S. pyogenes candidate vaccine

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RF and RHD

**Streptococcus pyogenes**

- Polyarthritis (90%)
- Subcutaneous Nodules
- Eritema marginatum
- Carditis (30-45%)

**Auto-immune Reactions**

(3 a 4%)

1840: “Rheumatic fever licks the articulations and bits the heart.” (Jean Baptiste Bouillot)

Reviewed by Steer et al, 2007

(Decourt, 1972); (Kaplan, 1979)
Pattern of Valvular Lesions of Rheumatic Fever/Rheumatic Heart Disease Patients Based on Cardiology Auscultation

311 out of 439 patients
63.6%
<table>
<thead>
<tr>
<th></th>
<th>Number - Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rheumatic Fever Surgeries</td>
<td></td>
</tr>
<tr>
<td>Valvar Replacement</td>
<td>3873</td>
</tr>
<tr>
<td>Valvar Plastic</td>
<td>1131</td>
</tr>
<tr>
<td>Total</td>
<td>5004</td>
</tr>
</tbody>
</table>

49.5 % of cardiac surgeries performed in 25 years

Pomerantzeff PMA, Brandao CM, et al. Valve Reconstruction in the Heart Institute of São Paulo, Brazil.; Semin Thorac Cardiovasc Surg, 2002
Medical Care

• Ambulatory
  
  2014
  
  – Following-up: approximately 12000 patients;
  
  – Average monthly attendance: 1600 patients;

May/2015:

  • New cases of VHD: 81 patients
  
  • Total: 1604 patients
Medical Care - Teaching

- 28 bed ward for valvular heart disease;
- **In 2014:** 615 surgeries (25% of all surgeries);
- **May 2015:** 57 valvular heart surgeries (20% of all surgeries);
Latin American / European / North American guidelines have a few differences and a lot of similarities:

• What is the Latin America (Brazilian) reality?
  – Higher prevalence of *rheumatic fever* (up to 70% of all surgical cases of valvular disease).
  – Incidence of rheumatic heart disease at school age is: 1 to 7/1000 children in Brazil versus 0.1 to 0.4/1000 in the USA.
  – Higher incidence of valvular disease in young people.

• What are the European and USA realities?
  – Higher prevalence of *degenerative valve disease* (aortic stenosis and mitral regurgitation)
| • ~ 600 outpatients / month |
| • ~ 2000 patients waiting for valvular surgery |
| • 38% of surgeries are in young patients |
HLA – Class II alleles
Serology, Mol Biol

MBL-2- Alleles A / O
TLR-2
FCN-2
FcRII A

Genetic Susceptibility

Adaptive IR

Cytokines
TNF- alfa
TGF-beta
IL-1 Ra
IL-10

Innate IR
SNP
**RHD- Autoimmune Reactions**: Peripheral T-cells and M protein Response

**Throat**

**A - Periphery**

- Macrophage
- peptide
- HLA DR/DQ
- CD4+T Cell
- Citokines
- B Cell
- Antibodies anti-streptococci
- Streptococci primed CD4+ T Cell

**Responders to M5(81-96) peptide**

- % Responders
- HLA class II

<table>
<thead>
<tr>
<th></th>
<th>DR7+</th>
<th>DR7-</th>
<th>DR53+</th>
<th>DR53-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe RHD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild RHD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

- 70% of RHD patients recognized Heart-tissue proteins

Rheumatic Valvulitis – Mitral v.

