A Transcriptomic-Based Tool to Predict Gemcitabine Sensitivity in Advanced Pancreatic Adenocarcinoma

Pancreatic ductal adenocarcinoma (PDAC) incidence has increased over the last 30 years.[1] When diagnosed at advanced stages, representing approximately 85% of cases, systemic therapy is the only treatment able to improve patient outcomes. For patients with a good performance status, FOLFIRINOX is the preferred choice, but has a high level of toxicity.[2] For unfit patients, gemcitabine administrated alone or combined with nab-paclitaxel remains the standard treatment.[3,4] Treatment choice is currently based on physician evaluation; using tumor molecular analysis to select the most effective and least toxic chemotherapy regimen would represent major progress.

In recent years, we and others have described RNA signatures associated with gemcitabine sensitivity. Tiriac et al[5] found that RNA signatures derived from organoids could determine chemotherapy sensitivity. We reported *GemPred*, a gemcitabine RNA signature containing thousands of transcripts and validated in a retrospective cohort of 435 patients.[6] As *GemPred* predictions were associated with the basal-like and classical PDAC subtypes that relate to patient prognosis, organoid models were included in the signature identification strategy. This allowed us to overcome the prognostic limitations of *GemPred* and generate an *improved GemPred* signature.[7] Finally, using a strategy based on the selection of a reduced number of transcriptomic-concordant in vitro and in vivo PDAC models, we identified *GemCore*, a gemcitabine sensitivity signature that has the advantage of containing fewer than 100 transcripts and that has been validated in 2 clinical cohorts of 80 and 305 patients.[8] As these signatures were all validated in retrospective cohorts of localized tumors on resected formalin-fixed, paraffin-embedded tissues samples, we decided to analyze their ability to predict gemcitabine sensitivity in advanced PDAC on formalin-fixed, paraffin-embedded microbiopsies from primary tumors and metastatic sites.

One hundred and seven patients with advanced PDAC were retrospectively included from 3 hospitals. All patients were treated with gemcitabine as monotherapy in the first line. One hundred and one assessable samples were obtained from 93 patients before treatment (57 unpaired from primary tumors, 28 unpaired from metastatic sites and 16 paired samples).

First, we analyzed primary tumors from 65 patients. Five patients (7.7%) had locally advanced disease and 60 (92.3%) had metastatic disease. Median overall survival (OS) was 5.7 months (95% CI, 4.62–8.52 months) and median progression-free survival (PFS) was 2.3 months (95% CI, 1.38–3.44 months). *Gem-Tiriac et al*, *GemPred*, and *improved GemPred* performed poorly in identifying gemcitabine sensitivity (Figure 1*A*–*F*). *Improved GemPred* revealed a significant association between PFS and gemcitabine sensitivity, with a hazard ratio (HR) of 0.57 (95% CI, 0.34–0.95; $P = .032$) (Figure 1*F*). Of all signatures, *GemCore* achieved the best performance, classifying 29 patients (44.6%) as *GemCore*+ and 36 (56.4%) as *GemCore*– (Figure 1*G* and *H*). *GemCore*+ patients displayed a median OS of 13.9 months (95% CI, 9.51–17.18 months) and a median PFS of 4.85 months (95% CI, 4.29–8.07 months). *GemCore*– patients had a median OS of 3.1 months (95% CI, 2.33–4.79 months) and a median PFS of 1.15 months (95% CI, 0.49–1.87 months). *GemCore* was also the only signature to show a significant association with objective response in primary tumors (Table 1 and Supplementary Table 1). In the univariate Cox model, *GemCore*+ patients showed an OS HR of 0.19 (95% CI, 0.10–0.34; $P < .001$) and a PFS HR of 0.12 (95% CI, 0.06–0.25; $P < .001$). When we contrasted the *GemCore* signature prediction with clinicopathological variables and transcriptomic RNA biomarkers, we found that 5 variables were statistically significant predictors of OS and PFS ($P < .05$) (Supplementary Table 2): World Health Organization performance status score ≥ 2, presence of hepatic metastasis, carbohydrate antigen 19-9 levels 59 times higher than the upper limit and poor differentiation were significant for both OS and PFS, whereas number of metastases was only significant for OS and weight loss only for PFS. *GemCore* was significantly associated with hepatic metastases and the degree of tumor differentiation (Table 1). Despite the observed enrichment of the *GemCore* stratification with the clinicopathological variables mentioned, *GemCore*+ remained a predictor of OS (HR, 0.18; 95% CI, 0.09–0.35; $P < .001$) and PFS (HR, 0.11; 95% CI, 0.04–0.26; $P < .001$) in a Cox multivariate model (Supplementary Table 2).

