Low-Energy-Electron Irradiation

A game changer for inactivation of pathogens
The Importance of Vaccines

- Vaccines have been used for over one century to eradicate some of the most severe diseases in the world.

- Currently, COVID-19 is the best evidence why vaccines have a significant position in worldwide healthcare architecture.
Bringing Inactivated Vaccine Technology to the Next Level

- KyooBe seeks to revolutionize vaccine manufacturing through advanced technology approaches
- Rapid and safe without toxic components
- Protecting important antigen structures more effectively
Who we are

Interdisziplinary
Close to the Customer
Extraordinary
Innovative

StartUp company formed Dec. 2019 as part of Bausch+Ströbel Group:
Our Network
Together for the best solutions
Part 1 - Core Technology
The Use of accelerated electrons

Applications and technologies – industrial scale

The technology meets the following criteria:

- LEEI is based on *cathode ray technology* (CRT) which has been used for decades
- Safe application in various business fields, including printing and packaging industry
- Manufacturing set up can be designed as a continuous or batch-driven process

Applications and technologies:

- Television
- Ink Crosslinking
- Packaging / Sterilization
- Vaccines (starting 2023)
The Use of accelerated electrons

Applications and technologies – industrial scale

Low doses (up to 1 kGy)
Medium doses (up to 10-30 kGy)
High doses (over 25 kGy)
How it works
Low-Energy Electron Irradiation (LEEI)

Benchmarking against inactivation techniques and procedures

<table>
<thead>
<tr>
<th>Inactivation</th>
<th>Chemical *</th>
</tr>
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<tbody>
<tr>
<td>Process architecture</td>
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*Currently the only way to produce inactivated vaccines

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eBeam-based effects: molecular level

(1) Direct action
- deposition of energy by accelerated electron in the target molecule
- eliminating H atom and leading to a radical

(2) Indirect action
- Reactive species are formed in the surrounding of the target molecule
- OH radicals interact with the target resulting in strand breaks

Reference:
eBeam-based effects: pathogen inactivation

- eBeam mainly addresses large molecules like nucleic acids
- maintaining the antigen structure of the pathogen
KyooBe’s vision on technology
Adaptive inactivation platform for commercial manufacturing

ELLI300 (Fraunhofer IZI)

Pathogen Inactivation Platform (PIP) / 2023
KyooBe’s vision on technology
Adaptive inactivation platform for commercial manufacturing

ELLI300 (Fraunhofer IZI)

Pathogen Inactivation Platform (PIP) / 2023
PIP will be designed as a full radiation protection system with biological containment!
Pathogen Inactivation Platform (PIP)

Core functions

1. Irradiation chamber
2. ebeam accelerator
3. Liquid roll-system
4. Rotating roll
5. Thin liquid film
6. Squeegee / wiper lip
7. Peristaltic pump
8. Pathogen solution
9. Inactivated solution
Key characteristics of the process
Controlling critical process parameters

Schematic layer set-up

Detailed view on liquid film environment

1 Ebeam accelerator

2 Electrons travelling through defined Ti foil into aseptic space in cassette

3 Gaps - consisting of defined inert gas atmosphere

4 Gaps - separating the defined inert gas atmosphere

5 Liquid pathogen film irradiated by LEEI beams – thickness up to 150 µm, depending on the process

6 Metal roll for fluid transport – also source of Bremsstrahlung
Key characteristics of the process

Controlling critical process parameters

\[ D = Y \cdot \frac{I_B}{v_L \cdot b_B} \]

- \( D \) = Irradiation dose [kGy]
- \( Y \) = Dose constant [kGy \cdot m^2 \cdot mA^{-1} \cdot min^{-1}]
- \( v_L \) = Rotational speed [m \cdot min^{-1}]
- \( I_B \) = Current [mA]
- \( b_B \) = Beam width [m]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Impact on</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid film thickness</td>
<td>~ 120 µm</td>
<td>throughput</td>
</tr>
<tr>
<td>Acceleration voltage</td>
<td>200 keV</td>
<td>penetration depth</td>
</tr>
<tr>
<td>Ebeam current</td>
<td>10 mA</td>
<td>radiation dose</td>
</tr>
<tr>
<td>Thickness Titaniumium window</td>
<td>15 µm</td>
<td>penetration depth</td>
</tr>
<tr>
<td>Air gap distance</td>
<td>12 mm</td>
<td>penetration depth</td>
</tr>
<tr>
<td>Ebeam accelerator window</td>
<td>240 x 60 mm</td>
<td>irradiated area</td>
</tr>
<tr>
<td>Irradiated area</td>
<td>160 x 60 mm</td>
<td>throughput</td>
</tr>
<tr>
<td>Performance</td>
<td>max. 2 kW</td>
<td>current and voltage</td>
</tr>
<tr>
<td>Soll dose in target</td>
<td>~ 60 kGy</td>
<td>pathogen inactivation</td>
</tr>
<tr>
<td>Rotational speed</td>
<td>~ 9 m/min</td>
<td>throughput; radiation dose</td>
</tr>
<tr>
<td>Revolutions per minute</td>
<td>~ 19.1 RPM</td>
<td>throughput; radiation dose</td>
</tr>
<tr>
<td>Throughput</td>
<td>~ 10 L/h</td>
<td>Rotational speed; ebeam current</td>
</tr>
</tbody>
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Schematic layer set-up
Key characteristics of ebeam

