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Phase II study evaluating the association of gemcitabine, trastuzumab and erlotinib as first-line treatment in patients with metastatic pancreatic adenocarcinoma (GATE 1)

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Abstract

In a previous phase II study (THERAPY), cetuximab and trastuzumab combination, as second-line after progression with gemcitabine, showed disease stabilization in 27% of 33 patients with pancreatic carcinoma. In the present phase II multicenter study, we assessed the efficacy and tolerance of gemcitabine, trastuzumab plus erlotinib as first-line treatment of metastatic pancreatic cancer. The primary endpoint was disease control rate (DCR, RECIST v.1); secondary endpoints were progression-free (PFS), overall (OS) survival and toxicity (NCI-CTCAE v3.0). Ancillary study addressed the predictive value of both EGFR/HER2 expression and *KRAS* mutational status. Sixty-three patients from four centers were included (62 evaluable for toxicity, 59 for efficacy), median age was 62 years (35-77), 59.7% men. The median treatment duration was 16.1 weeks (2.1-61). Eleven patients (19%) reported a partial tumor response, and 33 (56%) disease stabilization. DCR was 74.6% (95%CI: 61.8-85.0; 44/59 patients). After a median follow-up of 23.3 months (0.6-23.6), median PFS was 3.5 months (95%CI: 2.4-3.8) and median OS 7.9 months (95%CI: 5.1-10.2). PFS was significantly longer in patients with grade ≥ 2 cutaneous toxicities vs patients with grade 0-1 toxicities (HR = 0.55, 95%CI: 0.33-0.92, *P* = .020). Expression of EGFR and

Abbreviations: 5-FU, 5-fluorouracil; 95% CI, 95% confidence interval; CA 19-9, carbohydrate antigen 19.9; CAE, carcinoembryonic antigen; CT-scan, computed tomography scan; DCR, disease control rate; ECOG, European Cooperative Oncology Group; EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; HR, hazard ratio; IV, intravenous; KRAS, Kirsten Rat Sarcoma Viral Oncogene Homolog; MRI, magnetic resonance imaging; NCI-CTCAE, National Cancer Institute—Common Terminology Criteria for Adverse Events; NCI-CTG, National Cancer Institute of Canada, Clinical trials group; OS, overall survival; PFS, progression-free survival; PS, performance status; RECIST, response evaluation criteria in solid tumors; WHO, World Health Organization.

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HER2 was correlated with PFS and OS in multivariate analysis; HER2 expression was correlated with the tumor response. Main severe toxicities were neutropenia (32%), cutaneous rash (37%) and thrombosis/embolisms (35.5%). This triplet combination is effective in terms of disease control, PFS and OS, and acceptable for safety. A larger study to investigate this combination compared to the standard regimen should be discussed.

KEYWORDS

combination therapy, efficacy, pancreatic cancer

1 | INTRODUCTION

Gemcitabine has been the mainstay of metastatic pancreatic cancer treatment for many years, after the positive results of the randomized trial of gemcitabine alone vs 5-fluorouracil (5-FU).[1] It remains the treatment of choice for patients with metastatic pancreatic cancer with an ECOG performance status (PS) of 1 or 2, in older patients or in case of hyperbilirubinemia. During 10 years, more than 20 randomized trials are compared gemcitabine alone *vs* gemcitabine in combination with either cytotoxic agents or targeted therapies. They have failed to show any clinical benefit in patients with advanced pancreatic cancer.[2-4] In 2011, a phase II-III randomized trial in patients with treatment-naïve metastatic pancreatic cancer with good ECOG PS 0-1 showed that the combination of 5-FU, folinic acid, irinotecan and oxaliplatin (FOLFIRINOX) resulted in a better survival rate, but increased toxicity compared to gemcitabine alone.[5] In 2013, a phase III study of albumin-bound paclitaxel (nab-paclitaxel) plus gemcitabine vs gemcitabine monotherapy in patients with metastatic pancreatic cancer reported median progression-free and overall survival of 5.5 and 8.5 months in the nab-paclitaxel-gemcitabine group, significantly longer than in the gemcitabine alone group (3.7 and 6.7 months, respectively).[6]

However, to date, there are no identified predictive biomarkers to assess response to conventional chemotherapy treatment and, in practice, as a result of this unmet clinical need, pancreatic cancer remains a disease with an extremely poor prognosis (5-year survival of 3%-5%).[7-9]

Preclinical evidence supports the involvement of the epidermal growth factor receptor (EGFR) in the biology of pancreatic cancer.[10,11] Overexpression of type 1 EGFR (ErbB1/HER1) is reported in >90% of pancreatic cancers and is associated with a poorer prognosis.[12] In this context, a double-blind randomized phase III trial conducted by the National Cancer Institute of Canada, Clinical trials group (NCIC-CTG), showed that the combination of gemcitabine and erlotinib significantly improved progression-free survival (hazard ratio [HR] 0.77; 95% CI: 0.64-0.92, P = .004) and overall survival (HR 0.82; 95% CI: 0.69-0.99, P = .038) compared to gemcitabine plus placebo.[13] Median survival times were 6.24 months for the gemcitabine/erlotinib arm vs 5.9 months for the gemcitabine/placebo arm.

