EU – Latin American AIDS / TB networking event
Medellin - Colombia

Brazilian Tuberculosis Research Network:
Multidisciplinary Collaborative Project
REDE-TB

Afranio Kritski - Nov 03, 2008
Outline Presentation


2. Creation of REDE-TB and Coordination Areas on TB Research, 2001

3. Short Reports from each Coordination Area

4. Final Remarks
WHO Strategy for TB Control 1993

USING EXISTING TOOLS IN TB CONTROL

TB PROGRAMS – ADOPT DOTS STRATEGY:
  – To detect 70% of the Smear Positive + Pulmonary TB Cases with bacilloscopy
  – To achieve cure rate of 85% with first line regimen (RIF+INH+PZA)
  – Continue to use BCG vaccination at birth

• WAS NOT CONSIDERED PRIORITY
  – Research
  – Interaction with AIDS Program

Stop TB/WHO Global Plan
2006-2015

TOOLS FOR TB CONTROL PROGRAMS:

– Expand DOTS
– Increase Case Detection
– Emphasis on TB-MDR and TB-XDR
– Interaction between TB and HIV Control Program
– Inclusion of Civil Society (NGOs, Private Sector)
– Research

TUBERCULOSIS CONTROL ACTIVITIES (Brazil, 1988-2004)

MST: Ministry of Science and Technology; MoH: Ministry of Health; MEC: Ministry of Education; MIT: Ministry of Industry and Trade; Civil Society: NGOs, Health Council, Professional Associations.
SCIENTIFIC AND TECHNOLOGICAL PRODUCTION IN BRAZIL 1986 – 2006
TUBERCULOSIS

Kritski et al. Revista Saude Publica, 2007, 41 (supl 1)
TB publications according to the study design (1986 – 2006)

<table>
<thead>
<tr>
<th>Study design</th>
<th>Approach</th>
<th>Quantitative</th>
<th>Qualitative</th>
<th>Total</th>
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<tbody>
<tr>
<td>Descriptive</td>
<td></td>
<td>291</td>
<td>27</td>
<td>318</td>
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<tr>
<td>Review</td>
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<td>78</td>
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<td>78</td>
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<td>Case Report/ Case series</td>
<td></td>
<td>90</td>
<td>0</td>
<td>90</td>
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<tr>
<td>Applied Basic research</td>
<td></td>
<td>177</td>
<td>0</td>
<td>177</td>
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<tr>
<td>Basic Research</td>
<td></td>
<td>141</td>
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<td>Cross Sectional</td>
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<td>58</td>
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<td>Case control</td>
<td></td>
<td>43</td>
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<td>44</td>
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<tr>
<td>Cohort</td>
<td></td>
<td>32</td>
<td>3</td>
<td>33</td>
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<td>Operational/Effectiveness</td>
<td></td>
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<td>4</td>
<td>44</td>
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<td>Experimental / Clinical Trials</td>
<td></td>
<td>24</td>
<td>2</td>
<td>26</td>
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<td>Mathematical Modelling</td>
<td></td>
<td>15</td>
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<td>15</td>
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<tr>
<td>Echoloiogical</td>
<td></td>
<td>13</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>Tecnology / patents</td>
<td></td>
<td>23</td>
<td>0</td>
<td>23</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>1017</td>
<td>37</td>
<td>1054</td>
</tr>
</tbody>
</table>

Kritski et al. Revista Saude Publica, 2007, 41 (supl 1)
• Characterized by physical, human and technological resources
• Integrated by thematic areas
• 44 Institutions
• U$ 1,500 million/year

Millennium Institute Project - 2002. Approval by Ministry Science Technology
The REDE-TB mission is the innovation in the health area and to capacitate the country for the development of new strategies, technologies and products necessary to the treatment and control of tuberculosis through a multidisciplinary and multi-institutional approach acting in a network.

Collaborating in the public policies revision necessary to the control of tuberculosis.

Instructing high level human resources in all pertinent areas of the project.

Developing effective knowledge mechanisms and technology transfer to society.
Coordination Areas
(Rational approach to developing drugs to treat and vaccines to prevent TB, and diagnostic tools to detect *Mycobacterium tuberculosis*)

**Drugs**
- **(Rational design - defined molecular targets)**
  - Virtual Screening (dry-lab) (CPBMF* and Walter Azevedo******)
  - IQGs (a defined hit)
  - Chemical synthesis (Luiz Lopes**)
  - Pre-clinical studies (CPBMF* and Calixto***)
  - Pharmacological tests
  - Toxicology
  - Pharmacodynamics
  - Pharmacokinetics
  - Pharmacology
  - *in vitro* safety studies
  - *in vivo* toxicology
  - Acute
  - Chronic

