ProbioSatys®, Naturally modulating the appetite via the microbiome

Gregory Lambert, CEO
Rotterdam, May 2019
Executive summary

- Based on 15 years of research at Inserm (University of Rouen, France)
- Offices in Longjumeau, Paris area & Labs in Rouen, Normandy
- Financing
  - Raised series A of 6.7 M€ in April 2016 & March 2017
  - Obtained 1.3 M€ in non-dilutive financing
- IP: 9 patent families and 6 trademarks
- Platform delivers both therapeutic and nutraceutical products
Discovery timeline – conceptual evolution from neuropeptides and autoimmunity to direct role of gut bacteria in appetite control

- **2002**
  - Autoantibodies against neuropeptides regulating appetite
  - PNAS 2002: Autoantibodies against α-MSH, ACTH, and LHRH in anorexia and bulimia nervosa patients
  - Taylor & Francis 2004: Autoimmune component in anorexia and bulimia nervosa

- **2008**
  - Curr Opin Clin Nutr Metab Care, 2008: The putative role of neuropeptide autoantibodies in anorexia nervosa.
  - Appetite, 2009: Regulation of food intake and anxiety by α-MSH reactive autoantibodies

- **2009**
  - Nutrition, 2009: In search of the missing link in the regulation of appetite and body weight
  - Psychoneuroendocrinology, 2009: Regulation of feeding and anxiety by alpha-MSH reactive autoantibodies.

- **2011**

- **2014**
  - Neuropeptides, 2014: Effects of rabbit anti-α-melanocyte-stimulating hormone (α-MSH) immunoglobulins on α-MSH signaling related to food intake control.

- **2016**
  - Cell Metabolism, 2016: Gut Commensal E.coli Proteins Activate Host Satiety Pathways Following Nutrient-Induced Bacterial Growth
  - Nature Reviews, 2017: Role of the gut microbiota in host appetite control: bacterial growth to animal feeding behaviour

- **2017**
  - Biol Aujourd'hui, 2017: Involvement of gut bacteria in appetite control.
  - Nature Medicine, 2016: Metabolism in mind: New insights into the ‘gut-brain axis’ spur commercial efforts to target it
The concept of molecular mimicry
ClpB / α-MSH Molecular mimicry

- Strong sequence homology between an exposed loop on the surface of ClpB and α-MSH\(^1\)
- Confirmation in HRM Mass Spectrometry after immunoprecipitation of enterobacteria proteins with α-MSH antibody\(^2\)
- ClpB is a MCR agonist\(^3\)

\(^1\) Tennoune et al., Transl Psy, 2014
\(^2\) Internal report from Biognosys, 2018
\(^3\) Ericson et al., Bioorg Med Chem Letters, 2015
Naturally regulating the appetite via the Microbiome

**Key ProbioSatys® benefits**

- Reduction in body weight
- Reduction of food intake
- Improvement of body composition
- Activation of lipolysis
- Decrease of fasting glycemia (increase of insulin tolerance)
- Activation of central satiety pathways

Fetissov, Nature reviews Endocrinology, 2016
Breton, Cell Metabolism, 2016
Plasma ClpB levels correlate with ClpB DNA in gut microbiota in rats

ClpB is present in human plasma

ClpB DNA in feces inversely correlates with Body Mass Index in 1128 individuals from MetaHIT cohort.

From: Breton et al., Int J Eat Dis 49: 805-808, 2016

From: Pons, Le Chatelier and Ehrlich, Internal unpublished report, INRA-MGP, 2017
ClpB – effect in the gut
Stimulation of PYY release

Bacterial growth

ClpB Concentration

Proteomics
Exp. Phase (a)  Stat. Phase (b)

Primary cultures
Rat colon mucosa

Rat colon infusion

Manon Dominique’s PhD thesis data (unpublished)


WWW.TARGEDYS.COM
E. coli K12 / E. coli K12 ΔClpB
Oral gavage - Normal mice

Effects on body weight and food behaviour are linked to the production of ClpB

Same strain with and without the ClpB producing gene (ΔClpB strain)
Effects on food intake, body weight and fat mass

1-way ANOVA, Tukey’s post-test, $p<0.05$, $$p<0.001
**Hafnia alvei 4597**  
(Isolated in Raw Milk)

- **Proteobacteria** phylum  
- **Gammaproteobacteria** class  
- **Enterobacteriales** order  
- **Enterobacteriaceae** family

Gram-negative  
Facultative Anaerobic

INRA and TargEDys confirms that *Hafnia alvei* is naturally present as commensal in human gut flora.

