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Original research

# A randomized phase II trial of Captem or Folfiri as second-line therapy in neuroendocrine carcinomas

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ABSTRACT

*Background:* Neuroendocrine Carcinomas (NECs) prognosis is poor.No standard second-line therapy is currently recognized after failure of platinum-based first-line treatment. FOLFIRI and CAPTEM regimens have shown promising activity in preliminary studies. We aimed to evaluate these regimens in metastatic NEC patients.
*Methods:* This is an open-label, multicenter, randomized non-comparative phase II trial to evaluate the activity and safety of FOLFIRI or CAPTEM in metastatic NEC patients. Primary endpoints were the 12 weeks-Disease Control Rate (12w-DCR) by investigator assessment per RECIST v1.1 and safety per CTCAE v5.0. Additional endpoints included overall response rate (ORR), progression-free survival (PFS) and overall survival (OS).

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Patients' serum samples were subject to NGS miRNome profiling in comparison with healthy donors to reveal differentially expressed miRNAs as candidate circulating biomarkers.
*Results:* The study was halted for futility at interim analysis, as the minimum 12w-DCR threshold of 10 out of 25 patients required for the first step was not reached. From 06/03/2017 to 18/01/2021, 53 out of 112 patients were enrolled. Median follow-up was 22.6 months (range: 1.4–60.4). The 12w-DCR was 39.1 % in the FOLFIRI arm and 28.0 % in the CAPTEM arm. In the FOLFIRI subgroup the 12-months OS rate was 28.4 % (95 % CI: 12.7–46.5) while in the CAPTEM subgroup it was 32.4 % (95 % CI: 14.9–51.3). The most common G3-G4 side effects were neutropenia ($n = 5$, 18.5 %) and anemia ($n = 2$, 7.4 %) for FOLFIRI and G3-G4 thrombocytopenia ($n = 2$, 8.0 %), G4 nausea/vomiting ($n = 1$, 4.0 %) for CAPTEM. Three microRNAs emerged as NEC independent predictors. High expression values were found to be significantly associated with decreased PFS and OS.
*Conclusion:* The safety profile of FOLFIRI and CAPTEM was manageable. FOLFIRI and CAPTEM chemotherapy showed comparable activity in the second-line setting after progression on etoposide/platinum.
*ClinicalTrials.gov identifier:* NCT03387592

## 1. Introduction

Neuroendocrine Carcinomas (NECs) are very rare malignancies, representing only 5 %– 10 % of Neuroendocrine Neoplasia (NENs). [1–3] These tumors are characterized by aggressive histological features (high Ki-67 index, extensive necrosis, and nuclear atypia) and are classified as grade (G)3 NECs according to the new World Health Organization (WHO) classification.[4]

Platinum-based combinations are the gold standard for the treatment of NECs, and several studies published in the 1990s reported substantial antitumor activity and high response rates (41 %–67 %). [5] However, responses are usually short-lasting, with a median progression-free survival of 9 months and a median overall survival of 15–19 months. When progression occurs after first-line chemotherapy, the disease is usually very aggressive and patients succumb rapidly. [6].

Given the rarity of this disease, prospective clinical data are lacking and treatment recommendations are essentially expert-based opinions. For this reason, several efforts have recently focused on the identification of new treatment options for these patients. A French multicenter prospective phase II trial investigating the efficacy of the bevacizumab-FOLFIRI combination after progression on platinum-etoposide has been recently completed and data presented. [7] Different second-line chemotherapy combinations have been evaluated showing modest results. [6,8,9] In a single-institution retrospective clinical trial, Hentic et al. observed an objective response in 31 % of patients with extrapulmonary NECs treated with FOLFIRI as second-line therapy and a disease control rate (DCR) of 62 %. Median progression-free survival (PFS) and overall survival (OS) were 4 and 18 months, respectively.[10] In another retrospective study, a 71 % DCR was obtained with temozolomide-based chemotherapy. A OS of 22 months was reported in patients who responded to treatment or showed stable disease (SD), whereas OS was only 8 months in non-responders. The authors observed a higher response rate in patients with Ki-67 $\leq$ 60, in the group with high uptake in somatostatin receptor scintigraphy (SRS) and in those with positive staining for chromogranin A (CgA), often associated with more differentiated tumors.[11] Published results on lung NECs in progression after first-line chemotherapy are based on small patient series. [12].

The last WHO classification recognized a further group called G3 NETs as having intermediate features between NETs and NECs.4 The 2 subgroups display a distinct prognosis and different sensitivity to chemotherapy. [13–15] Therefore, there is also an urgent need for more accurate biomarkers to help diagnostic assessment of NEC and identify patients that will most likely respond to chemotherapy. In a study recently published on GEP-NEC patients undergoing first-line platinum-based therapy, median PFS was 19.3 months and 6.3 months ($P < 0.01$) in patients with Ki-67 % $<$ 50 % or $>$ 50 %, respectively. [16] Median (m)OS was 8.1 months in the latter group but was not reached in the former group ($P = 0.039$). Our previously published data highlighted that $^{68}$Ga-PET/CT positivity may be a discriminating factor in predicting prognosis, especially in the metastatic setting where histological material is not always available.15,16 Also $^{18}$fluorodeoxyglucose ($^{18}$FDG)-PET/CT could be useful to discriminate patients with different prognosis. [17] Micro(mi)RNAs, a class of small, non-coding, single--stranded RNAs, are also known to show specific expression patterns in several types of tumors, including NETs.[18–20] MiRNA are very promising biomarkers as they allow for non-invasive and continuous tumor sampling with implication for early diagnosis and monitoring of disease progression and treatment outcomes. However, little is known about differential miRNA profiles in NEC patients. [21].

Given the above premises, we decided to investigate the efficacy and safety of second-line FOLFIRI or CAPTEM in patients with GEP and lung NECs in progression after first-line platinum-based treatment. The miRNome profiling of serum samples from enrolled patients in comparison with healthy donors was performed to identify potential circulating biomarkers.

## 2. Methods

### 2.1. Study design, inclusion and exclusion criteria and treatment

The SENECA study (ClinicalTrial.gov identifier: NCT03387592) is a multicenter, randomized, non-comparative, phase II study (Figure 1). Patients with metastatic neuroendocrine carcinomas of different origin (lung or gastroenteropancreatic tract) progressive on first-line treatment were randomized to receive FOLFIRI every 14 days for a maximum of 12 cycles or until progression or unacceptable toxicity, or CAPTEM every 28 days for a maximum of 6 cycles or until progression or unacceptable toxicity.

The treatment arms were as follows:

FOLFIRI regimen.

- Irinotecan 180 mg/m2, given as a 60 min intravenous infusion on day 1 every 2 weeks followed by
- Leucovorin 200 mg/m2, given as a 2 h intravenous infusion on day 1 every 2 weeks followed by
- 5-fluorouracil (5-FU) 400 mg/m2 given as bolus, and then 5-FU 2400 mg/m2 given as a 48 h continuous infusion on day 1, every 2 weeks.

CAPTEM regimen.

- Capecitabine 750 mg/m2 two times a day on days 1–14
- Temozolomide 200 mg/m2 daily on days 10–14, every 4 weeks

The study included patients aged $\geq$ 18 years with a histological diagnosis of GEP- or lung neuroendocrine carcinoma. Small cell lung neuroendocrine carcinoma and mixed tumours were excluded. All enrolled patients had measurable disease according to Response evaluation criteria in solid tumors (RECIST) 1.1 criteria and an Eastern Cooperative Oncology Group (ECOG) performance status $\leq$ 2 with a life expectancy $>$ 3 months. Progression on or after platinum-based

chemotherapy (cisplatin/etoposide, carboplatin/etoposide, FOLFOX or CAPOX) was required for study eligibility. Adequate haematological, liver and renal function was required, and written informed consent was obtained from all patients before enrollment in the study.

