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Original Research

Erythrocyte-encapsulated asparaginase (eryaspase) combined with chemotherapy in second-line treatment of advanced pancreatic cancer: An open-label, randomized Phase IIb trial

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KEYWORDS
Erythrocyte;
Pancreatic adenocarcinoma;
Survival;
Asparaginase;
Asparagine synthetase

Abstract ***Purpose:*** This Phase IIb (NCT02195180) open-label study evaluated erythrocyte-encapsulated asparaginase (eryaspase) in combination with chemotherapy in second-line advanced pancreatic adenocarcinoma.
Methods: Eligible patients were randomized 2:1 to either eryaspase in combination with gemcitabine or mFOLFOX6 (eryaspase arm), or to gemcitabine or mFOLFOX6 alone (control arm). Co-primary endpoints were overall survival (OS) and progression-free survival (PFS) in patients with low asparagine synthetase (ASNS) expression. Secondary endpoints included OS and PFS in the entire population.
Results: 141 patients were randomized (eryaspase arm, n = 95; control arm, n = 46). Median OS and PFS in patients with low ASNS expression were 6.2 months (95% CI, 5.1–8.8) in the eryaspase arm versus 4.9 months (3.1–7.1) in the control arm (HR, 0.63; 95% CI, 0.39–1.01; $P = 0.056$) and 2.0 months (95% CI, 1.8–3.4) in the eryaspase arm versus 1.8 months (1.4–3.8) in the control arm (HR, 0.67; 95% CI, 0.40–1.12; $P = 0.127$), respectively. In the entire population, median OS and PFS for the eryaspase arm versus control were 6.0 months versus 4.4 months (HR, 0.60; $P = 0.008$) and 2.0 months versus 1.6 months (HR, 0.56; 95% CI, 0.37–0.84; $P = 0.005$), respectively. The combination of eryaspase and chemotherapy was well tolerated. The most frequent Grade 3/4 adverse events in the eryaspase arm (n = 93) were gamma-glutamyltransferase increase (16 [17.2%]), neutropenia (12 [12.9%]), and physical health deterioration (12 [12.9%]).
Conclusion: Eryaspase in combination with chemotherapy is associated with improvements in OS and PFS, irrespective of ASNS expression in second-line advanced pancreatic adenocarcinoma. A Phase III trial is underway.
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1. Introduction

Pancreatic adenocarcinoma is a leading cause of cancer-related deaths in the Western world and is of increasing incidence [1]. Despite the introduction of new agents, prognosis remains generally poor with a 5-year survival of less than 5% [1]. First-line chemotherapy for metastatic disease is still primarily oxaliplatin and folinic acid with 5-fluorouracil (5-FU) or gemcitabine with nab-paclitaxel [2,3]. Although 40–50% patients failing on first-line therapy are suitable for second-line treatment, there is a lack of consensus on optimal therapy for these patients [1]. Although gemcitabine after first-line oxaliplatin is an option, outcomes are suboptimal with disease control achieved in only one in five patients [4].

An important feature of pancreatic adenocarcinoma is the high prevalence of KRAS mutations, which occur in 90% of patients, and result in the constitutive activation of RAF/MEK/ERK and PIK3/AKT-mTOR pathways [5]. Constitutive KRAS signalling is associated with the dysregulation of metabolic pathways leading to addictions to metabolites, such as glutamine and asparagine, used by non-canonical metabolic pathways [5,6]. Indeed, glutamine deprivation and/or inhibition of enzymes downstream of KRAS results in suppression of pancreatic adenocarcinoma cell growth [9]. Thus, modulation of glutamine and asparagine levels may represent a critical vulnerability of these cells [6,7].

Cellular synthesis of asparagine from aspartate and glutamine is catalysed by asparagine synthetase (ASNS). Resistance to asparaginase (ASNase) treatment in acute lymphoblastic leukaemia (ALL) has been associated with changes in ASNS expression [8]. As pancreatic tumours are notoriously hypovascular and enhanced ASNS expression is involved in adaptation responses to hypoxia and glucose deprivation, it is hypothesized that ASNS expression may be a predictive factor for ASNase susceptibility in pancreatic carcinomas [9].

Although ASNase is a key component of chemotherapy for ALL, clinical studies in solid tumours have been limited by their narrow therapeutic index and associated toxicities. A novel approach to delivering ASNase at therapeutic doses with reduced propensity for toxicities is from encapsulation of ASNase within erythrocytes (eryaspase) through a proprietary process. The RBCs used for the production of eryaspase are leukoreduced packed RBC units, which are manufactured and qualified by an approved blood bank

according to current approved practices in Europe and the USA, and therefore, these follow all the safety measures put in place by health authorities for the preparation of blood products for transfusion.

The encapsulated ASNase remains biologically active, with a half-life of approximately 2 weeks. A Phase I study of single-agent eryaspase in metastatic pancreatic adenocarcinoma showed it to be well tolerated, with a recommendation for a Phase II eryaspase dose of 100 U/Kg [10].

This open-label, multicenter, randomized, Phase IIb trial assessed the efficacy and safety of eryaspase as second-line therapy in combination with chemotherapy in patients with advanced pancreatic adenocarcinoma.

2. Methods

2.1. Study design

This multicenter, open-label, randomized, Phase IIb trial, sponsored by ERYtech Pharma, was conducted at 16 centres in France under the auspices of the GERCOR (Groupe Coopérateur Multidisciplinaire en Oncologie). The trial was conducted in accordance with the protocol and principles of the International Conference of Harmonization Good Clinical Practices and Declaration of Helsinki and was approved by an independent ethics committee. Eligible patients were randomized in a 2:1 ratio to receive either eryaspase in combination with gemcitabine or mFOLFOX6 (eryaspase arm), or gemcitabine or mFOLFOX6 alone (control arm). Chemotherapy choice was determined by prior first-line therapy (gemcitabine or mFOLFOX6). Random assignment was stratified according to the first-line therapy. Co-primary endpoints in the study were OS and PFS in patients with low ASNS expression (ASNS 0/1+). Secondary endpoints included overall survival (OS) and progression-free survival (PFS) in the entire population, objective response rate (ORR), disease control rate (DCR), treatment compliance, safety, and quality of life (QoL).

