ProbioSatys – Naturally modulating the appetite via the microbiome

Gregory Lambert, CEO

6th MICROBIOME R&D & BUSINESS COLLABORATION FORUM
3rd PROBIOTICS CONGRESS: USA
Our DNA
We are a Probiotic 2.0, commercial stage nutraceutical company but with a pharmaceutical DNA. Our commercial dietary supplement is differentiated by a well understood and published molecular level mechanism of action, backed by strong pre-clinical data and currently clinically tested. All our developments have the opportunity to be transposed to therapeutic.

Our mission
is to control body weight and metabolic disease by modulating the appetite through an intervention on the microbiome

Our approach
is to use the concept of molecular mimicry to physiologically bind well-described pharmacological targets such as melanocortin receptors (MCR) with bacterial metabolites and derived small molecules
Discovery timeline – conceptual evolution from neuropeptides and autoimmunity to direct role of gut bacteria in appetite control

**2002**
Autoantibodies against neuropeptides regulating appetite

**2004**
Autoimmune component in anorexia and bulimia nervosa.

**2005**
Autoantibodies against neuropeptides are associated with psychological traits in eating disorders.

**2008**
Autoantibodies against α-MSH, ACTH, and LHRH in anorexia and bulimia nervosa patients.

**2010**
In search of the missing link in the regulation of appetite and body weight.

**2011**
The putative role of neuropeptide autoantibodies in anorexia nervosa.

**2014**
Regulation of food intake and anxiety by α-MSH reactive autoantibodies.

**2016**
The new link between gut-brain axis and neuropsychiatric disorders.

**2018**
Gut Commensal E.coli Proteins Activate Host Satiety Pathways Following Nutrient-Induced Bacterial Growth

**2018**
Role of the gut microbiota in host appetite control: bacterial growth to animal feeding behaviour

**2019**
Involvement of gut bacteria in appetite control.

**2020**
Bacterial protein mimetic of peptide hormone as a new class of protein-based drugs.

**2022**
Probiotics & Postbiotics for appetite dysfunction
The concept of molecular mimicry
ClpB / $\alpha$-MSH Molecular mimicry

- Strong sequence homology between an exposed loop on the surface of ClpB and $\alpha$-MSH\textsuperscript{1}
- Confirmation in HRM Mass Spectrometry after immunoprecipitation of enterobacteria proteins with $\alpha$-MSH antibody\textsuperscript{2}
- ClpB is a MCR agonist\textsuperscript{3}

\textbf{ClpB is the only protein with $\alpha$-MSH aa pattern}

\textbf{ClpB is one of the most abundant proteins amongst candidates}

1. Tennoune et al., Transl Psy, 2014
2. Internal report from Biognosys, 2018
A new model for gut-brain appetite regulation
Preclinical POC

ProbioSatys®

Key technology benefits

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

Fetissov, Nature reviews Endocrinology, 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**
  - Exp. Phase (a)
  - Stat. Phase (b)

- **ClpB Concentration**
  - Primary cultures
  - Rat colon mucosa

- **Proteomics**
  - Manon Dominique’s PhD thesis data (unpublished)

- **Rat colon infusion**
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

ANOVA, Tukey’s post-test, $p<0.05$, $$$p<0.001
First identified in 1954 (Moller)

1960-1990: *H. alvei* is widely found in raw milk cheese (Camembert up to $10^8$ CFU/g)\(^1\)

1990’s: Listeria contamination in raw milk cheeses

⇒ milk pasteurisation and loss of the traditional cheese taste

⇒ *Hafnia alvei* is used as a lactic ferment to bring back cheese taste\(^1\)

1998: Pr. Grimont safety review, *H. alvei* is non-pathogenic\(^1\)

**TargEDys**: *H. alvei* is a commensal bacteria, and *H. alvei* 4597 is naturally found in human microbiota (MetaHIT)\(^2\)

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**About Hafnia alvei**

(INRA reports for TargEDys)

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

Gram-negative rod-shaped bacillus
Facultatively anaerobic

**Hafnia alvei** 4597
(raw milk cheese origin)

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**Long term scientific knowledge, well characterised and safe *H. alvei* strains**

*H. alvei* strains are also found in traditional fermented food (kimchi, chorizo, etc.) and also used for bioprotection of meat against STEC for example.
Treatment with *Hafnia alvei* decreases food intake and induces a significant decrease in body weight gain as compared to untreated obese controls.

Two-way ANOVA, Bonferronni post-test, **p<0.01; *p<0.05
In addition to body weight, *Hafnia alvei* improves glycemia, OGTT and hepatic markers.
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** has 40% of the effect of Orlistat on **body weight** treat & no side effects

**Hafnia alvei** reduces food intake while Orlistat increases it
**Hafnia alvei** vs Orlistat

**Hybrid Model HF-HSD – ob/ob**

**OGTT results**

**Fasting and basal glycemia**

*Hafnia alvei* tends to improve glucose tolerance while Orlistat worsens it

*Hafnia alvei* reduces basal glycemia while Orlistat increases fasting glycemia
Ongoing Clinical Trial

- 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
Exploratory toxicity study done at CITOXLAB, Denmark
- 32 rats (16 males/16 females) – 3 groups
- 14 days + 8 days recovery for 8 animals
- Daily gavage up to 4E11 CFU – 1,4E12 cells
- Behaviour and histopathology observations

In conclusion, 2 week - daily administration of ProbioSatys® 4E11 CFU – 1,4E12 cells / day by oral route to rats did not cause any treatment related side effects or histopathologic changes.
H. alvei 4597 resists to digestion under fed conditions (not sensitive to bile acids)

H. alvei 4597 is able to adhere to the mucosa

H. alvei 4597 growth and is metabolically active in the colon (pH decreases)
ClpB protein-related product characterizations

Strain

- Petri dish cell count (CFU)
- Flow Cytometry method
  - Total biomass including cultivable, intermediate and dead cells

Protein and fragments characterization

- ELISA method to quantify the α-MSH mimetic protein area
- Western Blot method to quantify ClpB and fragments
EnteroSatys Probiotic 2.0 now going to market in Europe
Clinical data will boost sales 1 year after market introduction
TargEDys continues to develop:
  • Development of the Therapeutic version of EnteroSays
  • Discovery of new mimetic approaches in the gut-brain axis

We look for
  • Investors for our series A1
  • Partners / Licensees for EnteroSatys in USA, Asia and other territories
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MICROBIOME
Scientific
INNOVATIVE
NATURAL
Proven
Effective
PHYSIOLOGICAL