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RESEARCH ARTICLE

The Co-expression of HER Family Members and CD109 is common in Pancreatic Cancer.

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ABSTRACT

In the absence of biomarkers for the early detection of pancreatic cancer and the development of more effective therapeutic interventions, pancreatic cancer is projected to become the second leading cause of death from cancer in the Western world. In the past four decades, aberrant expression and activation of human epidermal growth factor receptor (EGFR, also called HER) family members have been reported in a wide range of human cancers. Currently, of the various types of drugs targeting one or more members of the HER family, only the EGFR-specific tyrosine kinase inhibitor erlotinib and more recently Zenocutuzumab, a HER-2/HER3 bispecific antibody, were granted breakthrough therapy designation by the FDA for the treatment of patients with pancreatic cancer. However, the therapeutic benefit may be modest in some patients. Hence there is an urgent need for the identification of biomarkers as prognostic indicators, therapeutic targets, and for the response to therapy and the development of more effective therapeutic agents for this highly heterogeneous cancer. In this study, for the first time, we investigated the relative expression and prognostic significance of all members of the HER family, the type-III EGFR mutant (EGFRvIII), and CD109 in tissue microarrays (TMAs) and whole tumour specimens (WTS) from pancreatic cancer patients by immunohistochemistry. We found the positive expression of wild-type EGFR (wt-EGFR) (63%, 4.7%), HER2 (75%, 14%), HER3 (none, 14%), HER4 (45%, 21%), EGFRvIII (5%, 3%), and CD109 (67%, 55%) in TMAs (USA) and WTS (UK) from pancreatic cancer patients respectively. Our results also show that the co-expression of HER family members with CD109 occurs frequently in patients with pancreatic cancer. In addition, the co-expression of HER4/CD109 may also be associated with poorer overall survival in such patients. In this article, we shall discuss these findings and their implications and future opportunities for more effective targeting of HER positive pancreatic cancer using the HER inhibitors in combination with other drugs and therapeutic interventions.

Introduction

Pancreatic cancer is one of the most aggressive and deadliest types of human cancer. Globally, there were an estimated 495,773 new cases of pancreatic cancer and 466,003 pancreatic cancer deaths in 2018[1]. In the USA, there will be an estimated 64,050 new cases of pancreatic cancer and 50,550 pancreatic cancer-related deaths in 2023, with the incidence increasing by 1% every year[2] While surgical resection is the only potentially curative treatment that may prolong survival and provide a good prognosis[3], around 80-90% of patients have a metastatic/ unresectable tumour at the time of presentation and despite aggressive treatments with surgery, chemotherapy, and radiotherapy have a 5-year survival rate of only 11%[4,5]. Therefore, there is an urgent need to identify biomarkers of diagnostic, prognostic, and predictive value and the development and application of more effective approaches for the treatment of patients with pancreatic cancer[5,6].

In the past few decades, the aberrant expression and activation of the human epidermal growth factor receptor family (also called ErbB/HER family) have been reported in numerous human malignancies, and in some studies, it has been associated with tumour aggressiveness and a poorer prognosis in patients with pancreatic cancer[7-11]. HER family members include EGFR, HER2, HER3, and HER4. Upon ligand binding, conformational changes in the extracellular domain of the receptor result in the formation of homodimers or heterodimers which then leads to the activation of various downstream signalling pathways such as PI3K-AKT, RAS/RAF/MAPK, JAK-STAT, and PLC-γ1 that regulates proliferation, metabolism, angiogenesis, cell progression, and survival [11-13]. Until June this year, of various types of monoclonal antibody (mAb)-based drugs and small molecule tyrosine kinase inhibitors (TKIs) targeting one or more members of the HER family, only the reversible EGFR-specific erlotinib has been approved by the US FDA for the treatment of locally advanced, unresectable, or metastatic pancreatic cancer when used in combination with gemcitabine[14]. In June 2023, the FDA granted breakthrough therapy designation to another HER inhibitor Zenocutuzumab, which is a HER-2/HER3 bispecific antibody, for the treatment of patients with NRG1+ pancreatic cancer[15]. While treatment with these drugs improved survival in some patients, many patients with pancreatic cancer do not benefit from such treatments, with tumour heterogeneity and primary and secondary resistance as the contributing factors[6,11,14]. In some studies, crosstalk between the HER-family members has been associated with a poorer response or resistant to the treatment with the HER inhibitors, supporting the need for investigation of the relative expression and prognostic significance of all members of the HER family in patients with pancreatic cancer.

Of various types of other biomarkers, CD109 is a glycosylphosphatidylinositol-anchored cell surface glycoprotein, a member of alpha 2 macroglobulin/ C3, C4, and C5 family of thioester-containing proteins[16-18]. Like increased expression and activation of EGFR and HER2, overexpression of CD109 has also been reported in various cancers and in some cases, it has been associated with unfavorable prognosis[19-31]. Interestingly, in some studies, it

has been shown that CD109 can form a complex with EGFR to positively regulate EGF signalling and tumour progression[32-34]. However, to our knowledge, there is currently no comprehensive study examining the expression pattern and prognostic significance of all members of the HER family along with type-III EGFR mutant (i.e., EGFRvIII), which is a ligand-independent and constitutively active form of the EGFR[35], and CD109 in pancreatic cancer. Therefore, in the present study, we examined the relative expression and cellular location of all members of the HER family, EGFRvIII, and CD109 by immunohistochemistry and their association with clinicopathological features and overall survival in patients with pancreatic cancer. We discussed this data as well as some of the challenges and future opportunities for more effective targeted therapy of HER positive pancreatic cancer patients.

Materials and Methods

In this retrospective study, tumour specimens from forty-three patients with pancreatic ductal adenocarcinoma who underwent Whipple's procedure or distal pancreatosplenectomy at the Royal Surrey County Hospital (Guildford, UK) between 2011 and 2018 were included. Ethical approval was obtained from the Research and Development Committee of the Royal Surrey County Hospital. As only achieved tumour specimens were included in this study, the ethics committee waived the need for patient consent and patient record/information were analysed anonymously. Tumour blocks with different tumour types, insufficient tumour, and no follow-up information were excluded from this study. Complete clinicopathological information including patient gender, tumour stage, and grade was available for each patient. In addition to whole pancreatic tumour specimens above, tissue microarrays (Cat. No. PA1002b, Biomax USA) from pancreatic cancer patients were also examined in this study.

Immunohistochemistry

Formalin-fixed paraffin-embedded sections from forty-three whole tumour specimens (3μM) were cut in serial sections, which along with 40 human pancreatic cancer TMAs were subjected to antigen retrieval (Tri-EDTA based CC1, pH 7.8 or citrate based CC2, pH 6.5) and incubated with the following primary antibodies: mouse anti-wild type EGFR (DAK-H1-WT 1:100 Dako, UK) for 44 minutes, mouse anti-HER2/neu (3B5 1:200, Santa Cruz Biotechnology, UK) for 32 minutes, rabbit anti-HER3 (SP71 1:50, Abcam, UK) for 1 hour, mouse anti-HER4 (HFR1 1:100, Santa Cruz Biotechnology, UK) for 28 minutes, mouse anti-EGFRvIII (DH8.3, 1:500, Novus Bio, UK) for 32 minutes and mouse anti-CD109 (KU42.33C, Kingston University, UK) for overnight as described previously[36,37]. The staining of slides containing serial sections of whole tumour specimens and TMAs was carried out on a Ventana Benchmark XT autostainer using the Ultra View DAB kit (Roche, UK). Ultimately, all slides were rehydrated using ethanol solutions (50% for 3 minutes, 80% for 3 minutes, 90% for 3 minutes, 100% for 3 minutes, and Histo clear for 3 minutes) and counterstained using haematoxylin and bluing agent, mounted and cover slipped.

Scoring system

In the present study, the immunostaining of the tumour sections was scored based on the

percentage of tumour cells with positive immunostaining at different cut-off values (i.e., >5%, >10%, >20%, and >50%), the intensity of immunostaining (i.e., 0 = negative, 1+ = weak, 2+ = moderate, and 3+ = strong) and their cellular location (membranous, cytoplasmic, and nuclear) as described in the previous studies with other cancers[37,38]. The immunostaining was also scored depending on the two independent observers (including a consultant histopathologist) without prior knowledge of clinicopathological features conducted the scoring and any disparity in scoring was resolved by simultaneous reassessment of the staining by both observers.

