Pharmacodynamic Characterization of eryaspase (L-asparaginase Encapsulated in Red Blood Cells) in Combination with Chemotherapy in a Phase 2/3 Trial in Patients with Relapsed Acute Lymphoblastic Leukemia (NCT01518517)

Iman El Hariry, MD, PhD[1], Karine Aguera, MSc[1], Frank Hoke, PhD[1], Kenneth Swart, PhD[2] Adam Hamm, PhD[3], Richard Kay, PhD[4], and Philip L. Lorenzi, PhD[5]

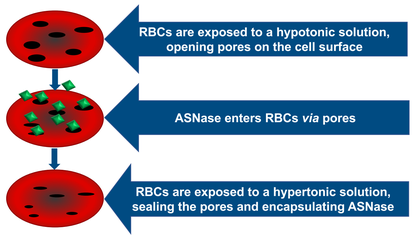
[1] ERYTECH Pharma, Cambridge, MA, [2] PAREXEL International Clinical Research Organization, Durham, NC, [3] Cytel Inc, Cambridge, MA, [4] School of Pharmacy and Pharmaceutical Medicine, Cardiff University, Cardiff, Wales, UK, [5]The Proteomics and Metabolomics Core Facility, Department of Bioinformatics and Computational Biology, The University of Texas MD Anderson Cancer Center, Houston, TX

Abstract 7049

Background

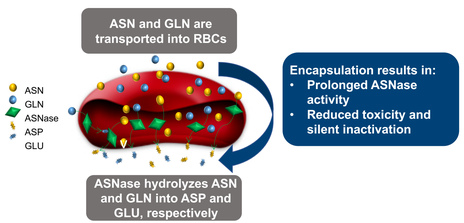
- L-asparaginase (ASNase) is an integral component of multi-agent chemotherapy regimens used during induction and consolidation phases for the treatment of acute lymphoblastic leukemia (ALL).[1]
- ASNase is an enzyme that hydrolyzes asparagine (ASN), and to a lesser extent, glutamine (GLN), both are amino acids that are necessary for cellular nucleic acid and protein synthesis. Leukemia cells are sensitive to depletion of both amino acids, thus ASNase is an effective anti-leukemic therapy.[2]
- The clinical benefit of integrating ASNase therapy in ALL treatment in pediatric and adult patients with ALL is partially offset by the development of frequent and potentially severe adverse reactions, which occur in up to 30% of patients, especially hypersensitivity reactions, coagulopathy, as well as hepatic and pancreatic toxicities.[3,4]
- Red blood cells (RBCs) are considered the most biocompatible carrier systems for various agents.[5] The encapsulation of ASNase in RBCs was shown to reduce toxicity associated with the native ASNase and silent inactivation, while prolonging its activity.[6]
- eryaspase is a dispersion for infusion of *E-coli*-derived ASNase-encapsulated in homologous RBCs (**Figure 1**). The main mode of action of eryaspase is the depletion of circulating ASN and GLN through active transport into RBCs where it is hydrolyzed by the encapsulated ASNase into aspartic acid (ASP) and glutamic acid (GLU), respectively (**Figure 2**).[7]
- No critical minimum value of circulating ASN has yet been established for anti-leukemic efficacy, and ASN levels remain difficult to measure accurately due to *ex vivo* depletion that occurs during processing of blood to plasma.[1]
- ***Main objectives of the current work:***
  1. ***To characterize the pharmacodynamic of eryaspase in a phase 2/3 trial in patients with relapsed ALL***
  2. ***To evaluate the ex-vivo depletion of ASN with naked (free) ASNase.***

Figure 1. ERYCAPS Platform Encapsulation Process



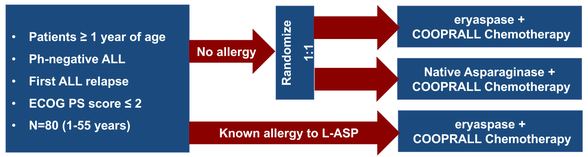
ASNase: asparaginase; RBCs: red blood cells

Figure 2. Activity of Encapsulated ASNase in RBCs



ASN: asparagine; GLN: glutamine; ASNase: asparaginase; ASP: aspartate; GLU: glutamate

Pharmacodynamic Characterization of Eryaspase in a Phase 2/3 Study in Relapsed ALL



Study Design

- This was an open-label, randomized, international phase 2/3 trial, evaluating the safety, efficacy, and pharmacodynamics of eryaspase in combination with chemotherapy for the treatment of patients with ALL
- The study enrolled male or female patients ages ≥ 1 year of age with relapsed Ph-negative ALL with an ECOG PS of ≤ 2

Co-primary Endpoints

- Duration of ASNase activity >100 U/L in whole blood, and incidence of allergic reactions during the induction phase

Key Secondary Endpoints

- Safety, complete remission rate, pharmacokinetics and pharmacodynamics.

