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Plasma miR-379 can predict treatment response to FOLFIRINOX and gemcitabine-*nab*-paclitaxel in advanced pancreatic cancer

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ABSTRACT

Background: Novel biomarkers, such as plasma microRNAs (miRs), are needed to help guide clinical decision-making for the type of chemotherapy to use in patients with advanced pancreatic ductal adenocarcinoma (PDAC). This study assessed the ability of plasma miRs to predict optimal treatment response from FOLFIRINOX or gemcitabine-*nab*-paclitaxel in these patients.

Methods: Next-generation sequencing (NGS) was performed for biomarker discovery in pre-treatment plasma samples from advanced PDAC patients subsequently treated with FOLFIRINOX (n = 12) or gemcitabine-*nab*-paclitaxel (n = 12). Selected candidate biomarkers were validated in 40 patients with advanced PDAC using RT-qPCR. Cox regression was then used to assess the predictive value of plasma miRs for either FOLFIRINOX or gemcitabine-*nab*-paclitaxel.

Results: In the validation cohort, high plasma miR-379 expression was strongly predictive of treatment response ($P_{interaction}$ = 0.0004). Overall survival (OS) was significantly better with FOLFIRINOX vs. gemcitabine-*nab*-paclitaxel in those patients with lower plasma miR-379 expression (hazard ratio, 0.32 [95% confidence interval, 0.08 to 0.98]; P = 0.046). However, gemcitabine-*nab*-paclitaxel was associated with superior OS in patients with higher plasma miR-379 (hazard ratio, 0.28 [0.10 to 0.86]; P = 0.027). In contrast, miR-127, miR-155, and miR-200 showed no predictive value for treatment response for either chemotherapy regimen ($P_{interaction}$ = 0.12, $P_{interaction}$ = 0.83 and $P_{interaction}$ = 0.12, respectively).

Conclusions: Plasma miR-379 appears clinically useful as a predictive biomarker to identify which patients with advanced PDAC benefit most from treatment with FOLFIRINOX or gemcitabine-*nab*-paclitaxel. Further validation in larger studies and clinical trials is now warranted.

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1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a very aggressive malignancy which is typically diagnosed at an advanced disease stage [1]. A small subset of patients is eligible for curative-intent surgical resection, but patients frequently present with early local recurrence and metastatic relapse despite adjuvant chemotherapy [2]. The 5-year survival rate of patients with PDAC is approximately 10%, while the incidence is increasing [1,3,4]. By 2030, PDAC is predicted to become the second leading cause of cancer-related death in Europe [3,4]. Currently, clinical management of patients with advanced PDAC consists of gemcitabine or multi-agent systemic therapy (i.e., FOLFIRINOX [fluorouracil, oxaliplatin, leucovorin and irinotecan] or gemcitabine plus nab-paclitaxel) [5–7]. Although patients with good performance status can benefit from multi-agent systemic therapy, success of treatment is often strongly limited by toxicity and drug resistance [8]. Predictive biomarkers are needed to help physicians decide which treatment to provide, and ultimately improve therapeutic success whilst avoiding adverse events.

Currently, the decision between first-line chemotherapeutic regimens is based on performance score, individual toxicity profiles, and shared decision making [5]. Patients with a good performance score and under the age of 75 years are preferably treated with FOLFIRINOX [9–12]. This recommendation is primarily based on the results of the PRODIGE 4/ACCORD 11 randomized clinical trial, which randomly assigned patients with advanced PDAC to FOLFIRINOX or gemcitabine, which was the standard of care before the trial. Compared to gemcitabine, the FOLFIRINOX arm showed longer overall survival (OS; hazard ratio, 0.57 [95% confidence interval, 0.45 to 0.73]; $P < 0.001$), but had a less favourable safety profile than gemcitabine [9]. In a meta-analysis of 9 studies comparing FOLFIRINOX with gemcitabine-*nab*-paclitaxel treatment for PDAC, overall objective response rates were limited, 24% and 25%, respectively [13], emphasizing the need to identify patients who will benefit most from a specific chemotherapy regimen.

