Development and Clinical Evaluation of New Leprosy Skin Test Antigens as Diagnostic-Epidemiological Tools
A <25 Year Study; Partners over that Period

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Our Goal in the 1980s: Development of a Simple Test Based on CMI Response to Address Early Pre-Symptomatic Leprosy to Complement PGL-I Based Ab Assays

Skin Test for Leprosy? + or = Comprehensive, useful, facile diagnostic tests for leprosy

• 94% of MB leprosy patients have antibodies to PGL-I
• 40% of PB leprosy patients have antibodies to PGL-I
• >70% of PB and early leprosy may be positive in a T cell-based test
T-Cell Based Diagnostic Test Options

Criteria
• Measure early/specific response
• Simple to implement in the field
• Inexpensive

Why a skin test?
Tuberculin PPD as the Archetype
• Measures CMI
• Low cost
• Rapid to implement
• Safe
• Easy to apply in large populations

Test method options
• Interferon gamma assay (PBMC vs whole blood)
• PCR
• Skin Test

Shortcomings
• Cannot differentiate protection from infection
• Requires return visit for reading
• Cross-reactive with environmental mycobacteria
• Often positive with prior BCG vaccination
Antigen Options

**M. leprae** specific antigens available in 1980s →
- PGL-I
- WHO/IMMLEP *E. coli* recombinant proteins (1990s)
- A few major native proteins
- Fractionated *M. leprae* from armadillo (cytosol, membrane, cell wall)

Prior skin test antigens
- Leproma (1919 to 1975): human nodules, macerated and delipidated
- Lepromin A (1975→): armadillo derived; heat killed; no FDA approval
- Convit’s antigen (1984): armadillo derived *M. leprae*, cytosol and membrane
- Rees antigen (1984): cytosol from armadillo derived *M. leprae* (called MLSA)

- Neither Convit’s or Rees antigens had FDA approval
- All early skin test antigens lacked adequate sensitivity and specificity.

Could We Dramatically Improve Rees/Convit STAs by Removal of Cross-Reactive Lipoglycans (e.g. LAM was found to inhibit the immunological response)?
Research Timeline

1992

- Discovery (late 1980s-1992)

1996

- Mfg (May 12, 1997)
- IND (Sept 23, 1998)

2000

- Phase I IIA Trial (Apr 30, 2002)

2004

- Phase I IIB Trial (May 11, 2003)

2008

- Phase IIC-1a Trial (Dec 12, 2006)

- Phase IIC-1b Trial (May 27, 2009)

2012

- Final Report (Feb 17, 2012)
Investigational New Drug Application, Clinical Protocols, Informed Consent Forms
Pilot Scale Manufacturing

cGMP = current Good Manufacturing Practices
Code of Federal Regulations, Title 21, Sections 210 and 211
255 Standard Operating Procedures written/approved
Skin Test Antigen Yields

Material
Armadillo Tissue: 242 g
M. leprae: 128.4 mg (0.05%)
MLSA-LAM: 4.6 mg (3.57%)
MLCwA: 5.0 mg (3.88%)

Coded Antigens

MLSA-LAM and MLCwA
Batch 23, Lot 051297

Caution: New Drug-Limited by Federal Law to Investigational Use

Vialled in 2 ml Wheaton Vials (1 ml @ 0.1, 1.0, 2.5 μg/100 μl; batch 23, lot # 051297; relabeled at Fisher Bioservices, NIH Repository)
Clinical Trials

Phase I – non-endemic region

Hartshorn Health Services, CSU
Fort Collins, CO USA

Phase II, Stages A-C: endemic region

Anandaban Hospital
Kathmandu, Nepal
The Phase II clinical trial was the first time MLSA-LAM and MLCwA antigens were tested in an endemic region for leprosy, against 260 volunteers, where the majority of the population had been BCG vaccinated and where sensitization with environmental mycobacteria was a specific concern. Both antigens at a high (1.0 µg) and low (0.1 µg) dose were found to be safe.
On-Site – Phase II Clinical Trial; Anandaban Leprosy Hospital, Katmandu

Antigen administration
Kapil Neupane

Injection site observation

- cytosol
Efficacy Results - Skin Test Induration

Scatterplot

Mean & Standard Error
### Stage C-1 (low dose) Induration

#### Day 3 and 7 Summary

<table>
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<tr>
<th>Study Group</th>
<th>Antigen</th>
<th>Visit Day</th>
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<td>BL/LL Leprosy</td>
<td>MLCwA 0.1 µg/ml</td>
<td>(0/19)</td>
<td>(0/18)</td>
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<tr>
<td></td>
<td>MLSA-LAM 0.1 µg/ml</td>
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<td>(0/18)</td>
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<tr>
<td></td>
<td>PPD</td>
<td>18.5 (7/19)</td>
<td>16.8 (9/18)</td>
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<td>BT/TT Leprosy</td>
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<td>11.4 (5/18)</td>
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<td>MLSA-LAM 0.1 µg/ml</td>
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<td>Contacts</td>
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<td>Tuberculosis</td>
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<td>MLSA-LAM 0.1 µg/ml</td>
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<td>PPD</td>
<td>20.7 (19/20)</td>
<td>18.8 (20/20)</td>
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Summary; What Next?

- Both MLSA-LAM and MLCwA safe at 0.1 and 1 μg doses
- Both show some specificity at 0.1 μg dose, but poor sensitivity
- But very small numbers of subjects; little hope for larger trials
- DTH/immunological anergy at BL/LL spectrum again dramatically demonstrated; what is the immunological basis?
- More immediate aspirations: incorporation of present STAs into the IDEAL/NLR/COLEP-II large chemo/immunoprophylaxis trial involving M. leprae-specific proteins/peptides Quantiferon-type tests and molecular epidemiology (SNP, VNTR), PGL-I serology and other leprosy biomarkers
- Try to generate interest in the immunological basis of leprosy anergy