What's new?

Despite extensive investigation into gemcitabine-based combination therapies for pancreatic cancer, significant need remains for novel strategies with improved clinical benefit. A promising approach is the triplet combination gemcitabine, trastuzumab, and erlotinib, which the present pilot multicenter phase II trial identifies as an effective strategy for disease control and survival when used as a first-line regimen. In particular, pancreatic cancer patients with grade 2 or worse cutaneous toxicity showed superior progression-free survival compared to patients with grade 0-1 cutaneous toxicities. In multivariate analyses, progression-free and overall survival were correlated EGFR and HER2, while HER2 expression was linked to tumor response.

HER2 is a tyrosine kinase-related receptor encoded by a proto-oncogene. Once activated, it promotes cellular proliferation, survival and migration through activating cascades. HER2 overexpression in various tumor cells, up to 45% in patients with pancreatic cancer,[14] has not only been associated with a poor prognosis; it also offers the therapeutic option of receptor targeting therapies. Indeed, two preclinical studies showed encouraging results of HER2 targeting therapies in pancreatic cancer cell lines and a xenograft mouse model.[15,16]

A phase II study in 17 patients with HER2 overexpressing metastatic pancreatic cancer investigated the efficacy and toxicity of the anti-HER2 antibody, trastuzumab combined with capecitabine. Although the therapy was well tolerated, PFS and OS did not perform favorably compared to standard chemotherapy. Consequently, the authors did not recommend further evaluation of anti-HER2 treatment in these patients.[17]

In oncology, therapeutic associations are very common. Adopting a combinatory strategy including mono- or bi-specific anti-HER family antibodies and tyrosine kinase inhibitors to increase the inhibition of more than one signaling pathway, and thus overcome treatment resistance, is a key to reach treatment efficacy. We have already shown that the combination of cetuximab (anti-EGFR) with trastuzumab

(binding to domain IV of HER2) induced a therapeutic synergy, efficient in immunodeficient mice with human pancreatic carcinoma.[18] In a first clinical trial combining cetuximab and trastuzumab administered in second-line, in 33 patients with pancreatic adenocarcinoma who progressed under gemcitabine, we reported a stable disease in 27% of patients, with a significant correlation between cutaneous toxicity and survival.[19]

The objective of this open-label multicenter phase II study was to assess the efficacy and tolerance of the combination of a conventional chemotherapy, gemcitabine, with two targeted therapies, trastuzumab and erlotinib, as first-line treatment of patients with metastatic pancreatic cancer.

2 | PATIENTS AND METHODS

2.1 | Study design and patients

We conducted a phase II open-label single-arm multicenter study. Patients ≥18 years old, with life expectancy >3 months, with metastatic histologically proven pancreatic adenocarcinoma were eligible to participate in the study. Patients had to be WHO performance status ≤1, with hematologic, renal and hepatic normal functions. Patients could have received previous gemcitabine treatment in adjuvant setting, with at least 6 months between the end of adjuvant chemotherapy and the diagnosis of recurrent metastatic disease. Patients were excluded from the study if they had nonmetastatic disease, if they presented with brain metastases or symptoms of leptomeningic carcinomatosis, if they had received previous erlotinib or trastuzumab treatment, or if they had significant comorbidities (vascular, hepatic, renal, medullar or infectious comorbidities).

2.2 | Treatment

Patients received 1000 mg/m^2 intravenous (IV) gemcitabine, 30 minutes infusion, on days 1, 8, 15, 22, 29, 36 and 43, during the first 8 weeks of treatment, then on days 1, 8 and 15, 3 weeks out of a 4-week cycle. They also received weekly IV trastuzumab, 4 mg/kg 90-minutes infusion on Day 1, 2 mg/kg on Days 8 and 15, 30-minutes infusion, and 100 mg/day erlotinib *per os*. Treatment was administered until disease progression, occurrence of unacceptable toxicity, patient withdrawal or decision of the investigator.

2.3 | Tolerance and follow-up

Toxicities were graded and reported according to the National Cancer Institute—Common Terminology Criteria for Adverse Events (NCI-CTCAE) v3.0.[20] An intermediate analysis of tolerance was performed after the two first cycles of treatment, for the first six patients included in the study. Follow-up was performed every 8 weeks and at the end of treatment, and comprised clinical exam, CT-scan or MRI, and CAE and CA 19.9 levels assessment.

