A Phase 2b clinical trial of eryaspase in combination with low-dose cytarabine as first-line therapy in elderly patients with acute myeloid leukemia (ENFORCE - NCT01810705)

X. Thomas[1], F. Orsini-Piocelle[2], P. Chevallier[3], E. Tavernier-Tardy[4], JP. Marolleau[5], M. Hunault[6], B. de Renzis[7], C. Bonmati[8], MP. Ledoux[9], F. Larosa[10], G. Guillerm[11], S. Chantepie[12], M. A. Sanz-Alonso[13], K. Porkka[14], F. Pane[15], B. Gjertsen[16], B. Quesnel[17], Y. Hichri[18], E. Jourdan[19], S. Lepretre[20], C. Recher[21], T. Leguay[22], T. Cluzeau[23], B. Steffen[24], JB. Bertrand[25], A Hamm[26], R Kay[27], I. El-Hariry[25]

[1]Centre Hospitalier Lyon Sud, Pierre-Bénite, [2]Centre Hospitalier de la Région d'Annecy, Pringy, [3]Hôtel Dieu, Nantes, [4]Institut de Cancérologie de la Loire, Saint-Priest-en-Jarez, [5]Groupe Hospitalier Sud, Amiens, [6]Centre Hospitalier Universitaire d'Angers, Angers, [7]Centre Hospitalier Universitaire Estaing, Clermont-Ferrand, [8]Hôpital de Brabois, Vandoeuvre-lès-Nancy, [9]Hopitaux Universitaires de Strasbourg, Strasbourg, [10]Hôpital Jean Minjoz, Besançon, [11]Centre Hospitalier Universitaire Morvan, Brest, [12]Centre Hospitalier Côte de Nacre, Caen, France, [13]Hospital Universitario y Politécnico la Fe, Valencia, Spain, [14]Helsinki University Hospital, Helsinki, Finland, [15]Universita' di Napoli Federico II, Napoli, Italy. [16]University of Bergen & Haukeland University Hospital, Bergen, Norway, [17]Hôpital Claude Huriez, Lille, [18] Hôpital Saint Eloi, Montpellier, [19]Groupe Hospitalo-Universitaire Carémeau, Nimes, [20]Centre Henri Becquerel, Rouen, [21]Institut Universitaire du Cancer de Toulouse, Toulouse, [22]Hôpital Haut-Lévêque, Pessac, [23]Hôpital L'archet, Nice, France, [24]University Hospital Frankfurt, Frankfurt, Germany, [25]Erytech Pharma, Lyon, France, [26]Cytel, Inc, Boston, United States, [27]Cardiff University, Cardiff, United Kingdom

Background

L-asparaginase (ASNase) is an enzyme that hydrolyzes asparagine (ASN), and to a lesser extent, glutamine (GLN), both of which are amino acids 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.[1] A synergistic effect of ASNase has been demonstrated in adult patients with acute myeloid leukemia (AML) when given in combination with chemotherapy, especially cytarabine.[2] Elderly patients represent the majority of the population with acute myeloid leukemia.[3] However, ASNase is poorly tolerated in elderly patients due to severe toxicities, which has limited its use in older populations.[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]

AML cells are dependent on glutamine for protein synthesis downstream of the mammalian target of rapamycin (mTOR).[8] ASNase can be used to target this dependence through its effects on glutamine depletion that result in inhibition of downstream mTOR signaling, inhibition of protein synthesis and, thus, loss of cell viability.[8] In a study of ASNase as a component of the HAMA regimen (cytarabine, mitoxantrone and ASNase) in elderly patients with AML, a 33% response rate was observed in patients ≥60 years, with acceptable toxicity.[9]

Figure 1. ERYCAPS platform encapsulation process

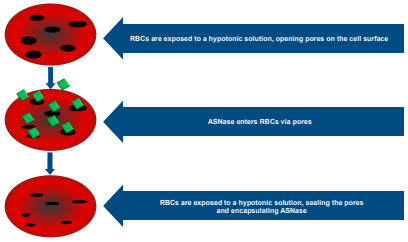
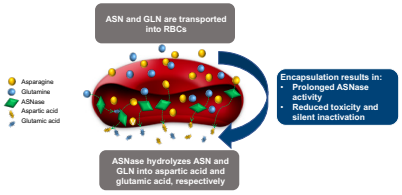


