mRNA hub meeting
Cape Town, April-2023
Argentina in the world

Buenos Aires
34º 36´

Cape Town
33º 55´
Global South
Our History: Sinergium Biotech S.A.

Biggest pharma lab in Argentina

Larger FMD veterinarian vaccine in the World

Cuidamos la salud, preservamos la vida
Biotech Hub: 20,000 sqm, 1,500 specialized employees, three companies.
### Sinergium Biotech in numbers

<table>
<thead>
<tr>
<th>Category</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Million doses produced</td>
<td>200</td>
</tr>
<tr>
<td>Batches manufactured</td>
<td>&gt;700</td>
</tr>
<tr>
<td>Rejected batches</td>
<td>0</td>
</tr>
<tr>
<td>Direct employees</td>
<td>350</td>
</tr>
<tr>
<td>Indirect employees</td>
<td>150</td>
</tr>
<tr>
<td>Women in our staff</td>
<td>45%</td>
</tr>
<tr>
<td>Agreements with multinational pharma companies</td>
<td>7</td>
</tr>
<tr>
<td>Successful tech-transfers</td>
<td>8</td>
</tr>
<tr>
<td>Public-private R&amp;D projects</td>
<td>6</td>
</tr>
</tbody>
</table>

*Cuidamos la salud, preservamos la vida*
Vaccines Portfolio

**VIRAFLU – 4FLU**
Inactivated Seasonal Trivalent and Quadrivalent influenza

**FLUXVIR**
Inactivated Seasonal Trivalent Influenza with adjuvant MF59C.1

**PREVENAR 13**
13-Valent Pneumococcal Vaccine
Vaccines Portfolio

MSD

SILGARD VPH
Human Papillomavirus (HPV) Vaccine
Type 6, 11, 16, 18

CARCIVAC
Onco BCG Vaccine Bacillus Calmette Guerin

SINOVAC
VAXIPAT
Inactivated Hepatitis A Vaccine
# mAbs Portfolio

## mAbxience

**NOVEX (Rituximab)**
- 100 mg
- 500 mg

**LUMIERE (Bevocizumab)**
- 0.2 ml with 5 mg Bevacizumab

**BEBAX (Bevocizumab)**
- 100 mg
- 400 mg

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Cuidamos la salud, preservamos la vida
Manufacturing site
The Production Site
Strong know-how in formulation and filling

Pre-filled syringes line
- Capacity for **50 million doses**
- 60% available capacity (30 million doses)

Vials line
- Capacity for **400 million doses** (estimated for 10-dose vials)
- Project under execution – 100% available for future projects from 2024

Site and industrial area
- Biotech Hub (Sinergium, Biogenesis Bago, Mabxience)
- 1,500 employees in the area
- 20,000 sq meters (only Sinergium site)

Antigen production
- Mammalian/insect cells capacity
- Adenovirus production
- **24,000 L** biorreactor capacity
The Production Site
Formulation areas - Grade C

- Fixed stainless Steel 316 AISI tank - Manufacturer: GEA Diessel GmbH (Germany)
  - 400 Liter capacity

- Mobile stainless steel 316 AISI tank - Manufacturer: GEA Diessel GmbH (Germany)
  - 200 Liter capacity

- Expertise in single use technology for vaccines & mABS

- Double jacket / Rupture disk included / Vesta Sterile Valve
  GEA / Temperature sensor / Preassure sensor

- Filter Integrity test equipment: Millipore IT5 / Biowelder and Biosealers: Sartorius

- Automatic CIP/SIP and formulation récipe : Simatic Batch (SIEMENS)
Quality Control

- HPLC/UPLC
- Capillary Electrophoresis
- UV
- QPCR
- Osmometer
- TOC
- Cell Bioassay Lab. (BSC, Incubators, Plate readers)
- Full Microbiology Lab. (BSC, Strain identification ViTek, particle counter)
- Sterility Test Area
- Hygienic control Area
- Physicochemical / Raw Material Lab. (pH meter, Conductivity meter, IR)
- Stability Areas (5±3°C / 25±5°C / -15±5°C)
Process development team

- 2 R&D centers, 1 dedicated tech-transfer team for new products
- 27 dedicated people
- AMBR High Throughput Microbioreactors Technology.
- 1 to 10L Bench Scale reactors.
- ATF Systems
- TFF, Chromatography and Filtration Scale Down Technology.
- Independent Analytical capacity
Sinergium Strategic Vision

Our objectives

• Foster strong long-term partnerships.

• Expand our reach globally.

• Expand our portfolio.
  New partnerships.
  New products.