When possible, biopsies from metastatic sites are frequently used for diagnostic purposes. Therefore, we analyzed the 4 signatures in 36 biopsies from PDAC metastases. Median OS was 3.5 months (95% CI, 2.39–6.00 months) and median PFS was 1.15 months (95% CI, 0.66–2.39 months). As in primary tumors, *GemCore* was better able to stratify gemcitabine sensitivity in metastasis samples. *GemCore*+ patients (n = 19 [52.78%]) had a median OS of 6.6 months (95% CI, 4.72–16.13 months) and a median PFS of 2.95 months (95% CI, 1.38–4.36 months). *GemCore*– patients (n = 17 [47.22%]) displayed a median OS of 2.1 months (95% CI, 1.64–3.48 months) and a median PFS of 0.36 months (95% CI, 0.00–1.34 months). The univariate Cox model confirmed the predictive capability of *GemCore* to discriminate gemcitabine-sensitive patients. *GemCore*+ showed an HR of 0.14 (95% CI,

Abbreviations used in this paper: **HR, hazard ratio; OS, overall survival; PDAC, pancreatic ductal adenocarcinoma; PFS, progression-free survival.**

Most current article

0016-5085
https://doi.org/10.1053/j.gastro.2022.11.035

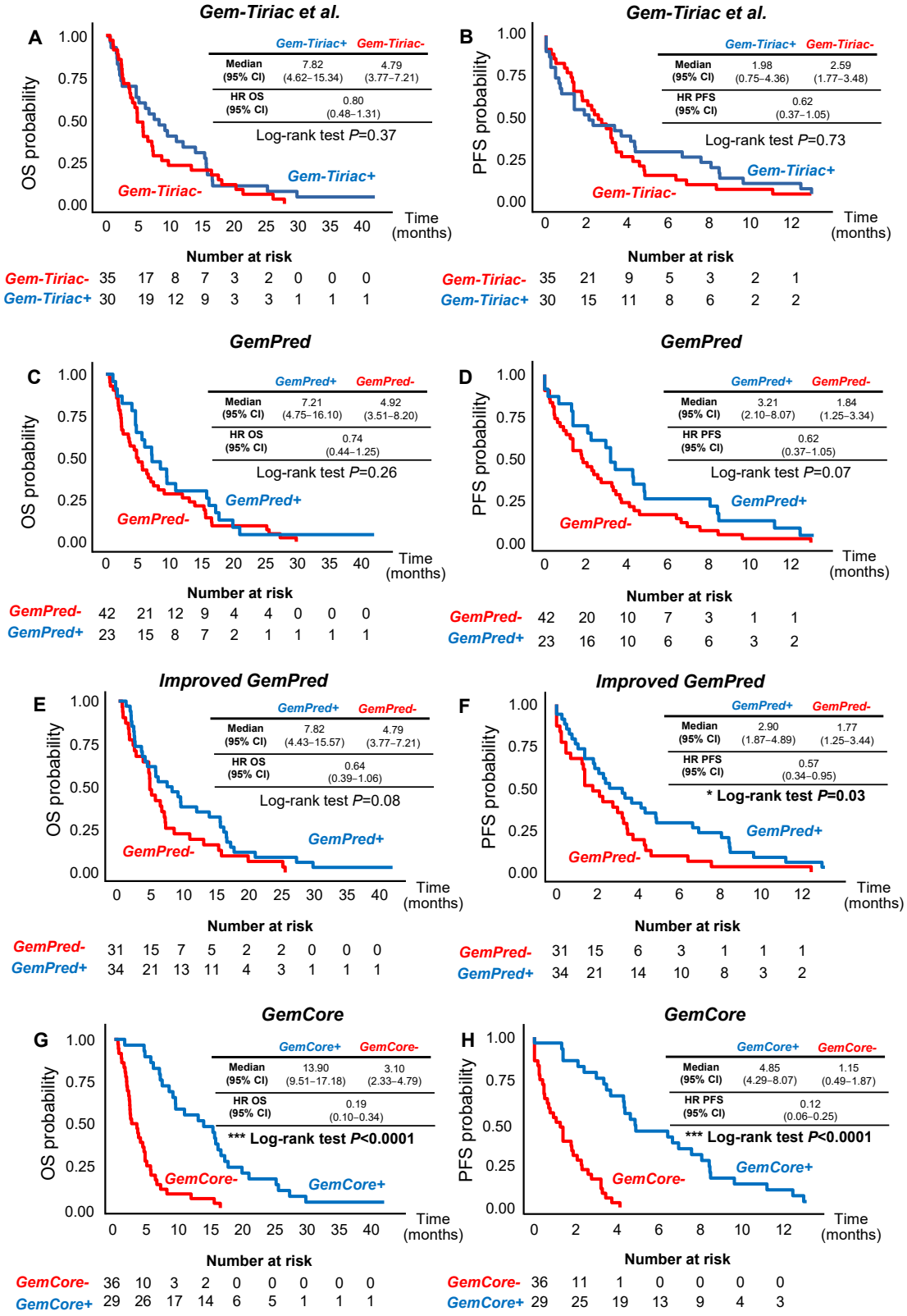

Figure 1. Comparison of gemcitabine sensitivity signatures in patients with advanced PDAC on samples obtained by endoscopic ultrasound fine-needle aspiration biopsy from primary tumors. Kaplan–Meier curve for OS and PFS stratified by gemcitabine sensitivity prediction for the different signatures: (*A, B*) *Gem-Tiriac et al*, (*C, D*) *GemPred*, (*E, F*) *improved GemPred*, and (*G, H*) *GemCore* signatures.