2.4 | Statistical considerations

The study primary endpoint was the disease control rate (DCR, i.e., complete and partial responses, and stable disease) according to the RECIST v1.1 criteria. Secondary endpoints were tolerance, progression-free and overall survival. With a Fleming single-stage design, α = 5%, β = 7.5%, p0 (the probability of inefficiency maximum) = 40% and p1 (the probability of minimum efficiency) = 60%, 57 evaluable patients were required (60 patients, with 5% non-evaluable patients). The association was to be considered sufficiently effective if there were at least 29 successes (disease control) among

TABLE 1 Patients' characteristics at baseline

	n = 62
Age (years), median [range]	62.0 [35–77]
Gender, n (%)	
Male	37 (59.7)
Female	25 (40.3)
WHO performance status, n (%)	
0	27 (43.5)
1	35 (56.5)
Location of primary tumor, n (%)	
Head of pancreas	25 (40.3)
Body of pancreas	22 (35.5)
Tail of pancreas	15 (24.2)
Primary tumor surgery, n (%)	10 (16.1)
Primary tumor radiotherapy, n (%)	4 (6.5)
Previous chemotherapy treatment, n (%)	
Adjuvant chemotherapy	6 (9.7)
With gemcitabine	5
With gemox	1
Number of metastatic sites, n (%)	
1	25 (40.3)
2	22 (35.5)
≥3	15 (24.2)
Metastatic sites location, n (%)	
Liver-only metastases	21 (33.9)
Other locations	41 (66.1)
Synchronous metastases, n (%)	52 (83.9)
Serum CA 19.9 level	
Median [range]	943 [0.9-45 111]
<65 UI/mL	15 (24.6)
≥65 UI/mL	46 (75.4)
Missing	1
KRAS status, n (%)	
WT	2 (6.1)
Mutated	31 (93.9)
Missing	8

Abbreviation: WHO, World Health Organization.

57 evaluable patients. Descriptive analyses were reported with median and range for continuous parameters, and frequency and percentage for categorical parameters. Progression-free survival was calculated from the inclusion until disease progression or death. Patients alive without progression were censored at the time of last contact. Overall survival was calculated from inclusion until death of any cause. Patients alive or lost to follow-up were censored at the time of last contact. The Kaplan-Meier method was used to estimate PFS and OS. Univariate and multivariate analyses were performed using Cox proportional hazard model. Hazard ratios (HR) are given with their 95% confidence interval (95% CI). A *P*-value of 0.05 was considered to indicate statistical significance. All statistical analyses were performed with STATA 13.0 software (StataCorp, College Station, Texas).

2.5 | Ancillary study

Expression of both HER1 and HER2 was analyzed by immunohistochemistry, as previously described.[19,21] HER1 and HER2 expression levels were scored using the H-Score method[22] by which the intensity of membrane staining (with a value of 0 [no staining], 1 [weak staining], 2 [moderate staining] or 3 [intense staining]) is multiplied by the percentage of stained tumor cells (from 0% to 100%) to give a

