Human Leptospirosis Vaccine Development Through Animal Health Product Development

Luna Bioscience, Inc.

Genomics-Driven Solutions for Infectious Diseases
Leptospirosis: Devastating Human Health Threat, No Human Vaccine

- A zoonotic disease, transmitted to humans through the environment (infected animals shed leptospires via urine)
- Caused by many different bacterial spirochetes of genus, _Leptospira_

1 Million infected 60,000 Dead 5-20% 45.7%
- Human infected globally, many remain undetected
- Annual global deaths due to complications
- Fatality rate
- Rodents as pathogenic carriers on USVI

Current Treatments

**Vaccines**
- **Bacterin** (current products)
  - Limited to selected serovars
  - Safety Concerns
- **Outer-envelope (not proven)**
  - Better safety profile
  - Serogroup-specific protection

**Antibiotics**
- Only Curative Treatment
- Potential for antibiotics resistance

**Supportive Care**
- For severe human cases

Recombinant protein
- Luna Bio has proof-of-principle
- Antigenically conserved
- Improved cross-protective immunity

IVI’s letter to FDA and WHO to urge adding Leptospirosis to the current list of tropical diseases.

March 27, 2022
US Food and Drug Administration
Re: Docket No. FDA-2008-N-0567-0148, Adding Leptospirosis to the Current List of Tropical Diseases in the Federal Food, Drug and Cosmetic Act

5-20% Fatality rate
60,000 Dead Annual global deaths due to complications
1 Million infected Human infected globally, many remain undetected
45.7% Rodents as pathogenic carriers on USVI
Pathway to Human Vaccine is through *Luna Bio’s* Animal Vaccine: Competing Products are Only for Animals, No Vaccine for Humans

Animal Vaccines Use 1960s Technology and are Deficient & Expensive

**Major Market Competitors**
- MERCK
- Boehringer Ingelheim
- Elanco
- Zoetis

**Minimal Product Differentiation**
Competitors share highly similar product profiles.

**Lack of Innovation**
Licensed inactivated vaccines against canine leptospirosis have been on the market since the 1960s, which remain the main product type.

**Insufficient Efficacy**
Does not prevent the spread of disease, short-term protection.

**Limited Coverage**
Few disease-causing serovars are included in any existing products, which limits vaccine efficacy.

**Current products expensive to manufacture; margins low**
Our Animal Vaccine is Better: A Pan-Leptospirosis Vaccine Designed for Longer Protection (ask me for details)

<table>
<thead>
<tr>
<th></th>
<th>Coverage</th>
<th>Protection Time</th>
<th>Current Products</th>
<th>Market Players</th>
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<tbody>
<tr>
<td>Canine</td>
<td>2 - 4 serovars</td>
<td>6-12 months</td>
<td>6 Lepto serovar-specific vaccines</td>
<td>MERCK Boehringer Ingelheim Elanco</td>
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<td>10 combo vaccines</td>
<td>durvet zoetis</td>
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<td></td>
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<td>10 combo vaccines</td>
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<tr>
<td>Bovine</td>
<td>1 - 5 serovars</td>
<td>6-12 months</td>
<td>3 Lepto serovar-specific vaccines</td>
<td>MERCK Boehringer Ingelheim Elanco</td>
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<td>Swine</td>
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<td>1 Lepto serovar-specific vaccine</td>
<td>MERCK Elanco</td>
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<td>2 combo vaccines</td>
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<tr>
<td>Equine</td>
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<td>1 Lepto serovar-specific vaccine</td>
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<tr>
<td>Luna Bio</td>
<td>Covers all</td>
<td>&gt;&gt; 1 year</td>
<td>Applicable to canine, equine, livestock and wild</td>
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<tr>
<td></td>
<td>Lepto serovars</td>
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<td>animals;</td>
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Non-confidential
$6 Billion Global Market (TAM) for Animal Vaccine

Very Conservative Estimation – Total SOM for Luna Bio:
~$0.8 Billion (2022) and ~$1.2 Billion (2028)
Majority in North America and EU

