#LBN 14

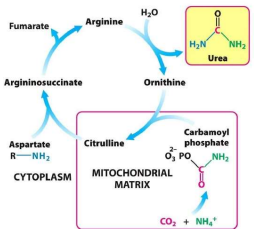
Eryminase, Arginine Deiminase-Encapsulated Red Blood Cells Effectively Lower Blood Arginine Levels in a Mouse Model of Inducible Hyperargininemia

E. Dufour, L. Ballantyne[&], A. Meyzaud[¶], F. Horand[¶], A. Scheer[¶] and C. D. Funk[&]*

**Erytech pharma Inc., Cambridge MA, USA; [&]Queen's University, Kingston, ON, CANADA; [¶]Erytech pharma, Lyon, France*

1 - Background

Arginase deficiency is a rare genetic disorder resulting from the loss of Arginase 1, affecting the final step of the urea cycle in liver. Arginase is of central importance in the detoxification of ammonia in mammals.



Symptoms generally appear in early infancy and include intellectual disability, non-ambulatory muscle stiffness and seizures. There are currently no cures and treatment outcomes are usually poor with a low-protein diet and/or nitrogen scavenger drugs. The main biochemical feature is accumulation of arginine leading potentially to toxic levels of guanidino compounds and nitric oxide.

As an alternative treatment approach to reduce the toxic accumulation of arginine and its metabolic side products, Erytech envisioned an innovative enzyme therapy: **Eryminase**, Arginine Deiminase (ADI) entrapped in red blood cells.

2 - Eryminase product presentation

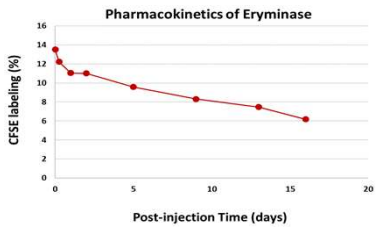
Eryminase product, Arginine Deiminase (ADI; EC number 3.5.3.6) entrapped in red blood cells (RBC), is obtained using the Erytech's proprietary ERYCAPS technology platform. The entrapment of therapeutic enzymes into red blood cells can provide effective, long-acting therapeutic activity with reduced toxicity.

Arginine Deiminase from *Mycoplasma arginini* is a homodimer composed of two subunits of 46.5 kDa each. It metabolizes L-arginine into L-citrulline and ammonia (NH_3).

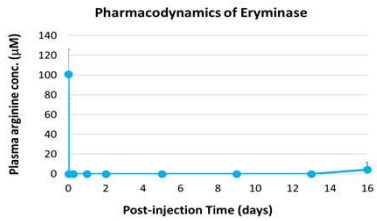
L-arginine + H_2O ⇌ L-citrulline + NH_3

Eryminase PK/PD in healthy C57BL/6 mice was explored:

Pharmacokinetics



Pharmacodynamics



CFSE-labeled RBC encapsulating ADI (CFSE-labeled **Eryminase**) was intravenously injected at a dose volume of 8 mL/kg. Sixteen (16) days after administration, 43 % of the injected **Eryminase** product is still in mouse blood circulation. Half-life of labeled Red Blood Cells in blood circulation is estimated between 17 and 20 days.

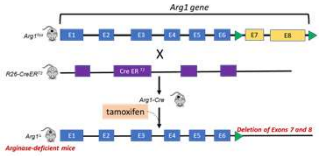
Free form of Arginine Deiminase injected to mice has a half-life of ~5 hours.

Eryminase was intravenously administered at a dose volume of 8 mL/kg. Blood sampling was performed at Day 0+15min, Day 0+6h, Day 1, Day 2, Day 5, Day 9, Day 13, and Day 16. A complete plasma L-arginine depletion is observed 15 minutes after administration and for 13 days.

ADI being entrapped in the RBC, the hypothesis is that L-arginine crosses the RBC membrane and is metabolized by ADI inside the RBC.

3 – Arginase-deficient mouse model

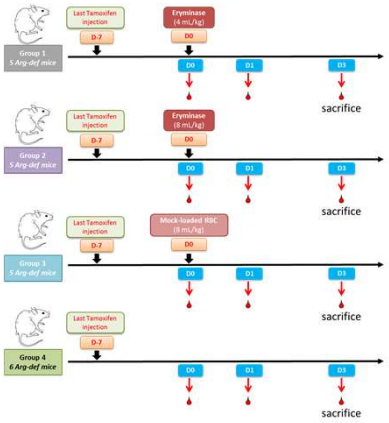
Recently, an inducible Arginase-deficient mouse model was generated using the Cre/loxP-directed conditional targeting strategy (Sin *et al.*, PLOS One, 8(11). This strategy allows a spatial and temporal control of Arginase gene deletion in mice. After 5 intraperitoneal injections of tamoxifen for 5 consecutive days, excision of exons 7 and 8 of *Arg1* gene is achieved.