Vegetations

Valve Cross Reactive Proteins
Vimentin, Collagen VI, Lumican

Guilherme, L et al,

Martins C, Guilherme, L et al, 2014

Intralesional Auto-reactive T cell clones
Acute Phase – 67%
Chronic Phase – 20 to 30%

Vegetations

Acute Phase – 67%
Chronic Phase – 20 to 30%
Cytokines in RHD

Th1

Inflammatory
- TNFa, IFNg
- IL-17, IL-23

Regulatory
- IL-10
- IL-4 – Low numbers

- Progression of RHD lesions
- Permanent valvular damage

* P < 0.02; O.R. = 15.8

Streptococcus pyogenes

More than 200 strains

(Fischetti et al., 1991)

(Smeesters, et al., 2010)
Vaccine Development
M protein- C-terminal Region: T and B Epitopes

Identical Region

253 KGLRRDLDSREAKKQLEAEQQ
288 EASR KGLRRDLDSREAKKQVEKA

T epitope

B epitope

PepVac/Rec.Prot – StreptInCor 55 aa

253 KGLRRDLDSREAKKQLEAEQQKLEEQNKISEASRKGLRRDLDSREAKKQVEKA

Sequence data bank
PDB ID 2KK9
RCSB 101224

Patents
INPI, BR – 0501290 / 0604997-4,
International: China, Korea, Japan, USA

Therapeutic Effect: USA

Guilherme L, Kalil, J et al, Clin Dev Immunol, 2006, Methods, 2009,
J Biol Chem, 2011
HLA Class II – Binding Prediction

Afinity

P1 - L, I
P4 - D, S, E, A, Q, N
P6 - D, E, Q, R,
P9 - R, K, E, L,

Binding Prediction

Pept. | HLA-II
--- | ---
A | DR-03, DR52
B | DR-04, DR-04
C | DR-04, DR-53
D | DR-06, 07, DR-52, 53
E | DR02, 05, DR-51, 52

Class II MHC
Human Humoral and Cellular Reactivity

Experimental Assays

• Mice (Balb-C, C57BL6, Swiss, HLA-class II transgenic mice)

• Mini pigs (25-30Kg)
StreptInCor induces high and specific IgG antibodies.
No crossreactivity against cardiac myosin was observed.

Postol E, Guilherme L, Plos One, 2013
Survival after emm1 *S. pyogenes* challenge

- Immunized mice: 87%
- Controls: 53%

*p = 0.05*

Postol E, Guilherme L, Plos One, 2013/ 2014
Adhesion/Invasion Inhibition – Hep-2 cells

Adhesion - *S. pyogenes*  

Sera from StreptInCor immunized mice

*S. pyogenes* – M1  Adhesion/Invasion Inhibition

- BALB/c (N=5)  
  95.0 %
- C57BL6 (N=7)  
  92.0 %
- Swiss (N=3)  
  98.5 %

UFC without sera > 200,000
Anti-StreptInCor antibodies induce Neutralization of several *S. pyogenes* strains

De Amicis MK, Guilherme L, Vaccine, 2013/2014
Anti-StreptInCor antibodies induce opsonophagocytose and *S. pyogenes* death

De Amicis MK, Guilherme L, Vaccine, 2013/14
Opsonization, phagocytose/death of M1 induced by anti-StreptInCor antibodies

Pré-immune sera

Hiperimmune sera

S.pyogenes

Phagocytosis of S.pyogenes by APC

De Amicis MK, Guilherme L, Vaccine, 2013/14
HLA Class II Tg Mice Model

• DRB1 (DR2, DR4)

• DQ6 and DQ8

Prof Chella David, Clinic Mayo, USA
HLA class II Tg Mice
StreptInCor antibodies recognize heterologous protein without crossreactivity against cardiac myosin

Guerino, T; Guilherme L, Vaccine, 2011
HLA-Class II - Transgenic mice: StreptInCor + ALUM

