L-Asparaginase Loaded Inside Red Blood Cells as a New Cell Based Medicinal Product

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Red cell as a vehicle: Overview of the technology

A new cell based Medicinal Product* with innovative mode of administration and action

Human red cells

Molecule / enzyme / drug

Sources

ERYtech Pharma
Alone or with Partners

Partners

Markets

* EMEA & FDA classification
Encapsulation Principle

Encapsulation of the drug involves hypotonic stress followed by hypertonic stress for the red cells. Before and after encapsulation, the process requires washing steps with standard machines and solutions mainly used in dialysis and blood fields.

No modification of the characteristics of the red cells have been observed during Graspa Phase I/II clinical trial.

The cells maintain the same half life and same phenotype (antigen surface).

- Pores up to 400nm
- ~100% of red cells are loaded
- Product dosage adapted with transfusion volume
- Great reproducibility
Red cell unit coming from Blood Bank

1st Step: Cell wash with NaCl (3 cycles)

2nd Step: Mix with active drug (protein, antigen, lysate)

3rd Step: Hypotonic dialysis

4th Step: Resealing solution

5th Step: Cell wash with NaCl/Glucose (3 cycles)

6th Step: Resuspension with preservative solution

7th Step: Medicinal product in blood compatible PVC bag

Quality control / Qualification

shipment to the hospital

Osmotic Fragility
Haematocrit
Active Drug

Adjustment of hypotonic dialysis parameters

Reproductibility
Stability
Entrapment control

Diagram of Encapsulation Process and Steps

Encapsulation process
Final Product Controls

- **Osmotic Fragility**
- **Blood Count** (MCV, MCHC…)
- **L-asparaginase Assay**
  - Quick and Automated Method
  - Developed and full validated by ERYtech Pharma
  - LOQ : 0.05 IU/mL
  - Intra and Extra cellular L-asparaginase
- **Hemoculture**
Diagram of Process Manufacturing

Phase A
Reception of prescription
Reception of compatible RBC

Phase B
Delivery and Shipment

Clinician

Qualified Person Office

Step 1
Definition of encapsulation parameters

Step 2
Product order

Production Box 1
Production Box 2
Production Box 3
Production Box 4
Production Box 5
Production Box 6

Validation Batch

Step 3
Sample of the manufactured batch is sent to Quality Control for control

Step 4
Quality Control validation and report redaction

Quality Control

Product order sent electronically to ERYcaps machines according to specified parameters

Traceability of blood

RBC = packed Red Blood Cells

1 Production Box = 1 batch = 1 qualified operator for all the entrapment steps
Logistic - Centralized cGMP manufacturing

1. Prescription by Physician

2. Compatible Packed RBC (autologous or homologous) are ordered to the Blood Bank

Patient

ERYtech Pharma
cGMP Facility

ERYcaps®
Within 2 h

3. Clinical batches are manufactured in cGMP facility

4. After quality control, clinical batches are shipped to hospital

Patented technology
<table>
<thead>
<tr>
<th>Assays</th>
<th>Specifications</th>
<th>Clinical Batches</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean.</td>
</tr>
<tr>
<td>Extracellular Haemoglobin</td>
<td>&lt; 0.4 g/dL</td>
<td>0.1</td>
</tr>
<tr>
<td>Osmotic Fragility</td>
<td>&lt; 3.5 g/L</td>
<td>1.4</td>
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<tr>
<td>Mean Corpuscular L-asparaginase activity</td>
<td>78-146 IU/mL</td>
<td>99.8</td>
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<tr>
<td>MCV</td>
<td>70-95 fL</td>
<td>77.5</td>
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<tr>
<td>MCHC</td>
<td>23-35 g/dl</td>
<td>30.4</td>
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<tr>
<td>Extracellular L-asparaginase</td>
<td>ND</td>
<td>0.4</td>
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</tbody>
</table>
FDA, EMEA regulates blood; similar to drugs and living tissues

- Blood center and Blood Banks are subject to GMP by FDA or EMEA

- Good manufacturing practices for blood and blood components

- Quality system management for tracability

- Dedicated Refrigerated Blood Transporteurs

- Biovigilance Program: Heamovigilance program
Asparagine is a tumor growth factor.

Asparaginase antitumoral activity is based on asparagine degradation in plasma.

CURRENT LIMITATIONS

Asparaginase enzyme has great efficacy but due to bacterial origin, its administration is associated with:
- high immunogenicity
- severe side effects

ADVANTAGES:
- Asparaginase is active during longer period while being protected from circulation by red cell membrane
- Antibodies formation and allergic reactions are prevented
- Side effects are greatly reduced
## Phase II clinical results

<table>
<thead>
<tr>
<th>Depletion duration with a single injection</th>
<th>Control Group with L-Asparaginase</th>
<th>Graspa Group</th>
</tr>
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<tbody>
<tr>
<td>6 patients</td>
<td>3 days</td>
<td>18 patients*</td>
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</table>

<table>
<thead>
<tr>
<th>Allergic reaction Grade III and IV</th>
<th>50 % (3/6 patients) 2/6 patients</th>
<th>6% (1/18 patients) 0/6 patients</th>
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</thead>
<tbody>
<tr>
<td>Coagulation disorders: ATIII decrease with significant clinical change</td>
<td>67 % (4/6 patients)</td>
<td>6% (1/18 patients)</td>
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<tr>
<td>Pancreatitis</td>
<td>17 % (1/6 patients)</td>
<td>22% (4/18 patients)</td>
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<tr>
<td>Hepatic disorders</td>
<td>83 % (5/6 patients)</td>
<td>50% (9/18 patients)</td>
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<tr>
<td>Hypoalbuminemia</td>
<td>67 % (4/6 patients)</td>
<td>11% (2/18 patients)</td>
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</table>

*Phase I/II in ALL relapse patient (ERYtech pharma study)

Significant improvements were observed:

- Longer biological efficacy
- Strong reduction in allergic reactions
- Reduced number of coagulation disorders
Development of a companion test

Preliminary data: ASNS expression in tumour cells

- Low ASNS expression (the market)
- High ASNS expression
Graspa Strategic plan is based on key principles:

1/ MAA as fast as possible

2/ Market penetration in niche indications with selected patients

3/ High price product, with companion test & assay

4/ Clear roadmap to build a broad oncology franchise:
   A/ From ALL to Selected Heamatological Cancers
   B/ From Onco-Haematology to Specific Solid Tumours
## Other applications

<table>
<thead>
<tr>
<th>Products</th>
<th>Indications</th>
<th>Discovery</th>
<th>POC Preclinical</th>
<th>P I</th>
<th>P II</th>
<th>P III</th>
<th>First sales</th>
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<tbody>
<tr>
<td>Graspa®</td>
<td>ALL Relapse &amp; Allergy</td>
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<td>Codev 2010</td>
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Thanks a lot!

Manufacturing team

Europe
Jerome Bailly
Veronique Sezanne
Angeline Chanel
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Fanny Roure

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The American Red Cross Cell Therapy’s Team

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