Previous studies have identified liquid biopsy-based microRNAs (miRs) as promising candidates for several applications, including: early and minimally invasive diagnosis, prognostication, and monitoring of chemotherapy response. miRs have also emerged as important players in therapy resistance in PDAC [14–18]. miRs are small non-coding RNA molecules that post-transcriptionally modulate gene expression by complementary binding to target messenger RNA (mRNA) transcripts, causing degradation of mRNA or preventing translation into a protein [19]. miRs are readily measurable in bodily fluids, suggesting that miRs could potentially be used as biomarkers to improve current treatment decision algorithms [20]. Therefore, the objective of this study was to identify miRs that can predict optimal response in patients with advanced PDAC to treatment with FOLFIRINOX or gemcitabine-*nab*-paclitaxel, in order to facilitate individualized clinical decision-making for these first-line chemotherapy options.

2. Materials and methods

2.1. Patient selection

In this study, consecutive patients with advanced PDAC were included between January 2018 and November 2020 as a discovery cohort. Patients from another university hospital were included between January 2017 and August 2020 as a validation cohort. Patients in the discovery cohort who received FOLFIRINOX as first-line treatment were matched based on age and sex to those treated with gemcitabine-*nab*-paclitaxel. This study was approved by the local medical ethics committees. All patients included in this study provided written informed consent.

Diagnosis of PDAC was based on radiological findings and histopathological proof in all patients. Overall survival (OS) was calculated as the time between the date of diagnosis and the date of censoring or death. Patients were followed until January 2023.

2.2. RNA extraction

Pre-treatment peripheral venous blood was collected at diagnosis. Blood samples were centrifuged directly after sample collection for 10 min at 5000g. Plasma was stored at −20 °C, and RNA was isolated using the Small RNA isolation kit (Exiqon) according to the manufacturer's protocol.

2.3. Next generation sequencing (NGS)

NGS of plasma RNA samples from the discovery cohort was performed by GenomeScan (Leiden, the Netherlands). Briefly, samples were processed using the NEBNext Multiplex Small RNA Library Prep Kit for Illumina, followed by sample preparation according to GenomeScan's standard operating procedure. Fragment Analyzer was used to assess the quality and yield following sample preparation. NovaSeq6000 was used to perform clustering and sequencing in accordance with the manufacturer's protocols.

The discovery cohort of 24 patients treated with FOLFIRINOX (n = 12) or gemcitabine-*nab*-paclitaxel (n = 12) was used. Patients in each group were divided based on progression-free survival (PFS) into a good response (i.e. PFS higher than median), and poor response group (i.e. PFS lower than median).

2.4. Validation of microRNA candidates

Plasma miR expression in the validation cohort was quantified RT-qPCR using miRCURY LNA Polymerase chain reaction primer sets (Exiqon). In total, four plasma miRNAs were validated: miR-127, miR-155, miR-200, and miR-379. These miRNAs were selected based on their performance (i.e. the $\log_2$ fold change and P value) in the discovery cohort, previous importance in literature, and the availability of primers. Based on previous literature, miR-16 was used as a reference miR for normalization [21,22].

2.5. miRNA target prediction

Three databases were used to identify potential targets of validated miRs: miRTarBase, TarBase v.8, and miRDB. Selection criteria were: gene targets supported by strong experimental evidence (i.e. reporter assay or Western blot); gene targets with a prediction score higher than 0.800; and gene targets with a Target Score higher than 0.85.

2.6. Statistical analysis

The predictive value of miRs for response to FOLFIRINOX or gemcitabine-*nab*-paclitaxel was assessed in the validation cohort using Cox regression, with OS (defined as time from diagnosis to either death or follow-up) as the outcome. The Cox regression model included the miR variable, the treatment assignment variable, and the interaction term between the miR and treatment assignment variable. The significance of the interaction term was tested using a likelihood ratio test to assess potential effect modification on the relative (i.e. hazard ratio) scale. Confounding by age, sex, WHO performance status, and tumour stage was addressed by including these variables as covariates in the model with Firth's bias correction to prevent potential sparse data bias. The proportional hazards assumption was assessed using the Grambsch-Therneau test and visual inspection of Schoenfeld residuals. Nonlinearity of continuous variables was assessed using Martingale residuals.