### 2.2. Outcomes

The primary endpoint of the study was the DCR of each treatment, defined as the percentage of patients who have achieved complete or partial response or stable disease by RECIST version 1.1 criteria for at least 12 weeks from the start of therapy. Acute and late toxicity were evaluated by CTCAE Version 4.03, the latter defined as toxicity occurring at least 30 days after the end of the last treatment cycle. Secondary endpoints were the evaluation of OS, calculated from the start of treatment to death from any cause and PFS, calculated from the start of treatment to the date of the first documented evidence of disease progression or of death from any cause. For quality of life analysis, the QLQ-C30 questionnaire was administered. It is composed of both multi-item scales and single-item measures. These include five functional scales, three symptom scales, a global health status/QoL scale, and six single items. Each of the multi-item scales includes a different set of items - no item occurs in more than one scale.

Bryant and Day design was used to estimate a sample size which takes into account both activity and toxicity. An α level of 0.10 (for both toxicity and DCR) and a power of 90 % were adopted. A 12 weeks DCR rate $\geq$ 60 % and a toxicity rate $\leq$ 20 % were considered acceptable rates while a DCR rate $\leq$ 40 % and a relevant toxicity rate $\geq$ 40 % were considered inacceptable rates. Given these hypotheses, the first step of the study required 25 patients. If $\geq$ 10 patients with a DCR were observed and $\geq$ 15 patients did not have relevant toxicity, the study could enroll patients in the subsequent step. A total of 53 patients would have been then enrolled. If $\geq$ 25 patients with DCR and $\geq$ 36 patients without any relevant toxicity were observed, treatment would have been considered active and non-toxic. Taking into account a 5 % dropout rate, 56 patients had to be enrolled in each arm (total 112 patients). G3–4 gastrointestinal toxicity, G4 thrombocytopenia, prolonged G3-G4 neutropenia (>7 days) and drug-related hospitalizations were considered relevant toxicity. The stratification factors of this study included Ki-67 (21 %−55 % versus >55 %) and site of primary tumor (lung versus GEP).

Complete response, partial response or stable disease for at least 12 weeks were considered as the DCR.

This study was performed in full collaboration with several Italian Centers, some of them belonging to EURACAN or ENETS center of excellence. The protocol was approved by the relevant institutional review boards and ethics committees and was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice Guidelines. All patients provided written informed consent before study enrollment.

### 2.3. MiRNAs profiling

For miRNA analysis serum samples were obtained from 18 patients enrolled in the SENECA study and 20 healthy donors. All participants signed a specific informed consent.

Purification of cell-free total RNA, which primarily includes small RNAs including miRNAs, was performed from serum using miRNeasy Serum/Plasma Kit (Qiagen), according to manufacturer's protocol. Libraries were then prepared starting from 5µl of RNA using QIAseq® miRNA Library Kit, containing integrated unique molecular indices (UMIs) to enhance differential expression analysis. Briefly, adapters were ligated sequentially to the 3′ and 5′ ends of miRNAs. Subsequently, universal cDNA synthesis with UMI assignment, cDNA cleanup, library amplification, and library cleanup were performed. Resulting libraries were then checked for quality using Agilent Bioanalyzer 2100 to evaluate the presence of the ≈ 180 bp peak, and concentration was determined using a Qubit Fluorimeter. The samples were pooled in equimolar ratios and the resulting pooled library was then diluted at a final concentration of 1.6pM and sequenced using NextSeq™ 550Dx High Output Reagent Kit v2.5 (75 cycles). Primary data analyses (UMIs count and miRNA sequences mapping) were then performed with proprietary online tool available at geneglobe.qiagen.com.

### 2.4. Statistical analysis

The primary objective of this study was to assess the DCR of FOLFIRI and CAPTEM regimens in patients with metastatic NECs of GEP or lung origin after failure of a first-line platinum-based treatment. Safety analysis was a co-primary objective. Analysis on primary objective was performed on the Intention-to-treat (ITT) population, defined as the

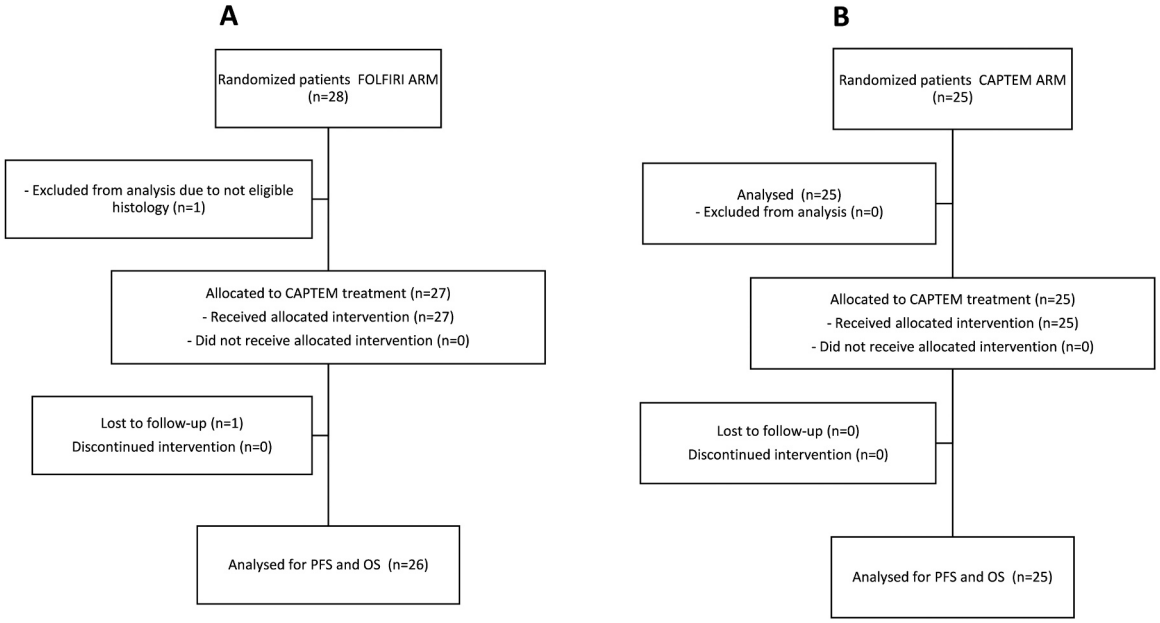


**Fig. 1.** Study flow chart: (A) FOLFIRI; (B) CAPTEM.

population of all enrolled patients. Toxicity evaluation was assessed on safety population (SP) which was defined as all patients who received, in each treatment group, at least one cycle of treatment. Continuous variables were presented as median (range) and categorical variables were presented as absolute or relative frequencies. Time-to-event data (PFS, OS) were calculated using the Kaplan-Meier curves [22] and compared using the log-rank test. Ninety-five percent confidence intervals (95 % CI) were calculated by non-parametric methods. Estimated HRs and their 95 % CI were calculated using univariate Cox proportional hazard models. The DCR was calculated with an exact 95 % CI using standard methods based on binomial distribution. Scoring of global health status, functional scales and symptom scales were calculated according to EORTC QLQ-C30 Scoring Manual. Descriptive data as mean and standard deviation was used to show QLQ-C30 scores between baseline and 3 months visit.