- **Canine Global TAM**
  - NA+EU+BRIC: 93%
  - $2.4 Billion
  - Very Conservative Estimation
  - Total SOM for Luna Bio: ~$0.8 Billion (2022) and ~$1.2 Billion (2028)
- **Swine Global TAM**
  - NA+EU+BRIC: 98%
  - $847 Million
  - Major in North America and EU
- **Bovine Global TAM**
  - NA+EU+BRIC: 89%
  - $788 Million
  - Majority in North America and EU
- **Equine Global TAM**
  - NA+EU+BRIC: 83%
  - $2.0 Billion
  - 2022 TAM SAM SOM

*Current market pricing used for all estimates
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Top Animal Vaccine Companies Eager to Partner with Us

Value to Top Players

- **Cost-saving** in manufacturing
- **Product differentiation** (for market lead)
- **Broad** coverage for major animals

Currently Active Traction

- MTA negotiation and development conversations with 3 of the top 5 market players
- In diligence process with top animal health accelerator
Conserved Antigen(s) Identified in Pathogenic \emph{Leptospira} ($15$ million NIH funding to date): IP Protected (Yale)

All \emph{Leptospira} species: 9 known pathogens, 5 intermediates, and 6 saprophytes

Total of 438 strains, combo of 454 and Illumina

$>$12 countries and organizations

Global \emph{Leptospira} Genome Project

Key Discovery protected by patents
- VM proteins Ricin B-type lectins, known MOA
- \emph{L. interrogans}, most important leptospiral pathogen; cross-\emph{Leptospira spp.} anti-VM protein Ab reactivity

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Vaccination with Full-Length VM Protein-Based Antigen(s) Protects Mice from Infection and Death

- Pan-leptospirosis protection in mice
- Protection across all medically important *Leptospira*

![Graph showing body weight change over time](image)

*Adjuvant: GLA-SE, Glucopyranosyl lipid adjuvant–stable emulsion*

**A:**
- G-I
- G-II
- G-III
- G-IV

**B:**
- Liver
  - Log of CFU/g tissue
  - **p = 0.0060**
  - *p = 0.0210*

**C:**
- Kidney
  - Log of CFU/g tissue
  - ****p < 0.0001****
  - **p = 0.0054**
  - ns

Chaurasia et al, Frontiers, 2022
Low-Risk, Clear USDA Approval Pathway for Animal Vaccines: First Product to Market in 2-3 Years

1. Dog
2. Bovine Equine
3. Swine

- **Meeting with USDA Licensing Reviewer**
  - Identify regulatory consultant

- **Discuss Pivotal Study Design with USDA**
  - Prior to implementing pivotal study while conducting hamster potency study

- **US Veterinary Biologics Product License + Establishment License**
  - Work with Diamond Animal Health for Product License approval
    - Identify and work with large distributors and manufacturers for Establishment License

- **Work with Regulatory Consultant on International Approvals**
  - EU, CAN, BRIC countries
World-Leading Team Expertise in Animal and Human Business Development and Vaccine Science

Management Team

Richard Squires, DVM, PhD
Assoc. Prof., James Cook University Chair, World Small Animal Veterinary Assn Vaccination Guidelines Committee (Animal vaccine)

Richard Marconi, PhD
Professor, VCU. Inventor of canine Lyme vaccine (Animal vaccine)

Paul Dick, DVM
President and Partner Paul Dick Associates and Vet Venture Capital

Jerome Kim, MD
Director General International Vaccine Institute (Human vaccine)

Carla Devillers, MBA
CEO and Co-Founder Wharton MBA in finance Experience in operations

Joseph Vinetz, MD
Co-Founder Professor of Medicine Yale School of Medicine

Yi Wang, PhD, MBA
Entrepreneurial Fellow, Yale Ventures VP of Business Development, Isolere Bio Angel Investor

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Genomics-Driven Platform: De-Risked Portfolio w/ Differentiated Products & Fast, Separate Return

