprays
- Transdermal: chemical modifiers
- Inhaler nanopowders
- Tablet coatings
- Oral nanotechnologies (hydrogels, dendrimers, liposomes etc.)
- Peptide drugs
- Proteins + Sustained release
- Nanotechnology based directed delivery (e.g., dendrimers, microencapsulation)
- Virus vector directed delivery

Time to market from 2005 (years)
Collaborators

• **Consortium members**
  - CSIR
  - University of Stellenbosch
  - UNISA
  - TUT
  - University of Pretoria

• **National collaborators**
  - Dr Anna Glober, Prof Kotze (North-West University, Potch Campus)
  - Dr Karen Weyer, Mr Kobus Venter, (MRC Pretoria)
  - Prof Peter Smith and Dr Jacobs (UCT)
  - CSIR, Biosciences
Collaborators

• **International collaborators**
  • EPFL
    • Prof Hubbell
  • Nottingham University
    • Dr Alexander
  • University of London
    • Prof Alpar
  • Cardiff University
    • Dr Jones
    • Prof Duncan
  • Nation Jewish Medical Research Centre and Aktiv-dry LCC
    • Dr Kisich
    • Dr Seivers
  • PGIMER
    • Prof Khuller
Funding

SA Department of Science and Technology
- 2005/6: R4M: Infrastructure and HCD
- 2006/7: R4M: Establishing the technology and HCD
- 2007/8: R3M: Optimisation and HCD
- 2008/9: R6M: Pre-clinical studies

CSIR
- 2007/8: R2.5M: Preliminary preclinical studies

NRF: Bilateral;
- UK-SA
- IBSA
THANK YOU