TargEDys uses *Hafnia alvei* as a probiotic strain in a dietary supplement to regulate appetite.

- **First identified** (Möller)  
  - 1954-1960
- **INRA** and TargEDys confirms that *Hafnia alvei* is naturally present as commensal in human gut flora.
- **TargEDys** uses *Hafnia alvei* as a probiotic strain in a dietary supplement to regulate appetite.

- **Listeria contamination episodes** in raw milk cheese led to milk pasteurisation and pasteurised milk cheeses.

- **End users complain** about loss of cheese traditionnal taste.  
  - => *Hafnia alvei* was identified as a key element to adding flavor and therefore starts to be used as part of the lactic ferments.

- **Hafnia is widely documented** in raw milk cheese (Camembert, up to $10^8$ UFC/g).
Treatment with **Hafnia alvei** decreases food intake and induces a significant decrease in body weight gain and improves metabolism as compared to untreated obese controls.

Two-way ANOVA, Bonferroni post-test, **p<0.01; *p<0.05**

Student's t-test, *p<0.05
Treatment with *Hafnia alvei* significantly decreased the body weight of HFD mice.

It also improves body composition.
Hafnia alvei vs Orlistat
Hybrid Model HF-HSD – ob/ob

Hafnia alvei reduces food intake and glycemia while Orlistat increases it.

Control of appetite is one key to achieve sustainable weight and metabolic disease management.
Toxicity study CITOXLAB, Denmark
- 32 rats (16 males/16 females) – 3 groups
- 14 days + 8 days recovery (8 animals)
- Oral gavage up to $4 \times 10^{11}$ CFU / $1.4 \times 10^{12}$ cells (2000 human dose/ kg)
- Behaviour and histopathology observations

- No side effect attributed to product
- Tested 2000 fold human dose / kg
- *H. alvei* 4597 resists digestion (low sensitivity to pH and enzymes)
- *H. alvei* 4597 adheres to mucus
- *H. alvei* 4597 grows and is metabolically active in colon
Industrial process
Optimisation of ClpB concentration

[Graph showing ClpB concentration in mg/g of material across different volumes: 1L, 30L, 5000L]

[Images of industrial equipment and laboratory settings]
Strain

- Petri dish cell count (CFU)
- Flow cytometry
  - Total cell count (bacteria)
  - Bacteria in intermediate stage
  - Cultivable bacteria

Protein and fragments characterization

- ELISA method to quantify $\alpha$-MSH pharmacophore
- Western Blot to quantify & characterize degradation

All non-lysed bacteria alive or not Contain and protects ClpB.

Molecular mimicry is present even after partial degradation
Comparison of 2 GI-resistant formulations (DRCAPS™ et HPMC) in ex-vivo GI model:

- Study of the release of the bacteria
- Study of the ClpB integrity

DRCAPS™ provide a delayed bacteria release & protection of the ClpB from degradation.

First stability data on the first commercial batch

25°C / 60% HR
The first ever Probiotic with a fully elucidated molecular mechanism of action in the gut-brain axis!

Naturally regulating the appetite through the Microbiome!

