Bacterium-like particles as delivery vehicles for multimeric antigens

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Mucosis BV.

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BACTERIUM-LIKE PARTICLES AS DELIVERY VEHICLES FOR MULTIMERIC ANTIGENS

Kees Leenhouts
CSO Mucosis B.V.
Vaccine Technology IV
Albufeira, Portugal
24 May 2012
# Snapshot

<table>
<thead>
<tr>
<th>Products</th>
<th>Innovative mucosal vaccines for respiratory tract infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Technology</td>
<td>Mimopath® vaccine platform</td>
</tr>
<tr>
<td>Based in</td>
<td>Groningen (NL) &amp; Rockville (USA)</td>
</tr>
<tr>
<td>Employees</td>
<td>20 FTE</td>
</tr>
<tr>
<td>Privately Held</td>
<td>MedSciences, BioGeneration, NV-NOM</td>
</tr>
<tr>
<td>Funding</td>
<td>~$16M from equity, credits &amp; grants</td>
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<tr>
<td>Partners</td>
<td>14 partners in US, Asia and EU</td>
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</tbody>
</table>

**Mucosis**

Vaccines that mimic nature
Mucosis is leveraging its Mimopath® platform to revolutionize vaccination by developing novel mucosal vaccines that provide optimal protection in the mucosa, the site where >90% of pathogens enter the human body.
# Mucosis’ innovative vaccine platform technology

## Current limitations in vaccine development
- Poor delivery methods
- Complex adjuvant technologies
- Systemic immune response primarily

## Mimopath® vaccine technology
- Delivery through natural route (mucosa)
- Straightforward, with well-understood mode of action
- Natural, balanced immune response: systemic and in the mucosa
Mimopath®: a versatile vaccine technology platform

Innovative & differentiated vaccines:
- Robust systemic and mucosal responses
- Multiple routes of administration
- Suited for complex antigens

- Mixed
  - Ag + BLP
  - e.g. FluGEM®

- Bound
  - Ag-Protan + BLP
  - e.g. PneuGEM® SynGEM®
Mimopath®: a versatile vaccine technology platform

Nearly any antigen

Any peptide or protein (> 50 done in house)
- Viral, bacterial, parasitic
  - No size limitation
  - Including complex glycosylated multimeric proteins
  - Potentially also tumor and allergy antigens
- Multiple production platforms
  - Bacterial (e.g. E. coli)
  - Mammalian (e.g. CHO)
  - Insect (e.g. S2)
  - Yeast (e.g. Pichia)
Binding of Ag-Protan is easy 1 step process

A
- wild-type *Lactococcus lactis*
- acid treatment & washes with buffer to remove acid and breakdown products

B
- introduce Ag-Protan in expression system
  - e.g. bacteria
  - eukaryotic cells
  - overexpression
  - removal of producer cells

C
- Ag-Protan fusion
  - mixing results in instant & strong non-covalent binding
  - washes with buffer

BLP-Ag vaccine
Robust BLP manufacturing process

**cGMP Manufacturing process:**
- Fermentation of *L. lactis* according to standard procedures
- Concentrate and wash with WFI
- Acid treatment at elevated Temp
- Concentrate and wash with PBS
- Final formulation in PBS

**BLP bulk material stability:**
- GLP bulk material stability study since Feb 2009
- cGMP bulk material stability study since Nov 2009
- Storage conditions: T=5°C and T=25°C
- Material is stable at both storage Temps

Large scale cGMP manufacturing feasible
Mimopath® is safe and well tolerated in man

BLPs derived from *Lactococcus lactis*,
a safe bacterium used for food production

- toxin-free (*no LPS*)
- no recombinant DNA
- non-living (*no dissemination*)
- qualified by FDA as G.R.A.S.

Mucosal administration:
- no local side effects
- no systemic side effects
- excellent safety & tolerability confirmed in man
How does it work?
Addressing innate immunity is key

Stimulation & Uptake

Resting innate immune cells

BLP TLR

Activation Migration

Activated innate immune cells

Priming adaptive immune response

CD4+ T cells help B-cells
CD8+ T-cells

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BLPs are a TLR2 agonist

in vitro

BLPs activate hDCs which stimulate T-cells in vitro

Adult human DCs

<table>
<thead>
<tr>
<th></th>
<th>Untreated</th>
<th>BLPs</th>
<th>TNFα</th>
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</thead>
<tbody>
<tr>
<td>CD86</td>
<td>30.59%</td>
<td>98.78%</td>
<td>72.46%</td>
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<tr>
<td>CD80</td>
<td>70.20%</td>
<td>99.34%</td>
<td>90.30%</td>
</tr>
<tr>
<td>CD83</td>
<td>4.37%</td>
<td>94.17%</td>
<td>75.64%</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>58.97%</td>
<td>99.44%</td>
<td>93.66%</td>
</tr>
</tbody>
</table>