**Vaccines**
- Defined mutant strains
  - *M. tuberculosis* (CPBMF*)
  - *M. Bovis BCG Moreau RJ* (Castello Branco****)
  - Gene knockout (auxotroph mutants)
  - Gene knockin (super-expression of antigens)
  - Protection and immunology studies (CPBMF*, Calixto***, and Castello-Branco***)
  - *M. Bovis BCG Moreau RJ*: oral (Castello Branco****)

**Who is Who?**
- * CPBMF-PUCRS – PUCRS-RS
- ** Luiz Lopes – UFC-CE
- *** João B. Calixto – UFSC-SC
- **** Castello-Branco – FIOCRUZ-RJ
- ***** José Aparício Funck – PUCRS-RS
- Walter F. Azevedo Jr – PUCRS-RS
(Rational approach to developing drugs and vaccines to treat TB, and diagnostic tools to detect and differentiate *Mycobacterium tuberculosis*)

**Immunopathogenesis Area**
- Gene-gene and Gene Environment Interaction Bioinformatic (DeGrave/Suffys-FioCruz)
- Ferrazoli - IAL-SP Rossetti – FEEPS
- Oliveira – UFRJ
- Immunological Drug Sensitive TB, Drug Resistant TB, TB close contacts
  1. Arruda-FioCruz SES Bahia
  2. Kritski-UFRJ SMS-RJ - Itaborai
  3. Picon SES-RS
  4. Dietze
- Immunogenetic studies Oliveira/Kritski – UFRJ
- Development of molecular prototype for TB infection and TB disease Markers Oliveira – UFRJ
- Development of molecular prototype for XDR-TB and most virulent Strain (MLPA) Oliveira/Suffys UFRJ-FioCruz
- Cohort Studies TBHIV-, TBHIV+ close contacts and volunteers vaccinated by BCG
  1. Arruda/FioCruz SES/Bahia
  2. Kritski-UFRJ SMS-RJ - Itaborai
  3. Picon SES-RS
  4. Dietze

**Diagnosis Area**
- Development of immunoserology prototype for (ELISA)
- Development of KIT for Drug Sensitive TB (ELISA) With Industry Interaction Rossetti - FEEPS
- Development of molecular KIT for Drug Sensitive TB (ELISA) With Industry Interaction Rossetti - FEEPS
- Development of KIT for TB infection and TB disease Markers Oliveira – UFRJ
- Lipidomic Platform Oeleman – UFRJ
- Proteomic Platform Lima/DeGrave - Fiocruz
- Development of skin test (new PPD) Soccol - FEEPS
- Development of molecular KITt for Drug Sensitive TB (Dot-Blot; ELISA) With Industry Rossetti – FEEPS
- Development of molecular KIT for Drug Sensitive TB (ELISA) With Industry Rossetti – FEEPS
- Development of immunoserology prototype for (ELISA) Kipnis - UFGO, Oeleman – UFRJ Soccol - UFRJ
- Development of immunoserology prototype for (lateral flow) Buhrer - UFGO
- Development of skin test (new PPD) Soccol - FEEPS
- Recombinant Protein Production Platform Soccol- TecPar

**Vaccine Area**
- Immunogenetic studies Oliveira/Kritski – UFRJ
- Lipidomic Platform Oeleman-UFRJ
- Proteomic Platform Lima/DeGrave - Fiocruz
- Development of immunoserology prototype for (lateral flow) Buhrer - UFGO
- Development of membrane filtration system Palaci ND-UFES
- Vaccine Area

Diagnostic tool validation – Phase II/III and IV – Kritski-Mello-Dalcolmo-Durovni-Fonseca-Trajman-Rolla (RJ), Dietze (ES), Picon (RS), Galesi (SP), Marinho (BA), Barreto (CE), Rabahi (GO), Spindola Miranda (MG), Gabardo (MG)
Institutions involved in the Basic Research area

**Amazonas**
INPA  Instituto Nacional de Pesquisas da Amazônia

**Espírito Santo**
UFES  Universidade Federal do Espírito Santo

**Pernambuco**
EIOCruz-PE  Fundação Oswaldo Cruz

**Rio de Janeiro**
EIOCruz-RJ  Fundação Oswaldo Cruz
UFRJ  Universidade Federal do Rio de Janeiro
UENF  Universidade Estadual Norte Fluminense

**Rio Grande do Sul**
LACEM-RS  Laboratório Central

**São Paulo**
UNIFESP  Universidade Federal de São Paulo
FMRP-USP  Faculdade de Medicina de Ribeirão Preto USP
FCFRP-USP  Faculdade de Ciências Farmacêuticas de Ribeirão Preto USP
IB  Instituto Butantan SP