2.2. Patients

Eligible patients were $\geq$18 years with histologically confirmed, non-resectable, metastatic pancreatic adenocarcinoma who had progressed during, or following, the first-line therapy. They had also received one prior systemic therapy for advanced disease and had measurable disease by RECIST version 1.1 and a European Cooperative Oncology Group performance status (ECOG-PS) of 0/1. Patients were excluded for known hypersensitivity or prior exposure to any form of ASNase or presence of inadequate organ function. All patients provided written informed consent before screening.

2.3. Treatment and study assessments

In the 28-day treatment cycle, eryaspase 100 U/kg was administered by intravenous (IV) infusion on Days 3 and 17, gemcitabine 1000 mg/m^2 by 30-min IV perfusion on Day 1 weekly for 3 weeks, and mFOLFOX6 (oxaliplatin 85 mg/m^2 IV on Day 1, leucovorin 400 mg/m^2, 5-FU 400 mg/m^2 by IV bolus, and continuous IV infusion of 5-FU 2400 mg/m^2 by continuous IV infusion over 46 h) every 2 weeks. Dose modifications were permitted according to protocol-specified algorithms.

Locally performed radiological (magnetic resonance imaging [MRI] or computed tomography [CT] scan) tumour assessments were undertaken every 8 weeks from randomization until disease progression, initiation of a new cancer treatment, or death, according to RECIST version 1.1. These results were centrally reviewed by independent radiologists. Adverse events (AE) were graded using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE). An independent data safety monitoring board conducted interim safety assessments (when six and then 24 patients had received eryaspase with gemcitabine, and six patients had received eryaspase with mFOLFOX6). Clinical assessments (physical examination, vital signs, and ECOG-PS) were performed 4 weekly and standard laboratory assessments every 2 weeks. QoL, assessed using the European Organization for Research and Treatment of Cancer Quality-of-Life Core Questionnaire (EORTC-QLQ-C30) version 3 and the EORTC-QLQ-PAN26 was assessed at baseline, at Weeks 2 and 4 of treatment cycle 1, and at the end of each subsequent cycle.

ASNS expression was determined prior to randomization by immunohistochemistry using the automated IHC Ventana® slide staining system (BenchMark GX). In brief, paraffin-embedded slides from archival tissue samples were labelled with anti-ASNS polyclonal antibody (ref. HPA029318, Sigma-Aldrich). ASNS scoring was based on staining intensity, graded using a four-point scale: 0: not detected; 1: weak; 2: moderate; 3: strong.

2.4. Statistical analyses

The primary analysis population was the ASNS 0/1+ subgroup. Key secondary analyses were performed in all comers. The original primary endpoint was PFS at 16 weeks with sample size calculated assuming the null hypothesis H0: $P \leq 25\%$ against the one-sided alternative hypothesis HA: $P > 40\%$, in ASNS 0/1+ patients who were randomized to eryaspase. For 80% power, 62 ASNS 0/1+ patients would be required, assuming a PFS rate at 16 weeks of 40% in that arm. It was expected that between 70% and 80% of eligible patients would be ASNS 0/1+ and, to additionally incorporate a 2:1 randomization, the total sample size was predicted to be