BLPs act through TLR2

*T- and B-cells*

**IFN-γ T-cell Elispot**

**Spleen**

Mouse (TLR2) k.o. WT

**DL**

k.o. WT

---

**B-cell Elispot**

**Spleen**

HA+BLP

**DL**

HA+BLP

Broere et al. (2011) unpublished
BLPs act through TLR2

serum IgG and isotype ratio

Intramuscular administration

IgG2c/IgG1 ratio
(Th1/Th2 ratio)

Broere et al. (2011) unpublished

Mucosis
Vaccines that mimic nature
BLPs act through TLR2

*secreted IgA*

**Secreted IgA (nose)**

**Secreted IgA (vagina)**

Broere et al. (2011) unpublished
Mimopath® suitable for repeated vaccination

αB-IgG response of BLP-B vaccine after prior intranasal immunization with PBS or BLP-A vaccine

Audouy et al. (2006) Vaccine 24:5434-5441
Mimopath®
Proof of Concept
Rationale for influenza (FluGEM® program) as proof-of-concept model for Mimopath®

- Well-established correlates of protection
  - Early assessment of potency of the platform technology in human trials (e.g. Hemagglutination titer > 40)

- Challenge models in animals readily available
  - Allows for assessment of potency and efficacy of the vaccines

- Wide range of reagents available
  - Characterisation of systemic responses
  - Assessment of mucosal responses
Mimopath® provides improved and lasting systemic immune response

- **2log HI titers in the serum**
- **FluGEM®**
- **HA i.m. (Benchmark)**
- **HA**
- **FluGEM®**
Immune response is well balanced Th1/ Th2

Cytokine release profile in spleen cells

Serum IgG subtypes

Mimopath® elicits robust local and distant mucosal immunity
Mimopath® provides superior protection & cross protection

**Viral replication**
*Titers in lung 5 days post PR8 challenge*

**Disease score**
*Weight development and survival after PR8 challenge*

De Haan et al. (2012) Vaccine: accepted

*Heterologous challenge*
Mimopath® elicits improved systemic & local α-flu immune responses in healthy volunteers

Healthy adults (n=15) were vaccinated intranasally at d=0 and d=21 3 weeks post each vaccination, HI titers (A/California/7/2009) and nasal IgA were determined.
Mimopath® elicits an improved α-flu CMI responses in healthy volunteers

Days 0 21 28
IN vaccinations

Days
0 21 28
PBMC sampling

A/California/7/2009 (H1N1)

A/Perth/16/2009 (H3N2)

Fold increase compared to baseline (day 0)

Fold increase INFγ producing PBMCs at day 28
<table>
<thead>
<tr>
<th><strong>Mimopath® conclusions</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proof of concept</strong></td>
</tr>
<tr>
<td><strong>Safety</strong></td>
</tr>
<tr>
<td><strong>Mucosal response</strong></td>
</tr>
<tr>
<td><strong>Systemic response</strong></td>
</tr>
<tr>
<td><strong>Protection</strong></td>
</tr>
</tbody>
</table>
Mimopath®
Display of complex multimeric antigens on BLPs

Proof of Concept
Rational for use of multimeric antigens

- Many important (viral) surface antigens are multimeric membrane glycoproteins
- Native multimeric Ags elicit most effective immune responses*

Wei et al. J. Virol. 82 [2008] 6200-6208
Bosch et al. J. Virol. 84 [2010] 10366-10374
Proof of Concept: Influenza HA

- Trimeric HA protein from subtype A H1N1/Cal/7/2009
- Production on HEK293 cells
HA\text{tri} has high affinity for BLPs

Mix and wash

Medium+HCP

BLP

HA\text{tri}

Medium+HCP

Anti-A/Cal/7/2009 Ab

Fluorescence

Light

A/Cal/7/2009 HA\text{tri} bound to BLPs

BLPs only

Slide 30
HA_{tri} displayed on BLP is functional

Slide 31
HA^{tri} displayed on BLP is stable

HA^{tri} bound to BLP

Biological activity (HAU)
HA<sup>tri</sup> displayed on BLP is highly immunogenic