0.06–0.35; $P < .001$) for OS and 0.17 (95% CI, 0.07–0.42; $P < .001$) for PFS. Among the clinicopathological variables and transcriptomic RNA biomarkers, tumor thickness was the only variable to predict OS in a univariate Cox model (HR, 1.03; 95% CI, 1.00–1.06). In addition, tumor thickness was significantly lower in *GemCore+* than in *GemCore–* patients (33.9 ± 11.8 vs 44 ± 12.4; $P = .017$) (Table 1). There was a significant association between *GemCore–* patients and the number of metastases being ≥2 ($P = .048$) (Table 1). Finally, our analysis of the paired primary tumor and metastasis samples revealed that the *GemCore* signature gave a matched prediction in 87.5% of cases (57% of samples were *GemCore–*, 43% were *GemCore+*).

Table 1. Association of *GemCore* With Clinicopathological and Transcriptional Biomarkers: Samples From Primary Tumors and Metastases

Variable	*GemCore* Positive	*GemCore* Negative	*P value*
Primary tumors			
Age, n (%)			1
≤65 y	6 (9.2)	7 (10.8)	
>65 y	23 (35.4)	29 (44.6)	
Sex, n (%)			1
Female	15 (23.1)	18 (27.7)	
Male	14 (21.5)	18 (27.7)	
WHO PS score, n (%)			.436
Unknown	1 (1.5)	0 (0.0)	
0	5 (7.7)	3 (4.6)	
1	8 (12.3)	14 (21.5)	
≥2	15 (23.1)	19 (29.2)	
Clinical stage, n (%)			.649
Locally advanced	2 (3.1)	3 (4.6)	
Metastatic	34 (52.3)	26 (40.0)	
Weight loss, *kg*, mean (SD)	8.24 (6.65)	8.03 (5.62)	1
Primary tumor location, n (%)			.617
Head	12 (18.5)	18 (27.7)	
Other	17 (26.1)	18 (27.7)	
Tumor thickness, *mm*, mean (SD)	41.9 (18.0)	39.3 (16.0)	.595
Pulmonary metastases, n (%)			1
No	21 (32.3)	26 (40.0)	
Yes	8 (12.3)	10 (15.4)	
Hepatic metastases, n (%)			.001
No	17 (26.1)	7 (10.8)	
Yes	12 (18.5)	29 (44.6)	
No. of metastatic sites, n (%)			.064
1	23 (35.4)	20 (30.1)	
≥2	6 (9.2)	16 (24.6)	
Level of CA19-9, n (%)			1
Unknown	5 (7.7)	10 (15.4)	
Normal	4 (6.1)	5 (7.7)	
<59× ULN	18 (27.7)	18 (27.7)	
>59× ULN	2 (3.1)	3 (4.6)	
Differentiation, n (%)			.005
Unknown	2 (3.1)	2 (3.1)	
1 (well)	25 (38.5)	23 (35.8)	
2 (moderately)	2 (3.1)	2 (3.1)	
3 (poor)	0 (0.0)	9 (13.8)	
Objective responses, n (%)			.026
Unknown	3 (4.6)	15 (23.1)	
Partial response	6 (9.2)	3 (4.6)	
Stable disease	15 (23.1)	6 (9.2)	
Progressive disease	5 (7.7)	12 (18.5)	
PurIST, n (%)			1
Basal-like	2 (3.1)	3 (4.6)	
Classical	27 (41.5)	33 (50.8)	
CDA, mean (SD)	2.29 (2.40)	2.70 (2.26)	.405
DCK, mean (SD)	0.71 (1.45)	0.61 (1.55)	.468
SLC29A1 (hENT1), mean (SD)	2.58 (2.54)	2.47 (2.79)	.967
hENT1/CDA ratio, mean (SD)	2.00 (2.79)	1.89 (3.71)	.708
Metastases			
Age, n (%)			.434
≤65 y	3 (8.3)	5 (13.8)	
>65 y	16 (44.4)	12 (33.3)	
Sex, n (%)			.335
Female	10 (27.8)	6 (16.7)	
Male	9 (25.0)	11 (30.6)	
WHO PS score, n (%)			.714
Unknown, n (%)	1 (2.8)	1 (2.8)	
0	1 (2.8)	1 (2.8)	
1	6 (16.7)	3 (8.3)	
≥2	11 (30.6)	12 (33.3)	
Primary tumor location, n (%)			.516
Head	9 (25.0)	6 (16.7)	
Other	10 (27.8)	11 (30.6)	
Tumor thickness, *mm*, mean (SD)	33.9 (11.8)	44.7 (12.4)	.017
No. of metastatic sites, n (%)			.048
1	11 (30.6)	4 (11.1)	
≥2	8 (22.2)	13 (36.1)	
Level of CA19-9, n (%)			.555
Unknown	2 (5.6)	7 (19.4)	
Normal	2 (5.6)	2 (5.6)	
<59× ULN	13 (36.1)	7 (19.4)	
>59× ULN	1 (2.8)	2 (5.6)	
Differentiation, n (%)			1
Unknown	2 (5.6)	3 (8.3)	
1 (well)	9 (25.0)	8 (22.2)	
2 (moderately)	4 (11.1)	3 (8.3)	
3 (poor)	4 (11.1)	3 (8.3)	
Objective responses, n (%)			.493
Unknown	6 (16.7)	14 (38.9)	
Partial response	2 (5.6)	1 (2.8)	
Stable disease	4 (11.1)	0 (0.0)	
Progressive disease	7 (19.4)	2 (5.6)	
PurIST, n (%)			.167
Basal-like	1 (2.8)	4 (11.1)	
Classical	18 (50.0)	13 (36.1)	
CDA, mean (SD)	3.24 (2.62)	4.55 (2.62)	.181
DCK, mean (SD)	0.99 (1.10)	0.86 (0.81)	.871
SLC29A1 (hENT1), mean (SD)	3.83 (2.19)	3.89 (2.37)	1
hENT1/CDA ratio, mean (SD)	2.18 (3.83)	0.79 (0.43)	.484

