Beyond the gut: Next generation probiotics for Cardiovascular Health \textit{Lactobacillus plantarum (LP}_{LDL}^{\textregistered})}

Stephen OHara  
CEO and Founder
Cardiovascular disease is a global health concern

Number of deaths, World - 2017

<table>
<thead>
<tr>
<th>Condition</th>
<th>Deaths (Thousands)</th>
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<tbody>
<tr>
<td>Cardiovascular disease</td>
<td>17,790.00</td>
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<tr>
<td>Respiratory diseases</td>
<td>9,560.00</td>
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<tr>
<td>Dementia</td>
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<td>Neonatal disorders</td>
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<td>Diabetes</td>
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<td>Road injuries</td>
<td>1,780.00</td>
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<td>HIV/AIDS</td>
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<td>Malaria</td>
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<td>Parkinson disease</td>
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<td>Poisonings</td>
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<td>Terrorism</td>
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<tr>
<td>Natural Disasters</td>
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<td>193.64</td>
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<td>53.35</td>
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<td></td>
<td>26.45</td>
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<tr>
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<td>9.60</td>
</tr>
</tbody>
</table>

Source: IHME; Global burden of disease
Cardiovascular disease is a major cause of deaths in the USA.

18 million people in the U.S. need additional support managing their cholesterol:
- 9.6 million not taking medication, primarily due to tolerability concerns about statins
- 8.7 million taking medication (statins) and not meeting their cholesterol-lowering goals

Globally, at least one-third of patients stop taking statins within one year of starting.

‘There is an unmet market need for a safe and effective product which reduces cholesterol and has no known side effects’

Source: CDC National Vital Statistics Reports Volume 70, Number 9 July 26, 2021 Deaths: Leading Causes for 2019
A systematic and pharma-based approach to strain rationale, identification and selection

- **Biomarker**
  - Target biomarker
  - Cholesterol

- **Mechanism**
  - Target Mechanism of Action
  - MoA: Bile Salt Hydrolase

- **Screening**
  - High throughput screening
  - 4,000 strains for BSH activity
  - 353 strains (all Lactobacilli)

- **Screening**
  - Quantitative determination BSH
  - In vitro cholesterol reduction
  - 3 strains met criteria

- **Pilot scale**
  - Gut survival
  - Bacterial growth optimisation, freeze dry effects, pilot scale production
  - Final Selection \( \text{LP}_{\text{LDL}} \)

- **Human Studies**
  - Human intervention studies \( \text{LP}_{\text{LDL}} \)
  - Randomized, dbl-blind, placebo-controlled, parallel-group trials

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Better Science, Better Health

optibiotix.com
Pre Clinical Studies on LPL_{LDL}®
High Throughput Screening Strain selection Criteria

• 4000 strains were identified and evaluated as potential producers of BSH activity across multiple genera
  ✓ Genera/species that were not probiotics or did not have a well documented history of safe human consumption were excluded

• Lactobacillus were selected as the primary target genus as:
  ✓ They are recognised producers of BSH
  ✓ Are established probiotics with a history of safe consumption with multiple health benefits

• Selected a broad range of species within the Lactobacillus genus

• Chosen strains had highest level of BSH activity, good cholesterol reduction activity, good gut survival, and good manufacturing characteristics in pre clinical studies
$\text{LP}_{\text{LDL}} \text{ BSH activity in pure culture}$

<table>
<thead>
<tr>
<th></th>
<th>Glycocholate</th>
<th>Glycodeoxycholate</th>
<th>Taurocholate</th>
<th>Taurodeoxycholate</th>
</tr>
</thead>
<tbody>
<tr>
<td>$L. \text{ fermentum} \ 30226$</td>
<td>2</td>
<td>100</td>
<td>200</td>
<td>300</td>
</tr>
<tr>
<td>$L. \text{ fermentum} \ 11976$</td>
<td>2</td>
<td>100</td>
<td>200</td>
<td>300</td>
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<tr>
<td>$L. \text{ casei} \ 30185$</td>
<td>2</td>
<td>100</td>
<td>200</td>
<td>300</td>
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<tr>
<td>$L. \text{ plantarum} \ 30187$</td>
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<td>100</td>
<td>200</td>
<td>300</td>
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<tr>
<td>$L. \text{ rhamnosus} \ 30224$</td>
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<td>100</td>
<td>200</td>
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<tr>
<td>$L. \text{ salivarius} \ 30188$</td>
<td>2</td>
<td>100</td>
<td>200</td>
<td>300</td>
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<tr>
<td>$L. \text{ delbrueckii} \ 11741$</td>
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<td>$L. \text{ acidophilus} \ 30184$</td>
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<td>100</td>
<td>200</td>
<td>300</td>
</tr>
</tbody>
</table>
LP_{LDL}® cholesterol removal activity in pure culture

![Graph showing cholesterol removal activity in different bacterial strains.]