• Develop new products under different platforms
  • Projects ongoing:
    • Baculovirus recombinant platform
    • mRNA platform
Advances in mRNA platform Development.
Sinergium Biotech in mRNA Program: Key events

- **Jul-2021**: mRNA hub lunch (Afrigen-Biovac-WHO-MPP)
- **Dec-2021**: Kick off Tech transfer Meeting-WHO-PAHO
- **Aug-2021**: WHO-EOI for mRNA technology
- **Sept-2021**: Sinergium Selected as a “Partner”
- **Mar-2022**: Training in RNA tech at UBA
- **Mar-2022**: Training on Afrigen
- **Apr-2022**: Starts uL tests. mRNA for research grade
- **Feb-2023**: WHO-PAHO-MPP visit to Argentina
- **Apr-2023**: Package 1a
Sinergium Biotech in mRNA Program: Platform technologies

1st generation mRNA technology – focusing on being able to demonstrate region capabilities to developed mRNA vaccines – base on mRNA Technology Transfer HUB Program (WHO/MPP)
  • Traditional Approach

2nd generation mRNA technology – Intensified Continuous manufacturing.

**Flowchart**

1st Platform Generation → Introduction to mRNA Technology training. → Technology Transfer Agreement

2023: Need to define Workplan for Sinergium

2nd Platform Generation → Quantom equipment Assessment

Define a new facility layout for R&D and GMP production → QC & manufacturing Equipment acquisitions Q2-Q3 2023
mRNA Platform development: Process flow (mL scale)

**Analytical Test**
- Integrity test agarose gel
- RNA concentration HPLC

**Process Flow**
- Linear DNA THAWING - CONDITIONING
- IVT
- Ultrafiltration UF/DF
  - Oligo dt MONOLITH CROMATOGRAPHY (90-95% de rendimiento)
- DENATURATION
- POST TRANSCRIPTIONAL CAPPING
- HIC MONOLITH CROMATOGRAPHY (Polishing mRNA)
- TFF (UF/DF)
- FINAL FILTRATION
- mRNA FREEZING

**Lab Scale**
- (20 & 100µL)
  - pDNA
  - mRNA
  - LNP
- (0.5-1 ml)
  - pDNA
  - mRNA
  - LNP
- (TBD ml)
  - pDNA
  - mRNA
  - LNP

*Cuidamos la salud, preservamos la vida*
mRNA Platform development: IVT optimization

• From kit to bulk reagents: need to optimise cc of reagents in IVT mix (500 µl) to get high yields.

\[ [\text{Mg}^2+] : 6\text{mM to 70mM} \]

\[ [\text{Mg}^2+] = 40\text{mM final (ratio Mg}^2+:\text{NTPs} = 1) \text{ shows the highest yield (\~9.22 \text{ug/uL})} \]
mRNA Platform development: Downstream process

IVT

Oligo dT

Unsuccessful results

IVT

DEAE

Unsuccessful results

IVT

TFF

Oligo dT

Successful results
mRNA Platform development: Downstream process

OligoDT chromatography

➢ Better % Recovery & mRNA shows the predicted size.

<table>
<thead>
<tr>
<th>Chromatography A (load of 500 µL)</th>
<th>% Recovery</th>
<th>% Recovery Eluate + FT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Load A</td>
<td>70</td>
<td>85</td>
</tr>
<tr>
<td>FT A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eluate A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ribonucleic</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chromatography B (load of 1000 µL)</th>
<th>% Recovery</th>
<th>% Recovery Eluate + FT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Load A</td>
<td>61</td>
<td>72</td>
</tr>
<tr>
<td>FT A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eluate A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ribonucleic</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
mRNA Platform development: LNP manual formulation

- Pipetting method
- Formulation Volume= 0,25mL
- N:P= 6
- Aquose phase: Organic phase= 3
- Lipids mix concentration= 12,5mM vs 25mM

<table>
<thead>
<tr>
<th>Lipids mix concentration</th>
<th>12,5mM</th>
<th>25mM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DLS Parameter</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z (nm)</td>
<td>165.3</td>
<td>136.8</td>
</tr>
<tr>
<td>IP</td>
<td>0.084</td>
<td>0.099</td>
</tr>
<tr>
<td><strong>mRNA-LNP</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z (nm)</td>
<td>159.3</td>
<td>132.7</td>
</tr>
<tr>
<td>IP</td>
<td>0.095</td>
<td>0.120</td>
</tr>
<tr>
<td><strong>mRNA-LNP diluted</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z (nm)</td>
<td>165.7</td>
<td>133.6</td>
</tr>
<tr>
<td>IP</td>
<td>0.094</td>
<td>0.099</td>
</tr>
<tr>
<td><strong>mRNA-LNP concentrated</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z (nm)</td>
<td>194.6</td>
<td>181.1</td>
</tr>
<tr>
<td>IP</td>
<td>0.142</td>
<td>0.134</td>
</tr>
</tbody>
</table>