- **Vaccine for Dogs & Livestock**
  - Composition of Matter: 2 PCTs converted (Aug 2022)
  - Global, exclusive license from Yale
  - High fidelity of POC + Low regulatory risk

- **Oral Delivery to Eliminate Reservoir in Wild Animals**
  - ~$10 Billion Global Market
  - Composition of Matter: 1 provisional filed
  - Proven success in oral Rabies vaccines delivery to raccoons
  - Proven govt purchasers and distributors (US+EU+CAN)

- **Two Independent, Sequential Exits**
  - Investors of animal products can exit before fundraising and development for human product begin
  - Accelerated path to market: human product development will leverage off of animal data
Blavatnik Funding Enables Advancement to Key Inflection Point: Target Animal (Dog) Pivotal Clinical Trial

Current stage
• Small-scale POC: hamster challenge study

Use of Blavatnik Funds ($300K)
• PHASE I: Pilot lot production of antigen (Lonza) ($100K)
  • Outcome: optimized antigen, delivery system
• PHASE II: Comprehensive hamster study (Diamond CDMO) ($200K)
  • Outcome: PoC ➔ USDA regulatory pathway
  • Direct to target animal vaccine trials
  • Enables long-term manufacturing, regulatory relationships
• Phase I and II lead to higher valuation; required by top animal health companies for corporate partnership/deals
• Human vaccine a goal; we have a plan

Solid IP Firewalls; Patents Pending
• Composition of Matter (program for animal vaccine): 2 PCTs converted (Aug 2022)
• Composition of Matter (program for eliminating reservoir in wild animals: 1 provisional filed (Oct 2022)
Supplementary Data
Animal health industry has major interests in an alternative to bacterin-based leptospirosis vaccines:

“We agree that current bacterin-based leptospirosis vaccines can be improved. The animal health industry could benefit from having a recombinant protein or other protein-delivery-based leptospirosis platform that would be active against multiple strains/serovars and species of *Leptospirosis*. These leptospiral strains are of substantial human health importance as well. A recombinant protein, subunit-based pan-leptospirosis vaccine that is safe, effective and can be produced at an acceptable cost could replace at least in part the current range of bacterin vaccines.”
Mechanistic explanation for advantage of recombinant protein-based leptospirosis vaccine over bacterins*

<table>
<thead>
<tr>
<th>Bacterins (whole killed cell vaccine)</th>
<th>Recombinant protein/DNA/mRNA-based vaccine delivery</th>
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<tbody>
<tr>
<td>T-independent antigen (LPS-carbohydrate)</td>
<td>T-dependent antigen</td>
</tr>
<tr>
<td>- No immunological memory</td>
<td>- Memory T and B cells</td>
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<tr>
<td>- Low affinity/avidity antibodies</td>
<td>- High affinity/avidity antibodies</td>
</tr>
<tr>
<td>- Short-term vaccine efficacy</td>
<td>- Longer-term vaccine efficacy</td>
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*Precedents
- Current carbohydrate-based bacterial vaccines, such as Pneumococcal, Meningococcal vaccines
- Conjugate vaccines attract enormous capital investment because of their immunological characteristics
MOA Demonstrated

Cell surface binding

Recombinant Antigen (VM protein) are cytotoxins

Trafficking to nucleus

C-terminal DNase activity

Chaurasia et al, Frontiers, 2022
Vaccine for Dogs & Livestock

- Composition of Matter: 2 PCTs converted (Aug 2022)
- Global, exclusive license from Yale
- High fidelity of POC + Low regulatory risk

Current Traction

- MTA negotiation and development conversations with 3 of the top 5 market players
- Diligence process with top animal health accelerator
- Eligible for Priority Review Voucher if leptospirosis is listed as a tropical disease

Differentiated Portfolio (2-3 years to market)

Technical Barrier

Expanded Market

Traction

Leverage

Oral Delivery to Eliminate Reservoir in Wild Animals

- $15 Million Existing Market; will expand on demand
- Composition of Matter: 1 provisional file
- Proven success in oral Rabies vaccine delivery to wolves, foxes, coyotes, raccoons
- Proven government purchasers and distributors (US+EU+CAN)