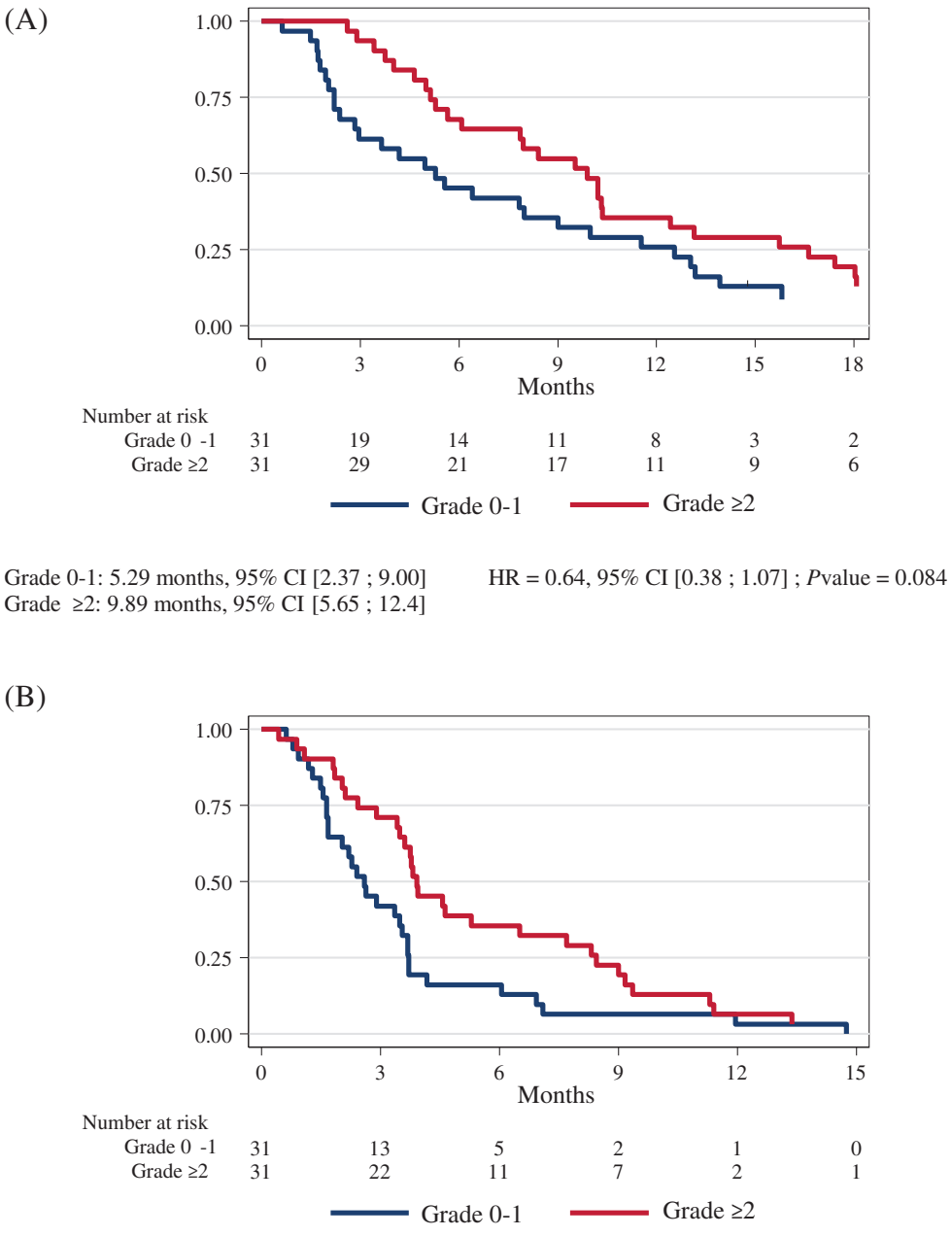

FIGURE 1 A, Overall (OS) and B, progression-free (PFS) survival according to the occurrence of cutaneous toxicities

score in the range of 0 to 300. In accordance with the aim of the study to identify predictive biomarkers of response to therapy and to erlotinib, we only analyzed the expression of EGFR receptor at the membrane level. Indeed, tyrosine kinase activation of EGFR is dependent on ligand binding when the receptor is located at the membrane. *KRAS* mutation status in tumor tissues was determined by combining high resolution melting analysis and direct sequencing.[19,23]

After macrodissection, DNA was extracted from 7-μm-thick sections by using the DNA QIAamp DNA Extraction Kit (Qiagen) following the manufacturer's instructions.

For the HRM screening of KRAS exon 2, a 84-bp fragment was PCR amplified using a Rotor-Gene 6000 instrument (Qiagen) and the LightCycler 480 High-Resolution Melting Master Reaction Mix (Roche Diagnostics). Briefly, 25 ng of purified genomic DNA were reacted with forward and reverse primer (primer sequences are available upon request). The cycling conditions were as follows: 95°C for 5 minutes, followed by 50 cycles of 95°C for 15 seconds, 63°C for 25 seconds with an initial 11 cycles of touchdown (0.5°C/cycle), and 72°C for 25 seconds. The melting conditions included one cycle of 95°C for 1 minute, one cycle of 40°C for 1 minute and one cycle of 65°C for 2 seconds, followed by a melt from 65°C to 95°C that increased 0.1°C per second. The HRM data were analyzed using Rotor-Gene 6000 software (v1.7).

Sequencing of KRAS exon 2, exon 3 and exon 4 was performed after PCR amplification of each individual exon. Briefly, all amplifications were performed in a volume of 50 μL with 5 units of AmpliTaq Gold DNA Polymerase (Applied Biosystems, Courtaboeuf, France), 200 ng of genomic DNA and specific primers (primer sequences available upon request. The thermal cycling conditions included a 10-minutes denaturation step at 94°C, 40 cycles of 94°C for 30 seconds, 60°C for 30 seconds and 72°C for 1 minute, and a final extension at 72°C for 7 minutes. The PCR products were purified by exonuclease I digestion (Amersham Biosciences, Little Chalfont, United Kingdom) and shrimp alkaline phosphatase (Roche Applied Sciences, Penzberg, Germany), according to the manufacturer's instructions. Direct sequencing of the amplicons was performed with both the forward and the reverse PCR primers using the BigDyeTM Terminator v3.1 Cycle Sequencing Kit with the ABI PRISMTM 3100 Genetic Analyzer (Applied Biosystems, Foster City, California).

3 | RESULTS

3.1 | Patients' characteristics

Between June 2010 and July 2013, 63 patients from four participating centers were included in the study; analyses were performed in 62 patients (one patient did not meet one major inclusion criteria). At baseline, patients were 59.7% male, with a median age of 62.0 years (range: 35-77; Table 1). The WHO performance status was 0 and 1 in 43.5% and 56.5% of patients. All patients presented with a pancreatic adenocarcinoma, mostly (91.4%) ductal adenocarcinomas. The primary tumor was localized in the pancreas head, body or tail in 40.3%, 35.5% and 24.2% of cases, respectively. Patients presented with synchronous metastases in 83.9% of cases, mostly hepatic (83.9%), and had 1, 2 and 3 or more metastatic sites in 40.3%, 35.5% and 24.2% of cases. *KRAS* was mutated in almost all of the 41 evaluable tumors (93.9%).