PDI ≤0.1 highly monodisperse
PDI de 0.1-0.4 slightly polydisperse
PSD > 0.4 highly polydisperse

<table>
<thead>
<tr>
<th>Vol. Total (ml)</th>
<th>0,25 ml</th>
<th>Vol. Total (ml)</th>
<th>0,25 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>mRNA-LNP</td>
<td></td>
<td>mRNA-LNP</td>
<td></td>
</tr>
<tr>
<td>mRNA:Etanol volum ratio v/v</td>
<td>3</td>
<td>mRNA:Etanol volum ratio v/v</td>
<td>3</td>
</tr>
<tr>
<td>vol total partes</td>
<td>4</td>
<td>vol total partes</td>
<td>4</td>
</tr>
<tr>
<td>mRNA:total ratio v/v</td>
<td>0,75</td>
<td>mRNA:total ratio v/v</td>
<td>0,75</td>
</tr>
<tr>
<td>Ethanol:total ratio v/v</td>
<td>0,25</td>
<td>Ethanol:total ratio v/v</td>
<td>0,25</td>
</tr>
<tr>
<td>Cc Mix lipidos (mM)</td>
<td>25</td>
<td>Cc Mix lipidos (mM)</td>
<td>12,5</td>
</tr>
<tr>
<td>Volumen de mRNA (ml)</td>
<td>0,188 ml</td>
<td>Volumen de mRNA (ml)</td>
<td>0,188 ml</td>
</tr>
<tr>
<td>Volumen de Etanol o Mix lipidos (ml)</td>
<td>0,063 ml</td>
<td>Volumen de Etanol/ Mix lipidos (ml)</td>
<td>0,063 ml</td>
</tr>
<tr>
<td>Cantidad de lipidos totales en mix (µmol)</td>
<td>1,5625</td>
<td>Cantidad de de lipidos totales en mix (µmol)</td>
<td>0,7813</td>
</tr>
<tr>
<td>N:P ratio (mol a mol)</td>
<td>6</td>
<td>N:P ratio (mol a mol)</td>
<td>6</td>
</tr>
<tr>
<td>mol P necesario</td>
<td>0,13</td>
<td>mol P necesario</td>
<td>0,07</td>
</tr>
<tr>
<td>MW RNMP promedio (g/mol)</td>
<td>321,48</td>
<td>MW RNMP promedio (g/mol)</td>
<td>321,48</td>
</tr>
<tr>
<td>MASA de mRNA para formular (mg)</td>
<td>0,04</td>
<td>MASA de RNA para formular (mg)</td>
<td>0,02</td>
</tr>
<tr>
<td>MASA de lipidos para formular (mg) usando Master Mix 25 mM</td>
<td>0,97</td>
<td>MASA de lipidos para formular (mg) usando Master Mix 12,5 mM</td>
<td>0,48</td>
</tr>
</tbody>
</table>
mRNA analytical tools development: RNA quantification using HPLC

- Testing made with PrimaS analytic monolith (Sartorius)
- Optimization of initial washing steps to reduce “noise”
- High quality salts and buffers needed

**Table 1: Method details and gradient.**

<table>
<thead>
<tr>
<th>Time [min]</th>
<th>MPA [%]</th>
<th>MPB [%]</th>
<th>MPC [%]</th>
<th>MPD [%]</th>
<th>Flow [mL/min]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>0.10</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>1.10</td>
<td>55</td>
<td>45</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>1.80</td>
<td>55</td>
<td>45</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>1.82</td>
<td>0</td>
<td>40</td>
<td>60</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>2.50</td>
<td>0</td>
<td>40</td>
<td>60</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>2.52</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>3.40</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>3.42</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>2</td>
</tr>
<tr>
<td>3.52</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>2</td>
</tr>
<tr>
<td>3.54</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>8.00</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