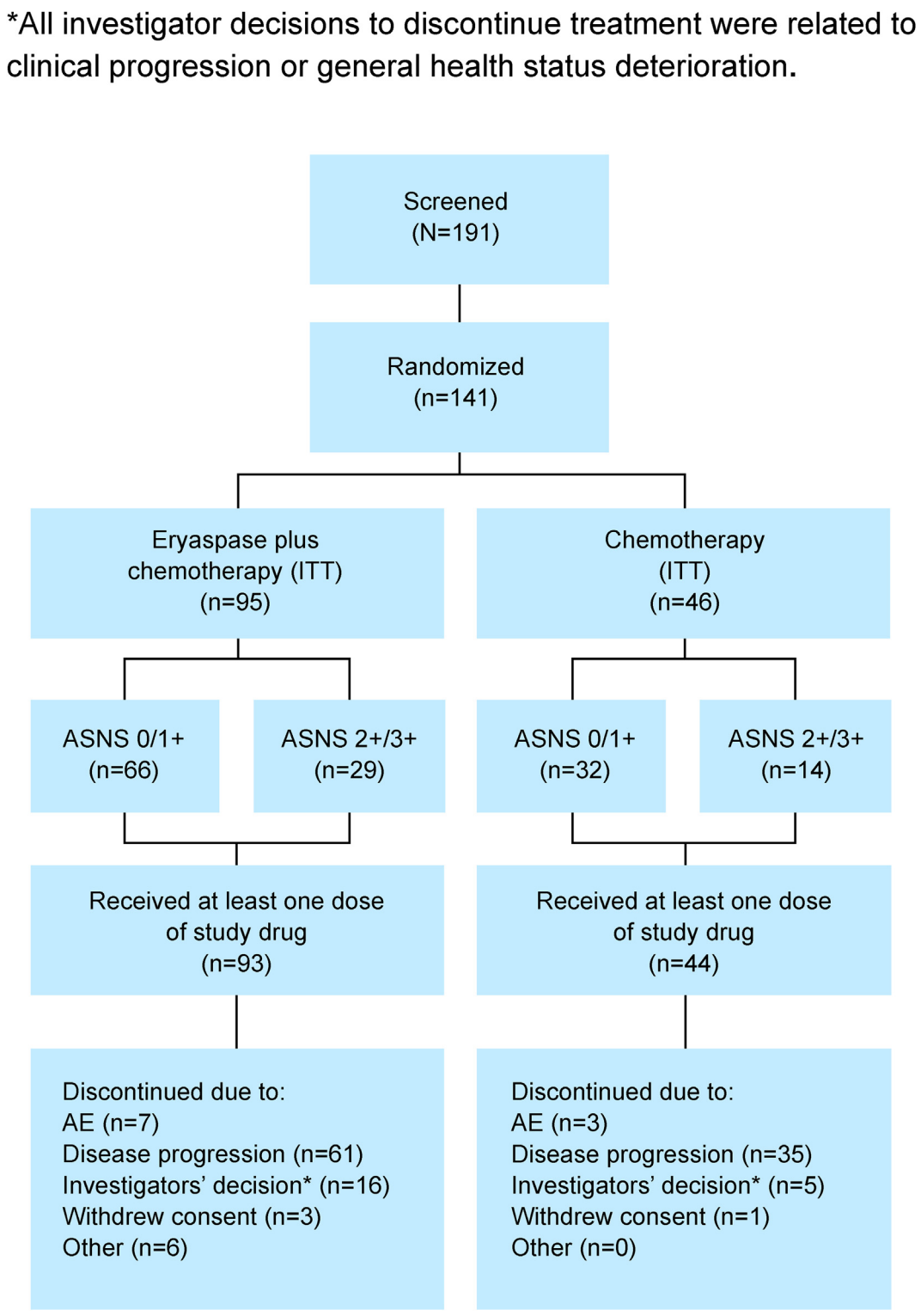

Fig. 1. Trial profile.

between 116 and 133. For an agent such as eryaspase that targets one or more metabolic pathways and in the absence of precedence of demonstrable clinical activity with similar agents in pancreatic cancer, it was unknown whether the disease-modifying effects could be observed early or late during the disease course. In addition, a review of literature indicated that effects could be seen in terms of either PFS or OS, or both. Therefore, the primary endpoint was amended (October 2016) with PFS and OS in the ASNS 0/1+ subgroup as co-primary endpoints. This was considered more appropriate as opposed to a landmark PFS rate at 16 weeks. Based on

discussions with clinical experts, an hazards ratio (HR) for either OS or PFS < 0.85 was to be viewed as an encouraging signal of activity. The sample size was not modified, as the focus was on the point estimates of the HRs rather than on formal statistical significance. OS was defined as the interval from randomization until death; PFS was defined as the interval from randomization until disease progression, including clinical progression, as per RECIST version 1.1, or death. Time-to-event data were summarized using the Kaplan–Meier methodology and HRs were obtained from the stratified Cox proportional hazards model, stratified by a background chemotherapy regimen. ORR and DCR were compared using a stratified Cochran–Mantel–Haenszel test. All QoL endpoints from the EORTC-QLQ-C30 and EORTC-PAN26 questionnaires were summarized at baseline and for each visit, along with change from baseline. Safety analyses were based on the safety population, which included all patients who received ≥one dose of study drug. All statistical analyses were performed with SAS, version 9.2 or higher.

3. Results

3.1. Baseline

Between July 2014 and October 2016, 141 patients were randomized to the eryaspase (n = 95) or control (n = 46) arms (Fig. 1). As first-line chemotherapy had been principally fluoropyrimidine-based, 84 (88.4%) and 41 (89.1%) patients in the eryaspase and control arms, respectively, received gemcitabine. Demographic and baseline characteristics were generally comparable between treatment arms (Table 1).

Table 1
Baseline demographic and disease characteristics for the intent-to-treat population.

Status[a]	Eryaspase plus chemotherapy (N = 95)	Chemotherapy alone (N = 46)	Total (N = 141)
Gender, n (%)			
Male	53 (55.8)	30 (65.2)	83 (58.9)
Female	42 (44.2)	16 (34.8)	58 (41.1)
Age at randomization, years			
Mean (SD)	62.7 (10.2)	62.4 (8.7)	62.6 (9.7)
Median	63	63	63
Range	(37–84)	(43–80)	(37–84)
ECOG-PS, n (%)			
0	29 (31.5)	11 (25.6)	40 (29.6)
1	63 (68.5)	32 (74.4)	95 (70.4)
CA19–9			
Mean (kU/L)	13268.7	10420.4	12,426.6
(SD)	(40730.7)	(21448.3)	(36042.7)
Time interval from initial diagnosis of advanced disease to randomization, months			
Mean (SD)	10.7 (10.5)	10.7 (7.9)	10.7 (9.7)
Median	8	9	8
Range	(2–87)	(3–39)	(2–87)
Stage at initial diagnosis, n (%)			
I/II	15 (15.8)	8 (17.3)	23 (16.2)
III	8 (8.4)	5 (10.9)	13 (9.2)
IV	72 (75.8)	33 (71.7)	105 (74.5)
Main sites of metastasis, n (%)			
Liver	73 (77.7)	37 (80.4)	110 (78.6)
Lung	23 (24.5)	8 (17.4)	31 (22.1)
Peritoneum	21 (22.3)	9 (19.6)	30 (21.4)
Number of metastatic sites, n (%)			
0–1	60 (63.2)	35 (76.1)	95 (67.4)
2	30 (31.6)	9 (19.6)	39 (27.7)
≥3	5 (5.3)	2 (4.3)	7 (5.0)
ASNS scoring intensity, n (%)	95	46	141
0/1+	66 (69.5)	32 (69.6)	98 (69.5)
2+/3+	29 (30.5)	14 (30.4)	43 (30.5)
Prior systemic therapy, n (%)	95	46	141
Yes	94 (98.9)[b]	46 (100.0)	140 (99.3)
Prior best overall response, n (%)			
Objective response and stable disease	63 (66.3)	30 (65.2)	93 (65.9)
Progressive disease	30 (31.6)	16 (34.8)	46 (32.6)
Non-evaluable	1 (1.1)	0	1 (0.7)

ASNS, asparagine synthetase; CA19-9, cancer antigen 19-9; ECOG-PS, Eastern Cooperative Oncology Group Performance Status; SD, standard deviation.