Previous treatments were administered to 11 patients (17.7%): 10 patients (16.1%) had undergone surgery for their primary tumor, including eight R0 and two R1 resections; six patients (9.7%) had received adjuvant chemotherapy for a median duration of 3.9 months (range: 1.1-6.0); four patients (6.5%) had received radiotherapy, for a median duration of 1.3 months (range: 1.1-1.5).

3.2 | Treatments

The median treatment duration was 16.1 weeks (range: 2.1-61). Patients received a median of three treatment cycles (range: 1-9). Doses of gemcitabine, trastuzumab and erlotinib received and relative dose-intensities are detailed in Table S1. Relative dose-intensities were 82.7% for gemcitabine, 83.5% for trastuzumab and 86.1% for erlotinib. Treatments were prematurely stopped for the following reasons: disease progression (69.4% of patients), death of other causes (12.9%, n = 8, including on 1 cardiac arrest, 3 thromboembolic events, 1 skeptic choc, 3 general physical health deterioration), toxicity (1.6%), treatment cycle delay (3.2%) and investigator's decision (12.9%). Doses reductions and treatment discontinuations rules are detailed in Table S2.

TABLE 2 Mild and severe toxicities reported

Toxicities, n (%)	Mild[a] toxicities		Severe[b] toxicities	
Cutaneous				
Rash/Acne	39	62.9%	23	37.1%
Paronychia	57	91.9%	5	8.1%
Hematological				
Neutropenia	42	67.7%	20	32.3%
Anemia	55	88.7%	7	11.3%
Thrombocytopenia	54	87.1%	8	12.9%
Gastrointestinal				
Mucositis/stomatitis	58	93.6%	4	6.4%
Anorexia	44	71.0%	18	29.0%
Diarrhea	54	87.1%	8	12.9%
Constipation	60	96.8%	2	3.2%
Abdominal pain	52	83.9%	10	16.1%
Nausea/vomiting	60	96.8%	2	3.2%
Cardiovascular				
Thrombosis/embolism	40	64.5%	22	35.5%
Others				
Asthenia	52	83.9%	10	16.1%
Fever	61	98.4%	1	1.6%

[a]Grades 0 and 1 for cutaneous toxicities and 0, 1 and 2 for all other toxicities;
[b]Grades ≥2 for cutaneous toxicities and ≥3 for all other toxicities.

3.3 | Efficacy

Efficacy analyses were performed in 59 patients (three patients were not evaluable for efficacy because of missing evaluation data). No complete response was reported. The response was partial in 11 patients (18.6%) and 33 patients (56.0%) had a stable disease. Disease progression was reported in 15 (25.4%) patients. The overall disease control (complete or partial responses, or stable disease) rate was 74.6% (95% CI: 61.6-85.0). Considering only the first 57 evaluable patients, according to the study design, 42 successes, that is, disease control, were reported, accounting for a success rate of 73.7% (95% CI: 60.3-84.5). The CA19-9 biologic response is presented as a Waterfall plot (Figure S2).

TABLE 3 Biological parameters

	n = 41
EGFR positive tumor cells (%), median [range]	40 [0-90]
Missing	4
EGFR QS, median [range]	60 [0-270]
Missing	4
≤60, n (%)	19 (51.4%)
>60, n (%)	18 (48.7%)
HER2 positive tumor cells (%), median [range]	40 [0–90]
Missing	5
HER2 QS, median [range]	60 [0–270]
Missing	5
≤40, n (%)	15 (41.7%)
>40, n (%)	21 (58.3%)

Abbreviation: QS, Quirk Score.

3.4 | Survival

After a median follow-up of 23.3 months (range: 0.6-23.6), 58 deaths (93.5%) and 61 progressive disease (98.4%) were reported. Median PFS was 3.5 months (95% CI: 2.4-3.8) and median OS was 7.9 months (95% CI: 5.1-10.2). The occurrence of cutaneous toxicities was correlated with overall survival, but not with the number or location of metastatic sites, nor with the presence of synchronous metastases. Indeed, PFS was significantly longer in patients who presented grades ≥2cutaneous toxicities vs patients who reported grade 0-1 cutaneous toxicities, HR = 0.55 (95% CI: 0.33-0.92, *P* = .020), and a trend, although not significant, was observed for OS (HR = 0.63 [95% CI: 0.38-1.07], *P* = .084; Figure 1). The occurrence of treatment premature discontinuation in eight patients (12.9%) was inversely correlated with the occurrence of skin toxicity (*P* = .023; seven patients with grade 0-1 vs 1 patient with grade ≥ 2).

3.5 | Toxicity

Main grade 3 to 4 treatment-related toxicities included neutropenia (32.3%), anorexia (29.0%), thrombosis and embolism (35.5%), asthenia (16.1%) and grade 2 to 3 cutaneous rash (37.1%) (Table 2).