Two Independent, Sequential Exits

- Investors of animal products can exit before fundraising and development for human product begin
- Accelerated path to market: human product development will leverage past animal data
Variable presence of 11 mutated pathogen/intermediate *Leptospira*-specific coding genes throughout genus

<table>
<thead>
<tr>
<th>Protein</th>
<th>Accession</th>
<th>Function</th>
<th>P19 Mutation</th>
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<tbody>
<tr>
<td>LA_4008</td>
<td>NP_714188.1</td>
<td>adenylate/guanylate cyclase</td>
<td>L46F</td>
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<td>LA_3777</td>
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<td>LA_1056</td>
<td>NP_711237.2</td>
<td>hypothetical protein</td>
<td>G401A</td>
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<td>NP_711714.2</td>
<td>thymidylate synthase</td>
<td>FRAMESHIFT</td>
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<td>LA_1568</td>
<td>NP_711749.1</td>
<td>beta-propeller repeat protein</td>
<td>N16T</td>
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<td>LA_1594</td>
<td>NP_711775.1</td>
<td>methyltransferase</td>
<td>F382L</td>
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<td>LA_1765</td>
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<td>cytoplasmic membrane protein</td>
<td>K2039Q; V2040F</td>
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<td>LA_3388</td>
<td>NP_713568.1</td>
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<td>LA_3490</td>
<td>NP_713670.1</td>
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<td>Y434D</td>
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Group 1 *Leptospira* (P), notably *L. interrogans*, have PF07598 paralog expansion.

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<td>LA_3271</td>
<td>NP_713451.1</td>
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</table>
Acquisition and Expansion of VM Protein Family Corresponds with *Leptospira* Virulence
Initial Structural Modeling Searches of VM Proteins
Suggested N-terminal Ricin B chain-like Lectin Domain; Secretory Signal Peptides

**Ricin A chain**: (toxin) N-glycoside hydrolase, inactivates ribosomes

**Ricin B chain**: (homing domain) binds terminal galactoses on glycoproteins on mammalian cell surfaces

http://www.sbg.bio.ic.ac.uk/phyre2/
Leptospirosis pathogenesis:

1. Cellular dysfunction by local effect;
2. Shock by affecting systemic endothelial cells
LAL negative, Endotoxin-free
Cytopathic effect of recombinant VM protein, LA3490, on HeLa cells.

Time lapse over 5 hr compressed to 11 sec
Cytopathic effect of recombinant VM protein, LA3490, on HeLa cells. (endotoxin-free)

Live/dead staining
Full length LA3490 traffics to nucleus
VM proteins have C-terminal DNase activity
Is leptospirosis pathogenesis a “vasculitis,” an endotheliopathy, or more…?
Effect of Recombinant LA3490 on Human Primary Pulmonary Endothelial Cell Barrier Function

TEER = Transendothelial electrical potential (continuous)

Pierce, Chaurasia, Pober, Vinetz, unpublished
## Treatment with t3490

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<th>DAPI</th>
<th>t3490-mCherry</th>
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<th>VE-Cadherin</th>
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Vaccination with Full-Length Lai-derived VM Protein-Based Antigen(s) Protects Mice from Canicola Infection and Death

C3H-HeJ Mice, Challenge $10^5$ low passage L. interrogans sv Canicola

Chaurasia et al, Frontiers, In press, 2022
Summary and Conclusions

• Clinical, epidemiological and environmental studies indicate that *L. interrogans* is the most important leptospiral pathogen and causes vast majority of deaths
  • More data needed

• PF07598 gene family-encoded VM proteins expanded in *L. interrogans*
  • VM proteins are cytotoxins, including on primary pulmonary endothelial cells
  • MOAs include cell surface binding, trafficking to nucleus, DNase activity

• Biologically plausible to develop serovar-independent vaccine
  • For animals
  • For humans