”Use of oral inactivated Mycobacterium manresensis to reduce the risk of TB”

Prof. Pere-Joan Cardona
IGTP
Badalona. Catalonia
Why an injury of 1 mms is transformed into a 10 mm injury?
TB: Dx & treatment Paradigm.

LTBI

Active TB

< $10^5$ cfu

10$^5$ cfu

20 years
The usual approach on the Evolution from infection to disease: Size no matters!

Kaufmann et al Nat Rev Micr 2015
Evolution from infection to disease: Size matters!

The “usual” approach

The “clinical” approach
MECHANISMS OF PATHOGENESIS

Damaging role of neutrophilic infiltration in a mouse model of progressive tuberculosis

Elena Marzo a, Cristina Vilaplana a, Gustavo Tapia b, Jorge Diaz a, Vanessa Garcia a, Pere-Joan Cardona a, *
Evolution from infection to disease: Progression of the lesions and coalescence
Evolution from infection to disease:
Initial infiltration with PMNs that allows the extracellular grown of the bacilli.

This allows a quick progression of the lesion to become Exudative.
Evolution from infection to disease: Progression towards exudative lesions
Evolution from infection to disease: NEUTROPHILES are the key cells!
Evolution from infection to disease:
*Human TB lesions in the pre-antibiotic times.*

Exudative Lesions
- PMNs!
- Active TB

Proliferative Lesions
- No PMNs!
- LTBI

*Medlar 1954*
Evolution from infection to disease: The “Bubble model”

TB is generated thanks to neutrophilic infiltration; generation of new “daughter” lesions and coalescence

Prats et al
2016
Evolution from infection to disease: The “Bubble model”

TB is generated thanks to neutrophilic infiltration; generation of new “daughter” lesions and coalescence

Prats et al
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Evolution from infection to disease: The “Bubble model”
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Prats et al 2016
Evolution from infection to disease: The “Bubble model”

TB is generated thanks to neutrophilic infiltration; generation of new “daughter” lesions and coalescence
Evolution from infection to disease: 

**The Hypothesis:** Th17 response keeps the neutrophil infiltration in the immune phase

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The induction of Exudative lesions
Evolution from infection to disease:  
**The Hypothesis:** Treg response is able to balance Th17

The induction of Proliferative lesions
Treg induction: Treg can be induced by daily oral administration of heat-killed mycobacteria for 14 days.

Induction of PPD-specific memory Tregs.
We obtained environmental mycobacteria (M. fortuitum complex) from natural water in the Cardener river. Manresa
Mycobacterium manresensis belongs to the M. fortuitum complex. (Rech et al 2015)
Oral Administration of Heat-Killed *Mycobacterium manresensis* Delays Progression toward Active Tuberculosis in C3HeB/FeJ Mice

Paula Cardona\(^{1\dagger}\), Elena Marzo-Escartin\(^{1\dagger}\), Gustavo Tapia\(^2\), Jorge Díaz\(^1\), Vanessa García\(^1\), Ismael Varela\(^1\), Cristina Vilaplana\(^1\) and Pere-Joan Cardona\(^1\*)
Administration of heat-killed mycobacteria. Increases PPD-specific memory Tregs and survival.

C3HeB/FeJ mice
Administration of heat-killed mycobacteria decreases IL-17 and increases IL-10.

Administration of heat-killed *Mycobacterium manresensis* allows the increase of Treg response, reduction of Th17 and control of the progression towards disease.
Head-killed *Mycobacterium manresensis* has been developed by a spin-off of the IGTP “MANREMYC”
In the Galenic form “Nyaditum resae®”
1st day of NYADATREG clinical trial.

Phase I Clinical Unit Hospital Germans Trias i Pujol.
RESEARCH ARTICLE

Pilot, double-blind, randomized, placebo-controlled clinical trial of the supplement food Nyaditum resae® in adults with or without latent TB infection: Safety and immunogenicity

Eva Montané1,2, Ana Maria Barriocanal2,3, Ana Lucía Arellano1,2, Angelica Valderrama1, Yolanda Sanz1, Nuria Perez-Alvarez4,5, Paula Cardona6, Cristina Vilaplana6, Pere-Joan Cardona6*

1 Department of Clinical Pharmacology, Hospital Universitari Germans Trias i Pujol, Badalona, Catalonia, Spain, 2 Department of Pharmacology, Therapeutics and Toxicology, Universitat Autònoma de Barcelona, Catalonia, Spain, 3 Fundació Institut Germans Trias i Pujol, Badalona, Catalonia, Spain, 4 Lluita Contra la Sida Foundation, Badalona, Catalonia, Spain, 5 Statistics and Operations Research Department, Universitat Politècnica de Catalunya- BarcelonaTech, Barcelona, Catalonia, Spain, 6 Unitat de Tuberculosis Experimental, Universitat Autònoma de Barcelona, CIBERES, Fundació Institut Germans Trias i Pujol, Badalona. Catalonia. Spain
Nyadatum resae® has a very good Safety Profile