TABLE 4 Univariate and multivariate analyses of biological parameters of progression-free (PFS) and overall (OS) survival

	Progression-free survival				Overall survival			
	Univariate		Multivariate		Univariate		Multivariate	
	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI
KRAS status								
WT	1				1			
Mutated	1.36	[0.32-5.80]			0.45	[0.10-2.02]		
	P = .662					*P* = .345		
QS EGRF								
≤60	1				1			
>60	1.82	[0.93-3.56]			1.40	[0.71-2.77]		
	P = .083				*P* = .328			
QS HER2								
≤40	1				1			
>40	2.66	[1.22-5.81]			2.98	[1.28-6.97]		
	P = .010				*P* = .008			
QS EGFR and HER2								
Others	1		1		1		1	
EGFR >60 and HER2 > 40	2.86	[1.34-6.11]	2.86	[1.34-6.11]	3.26	[1.44-7.40]	3.26	[1.44-7.40]
	P = .008		*P* = .008		*P* = .005		*P* = .005	

Abbreviations: 95% CI, 95% confidence interval; HR, hazard ratio; QS, quirk score; WT, wild-type.

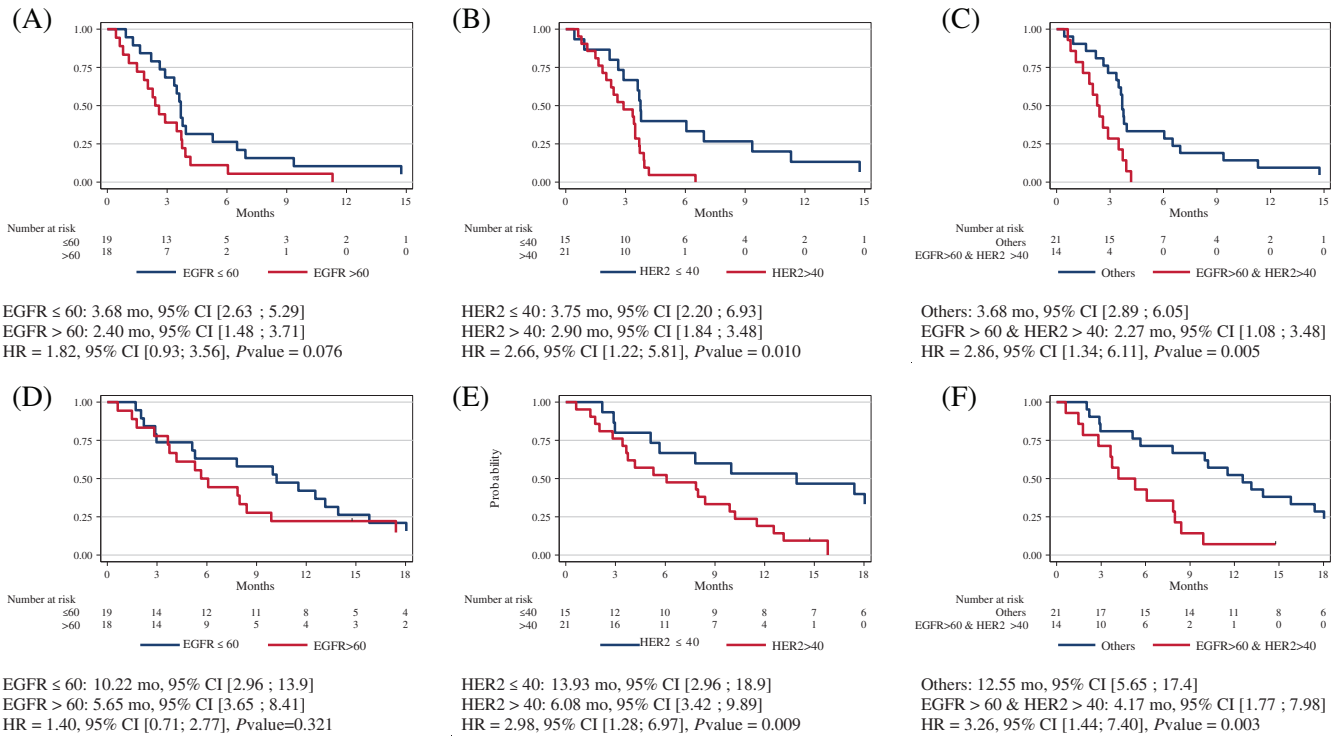

FIGURE 2 Progression-free (PFS, left) and overall survival (OS, right) according to the significant biological parameters: EGFR Quick Score (A, D), HER2 Quick Score (B, E) and EGFR and HER2 Quick Score (C, F)

3.6 | Ancillary studies

Clinical material (biopsies or surgical specimens) to evaluate EGFR and HER2 expression and *KRAS* mutation status was available for 41/62 patients (66%; Table 3). Our results showed that HER 2 expression and the combined expression of EGFR and HER2 were correlated to PFS and OS in univariate and multivariate analysis (Table 4 and Figure 2). A cutoff of 40 for HER2 and 60 for EGFR H-Scores was determined. In multivariable analysis, the expression of both EGFR and HER2 was independently correlated with PFS (HR = 2.86 [95% CI: 1.34-6.11], *P* = .008) and OS (HR = 3.26 [95% CI: 1.44-7.40], *P* = .005). Beyond this prognostic role, HER2 expression was also correlated with the objective tumor response (*P* = .01; Figure S1). No correlation was found with the *KRAS* status.