Millennium Institute Project · 2002
Approval by Ministry Science Technology
The bacillus study and the host-parasite relationship have allowed the discovery of molecular targets for vaccines, medicines and diagnosis tests.
• Genetic mechanisms associated with resistance to anti-TB drugs
• Molecular epidemiologic studies pursuing the analysis of Mtb genetic variability and virulence in different regions of Brazil
• Virulence studies using animal models (Brazil and Mexico)
• Characterization of new antigens for vaccines and diagnostic tests
• Identify markers related to response for anti-TB treatment and relapse
• Analysis of TB patients (pulmonary and extrapulmonary) and their close contacts in order to identify immunologic and genetic markers associated with the development of TB disease and TB infection
Impact of revaccination with BCG among medical students:

a) Control group, negative PPD negatives, non revaccination with BCG;

b) Revaccinated group, with IFN-gama low production;

c) Revaccinated group, with IFN-gama high production

Preliminary Results: differential pattern of antigen recognition, high correlation with the IFN-gama production profile. Mass spectometry evaluation of proteic spot under analysis.
To Identify biomarkers of TB infection, disseminated TB and TB relapse aiming the development of molecular prototype in nylon membrane

Phase I: Exploratory Study using retrospective cohort

The following cytokines or chemokines associated with TB pathogenesis has been assessed: IL-4, IL-6, IL-8, IL-10, TGF-β, IL-12, IFN-γ, TNF-α, IL-1β, MCP-1, NRAMP1, TLR-2, TLR-4, MBL, vitamin D receptor, Nod2/Card15, IRAK-M, SOCS1/3.

Phase II: Prospective cohort

Single nucleotide polymorphisms (SNPs): 1) previously been reported to be associated with TB or 2) are in genes biologically relevant to TB pathogenesis will be assessed. There will be 42 SNPs and 8 microsatellites for the former and approximately 30,000 SNPs in 64 genes for the latter.

The Targeted Genotyping platform from Affymetrix. This platform offers a directed and customized assay for fine mapping of SNPs and is tailored to the analysis of functional SNPs.
### Contacts of Pulmonary TB cases: Preliminary results – Phase I

**Cohort of 525 contacts / Median follow-up was 52.8 months.**

Positive TST, TST conversion and TB disease among Close contacts

<table>
<thead>
<tr>
<th>Variable</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final Positive TST (1)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>273 (64.7%)</td>
</tr>
<tr>
<td>No</td>
<td>149 (35.3%)</td>
</tr>
<tr>
<td>TST conversion (2)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>23 (13.1%)</td>
</tr>
<tr>
<td>No</td>
<td>154 (87.0%)</td>
</tr>
<tr>
<td>TB Disease (3)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>44 (12.2%)</td>
</tr>
<tr>
<td>No</td>
<td>317 (87.8%)</td>
</tr>
</tbody>
</table>

n= (1) 422; (2) 177; (3) 361; TST: tuberculin skin test

TB disease was evaluated among those with at least 12 months of follow-up
Table. Multivariate logistic regression analysis of variables associated with TST conversion and TB disease in close contacts of pulmonary TB patient

Phase I – Exploratory Study

<table>
<thead>
<tr>
<th>TST conversion</th>
<th>Variables in the final model</th>
<th>OR (I.C. 95%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Close contact is not a family member</td>
<td>5.17 (1.64 – 16.35)</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>Presence of BCG scar</td>
<td>2.03 (0.66 – 6.24)</td>
<td>0.214</td>
</tr>
<tr>
<td></td>
<td>Heterozygous TLR4 Thr399Ile genotype (CT)</td>
<td>4.21 (1.27 – 13.93)</td>
<td>0.019</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TB disease</th>
<th>Variables in the final model</th>
<th>OR (I.C. 95%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age (year) *</td>
<td>0.95 (0.93 – 0.98)</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>Presence of BCG scar</td>
<td>0.35 (0.13 – 0.92)</td>
<td>0.033</td>
</tr>
<tr>
<td></td>
<td>Index case with lung cavitation on chest X-ray</td>
<td>1.63 (0.64 – 4.15)</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>Heterozygous TLR4 Thr399Ile genotype (CT)</td>
<td>5.32 (1.83 – 15.46)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

*Estimates of the OR and 95% CI per 1 yr increase in age.
Despite the concomitant heightened levels of Th1-type mediators, immune activation is rendered ineffectual by high levels of intracellular (SOCS family members) and extracellular (TGF-β RII) suppressive mediators.

The marked elevation of suppressive mediators supports the hypothesis that *M. tuberculosis* actively promotes these modulators to counteract Th1-type and innate immunity as an immunopathological strategy.