Statistical Analysis

The statistical analysis was carried out using the Statistical Package for the Social Sciences software (IBM®, SPSS statistics version 26, UK). The association between the immunohistochemistry scores and the patient's clinicopathological features was determined using the chi-squared test (Pearson Chi-square) and Fisher's exact test. Kaplan-Meier survival plots were used to carry out univariate analysis and the difference between the individual groups was determined using a log-rank test. Cox-regression univariate and multivariate analysis was also conducted to confirm its significance and $p<0.05$ was considered statistically significant.

Results

IMMUNOHISTOCHEMICAL EXPRESSION OF HER FAMILY MEMBERS AND EGFRVIII IN PATIENTS WITH PANCREATIC CANCER:

The expression level of HER family members (wt-EGFR, HER2, HER3, and HER4), and EGFRvIII were determined in 40 cases of pancreatic cancer TMAs. Overall, except for HER3 staining, the expression of the rest of the HER family members (EGFR, HER2, and HER4) was much higher in TMAs compared to whole tumour specimens. At the cut-off value of >5% of tumour cells with positive immunostaining, the expression of wt-EGFR was seen in 25/40 (62.5%) of the TMAs. The cellular location of wt-EGFR immunostaining was both membranous and cytoplasmic in 21/40 (52.5%) of the TMAs. Of the wt-EGFR+ cases, 24/40 (60%) showed weak wt-EGFR expression and only 3/40 (7.5%) showed moderate wt-EGFR expression (Table 1, Figure 1, Supplementary Table 1).

Interestingly, at the same cut-off value of >5% immunostaining, the vast majority of TMAs (75%) were HER2-positive. The cellular location of HER2 staining was found to be membranous in 19/40 (47.5%) and cytoplasmic in 30/40 (75%) of the pancreatic cancer TMAs. In addition, moderate HER2 staining intensity (2+) was present in 12/40 (30%) of the cases examined (Table 1), supporting their potential as a target for therapy with various types of anti-HER-2 antibodies. At the same cut-off value, while none of the cases in the TMAs showed HER3 staining, 45% of the cases were HER4 positive. The cellular location of HER4 staining was predominantly nuclear (47.5%) with 20% of the cases showing cytoplasmic HER4 staining. Finally, at the same cut-off value, only 2/40 (5%) of the cases exhibited weak cytoplasmic EGFRvIII staining (Table 1, Figure 1, Supplementary Table I). The results of immunostaining with anti-HER antibodies at other cut-off values (i.e. >10%, 20%, 50%) are also summarised and presented in Table 1.

Table 1: Immunohistochemical expression of HER family members, EGFRvIII, and CD109 in TMAs (A) and whole pancreatic tumour specimens (B) in patients with pancreatic cancer.

Scoring criteria	wt-EGFR	HER-2	HER-3	HER-4	EGFRvIII	CD109
(A) Pancreatic cancer TMAs						
Percentage of positive tumour cells (%)						
>5%	25(62.5)	30(75.0)	0	22(45.0)	2(5.0)	26(66.7)
>10%	23(57.5)	30(75.0)	0	15(37.5)	1(2.5)	20(51.3)
>20%	17(42.5)	29(72.5)	0	8(20.0)	0	13(33.3)
>50%	15(37.5)	27(67.5)	0	6(15.0)	0	8(20.5)
Intensity						
1+	24(60.0)	24(60.0)	0	21(52.5)	2(5.0)	20(51.3)
2+	3(7.5)	12(30.0)	0	1(2.5)	0	6(15.4)
3+	0	0	0	0	0	0
Sub-cellular localisation						
Membranous	21(52.5)	19(47.5)	0	0	0	13(33.3)
Cytoplasmic	21(52.5)	30(75.0)	0	8(20.0)	2(5.0)	26(66.7)
Nuclear	0	0	0	19(47.5)	0	0
(B) Whole pancreatic tumour specimens						
Percentage of positive tumour cells (%)						
>5%	2 (4.7)	6 (14.0)	6 (14.0)	9 (20.9)	1 (2.3)	24(55.8)
>10%	1 (2.3)	5 (11.6)	4 (9.3)	5 (11.6)	1 (2.3)	19(44.2)
>20%	0	2 (4.7)	3 (7.0)	4 (9.3)	1 (2.3)	14(32.6)
>50%	0	1 (2.3)	3 (7.0)	1 (2.3)	1 (2.3)	8(18.6)
Intensity						
1+	2 (4.7)	5 (11.6)	2 (4.7)	8 (18.6)	1 (2.3)	22(51.2)
2+	0	1 (2.3)	6 (14.0)	1 (2.3)	1 (2.3)	2(4.7)
3+	0	0	1 (2.3)	0	0	0
Sub-cellular localisation						
Membranous	1 (2.3)	0	6 (14.0)	0	0	1(2.3)
Cytoplasmic	2 (4.7)	6 (14.0)	6 (14.0)	0	1 (2.3)	24(55.8)
Nuclear	0	0	0	9 (20.9)	0	0

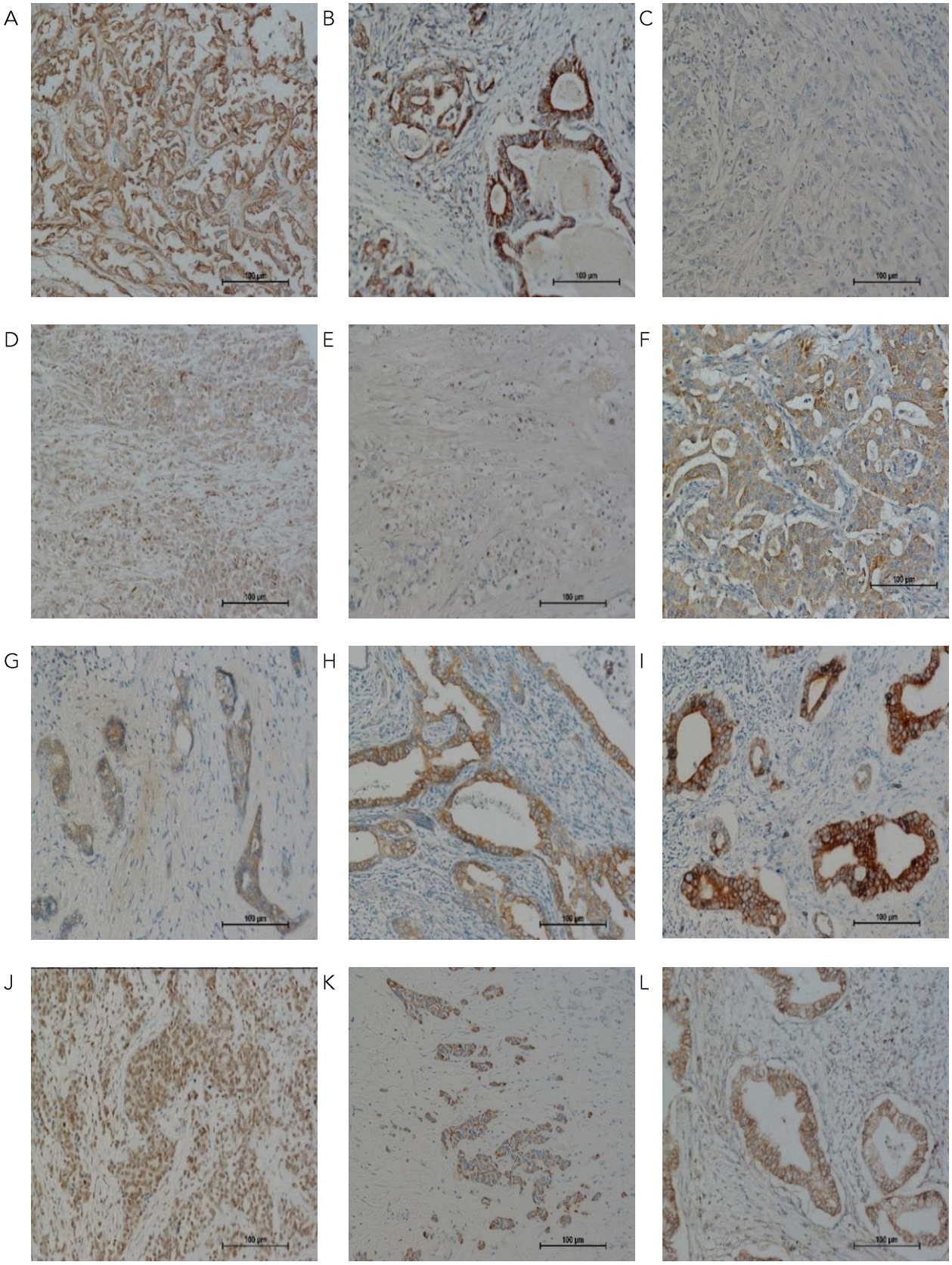

Figure 1. Immunohistochemical staining of pancreatic cancer TMAs (A-F) and whole pancreatic tumour specimens (G-K) from patients with pancreatic cancer. (A) wt-EGFR 1+ cytoplasmic/2+ membranous, (B) HER2 2+ cytoplasmic, (C) HER3 0, (D) HER4 1+ cytoplasmic/nuclear, (E) EGFRvIII 1+ cytoplasmic, (F) CD109 2+ cytoplasmic, (G) wt-EGFR 1+ cytoplasmic, (H) HER2 2+ cytoplasmic, (I) HER3 3+ membranous/cytoplasmic (J) HER4 2+ nuclear, (K) EGFRvIII 1+/2+ cytoplasmic, (L) CD109 2+ membranous/cytoplasmic, Magnification x200.