CA19–9, cancer antigen 19–9; CDA, cytidine deaminase; DCK, deoxycytidine kinase; hENT1, human equilibrative nucleoside transporter 1; PurIST, Purity Independent Subtyping of Tumours; SLC29A1, solute carrier family 29 member 1; ULN, upper limit of normal; WHO PS, World Health Organization performance status score.

A weakness associated with drug-response RNA signatures is that they frequently capture the basal-like or classical transcriptomic landscape that is related to the patient's prognosis. However, *GemCore* did not correlate with any PDAC subtype and was the main OS and PFS predictor in the multivariate Cox analysis (Supplementary Table 2 and Table 1). These observations suggest that *GemCore* has a predictive, not prognostic, capacity.

Gemcitabine is the main drug used in unfit patients with metastatic PDAC because it has reduced infusion times and fewer adverse effects than polychemotherapy regimens (ie, FOLFIRINOX and gemcitabine/nab-paclitaxel). To avoid any potential biases derived from a combined treatment, here we focused on patients treated with gemcitabine alone. However, further validation of *GemCore* is needed in patients treated with gemcitabine plus nab-paclitaxel to enlarge the scope of this signature.

We noted that the median OS of *GemCore*+ patients with biopsied primary tumors was longer than that of those with biopsies from metastatic sites. Although *GemCore* was able to identify responders to gemcitabine in both, the difference in the median OS is suspected to be because of the small number of patients in the metastatic group and/or because the biopsies of metastatic tissue correspond to those patients with the most advanced disease; further validation on larger metastasis cohorts is needed to elucidate this discrepancy.

Development of predictive signatures is challenging and in permanent evolution. These predictors depend on the technology used for RNA sequencing and even more on the site from which the biopsy is taken. In this work, we challenged in a multicentric cohort of advanced PDAC patients the *GemCore* signature alongside 3 other signatures previously validated for gemcitabine as adjuvant treatment for patients who have undergone surgery. *GemCore* represents the RNA-based signature best able to predict gemcitabine response not only in resected but also in advanced PDAC patients and in all types of samples (ie, resections or microbiopsies from primary tumors and metastatic sites).

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at https://doi.org/10.1053/j.gastro.2022.11.035.