[a] *n* in each summary is the number of patients with non-missing data for the category – percentages are calculated with the number of randomized patients with non-missing data in each group as denominator.

[b] One patient was randomized outside the inclusion criteria.

3.2. Efficacy

Median patient follow-up was 6.1 months in the eryaspase arm and 4.7 months in the control arm. In patients with ASNS 0/1+, median OS was 6.2 months (95% CI, 5.1–8.8) in the eryaspase arm and 4.9 months (95% CI, 3.1–7.1) for the control (HR, 0.63; 95% CI, 0.39–1.01; $P = 0.056$) (Table 2; Fig. 2A). Median PFS in this ASNS group was 2.0 months (95% CI, 1.8–3.4) in the eryaspase arm and 1.8 months (95% CI, 1.4–3.8) for the control (HR, 0.67; 95% CI, 0.40–1.12; $P = 0.127$) (Table 2; Fig. 2B).

In the entire population (ASNS 0/1+ and ASNS 2+/3+), median OS was 6.0 months (95% CI, 4.8–6.6) and 4.4 months (95% CI, 3.0–5.0) in the eryaspase and control arms, respectively (HR, 0.60; 95% CI, 0.41–0.87; $P = 0.008$) (Table 2; Fig. 2C), with respective median PFS values of 2.0 months (95% CI, 1.8–3.4) and 1.6 months (95% CI, 1.4–1.8) (HR, 0.56; 95% CI, 0.37–0.84; $P = 0.005$) (Table 2; Fig. 2D).

In patients with ASNS 2+/3+, median OS was 4.8 months (95% CI, 3.4–6.8) in the eryaspase arm and 2.7 months (95% CI, 1.6–4.5) for the control (HR, 0.52; 95% CI, 0.26–1.04; $P = 0.063$) (Table 2; Fig. 2E), with respective median PFS values of 1.9 months (95% CI,

Table 2
Summary of efficacy measures by population and treatment group.

	Entire population		ASNS 0/1+		ASNS 2+/3+	
	E + CT (N = 95)	CT (N = 46)	E + CT (N = 66)	CT (N = 32)	E + CT (N = 29)	CT (N = 14)
OS						
Event rate, n (%)	82 (86.3)	42 (91.3)	55 (83.3)	28 (87.5)	27 (93.1)	14 (100.0)
Median OS, months	6.0	4.4	6.2	4.9	4.8	2.7
95% CI	4.8–6.6	3.0–5.0	5.1–8.8	3.1–7.1	3.4–6.8	1.6–4.5
HR	0.60		0.63		0.52	
95% CI	0.41–0.87		0.39–1.01		0.26–1.04	
p	0.008		0.056		0.063	
6-month OS, %	50.6	35.8	53.3	46.0	44.4	14.3
PFS[a,b]						
Event rate, n (%)	70 (73.7)	36 (78.3)	50 (75.8)	23 (71.9)	20 (69.0)	13 (92.9)
Median PFS, months	2.0	1.6	2.0	1.8	1.9	1.4
95% CI	1.8–3.4	1.4–1.8	1.8–3.4	1.4–3.8	1.6–3.4	1.0–1.6
HR	0.56		0.67		0.38	
95% CI	0.37–0.84		0.40–1.12		0.18–0.83	
p	0.005		0.127		0.015	
6-month PFS, %	17.2	2.9	20.4	4.5	9.5	0.0
Response[a,b]						
ORR, n (%)	12 (12.6)	3 (6.5)	10 (15.2)	3 (9.4)	2 (6.9)	0 (0.0)
95% CI	6.7–21.0	1.4–17.9	7.5–26.1	2.0–25.0	0.8–22.8	0.0–23.2
SD, n (%)	34 (35.8)	8 (17.4)	21 (31.8)	7 (21.9)	13 (44.8)	1 (7.1)
PD, n (%)	42 (44.2)	31 (67.4)	32 (48.5)	18 (56.3)	10 (34.5)	13 (92.9)
NE[c], n (%)	7 (7.4)	4 (8.7)	3 (4.5)	4 (12.5)	4 (13.8)	0 (0.0)
DCR[d], n (%)95% CI	46 (48.4)	11 (23.9)	31 (47.0)	10 (31.3)	15 (51.7)	1 (7.1)
	38.0–58.9	12.6–38.8	34.6–59.7	16.1–50.0	32.5–70.6	0.2–33.9

ASNS, asparagine synthetase; CI, confidence interval; DCR, disease control rate; CT, chemotherapy; E + CT, eryaspace plus chemotherapy; HR, hazard ratio; NE, no follow-up scans; ORR, objective response rate; OS, overall survival; PD, progressive disease; PFS, progression-free survival; SD, stable disease.

[a] Based on independent review.

[b] Per RECIST criteria, version 1.1.

[c] 4 consent withdrawal, 4 randomized but not treated, 1 fatal event, 1 target lesions unassessed, 1 treated but discontinued treatment before follow-up scans. NE was similar between investigator and independent review.

[d] CR + PR + SD.