Uniformity, profile and robustness

Penetration depth in material (water) at 200 keV

- one Titan window
- 0 window (water in vacuum)
- two Titan windows

Ebeam Accelerator Window
X = 240 mm

Width B = 160 mm

Role ø = 150 mm
Key characteristics of ebeam

Uniformity, profile and robustness

ISO/ASTM 51818:2009
Practice for dosimetry in an electron beam facility for radiation processing at energies between 80 and 300 keV
Key characteristics of ebeam

Uniformity, profile and robustness

Penetration depth in material (water) at 200 keV

Ebeam Accelerator Window
- X = 240 mm
- Y = 60 mm
- Width B = 160 mm
- Role ø = 150 mm

Ebeam Accelerator Window

Axial length (diameter)
- 60 mm
- 150 mm

Longitudinal width
- 160 mm
- 240 mm

Dose [kGy]

Depth [µm]
Substantiating the technology

- **Process Control**
  - Liquid film thickness (already started)
  - Inline & real-time dosimetry

- **Experiments and Test Cases**
  - Generating liquid films
  - Controlling liquid film thickness
  - Transporting fluids within the system
  - Ebeam irradiation with Ebeam supplier (ECAB)
  - Investigating continuous dosimetry

*Liquid film thickness – test case set-up; validation by a confocal sensor (not shown here)*
In a nutshell – Status quo

- PIP = fridge-sized platform

- PIP will include an enclosed box for aseptic handling of pathogens (containment container)

- 10 L/h* Throughput

- **Within sub-seconds** can pathogens be inactivated

- **60 kGy** effective inactivation dose

- Ebeam accelerator for next-gen prototype platform
  - Dimensions: 700 mm x 560 mm x 340 mm
  - 200 kV; 10 mA; 2 kW

*Not yet tested
Ebeam in vaccine manufacturing

Technology Summary

The technology meets the following criteria:

- Approved Technology
- Scalable Process
- Safe Applifaction
- Pathogen Inactivation
Part 2
The Application
Traditional and novel process chains
A disruptive change is about to happen

Supply/Testing of Raw Material

~2 weeks
Cultivation process/Harvest

~52 weeks
Purification process

Inactivation

~1 week
Formulation

Fill, Finish, Batch Release, Transport

Reference: Lücke, Baedeker, Hildinger, BCG. Biotechnology report 2016
Traditional and novel process chains
A disruptive change is about to happen

~ 2 weeks
Supply/Testing of Raw Material

~ 52 weeks
Cultivation process/Harvest

Purification process

Inactivation

Formulation

Fill, Finish, Batch Release, Transport

~ 2 weeks
Supply/Testing of Raw Material

~ 7 weeks
Cultivation process/Harvest

Inactivation

Purification process

Formulation

Fill, Finish, Batch Release, Transport

~ 1 week
Supply/Testing of Raw Material

~ 1 week
Cultivation process/Harvest

Purification process

Inactivation

Formulation

Fill, Finish, Batch Release, Transport
Traditional and novel process chains
A disruptive change is about to happen

- **Supply/Testing of Raw Material**
  - ~2 weeks
  - Cultivation process/Harvest
    - Purification process
      - Inactivation
        - Formulation
          - Fill, Finish, Batch Release, Transport

- **Traditional process**
  - ~52 weeks

- **Novel process**
  - ~2 weeks
  - Supply/Testing of Raw Material
    - Cultivation process/Harvest
      - Inactivation
        - Purification process
          - Formulation
            - Fill, Finish, Batch Release, Transport

- **Supply/Testing of Raw Material**
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- **Supply/Testing of Raw Material**
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# Application of LEEI Technology