- L. fermentum 30226
- L. fermentum 11976
- L. casei 30185
- L. plantarum 30187
- L. helveticus 30224
- L. rhamnosus 30188
- L. salivarius 11741
- L. delbrueckii 30186
- L. salivarius 30225

% Cholesterol removal

- 0h
- 8h
- 12h
- 24h

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LP_{LDL}\textsuperscript{®} activity in faecal batch culture

- Comparable findings to pure cultures
Human intervention studies on LP_{LDL}®
I. LP_{LDL}® in normal to mildly hypercholesterolemic adults: Study design

Single-centre, randomised, double-blind, placebo-controlled, parallel human study carried out at the University of Reading, UK

49 male & female subjects, 30–65 years, with normal to mild hypercholesterolemia (TC≤6mM)

- Duration: 12 weeks + 4-week washout
- Dose: 4x10^9 cfu LP_{LDL}®/day encapsulated
- Participants advised not to change their regular diet throughout the trial period.
- No other cholesterol management medication, pre/pro/synbiotics allowed
1. Human trial $LPLDL^\text{®}$ in normal to mildly hypercholesterolemic adults:

49 male & female subjects, 30–65 years mildly hypercholesterolemic

Statistically significant reductions after 12 weeks $LPLDL^\text{®}$ vs placebo

- Blood Pressure: -5.1%
- HDL: +4.5%
- LDL: -13.9%
- Total Cholesterol: -36.7%
II. \( \text{LP}_{\text{LDL}} \) in hypercholesterolemic adults: Results

16 male & female subjects, 30–65 years, with hypercholesterolemia (TC>6mM)

Statistically significant reductions after 3 & 6 weeks \( \text{LP}_{\text{LDL}} \) vs placebo

- TC: -34.6%
- LDL-C: -26.4%
- Non-HDL-C: -26.4%
- ApoB: -28.6%

6 weeks

% Change
Comparison to existing treatments

Statistically significant reductions (P<0.05) as early as 3 and 6 weeks of LP\textsubscript{LDL} \textsuperscript{®} intake in:

- Total cholesterol (TC) (P\textsubscript{6w}= 0.001)
- LDL-C (P\textsubscript{6w} = 0.001)
- Non-HDL-C (P\textsubscript{6w} = 0.001)
- ApoB (P\textsubscript{6w} = 0.008)

<table>
<thead>
<tr>
<th>Reductions</th>
<th>LP\textsubscript{LDL} \textsuperscript{®} \textsuperscript{1} (6weeks)</th>
<th>Bempedoic acid\textsuperscript{2} (12 weeks)</th>
<th>Lipitor (2.5mg)\textsuperscript{3} (6weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>34.6%</td>
<td>9.9%</td>
<td>17.3%</td>
</tr>
<tr>
<td>LDL-C</td>
<td>26.4%</td>
<td>15.1%</td>
<td>25%</td>
</tr>
<tr>
<td>Non HDL-C</td>
<td>17.6%</td>
<td>10.8%</td>
<td>NA</td>
</tr>
<tr>
<td>ApoB</td>
<td>28.6%</td>
<td>9.3%</td>
<td>16%</td>
</tr>
</tbody>
</table>

2. CLEAR Wisdom Trial 2019
3. https://www.ahajournals.org/doi/full/10.1161/01.atv.15.5.678
4. You need 30-40g of Benecol per day for a 7-10% reduction in TC
III. CholBiome BP® for blood pressure & cholesterol management: Study design

• Single centre, open label study carried out at the University of Pavia, IT

40 adults with high normal blood pressure  (pre-hypertension: SBP 130-139 mmHg and/or DBP 85-89 mmHg)

• Duration: 12 weeks

• Formulation:
  - \( \text{LP}_{\text{LDL}} ^\circ : 4 \times 10^9 \text{ cfu/d} \)
  - Arginine & Vitamin B1
  - Coenzyme Q10

Triple layer tablet to support stability, survivability & effectiveness
III. CholBiome® BP for blood pressure & cholesterol management

Significant reduction:
- systolic & diastolic blood pressure
- total cholesterol (TC)
- LDL-C