NICOLAS FRAUNHOFFER*
Cancer Research Center of Marseille (CRCM)
INSERM, CNRS
Institut Paoli-Calmettes
Aix-Marseille University
Marseille, France

BRICE CHANEZ*
Cancer Research Center of Marseille (CRCM)
INSERM, CNRS
Institut Paoli-Calmettes
Aix-Marseille University
Marseille, France, *and*
Medical Oncology Department
Institut Paoli-Calmettes
Marseille, France

CARLOS TEYSSEDOU
Cancer Research Center of Marseille (CRCM)
INSERM, CNRS
Institut Paoli-Calmettes
Aix-Marseille University
Marseille, France, *and*
Endocrine and Visceral Surgery Department
University Hospital Angers
Angers, France, *and*
Haut Anjou Hospital Center
Château-Gontier, France

PDAC CHEMO SENSITIVITY PREDICTION WORKING GROUP

JUAN L. IOVANNA
EMMANUEL MITRY[§]
Cancer Research Center of Marseille (CRCM)
INSERM, CNRS
Institut Paoli-Calmettes
Aix-Marseille University
Marseille, France, *and*
Medical Oncology Department
Institut Paoli-Calmettes
Marseille, France

NELSON J. DUSETTI[§]
Cancer Research Center of Marseille (CRCM)
INSERM, CNRS
Institut Paoli-Calmettes
Aix-Marseille University
Marseille, France

References

1. Siegel RL, et al. CA Cancer J Clin 2020;70:7–30.
2. Conroy T, et al. N Engl J Med 2011;369:1817–1825.
3. von Hoff DD, et al. N Engl J Med 2013;369:1691–1703.
4. Burris HA, et al. J Clin Oncol 1997;15:2403–2413.
5. Tiriac H, et al. Cancer Discov 2018;8:1112–1129.
6. Nicolle R, et al. Ann Oncol 2021;32:250–260.
7. Nicolle R, et al. Transl Oncol 2022;16:101315.
8. Fraunhoffer NA, et al. Cancer Commun 2022;42:1212–1216.

[*]Authors share co-first authorship; [§]Authors share co-senior authorship.

Received September 29, 2022. Accepted November 30, 2022.

Correspondence
Address correspondence to: Nelson Dusetti, PhD, Cancer Research Center of Marseille, 163, Av de Luminy, Case 915, 13288 Marseille Cedex 09, France. e-mail: nelson.dusetti@inserm.fr; or Juan Iovanna, MD, PhD, Cancer Research Center of Marseille, 163, Av de Luminy, Case 915, 13288 Marseille Cedex 09, France. e-mail: juan.iovanna@inserm.fr.

Acknowledgments
PDAC Chemo Sensitivity Prediction Working Group includes Martin Bigonnet,[1] Claire Bongrain,[2] Emilie Lermite,[3] Patrick Pessaux,[4] Fabio Giannone,[4] Marie-Pierre Chenard,[4] Sophie Michalak,[5] Rémy Nicolle,[6] Marion Rubis,[2] Flora Poizat,[7] Marc Giovannini,[7] Fabrice Caillol,[7] and Philippe Rochigneux[7]; from [1]Predicting Med, Luminy Science and Technology Park, Marseille, France; [2]Cancer Research Center of Marseille (CRCM), INSERM, CNRS, Institut Paoli-Calmettes, Aix-Marseille University, Marseille, France; [3]Endocrine and Visceral Surgery Department, University Hospital Angers, Angers, France; [4]Department of General, Digestive, and Endocrine Surgery, Nouvel Hôpital Civil, Strasbourg, France; [5]Department of Pathology, University Hospital Angers, Angers, France; [6]Université Paris Cité, Centre de Recherche sur l'Inflammation (CRI), INSERM, U1149, CNRS, ERL 8252, Paris, France; and [7]Medical Oncology Department, Institut Paoli-Calmettes, Marseille, France.

CRedit Authorship Contributions
Nicolas Fraunhoffer, PhD (Data curation: Lead; Formal analysis: Lead; Investigation: Lead; Methodology: Lead; Software: Lead; Validation: Lead; Visualization: Lead; Writing – original draft: Lead).

Brice Chanez, MD, PhD (Data curation: Lead; Formal analysis: Supporting; Investigation: Supporting; Methodology: Supporting; Resources: Lead; Validation: Lead; Visualization: Supporting; Writing – review & editing: Equal).

Carlos Teyssedou, MD (Data curation: Lead; Resources: Lead) Martin Bigonnet, Mr (Methodology: Equal).

Claire Bongrain, MD (Methodology: Supporting).

Emilie Lermite, MD (Resources: Supporting).

Patrick Pessaux, MD (Resources: Supporting).

Fabio Giannone, MD (Resources: Supporting).

Marie-Pierre Chenard, MD (Resources: Supporting).

Sophie Michalak-Provost, MD (Resources: Supporting).

Rémy Nicolle, PhD (Formal analysis: Supporting; Validation: Supporting).

Marion Rubis, Ms (Methodology: Supporting).

Flora Poizat, MD (Methodology: Supporting; Resources: Supporting).

Marc Giovannini, MD (Resources: Supporting).

Fabrice Caillol, MD (Resources: Supporting).

Philippe Rochigneux, MD (Resources: Supporting).

Juan L. Iovanna, MD, PhD (Conceptualization: Lead; Data curation: Lead; Formal analysis: Lead; Funding acquisition: Lead; Investigation: Lead; Supervision: Lead; Validation: Lead; Visualization: Lead; Writing – review & editing: Lead).