Guerino, T Guilherme et al, Vaccine, 2011
<table>
<thead>
<tr>
<th>Overlapping Peptides Sequences (20 aa residues)</th>
<th>Transgenic Mice Bearing HLA Class II Alleles</th>
<th>Humoral Immune Response (IgG)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KGLRRDLDAWAREKQEKL</td>
<td>DR2</td>
<td>6+/6</td>
</tr>
<tr>
<td>KGLRRDLDAWAREKQEKL</td>
<td>DR2</td>
<td>5+/6</td>
</tr>
<tr>
<td>GLRRDLDAWAREKQEKL</td>
<td>DR2</td>
<td>5+/6</td>
</tr>
<tr>
<td>LDASBEAKQLEAEQQKLE</td>
<td>DR2</td>
<td>4+/6</td>
</tr>
<tr>
<td>KLEQNKISEASRKGLRDL</td>
<td>DR2</td>
<td>5+/6</td>
</tr>
<tr>
<td>KISEASRKGLRDLASFRE</td>
<td>DR2</td>
<td>5+/6</td>
</tr>
<tr>
<td>SEASRKLRRDLASFRE</td>
<td>DR2</td>
<td>5+/6</td>
</tr>
<tr>
<td>ASRKLRRDLASFRE</td>
<td>DR2</td>
<td>4+/6</td>
</tr>
</tbody>
</table>

Guerino, T; Guilherme L, Vaccine, 2011,
StreptInCor did not Induce Heart-tissue Proteins Crossreactive Antibodies

1 (+) mouse anti-myosin sera
2 (-) non-immunized sera
3 DR2 tg mice sera
4 DR4 tg mice sera
5 DQ6 tg mice sera
6 DQ8 tg mice sera

Human myocardium tissue, Cadaveric donor

Guerino, T; Guilherme L, Vaccine, 2011
StreptInCor vaccine did not induce autoimmune reactions

1 year post-vaccination

Guerino, T; Guilherme L, Vaccine, 2011
## Autoimmunity Control

### RHD - Heart tissue infiltrating cells

<table>
<thead>
<tr>
<th></th>
<th>StreptInCor (Several peptides)</th>
<th>T cell lines T = 29 *</th>
<th>T cell clones N=49 (5 T cell Lines)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Negative</strong></td>
<td>23/29</td>
<td>79.3</td>
<td>42/49</td>
</tr>
<tr>
<td><strong>Positive</strong></td>
<td>6/29</td>
<td>20.7</td>
<td>7/49</td>
</tr>
</tbody>
</table>

* 14 valves and 15 myocardium

Guilherme L, et al, unpublished data

- **IL-10**
  - T-reg cells?

Guilherme L, et al, unpublished data
T regulatory (Treg) Cells

Are defined by several cell markers and are important tools as immunotherapy in organ transplantation and autoimmune diseases.

C-terminal M protein epitope- StreptInCor has a potential to induce:

• Protection against *S. pyogenes* (vaccine)

• T reg cells that regulate autoimmune reactions (therapeutic effect)
StreptInCor: Potential Therapeutic Effect
T regulatory cells: Peripheral blood of RHD patients

Flow Cytometry

Absolute number of cells

CD3⁺ CD4⁺ CD25<sup>high</sup> CD127⁻ Foxp3⁺

StreptInCor (vaccine candidate epitope) increases the numbers of Natural T Reg cells

Köhler KF, Guilherme L, et al, In Preparation
Summary

• The social-economic impact of RF/RHD in Brazil is still important

   In the last 20 years - our studies lead to:

1. Better understanding of the autoimmune and inflammatory mechanisms leading to the rheumatic heart lesions

2. C-terminal M protein epitope - StreptInCor has a potential to induce:
   • Protection against *S. pyogenes* (vaccine)
   • Cells that regulate autoimmune reactions (therapeutic effect)

Both Properties of StreptInCor Certainly will contribute to a better life of RF/RHD patients and to prevent new infections.
Clinical Phase I Assays / Design of the Study

- **Clinical Phase I**: random, double-blind, controlled with placebo, sequential dosing of StreptInCor (50 µg, 100µg, 200 µg - 2 doses with 28d interval); 6 months boost.

- **Healthy Volunteer**: individual without confirmed disease diagnosis or infection that would compromise the immune response, with ages between 18 and 45 years old.
Next Steps

• GMP production

• Phase I/IIa Clinical Trials – 2015/2016

• ANVISA and FDA registration

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RF/RHD Mechanism of Pathogenesis
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