Drug discovery for Tuberculosis

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Introduction
Part 1.

TUBERCULOSIS AND DISEASE PROGRESSION
Facts about TB (2017)

- 10.4 million infections
- 1 million children
- 140,000 Children
- 1.6 million deaths
- 400,000 HIV+
- 1 million HIV+
- 1.1 million HIV+
- 1.6 million infections
- 1.1 million HIV+
- 58% in SE Asia
- 1st killer of HIV patients
- 480,000 MDR-TB
- 3x increase since 2009
- 50% success rate
- 10% XDR
Disease progression

Primo infection

1/3 of the global population!

1.6 million deaths

Boshoff et al. 2005 Nat. Rev. Microbio. 3: 70-80
Phagosomal escape

Failure of bacterial killing by macrophages
- Bacterial replication
- Cell death
- Inflammation
- Large influx of cells
  - Mostly macrophages and neutrophils
- High Local pressure on epithelium and capillary
- Collapsing of capillary and alveolus
Granuloma

Granuloma with a necrotic core
Necrotic core surrounded by foamy macrophages

Stage III
31 days in guinea pig model
Large amount of lymphocytes in the edge
Bacterial propagation stopped

Collagen deposition
Necrotic, hypoxic core
Lymphocytes
Foamy macrophages

* Lymphocytes

Turner et al. 2003 Infect Immun (71) 864-871

10% of TB cases: cavitation and propagation

Boshoff et al. 2005 Nat Rev Microbio (3) 70-80

Kaplan et al. 2003 Infec Immun (71) 7099–7108

Case courtesy of Dr Frank Gaillard, Radiopaedia.org

cav, cavity
nec, necrotic area
90% of cases: Granuloma calcification

Mature granuloma with a mineralized core
Surrounded by foamy macrophages and lymphocytes

Stage IV
93 days in guinea pig model
Fibrosis and mineralization, healing process by calcification

Mineralized, hypoxic core
Lymphocytes
Network of fibrosis
Foamy macrophages
Necrotic ribbon = acellular rim

Turner et al. 2003 Infect Immun (71) 864-871

c, mineralized core
f, fibrosis
a, artery
b, bronchiole

Necrotic debris

100 µm

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Part 1.

DRUG DISCOVERY IN TB FIELD
Screening strategies

Target-based screening
- Enzymatic assays
- Fragment / in silico approaches
- Need a protein/crystal/model!

Target-free / Phenotypic screening
- Bacterial replication / survival assays
- Infected cells

$IC_{50} = 12 \mu M$

$IC_{50} = 330 \mu M$

$IC_{50} = 3.7 \text{nM}$
Drug discovery pipeline

Target-based screening

Target ID Validation → HTS → Hit selection → Lead optimization → Preclinical selection → Preclinical candidate → Candidate Drug (IND)

Enzyme inhibition, MTB growth inhibition, Efficacy in animals
Hit selection

Primary screening
• Single dose
• Select compounds with activity > Threshold

Secondary screening
• Cherry-picking & validation by dose-response curve (DRC)
• Repurchase & Resynthesize, validate again by DRC

Dose-response curves

Hits
• Structure and activity confirmed
• Clustering & prioritization
Hit-to-lead

- **HITS**
  - **M. tuberculosis in vitro**
  - **M. tuberculosis in macrophage**

- **SAR**
  - **in vitro activity**
  - **Physicochemical properties**
    - Solubility, pKa, LogD
  - **Early ADMET (in vitro)**
    - Cytotoxicity
    - Plasma, microsomal, hepatocyte stability
    - CYP inhibition
    - Plasma Protein Binding assay
    - hERG assay
    - Genotoxicity
    - Transporter inhibition
  - **in vivo PK**
    - AUC, clearance, bioavailability

- **LEAD**
  - **in vivo efficacy testing (mice model)**

- **MOA**
  - **PCC**
Phenotypic screening assays for Tuberculosis research
Phenotypic assays – Quantification?