Almeida A et al. Submitted, 2008
Evaluate the impact of different Mtb genotype in
The occurrence of TB disease and TB infection
with the close contact cohort

And, the occurrence of disseminate TB, of non-
response to anti-TB treatment and of high relapse rate
Institutions involved in the Vaccine area

Espírito Santo
UFES  Universidade Federal do Espírito Santo

Minas Gerais
UFMG – Universidade Federal de Minas Gerais

Pernambuco
FIOCRUZ-PE  Fundação Oswaldo Cruz

Rio de Janeiro
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FCFRP-USP  Faculdade de Ciências Farmacêuticas de Ribeirão Preto  USP
IB  Instituto Butantan  SP
UNICAMP – Universidade Estadual de Campinas
UNESP – Universidade Estadual de São Paulo – Botucatu SP
FMUSP – Faculdade de Medicina da USP SP
Research and development of new vaccines for tuberculosis

Basic Research → Development → Clinical Studies

Types of vaccines and concepts in development

DNA Vaccines

Subunit vaccines

recombinant BCG

Antigens: HSP65, MCE2, ESAT6, 85A, MPB83, MPB70, TPX, PLCa
“Prime Boost Concept”
Fusion Proteins (genic or subunits)
Vaccine Area- Activities

• Construction by gene replacement (homologous recombination) and characterization of defined mutant strains of *M. tuberculosis* H37Rv for the following genes: *pyrE* (orotate phosphoribosyltransferase), *cdd* (citidine deaminase), *hns* (histone-like nucleoid structuring protein), and *gpsI* (polynucleotide phosphorylase).

• Construction by gene replacement (homologous recombination) and characterization of defined mutant strains of *M. bovis* BCG Moreau – Rio de Janeiro, for instance *cdd* (citidine deaminase).
Institutions involved in R&D of new drugs for TB

**Amazonas**
INPA Instituto Nacional de Pesquisas da Amazônia

**Ceará**
UFCE Universidade Federal do Ceará

**Pernambuco**
UFPE Universidade Federal de Pernambuco

**Rio Grande do Sul**
PUC/RS – Pontifícia Universidade Católica RS
IB/UFRGS Universidade Federal do Rio Grande do Sul

**Santa Catarina**
UFSC Universidade Federal de Santa Catarina

**São Paulo**
FMRP-USP Faculdade de Medicina de Ribeirão Preto USP
FCFRP Faculdade de Ciências Farmacêuticas de Ribeirão Preto USP
UNESP Universidade Estadual de São Paulo Rio Claro
UNESP Universidade Estadual de São Paulo São José do Rio Preto
UNICAMP Universidade de Campinas
UFSC Universidade Federal de São Carlos
IQUSP Instituto de Química da USP
IB – Instituto Butantan

Millenium Institute Project - 2002 - Approval by Ministry Science Technology
Discovery of New anti-TB compounds using screening strategy

Evaluation of specific molecular targets for new Drugs
Some compounds are under final evaluation on pre-clinical phase

- *in vitro* determination of Minimum Inhibitory Concentration (MIC) of promising chemical compounds (enzyme inhibitors) against *M. tuberculosis* H37Rv strain in defined culture medium.

- *in vitro* determination of Minimum Inhibitory Concentration (MIC) for IQG607 against isoniazid-resistant *M. tuberculosis* strains harboring katG- and/or inhA-structural gene mutations, and inhA-operon promoter mutations.
Institutions involved in the Diagnostic area

**Amazonas**
INPA - Instituto Nacional de Pesquisas da Amazônia
UFAM - Universidade Federal do Amazonas

**Espírito Santo**
NDI/UFES - Universidade Federal do Espírito Santo

**Minas Gerais**
UFMG/MG - Universidade Federal de Minas Gerais MG

**Pernambuco**
UFPE/PE - Universidade Federal de Pernambuco
CpqAM-FIOCRUZ – Fundação Oswaldo Cruz PE

**Rio de Janeiro**
IOC/FIOCRUZ – Instituto Oswaldo Cruz RJ
UGF/RJ – Universidade Gama Filho RJ
NES/P/UFPR – Universidade Federal do Rio de Janeiro RJ
IDT/UFJ – Universidade Federal do Rio de Janeiro
FAC FARMACIA – Universidade Federal do Rio de Janeiro RJ
IM/UFJ Instituto de Microbiologia RJ
IBCCF/UFJ – Universidade Federal do Rio de Janeiro RJ

**Rio Grande do Sul**
PUC/RS – Pontifícia Universidade Católica RS
CDCT/FEPPS/RS
UFGRS – Universidade Federal do Rio Grande do Sul RS

**São Paulo**
FMU/RP/USP – Faculdade de Medicina de Ribeirão Preto - USP
CRT-AIDS/SES/SP – Secretaria Estadual de Saúde SP
IAL/SES/SP – Secretaria Estadual de Saúde SP
IEER/SES/SP – Secretaria Estadual de Saúde SP

International collaboration:
USA, Canada, South Africa, Norway, Netherlands, Russia, Vietnam, Mexico

Millenium Institute Project - 2002 - Approval by Ministry Science Technology
A guide for diagnostic evaluations

Rosanna W. Peeling, Peter G. Smith and Patrick M. M. Bossuyt
Nature, S1-S6, September 2006