Emmanuel Mitry, MD, PhD (Data curation: Supporting; Formal analysis: Supporting; Investigation: Supporting; Supervision: Supporting; Validation: Supporting; Writing – review & editing: Equal).

Dusetti J. Nelson, PhD (Conceptualization: Lead; Data curation: Lead; Formal analysis: Lead; Funding acquisition: Lead; Investigation: Lead; Methodology: Lead; Supervision: Lead; Validation: Lead; Visualization: Lead; Writing – original draft: Lead; Writing – review & editing: Lead).

Conflicts of interest
These authors disclose the following: Nelson J. Dusetti and Juan L. Iovanna are founders of PredictingMed. The remaining authors disclose no conflicts.

Funding
This work was supported by INCa (grants 2018-078, 2018-079, and 2019-037]; Canceropole PACA; Amidex Foundation; ARC Foundation, and the Institut National de la Santé et de la Recherche Médicale.

Supplementary Material and Methods

Patient Cohorts

This study retrospectively included patients from 3 hospitals using the following as inclusion criteria: confirmed diagnosis of PDAC at an advanced stage; treated with gemcitabine monotherapy in the first line; and tumor sample availability (formalin-fixed, paraffin embedded [FFPE] tissues). A total of 107 consecutive patients, diagnosed during 2010–2021, were included from 3 hospitals (95 from the Institut Paoli-Calmettes [IPC], 6 from the University Hospital Angers and 6 from the Nouvel Hôpital Civil, Strasbourg). Fourteen patients (13.1%) were excluded, as samples had poor RNA quality, leaving 93 assessable patients (101 samples). All samples were collected before any treatment. Samples were distributed as follows: 57 unpaired from primary tumors, 28 unpaired from metastatic sites, and 16 paired primary tumor and metastasis samples from 8 patients. The IPC patients were recorded using the ConSoRe (Continum Soin Recherche) clinical data mining interface, using the following keywords: "pancreas adenocarcinoma" as primary tumor and "gemcitabine" as chemotherapy regimen to identify consecutive patients. Patients from the University Hospital Angers and the Nouvel Hôpital Civil, Strasbourg were manually selected. The IPC Internal Review Board approved the study as no. IPC2021-070 (*Gempred*-Retro), and the research was conducted in accordance with the Helsinki Declaration. The consent forms of informed patients were collected according to ethics principles.

RNA Extraction and RNA-Sequencing Analysis

Total RNA was extracted from FFPE tissue sections using the RNeasy FFPE kit (Qiagen, Hilden, North Rhine-Westphalia, Germany) following the manufacturer's instructions. Briefly, the presence of neoplastic cells and the percentage of cellularity were evaluated by a pancreatic pathologist using H&E staining. Only nucleated cells were considered for the calculation of cellularity (red blood cells were not considered). From each FFPE block, between 4 and 5 sections of 10 μm were cut and manually macrodissected to enrich for neoplastic cells. Samples with neoplastic cellularity of $>$10% and that produced $>$30 ng of total RNA were used for transcriptomic analysis. The quality of FFPE-derived RNA was measured by the proportion of fragments above 200 base pairs (DV200) and ranged from 17% to 77% (mean 51.3%) assessed by use of the Agilent 2100 Bioanalyzer System. RNA libraries were prepared with the QuantSeq 3′ mRNA-Seq kit (Lexogen, Vienna, Austria) and run on the Illumina NovaSeq 6000 for 50 base pair single-end reads. The expression matrix was obtained using the Rsubread R package. Then, the RNA reads were normalized using trimmed mean of M-values and $\log_2$ transformed.

Gemcitabine Sensitivity Signature Analysis

Different gemcitabine RNA signatures were assessed using the parameters defined by the original description. For the *Gem-Tiriac et al*, signature, the transcripts positively correlated with sensitivity ($r < -0.38$) were selected to compute the mean *z*-score. *GemPred* and improved *GemPred* were computed using the web application (https://app.gebican.fr/pdac-gempred/). Finally, *GemCore* stratification was calculated through a binary classifier defined by logistic regression.

Statistical Analysis

OS was defined as the time from diagnosis to death. PFS was measured from the date of first gemcitabine injection to the time of disease progression or death. Objective responses were assessed by using the Response Evaluation Criteria in Solid Tumours, version 1.1 criteria. Survival curves were estimated using the Kaplan–Meier technique and compared with the log-rank test. Qualitative variables were compared with χ^2 test or Fisher test, and quantitative variables with the use of Student *t* test or a nonparametric Wilcoxon test. Normality was tested with the Shapiro–Wilk test. For each test, statistical significance was set at a 2-sided *P* value of $<$.05. Univariate and multivariate Cox regression analyses and Kaplan–Meier curves were computed using the survival R package. Variable selection for the Cox multivariate analysis was performed applying Lasso regression with lambda cross-validation. Variables with non-zero coefficients were selected. The Cox proportional hazard regression model was used for univariate and multivariate analyses to estimate the hazard ratio with a 95% CI. Proportional hazards assumption was tested using the Schoenfeld residuals.