To study the role of biomarkers, the Shapiro-Wilk test was used to test the normality distribution of each investigated biomarker, and nonparametric tests including Wilcoxon Mann-Whitney or Chi-Square test, as appropriate, were used when the normality distribution assumption was not respected to analyze the relationship between the serum levels of each marker, considered as continuous variables, and the different subgroups. ROC (Receiver Operating Characteristics) curves were used to identify optimal cut-offs of biomarkers for predicting OS.

The analysis on all endpoints were performed separately on each group of treatment.

Statistical significance for all tests was defined as $P < 0.05$ (two-sided). Statistical analysis was carried out using Stata/SE version 15.1 for Windows (StataCorpLP, College Station, TX, USA).

### 2.5. Randomization

Participants were randomly assigned (1:1) to receive FOLFIRI or CAPTEM using the blocked randomization method, with random block sizes. Study statistician had generated the allocation sequence using a computer software (nQuery) and the patient's attribution was done through a phone call by a physician to the central coordinating office for each patient assignment at the patient's study inclusion time, to ensure concealed allocation.

Randomization will be stratified according to the ki67 value (21–55 % versus >55 %), morphology and site of primary tumor (Lung+other versus GEP). Neither patients nor investigators were masked to group assignment.

## 3. Results

### 3.1. Patients

From 06/03/2017 to 18/01/2021, 53 out of 112 patients initially planned were enrolled in 17 of 23 participating centers. The main characteristics of enrolled patients are shown in Table 1.

**Table 1**
Patients' characteristics.

| Variable | Arm A: FOLFIRI N = 28 (%) | Arm B: CAPTEM N = 25 (%) | Overall N= 53 (%) |
|---|---|---|---|
| Median age (range) | 63 (36-79) | 62 (30-80) | 62 (30-80) |
| Sex | | | |
| Male | 15 (53.6) | 17 (68.0) | 32 (60.4) |
| Female | 13 (46.3) | 8 (32.0) | 21 (39.6) |
| PS ECOG | | | |
| 0 | 14 (50.0) | 15 (60.0) | 29 (54.7) |
| 1 | 12 (42.9) | 8 (32.0) | 20 (37.7) |
| 2 | 2 (7.1) | 2 (8.0) | 4 (7.6) |
| Site of disease | | | |
| Lung | 3 (10.7) | 4 (16.0) | 7 (13.2) |
| GEP tract | 20 (71.4) | 19 (76.0) | 39 (73.6) |
| Other | 5 (17.9) | 2 (8.0) | 7 (13.2) |
| Site of metastases | | | |
| Liver | 19 (67.9) | 19 (76.0) | 38 (71.7) |
| Bone | 6 (21.4) | 8 (32.0) | 14 (26.4) |
| Lung | 1 (3.6) | 8 (32.0) | 9 (17.0) |
| Lymph nodes | 12 (42.9) | 10 (40.0) | 22 (41.5) |
| Other | 4 (14.3) | 6 (24.0) | 10 (18.9) |
| Previous surgery | 15 (53.6) | 15 (60.0) | 30 (56.6) |
| Smoke | | | |
| Yes | 12 (50.0) | 14 (70.0) | 26 (58.1) |
| No | 12 (50.0) | 6 (30.0) | 18 (40.9) |
| Unknown | 4 | 5 | 9 |
| PET/CT scan with 68Ga-dota-peptides | | | |
| Performed | 7 (25.0) | 6 (24.0) | 13 (24.5) |
| Not performed | 21 (75.0) | 19 (76.0) | 40 (75.5) |
| 68Ga-PET/CT | | | |
| Positive | 5 (71.4) | 5 (100.0) | 10 (83.3) |
| Negative | 2 (28.6) | 0 (0.0) | 2 (16.7) |
| PET/CTscan with 18 F-FDG | | | |
| Performed | 9 (32.1) | 5 (20.0) | 14 (26.4) |
| Not performed | 19 (67.9) | 20 (80.0) | 39 (73.6) |
| 18FDG-PET | | | |
| Positive | 8 (88.9) | 5 (100.0) | 13 (92.9) |
| Negative | 1 (11.1) | 0 (0.0) | 1 (7.1) |
| Morphology | | | |
| Well differentiated | 1 (4.5) | 3 (14.3) | 4 (9.3) |
| Poorly differentiated | 21 (95.5) | 18 (85.7) | 39 (90.7) |
| Not specified | 6 | 4 | 10 |
| Ki-67 | | | |
| Median (range) | 80 (23-95) | 80 (22-95) | 80 (22-95) |
| Unknown | 1 | 0 | 1 |
| Ki-67 | | | |
| < 55 | 8 (29.7) | 7 (28.0) | 15 (28.9) |
| > 55 | 19 (70.3) | 18 (72.0) | 37 (71.1) |
| Unknown | 1 | 0 | 1 |
| Comorbidities | | | |
| Yes | 17 (60.7) | 17 (68.0) | 34 (64.2) |
| No | 11 (39.3) | 8 (32.0) | 19 (35.8) |

Median follow up was 22.6 (range 1.4-60.4) months.

### 3.2. FOLFIRI arm: efficacy and safety

At the date of interim analysis, 28 patients were enrolled in the FOLFIRI arm. Of these, 24 patients underwent the first disease evaluation at 3 months as per protocol. One patient performed a disease evaluation at 60 days due to local investigator's decision. Three patients progressed clinically and did not undergo radiological evaluation. The DCR was 39.1 %, with progression recorded as best response in 15 patients (60.9 %). The patient with a 60 days disease evaluation was stable. Four patients (16.7 %) obtained a partial response (Figure 2). As shown in Fig. 3A, the 6-months PFS rate was 34.6 % (95 % CI: 17.5–52.5) and 6-months and 12-months OS rate were 52.8 % (95 % CI: 32.0–69.9) and 28.4 % (95 % CI: 12.7–46.5) respectively (Fig. 3B). The mOS was 6.7 (95 % CI: 4.1–11.3) months. In the pre-planned subgroup analysis according to the Ki-67 index $\leq$ or > 55 %, the mPFS of the first group ($n = 8$) was 8.5 months (95 % CI: 2.0–13.6) with a 6- and 12-months PFS rate of 87.5 % (95 % CI: 38.7–98.1) and 25.0 % (95 % CI: 3.7–55.8) respectively. When Ki-67 > 55 % ($n = 18$) the mPFS was 2.9 months (95 % CI: 1.9–3.2) and the 6- and 12-months PFS rates were 11.1 % (95 % CI: 1.9–29.8) and 5.6 % (95 % CI: 0.4–22.4). The median OS was 13.5 months (95 % CI: 5.2-Not Estimable) in the first group and 4.3 (95 % CI: 2.8–6.7) months in the second one.

In the safety assessment for the interim analysis in the FOLFIRI arm, G3-G4 relevant toxicity was seen in 6 (21.4 %) of 28 patients. Three patients had a prolonged G3 neutropenia for more than 7 days (10.7 %), 1 patient a G3 anemia, 1 patient a G3 pneumonitis possibly related to the treatment and 1 patient a G3 herpes zoster infection.

Treatment-related AEs (TRAEs) occurred in 15 out of 27 patients (55.5 %) enrolled in the FOLFIRI arm. The most common TRAEs were G1 vomiting (22.2 %), G1 nausea (22.2 %) and G1 fatigue (22.2 %).