Table 1: Baseline Demographic Characteristics

	Non-Allergic Patients eryaspase n=26	Non-Allergic Patients Native ASNase n=28	Allergic Patients eryaspase n=26	Total n=80
Gender n (%)				
Male	16 (61.5)	18 (64.3)	18 (69.2)	52 (65.0)
Female	10 (38.5)	10 (35.7	8 (30.8)	28 (35.0)
Age (years)				
Mean (SD)	12.34 (10.45)	16.24 (13.22)	19.2 (15.9)	15.94 (13.48)
Median	8.69	12.54	12.8	10.62
Range	2.7 – 42.4	1.9 – 55.9	2.1 – 56	1.9 – 56.0
ECOG PS at Inclusion n (%)				
0	20 (76.9)	18 (64.3)	14 (53.8)	52 (65.0)
1	4 (15.4)	8 (28.6)	9 (34.6)	21 (26.3)
2	2 (7.7)	1 (3.6)	3 (11.5)	6 (7.5)
3	0	1 (3.6)	0	1 (1.3)

Table 2: Duration of Total ASNase Activity

	Non-Allergic Patients eryaspase n=26	Non-Allergic Patients Native ASNase n=28	Allergic Patients eryaspase n=26
Duration of ASNase activity* >100 U/L in whole blood (days)			
Mean (SD)**	18.9 (5.3)	8.5 (6.6)	17.2 (6.3)
CI (95%)	(16.7; 21.0)	(6.0; 11.1)	(14.6; 19.7)
Median	20.1	7.1	20.0
Range	8 – 29	0 – 23	5 – 27
Duration of ASNase activity >100 U/L in Plasma (days)			
Mean (SD)	0.3 (0.5)	8.1 (6.9)	0.0 (0.2)
CI (95%)	(0.1; 0.5)	(5.4; 10.8)	(0.0; 0.1)
Median	0.0	6.9	0.0
Range	0 – 2	0 – 23	0 – 1

* Asparaginase activity values are censored at the time point when the patient switched to any alternative asparaginase treatment.
** P value significant

Table 3: Asparagine Depletion and Complete Remission Rate

	Non-Allergic Patients eryaspase n=26	Non-Allergic Patients Native ASNase n=28	Allergic Patients eryaspase n=26
Duration of ASN depletion ≤2 µM (days)			
Mean (SD)	6.0 (5.0)	11.6 (7.3)	1.9 (2.7)
CI (95%)	(4.0; 8.0)	(8.8; 14.4)	(0.8; 3.0)
Median	5.0	8.0	0.5
Range	0 – 16	1 – 23	0 – 8
Maintenance of ASN Depletion ≤2 µM (N (%) patients)	N= 20	N=28	N=15
At 7 days	14 (70.0)	21 (75.0)	3 (20.0)
CR rate*-#	17 (65.4%)	13 (50.0%)	10 (35.7%)

*P-value significant
For patients who switched to any alternative asparaginase treatment, discontinued the study, or died before the complete remission assessment, complete remission and is censored was considered not achieved.

Table 4: Correlation Between CR and ASN Depletion – ROC Analysis

Statistic	Day +6 N=26; CR=17		
	Estimate	Lower 95% CL	Upper 95% CL
Optimal cut-off (%)	**7.55**	**5.21**	**16.87**
Sensitivity	0.82	0.71	1.00
Specificity	0.78	0.44	1.00
Positive predictive value	0.88	0.75	1.00
Negative predictive value	0.70	0.55	1.00

The optimal cut-off for the depletion of ASN was determined by Youden's Index, the cut-off corresponding to the maximum height of the ROC curve above the diagonal chance line. The 95% confidence intervals were estimated using a stratified bootstrap with 2000 repetitions.
(1) For ASN depletion, the 95% confidence interval for sensitivity is [1.0, 1.0] because all of the CR subjects have depletion levels of the lowest possible value (BLLQ = 0.52), so that for every bootstrap and threshold, the sensitivity is either 0 or 1.

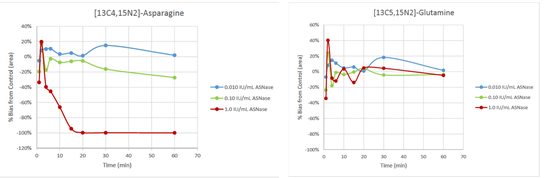
Discussion

- Treatment with eryaspase led to ASN depletion in the majority of the patients below the cut-off threshold ≤2 µM. However, non-inferiority was not demonstrated when the mean duration of ASN depletion was compared to that of the control arm.
- Analysis of biological effect on ASN depletion and correlation with CR in the study, using ROC approach showed that the optimal cut-off for ASN depletion is at 7.55 µM with a sensitivity of 0.82 and a specificity of 0.78.