A/California/7/09 HA

Serum IgG

HI titer

Nasal Secreted IgA

m = mixed
b = bound
Display of complex multimeric antigens: conclusions

- Replacement of transmembrane domain by heterologous multimerization domain feasible
- Plug & play cassette available
- Multimeric Protan fusions efficiently bind to BLPs
- Displayed proteins are functional, stable & immunogenic
- Applicable to multiple disease targets (Influenza, RSV, HIV, Herpes, SARS etc.)
SynGEM®
Lead product
Medical need
- severe respiratory disease in **children** and **elderly**
- annually 64 million infections
- mortality 160,000 (in elderly as severe as Influenza)
- 18,000 to 75,000 hospitalizations (children, USA)

Vaccine failure
- formaldehyde inactivated, alum adjuvanted vaccine (FI-RSV)
- enhanced disease in children upon natural infection
- cause: no neutralizing Ab
  - response skewed towards Th2
  - formaldehyde inactivation modification of (neutralizing) epitopes
  - lack of affinity maturation (poor TLR stimulation)=low-avidity Ab

No registered vaccine available
Rationale for SynGEM®

- Native (trimeric) F antigens bound to BLPs:
  - Native F antigens elicit most effective immune responses*

- BLPs induce protection at the port of entry for RSV infection:
  - Elicit robust mucosal immunity in respiratory tract
  - Demonstrated in preclinical and clinical Mimopath® POC program

- BLPs induce protection against RSV disease:
  - Elicit potent humoral immune responses
  - Importance of humoral immune responses demonstrated by the success of antibody-based therapy in reducing RSV-associated hospitalizations in high-risk infants

- BLPs prevent enhanced disease:
  - Th1-biased responses demonstrated in preclinical Mimopath® POC program
  - Contrasts with Th2-biased responses associated with enhanced disease observed with formalin-inactivated RSV vaccine

- BLPs provide safety advantages:
  - Safe and well tolerated in man
  - Non-living, non-replicating, and GRAS component

Wei et al. J. Virol. 82 [2008] 6200-6208
Bosch et al. J. Virol. 84 [2010] 10366-10374
SynGEM® characteristics

- BLPs displaying antigen as particle
- Stabilized (trimeric) F antigen
- Synagis-like immune response

Sources: [http://www.uct.ac.za/depts/mmi/stannard/syncytia.html](http://www.uct.ac.za/depts/mmi/stannard/syncytia.html)
SynGEM® displays stable trimeric F

- Trimeric F(usion) protein from subtype A
- Production on HEK, CHO or insect cells
- Stable display of F on BLPs

Slide 39
SynGEM® induces Synagis-like Ab

mice

Synagis competition ELISA

Incubate with mouse serum

Add Synagis

F-coated wells

Transfer non-bound Synagis

Detect Synagis

OD492

pre post
SynGEM® induces local and better systemic Ab  

mice

Slide 41
**SynGEM® challenge study design**

* cotton rats *

<table>
<thead>
<tr>
<th>Groups:</th>
<th>Vaccination 1</th>
<th>Vaccination 2</th>
<th>Vaccination 3</th>
<th>Challenge</th>
<th>Termination</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>Day 0</td>
<td>Day 14</td>
<td>Day 28</td>
<td>Day 42</td>
<td>Day 48</td>
</tr>
<tr>
<td>F</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SynGEM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Challenge:**
RSV/A/Long at $10^5$ pfu

**Readout**
Viral load in lungs (protection parameter )
Lung histopathology score (enhanced disease outcome parameter)
(FI-RSV; i.m., positive control enhanced disease)
SynGEM® elicits protective responses in cotton rats.
SynGEM® no enhanced disease in cotton rats

* The control group which received IM administration with FI-RSV followed by an RSV challenge showed enhanced disease as expected.

*Enhanced disease score*

- Interstitial Pneumonia
- Alveolitis

Lung Pathology Score

FI-RSV*  
PBS  
F  
SynGEM

* Slide 44
**SynGEM® conclusions**

<table>
<thead>
<tr>
<th>Display of RSV F&lt;sub&gt;tri&lt;/sub&gt; on BLPs</th>
<th>RSV F&lt;sub&gt;tri&lt;/sub&gt; is bound in native conformation &amp; recognized by Synagis®</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunogenicity</td>
<td>SynGEM® induces Synagis-like neutralizing antibodies in mice</td>
</tr>
<tr>
<td>Protection</td>
<td>SynGEM® provides protection in the cotton rat challenge model</td>
</tr>
<tr>
<td>Safety</td>
<td>SynGEM® does not induce enhanced disease</td>
</tr>
</tbody>
</table>
Acknowledgements

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