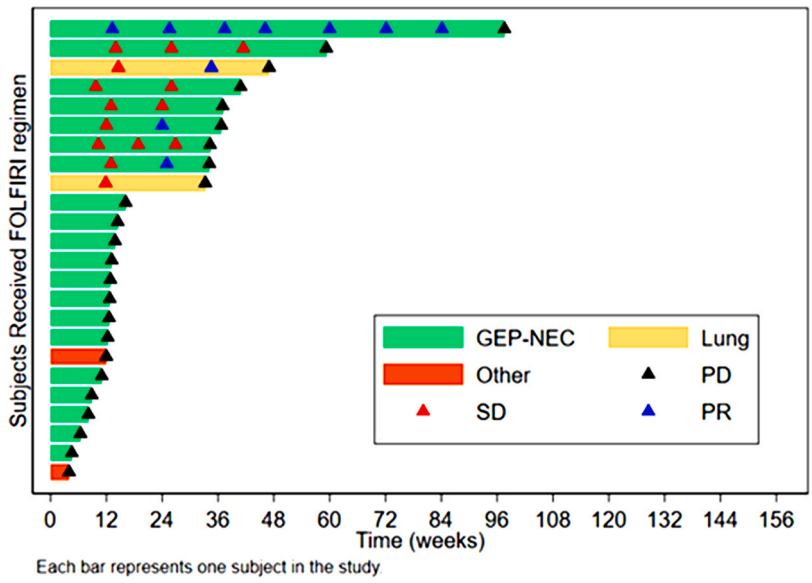


**Fig. 2.** Spider plot for FOLFIRI arm.

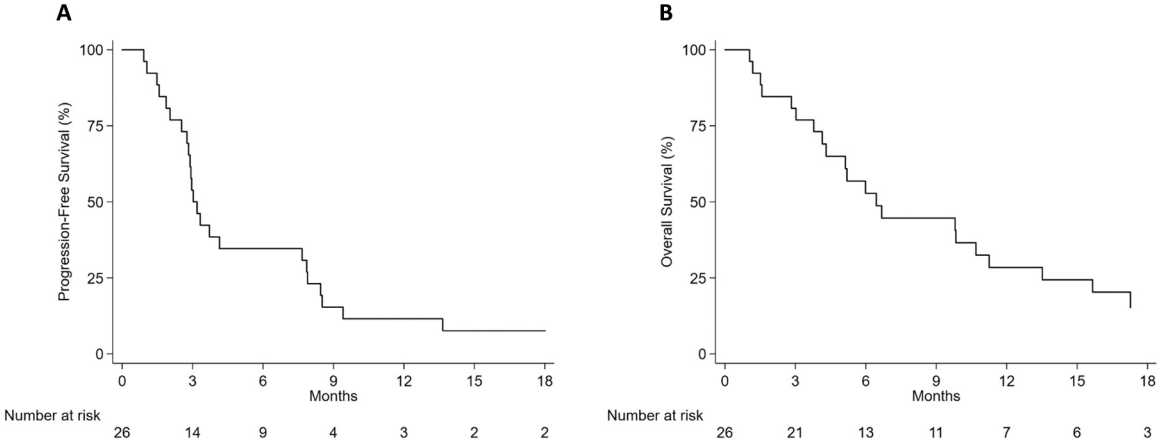


**Fig. 3.** . (A) PFS and (B) OS for FOLFIRI arm.

Grade 3 Neutropenia occurred in 4 cases (14.8 %) and Grade 4 neutropenia in 1 case (3.7 %). A grade 4 renal failure was recorded in the FOLFIRI arm, but was likely related to globally worsened clinical conditions rather than the treatment itself.

Only one patient interrupted the treatment with FOLFIRI because of unacceptable toxicities. Dose reductions were required in 9 of 27 patients (33.3 %). At least one treatment delay was recorded in 12 patients (44.4 %): in seven (25.9 %) for hematological toxicity and in 5 (18.5 %) for not hematological ones. The median time to first dose reduction was 4.6 weeks (range: 1.0–16.0).

### 3.3. CAPTEM arm: efficacy and safety

At the date of interim analysis, 25 patients were enrolled and 23 of them completed the first scheduled disease evaluation.

The DCR was 28.0 % and 3 patients (12.0 %) obtained a partial response (Figure 4). Sixteen patients had a progressive disease after the first radiological evaluation (64.0 %). Two patients clinically progressed (8.0 %). The 6-months and 12-months PFS rate was 8.0 % (95 % CI: 1.4–22.5) and 4.0 % (95 % CI: 0.3–17.0), respectively (Fig. 5A).

The mOS was 7.4 months (95 % CI: 3.7–12.5) while the 6-months and 12-months OS rate were 54.2 (95 % CI: 32.7–71.4) and 32.4 (95 % CI: 14.9–51.2), respectively (Fig. 5B).

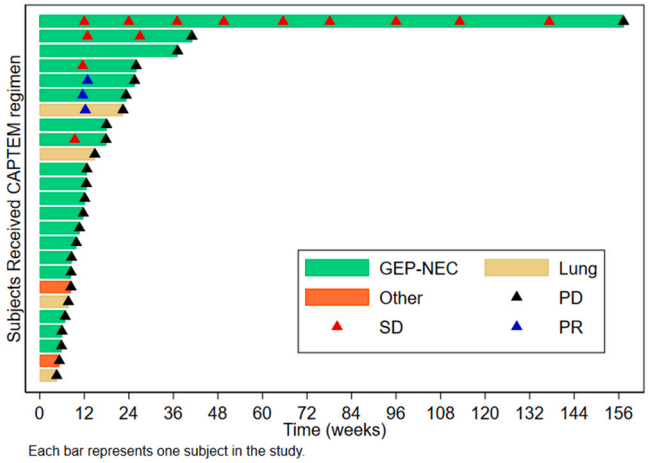


**Fig. 4.** Spider plot for CAPTEM arm.

In the CAPTEM arm patients with a Ki-67 value ≤of 55 % had mPFS of 4.1 (95 % CI: 1.1–9.4) months with a 6-months PFS rate of 28.6 % (95 % CI: 4.1–61.1). In patients with Ki-67 higher than 55 % mPFS was 2.0 (95 % CI: 1.6–2.9) months. The mOS was not reached in the Ki-67

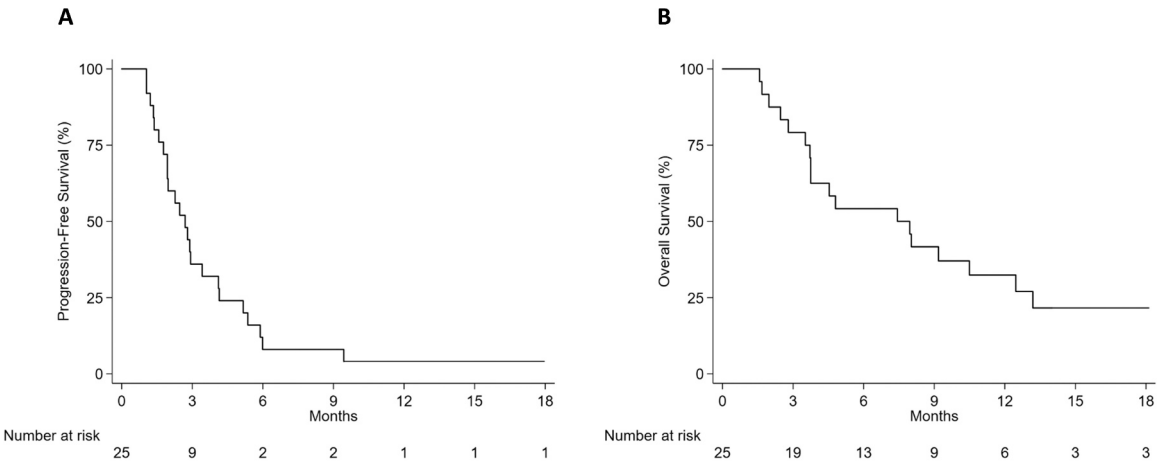


**Fig. 5.** (A) PFS and (B) OS for CAPTEM arm.

≤ 55 % group and 4.5 % (95 % CI: 2.8–9.2) months in the second group.