Next, the expression level of HER family members and EGFRvIII was determined in 43 whole tumour specimens from patients with pancreatic cancer. At the cut-off value of >5%, the expression of wt-EGFR was low in whole tumour specimens with only 4.7% of pancreatic cancer cases showing weak membranous and cytoplasmic EGFR staining. At the same cut-off value, HER2 expression was detected in 14% of the cases examined. Of the HER2+ cases, 11.6% and 2.3% had weak and moderate levels of HER2 expression respectively (Table 1, Figure 1). At the same cut-off value of >5% of tumour cells with positive immunostaining, 14% and 21% of the cases were HER3 and HER4 positive respectively (Table 1). The cellular location of HER3 staining was both membranous and cytoplasmic and the intensity of 2+ and 3+ was present in 14% and 2.3% of the cases examined. In contrast, the cellular location of HER4 was predominantly nuclear with only 2.3% of the cases exhibiting HER4 staining intensity of 2+. Finally, the expression of EGFRvIII was rare in whole tumour specimens from these patients with only 2.3% of the pancreatic cancer cases showing weak-moderate cytoplasmic EGFRvIII staining (Table 1, Figure 1).

IMMUNOHISTOCHEMICAL EXPRESSION OF CD109 AND ITS CO-EXPRESSION WITH HER FAMILY MEMBERS IN PATIENTS WITH PANCREATIC CANCER:

Overexpression of CD109 has been reported in multiple human malignancies and some studies also suggested its interaction with HER-family members in cancer progression. As a result, the expression level of CD109 was also determined in 40 TMAs and 43 whole tumour specimens from patients with pancreatic cancer. At the cut-off value of >5% of tumour cells with positive immunostaining, CD109 expression was detected in 26/40 (66.7%) and 24/43 (55.8%) of the TMAs and whole tumour specimens respectively (Table II, Figure 1). The expression of CD109 was mainly weak (1+) in both TMAs (51.3%) and whole tumour specimens (51.2%) (Table 2, Figure 1).

Next, the co-expression of HER family members, EGFRvIII, and CD109 was investigated at different cut-off values and the results are summarised in Table 2. For example, at the cut-off value of >5% of tumour cells with positive immunostaining, 45%, 40%, 48%, and 33% of the TMA cases had the co-expression of wt-EGFR/HER2, wt-EGFR/HER4, HER2/HER4, and wt-EGFR/HER2 /HER4 respectively. Interestingly, the co-expression of CD109 was frequently present with 39%, 56%, 39% of the cases having co-expression with wt-EGFR, HER2, and HER4 respectively. Moreover, 23% of the cases had co-expression of CD109 with wt-EGFR/HER2 /HER4 (Table 2A). In contrast, at the same cut-off value, the co-expression of CD109 with other members of the HER-family was present in between 4.7% and 14% of the whole tumour specimen cases examined (Table 2B).

Table 2: Co-expression of HER family members, EGFRvIII, and CD109 in TMAs (A) and whole pancreatic tumour specimens (B) in patients with pancreatic cancer.

Markers	Number of positive tumours (%)			
(A)*	>5% cut off	>10% cut off	>20% cut off	>50% cut off
wt-EGFR/HER2	18(45.0)	17(42.5)	10(20.5)	10(25.0)
wt-EGFR/HER4	16(40.0)	8(20.0)	4(10.0)	3(7.5)
Wt-EGFR/CD109	15(38.5)	10(25.6)	6(15.4)	4(10.3)
HER2/HER4	19(47.5)	14(35.0)	7(17.5)	6(15.0)
HER2/CD109	22(56.4)	18(46.2)	12(30.8)	6(15.4)
HER4/CD109	15(38.5)	7(17.9)	3(7.7)	1(2.6)
wt-EGFR/HER2/HER4	13(32.5)	7(17.5)	3(7.5)	3(7.5)
Wt-EGFR/HER2/CD109	12(30.8)	8(20.5)	5(12.8)	3(7.7)
Wt-EGFR/HER4/CD109	11(28.2)	3(7.7)	2(5.1)	1(2.6)
Wt-EGFR/HER2/HER4/CD109	9(23.1)	2(5.1)	2(5.1)	1(2.6)
(B)	>5% cut off	>10% cut off	>20% cut off	>50% cut off
HER2/HER4	2(4.7)	2(4.7)	0	0
HER3/HER4	3(7.0)	1(2.3)	0	0
wt-EGFR/CD109	2(4.7)	1(2.3)	0	0
HER2/CD109	2(4.7)	1(2.3)	0	0
HER3/CD109	3(7.0)	3(7.0)	3(7.0)	3(7.0)
HER4/CD109	6(14.0)	3(7.0)	3(7.0)	1(2.3)
Wt-EGFR/HER2/HER4/CD109	1(2.3)	0	0	0

* HER3 expression was negative in TMAs.

THE ASSOCIATION BETWEEN THE CLINICOPATHOLOGICAL PARAMETERS AND THE EXPRESSION OF HER FAMILY MEMBERS AND CD109 IN PATIENTS WITH PANCREATIC CANCER:

The association between the expression level of HER family members, EGFRvIII, and CD109 and the patient's clinicopathological parameters was investigated using the Fishers-Exact correlation test and the results are summarised in Table 3. No significant association was found between the expression level of the above markers and clinicopathological features of pancreatic tumour specimens ($p>0.05$). In contrast, in cases of pancreatic cancer TMA, some interesting correlations were found between patients' tumour grades and the expression level of HER family members and CD109. For instance, a significantly higher number of

high-grade tumours also had both nuclear HER4 (p=0.002) and HER4 immunostaining intensity of 1+ (p=0.024). Moreover, at the cut-off value of above 5%, the co-expression of wt-EGFR/HER2 (p=0.027), HER2/HER4 (p=0.022), HER2/CD109 (p=0.020), and wt-EGFR/HER2/HER4 (p=0.020) were found to be higher in G3 tumours compared to low-grade tumours. In contrast, a significantly higher number of low-grade tumours had the membranous expression of HER2 (p=0.024). Finally, at the cut-off value of >10% and >20%, a significantly higher number of high-grade tumours co-expression of HER2 with CD109 (p=0.025 and p=0.041 respectively) (Table 3).

Table 3: The association between HER family members, EGFRvIII, and CD109 and clinicopathological parameters of pancreatic cancer TMAs using the Chi-squared test (Fisher's exact test).

Receptors (sub-categories)	Number of TMAs with receptor expression		
	Clinicopathological parameters		Fisher Exact Test
	Grade		
	<G3	G3	P-value
wt-EGFR (>5%)	6	19	0.003
wt-EGFR (>10%)	5	18	0.003
wt-EGFR (>20%)	3	14	0.01
wt-EGFR (>50%)	3	12	0.046
wt-EGFR (1+ Intensity)	6	18	0.009
wt-EGFR (Membranous)	5	16	0.024
wt-EGFR (Cytoplasmic)	4	17	0.003
HER2 (Membranous)	12	7	0.024
HER4 (Nuclear)	3	16	0.002
HER4 (1+ Intensity)	5	16	0.024
HER2/HER4 (>5%)	3	13	0.022
wt-EGFR/HER2 (>5%)	4	14	0.027
HER2/CD109 (>5%)	3	14	0.020
wt-EGFR/HER2/HER4 (>5%)	2	11	0.020
HER2/CD109 (>10%)	5	16	0.025
HER2/CD109 (>20%)	8	19	0.041

THE ASSOCIATION BETWEEN THE CLINICOPATHOLOGICAL FEATURES OF PANCREATIC CANCER PATIENTS AND THEIR OVERALL SURVIVAL:

As there was no follow-up data for pancreatic cancer TMAs, the survival analysis could not be investigated. The median patient follow-up period for whole tumour specimens was 4 years, and the median age was 67 years. The overall survival was determined using Kaplan-Meier curves and a log-rank test (Kaplan 1983). The mean overall survival was 4.51±0.391 years and it was found to be significantly poorer in females compared to male patients (5.3±0.6 versus 7.5±0.6 years, p=0.027). In addition, patients with an advanced tumour stage (i.e., T3), grade (i.e., G3), lymph node involvement, and the

presence of lymphatic and vascular invasion had reduced overall survival. However, this reduction in overall survival was not statistically significant (p>0.05) (Table 4).