Figure 2 | The bench-to-bedside pathway of diagnostics development and evaluation. The development of a diagnostic test usually follows a path from identification of the diagnostic target and optimization of test reagents to the development of a test prototype that then undergoes a series of evaluations. Reproduced with permission from REF. 13.
Phased Trials for Diagnostics

- **Phase I** - pre-clinical evaluation, analytic study
- **Phase II** - proof of principle study distinguishing diseased from healthy persons in easily accessible populations (sensitivity/specificity/accuracy)
- **Phase III** - evaluation of test performance in populations of intended use (Lab and Field Trials) (effectiveness, predictive values)
- **Phase IV** - delineation of cost-effectiveness and societal impact of new tests in comparison with existing tools (Utility studies)

**Funding available**

**Approved by Regulatory Agency**

**Nobody cares**
• Evaluation performed by Ministry of Health
• 48 tests were registered since 2000
• 15 had valid registration
• 33 tests are still on the market !!!
• No information regarding to the type of clinical scenario/patients that those Diagnostic Tests have been commercialized in the country.
There is no surveillance on the commercialized tests for TB diagnosis

No interaction between:

- Regulatory Agency
- National TB Control
- Universities/Research Inst.
- Medical Associations
There was no test for TB diagnosis developed by Brazilian institutions.

Development of new molecular diagnostic tests for Drug sensitive TB
Develop of rapid diagnostic tests for M. tuberculosis in clinical isolates

Phase II trial. Evaluation of PCR-DOT for drug sensitive pulmonary TB

Amplified PCR products with
hybridization using biotinilated probes

Table 2  Sensitivity and specificity of PCR-AG, PCR dot-blot assays, smear and culture

<table>
<thead>
<tr>
<th>Method</th>
<th>No. of confirmed TB results</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Results</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>PCR-AG</td>
<td>Positive</td>
<td>38</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>4</td>
<td>37</td>
</tr>
<tr>
<td>PCR dot-blot</td>
<td>Positive</td>
<td>40</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>2</td>
<td>37</td>
</tr>
<tr>
<td>Smear*</td>
<td>Positive</td>
<td>23</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>2</td>
<td>32</td>
</tr>
<tr>
<td>Culture</td>
<td>Positive</td>
<td>33</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>9</td>
<td>38</td>
</tr>
</tbody>
</table>

* Insufficient material in 7 specimens.
PCR-AG = polymerase chain reaction-agarose gel.

Gold Standard: Clinical Evolution/ATS 2002

Interlaboratorial Validation started in 2005

Sperhacke, R et al. IJTLD 8 (3): 312-317, 2004
Validation of in house PCR dot blot in three reference TB laboratories carried out in 2005

The high accuracy results obtained in previous phase were not confirmed !!!

Research Laboratories need also to be certificate by external agencies
Question:

**How to scale-up the in house PCR technique developed by Universities ??**

Answer:

**We do need to interact with Industry**
Development of in house PCR for TB diagnosis

Interaction with Private Industry

Sample preparation and DNA purification

Resine in house (BOOM et al., 1990).

Amplification – PCR

Probe

ELISA

Concentração
Development of new molecular diagnostic tests for drug resistant TB

We have to take into account the diversity of drug resistance and Mtb genotypes
TOTAL 1456 MTB STRAINS

North - N=162  LAM=72%; Haarlem=22%

Northeast - N=176
LAM=76%; Haarlem=18%

South - N=234
LAM=71
Haarlem=18%

Southeast - N=884
LAM=65%; Haarlem=19%
Beijing 1%
Cohort of Close Contacts of MDR-TB and DS-TB in Brazil

<table>
<thead>
<tr>
<th>Index case</th>
<th>Contacts</th>
<th>TST+</th>
<th>active TB</th>
</tr>
</thead>
<tbody>
<tr>
<td>TB-MDR: 26</td>
<td>133</td>
<td>59 (44%)</td>
<td>6 (4%)</td>
</tr>
<tr>
<td>TB-DS: 52</td>
<td>231</td>
<td>86 (37%)</td>
<td>11 (4%)</td>
</tr>
</tbody>
</table>

RFLP – identical Mtb isolates (index cases and their close contacts)

<table>
<thead>
<tr>
<th>Group of selected index cases</th>
<th>N. case</th>
<th>Active TB /Total of Contacts</th>
<th>MIC</th>
<th>KatG mutant</th>
<th>315 mutant</th>
<th>Spoligotyping</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDR</td>
<td>5</td>
<td>8/16</td>
<td>4.0-8.0</td>
<td>Yes</td>
<td></td>
<td>Lam = 1 Haarlem= 4</td>
</tr>
<tr>
<td>MDR</td>
<td>5</td>
<td>0/28</td>
<td>4.0-16.0</td>
<td>Yes</td>
<td></td>
<td>Lam: 3 T2: 2</td>
</tr>
<tr>
<td>DS</td>
<td>7</td>
<td>11/37</td>
<td>-</td>
<td>No</td>
<td></td>
<td>Lam: 5 T1: 1 Haarlem: 1</td>
</tr>
<tr>
<td>DS</td>
<td>6</td>
<td>0/20</td>
<td>-</td>
<td>No</td>
<td></td>
<td>Lam: 6</td>
</tr>
</tbody>
</table>

MDR: multidrug-resistant; DS: drug sensitive

Next step: animal model -???
It would be desirable the development, validation and initial application of a simple assay allowing simultaneous genotyping and detection of drug resistance mutations in MTB.