A unique strain: *Hafnia alvei* 4597 Covered by 4 patent families
Double-blind, randomised, placebo-controlled study to evaluate benefit of ProbioSatys® on weight reduction in overweight subjects

Probiotic strain & dosage: *Hafnia alvei* 4597 – 1.10^{11} cells/day

12 weeks of treatment, 2 capsules/day – 5 visits

3 centres in Germany

2 arms / 120 subjects par arm - BMI: 25 kg/m2 – 29.9 kg/m2

Main endpoints:

- Body weight (kg and %)
- Body fat and fat free mass
- Waist and hip circumference
- Lipid metabolism parameters
- Glucose blood parameters
- Feeling of satiety
- General well-being parameters
- Safety

A Human POC for ProbioSatys® Nutra and Therapeutic products
Consumer inquiry
EnteroSatys®

Ongoing study – intermediate results (39 consumers)

72% responders on satiety
(meal size ↓, sweet cravings ↓, snacking ↓)

10 days

-2.32% average weight loss after 1 month

85% would recommend the product
## Competitor Analysis

### Probiotic Strains

<table>
<thead>
<tr>
<th>Strains</th>
<th>L. gasseri</th>
<th>Dupont Danisco</th>
<th>ADM Biopolis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ProbioSatys</strong></td>
<td>Probiotic strains</td>
<td><em>Hafnia alvei</em> 4597</td>
<td><em>Bifidobacterium lactis</em> B420™</td>
</tr>
<tr>
<td><strong>TargEdys</strong></td>
<td></td>
<td><em>Lactobacillus gasseri</em> SBT2055</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td><em>Lactobacillus gasseri</em> BNR17</td>
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### Mechanism of Action

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<tbody>
<tr>
<td><strong>ProbioSatys</strong></td>
<td>Probiotic strains</td>
<td>Molecular mimicry of α-MSH with a bacterial protein</td>
<td>Not clear</td>
</tr>
<tr>
<td><strong>TargEdys</strong></td>
<td></td>
<td>Not clear</td>
<td></td>
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### Expected Effect

- Increase of satiety
- Lose body weight
- Improve body composition
- Reduce of glycemia and insulin resistance

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<tr>
<td><strong>ProbioSatys</strong></td>
<td>Probiotic strains</td>
<td>• Decrease abdominal fat</td>
<td>• Lose body weight</td>
</tr>
<tr>
<td><strong>TargEdys</strong></td>
<td></td>
<td>• Lose body weight</td>
<td></td>
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</table>

### Animal Data

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<thead>
<tr>
<th>Strains</th>
<th>L. gasseri</th>
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<tbody>
<tr>
<td><strong>ProbioSatys</strong></td>
<td>Probiotic strains</td>
<td><strong>Body weight:</strong> -2.5% vs control after 18 days <em>p&lt;0.001</em> Significant from day 11</td>
<td>Not clear</td>
</tr>
<tr>
<td><strong>TargEdys</strong></td>
<td></td>
<td><strong>Food intake:</strong> -15% vs control after 18 days <em>p&lt;0.10</em> Significant from day 10</td>
<td></td>
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</tbody>
</table>

### Human Data

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<tbody>
<tr>
<td><strong>ProbioSatys</strong></td>
<td>Probiotic strains</td>
<td><strong>Body weight:</strong> -1.4% after 3 months (significant)</td>
<td><strong>Body weight:</strong> -1.2% vs placebo after 6 months <em>p=0.15</em> (not significant)</td>
</tr>
<tr>
<td><strong>TargEdys</strong></td>
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### Consumer Survey

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<tbody>
<tr>
<td><strong>ProbioSatys</strong></td>
<td>Probiotic strains</td>
<td><strong>Body weight:</strong> -3.7% at 1 month</td>
<td><strong>Body weight:</strong> no difference vs placebo</td>
</tr>
<tr>
<td><strong>TargEdys</strong></td>
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Aims

- Regulatory deployment in EU and USA
- Accelerate marketing agreements discussions
- Break-in supply chain
- Measure end user satisfaction in real life

EnteroSatys® French sales
update as of May 17, 2019
TargEDys Development axis

**Dietary supplement**
- EnteroSatys®
  - On the market

**Functional foods**
- Looking for

**Pet Care**
- Ongoing

**Therapeutics**
- POC in animal

**Probiotic**
- EnteroNutrys®
  - POC in animal

**Discovery**
- Ongoing research work

**Other mimetics**
Contact information

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