The association between the expression level of each miR and other baseline characteristics (e.g. age, tumour stage, and WHO performance score) was assessed using multivariable proportional odds logistic regression models, which make fewer distributional assumptions than conventional linear regression analysis. Nonlinearity of continuous variables was assessed using restricted cubic splines. However, as the overall nonlinearity Wald test was consistently not significant, all nonlinear

terms were removed from the proportional odds model. Likelihood ratio tests were performed to assess the statistical significance of covariates in the model. All proportional odds models were fitted on multiply imputed datasets. Missing data for covariates were handled using multiple imputation [23]. The imputation model included the exposure variable (i.e., FOLFIRINOX or gemcitabine-*nab*-paclitaxel), relevant covariates (age, sex, CA19-9, WHO performance score, and tumour stage), the event indicator, and the Nelson-Aalen estimate of the cumulative baseline hazard [24]. In total, 20 imputed datasets and 15 iterations were used. A two-sided *P* value lower than 0.05 was considered statistically significant. All analyses were performed in R, version 4.1.2.

3. Results

In total, 24 patients with advanced PDAC were included in the discovery cohort, and 40 patients with advanced PDAC were included in the validation cohort. Patient characteristics in the discovery and validation cohort are described in Table 1 and Table 2, respectively.

Table 1
Patient characteristics of the discovery cohort.

	FOLFIRINOX ($n = 12$)		Gemcitabine-*nab*-paclitaxel ($n = 12$)	
Age at diagnosis, median (IQR) – yr	67	(64–71)	70	(65–74)
Sex – *n* (%)				
Female	7	(58%)	7	(58%)
Male	5	(42%)	5	(42%)
Tumour stage – *n* (%)				
Stage III	0	(0%)	0	(0%)
Stage IV	12	(100%)	12	(100%)
WHO performance score – *n* (%)				
0	11	(92%)	5	(42%)
1	1	(8%)	7	(58%)
CA19-9, median (IQR) – U/mL	2220	(155–4594)	659	(283–1962)
CA19-9 – *n* (%)				
Normal (<37 U/mL)	0	(0%)	1	(8%)
Elevated	11	(92%)	11	(92%)
Number of cycles, median (IQR)	10	(6–12)	5	(3–8)
CEA, median (IQR) – µg/L	4.0	(3–30)	3.7	(2.8–4.4)
Bilirubin, median (IQR) – µmol/L	0.9	(0.7–6.0)	0.6	(0.6–0.7)
RECIST response – *n* (%)				
Partial response	3	(25%)	4	(33%)
Stable disease	4	(33%)	5	(42%)
Progressive disease	2	(17%)	2	(17%)

Categories of several variables do not add up to 100% due to missing data.

Table 2
Patient characteristics of the validation cohort.

	FOLFIRINOX ($n = 12$)		Gemcitabine-*nab*-paclitaxel ($n = 28$)	
Age at diagnosis, median (IQR) – yr	61	(58–66)	64	(58–69)
Sex – *n* (%)				
Female	6	(50%)	10	(36%)
Male	6	(50%)	18	(64%)
Tumour stage – *n* (%)				
Stage III	9	(75%)	6	(21%)
Stage IV	3	(25%)	21	(75%)
WHO performance score – *n* (%)				
0	9	(75%)	22	(79%)
1	1	(8%)	2	(7%)
2	1	(8%)	3	(11%)
3	1	(8%)	0	(0%)
CA19-9, median (IQR) – U/mL	907	(447–1884)	1014	(322–3384)
CA19-9 – *n* (%)				
Normal (<37 U/mL)	1	(8%)	3	(11%)
Elevated	8	(67%)	14	(50%)
RECIST response – *n* (%)				
Partial response	4	(33%)	12	(43%)
Stable disease	3	(25%)	6	(21%)
Progressive disease	1	(8%)	5	(18%)

Categories of several variables do not add up to 100% due to missing data.