**Mobile phase A (baseline)**

**mRNA sample purified by LiCl precipitation**
mRNA analytical tools development: RNA quantification using HPLC

### Calibration curve using RNA sample purified by LiCl and quantified by spectrophotometry:

<table>
<thead>
<tr>
<th>µL</th>
<th>µg</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0.45</td>
<td>250803</td>
</tr>
<tr>
<td>10</td>
<td>1.12</td>
<td>691430</td>
</tr>
<tr>
<td>20</td>
<td>2.24</td>
<td>1334608</td>
</tr>
<tr>
<td>30</td>
<td>3.36</td>
<td>2222670</td>
</tr>
<tr>
<td>40</td>
<td>4.48</td>
<td>2686709</td>
</tr>
<tr>
<td>60</td>
<td>6.72</td>
<td>4389118</td>
</tr>
</tbody>
</table>

\[ Y = 651699x - 65845 \]  \[ R^2 = 0.9958 \]

**ARN011 M2 CC = 0.112 ug/µL**

### RNA mass determination using calibration curve above: 10µL injection of 1/20 sample dilution

<table>
<thead>
<tr>
<th>Sample</th>
<th>Area</th>
<th>RNA mass PrimaS (µg)</th>
<th>RNA mass spectro (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARN012 ARN M3</td>
<td>2906471</td>
<td>4.56</td>
<td>4.61</td>
</tr>
<tr>
<td>ARN012 ARN M2</td>
<td>2210289</td>
<td>3.49</td>
<td>3.34</td>
</tr>
<tr>
<td>ARN012 ARN M1</td>
<td>0</td>
<td>0.10</td>
<td>0</td>
</tr>
<tr>
<td>ARN012 IVT M2</td>
<td>2583047</td>
<td>4.06</td>
<td></td>
</tr>
</tbody>
</table>

RNA samples purified by LiCl & quantified by spectrophotometry

RNA samples post IVT- non purified

CUIDAMOS LA SALUD, PRESERVAMOS LA VIDA
### 2023

**Upstream**
- Optimization IVT 500uL
  - Template pDNA production
  - Mixer/Incubator arrival
  - NEB Reagents (mAbxience)
  - Protocol
- Optimization IVT 1mL
  - NEB reagents arrival
  - mRNA: DWS Capture Test
  - LNP Formulation
  - Ribogreen Analytics
- Opt capping 1mL
  - RNA denatured
  - Protocol
- mRNA: DWS Polishing Test
- Capping efficiency analytics

**DWS 500uL- 1mL**
- Capture Croma Optimization
  - Protocol OligodT (Sartorius/RIA)
  - Buffers Preparation
  - Protocol OligoDF (Thermo)
  - Buffers Preparation
- Capture Croma Optimization
  - Protocol C4-HLD monolith
  - Buffers Preparation
- RNA: LNP Formulation

**Formulation**
- LNP Formulation
  - LNPc: LNPc: DLS analytics and Ribogreen
  - Ignite + syringes Arrival
  - Protocol

**Downstream**
- Ribogreen Optimization
  - Analyte to use CC: SB equipment
  - Protocol for RNA quantity
- Protocol for efficiency encapsulation of the mRNA
- DLS Optimization
  - RNAse H enzyme arrival and kit Monarch
  - Protocol
- Digestion with c/RNAsa
- Capping efficiency optimization
  - HPLC Mix equipment?
  - Column acquisition
  - Protocol

**Analytics**
- UBA
- SB

**Proof of concept in animals**
- Institute definition
- Commercial agreement/CDA

---

Cuidamos la salud, preservamos la vida
New mRNA capabilities
### New R&D / GMP RNA facility

<table>
<thead>
<tr>
<th>Option</th>
<th>Surface</th>
<th>Estimated cost of construction (USD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R&amp;D</td>
<td>350 m²</td>
<td>Aprox. 2 M</td>
</tr>
<tr>
<td>R&amp;D + pDNA manufacturing + mRNA manufacturing + LNP manufacturing (Process diagram 1)</td>
<td>900 m² manufacturing, 200 m² Warehouse y 100 m² annex areas.</td>
<td>Out of Budget</td>
</tr>
<tr>
<td>R&amp;D + mRNA manufacturing + LNP manufacturing (Process diagram 2)</td>
<td>~500 m²</td>
<td>TBD</td>
</tr>
</tbody>
</table>

**Process diagram 1**
- Process Flow Diagram & mass balance
- Equipment dimensions
- Conceptual layout

**Process diagram 2**
- pDNA linealization
- Quantoom equipment Midi
- Knauer system

*Cuidamos la salud, preservamos la vida*
New R&D and GMP RNA facility

- Start with a R&D layout (Q1 2023)
- Finish with a GMP mRNA layout (Q1 2023)
- Started construction: Ground preparations (March 2023)
- Detail engineering ongoing.
- Equipment quotations & acquisitions ongoing.
- Estimated end construction: (Q3 2024).
Thank you!

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Trinidad.Pomilio@SinergiumBiotech.com