Supplementary Table 1. Association of *Gem-Tiriac et al*, *GemPred,* and *Improved GemPred* With Objective Responses: Samples From Primary Tumors and Metastases

	Gem-Tiriac et al.			*GemPred*			*Improved GemPred*		
Variable	Positive, n (%)	Negative, n (%)	*P* value	Positive, n (%)	Negative, n (%)	*P value*	Positive, n (%)	Negative, n (%)	*P value*
Primary tumors			.747			.084			.662
Objective responses									
Partial response	3 (4.6)	6 (9.2)		3 (4.6)	6 (9.2)		6 (9.2)	3 (4.6)	
Stable disease	11 (16.9)	10 (15.4)		11 (16.9)	10 (15.4)		11 (16.9)	10 (15.4)	
Progressive disease	8 (12.3)	9 (13.8)		3 (4.6)	14 (21.5)		11 (16.9)	6 (9.2)	
Unknown	9 (13.8)	9 (13.8)		6 (9.2)	12 (18.5)		6 (9.2)	12(18.5)	
Metastases			.485			.906			.5134
Objective responses									
Partial response	1 (2.8)	2 (5.6)		1 (2.8)	2 (5.6)		3 (8.3)	0 (0.0)	
Stable disease	3 (8.3)	1 (2.8)		2 (5.6)	2 (5.6)		1 (2.8)	3 (8.3)	
Progressive disease	4 (11.1)	5 (13.9)		4 (11.1)	5 (13.9)		3 (8.3)	6 (16.7)	
Unknown	16 (44.4)	4 (11.1)		9 (25.0)	11 (30.5)		13 (36.1)	7 (19.4)	

NOTE. The objective response was determined according to the Response Evaluation Criteria in Solid Tumours, version 1.1.

Supplementary Table 2. Univariate and Multivariate Cox Analysis of Clinicopathological Variables to Determine Associations With Overall Survival and Progression-Free Survival: Samples From Primary Tumors

		Univariate		Multivariate	
Variable	Data	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Overall survival					
GemCore, n (%)			—		<.001
GemCore–	36 (55.4)	—		Ref	
GemCore+	29 (44.6)	—		0.18 (0.09—0.35)	
Age, n (%)			.743		—
≤65 y	13 (20.0)	Ref		—	
>65 y	52 (80.0)	1.11 (0.60–2.06)		—	
Sex, n (%)			.443		—
Female	33 (50.8)	Ref		—	
Male	32 (49.2)	0.82 (0.50–1.36)		—	
WHO PS score, n (%)					
Unknown	1 (1.5)	—	—	—	—
0	8 (12.3)	Ref	—	Ref	—
1	22 (33.8)	1.74 (0.77–3.93)	.184	1.62 (0.70–3.74)	.257
≥2	34 (52.3)	2.33 (1.05–5.15)	.037	14.00 (1.65–118.67)	.006
Clinical stage, n (%)			.335		—
Locally advanced	5 (7.7)	Ref		—	
Metastatic	60 (92.3)	1.57 (0.63–3.96)		—	
Weight loss, *kg*, n (%)	8.1 (6.0)	1.04 (0.99–1.08)	.110	—	—
Primary tumor location			.196		—
Head	30 (46.2)	Ref		—	
Other	35 (53.8)	1.40 (0.84—2.34)		—	
Tumour thickness, *mm*, mean (SD)	40.4 (16.8)	1.00 (0.99–1.02)	.541		—
Pulmonary metastases, n (%)			.950		
No	47 (71.9)	Ref		—	
Yes	18 (28.1)	1.02 (0.58–1.78)		—	
Hepatic metastases, n (%)			.012		.362
No	24 (36.9)	Ref		Ref	
Yes	41 (63.1)	1.94 (1.16–3.26)		1.31 (0.74–2.32)	
No. of metastatic sites, n (%)			.005		.012
0–1	43 (66.2)	Ref		Ref	
≥2	22 (33.8)	2.18 (1.27–3.75)		2.15 (1.18–3.89)	
Level of CA19-9, n (%)					—
Unknown	15 (23.1)	—	—	—	—
Normal	9 (13.8)	Ref	—	—	—
<59× ULN	36 (55.4)	1.67 (0.79–3.53)	.179	—	—
>59× ULN	5 (7.7)	3.70 (1.18–11.63)	.025	—	—
Differentiation, n (%)					
Unknown	4 (6.1)	—	—	—	—
1 (well)	48 (73.8)	Ref	—	—	—
2 (moderately)	4 (6.1)	1.31 (0.47–3.69)	.605	—	—
3 (poor)	9 (13.8)	3.33 (1.58–7.03)	.002	—	—
PurIST, n (%)					
Basal-like	5 (7.7)	Ref	—	—	—
Classical	60 (92.3)	0.85 (0.34–2.16)	.736	—	—
CDA, mean (SD)	2.5 (2.3)	1.04 (0.93–1.16)	.499	—	—
DCK, mean (SD)	0.7 (1.5)	1.07 (0.90–1.28)	.443	—	—
SLC29A1 (hENT1), mean (SD)	2.5 (2.7)	0.99 (0.89–1.09)	.778	—	—
hENT1/CDA ratio, mean (SD)	1.9 (3.3)	1.01 (0.91–1.13)	.846	—	—