<table>
<thead>
<tr>
<th>Total Adverse Events per subject, Median (IQR)</th>
<th>Non-gastrointestinal</th>
<th>Gastrointestinal</th>
<th>Total</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLACEDO</td>
<td>4 (2 - 6)</td>
<td>2 (1 - 3)</td>
<td>6 (4 - 9)</td>
<td>NS</td>
</tr>
<tr>
<td>Nyadatum resae® low dose</td>
<td>2,5 (1,3 - 4)</td>
<td>2 (1,3 - 4,5)</td>
<td>4,5 (4 - 9)</td>
<td>NS</td>
</tr>
<tr>
<td>Nyadatum resae® high dose</td>
<td>2 (1,0 - 3,5)</td>
<td>2 (1 - 4,5)</td>
<td>5 (2,5 - 7,5)</td>
<td>NS</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of subjects presenting possible or probable-related adverse events</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADVERSE EVENTS</td>
</tr>
<tr>
<td>Gastrointestinal</td>
</tr>
<tr>
<td>Abdominal pain</td>
</tr>
<tr>
<td>Faeces deposition Increased</td>
</tr>
<tr>
<td>Faeces consistency Decreased</td>
</tr>
<tr>
<td>Nausea</td>
</tr>
<tr>
<td>Diarrhoea</td>
</tr>
<tr>
<td>Dispepsia</td>
</tr>
<tr>
<td>Faeces deposition Increased</td>
</tr>
<tr>
<td>Faeces consistency Decreased</td>
</tr>
<tr>
<td>Vomits</td>
</tr>
<tr>
<td>Flatulonco</td>
</tr>
<tr>
<td>Constipation</td>
</tr>
<tr>
<td>Epigastralgia</td>
</tr>
<tr>
<td>Rectal Tenesmus</td>
</tr>
<tr>
<td>Non-Gastrointestinal</td>
</tr>
<tr>
<td>Hepatic alterations</td>
</tr>
<tr>
<td>Homatologic alterations</td>
</tr>
<tr>
<td>Cephalic, migraine</td>
</tr>
<tr>
<td>Respiratory Infection</td>
</tr>
<tr>
<td>Hyperglycemia</td>
</tr>
<tr>
<td>Others</td>
</tr>
</tbody>
</table>
NYADATREG

Ratio CD25+CD39+/CD25-CD39+
Nyaditum resae® induces PPD Specific Memory Tregs

Fig 7. Protection index as assayed after Dwyer [26] determined as a ratio between the data obtained by the ratio of stimulated and non-stimulated CD25+CD39+ and the obtained by the ratio of stimulated and non-stimulated CD25-CD39+ cells. Results divided according TST status: (A) TST-positive, (B) TST-negative. Treatment groups are represented in black, red and blue, corresponding to Placebo, low dose and high dose Nyaditum resae® respectively. P-values calculated by Wilcoxon matched pairs test. Plots are shown with median, IQR and minimum/maximum values.
Study to Evaluate the Tolerability and Immunogenicity of Nyaditum Resae® Probiotic Administered to Pediatric Population in Contact With Tuberculosis With or Without Latent Tuberculosis Infection

This study is currently recruiting participants. (see Contacts and Locations)

Verified July 2016 by Manresana de Micobacteriologia, SL

Sponsor:
Manresana de Micobacteriologia, SL

Information provided by (Responsible Party):
Manresana de Micobacteriologia, SL

ClinicalTrials.gov Identifier:
NCT02581579

First received: October 19, 2015
Last updated: July 15, 2016
Last verified: July 2016

Purpose

This is a double-blind, masked, compared with placebo clinical trial in pediatric population in contact with tuberculosis with or without tuberculosis infection. This trial aims to study the effect of the probiotic Nyaditum resae® at the level of specific Treg memory cells eight weeks after the first administration, and the global tolerability of the treatment.

Nyaditum resae® is a preparation in the form of capsules containing heat-killed environmental mycobacteria Mycobacterium manresensis. The overall objective of the study is the effect of Nyaditum resae® on immunity, which could reduce the risk of developing active tuberculosis.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Intervention</th>
<th>Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuberculosis</td>
<td>Dietary Supplement: Nyaditum resae® 10e5 of heat-killed Mycobacterium manresensis</td>
<td>Phase 1</td>
</tr>
<tr>
<td></td>
<td>Other: Placebo</td>
<td></td>
</tr>
</tbody>
</table>
EFFICACY CLINICAL TRIAL has started on May 2017

NYADAGEORG
Efficacy of Nyaditum Resae(R) Against Active TB in Georgia

This study is not yet open for participant recruitment. (see Contacts and Locations)
Verified September 2016 by Fundació Institut Germans Trias i Pujol

Sponsor:
Fundació Institut Germans Trias i Pujol

Collaborator:
National Center for Tuberculosis and Lung Diseases (NCTLD)

Information provided by (Responsible Party):
Fundació Institut Germans Trias i Pujol

NCT02897180

ClinicalTrials.gov Identifier:
NCT02897180
First received: September 7, 2016
Last updated: September 13, 2016
Last verified: September 2016

Purpose

The use of a supplement food like "Nyaditum resae" is a reliable opportunity to stop the progression towards active TB through the most updated knowledge of this disease: the induction of tolerance.

In order to demonstrate the percentage of efficacy of this approach, different studies must be run to elucidate the percentage of protection in different setting all over the world. The strategy is to establish its efficacy through a simple clinical trial, aimed just to know the incidence of TB in Placebo and NR treated contacts of active TB cases.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Intervention</th>
<th>Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Close Contacts of Active Tuberculosis</td>
<td>Dietary Supplement: <strong>Nyaditum resae</strong>(R)</td>
<td>Phase 2</td>
</tr>
<tr>
<td></td>
<td>Dietary Supplement: Placebo</td>
<td></td>
</tr>
</tbody>
</table>
1650 subjects

2 years

3%

5%

CLOSE CONTACTS >6 h/day

National Center of Tuberculosis and Lung Diseases (NCTLD).
Tbilisi, Georgia

PLACEBO
CONCLUSION:

Oral daily administration (14 days) of heat-killed *M. manresensis* can reduce the risk of progression towards active TB for at least 1 year.

Heat-inactivated *M. manresensis* is being registered as supplement food in Europe and Asia.
Aknowledgements

Paula Cardona  Lilibeth Arias  Cris Vilaplana