4 | DISCUSSION

Our results showed that combining gemcitabine, trastuzumab and erlotinib is effective in terms of disease control rate, progression-free and overall survivals. They appear consistent with the published results of studies assessing gemcitabine alone[1] or gemcitabine plus nab-paclitaxel,[6] but inferior to those reached with Folfirinox.[5] Besides, survival of patients with cutaneous toxicities or with high HER2/EGFR level expression compares favorably with that obtained with the gemcitabine and nab-paclitaxel combination. This triplet gemcibatine, trastuzumab and erlotinib combination of chemotherapy and targeted therapies did not induce unexpected toxicity results, except the occurrence of a high rate of thromboembolic complications (33%), higher than the rate usually described in metastatic pancreatic adenocarcinoma patients (15%-20%)[24] but similar to the one displayed by the gemcitabine nab-paclitaxel plus PEGPH20 combination. This could justify a prophylactic therapy[24,25] No increased cardiac toxicities (left ventricular dysfunction, congestive heart failure, myocardial ischemia, myocarditis, QT prolongation and arrhythmia) were reported.

Conventional chemotherapies (Folfirinox, gemcitabine and nab placitaxel), without the addition of targeted therapies, are still valuable options f for metastatic pancreatic cancer treatment. However, to date, there are no identified predictive biomarkers of response to these conventional, nontargeted treatments.

Skin rash has been identified as a good prognostic factor in advanced pancreatic cancer in several retrospective studies.[26-28] In metastatic patients, erlotinib was shown to be more effective in case of skin rash and it was purpose that skin rash could be an early clinical sign to predict efficacy. The Spanish Pantar trial by Aranda et al assessed the efficacy of the gemcitabine/erlotinib combination in 153 patients with locally advanced and metastatic pancreatic adenocarcinoma.[29] A clear advantage in terms of efficacy was found in patients with skin rash ≥grade 2 (25% patients) with a median OS of 11 months vs 5 months for patients with grade 1 or no skin rash; progression-free survival was 6 months vs 3 months, respectively. The efficacy of same combination was assessed in rash-positive patients

eligible for Folfirinox[30] in a prospective phase II study: 150 patients in 20 centers received the combination (run-in phase); then patients with skin rashes of any grade received the gemcitabine-erlotinib combination and rash-negative patients switched to Folfirinox. The primary endpoint was to achieve a 1-year survival rate ≥ 40% in rash-positive patients. Ninety patients were positive for skin rash by the end of the run-in phase, showing a 1-year survival rate of 40.0% (95% CI: 29.8-50.9), vs 48.1% (95% CI: 28.7-68.1) in the Folfirinox rash-negative patients (n = 27). Median OS and PFS were 10.1 months and 3.8 months in the rash-positive patients, vs 10.9 months and 6.6 months in the Folfirinox group; the overall response rate were 23.3% and 33.3%, respectively.

The question of a clear mechanistic explanation for this reported cutaneous toxicity remains. Indeed, as the EGFR signaling pathway is involved in epidermal homeostasis and hair follicle development, modulation of the EGF receptor/ligand system by anti-EGFR agents impacts the behavior of keratinocyte stem cells leading to cutaneous toxicities.[31] Moreover, a higher incidence of skin rash was described for patients aged <65 years and with better performance status.[26,32] Several authors discussed the effects of pharmacological and pharmacodynamics population-based variations, leading to different levels of erlotinib efficacy and toxicity.[33] Noll et al described an "exocrine like subtype" of pancreatic adenocarcinoma with higher P450-3A5 cytochrome activity, leading to resistance to small-molecule inhibitors such as erlotinib.[34] Other hypotheses were based on polymorphisms of the EGFR gene[35] or special HLA-types.[36]

To date, no biological predictive factor of the efficacy of erlotinib in pancreatic cancer was described.[37] We thus assessed EGFR and HER2 expression and *KRAS* mutation status as predictive factors in our population. Our results regarding the HER2 and EGFR expression levels are new and interesting. Indeed, they appear as a potential pretherapeutic factor predictive of response and correlated to survival under gemcitabine, erlotinib and trastuzumab combination. HER2 signaling was targeted in patients with HER2+ metastatic pancreatic adenocarcinoma, using trastuzumab, in at least two previous phase II trials.[17,38] None showed favorable results. Definition of HER2 status for trastuzumab administration is tumor dependent; indeed gastric and breast cancers have different HER2 expression evaluation criteria. We thus performed a nonbiased HER2 H-Score analysis in our cohort. In an observational study, HER2 was shown to be amplified only in 2% of 469 not previously treated pancreatic adenocarcinoma.[39] In our study, we found a correlation between HER2 expression levels using IHC and poorer prognosis. A high HER2 level alone or associated with a high EGFR level were correlated with shorter PFS and OS and a poorer tumor response. In contrast, EGFR expression using IHC was not found a prognostic factor.