1.6–3.4) and 1.4 months (95% CI, 1.0–1.6) (HR, 0.38; 95% CI, 0.18–0.83; $P = 0.015$) (Table 2; Fig. 2F).

In the pre-determined subgroup analyses of OS and PFS, the eryaspase arm was favoured across all subgroups assessed; there was no evidence of heterogeneity across the population as a whole (Fig. 3).

ORR and DCR (complete response, partial response, and stable disease) results are reported in Table 2. Complete responses were exhibited in two patients receiving eryaspase (Table 2; Fig. 4).

For QoL, mean change from baseline in the global EORTC-QLQ-C30 score at Week 4 of Course 1 was −4.0 (SD, 18.4) in the eryaspase arm (n = 48) and −6.4 (SD, 16.3) in the control arm (n = 22).

3.3. Adherence to treatment

Treatment compliance was similar between groups (mean values; eryaspase 86.2% [SD, 14.8] versus control 87.5% [SD, 16.0]). Twenty (21.1%) patients in the eryaspase arm and seven (15.2%) patients in the control arm completed six treatment cycles. Exposure to chemotherapy was longer in the eryaspase arm than the control arm (Supplementary Table 1). In the eryaspase/gemcitabine group (n = 83), 65.1% patients required a dose delay, and 27.7% a gemcitabine dose reduction. This compared with 59.0% and 17.9% patients, respectively, in the gemcitabine alone group. Of the 10 patients treated with eryaspase plus mFOLFOX6, eight required a dose delay and five required a dose reduction of at least one component of the mFOLFOX6 regimen, which was similar to that in the mFOLFOX6 alone arm.

3.4. Safety

Safety results are reported in Table 3. Overall, the incidence of AEs was generally similar between treatments. In both treatment arms, asthenia was the most frequent AE, followed by nausea. Haematological AEs were more frequent with combination therapy compared with chemotherapy alone; however, Grade 3/4 haematological AEs occurred with similar frequency between treatment arms. Discontinuations for treatment-related AEs were reported in 29% and 18.2% of patients in the eryaspase and control arms, respectively. One or more AEs with a fatal outcome were reported in 10 (10.8%) eryaspase-treated patients and in eight (18.2%) control patients (Supplementary Table 2). These fatal outcomes were not considered treatment-related in the eryaspase arm.

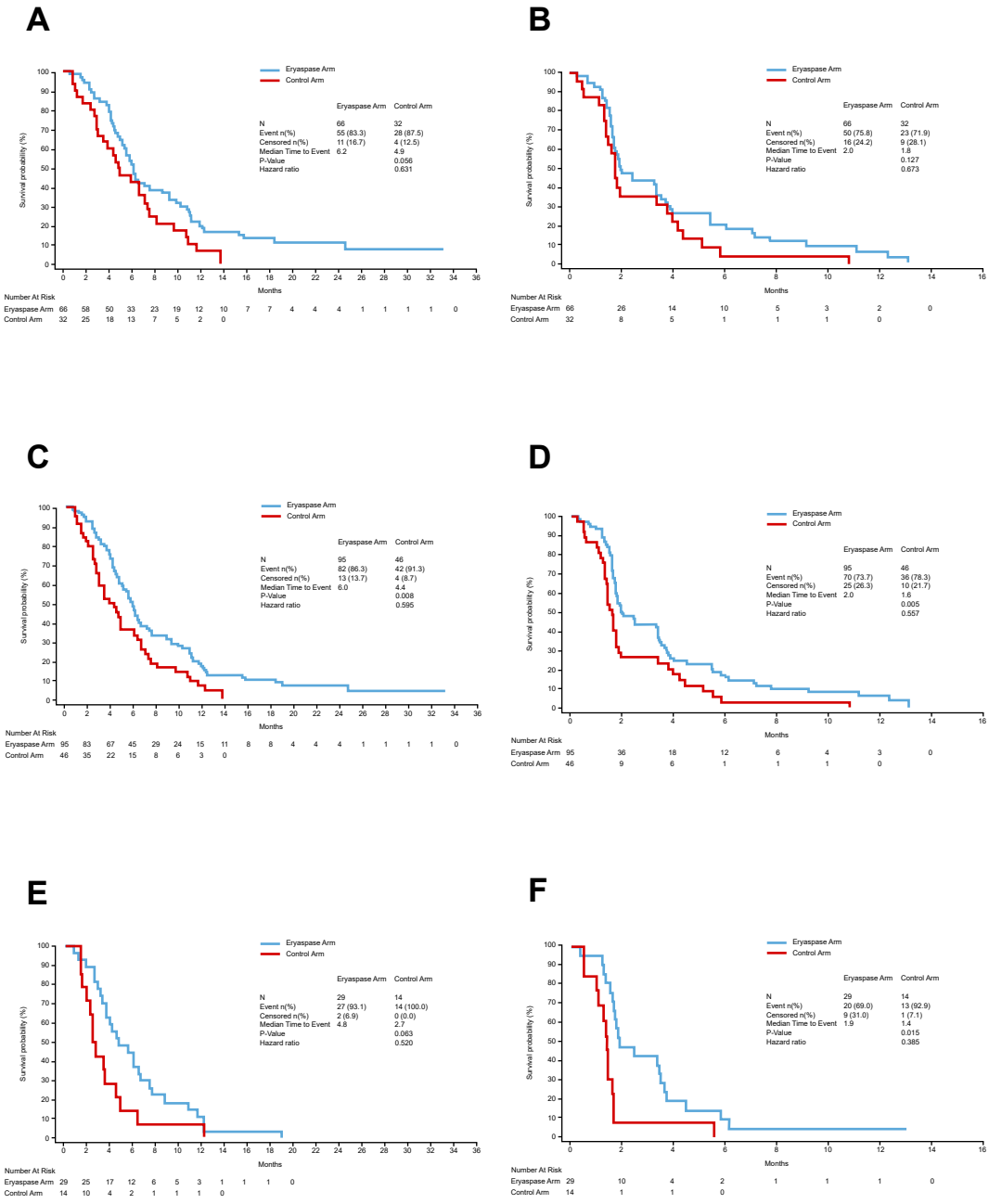

Fig. 2. Kaplan–Meier curves depicting OS in ASNS 0/1+ population (A); PFS in ASNS 0/1+ population (B); OS in entire population (C); PFS in entire population (D); OS in ASNS 2+/3+ population (E); PFS in ASNS 2+/3+ population (F).