In the safety assessment for the interim analysis in the CAPTEM group, G3-G4 relevant toxicities were seen in 6 (21.4 %) out of 28 patients. Only three patients experienced a prolonged G3 neutropenia for more than 7 days (10.7 %), 1 patient a G3 anemia, 1 patient a G3 pneumonitis possibly related to the treatment and 1 patient a G3 herpes zoster infection.

Overall, treatment-related AEs (TRAEs) occurred in 16 out of 25 patients (64.0 %) enrolled.

No patients interrupted the CAPTEM treatment due to unacceptable toxicity. Dose reductions were required in 2 out of 25 patients (8.0 %; patient decision and haematological toxicity in one patient respectively). At least one treatment delay was recorded in 8 patients (36.4 %): in 2 patients (8.0 %) because of hematological toxicity and in 1 patient (4.0 %) because of non-hematological adverse events. The median time to first dose reduction was 4.6 weeks (range: 1.0–16.0).

Overall, recorded adverse events were consistent with the known safety profile of both FOLFIRI and CAPTEM (Table 2).

### 3.4. Quality of Life

Twenty-four patients answered all items of EORTC QLQ-C30 questionnaires at enrollment and after 3 months of treatment. As shown in Figure 6, a worsening was observed for both functional and symptoms domains in patients who progressed within 3 months, while a reduction in symptoms was observed at 3 months for responding patients or those with stable disease.

### 3.5. Identification of circulating microRNAs

Serum samples of 18 patients enrolled in the study were subjected to NGS miRNome profiling in comparison with 20 healthy donors to reveal differentially expressed miRNAs as candidate circulating biomarkers. The median age was 34.9 years (range: 28.6–53.6) in the healthy group and 60.5 years (range: 35.8–78.4) in the patient group. Males and females were equally distributed (Table 3).

Twenty miRNAs were found to be upregulated in NEC patients compared to healthy subjects (Table 4 and Supplementary Fig. S1).

In order to take into account the differences in the age distribution of patients versus healthy donors that might influence miRNA expression, age was added in logistic models as covariate. Three miRNAs remained as NEC independent predictors and separated patients from healthy donors, after the introduction of age as covariate: miR-1246, miR-1290 and miR-320c (Fig. 7A). The receiver operating characteristics (ROC)

**Table 2**
Treatment-related AEs reported among patients with at least 1 cycle of treatment.

| | ARM A: FOLFIRI (N of patients=27) N of AE (%) | | | | B: CAPTEM (N of patients = 25) N of AE (%) | | | |
|---|---|---|---|---|---|---|---|---|
| | G0/G1 | G2 | G3 | G4/G5 | G0/G1 | G2 | G3 | G4/G5 |
| Neutropenia | 3 (11.1) | 4 (14.8) | 4 (14.8) | 1 (3.7) | 0 (0.0) | 3 (12.0) | 1 (4.0) | 0 (0.0) |
| Febrile Neutropenia | 0 (0.0) | 0 (0.0) | 1 (3.7) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Leukopenia | 0 (0.0) | 0 (0.0) | 1 (3.7) | 0 (0.0) | 1 (4.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Anemia | 4 (14.8) | 2 (7.4) | 2 (7.4) | 0 (0.0) | 1 (4.0) | 2 (8.0) | 0 (0.0) | 0 (0.0) |
| Thrombocytopenia | 0 (0.0) | 2 (7.4) | 1 (3.7) | 0 (0.0) | 2 (8.0) | 2 (8.0) | 1 (4.0) | 1 (4.0) |
| Asthenia/fatigue | 6 (22.2) | 3 (11.1) | 0 (0.0) | 0 (0.0) | 2 (8.0) | 3 (12.0) | 1 (4.0) | 0 (0.0) |
| Nausea | 6 (22.2) | 3 (11.1) | 0 (0.0) | 0 (0.0) | 3 (12.0) | 1 (4.0) | 0 (0.0) | 1 (4.0) |
| Vomiting | 6 (22.2) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 2 (8.0) | 2 (8.0) | 0 (0.0) | 1 (4.0) |
| Diarrhea | 5 (18.5) | 3 (11.1) | 0 (0.0) | 0 (0.0) | 1 (4.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Loss of appetite | 2 (7.4) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Erythema/rush | 1 (3.7) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (4.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Fever | 1 (3.7) | 1 (3.7) | 0 (0.0) | 0 (0.0) | 1 (4.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Anorexia | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 2 (8.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Mucositis | 2 (7.4) | 1 (3.7) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Liver toxicity | 4 (14.8) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 2 (8.0) | 3 (12.0) | 1 (4.0) | 0 (0.0) |
| Renal toxicity | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (4.0) | 0 (0.0) | 0 (0.0) |
| Nervous system disorder | 0 (0.0) | 1 (3.7) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Hand-foot syndrome | 1 (3.7) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Peripheral edema | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 2 (8.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Other | 9 (33.3) | 1 (3.7) | 2 (7.4) | 0 (0.0) | 6 (24.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |

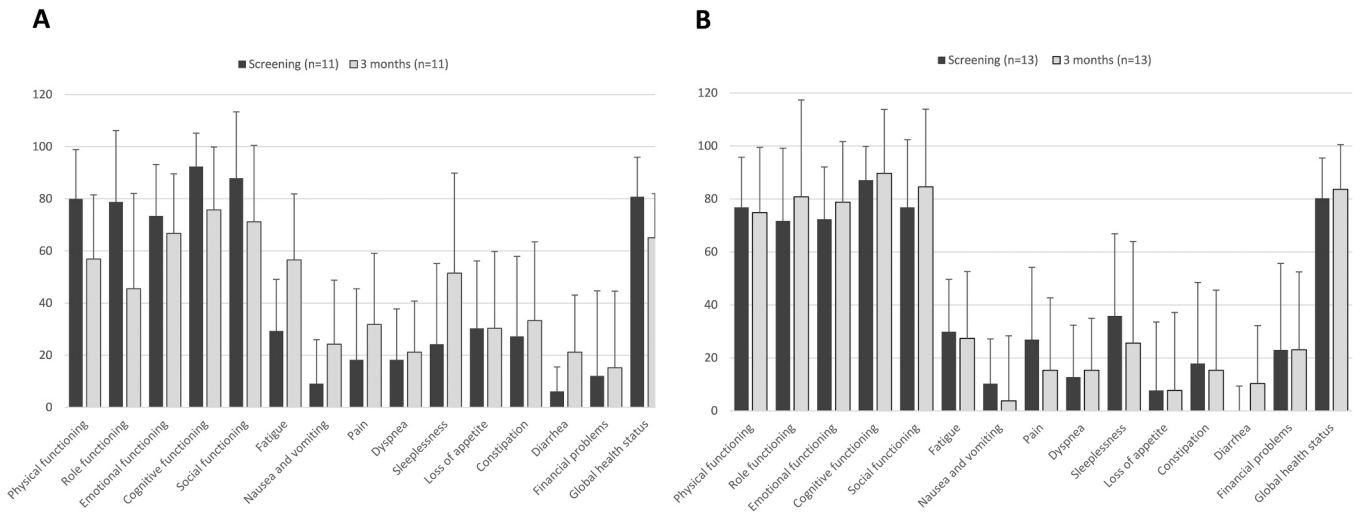


**Fig. 6.** Mean values for various domains and symptom score of the EORTC QLQ-C30 questionnaires: (A) Patients in PD; (B) Patients in SD/PR/CR.