Wild-Type bacteria
- CFU counting
  - Agar
  - Bactec MGIT
- OD reading
- Resazurin

Reporter strains
- Luminescence, fluorescence (GFP, RFP…)
- Constitutive vs stimuli-driven expression

Compound A

Dyes or antibodies
Multiparametric image analysis

Pictures analyzed by dedicated scripts

- Definition of two Populations: cells & bacteria
- Several parameters used to quantify compound efficiency:
  - Number of cells
  - Total area of bacteria (px)
  - Average area of bacteria per infected cells (px)
  - Ratio of infected cells
Phenotypic screening assays - overview

Genetic modulators (Host)
1. Reverse transfection
   - a. siRNA
2. Infection (adherent cells)
   - b. Transfection reagent
   - c. Cells

Chemical modulators
1. Compounds distribution
2. Infection (cells in suspension)

Genetic modulators (Bacteria)
1. Mutants distribution
2. Cells distribution

Automated image acquisition and image-based analysis

Host effectors

Bacterial effectors

New scaffolds for drug development
Part 1.

CHEMICAL MODULATORS
Chemical modulators - Screening process

Biological assay

- *M. tuberculosis* H37Rv-GFP culture
- Raw 264.7 Macrophages
- Batch infection (2h), washing
- *M. tuberculosis* H37Rv-GFP infected Raw cells

Compound preparation

- Dilution
- Transfer
- 5 days Incubation

Extracellular assay

- Fluorescence Reading

Intracellular assay

- Automated confocal microscopy readout

Christophe *et al.* 2010 Future Med. Chem. 2: 1283-1293
Chemical modulators: Q203

Q203

- Imidazo[1,2-a]pyridine amide (IPA) Family
- From a screening of 120,000 compounds
- Identified by infected macrophage assay (QIM)
- Hit compounds were active in the µM range

Optimizations gave preclinical candidate

Pethe et al. 2013 Nat. Med
Q203 - Overview

**Triggers ATP depletion**
- More potent than BDQ
- Active against MDR-XDR

**In vivo efficacy in mice**
- Acute model (GSK)
  - 3 days treatment

**Chronic model**
- Gavage, 5x per weeks

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**Graphs and Data**
- Comparison of ATP depletion
- In vivo efficacy in mice over time
- Chronic model treatment

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**Table**
- Comparison of MIC values for different strains and drugs
- Type, Family, INH, Rif, Strept, Oflox, MIC

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**Q203**
- 6.5 mpk
- 10 mpk
- 15 mpk

**BDQ**
- 6.5 mpk
- 15 mpk

**INH**
- 10 mpk
- 15 mpk

---

**Graphs**
- ATP depletion over time
- Lung CFU (log₁₀) vs Time (d)
- Treatment efficacy over 28 days
**Q203 & QcrB**

**Target identification**
- Generation of spontaneous resistant mutants

IC$_{50}$ = 10 nM  
IC$_{50}$ = 5 nM

**Whole genome sequencing: qcrB**

<table>
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<th>Strain</th>
<th>Accession</th>
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<th>Stop</th>
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</tbody>
</table>

**Mutation freq.**  
$2.4 \times 10^{-8}$

**Respiratory complex III (Cytochrome bc oxidase)**

Pethe *et al.* 2013 Nat. Med
Q203 & QcrB

Target purification

- Collaboration with Dr. E. Berry (Upstate Univ)
- Hybrid supercomplex
  - *M. smegmatis* cytochrome *aa*$_3$
  - + *M. tuberculosis* QcrCAB (bcc)

+, QcrB, Cox1; -, QcrC; *, QcrA, Cox2, Rieske

Kim *et al.* 2015 J Biol Chem
Chemical modulators - optimization

Adding value by combining screening strategies
- Finding compounds active in multiple models

Intracellular efficiency

Booster

Affinity with EthR (TS Assay)

Blondiaux et al. 2017 Science
Part 3.