Our data suggest a more complex biological regulation than expected. The HER family receptors expression is not static and may be induced by oncologic treatments, among which gemcitabine.[40] Also, dynamic interactions occur between different receptor subtypes among the HER family (EGFR, HER2, HER3) as homo- or heterodimers, attesting of the complexity of this signaling pathway. The cetuximab/trastuzumab combination showed a better efficacy than lapatinib or gemcitabine alone in preclinical studies[41] and a greater efficacy on tumor progression than pertuzumab alone or in combination with trastuzumab.

These combinative strategies show a greater efficacy in EGFR-HER2 or HER2-HER3 heterodimers disorganization, a more efficient and more stable inhibition of the Ras/Raf/MER/ERK and PI3K/AKT/mTOR signaling pathways, an increase of the targeted HER2 receptor degradation and an improvement of ADCC.[18,19,42,43] Indeed, the mechanisms leading to an enhanced activity after administration of a tyrosine kinase inhibitor with a monoclonal antibody have been described in other cancer cell models.[44,45] Lapatinib enhanced the effects of trastuzumab inducing HER2 accumulation at the cell surface of breast cancer cell lines,[46] and lapatinib was reported to induce accumulation of HER2 and EGFR on esophageal cancer cell lines evoking trastuzumab- and cetuximab-mediated ADCC.[47] The antitumor effect of drug combinations was more evident in ADCC experiments compared to cell viability experiments. In the Calu-3 xenograft model, the combination resulted in lower tumor growth, suggesting the involvement of NK activity as a determinant factor to improve the efficacy of the combined treatment.

Concerning mutational aspects, Wang et al showed that Chinese patients with EGFR mutation (L778P mutation especially) presented a higher disease control rate under gemcitabine plus erlotinib than under gemcitabine alone.[48] There was no correlation between KRAS and EGFR mutations in our study, unlike in lung cancer, where they are mutually exclusive. KRAS mutation did not predict outcome and response to treatment in our study and in ours, as it was already described.[49] Interestingly, Schultheis et al showed in a randomized phase II study that nimotuzumab, a new anti-epidermal growth factor receptor monoclonal antibody, combined with gemcitabine was safe and well-tolerated in pancreatic cancer patients.[44] The 1-year OS and PFS rates were encouraging, especially in patients with KRAS wildtype. Finally, in a recently published randomized phase II trial, dual (erlotinib and panitumumab) EGFR-directed therapy resulted in a trend of prolonged overall survival in patients with advanced adenocarcinoma of the pancreas but no predictive factor was found.[45] In this context, although our work brings new results and perspectives, it has some limits, among which its nonrandomized design and the low number of patients and samples evaluable for the ancillary study.

In conclusion, the gemcitabine/erlotinib/trastuzumab combination showed a favorable therapeutic effect in patients with metastatic pancreatic cancer. Toxicity was acceptable while the occurrence of thromboembolism events encourages the use of systematic prophylaxis. Comparing this triplet combination and gemcitabine associated with other cytotoxic or targeted drugs in these patients is now needed, with HER2 expression and skin toxicities monitoring.

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CONFLICT OF INTEREST

E. A. disclosed research funding from Bayer and Roche, honoraria from Novartis, BMS, Esai, Ipsen, Roche, Bayer, Sanofi and AAA and travel expenses paid by IPSEN and Bayer. L. M. disclosed honoraria from Roche and Bayer. C. L. B. disclosed conflicts of interest with Pfizer, Novartis, Keocyt, Ipsen, Sanofi and AAA. E. S. disclosed honoraria from Lilly, Amgen, Sanofi, Roche and Merck, research funding from Bayer and Merck, travel expenses paid by Ipsen and Roche. F. P. disclosed honoraria from AAA, Bayer, Ipsen and Servier, consulting and expertise for Amgen and Sanofi, research funding by Merck, travel expenses paid by Novartis and Servier. T. W. disclosed research funding from Roche, honoraria from Novartis, Keocyt, Ipsen, Sanofi and AAA, travel expenses paid by Ipsen. M. D. disclosed travel expenses paid by Ipsen and Novartis. M. Y. disclosed honoraria from Novartis, BMS, Merck, Servier, Roche, Bayer and Sanofi and travel expenses paid by BMS, Roche and Merck. T. M. disclosed research funding from Roche and Amgen, honoraria from Amgen, Sanofi and BMS, and travel expenses paid by Amgen. C. M., E. L. C., H. F., F. B. M. and A. H. P. C. had not conflict of interest to disclose.

DATA AVAILABILITY STATEMENT

The dataset supporting the conclusions of this article is available upon reasonable request at the Biometrics Unit of the Montpellier Cancer Institute.

ETHICS STATEMENT

The study was approved by the local ethics committee and conducted according to the Clinical Good Practice of the Helsinki Declaration (Clinical trial number: NCT01204372) and all patient has signed an informed consent form.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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