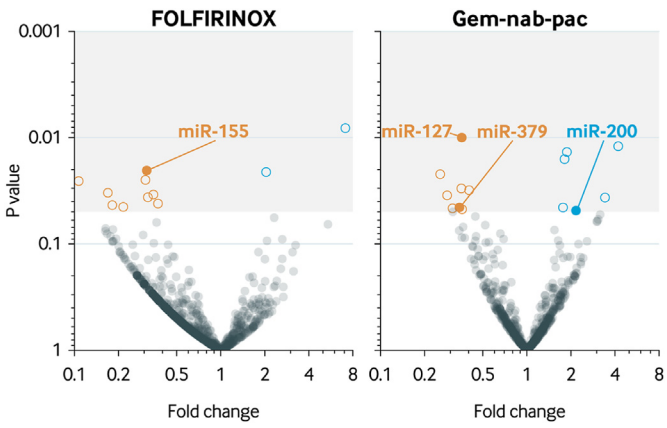

Fig. 1. Volcano plot. Grey region: $P < 0.05$. The miRs with $P < 0.05$, and which have been previously described in PDAC are highlighted.

3.1. Biomarker discovery

In total, 17 significantly downregulated and 8 significantly upregulated plasma miRs were identified by NGS (Fig. 1). Four significantly differentially expressed candidate biomarkers were selected for further validation based on their fold change, *P* value, and previous studies describing their predictive value for chemotherapy response (plasma miR-127, miR-155, miR-200, and miR-379; fig. S1, table S1). Association of candidate miRs with baseline characteristics are displayed in tables S2–4.

3.2. Predictive value of miR-127, miR-155, miR-200, and miR-379 in the validation cohort

In the validation cohort, there was no evidence for a difference in OS between patients treated with FOLFIRINOX compared to those treated with gemcitabine-*nab*-paclitaxel (hazard ratio, 0.85; 95% confidence interval [CI], 0.34 to 2.15; Fig. 2). In unadjusted analyses, there was no association between either plasma miR-155 or miR-200 expression and treatment response (interaction test, $P = 0.36$ and $P = 0.19$, respectively). However, plasma miR-127 was moderately predictive of treatment response (interaction test, $P = 0.036$; fig. S2A). Notably, after correcting for confounding by age, sex, WHO performance score, tumour stage, and CA19-9, the predictive value of plasma miR-127, miR-155, and miR-200 was attenuated (interaction test, $P = 0.12$, $P = 0.83$, $P = 0.12$, respectively; Fig. 3A).

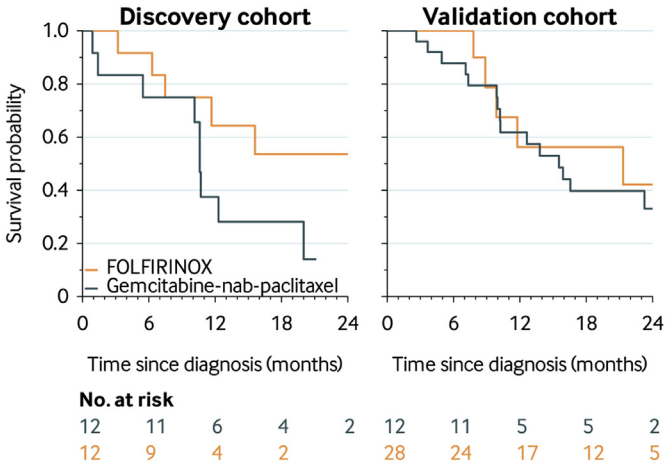

Fig. 2. Overall survival of patients in the discovery and validation cohort, stratified by therapy (gemcitabine-*nab*-paclitaxel, teal; FOLFIRINOX, gold). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