Figure 2. Eryaspase mechanism of action

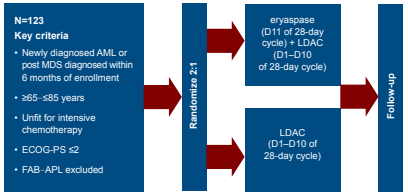


Study objective

The aim of this multicenter, open-label, randomized, controlled Phase 2b trial was to compare the efficacy and tolerability of eryaspase plus low-dose cytarabine (LDAC) with low-dose cytarabine alone in the treatment of patients 65 to 85 years old with AML who are unfit for intensive chemotherapy.

Study design

Figure 3. ENFORCE 1 study design



AML: acute myeloid leukemia; APL: acute promyelocytic leukemia; D: day; ECOG-PS: European Cooperative Oncology Group-Performance Status; FAB: French–American–British; LDAC: low-dose cytarabine; MDS: myelodysplastic syndrome

In this study, 123 patients (65 to 85 years old) with newly diagnosed AML, who were unfit for intensive chemotherapy, were randomized 2:1 to receive either eryaspase in combination with LDAC (n=83) or LDAC alone (n=40) for up to 24 months (Figure 3).

Patients in the experimental arm received an intravenous infusion of eryaspase (100 U/kg) on Day 11 of a 28-day cycle. Patients in both arms of the trial received subcutaneous LDAC (20 mg twice daily) on Days 1 to 10 of each cycle.

The primary endpoint was overall survival (OS). This was defined as the time elapsed between randomization and death from any cause. Key secondary endpoints included progression-free survival (PFS), objective response rate, (ORR), and the safety and tolerability of eryaspase.

The sample size calculation is based on the original primary endpoint of PFS. We assumed 75% improvement in median PFS in the eryaspase plus LDAC group compared with median PFS in the LDAC group. With a two-sided 5% level significance test and a power of 80%, taking into account the 2:1 randomization, a total of 117 patients should be enrolled to observe 117 events (78 in the eryaspase plus LDAC group and 39 in the LDAC alone group).

Results

Table 1. Patient disposition

Status	Eryaspase + LDAC N=83 (%)	LDAC alone N=40 (%)
Randomized (ITT population*)	**83 (100.0)**	**40 (100.0)**
Safety population†	**81 (97.6)**	**39 (97.5)**
Per protocol‡	**54 (65.1)**	**38 (95.0)**
Discontinued treatment, primary reasons, n (%)		
Adverse event	20 (24.1)	7 (17.5)
Consent withdrawal	1 (1.2)	1 (2.5)
Disease progression	31 (37.3)	22 (55.0)
Death	20 (24.1)	8 (20.0)
Censored for follow-up	2 (2.4)	2 (5.0)
Other¶	9 (10.8)	0
Discontinued study, primary reasons, n (%)		
Death	70 (84.3)	34 (85.0)
Consent withdrawal	1 (1.2)	1 (2.5)
Censored for follow-up	10 (12.0)	5 (12.5)
Other¶	2 (2.4)	0

ITT: intention-to-treat
*All patients randomized; †All patients who received at least one dose of study drug; ‡All patients in the ITT set who received at least one course of treatment and who did not have a major protocol deviation; ¶Patient decision to stop the treatment but not the study, wrongly enrolled, post-production incompatibility; ¶Wrongly enrolled.