Supplementary Table 2. Continued

Variable	Data	Univariate		Multivariate	
		HR (95% CI)	*P* value	HR (95% CI)	*P* value
Progression–free survival					
GemCore, n (%)			—		<.001
GemCore–	36 (55.4)	—		Ref	
GemCore+	29 (44.6)	—		0.11 (0.04-0.26)	
Age, n (%)			.9		—
≤65 y	13 (20.0)	Ref		—	
>65 y	52 (80.0)	1.04 (0.56–1.93)		—	
Sex, n (%)			.93		.273
Female	33 (50.8)	Ref		Ref	
Male	32 (49.2)	0.98 (0.59–1.61)		1.41 (0.76-2.62)	
WHO PS score, n (%)					
Unknown	1 (1.5)	—	—	—	—
0	8 (12.3)	Ref	—	Ref	—
1	22 (33.8)	2.08 (0.88–4.92)	.1	2.64 (1.01-6.88)	.047
≥2	34 (52.3)	2.50 (1.10–5.70)	.03	3.81 (1.56-9.27)	.003
Clinical stage, n (%)			—		—
Locally advanced	5 (7.7)	Ref	—	—	
Metastatic	60 (92.3)	1.25 (0.50–3.12)	.64	—	
Weight loss, *kg*, mean (SD)	8.1 (6.0)	1.05 (1.00–1.11)	.03	—	
Primary tumor location, n (%)					
Head	30 (46.2)	Ref	—	—	—
Other	35 (53.8)	0.70 (0.42–1.18)	.18	—	—
Tumor thickness, *mm*, mean (SD)	40.4 (16.8)	1.00 (0.98–1.01)	.54	—	—
Pulmonary metastases, n (%)					
No	46 (71.9)	Ref	—	Ref	—
Yes	18 (28.1)	0.84 (0.48–1.45)	.52	0.57 (0.28-1.15)	.116
Hepatic metastases, n (%)			.01		—
No	24 (36.9)	Ref		—	
Yes	41 (63.1)	2.00 (1.19–3.35)		—	
No. of metastatic sites, n (%)			.070		.099
1	43 (66.2)	Ref		Ref	
≥2	22 (33.8)	1.63 (0.96–2.75)		1.68 (0.91-3.10)	
Level of CA19-9, n (%)					
Unknown	15 (23.1)	—	—	—	—
Normal	9 (13.8)	Ref	—	—	—
<59× ULN	36 (55.4)	1.57 (0.72–3.41)	.25	—	—
>59× ULN	5 (7.7)	3.22 (1.02–10.20)	.05	—	—
Differentiation, n (%)					
Unknown	4 (6.1)	—	—	—	—
1 (well)	48 (73.8)	Ref	—	Ref	—
2 (moderately)	4 (6.1)	1.90 (0.67–5.37)	.23	1.85 (0.60-5.70)	.284
3 (poor)	9 (13.8)	5.16 (2.32–11.48)	<.001	2.39 (1.00-5.74)	.051
PurIST, n (%)					
Basal-like	5 (7.7)	Ref	—	—	—
Classical	60 (92.3)	0.79 (0.31–1.99)	.616	—	—
CDA, mean (SD)	2.5 (2.3)	1.03 (0.93–1.14)	.618	—	—
DCK, mean (SD)	0.7 (1.5)	1.03 (0.85–1.25)	.785	—	—
SLC29A1 (hENT1), mean (SD)	2.5 (2.7)	1.06 (0.96–1.18)	.245	—	—
hENT1/CDA ratio, mean (SD)	1.9 (3.3)	1.06 (0.96–1.16)	.281	—	—

CA19–9, cancer antigen 19–9; CDA, cytidine deaminase; DCK, deoxycytidine kinase; hENT1, human equilibrative nucleoside transporter 1; PurIST, Purity Independent Subtyping of Tumours; SLC29A1, solute carrier family 29 member 1; ULN, upper limit of normal; WHO PS, World Health Organization performance status.