**Table 3**
Eligible subjects for miRNA analysis.

| Variable | Healthy donor N= 20 % | Patients N= 18 % | Overall N= 38 % | *P*-value |
|---|---|---|---|---|
| Age at randomization | | | | |
| Median (range) | 34.9 (28.6-53.6) | 60.5 (35.8-78.4) | 49.1 (28.6-78.5) | < .001 |
| Sex | | | | |
| Male | 12 (60.0) | 10 (55.6) | 22 (57.9) | .782 |
| Female | 8 (40.0) | 8 (44.4) | 16 (42.1) | |
| Site of disease | | | | |
| Lung | - | 3 (16.7) | 3 (16.7) | - |
| GEP | - | 15 (83.3) | 15 (83.3) | |
| Ki-67 value | | | | |
| < 55 | - | 7 (38.9) | 7 (38.9) | - |
| > 55 | - | 11 (61.1) | 11 (61.1) | |

*P*-value from Wilcoxon Mann-Whitney test for age, and from chi-square for sex.

curve indicated a good diagnostic accuracy in separating patients versus healthy donors with an area under the curve (AUC) of 0.9417 (95 % CI: 0.83–1.00) for miR-1246, 0.9167 (95 % CI: 0.80–1.00) for miR-1290, and 0.9333 (95 % CI: 0.83–1.00) for miR-320c (Fig. 7B).

An explorative analysis was carried out and a specific cut off was found considering ROC curves for selected miR and 6-months PFS and OS as events. High expression values of these 3 miRNAs were also significantly associated with decreased mPFS and mOS (Table 5). Patients with an expression of miR-1246 < 690 expression ≥ 690 had a mPFS of 2.9 months (95 % CI: 2.0–7.8, P = 0.029). Similarly, patients with a miR-1290 expression < 241 had a mPFS of 7.9 months (95 % CI: 4.1-Not Estimable) respect to patients who had ≥ 214 that had 2.8 months (95 % CI: 1.0–7.9, P = 0.025). Finally, patients with a miR-320c expression < 4242 had a mPFS of 7.9 months (95 % CI: 2.8–9.4) against 2.7 months (95 % CI: 1.0–4.1) reported by patients with a miR-320c expression ≥ 4242, P = 0.016) (Fig. 8A). An area under the curve (AUC) of 0.7250 (95 % CI: 0.46–0.98) for miR-1246, 0.7375 (95 % CI: 0.47–0.99) for miR-1290, and 0.7500 (95 % CI: 0.51–0.99) for miR-320c were observed, taking account of 6-months PFS (Fig. 8B).

Regarding OS, expression values of miR-1246 were not significantly associated with OS, although a trend was observed: patients with a miR-1246 expression lower than 3407 had a mOS of 10.7 months (95 % CI: 7.4-Not Estimable) while patients with values higher than the cut-off had a mOS of 3.8 months (1.12-Not Estimable, P = 0.093). Patients with an expression of miR-1290 < 1734 had a mOS of 13.2 months (95 % CI: 9.2–23.9) while patients with an expression ≥ 1734 had a

**Table 4**
Median values for MIR expression on crude values.

| Variable | Healthy donor N= 20 (%) | Patients N= 18 (%) | Overall N= 38 (%) | *P*-value[#] |
|---|---|---|---|---|
| hsa-miR-1224-5p | 6.6 (0.0-81.9) | 458.6 (29.9-10449.8) | 41.8 (0.0-10449.8) | < .001 |
| hsa-miR-1246 | 206.6 (61.3-522.5) | 2596.1 (57.5-45681.5) | 418.3 (57.5-45681.5) | < .001 |
| hsa-miR-1290 | 38.6 (18.4-156.8) | 415.6 (11.9-9019.8) | 84.4 (11.9-9019.9) | < .001 |
| hsa-miR-141-3p | 9.9 (0.0-152.9) | 40.0 (6.7-981.9) | 24.2 (0.0-981.9) | < .001 |
| hsa-miR-200a-3p | 5.1 (0.0-52.3) | 40.0 (6.7-859.2) | 16.3 (0.0-859.2) | < .001 |
| hsa-miR-200b-3p | 7.7 (0.0-69.7) | 46.6 (0.0-846.7) | 20.1 (0.0-846.7) | < .001 |
| hsa-miR-200c-3p | 147.6 (30.6-535.0) | 370.3 (91.1-7987.3) | 200.9 (30.6-7987.4) | < .001 |
| hsa-miR-210-3p | 20.3 (0.0-60.9) | 96.0 (33.5-495.0) | 35.7 (0.0-495.0) | < .001 |
| hsa-miR-320b | 160.1 (78.4-409.9) | 380.7 (138.7-2639.8) | 210.7 (78.4-2639.8) | < .001 |
| hsa-miR-320c | 383.6 (178.1-1306.3) | 2884.8 (209.7-37222.5) | 610.8 (178.1-37222.5) | < .001 |
| hsa-miR-320d | 126.5 (52.9-305.7) | 1329.3 (91.0-24361.8) | 182.9 (52.9-24361.8) | < .001 |
| hsa-miR-375-3p | 312.6 (54.2-1898.5) | 11366.9 (779.9-930915.0) | 901.7 (54.2-930915.0) | < .001 |
| hsa-miR-4488 | 5.1 (0.0-152.9) | 60.3 (2.9-1103.5) | 19.1 (0.0-1103.5) | < .001 |
| hsa-miR-483-5p | 271.0 (27.6-1968.1) | 1424.8 (64.9-45341.8) | 481.8 (27.6-45341.1) | < .001 |
| hsa-miR-760 | 85.7 (24.0-229.3) | 246.1 (13.4-1664.4) | 122.2 (13.4-1664.4) | < .001 |
| hsa-miR-874-3p | 37.9 (0.0-122.5) | 106.8 (14.2-1425.6) | 51.9 (0.0-1425.6) | .006 |
| hsa-miR-92b-3p | 221.3 (51.5-625.2) | 460.8 (155.2-4795.6) | 325.7 (51.5-4795.6) | .001 |
| hsa-miR-95-3p | 4.3 (0.0-48.1) | 36.8 (0.0-980.4) | 14.9 (0.0-980.4) | < .001 |
| hsa-miR-10a-5p* | 98.8 (0.0-245.9) | 218.9 (73.6-2793.3) | 157.8 (0.0-2793.3) | < .001 |
| hsa-miR-184 * | 2.4 (0.0-211.4) | 13.4 (0.5-383.5) | 5.3 (0.0-383.5) | .001 |

Minimum and maximum values between parentheses. *P*-value based on Wilcoxon Mann-Whitney test.