Table 4: Patient clinicopathological features and their association with overall survival using Kaplan-Meier analysis and log-rank test in forty-three whole pancreatic tumour specimens.

Characteristics	Number of patients (%)	Overall survival in years (mean ± SE)	95% CI	P-value
Age				
<65	16 (37.2)	5.7 ± 0.8	4.2 - 7.2	0.131
≥65	27 (62.8)	7.1 ± 0.6	5.9 - 8.3	
Gender				
Female	18 (41.9)	5.3 ± 0.6	4.1 - 6.5	**0.027**
Male	25 (58.1)	7.5 ± 0.6	6.3 - 8.8	
T-stage				
<T3	41 (95.3)	6.5 ± 0.5	5.5 - 7.5	0.677
T3	2 (4.7)	3.5 ± 0.4	2.8 - 4.2	
N-stage				
N0	7 (16.3)	6.3 ± 1.4	3.6 – 8.9	0.923
N1&N2	36 (83.7)	6.5 ± 0.5	5.5 – 7.6	
Grade				
<G3	7 (16.3)	8.5 ± 0.6	7.3-9.7	0.088
G3	36 (83.7)	6.0 ± 0.5	5.0-7.1	
Lymphatic invasion				
Absent	8 (18.6)	7.2 ± 1.3	4.7 - 9.8	0.44
Present	35 (81.4)	6.3 ± 0.5	5.3 - 7.4	
Vascular invasion				
Absent	21 (48.8)	6.8 ± 0.6	5.5 - 8.0	0.341
Present	22 (51.2)	5.8 ± 0.7	4.5 - 7.1	
Perineural invasion				
Absent	4 (9.3)	4.0 ± 0.7	2.6 - 5.4	0.844
Present	39 (90.7)	6.5 ± 0.5	5.5 - 7.5	

THE PROGNOSTIC SIGNIFICANCE OF CD109 EXPRESSION AND ITS CO-EXPRESSION WITH HER FAMILY MEMBERS IN PATIENTS WITH PANCREATIC CANCER:

The association between the expression level of HER family members, EGFRvIII, and CD109 and the patient's overall survival was determined using Kaplan-Meier curves and log-rank test. There was no significant association between HER family members and EGFRvIII positive immunostaining at any cut-off values and overall survival in patients with pancreatic cancer. Interestingly, at the cut-off value of >50% of tumour cells with positive immunostaining, CD109 expression was associated with better overall survival (9.5±0.5

vs 6.0±0.5 years, p=0.021) in these patients (Figure 2A). However, the multivariate cox-regression analysis revealed that CD109 expression was not an independent predictor of better survival in these patients (p>0.05) (Table 5). Moreover, at the cut-off value of >5% of tumour cells with positive immunostaining, the co-expression of CD109 with HER4 was associated with a poorer patient's overall survival (4.7±1.1 vs 6.8±0.5 years p=0.032, Figure 2B). While the univariate analysis showed a 2.9-fold increased risk of poorer overall survival, the multivariate analysis showed that HER/ CD109 co-expression is not an independent predictor of poor survival (Table 5).

Table 5: Univariate and multivariate analysis of the association between sub-categories of various receptors and overall survival of patients with pancreatic cancer. A p-value of <0.05 was considered significant. Overall survival relative to the indicated features was determined by Cox regression analysis.

Variable	Overall Survival (Univariate)			Overall Survival (Multivariate)		
	HR	95% CI	P-value	HR	95% CI	P-value
CD109 >50%	0.166	0.02-1.27	0.084	0.249	0.03-2.10	0.201
HER4/CD109 >5%	2.953	0.95-9.23	0.063	3.410	0.86-13.49	0.080

HR: Hazard Ratio, 95% CI: 95% Confidence Interval, p-value <0.05.

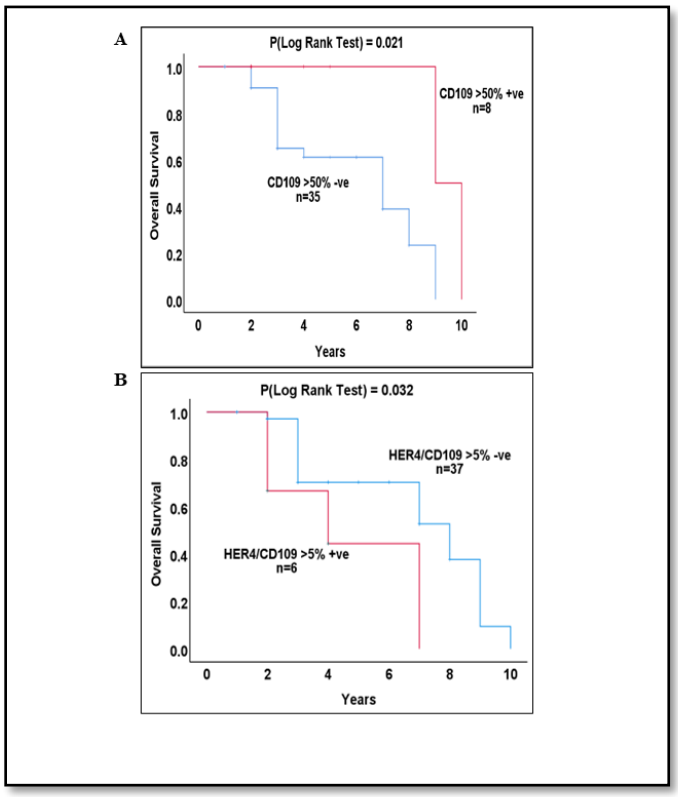

Figure 2: The association between CD109 and HER4/CD109 and overall survival of patients with pancreatic cancer. Kaplan-Meier survival curves showing the impact on patient overall survival with CD109 expression (A), and HER4/CD109 co-expression (B).

Discussion

Pancreatic cancer is currently one of the most aggressive and deadliest types of human cancer[5]. To reduce the mortality from pancreatic cancer, it is essential to identify biomarkers with high levels of expression and to determine their potential for use in the early diagnosis and as prognostic indicators, predictive biomarkers, and therapeutic targets. In the past few decades, the overexpression of HER family members has been reported in various human malignancies and, in some studies, it has been shown to have prognostic significance[8-10,39-44]. To date, of the HER family members, EGFR and HER-2 are important therapeutic targets in patients with a wide range of tumours. Of the various types of approved HER inhibitors, erlotinib was the only EGFR-specific reversible TKI that has been approved for the treatment of patients with pancreatic cancer when used in combination with gemcitabine[14]. In June 2023, FDA granted breakthrough therapy designation to Zenocutuzumab, which is a bispecific antibody inhibiting HER-2/HER3 heterodimerization, for the treatment of patients with advanced unresectable or metastatic NRG1 gene fusion-positive (NRG1+) pancreatic cancer following disease progression on previous systemic therapy or who have no alternative options available[15]. However, the great majority of patients with pancreatic cancer do not respond to such treatments and more effective therapeutic interventions are urgently needed.

We have previously found that the irreversible pan-HER TKI afatinib was more effective than the FDA-approved EGFR-specific TKI erlotinib in inhibiting the proliferation of human pancreatic cancer cell lines[45,46]. Indeed, several studies have reported the role of cross-talk between the HER family members in the progression of human cancer and resistance to the HER inhibitors. More recently, the importance of interaction between the HER-family members and CD109 has also been associated with tumorigenesis and metastasis in human cancers[47,48]. Therefore, in this comprehensive study for the first time, we investigated the expression and/or co-expression of all members of the HER family, EGFRvIII, and CD109 proteins by immunohistochemistry, which are the actual targets for therapy with antibody-based drugs, in patients with pancreatic cancer.