% Change after 12 weeks of treatment

- SBP: -8.5%
- DBP: -6.6%
- TC: -3.4%
- LDL-C: -3.0%

Derosa et al., 2020
CholBiome®x3 consumer study 2021

- **TOTAL CHOLESTEROL**
  - Reduced 96%
  - No change 4%

- **LDL-C**
  - Reduced 88%
  - I do not know 8%
  - No change 4%

- **HDL-C**
  - Increased 58%
  - do not know 29%
  - No change 13%

Source: Consumer survey September 2021
A patented probiotic CVD portfolio with human studies

LDL-Cholesterol reduction
Blood pressure reduction
Artery Plaque reduction
## Science, Industry, and Retail Awards and Nominations

<table>
<thead>
<tr>
<th>Category</th>
<th>Award</th>
<th>Year</th>
<th>Event</th>
<th>Winner/Finalist</th>
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</thead>
<tbody>
<tr>
<td>Best Scientific Abstract</td>
<td>Probiota 2018 &amp; 2019</td>
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<td>Winner</td>
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<tr>
<td>Probiotic Product of the Year</td>
<td>Nutra-Ingredients Europe 2018</td>
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<td>Finalist</td>
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<td>Editors Choice Award DS1</td>
<td>Supply Side West 2018</td>
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<td>Finalist</td>
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<tr>
<td>Best Nutraceutical Product of the Year</td>
<td>Food Matters Live 2018</td>
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<td>Best Nutraceutical Ingredient &amp; Product of the Year</td>
<td>Food Matters Live 2018 &amp; 2019</td>
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<td>Finalist</td>
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<td>Best Probiotic Product of the Year</td>
<td>Nutra-Ingredients Europe 2019</td>
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<td>Editors Award Functional Food Innovation Product of the Year</td>
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<td>ProBiotic Product of the Year</td>
<td>Nutra-Ingredients Asia 2021</td>
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<td>Finalist</td>
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</tbody>
</table>

*OptiBiotix*<sup>®</sup> HEALTH PLC  
**Better Science, Better Health**  
optibiotix.com
Targeted prebiotic development for cholesterol reduction: LPGOS

Aim: Synthesise a synergistic synbiotic for LP_{LDL} (LPGOS) using reverse enzyme that selectively enhances its growth and cholesterol reducing activity i.e a targeted prebiotic
LPGOS selectively increases Lactobacilli but not Bifidobacteria numbers

pH-controlled faecal batch cultures

LPGOS significantly increases \textit{Lactobacillus} compared to LPGOS alone

Use of LPGOS+LPLDL\(^{\circledast}\) significantly increases \textit{Lactobacillus} compared to LPGOS alone

\textit{Bifidobacterium} derived GOS (BGOS) has no impact on \textit{Lactobacillus}
LPGOS selectively increases bile salt hydrolysis

pH-controlled faecal batch cultures

<table>
<thead>
<tr>
<th></th>
<th>Control 0h</th>
<th>Control 24h</th>
<th>LPLDL® 0h</th>
<th>LPGOS 24h</th>
<th>LPLDL®+LPGOS 24h</th>
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</thead>
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<tr>
<td>Glycolate</td>
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<td>40</td>
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<td>Taurocholate</td>
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<td>Taurodeoxycholate</td>
<td>10</td>
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<td>40</td>
<td>50</td>
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</table>
LPGOS significantly enhances cholesterol reduction at pH-controlled faecal batch cultures.
Summary

- A systematic approach to strain rationale, identification and selection identified a strain of \textit{Lactobacillus} plantarum (LP_{LDL}) with the potential to reduce cholesterol.
- Human studies support in vitro findings showing significant reductions in a range of cardiovascular risk markers (Total cholesterol, LDL cholesterol, and Apo B) with no safety or tolerance issues.
- Customer post market surveillance provides further evidence of safety, tolerance and efficacy in every day use.
- A reverse enzyme approach has been used to create a highly selective prebiotic which:
  - Selectively increases Lactobacillus numbers
  - Enhances bile salt hydrolase activity
  - Enhances LPDLs ability to reduce cholesterol
- This creates the potential to:
  - Create next generation targeted prebiotics which selectively enhance the growth and effect of existing probiotics
  - Precision engineer the microbiome by creating products which selectively enhance the growth of specific bacteria (e.g. Roseburia, Propionibacteria, Faecalibacteria) in the gut to prevent, manage and treat disease.
Acknowledgments

Thank you for listening

Prof Bob Rastall
Prof Glenn Gibson
Prof Kim Watson
Irene Feliciotti (PhD candidate)

Dr Adele Costabile

Dr Oswaldo Hernandez