Table 2. Baseline demographic characteristics

Status	Eryaspase + LDAC N=83	LDAC alone N=40
Gender, n (%)	**83**	**40**
Male	46 (55.4)	24 (60.0)
Female	37 (44.6)	16 (40.0)
Age at randomization, n	**81**	**39**
Median	78	77
Range	(66, 85)	(65, 85)
ECOG-PS, n (%)	**83**	**40**
0	18 (21.7)	8 (20.0)
1	46 (55.4)	24 (60.0)
2/3	19 (22.9)	8 (20.0)
Site of disease, n	**83**	**40**
Missing	5	2
Medullary*, n (%)	73 (88.0)	35 (87.5)
Combined medullary and extramedullary*, n (%)	5 (6.0)	3 (7.5)
Bone marrow aspirate blasts, n	**81**	**40**
Missing, n	2	0
Median	60.0	40.5
Range	(6.0, 95.0)	(8.0, 88.0)
WHO classification, n (%)	**81**	**40**
Missing	2	0
Recurrent genetic abnormalities	0	0
Multilineage dysplasia	20 (24.7)	9 (22.5)
Myelodysplastic syndromes	1 (1.2)	1 (2.5)
Not otherwise categorized	60 (74.1)	30 (75.0)
Baseline leukocyte count, (10x9/L)	**83**	**40**
Median	4.3	3.1
Range	(0.7, 1990.0)	(0.5, 67.3)

ECOG-PS: European Cooperative Oncology Group- Performance Status
*One subject had a diagnosis based on results of blood/cytogenetic analyses conducted on bone marrow aspirate; the diagnosis of AML was confirmed upon flow cytometry analysis. A new bone marrow aspirate was collected, but the sample was unfortunately lost and the physician did not want to collect another sample. One subject had a myelogram done but hemodilution of the smear occurred, so the result could not be used.

Table 3. Overall survival in the ITT population

Status OS (months)	Eryaspase + LDAC N=83	LDAC alone N=40
Patients died, n (%)	70 (84.3)	34 (85.0)
Median OS (95% CI) (months)	4.8 (3.1, 7.0)	6.4 (3.6, 10.7)
p-value	0.827	
Unadjusted hazard ratio (95% CI)	1.05 (0.69, 1.59)	
OS by treatment group - Cox PH model with prognostic factors		
p-value	0.815	
Adjusted hazard ratio (95% CI)	0.95 (0.60, 1.49)	

CI: confidence interval; LDAC; low-dose cytarabine; OS: overall survival; PH: proportional hazards

The adjusted hazard ratio (HR) for the primary endpoint of OS was 0.98 (95% CI; 0.70, 1.61).

Table 4. Progression-free survival in the ITT population

Status PFS (months)	Eryaspase + LDAC N=83	LDAC alone N=40
Patients progressed/died, n (%)	77 (92.8)	37 (92.5)
Median PFS (95% CI) (months)	4.0 (3.0 , 5.7)	6.2 (3.6 , 7.9)
p-value	0.319	
Hazard ratio (95% CI)	1.22 (0.82, 1.82)	
PFS by treatment group - Cox PH model with prognostic factors		
p-value	0.667	
Adjusted hazard ratio (95% CI)	1.10 (0.71, 1.69)	

CI: confidence interval; LDAC; low-dose cytarabine; PFS: progression-free survival; PH: proportional hazards

Figure 4. Overall survival by treatment group in the ITT population

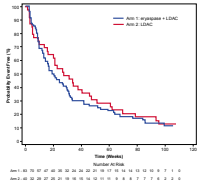
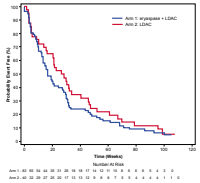


Figure 5. Progression-free survival by treatment group in the ITT population



ITT: intention-to-treat; LDAC: low-dose cytarabine

Table 5. Disease response in the ITT population

Status	Eryaspase + LDAC N=83	LDAC alone N=40
Overall response (CR+CRi*+PR), n (%)	20 (24.1)	9 (22.5)
Odds ratio, 95% CI	0.915 (0.373 , 2.242)	
p-value	0.845	
Best overall response		
CR, n (%)	13 (15.7)	7 (17.5)
CRi*, n (%)	5 (6.0)	1 (2.5)
PR, n (%)	2 (2.4)	1 (2.5)

CI: confidence interval; CR: complete remission; CRi: complete remission with incomplete recovery; ITT: intention-to-treat ; PR: partial response
*CR with residual neutropenia (<1.0 × 109/L [1000/µL]) or thrombocytopenia (<100 × 109/L [100,000/µL])

Figure 6. Forest plot of overall survival hazard ratios in prognostic factors for the ITT population

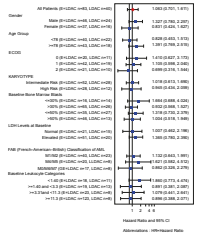
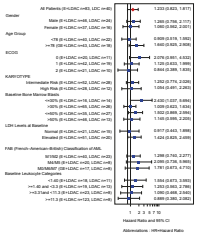