Challenges of measuring ASN depletion with free ASNase treatment

- Assessment of ASNase therapy is faced with major challenges: a) Asparaginase therapy aims to achieve asparagine depletion, but no critical minimum value for efficacy has yet been established [10]; b) ASN levels are difficult to measure accurately due *ex vivo* effect, even at very low subtherapeutic activity levels during the time required to harvest plasma from blood samples if theses samples are not immediately processed; and c) In patients previously exposed to ASNase, residual ASN concentrations in the presence of high ASNase activity has been reported with other formulations [11,12]. The lack of consensus in clinical practice regarding level of ASN depletion required and the challenges with *ex vivo* depletion has led to a shift in therapeutic drug monitoring based on ASNase activity.
- The current study highlights the challenges of comparing ASN depletion with an encapsulated and a naked ASNase formulation. The *ex vivo* depletion is expected to be minimal with the former compared to the latter. The extent of *ex vivo* depletion has never been assessed.
- We therefore conducted an *in vitro* study to assess the extent of *ex vivo* ASN depletion with a naked ASNase formulation:
  - A range of ASNase activities were spiked into whole blood solutions containing $^{13}C_4$-ASN and $^{13}C_5$-GLN, and a time course of conversion to the corresponding stable isotope labeled amino acid products was measured as a surrogate of *ex vivo* depletion.
  - The extent of *ex vivo* depletion of ^{13}C-ASN increased with increasing ASNase dosage and sample processing time. *Ex vivo* depletion of ^{13}C-ASN was minimal at and below ASNase concentration of 10 U/L. At typical ASNase concentrations observed in patients (100 U/L and above), *ex vivo* depletion of ASN was extensive—100 µM in 30 min at an ASNase concentration of 100 U/L; 500 µM in 15 min at an ASNase concentration of 1000 U/L, **Figure 3**

Figure 3: Estimate of *Ex Vivo* ASN Depletion by Typical ASNase Concentrations as a Function of Sample Processing Time



- *Ex vivo* depletion of ASN was examined over a range of ASNase activities (10, 100, and 1000 U/L) spiked into artificial whole blood solutions containing 0.50 mM $^{13}C_4$,$^{15}N_2$-Asn and 1.0 mM $^{13}C_5$,$^{15}N_2$-glutamine. Conversion to the corresponding stable isotope-labeled amino acid products was examined over a 60-minute time course to simulate a range of sample processing times.

Conclusions and Perspectives

- Eryaspase in combination with chemotherapy for the treatment of patients with ALL demonstrated sustained asparaginase activity in patients previously treated with *E-coli* derived ASNase.
- ASN Depletion ≤2 µM was maintained at Day 7 in 70% and 75% of patients in the eryaspase and native ASNase arms, respectively.
- The exploratory ROC analysis demonstrated that the exposure-response relationship was achieved and showed that a level of depletion to ≤2 µM may not be necessary with eryaspase treatment but rather the optimal cut-off for ASN depletion <7.55 µM, at day +6, with a high sensitivity and a specificity. These results may need to be further validated in future studies.
- The extent of *ex vivo* depletion of ASN under "typical" sample collection and preparation conditions is likely to be large. New and improved methods that properly account for unquenched enzyme activity are required to improve the accuracy of ASN measurements.
- In the Phase 2/3 trial, the highest ASNase concentration measured in a patient treated with native ASNase was 4000 U/L. That level of ASNase activity is sufficiently potent to yield *ex vivo* depletion >500 µM ASN within 15 min. At less extreme ASNase concentrations of 100 U/L, *ex vivo* depletion is still large, with approximately 100 µM ASN within 30 min sample processing time. With normal ASN levels in the circulation ranging between 50-60µM, even low concentrations of free ASNase is therefore expected to completely deplete any remaining ASN *ex vivo*.
- Overall, these findings from the *in vitro* study indicates that *ex vivo* depletion is a serious issue, because most clinical trials fail to quench ASNase instantaneously at the point of blood collection. Therefore, *ex vivo* depletion does occur, and it is clearly extensive if the ASNase concentration is greater than 100 U/L, which is very likely the case in most patients treated with naked ASNase enzyme or other forms of the free enzyme.
- We are developing and testing in preclinical and clinical settings a new method using blood collection tubes with stable isotope-labeled substrates of ASNase for the assessment of *ex vivo* depletion and back-calculation of the true concentration of ASN that was present at the point of blood collection. In addition, we are evaluating *ex vivo* depletion in clinical samples from ALL patients treated with naked ASNase enzymes.
- Eryaspase activity in relapsed ALL provides an alternative option for patients, particularly those who may have developed toxicities and/or silent inactivation to other asparaginase formulations.

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Corresponding author: IEH

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