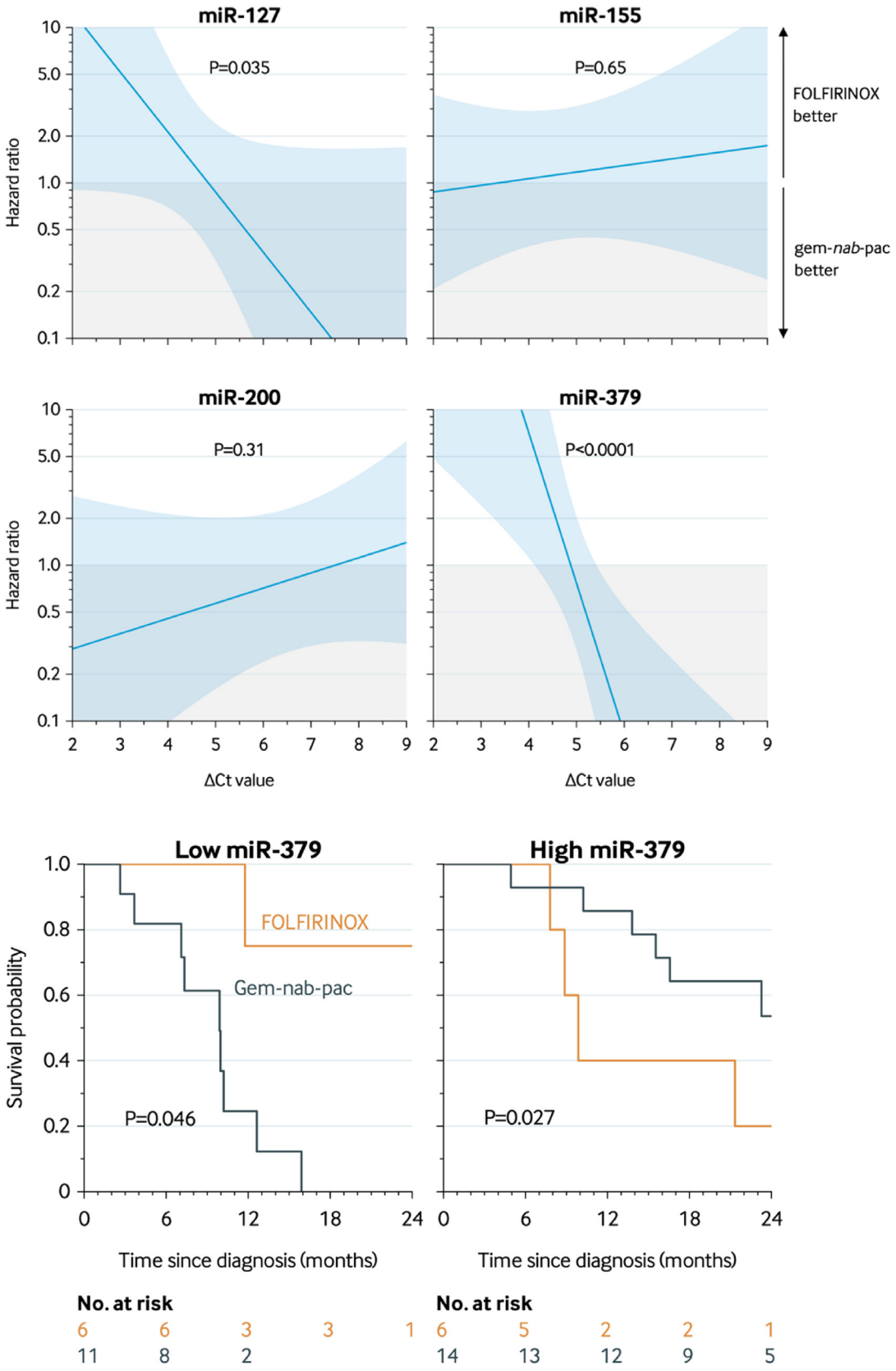

Fig. 3. A, The association between plasma miRs and treatment response of FOLFIRINOX vs. gemcitabine-*nab*-paclitaxel; B, Overall survival for patients treated with gemcitabine-*nab*-paclitaxel or FOLFIRINOX with low (i.e. lower than median) and high plasma miR-379. HR > 1, FOLFIRINOX better; HR < 1, gemcitabine-*nab*-paclitaxel better. Gem-*nab*-pac, gemcitabine-*nab*-paclitaxel.