mOS of 3.7 months (95 % CI: 1.2-Not Estimable, P = 0.010). Similarly, patients with a miR-320c expression < 4954 had a mOS of 13.2 months (95 % CI: 9.8-Not Estimable) and patients with miR-320c expression ≥ 4954 had a mOS of 3.8 months (95 % CI: 2.8-Not Estimable,

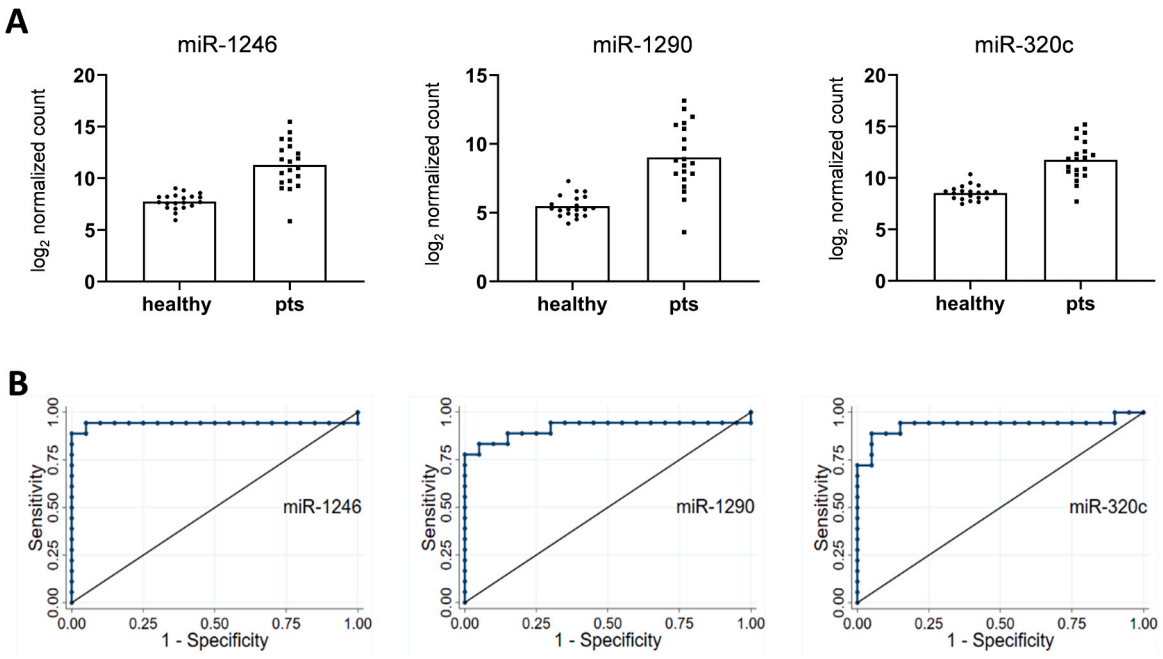


**Fig. 7.** (A) Box plot of $log_2$ distribution among healthy donors and patients for miR-1246, miR-1290 and miR-320; (B) ROC curves distinguishing among healthy donors and patients for miR-1246, miR-1290, miR-320.

**Table 5**
OS and PFS values for MIR selected values.

| PFS | | | | | OS | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | No. of patients | No. of PD | Median PFS (95 % CI) | *P*-value | | No. of patients | No. ofdeaths | Median OS (95 % CI) | *P*-value |
| All cases | 18 | 18 | 5.2 (2.7-7.8) | - | All cases | 18 | 14 | 10.5 (5.2-17.3) | - |
| hsa-miR-1246 | | | | | hsa-miR-1246 | | | | |
| < 690 | 4 | 4 | 7.9 (7.7-NE) | .029 | < 3407 | 10 | 7 | 10.7 (7.4-NE) | .093 |
| ≥ 690 | 14 | 14 | 2.9 (2.0-7.8) | | ≥ 3407 | 8 | 7 | 3.8 (1.1-NE) | |
| hsa-miR-1290 | | | | | hsa-miR-1290 | | | | |
| < 241 | 6 | 6 | 7.9 (4.1-NE) | .025 | < 1734 | 13 | 9 | 13.2 (9.2-23.9) | .010 |
| ≥ 241 | 12 | 12 | 2.8 (1.0-7.9) | | ≥ 1734 | 5 | 5 | 3.7 (1.2-NE) | |
| hsa-miR-320c | | | | | hsa-miR-320c | | | | |
| < 4242 | 11 | 11 | 7.9 (2.8-9.4) | .016 | < 4954 | 12 | 8 | 13.2 (9.8-NE) | .005 |
| ≥ 4242 | 7 | 7 | 2.7 (1.0-4.1) | | ≥ 4954 | 6 | 6 | 3.8 (2.8-NE) | |

PD, Progressive disease; NE, Not estimable from statistical software.

$P = 0.005$) (Fig. 8C). An area under the curve (AUC) of 0.9231 (95 % CI: 0.79–1.00) for miR-1246, 0.9077 (95 % CI: 0.87–1.00) for miR-1290, and 0.8615 (95 % CI: 0.64–1.00) for miR-320c were observed, considering 6-months OS (Fig. 8D).

## 4. Discussion

To date, there is no truly effective second-line chemotherapy for patients with NEC. The overall prognosis of these patients is poor, with an OS of approximately 5 months in the metastatic setting according to the SEER (Surveillance, Epidemiology, and End Results) database. [23] Our study confirms the poor prognosis of these rare and aggressive tumors and fails to identify an effective regimen for their second-line treatment.

Despite the fact that we did not reach the primary end-point and the trial was stopped prematurely due to futility, some information can be extrapolated by the results presented. In a recent published meta-analysis, second-line therapy for patients with advanced extra-pulmonary NEC had limited efficacy, and a high Ki-67 was associated with poor treatment outcomes, as reported previously in the NORDIC NEC study. [24].

Median response rate was 18 % (range 0–50; 0 % for single-agent everolimus, temozolomide, topotecan; 50 % with amrubicin). Median PFS was 2.5 months (range 1.2–6.0) and median OS was 7.6 months (range 3.2–22). In our study the mOS was 6.4 months in the FOLFIRI arm and 7.4 months in the CAPTEM arm, similarly to what reported in this analysis. Moreover, stratification of patients demonstrated different responses to treatments and different outcomes according to Ki67 values. In particular, the mOS differed substantially between patients with ki67 levels $\leq$ and $>$ of 55 % and it was longer in the first group than in the second one in both treatments' arms. [24] Similarly, in the NORDIC NEC study the mOS was 14 versus 10 months in patients with ki67 value $<$ or $\geq$ 55 %. [25].