4. Discussion

In this open-label, randomized, Phase IIb study of eryaspase in combination with chemotherapy for second-line treatment of advanced pancreatic adenocarcinoma, eryaspase demonstrated an encouraging signal of activity for the co-primary endpoints of OS and PFS in the ASNS 0/1+ population. In addition, eryaspase with chemotherapy significantly prolonged OS and PFS in the entire ASNS population compared with chemotherapy alone, with a 40% reduction in risk of death on average over time. In a pre-planned subgroup analyses, the effect of eryaspase on OS and PFS was maintained across all subgroups; there was no evidence of heterogeneity in the treatment effect. In addition, treatment with eryaspase led to encouraging improvements in ORR and DCR in the entire population, as well as in the ASNS 0/1+ subgroup.

To our knowledge, this study represents the largest cohort of patients treated with gemcitabine alone after fluoropyrimidine-based chemotherapy. Thus, our results are consistent with those for nanoliposomal irinotecan and also with those in the gemcitabine post-FOLFIRINOX setting [11,12].

ASNS=asparagine synthetase; CA19.9=cancer antigen 19.9; CI=confidence interval; CR=complete response; ECOG=European Cooperative Oncology Group; HR=hazard ratio; OR=objective response; PD=progressive disease; SD=stable disease.

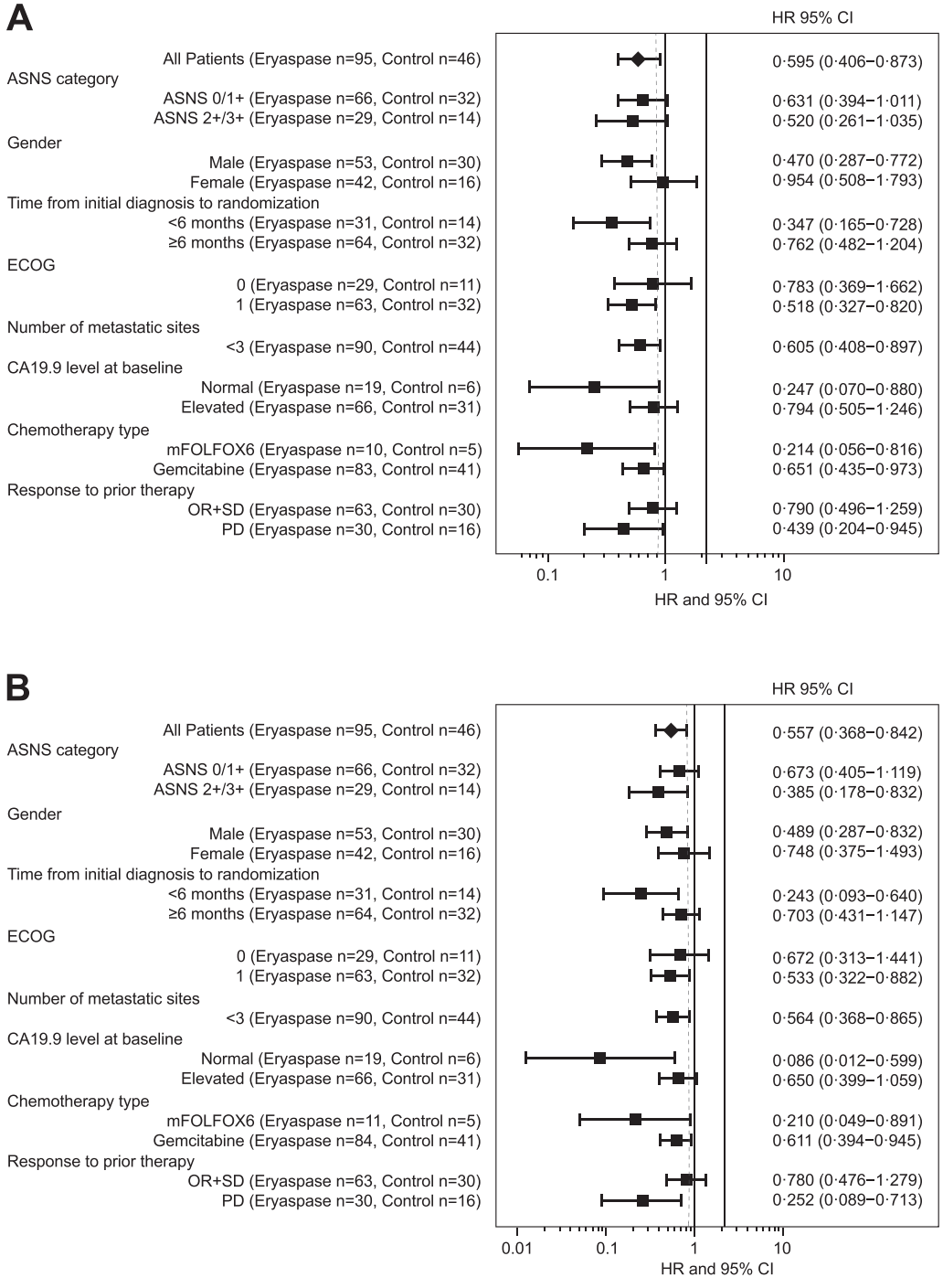

Fig. 3. Forest plot of overall survival (A) and progression-free survival (B; independent assessment) hazard ratios in prognostic factors (intention-to-treat population).