Identify important genetic markers, concerning:

1. genotype (IS6110, gyrA, katG, TbD1),
2. drug resistance (rpoB, katG, inhA, embB) and
3. possibly increased adaptive potential (mutT2, mutT4, ogt).

Determining all these aspects in one assay would be advantageous for transmission studies, molecular biology research and selection of optimal treatment regimens.
Question:

How to evaluate the performance (under field conditions) and the utility of new diagnostic techniques in Primary, Secondary and Tertiary Health Units?

Answer:

We do need to interact with TB control Program and Industry
Institutions involved in the Operational Clinical area

**São Paulo**
- FMRP-USP Faculdade de Medicina de Ribeirão Preto USP
- CRT/AIDS SP Centro de Treinamento
- HCFMRP-USP Hospital das Clínicas da FMRF-USP
- EPM-UNIFESP Universidade Federal de São Paulo
- FM-USP Faculdade de Medicina da USP
- FSP-USP Faculdade de Saúde Pública da USP
- SMS-SP Secretaria Municipal de Saúde São Paulo
- INSTITUTO DA CRIANÇA-USP
- EERP-USP Escola de Enfermagem de Ribeirão Preto USP
- UNICAMP
- EEFM-S.J.R.PRETO
- EE-USP Escola de Enfermagem da USP
- FMB-UNESP Faculdade de Medicina de Botucatu
- ICF-SP Instituto Clemente Ferreira
- HSP-SP Hospital do Servidor Público SP
- FELASP Federação das Ligas Anti-Tuberculose SP
- SES-SP Secretaria Estadual de Saúde SP
- FM-PUC SOROCABA
- FM-UNICAMP Fac. Medicina da UNICAMP
- I. ADOLFO LUTZ-SP
- FFRP-USP Faculdade de Filosofia de Ribeirão Preto USP

**Goiás**
- UFGO Universidade Federal de Goiânia
- SES-GO Secretaria Estadual de Saúde de Goiás
TUBERCULOSIS IN BRAZIL

• 85,000 TB cases/year.
• 6,000 TB deaths/year.
• 7ª of Hospital costs per admissions per infectious Diseases.
• 4ª causes of death per infectious diseases
TB incidence per State in Brazil 2005

Brasil -
44/100.000hab.
81,286 cases

30% TB cases
Hospitals
Linear Regression Analysis

Taxa de Incidência de Casos Novos Notificados e modelo regressivo log normal
Tuberculose, Brasil, 1980 a 2006

Queda média anual =1,8%

Impact of HIV infection

Fonte: Relatório da Avaliação Externa e Independente. M.L.Penna e col.
TB reported cases - 2006

- Total TB cases: 83,293 (41.7/100,000 hab)
- Reporting Site: 20-40% in Hospitals
- Cure rate: 62,802 (75.4%)
- Defaulting rate: 12.2%
- Mortality rate: 2 - 3%
- DOTS strategy coverage: 52%
Proportion of TB diagnosed among AIDS reported cases by National AIDS Program. Brasil, 1990-2006*

Fonte: SINAN e PN-DST/AIDS (*estimated)
Target population: TB/HIV - 2006

- TB/HIV cases reported : 6,995
- Culture for Mtb not performed : 4,896 (70.0%)
- Cure rate : 3,254 (46.5%)
- Defaulting rate : 1,685 (24.1%)
- Mortality rate : 1,369 (19.6%)
  (majority in hospitals with no TB control activities)
Reported cases of MDRTB by year, and trend
BRAZIL  2000 – 2006
n = 2443 new cases

Notificações de 1º tratamento  N = 2240
1995 – 2005

As of October 2006 = 203

40% from Rio

Source: Surveillance Database MDRTB – Reference Center  Hélio Fraga/ MoH
Target population: TB – MDR - 2006

- Retreatment TB cases : 9,598 / 83,293 (11.5%)
- Culture for Mtb not performed : 8,733 (91%)
- Drug suscep.testing not performed : 9,022 (94%)
- Low TB-MDR cases detection : 320/83,293 (0.39%)
  • Few close contact evaluation
Researches in Health Care Systems

Structure

Process

Provided Attention

Received Attention

PATIENT

Personal Services
Facilities - teams
Service types
Organization
Continuity
Accessibility
Financing
Accepted population