The BEVANEC study data were recently published. [7] In this randomized, multicentre, non-comparative phase II study, mOS was 7.0 months (95 % CI: 4.6–11.5) in the FOLFIRI + BEVACIZUMAB arm and 8.9 months in the FOLFIRI one. These results are similar to those described in our study in the FOLFIRI subgroup confirming the impact of this latter on the patients' outcomes. Our trial is the first to present prospective data on the efficacy of FOLFIRI and CAPTEM in a homogeneous population of NEC patients. Another phase II study with temozolomide monotherapy has been recently published with a very short PFS and a mOS of 7.0 months. However, the sample size was low and the primary tumor location of the patients enrolled was very

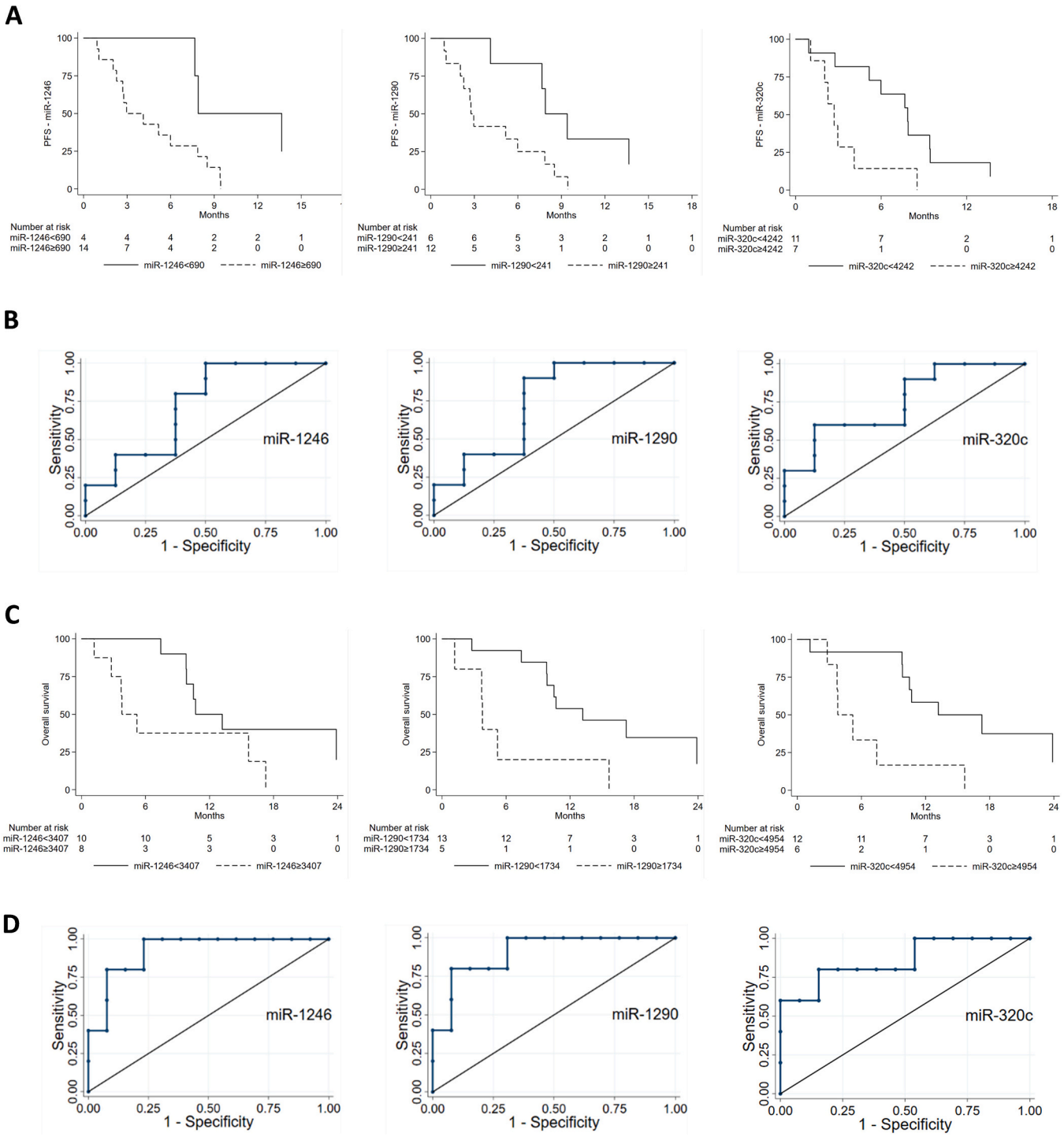


**Fig. 8.** (A) Kaplan-Meier curves of PFS for miR-1246, miR-1290 and miR-320; (B) ROC curves for PFS according to expression values of miR-1246, miR-1290, miR-320; (C) Kaplan-Meier curves of OS for miR-1246, miR-1290 and miR-320; (D) ROC curves for OS according to expression values of miR-1246, miR-1290, miR-320.

heterogeneous. [26].

Similarly, preliminary data of the randomized, multicentre phase II NET-2 trial about liposomal irinotecan (nal-IRI) and 5-fluorouracil (5-FU)/folinic acid or docetaxel as second-line therapy in patients with progressive poorly differentiated extra-pulmonary neuroendocrine carcinoma have been recently presented. The 6-months PFS rate was 32.1 (lower 95 % CI: 17.9) in 28 patients treated with nal-IRI/5-FU/folinic acid and 14.8 (lower 95 % CI: 5.2) in 27 patients treated with Docetaxel. Despite the data having not been cleaned and a direct comparison is not possible, the activity of Irinotecan seems to be confirmed while docetaxel fails as a possible therapeutic option in EP-NEC. However there are some differences between SENECA study and NET-= 2 in the study design and characteristics of enrolled patients. [27].

In addition, we conducted an exploratory analysis on circulating miRNAs in the enrolled patients to collect information on potential disease biomarkers with diagnostic and prognostic relevance. We identified 3 circulating miRNAs that are NEC independent predictors and separate patients from healthy subjects. High levels of these miRNAs were found to be significantly associated with worse disease outcomes in terms of PFS and OS. The 3 identified miRNAs are known to be

implicated in tumor stemness, aggressiveness and resistance to chemotherapy, and have been investigated as non-invasive biomarkers in various cancer types. [28–32] Selected circulating miRNAs could be used to discriminate patients from healthy individuals making them ideal tools for an earlier diagnosis. Moreover, miRNAs are reliable candidates for disease monitoring as they change throughout tumor progression. The identification of liquid biomarkers might introduce potential benefits in the patient management, especially for these rare tumors where biomarker-driven approaches are missing.

There are some limitations to the present study. The lack of benefit observed should also be considered in the context of the ambitious end point targeted considering the poor outcome of the disease. Infact, the impact of both regimens on overall survival is of value. We would point out that the absence of a single response prevented us from reaching the primary endpoint in the Folfiri arm, as we decided to not include a patient who obtained a RECIST SD at 60 days as per protocol. Another limitation is the lack of a centralized pathology report. However, this limitation was partially mitigated by the long-lasting expertise possessed by the most of enrolling centres.

## 5. Conclusion

Although we didn't reach the primary endpoint, our study suggests that chemotherapy with FOLFIRI or CAPTEM could be active and safe as second line therapy in NEC patients and are able to improve the quality of life of these patients. In particular, CAPTEM does not seem to be inferior to FOLFIRI as second-line treatment for patients with NECs (although there is no formal comparison here). As result, CAPTEM or FOLFIRI might be used interchangeably in the second-line setting, with the choice being driven by patient comorbidities/chemo regimen toxicity profile.

Finally, the miRNAs identified in this study could be useful to introduce new reliable biomarkers for diagnostic and prognostic purposes. Longitudinal analysis of the selected miRNAs is warranted to investigate their clinical usefulness.

"Non quia difficilia sunt, non audemus; sed quid non audemus, difficilia sunt" (It is not because things are difficult that we do not dare; but it is because we do not dare that things are difficult) (Seneca, *Epistulae Morales*, 104.26).

## Funding

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## Ethical statement

The protocol was approved by the institutional review boards and Ethics Committees of the centers participating in the study and was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice Guidelines.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### *Informed consent statement*

All patients provided written informed consent before study enrollment.

### *Consent for publication*

No identifiable images were included in the manuscript, therefore consent for publication is not applicable.

### *Data sharing statement*

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ejca.2024.114129.

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