## Key market

<table>
<thead>
<tr>
<th>Product type</th>
<th>Human Vaccines</th>
<th>Veterinary Vaccines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Application/ Proof of concept</td>
<td>Pathogen inactivation</td>
<td>Pathogen inactivation</td>
</tr>
<tr>
<td>TLR Score</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Market availability</td>
<td>2023</td>
<td>2023</td>
</tr>
</tbody>
</table>

## R&D / sidekicks

<table>
<thead>
<tr>
<th>Product type</th>
<th>Cell- Products</th>
<th>Blood Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Application/ Proof of concept</td>
<td>Irradiation of (immune) cell therapeutics</td>
<td>Irradiation of transfusion-medicine products/ Serum for cell culture applications</td>
</tr>
<tr>
<td>TLR Score</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Market availability</td>
<td>To be determined</td>
<td>To be determined</td>
</tr>
</tbody>
</table>
Review on Feasibility

Inactivation by LEEI works for:
- Bacteria
- Viruses
- Parasites
- Human & veterinary pathogens.

Dose range: 1 kGy and 33 kGy.

Inactivation is *inter alia* pathogen specific.

### Overview of Pathogens successfully inactivated by LEEI:

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Type</th>
<th>Veterinary / Human</th>
<th>Concentration</th>
<th>Dose for inactivation</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSV</td>
<td>ss RNA virus</td>
<td>Human</td>
<td>$2 \times 10^7$ TCID$_{50}$/ml</td>
<td>20 kGy</td>
</tr>
<tr>
<td>Influenza A (H3N8)</td>
<td>(-) ds RNA virus</td>
<td>Veterinary</td>
<td>$5 \times 10^5$ TCID$_{50}$/ml</td>
<td>22 kGy</td>
</tr>
<tr>
<td>ZIKV</td>
<td>ss RNA virus</td>
<td>Human</td>
<td>$5 \times 10^6$ TCID$_{50}$/ml</td>
<td>20 kGy</td>
</tr>
<tr>
<td>PRRSV</td>
<td>ss RNA</td>
<td>Veterinary</td>
<td>5.42 log TCID$_{50}$/ml</td>
<td>10.4 ±1 kGy</td>
</tr>
<tr>
<td>EHV-1</td>
<td>ds DNA Virus</td>
<td>Veterinary</td>
<td>3.89 log TCID$_{50}$/ml</td>
<td>10.4 ±1 kGy</td>
</tr>
<tr>
<td><em>R. pneumotropicus</em></td>
<td>Bacterium</td>
<td>Veterinary</td>
<td>$1 \times 10^5$ CFU/ml</td>
<td>20 kGy</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>Bacterium</td>
<td>Human</td>
<td>$1.67 \times 10^7$ CFU/ml</td>
<td>2.2 kGy</td>
</tr>
<tr>
<td><em>B. cereus</em></td>
<td>Bacterium</td>
<td>Human</td>
<td>$4.33 \times 10^6$ CFU/ ml</td>
<td>33 kGy</td>
</tr>
<tr>
<td><em>Eimeria tenella</em></td>
<td>Parasite</td>
<td>Veterinary</td>
<td>1.0–2.0 $\times 10^4$ Oozysten/ml</td>
<td>1 kGy</td>
</tr>
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</table>
KyooBe Tech GmbH

03.12.2020

Proof of Concept
Dose- Inactivation curves

→ Successful dose-dependent inactivation of bacteria and viruses

→ Doses required for inactivation comparable to those reported with other ionizing radiation technologies.

Correlation between genome size and irradiation dose required for complete inactivation: smaller genome size -> Higher irradiation dose.
High degree of conservation of the native antigen structure.

High reproducibility of antigen conservation.

Monoclonal antibody recognition of RSV F-Protein is not altered after LEEI-treatment.

Direct comparison to FI:
All animals developed significant levels of RSV-specific antibodies.

All animals developed significant amounts of virus neutralizing antibodies.

Animals immunized with 20 kGy-irradiated RSV have RSV-RNA levels close to detection limit.
Paving the way to market

- Safety
- GxP
- Radiation protection
- People
- Equipment
- Environment
- Market
- GxP
- Documentation
- Regulatory
- User
- Cost
- Scale
- Patient
- Demand
- Production environment
- Containment
- GxP
- Formulation
- Stability
- Shelf life
- Purity
- Effectiveness
- Product
- Procedures
- Processes
- KyooBe Tech GmbH

03.12.2020
Timeline and Milestones

- 2012
- 2014
- 2020
- 2021
- 2022
- 2023
Partnering with KyooBe Tech

- Development (sparring) partners
- Lead customers
- Regulatory experts with vaccine manufacturing background

Enthusiastic and motivated people who want to bring WIV vaccine manufacturing to the next level