Interestingly, while the expression for all HER family members was low to moderate in whole tumour specimens from pancreatic cancer patients, the expression of wt-EGFR, HER2, and HER4 was moderate to high in the TMAs from pancreatic cancer patients (Table 1). In comparison to HER3 expression in the whole tumour specimens, HER3 expression was found to be negative in the TMAs from patients with PADCs. In this study, we found no significant association between the expression level of the HER family members in whole tumour specimens and patient overall survival. It is noteworthy that the expression levels of EGFR, HER2, HER3, and HER4 in the literature ranged from 0-84%, 0-92%, 24-41%, and 9-82% respectively (Table 6). In one study, Te Velde and colleagues examined the expression level of HER family members in tissues of 45 resected pancreatic cancer patients and found no overexpression of EGFR and HER2 in these patients. In addition, they found while HER3 and HER4 are expressed in the normal pancreas, their

expression is lost in pancreatic cancer[49]. In another study, Bittoni and colleagues examined the expression and prognostic significance of three of the four members of the HER-family in tissue samples from 91 pancreatic cancer patients. They found overexpression of EGFR (45%), and HER3 (41%) but not HER2 in these patients. They found that only overexpression of HER3 was associated with an advanced TNM stage and poorer patient survival[50]. The expression level of HER family members was also examined in another study involving 70 patients with operable pancreatic cancer by Li and colleagues. They found positive expression of EGFR, HER2, HER3, and HER4 in 41.4%, 60.0%, 24.3%, and 65.7% of the cases respectively and only HER3 expression was an independent prognostic predictor of poor survival (p=0.001)[7]. They found there was no significant association between EGFR and HER2 expression and overall survival in such patients. Some of the contributing factors for the wide difference in receptor expression of different members of the HER family could be due to differences in sample size, patient population, tumour heterogeneity, and differences in antibody used and the scoring system employed[51]. Moreover, several studies have reported a higher expression of HER family members in the TMAs from pancreatic cancer patients compared to whole tumour specimens[52-58]. In this study, we examined the whole pancreatic tumour specimens and the TMAs which were from pancreatic cancer patients in the UK and the USA respectively. As pancreatic cancer is heterogenous in nature, further investigation of the relative expression of all members of the HER family in tumour specimens from a larger group of pancreatic cancer patients from different regions is warranted. Such studies should help to overcome some of the challenges in identifying the subgroup of pancreatic cancer whose tumours are HER-dependent and would benefit from the treatment with different types of the HER inhibitors.

In some studies, CD109 was found to form a complex with EGFR to promote tumour progression and CD109 expression has been associated with an unfavourable prognosis. In this study, we found that, using anti-CD109 mAb KU42.33C, at the cut-off value of >5% of tumour cells with positive immunostaining, 67% and 56% of TMAs and the whole tumour specimens from pancreatic cancer cases were CD109 positive (Table 1). In addition, 31% and 23% of the TMA cases had co-expression of CD109 with the wt-EGFR/HER2 and EGFR/HER2/HER4 respectively (Table 2). In addition, using the same anti-CD109 antibody in our previous study, we found that 94% of TMAs from another 65 pancreatic cancer patients were CD109 positive[18]. In another study, using an anti-CD109 antibody (C-9), Adachi and colleagues found CD109 expression in 53.3% of 92 pancreatic cancer patients. They also found that CD109 expression was associated with disease-free survival (p=0.003) and overall survival (p=0.002) and was an independent prognostic factor of both DFS (p=0.0173) and OS (p=0.0104) in these patients[82]. Likewise, a recent meta-analysis including 7 studies consisting of 1583 patients with different types of epithelial and mesenchymal cancers found that the CD109 expression was associated with unfavourable survival in these patients [83]. Interestingly, in this study, the

expression of CD109 in >50% of tumour cells was associated with better overall survival. However, the co-expression of CD109 with HER-4 was associated with poorer patient outcomes and both the univariate and multivariate analysis showed that CD109 expression and HER4/CD109 co-expression were not independent factors of patient outcome ($p>0.05$). Our results highlight that HER family members and CD109 could serve as potential therapeutic targets in patients with pancreatic cancer and warrant further investigation in a larger group of patients. Such studies should provide further evidence on the therapeutic potential of the HER inhibitors when used in combination with CD109 inhibitors, other targeted agents, and traditional therapy in pancreatic cancer[6,11,45,46,84].

Future Opportunities

Overexpressed cell surface antigens such as EGFR and HER2 are currently important targets for therapy with monoclonal antibody-based drugs in patients with a wide range of cancers. For example, anti-HER2 antibody trastuzumab has been approved initially for the treatment of patients with HER2 overexpressing (i.e. IHC score of 3+) metastatic or early-stage breast cancer[6,18]. Interestingly, it has been shown more recently that the treatment of patients with even HER2-low (or IHC2+/ISH-) metastatic breast cancer with anti-HER2 antibody-drug conjugate fam-trastuzumab deruxtecan-mxki (T-DXd) resulted in significantly longer progression-free survival and overall survival in such patients and its FDA approval in August 2022[85,86]. Therefore, pancreatic cancer patients with low level of HER-2 expression may also benefit from such drugs.

We have shown recently that treatment with a combination of irreversible pan-HER blocker afatinib with dasatinib resulted in synergistic tumour growth inhibition of all human pancreatic cancer cell lines examined, thereby supporting further investigation on the therapeutic potential of these combinations in future clinical trials in pancreatic cancer[45]. The triple combination of HER inhibitor (erlotinib), dasatinib, and gemcitabine demonstrated synergistic anti-tumour activity in pancreatic cancer with encouraging preliminary results shown in patients with advanced pancreatic cancer[87,88]. Indeed, several clinical trials investigating the effect of HER inhibitors alone and/or in combination with other agents have been completed, and others are still ongoing or will be starting in patients with pancreatic cancer (Table 7). A major challenge is to improve the drug efficacy and to reduce severe toxicity which resulted in the termination of some of the clinical trials. Another challenge is the complex biology and heterogenous nature of pancreatic cancer and as highlighted in our current study, for example, many patients with pancreatic cancer are HER negative and may not therefore benefit from therapy with the HER inhibitors. Therefore, it is essential to discover novel therapeutic targets and to develop more effective and less toxic therapeutic agents for the treatment of patients diagnosed at different stages of the disease. These are currently an area of active research by colleagues in both academia and industry[6].

Table 6: Summary table of studies of the expression level and prognostic significance of EGFR (A), HER2 (B), HER3 and HER4 (C) determined by immunohistochemistry in patients with pancreatic cancer in the literature.

Study (year of study)	Samples	Antibodies (source)	Percentage expression (%)	Other findings	Conclusion
(A) EGFR					
[59] (2003)	77	Anti-EGFR mAb (pre-diluted CONFIRM EGFR antibody, clone 5B7)	41.6%	EGFR expression was associated with gender, histological differentiation and metastatic TNM classification (p<0.01)	EGFR expression plays a role in metastasis, especially liver metastasis and recurrence of pancreatic cancer.
[60] (2004)	76	Anti-EGFR mAb (Zymed, clone 31G7	Cytoplasmic EGFR overexpression; invasive=62%, intraductal=25% Membranous EGFR overexpression; Intraductal=54%, invasive=14%		Cytoplasmic EGFR overexpression was associated with shorter OS (P=0.02
[52] (2006)	71 TMAs	EGFR PharmDx assay kit, (Clone 2-18C9, Dako)	69%		EGFR is overexpressed in pancreatic cancer but does not predict survival.
[53] (2006)	50 TMAs	Anti-EGFR mAb (Clone:31G7)	58%	EGFR overexpression was correlated with stage (P=0.001)	No prognostic significance of EGFR was studied
[61] (2006)	39	Anti-EGFR mAb (Zymed)	30.8%	High EGFR was correlated with the higher tumor grade (P = 0.021)	No correlation between EGFR expression and patient OS
[62] (2006)	74	Anti-EGFR mAb (Zymed, Clone 31G7)	30% cytoplasmic, 9% membranous		No prognostic significance of EGFR was studied
[63] (2007)	32	EGFR Pharm Dx kit (Dako)	66%		No prognostic significance of EGFR was studied
[64] (2008)	36	Anti-EGFR (Dako, Clone:H11)	50%	EGFR was associated with metastasis to lymph nodes and other organs	No prognostic significance of EGFR was studied
[49] (2009)	45	EGFR (NovocastraNcl-L-EGFR-r)	0%		No correlation between EGFR expression and patient OS