Exons deletion is checked by PCR genotyping and the absence of Arginase 1 activity is checked by quantifying the amount of urea produced in the liver.

These knockout mice exhibit progressive weight loss and a loss of motor functions. But in contrast to human patients, these mice die invariably by two weeks after the last tamoxifen injection.

4 – *In vivo* study Design with Arginase-deficient mice



Four (4) groups of 5/6 arginase-deficient mice were designed to address the **Eryminase** efficacy on blood arginine and serum ammonia levels.

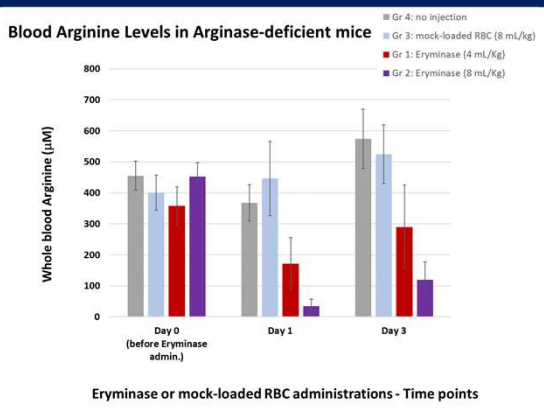
Eryminase or mock-loaded RBC products were intravenously injected 8 days after the last tamoxifen administration at two dose volumes (group 1: 4mL/kg and group 2: 8 mL/kg, respectively). Four (4) and 8 mL/kg doses correspond to 112 and 224 IU/kg doses, respectively.

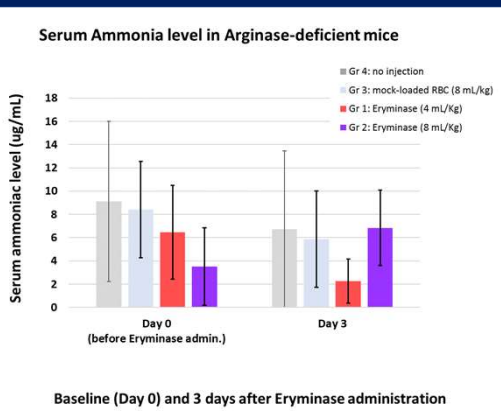
Mock-loaded RBC were injected at a dose volume of 8 mL/kg (group 3). One control group didn't receive any treatment (group 4).

Blood sampling (50 µL) was performed on each mouse at Day 0 (before **Eryminase** or mock-loaded RBC administrations), the following day (day 1) and 3 days after the injections (day 3). Mice were sacrificed at day 3.

The twenty one (21) blood samples were used to assay the blood arginine levels (Mass spectroscopy) at three different time points and serum was prepared to measure the amount of ammonia (µg/mL).

5 – Results





Eight (8) days after last tamoxifen injection, which effectively deletes exons 7 and 8 of the mouse *Arg1* gene, blood levels of arginine were approximately 4 times greater (400 µM) than at baseline in all 4 groups of the study.

Preliminary results show that **Eryminase** product injected at 2 doses (4 and 8 ml/kg; Groups 1 and 2; n=5 each) reduced blood arginine levels, 24h after administration, by 52 and 92 %, respectively, while mice injected with mock-loaded RBCs (Group 3; n=5) and non-RBC injected control (Group 4; n=6) showed unchanged or slightly elevated levels. Seventy-two (72) hours later, blood arginine levels remained suppressed in mice of Groups 1 and 2 by 19 and 73 %, respectively (left panel).

Ammonia, one of the products of Arginine Deiminase enzymatic reaction, was assayed in the serum (right panel). No significant differences were observed between all the groups of the study.

6 - Conclusion

Eryminase, entrapment of Arginine Deiminase inside Red Blood Cells, greatly improves the pharmacological properties of the enzyme. In healthy mice, plasma L-arginine depletion is complete 15 minutes after administration and is sustained for 13 days. When injected to Arginase-deficient mice, **Eryminase** has demonstrated a spectacular efficacy on the very high blood L-arginine concentrations displayed by these mice. Indeed, 24h after administration, blood L-arginine concentration was reduced by 52 and 92% when 4 or 8 mL/kg were injected, respectively. Three days after administration, blood L-arginine level remains reduced by 19 and 73%. Moreover, despite the production of ammonia by Arginine Deiminase, the serum level stayed comparable to the control arginase-deficient mice.

Based on these results, **Eryminase** may be an effective strategy to counteract the main biochemical defect of the rare genetic disorder of Arginase deficiency.

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Disclosure

E. Dufour, A. Meyzaud, F. Horand and A. Scheer are employees at Erytech pharma.