***Patients with new tumours whose best overall response was progressive disease were assigned a value of +150% to highlight the poorest 'best outcome'.**

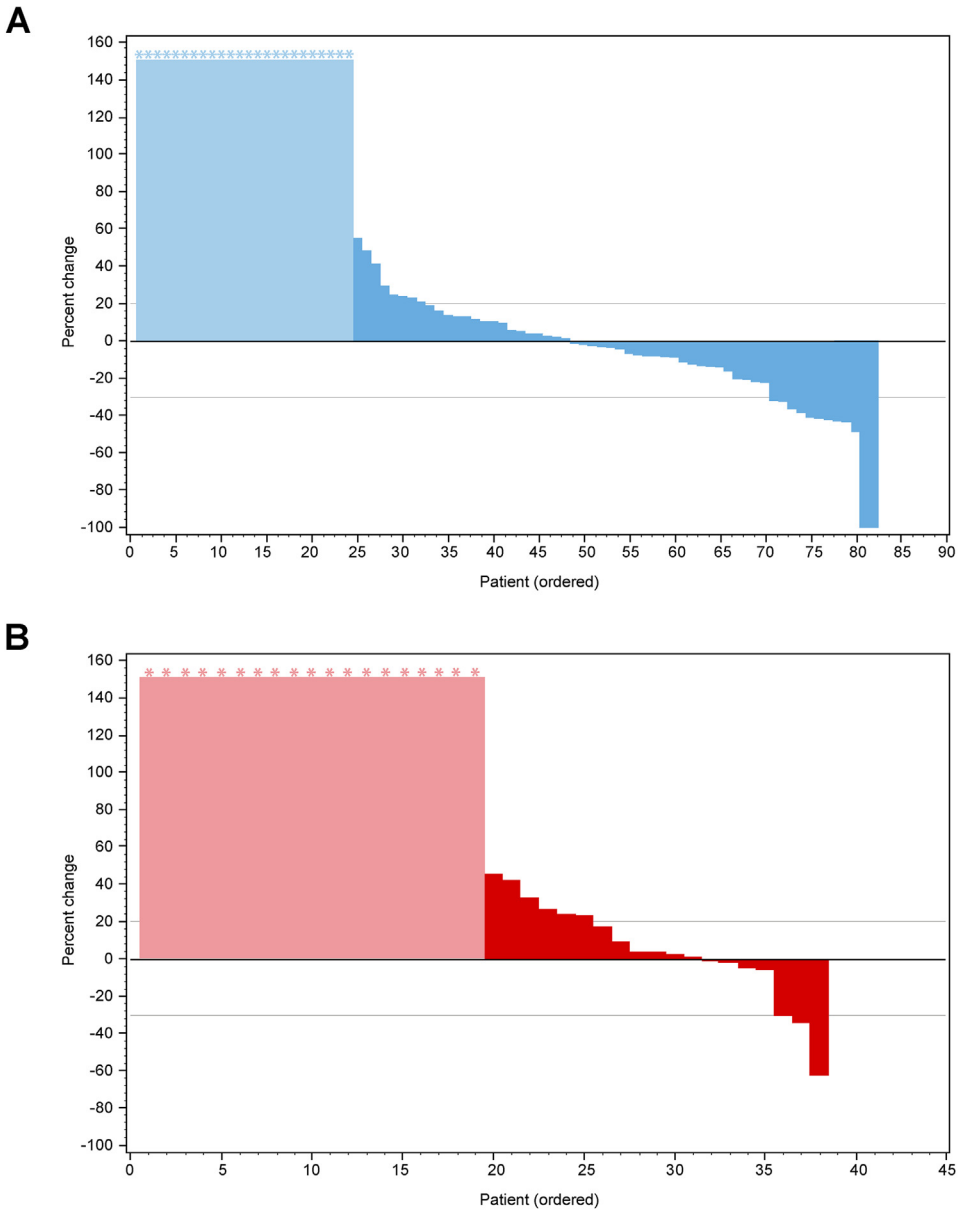

Fig. 4. Waterfall plot of best response in tumour percentage change from baseline in the sum of longest diameter, based on independent radiological review eryaspase arm (A) and control arm (B).

This is the first study to prospectively evaluate any association between ASNS protein expression and clinical outcomes in ASNase-treated metastatic pancreatic adenocarcinoma. These results appear counterintuitive, given previous reports suggesting that low ASNS expression in lymphoblastic cells renders them susceptible to asparagine depletion, and that upregulation of ASNS mRNA/protein levels are associated with resistance to ASNase [13–15]. However, being *in vitro* studies, in which various metabolic pathway products and equilibrium conditions could be altered, these findings may not be analogous to *in vivo* results [16]. Clinically, several studies have demonstrated a lack of correlation between ASNS mRNA/protein levels and ASNase sensitivity in ALL [17,18]. It is plausible that ASNS serves as a prognostic indicator (as opposed to predictive indicator) of ASNase sensitivity in ALL or other malignancies. In pancreatic cancer cells, enhanced

Table 3
Most frequent (≥10% of patients in either treatment arm) adverse events, regardless of relationship to study drug.