[65] (2010)	137	Anti-EGFR murine mAb (Novocastra)	42.3%		No prognostic significance of EGFR was studied
[66] (2011)	52	EGFR PharmDx™ kit (Dako)	57%		No prognostic significance of EGFR was studied
[54] (2011)	100 TMAs	EGFR PharmDx™ Kit (Dako)	84% membranous, 82% cytoplasmic	EGFR was significantly higher in grade 3 patients (P=0.036)	No correlation between EGFR expression and patient OS
[9] (2012)	90	EGFR PharmDx™ kit (Dako)	57% membranous, 68% cytoplasmic	Membranous EGFR overexpression was significantly correlated with lymph node positivity (P=0.03)	Membranous EGFR overexpression was associated with shorter PFS (P=0.02)
[42] (2012)	105	Anti-EGFR (5B7, CONFIRM)	30.4% membranous	EGFR was associated with lymph node metastasis (P=0.038)	High EGFR expression was significantly associated with shorter patient OS (P<0.001)
[10] (2012)	44 resected and 40 primary or metastatic pancreatic cancer	EGFR PharmDx™ kit (Dako)	EGFR overexpression in resected tumors: 30% membranous 23% cytoplasmic In primary or metastatic tumors: 48% membranous 33% cytoplasmic		Overexpression of membranous and cytoplasmic EGFR may be indicative of a more aggressive phenotype
[67] (2013)	72 primary tumours	Anti-EGFR (NovaCastra)	44% membranous	EGFR overexpression was associated with tumour type (P=0.04)	No prognostic significance of EGFR was studied
[68] (2013)	181	Anti-EGFR mAb (Clone 5B7 pre-diluted CONFIRM EGFR antibody)	49%		No prognostic significance of EGFR was studied
[55] (2014)	99 TMAs	EGFR	62% membranous	Membranous EGFR was associated with tumor dedifferentiation, mitotic activity, and abnormal expression of cancer cell adhesion protein	No correlation between EGFR expression and patient OS
[69] (2015)	131	Anti-EGFR (Abcam)	48.9%	EGFR was associated with differentiation (P=0.031)	No correlation between EGFR expression and patient OS

[50] (2015)	92	EGFR PharmDX™ (Dako)	45.1%		No correlation between EGFR expression and patient OS
[8] (2015)	88	EGFR (zymed)	35.5% Membranous 11.3% Cytoplasmic	Membranous EGFR was associated with male gender (P=0.03)	Membranous EGFR was correlated with reduced survival (P=0.04)
[70] (2015)	81	Anti-EGFR mAb (Clone 3C6, CONFIRM)	64.2%		No correlation between EGFR expression and patient OS
[71] (2016)	357	EGFR (Dako PharmDx™ kit)	45.4%	Tumour size was significantly correlated with OS in EGFR+ patients (P<0.001)	No correlation between EGFR expression and patient OS
[72] (2016)	137 TMAs	Anti-EGFR (Clone E30, Dako)	95%		No prognostic significance of EGFR was studied
[7] (2016)	70	Anti-EGFR rabbit pAb (sc-03)	41.4%		No correlation between EGFR expression and patient OS
[73] (2016)	92	Anti-EGFR mAb (Santa Cruz), anti-EGFRvIII mAb, anti-AREG antibody	13% EGFRvIII, 53.3% AR, 23.9% AR/EGFR co-expression	A significant association was found between AR/EGFR co-expression and poor tumour differentiation.	No prognostic significance of EGFR was studied
[43] (2019)	32	Anti-EGFR mAb (Clone DAK H1 WT, M7298, Agilent)	47% membranous		High EGFR expression was significantly associated with shorter OS (P=0.004)
[74] (2021)	174	Anti-EGFR (Proteintech)	72.5%		EGFR high expression was correlated with a poor survival rate of pancreatic cancer patients (P < 0.01).
Khan et al., current study	43 whole tumour specimens and 40 TMAs	Anti- wild-type EGFR mAb (Clone: DAK-H1-WT)	4.7% whole tumour specimens and 62.5% TMA		No correlation between EGFR expression and patient OS in whole tumour specimens. Follow-up data was not available for 40 TMA patients
(B) HER2					
[60] (2004)	76	Anti-HER2 rabbit pAb	Membranous HER2 overexpression; intraductal=20% invasive=3% Cytoplasmic HER2 overexpression;		No correlation between HER2 expression and patient OS.

			intraductal=16%, invasive=11%		
[39] (2005)	30	Anti-HER2 pAb (Dako)	17%		A poor survival in HER2 positive patients
[63] (2007)	28	HER2 (HercepTest kit Dako)	17%		No association between HER2 and patient OS
[64] (2008)	36	Anti-HER2 (Clone NCL-c-erbB-2-316, Novocastra)	66.7%		No association between HER2 and patient OS
[49] (2009)	45	HER2 (Hercep test Dako,)	0%		No association between HER2 and patient OS
[65] (2010)	137	Anti-HER2rabbit pAb (Dako)	62.8%		No association between HER2 and patient OS
[75] (2012)	207	Anti-HER2 primary antibody	59.9%		No association between HER2 and patient OS
[67] (2013)	72	Anti-HER2 (Dako)	1% membranous		No association between HER2 and patient OS
[76] (2013)	469	Anti-HER2 antibody	19.8%		No prognostic significance of HER2 was studied
[77] (2014)	79	Anti-HER2 rabbit pAb (Clone 4B5, Ventana)	7.6%		No association between HER2 and patient OS
[78] (2014)	45	Anti-HER2 rabbit mAb (clone 4B5, Ventana)	22%		No association between HER2 and patient OS
[50] (2015)	91	HER2 (HercepTest, Dako)	1.1%		No association between HER2 and patient OS
[79] (2015)	2072	Anti-HER2 rabbit mAb (Clone 4B5, PATHWAY)	0.7%		No association between HER2 and patient OS
[80] (2015)	30	Anti-HER2 rabbit pAb (Neomarkers)	3.33% weak, 33.3% moderate and 36.7% diffuse cytoplasmic staining		No association between HER2 and patient OS
[7] (2016)	70	Anti-HER2 rabbit pAb (sc-284)	60%		No association between HER2 and patient OS
[72] (2016)	137 TMAs	Anti-HER2 (A0485, Dako),	92%		No association between HER2 and patient OS

[81] (2018)	20 TMAs	Anti-HER2 (Cell signalling)	40%		No association between HER2 and patient OS
[44] (2021)	55 TMAs	Anti-HER2 (Clone 4B5, Ventana)	41.8%		HER2 positive expression was significantly associated with a poorer OS (p=0.027)
Khan et al., Current study	43 whole tumour specimens and 40 TMAs	Anti-HER-2 mAb (Clone: 3B5)	14% whole tumour specimens 75% TMAs		No association between HER2 and patient OS in whole tumour specimens. No follow up data was available for 40 TMA patients
(C) HER3 and HER4					
[49] (2009)	45	Anti-HER3 mAb (MS-725-P, Neomarkers), Anti-HER4 mAb (MS-637-P, Neomarkers)	HER3: 27% overexpression HER4: 82% cytoplasmic, 9% membranous	Cytoplasmic HER3 expression decreased from early to late stage (P=0.01)	No correlation between HER3, HER4 expression and with patient OS
[41] (2011)	126	Anti-HER3 mouse mAb (Nanotools)	41.3%		HER3 overexpression was independent prognostic indicator of poor survival
[78] (2014)	45	Anti-HER3 mouse mAb (Clone DAK-H3-IC, Dako)	27%		No prognostic significance of HER3 was studied
[50] (2015)	91	Anti-HER3 mAb (Clone DAK-H3-IC, Dako)	40.7%	HER3 overexpression was associated with advanced TNM stages	HER3 overexpression correlated with a shorter was an independent prognostic factor (P=0.03)
[7] (2016)	70	Anti-HER3 rabbit pAb (sc-285), Anti-HER4 rabbit pAb (sc-283)	HER3 24.3%, HER4 65.7%		HER3 expression was an independent prognostic factor of poor survival (P=0.001), Mean survival time was higher in HER4+ group than HER4- group (P=0.027)
Khan et al., Current study	43 whole tumour specimens and 40 TMAs	Anti-HER3 mAb (Clone: SP71) Anti-human HER4 mAb (Clone: HFR1)	HER3 14% whole tumour specimens and 0% TMAs HER4 20.9% whole tumour specimens and 45% TMAs		No association between HER3 and HER4 and patient OS in whole tumour specimens . No follow up data was available for the 40 TMA patients.