Problem Identification
Diagnosis
Treatment
Control

Use
Acceptance
Comprehension
Participation

Longevity
Activity
Wellness
Fulfilled expectations
Disease
Recovery

Environment

Clinical Operational

Result

Infrastructure

Support

Participants

Rede’s Mission
# Sensitivity analysis of costs based on screening of 1000 suspects comparing ZN plus Culture, ZN plus PCR dot-blot

<table>
<thead>
<tr>
<th>Component</th>
<th>Current situation</th>
<th>Adjustment</th>
<th>ZN plus Culture</th>
<th>ZN plus PCR dot-blot</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>TB prevalence</td>
<td>46%</td>
<td>No adjustment</td>
<td>U$448</td>
<td>U$429</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10%</td>
<td>U$2072</td>
<td>U$1947</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20%</td>
<td>U$1036</td>
<td>U$994</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40%</td>
<td>U$518</td>
<td>U$497</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60%</td>
<td>U$345</td>
<td>U$331</td>
<td>0.96</td>
</tr>
<tr>
<td>Sensitivity PCR dot-blot</td>
<td>85%</td>
<td>95%</td>
<td>U$408</td>
<td>U$383</td>
<td>0.94</td>
</tr>
<tr>
<td>Specificity PCR dot-blot</td>
<td>84%</td>
<td>95%</td>
<td>U$350</td>
<td>U$329</td>
<td>0.94</td>
</tr>
<tr>
<td>PCR dot-blot running costs*</td>
<td>U$12833</td>
<td>U$10000</td>
<td>U$448</td>
<td>U$403</td>
<td>0.90</td>
</tr>
</tbody>
</table>

In Teaching Hospital, cost effectiveness evaluation that incorporated

- Health Service Costs
- Labour Costs
- Patients Costs

Demonstrated the AFB+PCR had lower costs than AFB+culture for TB diagnosis algorithm
A randomized clinical trial, open and pragmatic

has been carried out at University Hospital and Health Center in Rio de Janeiro City, Brazil. Participants: Pulmonary TB (PTB) suspects with more than 18 years that signed the written informed consent. Interventions: comparison of MGIT960 arm, and the Lowenstein Jensen/LJ arm for PTB diagnosis.

**Outcomes:** Proportion of treatment changes after the test result and Clinical outcome.

**Results:** Anti-TB treatment changes occurred on MGIT960 arm in 8 cases (8.9%), and on LJ arm in 6 cases (7.8%). Among MGIT960 arm, 8/16 (50%) change treatment before 30 days.

Description of culture results during the study period

<table>
<thead>
<tr>
<th>Test Arm</th>
<th>0-15 days</th>
<th>16-30 days</th>
<th>31-56 days</th>
<th>57-60 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGIT 960</td>
<td>Pos: 13*</td>
<td>Pos: 3**</td>
<td>Neg: 73</td>
<td>-</td>
</tr>
<tr>
<td>Lowenstein Jensen</td>
<td>-</td>
<td>Pos: 2</td>
<td>Pos: 12+</td>
<td>Neg: 53</td>
</tr>
</tbody>
</table>

Anti-TB treatment changes: *n=5; **n=3; +n= 6; Pos: Positive; Neg: Negative

Rezende A, submitted for ATS Meeting, 2009
Clinical Assays Development
Local Network

- Moxifloxacin in the Initial Therapy of Tuberculosis: A Randomized, Phase 2 Trial
- INH vs RIF for LTBI trial
- Pragmatic Diagnostic Trials on TB (MGIT960 vs LJ)

Millenium Institute Project - 2002 - Approval by Ministry Science Technology
Área de Tuberculose e HIV

Coordenador
Valéria Cavalcanti Rolla

Vice-Coodenador
Leda Jamal
Integrate activities on TB and HIV Control (WHO priorities)

- Intensified TB case finding
- Infection Control
- Isoniazid Preventive Therapy

- To establish clinical trials sites for phase I, II, III and IV
- To compare the efficacy of regiments containing Efavirenz in different doses (600 X 800 mg)
- To evaluate the pharmacokinetics and pharmacodynamics of HAART and its association with adverse reactions, failure
- To describe the immunological and genetic aspects among patients with paradoxal reactions
- To evaluate the impact of different HIV sublineages and the occurrence and clinical outcome of TB
Human Resources Area

Coordenador
José Roberto Lapa e Silva

Vice-Coordenador
Anete Trajman
Strategies for Human Resources Training

TRAINING LEVELS

Level 1 - 1 week courses aiming at giving epidemiologic and scientific methodology notions to professionals working in the health systems, enabling them to participate in operational research projects.

Level 2 - 2 week courses using the Health System Research Method and small competitive help for field research.