In contrast, plasma miR-379 was strongly predictive of treatment response (interaction test, $P = 0.0004$; fig. S2), and remained predictive after correction for confounding by age, sex, WHO performance score, tumour stage, and CA19-9 (interaction test, $P = 0.016$; Fig. 3A). FOLFIRINOX was significantly better than gemcitabine-*nab*-paclitaxel in the subset of patients with plasma miR-379 lower than the median (adjusted hazard ratio, 0.32 [95% confidence interval, 0.08 to 0.98]; $P = 0.046$; Fig. 3B), while gemcitabine-*nab*-paclitaxel was superior in the subset of patients with higher than median miR-379 (adjusted hazard ratio, 0.28 [0.10 to 0.86]; $P = 0.027$). In target databases, EIF4G2 and PLAGL1 were identified as potential targets of miR-379 (table S5).

4. Discussion

In this study, we discovered and validated plasma miR-379 as a predictive biomarker for chemotherapy response in patients with advanced PDAC. FOLFIRINOX was superior compared to gemcitabine-nab-paclitaxel in terms of OS in the subset of patients with lower than median plasma miR-379 expression, whereas the opposite was true for patients with higher than median plasma miR-379. In contrast, there was no evidence for predictive value of plasma miR-155, miR-200, and miR-127.

miR-379 is associated with inhibiting cell migration and proliferation, and has a tumour-suppressive role by inhibiting epithelial-to-mesenchymal transition [25]. In particular, miR-379 has been described as a tumour suppressor in several cancer types, including breast cancer, endometrial cancer, and non-small cell lung cancer [25]. In addition, miR-379 has been shown to reduce chemoresistance to cisplatin, docetaxel, and paclitaxel in non-small-cell lung carcinoma through its role in regulating activity of insulin-like growth factor I, in line with our results [26–30]. However, the potential of plasma miR-379 as a predictive biomarker for chemotherapy response in pancreatic cancer has never been described in the literature.

The mechanism through which miR-379 may influence the efficacy of FOLFIRINOX or gemcitabine-*nab*-paclitaxel is currently not fully understood. However, miRNA target prediction databases indicated that EIF4G2 and PLAGL1 could be potential gene targets of miR-379. EIF4G2 has been shown to be involved in paclitaxel resistance in ovarian cancer, and miR-379-mediated suppression of EIF4G2 altered cisplatin chemosensitivity in non-small cell lung cancer cells [27,31]. In addition, PLAGL1 is a tumour suppressor gene that reduces progression and proliferation of PDAC cells [32]. Future experiments should include functional tests to identify pathways targeted by miR-379 in PDAC.

In this study, plasma miR-379 was validated as a predictive biomarker for chemotherapy response in advanced PDAC. Such markers are needed to individualize clinical decision-making by guiding chemotherapy choice based on the expected survival benefit of FOLFIRINOX compared to gemcitabine-*nab*-paclitaxel. Currently, this decision is based on a patient's fitness (e.g. age, performance status, and comorbidities), rather than predicted treatment benefits. However, selecting patients based on treatment benefits could result in patients eligible for FOLFIRINOX to receive gemcitabine-*nab*-paclitaxel instead. This approach could optimize patient outcomes by decreasing the occurrence of adverse events and dose adjustments, without compromising survival outcomes. Specifically, miR-379 levels could be measured in patient blood samples during routine clinical work-up after a diagnosis of advanced PDAC. These levels could subsequently be used in conjunction with clinical parameters to determine the optimal first-line treatment choice for an individual patient.