Table 7: Summary of clinical trials with HER family inhibitors completed or ongoing in patients with pancreatic cancer.

Clinical trials Identifier	Recruitment status (start date)	Official title	Participants	Phase
With EGFR-inhibitors in pancreatic cancer				
NCT00225784	Completed (Feb 2005)	Cetuximab, Radiotherapy and Twice Weekly Gemcitabine in Patients With Adenocarcinoma of the Pancreas	37	II
NCT00536614	Completed (May 2005)	A Randomized Phase II Study of Gemcitabine/Cisplatin With or Without Cetuximab to Evaluate the Efficacy in Patients With Locally Advanced or Metastatic EGFR-EGFR-Positive Pancreatic Cancer	86	II
NCT00243854	Completed (Nov 2005)	Pilot Study Using Neoadjuvant Chemo-Radiotherapy and EGFR-tyrosine Kinase Inhibitor for Potentially Resectable pancreatic cancer	8	I
NCT00260364	Completed (Nov 2005)	Dose Finding and Early Efficacy Study of Combination Therapy With Erlotinib (Tarceva), Gemcitabine, Bevacizumab (Avastin), and Capecitabine in Advanced Pancreatic Cancer	44	I/II
NCT00622674	Completed (Nov 2005)	Bortezomib (Velcade) and Cetuximab (Erbitux) for Patients With Solid Tumors Expressing EGFR	37	I
NCT00313560	Completed (Mar 2006)	Erlotinib (TarcevaTM) **Combined** With Chemoradiation and Adjuvant Chemotherapy in Patients With Resectable Pancreatic Cancer	48	II
NCT00497224	Completed (Nov 2006)	Erlotinib in Advanced Pancreatic Cancer	51	II
NCT00397384	Completed (Jan 2007)	Clinical and Biological Evaluation of Combined EGFR Blockade with Erlotinib and Cetuximab in Patients with Advanced cancer	43	I
NCT00383149	Completed (Jan 2007)	Open Label Trial of Ixabepilone Plus Cetuximab as First Line Therapy for Metastatic Pancreatic Cancer	58	II
NCT00480584	Completed (Apr 2007)	GemCap-T, Capecitabine in Combination with Gemcitabine and Erlotinib (Tarceva®) in Patients with Advanced pancreatic adenocarcinoma	20	I
NCT00696696	Completed, has results (Sep 2007)	Gemcitabine and Erlotinib Plus Sorafenib (GES) in Metastatic Pancreatic Cancer	45	II
NCT00565487	Completed (Dec 2007)	Combination of Capecitabine and Erlotinib Concurrent With Radiotherapy in Patients With Non-Operable Locally Advanced Pancreatic Cancer	15	I

Clinical trials Identifier	Recruitment status (start date)	Official title	Participants	Phase
NCT00614653	Completed (Jan 2008)	Preoperative Radiotherapy With Concurrent Bevacizumab, Erlotinib and Capecitabine for Locally Advanced Pancreatic Cancer	17	I
NCT00634725	Completed (Feb 2008)	Gemcitabine With or Without Chemoradiotherapy and With or Without Erlotinib	820	III
NCT00617708	Completed, has results (Mar 2008)	Gemcitabine + Erlotinib (NSC-718781) + IMC-A12 (NSC-742460) vs. Gemcitabine + Erlotinib as First-Line Treatment in Patients With Metastatic Pancreatic Cancer	134	I/II
NCT00652366	Completed, has results (May 2008)	A Randomized, Open-label, Dose-escalation to Rash Study to Assess the Effect of Tarceva in Combination With Gemcitabine on Overall Survival in Patients With Metastatic Pancreatic	467	II
NCT00674973	Completed, has results (Jun 2008)	Biomarker Identification Trial for Erlotinib (Tarceva®) in Patients With Advanced Pancreatic Carcinoma	207	II
NCT00761345	Completed, has results (Sep 2008)	Low-Dose Fractionated Radiotherapy as a Chemosensitizer for Gemcitabine and Erlotinib in Patients With Locally Advanced or Limited Metastatic Pancreatic Cancer	27	I
NCT00739453	Completed (Oct 2008)	Dose-escalation Study of OSI-906 and Erlotinib (Tarceva®) in Patients With Advanced Solid Tumors	95	I
NCT00837876	Completed, has results (Oct 2008)	Sorafenib and Erlotinib in Unresectable Pancreatic Cancer	37	II
NCT00769483	Completed, has results (Nov 2008)	Gemcitabine Plus Erlotinib Plus MK-0646; Gemcitabine Plus MK-0646 and Gemcitabine Plus Erlotinib for Patients With Advanced Pancreatic Cancer	81	I/II
NCT01181245	Completed (Dec 2008)	Safety and Bioactivity With FG-3019 in Combination With Gemcitabine and Erlotinib for Subjects With Locally Advanced or Metastatic Pancreatic Cancer	50	I
NCT00925769	Completed, has results (Jan 2009)	An Open Label Study to Evaluate the Safety and Effect on Disease Progression of Triple Combination Treatment With Erlotinib (Tarceva), Bevacizumab (Avastin), and Capecitabine (Xeloda) in Patients With Locally Advanced and/or Metastatic Pancreatic Cancer	32	I
NCT00962312	Completed (Jan 2009)	Lapatinib and Capecitabine in the Treatment of Metastatic Pancreatic Cancer	9	II
NCT00550836	Completed, has results (Mar 2009)	Panitumumab, Erlotinib and Gemcitabine vs. Erlotinib and Gemcitabine in Patients With Untreated, Metastatic Pancreatic Adenocarcinoma	104	II

Clinical trials Identifier	Recruitment status (start date)	Official title	Participants	Phase
NCT00810719	Completed (Apr 2009)	Gemcitabine and Intermittent Erlotinib in Advanced Pancreatic Cancer	30	II
NCT00733746	Completed, has results (Apr 2009)	Preoperative Gemcitabine and Erlotinib Plus Pancreatectomy and Postoperative Gemcitabine and Erlotinib for Patients With Operable Pancreatic Adenocarcinoma	123	II
NCT00922896	Completed (Jun 2009)	Erlotinib in Combination With Gemcitabine and Cisplatin in Metastatic Pancreatic Cancer	22	II
NCT01077986	Completed (Aug 2009)	Non-randomized, Feasibility/ Safety and Efficacy Study of the Combination of Everolimus, Cetuximab and Capecitabine in Patients With Metastatic Pancreatic Cancer	35	I/II
NCT00987766	Completed (Nov 2009)	Gemcitabine and Oxaliplatin (GEMOX) With Erlotinib in Patients With Advanced Biliary Tract Cancer.	28	IB
NCT01010945	Completed (Feb 2010)	Erlotinib in Combination With Gemcitabine and Nab-Paclitaxel in Patients With Previously Untreated Advanced Pancreatic Cancer	19	IB
NCT01210911	Completed (Aug 2010)	A Phase II, Randomized, Placebo Controlled Study to Evaluate the Efficacy of the Combination of Gemcitabine, Erlotinib and Metformin in Patients with Locally Advanced and Metastatic pancreatic cancer	120	II
NCT01222689	Completed, has results (Nov 2010)	AZD6244 Plus Erlotinib for the Second-Line Treatment of Advanced pancreatic adenocarcinoma	46	II
NCT01505413	Completed (Jan 2011)	Phase 2 Study of GEMOX-T in Previously Untreated Patients with Advanced pancreatic cancer	33	II
NCT01303029	Completed (Feb 2011)	Randomized Study to Evaluate the Efficacy of Gemcitabine-erlotinib Versus Gemcitabine-erlotinib-capecitabine in Patients With Metastatic Pancreatic Cancer	120	IIB
NCT01389440	Completed (May 2011)	Open, Not Randomized to Evaluate the Efficacy and Safety of Neoadjuvant Treatment With Gemcitabine and Erlotinib Followed by Gemcitabine, Erlotinib and Radiotherapy in Patients With Resectable Pancreatic Adenocarcinoma	24	II
NCT01693419	Completed (Aug 2011)	S-1 in Combination with Gemcitabine and Erlotinib in Patients with Advanced or Metastatic pancreatic cancer	37	II
NCT02154737	Completed (Jul 2013)	Open-label, Dose Escalation Study of Gemcitabine and Pulse Dose Erlotinib in Second Line Treatment of Advanced Pancreatic Cancer	24	I