Figure 7. Forest plot of progression-free survival hazard ratios in prognostic factors for the ITT population



AML: acute myeloid leukemia; CI: confidence interval; E: eryaspase; ECOG: European Cooperative Oncology Group; HR: hazard ratio; ITT: intention-to-treat; LDAC: low-dose cytarabine; LDH: lactate dehydrogenase

Safety

Table 6. Exposure to treatment (safety population)

Status	Eryaspase + LDAC N=81	LDAC alone N=39
Total treatment exposure, eryaspase, weeks		
Median	4.9	–
Range	(0.1, 100.3)	–
Total treatment exposure, LDAC, weeks		
Median	6.1	5.9
Range	(0.6, 101.6)	(0.9,106.3)

LDAC; low-dose cytarabine

Table 7. Subsequent anti-neoplastic agents (ITT population)

Status, n (%)	Eryaspase+ LDAC N=83	LDAC alone N=40
Number of subjects with anti-neoplastic medications, n (%)	42 (50.6)	20 (50.0)
Hydroxyurea	19 (22.9)	11 (27.5)
Cytarabine	17 (20.5)	5 (12.5)
Azacitidine	15 (18.1)	3 (7.5)
Mercaptopurine	5 (6.0)	2 (5.0)
Etoposide	3 (3.6)	1 (2.5)
Methotrexate	2 (2.4)	2 (5.0)
Daunorubicin	1 (1.2)	2 (5.0)
Decitabine	1 (1.2)	2 (5.0)
Fludarabine	1 (1.2)	1 (2.5)
Arsenic trioxyde	1 (1.2)	0
Novantrone	0	1 (2.5)
Retinoic acid	1 (1.2)	0
Selinexor (Clinical Trial)	1 (1.2)	0
Study Drug (New Clinical Trial)	0	1 (2.5)

Table 8. Incidence of grade 3/4 treatment-emergent adverse events occurring in ≥10% of patients in either treatment arm, regardless of relationship to study drug (safety population)

Preferred Term, n (%)	Eryaspase + LDAC N=81	LDAC alone N=39
Patients with at least one AE	79 (97.5)	39 (100.0)
Anemia	60 (74.1)	32 (82.1)
Thrombocytopenia	59 (72.8)	32 (82.1)
Leukopenia	40 (49.4)	19 (48.7)
Neutropenia	35 (43.2)	18 (46.2)
Hypokalemia	13 (16.0)	8 (20.5)
Asthenia	11 (13.6)	5 (12.8)
Pyrexia	13 (16.0)	2 (5.1)
Febrile neutropenia	11 (13.6)	3 (7.7)
Hyponatremia	11 (13.6)	3 (7.7)
Abnormal pancreatic enzymes	12 (14.8)	2 (5.1)
Atrial fibrillation	1 (1.2)	4 (10.3)

Conclusions

This is the largest prospective study to date evaluating the efficacy and safety of an ASNase in elderly patients presenting with AML, and who are unfit to receive intensive chemotherapy (Table 1).

In this difficult-to-treat patient population, the addition of eryaspase to LDAC did not improve OS or PFS (Figures 4, 5, 6 and 7; Tables 3 and 4) .

Of interest, the median age group was 78 and 77 years in the eryaspase and LDAC arms, respectively. This is higher than the average age levels in other clinical trials in this subset of patient population, and could explain the lack of effect with the addition of eryaspase. When OS was assessed as a function of age, the Forest plot (Figure 6) showed a tendency towards improved outcome in patients <78 years, with a HR of 0.82, as compared with HR of 1.39 in patients >78 years.

The addition of eryaspase did not exacerbate the toxicity of LDAC.

Patient selection is likely to be the most important reason for the study not meeting its primary endpoint. The study enrolled unfit patients who only remained briefly on treatment to achieve a potential drug effect. The median duration of treatment exposure was approximately 5 weeks in both treatment arms, which is generally shorter than what has previously been reported.

Individualized risk based on multiparameter assessment tools and comorbidity burden should be considered for elderly patients with AML participating in clinical trials.

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