This study has several limitations. First, miRs were only measured at baseline, precluding assessment of plasma miR-379 as a biomarker to monitor treatment response over time. Second, FOLFIRINOX and gemcitabine-*nab*-paclitaxel were not randomly assigned, and as such, the interaction between plasma miR-379 and treatment assignment could have been confounded by differences in potentially unmeasured baseline characteristics between patients with low and high plasma miR-379 values, and between patients treated with FOLFIRINOX or gemcitabine-*nab*-paclitaxel. Ideally, data from randomized clinical trials would be available as discovery and validation cohorts for predictive biomarkers. However, there are currently no randomized trials comparing the efficacy of FOLFIRINOX and gemcitabine-*nab*-paclitaxel for advanced PDAC, and, even if such trials were available, confounding can still occur in subgroup analyses (including predictive biomarker discovery analyses) [33].

Our study was underpowered to assess whether plasma miR-379 has added predictive value over routinely measured potential treatment effect modifiers, such as baseline performance score, pre-treatment CA19-9 levels, and the number and location of metastatic sites [6,9]. As subgroup analyses (including analyses to discover predictive biomarkers) are typically also substantially underpowered in randomized trials [34], large observational registries are necessary to further validate plasma miR-379, and assess whether it has incremental value over commonly available clinicopathological variables to estimate individualized treatment effects.

Strengths of this study include: (1) the use of unbiased NGS to discover potentially predictive plasma biomarkers in a comprehensive and reproducible way; (2) the inclusion of a validation cohort to assess the predictive value of candidate plasma miRs using RT-qPCR, and (3) the use of blood plasma samples to identify predictive biomarkers, as this minimally invasive approach is more likely to be tolerated by patients.

5. Conclusions

In conclusion, this study validated plasma miR-379 as a predictive biomarker for treatment response to FOLFIRINOX and gemcitabine-*nab*-paclitaxel in patients with advanced PDAC. Pending further validation of plasma miR-379 in large, observational cohort studies, monitoring plasma miR-379 has the potential to improve individualized clinical decision-making when choosing between these first-line chemotherapy regimens, and optimize survival outcomes for patients with advanced PDAC.

Ethics approval and consent to participate

This study was approved by all local medical ethics committees: the ethical board of the University of Pisa, the University Hospital of Parma and Carrara Civic Hospital (Italy). All patients included in this study provided written informed consent.

Consent for publication

Not applicable.

Availability of data and materials

Data are available from the corresponding author upon reasonable request and provision of a statistical analysis plan.

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Authors' contribution

LNCB, MA, JRP, and EG conceived the work that led to the submission, and acquired the data. All authors played an important role in interpreting the results, drafted or revised the manuscript, approved the final version, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Elisa Giovannetti reports financial support was provided by Dutch Cancer Society. Elisa Giovannetti reports financial support was provided by AIRC. Geert Kazemier reports financial support was provided by Dutch Cancer Society. Elisa Giovannetti reports financial support was provided by Bennink Foundation. Lenka NC Boyd, Mahsoem Ali, Jisce R Puik, Tessa YS Le Large, Geert Kazemier reports financial support was provided by Bennink Foundation. If there are other authors, they 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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None reported.

Abbreviations

CA19-9	carbohydrate antigen 19-9
CI	confidence interval
EIF4G2	eukaryotic translation initiation factor 4 gamma 2
FOLFIRINOX	fluorouracil, leucovorin, irinotecan, and oxaliplatin
miR	microRNA
mRNA	messenger RNA
OS	overall survival
PDAC	pancreatic ductal adenocarcinoma
PFS	progression-free survival
PLAGL1	PLAG1 (pleomorphic adenoma gene 1)-like zinc finger 1
RT-*q*PCR	reverse transcriptase quantitative polymerase chain reaction

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jlb.2024.100152.

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