Level 3 - Advanced Training Courses

Level 4 - PG

Training for Clinical, Operational and Health Service Research Areas

Human Resources

RESEARCH TRAINING
Participants in Research Training Courses
2005 -2007

Level 1 (8-40hours): Research awareness for the Community
(N= 492)

Level 2 (80hours): Fundamentals on Scientific Methodology - (Quantitative / Qualitative)
(n= 178)

Level 3: Advanced Training Courses: 47 (JHU/UCB)

Level 1 PhD: 24

User of Health System NGOs, Health Council

Health Professionals at: Family Health Program Health Center Unit Hospital Prison TB Laboratories
October 2005 – October 2007
RESEARCH TRAINING

Courses Level 2 (total: 178)
– Health Professionals: 134
– Users/Community leaders: 44

• 178 research projects developed
• 38 received awards due to the high quality
List of 8 research projects from those 38 that received award

<table>
<thead>
<tr>
<th>Participant</th>
<th>Institution</th>
<th>Professional category</th>
<th>Project title</th>
<th>Supervisors</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.Denise Freitas</td>
<td>City TB Control Program Itaborai - RJ</td>
<td>Physician</td>
<td>Impact of active finding of TB in the community using Family Health Program</td>
<td>R. Medronho,</td>
</tr>
<tr>
<td>10. Maria Martins</td>
<td>State TB Laboratory São Paulo</td>
<td>Biologist</td>
<td>How to decrease the time for DST results given to the Health Unit?</td>
<td>L. Fonseca, A. Ruffino</td>
</tr>
<tr>
<td>17. Maria Catarina Salvador Motta</td>
<td>Nurse School</td>
<td>Nurse</td>
<td>User's and Health Professional's perceptions on TB in the community - Itaborai</td>
<td>T. Scatena Villa Linda</td>
</tr>
<tr>
<td>22. Wilza Mary Souza Barreto</td>
<td>Ministry of Health Task Force -</td>
<td>Nurse</td>
<td>Effectiveness of DOTS in Itaborai and São Goncalo - RJ</td>
<td>R. Medronho,</td>
</tr>
<tr>
<td>32. Gloria Mizael</td>
<td>Fórum ONG</td>
<td>Psicologist</td>
<td>Capacity building of community volunteers in the active finding</td>
<td>K. Geluda</td>
</tr>
<tr>
<td>33. Luzia Araújo</td>
<td>Hospital - RJ</td>
<td>Nurse</td>
<td>Why nurse professionals do not use the available respirators in isolation rooms?</td>
<td>ML Bosi</td>
</tr>
<tr>
<td>37. Carla Damas</td>
<td>State TB Hospital RJ</td>
<td>Nurse</td>
<td>Why TB patients abandon the anti-TB treatment under DOTS?</td>
<td>K. Geluda,</td>
</tr>
</tbody>
</table>
Social Mobilization

Objectives

- Improve case detection, and creating demand for DOTS services through grass-roots participation and ownership, and integrated communication;

- Reduce stigma associated with the disease by helping TB patients and health care workers to be influential voices.

Activities

- Mobilizing local communities and patients
- Providing advocacy training to health officials
- Creating 1st NGOs for TB (2003)
- Brazilian TB Partnership/Stop TB (2004)
- Member of Executive Secretary of Global Fund TB
- Organization of National TB Meeting (I, II, III)
Clinical and Operational Research Agenda Priorities

• Evaluate the clinical utility, usefulness and cost-effectiveness of new technologies for TB diagnosis, especially for paucibacillary TB, TB /HIV and MDR-TB suspects

• Evaluate new strategies to diminish the MDRTB transmission in the community, in hospitals and in prisons

• Identify new strategies for the TB and HIV control activities (to diminish stigma, increase adherence)
Clinical and Operational Research Agenda Priorities

- Identify microbial factors in Tb virulence, persistence and latency/reactivation as potential biomarkers

- Identify host factors in susceptibility to TB (or development of symptom-free infection), and HIV/Tb interactions.

Information resulting from these studies may be utilized to improve TB vaccine intervention, and interrupting HIV production
• Successful establishment of the TB network with high capillarity of the **REDE TB in the Academic, Governmental Entrepreneurship environment, and Civil Society**;

• Strengthens of the **links with civil society, police makers involved in the control of TB**

• Establishment of interaction between Lab. and Health Units in the different fields for research (**Demonstration Areas**)  

• High level **research training** at different levels (professionals, technicians, researchers - Msc. PhD.)
• Changing Researcher’s Behavior from Lab and Clinical towards certification by National and International Agencies (Good Laboratory Practices, Good Clinical Practices, Documentation and Training);

• Intellectual property (vaccine, diagnostics and therapeutics) and technology domain by means of scale up developments.

• Initiate the Discussion inside Universities about the Technology transfer (the necessity of creation of star up and spin off).
Acknowledgments

Ministério da Ciência e Tecnologia - CNPq

Ministério da Saúde

Ministério da Educação - CAPES

Empresas Públicas e Privadas
Thank you
Thanks for your attention

kritskia@gmail.com