GENETIC MODULATORS
(BACTERIA SIDE)
Failure of lysosomal fusion with *M. tuberculosis* containing phagosomes

- Which bacterial effectors are implied?
- Use of a library of ~11,000 transposon mutants build in *M. tuberculosis* Beijing GC1237 strain
- Follow lysosomal fusion using the pH-sensitive Lysotracker probe
- Read-out: number of bacteria in acidified compartments
Genetic modulators - Screening principle

Validation using H37Rv and Beijing GC1237 fluorescent (RFP) strains

- Live and heat killed (HK) bacteria
- Increase in the number of acidified compartment with the HK strains

Brodin et al. 2010 Plos Pathogen 6(9): e1001100
Genetic modulators - Quantification

Image analysis
- Detection of cell nuclei
- Detection of acidified compartment
  - Detection of bacteria is optional,
  - Quantification gave similar results

Brodin et al. 2010 Plos Pathogen 6(9): e1001100

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Genetic modulators - Process and screening

**Process:**
- Mutant distribution
- Cell distribution
- 2h incubation
- Staining, fixation, reading

**Results:**
- Two extreme phenotypes:

![Lysosomes](image)

- Attenuated mutant
- Virulent mutant

**Graph:**
- Mean ± 3s.d.
- Cell nuclei number
- Surface of lysosomes proximal to cell nuclei

**Images:**
- Primary macrophages (BMDM)
- Transfer
- Mutant library
- Lysotracker assay
- Automated confocal microscopy readout

**References:**
Brodin et al. 2010 Plos Pathogen 6(9): e1001100

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Genetic modulators – Results

Set of 10 mutants with defect in escaping lysosomal fusion

<table>
<thead>
<tr>
<th>Mutant id</th>
<th>Gene (nt)</th>
<th>Putative function</th>
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<td>P69D07</td>
<td>pstS3 (482)</td>
<td>Phosphate transport</td>
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<tr>
<td>P117C08</td>
<td>Rv1503c (186)</td>
<td>TDP-4-oxo-6-deoxy-D-glucose transaminase (glycosyl aminotransferase)</td>
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<td>Rv1506c (211)</td>
<td>Methyltransferase</td>
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<td>lppM (272)</td>
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<td>P1E07</td>
<td>Rv2295 (-35)</td>
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<td>fadD28 (1252)</td>
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<td>moaC1 (507)</td>
<td>Molybdopterin biosynthesis</td>
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<tr>
<td>P39E07</td>
<td>Rv3880c (269)</td>
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</tbody>
</table>

Defect in DAT production

Brodin et al. 2010 Plos Pathogen 6(9): e1001100
Part 3.

GENETIC MODULATORS
(HOST SIDE)
Objectives:
- To identify essential pathways manipulated by the bacteria

Method:
- Bacterial replication assay in siRNA transfected cells

Reverse transfection

1. Gene silencing
2. 8,092 siRNA transfection
3. Raw 264.7
4. 3 days gene knockdown
Strategy can be applied to different cell types

- Macrophages
- Epithelial cells

Validation process:

Primary screening (16,532)

Data normalization (Z-score)

Cell number

Z-score > -3

Multi-parameters

Z-score > +5

124 hits

Secondary screening (Quadruplicate)

Data normalization (Z-score)

Cell number

Z-score > -3

Multi-parameters

Z-score > +2

Reproducibility ≥ 50%

79 hits

Z-score > +5

44%

23%

4%

3%

6%

6%

3%

5%

1%

1%

4%

Other

Cytokine

Enzyme

GPCR

Growth factor

Kinase

Peptidase

Phosphatase

Transcription regulator

Transmembrane receptor

Transporter

Other
Identification of CISH

CISH is required for efficient *Mtb* replication in macrophages

Queval et al. 2017 Cell Reports
CISH expression pattern

(a) mRNA relative copy number

(b) Relative expression

Queval et al. 2017 Cell Reports
CISH interferes with phagosome acidification

Queval et al. 2017 Cell Reports
CISH targets V-ATPase

Expression vector: pcDNA 3.1

Cish

Ubiquitin ligase complex

Nter SH2 domain SOCS box Cter

ΔSOCS box

Nter SH2 domain Cter

Ubiquitinated proteins Pull-down

Proteomic analysis

scramble

siCISH

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Queval et al. 2017 Cell Reports
CISH – A summary

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Queval et al. 2017 Cell Reports
Thank you!

Q & A

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