Preferred term	Eryaspase plus chemotherapy (N = 93)		Chemotherapy (N = 44)	
	All grades	Grades 3/4	All grades	Grades 3/4
Patients with ≥1 AE	93 (100.0)	73 (78.5)	44 (100.0)	38 (86.4)
Asthenia	64 (68.8)	6 (6.5)	29 (65.9)	12 (27.3)
Nausea	58 (62.4)	4 (4.3)	26 (59.1)	1 (2.3)
Anaemia	42 (45.2)	8 (8.6)	22 (50.0)	5 (11.4)
Vomiting	41 (44.1)	4 (4.3)	15 (34.1)	2 (4.5)
Thrombocytopaenia	40 (43.0)	9 (9.7)	16 (36.4)	4 (9.1)
Abdominal pain	33 (35.5)	7 (7.5)	17 (38.6)	4 (9.1)
Diarrhoea	37 (39.8)	3 (3.2)	13 (29.5)	0 (0.0)
Decreased appetite	28 (30.1)	2 (2.2)	16 (36.4)	5 (11.4)
Pyrexia	28 (30.1)	1 (1.1)	12 (27.3)	0 (0.0)
Constipation	25 (26.9)	1 (1.1)	12 (27.3)	1 (2.3)
Neutropenia	23 (24.7)	12 (12.9)	7 (15.9)	5 (11.4)
GGT increased	18 (19.4)	16 (17.2)	11 (25.0)	11 (25.0)
Physical health deterioration	17 (18.3)	12 (12.9)	7 (15.9)	2 (4.5)
Antibody test positive	16 (17.2)	1 (1.1)	0 (0.0)	0 (0.0)
Weight decreased	15 (16.1)	1 (1.1)	9 (20.5)	0 (0.0)
Peripheral oedema	16 (17.2)	0 (0.0)	7 (15.9)	0 (0.0)
Upper abdominal pain	14 (15.1)	2 (2.2)	9 (20.5)	3 (6.8)
Stomatitis	15 (16.1)	0 (0.0)	5 (11.4)	0 (0.0)
ALT increased	14 (15.1)	6 (6.5)	2 (4.5)	2 (4.5)
Hypokalemia	13 (14.0)	4 (4.3)	3 (6.8)	1 (2.3)
Neuropathy peripheral	13 (14.0)	2 (2.2)	9 (20.5)	3 (6.8)
Fatigue	12 (12.9)	3 (3.2)	8 (18.2)	4 (9.1)
Back pain	11 (11.8)	0 (0.0)	5 (11.4)	0 (0.0)
Cough	11 (11.8)	0 (0.0)	3 (6.8)	0 (0.0)
Mucosal inflammation	11 (11.8)	0 (0.0)	3 (6.8)	1 (2.3)
Alopecia	11 (11.8)	1 (1.1)	1 (2.3)	0 (0.0)
Hyperthermia	11 (11.8)	1 (1.1)	0 (0.0)	0 (0.0)
Lymphopenia	10 (10.8)	3 (3.2)	3 (6.8)	2 (4.5)
Hyperglycemia	10 (10.8)	7 (7.5)	3 (6.8)	2 (4.5)
AST increase	10 (10.8)	3 (3.2)	1 (2.3)	1 (2.3)
Anxiety	9 (9.7)	1 (1.1)	6 (13.6)	0 (0.0)
Hypoalbuminemia	8 (8.6)	1 (1.1)	8 (18.2)	1 (2.3)
Insomnia	8 (8.6)	0 (0.0)	6 (13.6)	0 (0.0)

AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase.

ASNS expression may convey protection against apoptosis induced by glucose deprivation and cisplatin, indicating a shift in cellular metabolism [9]. Our study appears to indicate that high ASNS expression could be an indicator of poor prognosis, as reflected by the differing OS outcomes between ASNS groups. However, some caution should be applied because, in this study, ASNS testing was performed on archival tissues.

The combination of eryaspase and chemotherapy was generally well tolerated; no unexpected safety findings were reported, and eryaspase did not add substantially to the toxicity of chemotherapy.

Potential limitations of the study are from the change in the primary endpoint and no formal control of multiplicity. All analyses were planned *a priori*, and the change was made prior to data unblinding and final analyses. However, multiple sensitivity analyses suggest that the findings are robust (Supplementary Tables 3 and 4). As a proof-of-concept study, the co-primary endpoints relied on numerical values, rather than statistical significance. Consistency of the clinical effect across all subpopulations supports the external validity of these results.

In conclusion, addition of eryaspase to chemotherapy confers clinical benefit, irrespective of ASNS tumour-expression status, when used in the second-line treatment of advanced pancreatic adenocarcinoma. To our knowledge, this is the only Phase IIb study investigating ASNase in pancreatic cancer or in any other solid tumour. Furthermore, this is the only prospective study assessing the predictive value of ASNS expression. A confirmatory Phase III study will assess the OS benefit of eryaspase in combination with gemcitabine and nab-paclitaxel versus nanoliposomal irinotecan-based chemotherapy in second-line advanced pancreatic cancer.

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Conflict of interest statement

PH has received personal fees from Amgen, AstraZeneca, Celgene, Halozyme, Merck Serono, Shire, Sanofi, Servier, and non-financial support from AstraZeneca, Celgene, and Halozyme. JBB has received personal fees from Amgen, Bayer, Celgene, Merck Serono, Roche, Sanofi, Servier, and non-financial support from Amgen, Merck Serono, and Roche. TA has received honoraria from Lilly, Sanofi Aventis, and Yakult. OB reports grants from Roche and Pierre Fabre and personal fees outside the submitted work from Roche, Pierre Fabre, Amgen, Bayer, Lilly, Merck, and Novartis. LM, CDLF, PF, DT, RG, AH, JC, RK, RF, CT, CL, and TL have no interests to declare. AN and IEH are employees of ERYtech Pharma.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2019.10.020.

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