Clinical trials Identifier	Recruitment status (start date)	Official title	Participants	Phase
NCT03403049	Completed (Apr 2016)	Clinical Trial of Carbon Ion Radiation Therapy for Locally Advanced, Unresectable Pancreatic Cancer	14	I
NCT03319459	Completed (Jan 2018)	FATE-NK100 as Monotherapy and in Combination With Monoclonal Antibody in Subjects With Advanced Solid Tumors	44	I
NCT03989115	Completed (Jul 2019)	Open-Label, Multicenter Dose-Esc & Dose-Exp Study of Combo RMC4630 & Cobimetinib in Participants w/Relapsed/Refractory Solid Tumors & Ph1b Study of RMC4630 w/Osimertinib in Participants w/EGFR Mutation +,Locally Adv or Meta NSCLC	113	IB/II
NCT05039177	Recruiting (Sep 2021)	Agents Targeting the Mitogen-Activated Protein Kinase Pathway in Patients With Advanced Gastrointestinal Malignancies	200	IB/II
NCT05704985	Recruiting (Apr 2023)	Evaluating Safety and Biomarkers Using DK210 (EGFR) for Inoperable Locally Advanced and/or Metastatic EGFR+ Tumors With Progressive Disease Failing Systemic Therapy	60	I
NCT00878163	Active, not recruiting (Mar 2009)	Combination of Vismodegib GDC-0449 and Erlotinib +/- Gemcitabine	55	I
NCT01013649	Active, not recruiting (Nov 2009)	Evaluating Both Erlotinib (Ph II-R) and Chemoradiation (Ph III) as Adjuvant Treatment for Patients With Resected Head of Pancreas Adenocarcinoma	545	II/III
NCT01660971	Active, not recruiting (Jul 2012)	Gemcitabine, Dasatinib and Erlotinib in Patients With Advanced Pancreatic Carcinoma	19	I
NCT03878524	Active not Recruiting (Apr 2020)	Serial Measurements of Molecular and Architectural Responses to Therapy (SMMART) Trial: PRIME	2	I
NCT05316480	Not yet recruiting (May 2022)	Preliminary Exploration of Therapeutic Efficacy of Nimotuzumab in EGFR Highly Expressed Pancreatic Neuroendocrine Neoplasms	42	II
With HER2 inhibitors in pancreatic cancer				
NCT01728818	Unknown (Apr 2013)	Gemcitabine in Combination with the Oral Irreversible ErbB Inhibitor afatinib Versus Gemcitabine Alone in Patients with Metastatic pancreatic cancer	119	II
NCT02450656	Unknown (Jun 2015)	Phase I/II Study with the Combination of afatinib and Selumetinib in Advanced KRAS Mutant Positive and PIK3CA Wildtype Non-small Cell Lung cancer	320	I/II

Clinical trials Identifier	Recruitment status (start date)	Official title	Participants	Phase
NCT02451553	Completed (Nov 2015)	Multi-center Study of Irreversible EGFR/HER2 Tyrosine Kinase Inhibitor afatinib (BIBW2992) in Combination with Capecitabine for Advanced Solid tumors and Pancretico-Biliary cancers	41	I/IB
NCT02975141	Unknown (Sep 2016)	Gemcitabine and Nab-Paclitaxel Combined with the Oral Irreversible ErbB Family Blocker afatinib in Patients with Metastatic pancreatic cancer	12	I
NCT02999672	Completed (Dec 2016)	Exploratory, Multicenter, Non Randomized, Single Agent Cohort Study to Determine Best Tumor Response With Trastuzumab Emtansine in HER2 Overexpressing Solid Tumors	20	II
NCT03785249	Recruiting (Jan 2019)	Multiple Expansion Cohort Trial of MRTX849 in Patients With Advanced Solid Tumors With KRAS G12C Mutation KRYSTAL-1	822	I/II
NCT03919292	Recruiting (May 2019)	Neratinib and Divalproex Sodium (Valproate) in Advanced Solid Tumors, With an Expansion Cohort in Ras-Mutated Cancers	113	I/II
NCT02465060	Active, not recruiting (Aug 2015)	Molecular Analysis for Therapy Choice (MATCH)	6452	II
NCT04482309	Active, not recruiting (Aug 2020)	Multicenter, Open-label Study to Evaluate the Efficacy and Safety of Trastuzumab Deruxtecan (T-DXd, DS-8201a) for the Treatment of Selected HER2 Expressing Tumors	468	II
With Bispecific antibodies in pancreatic cancer				
NCT01420874	Completed (Aug 2011)	Treatment of Advanced Colorectal or Pancreatic Cancer With Anti-CD3 x Anti-EGFR-Armed Activated T-Cells	15	IB
NCT02620865	Completed (Dec 2015)	treatment of Advanced Pancreatic Cancer With Anti-CD3 x Anti-EGFR-Bispecific Antibody Armed Activated T-Cells (BATs) in Combination With Low Dose IL-2 and GM-CSF	2	IB/II
NCT02912949	Recruiting (Jan 2015)	MCLA-128, a Full Length IgG1 Bispecific Antibody Targeting HER2 and HER3, in Patients With Solid Tumors	250	I/II
NCT03269526	Active not recruiting (Jul 2017)	Treatment of Advanced Pancreatic Cancer With Anti-CD3 x Anti-EGFR-Bispecific Antibody Armed Activated T-Cells (BATs)	22	IB/II
NCT04137536	Active not recruiting (Oct 2019)	Treatment of Advanced Pancreatic Cancer With Anti-CD3 x Anti-EGFR-Bispecific Antibody Armed Activated T-Cells (BATs)	7	IB

Finally, owing to the ongoing advances in drug development, it has been possible to develop various types of drugs targeting one or more members of the HER family (e.g. monoclonal antibodies, antibody-drug conjugates, bispecific antibodies, CAR T cells, and small molecules tyrosine kinase inhibitors). Such advances together with a better understanding of the complex and heterogeneous nature of pancreatic cancer, the discovery of symptoms and biomarkers for the early stage of pancreatic cancer, as well as identification of more specific therapeutic targets should ultimately help to reverse the projection of pancreatic cancer becoming the second leading cause of death from cancer by 2030.

Conclusion

Pancreatic cancer (PC) is one of the most aggressive and heterogeneous types of human cancer. There is an urgent need for the discovery of novel therapeutic targets and development of novel and more effective therapeutic agents. Here, we investigated the relative expression and prognostic significance of all members of the HER-family and CD109 in patients with PC. We found that the co-expression of HER-family members and CD109 occurs in patients with PC and can be associated with poorer overall survival. These results provide the rationale for the study of the therapeutic value of co-targeting of HER-family members and CD109 in patients with PC.

Conflict of Interest Statement:

The authors declare that they have no conflict of interest.

Acknowledgement Statement:

H.M conceived the original research idea and designed the experiments for TK's PhD project. T.K collected patient's tumour blocks, follow-up data, performed the experiment and the data analysis. I.B and T.K carried out the scoring of immunohistochemistry staining. AMS, SA, IB, GD and IB were the other supervisors on the project. T.K wrote the transcript and all authors have read and approved the manuscript.

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Supplementary Materials

Supplementary Table 1 has been included before references.

Table S1: Patient characteristics, the expression level and cellular location of the HER-family members, EGFRvIII, and CD109 in the tissue microarray from pancreatic cancer and normal pancreas tissues (Cat No. PA1002b, Biomax USA).

No.	Age (Sex)	Pathology diagnosis	TNM	Grade	Stage	wt-EGFR	HER2	HER3	HER4	EGFRvIII	EGFR113	CD109
1	41(F)	Ductal adenocarcinoma	T3N1M0	1	IIB	1+ m	1+ c/m	negative	1+ c	negative	negative	1+ c
2	65(M)	Ductal adenocarcinoma	T3N0M0	1	IIA	negative	1+ c/m	negative	negative	negative	negative	1+ c
3	58(F)	Ductal adenocarcinoma	T2N0M0	1	IB	negative	2+ c/m	negative	1+ n	negative	negative	1+ c
4	72(F)	Ductal adenocarcinoma	T3N0M0	1	IIA	negative	2+ c/1+ m	negative	negative	negative	negative	2+ c/m
5	60(M)	Ductal adenocarcinoma	T3N0M0	1	IIA	negative	1+ c/m	negative	negative	1+ c	negative	2+ c/m
6	52(M)	Ductal adenocarcinoma	T3N1M0	1	IIB	1+ c/m	2+ c/ 1+ m	negative	negative	negative	negative	1+ c
7	65(M)	Duct adenocarcinoma with cataplasia	T3N1M0	2	IIB	negative	negative	negative	negative	negative	negative	negative
8	41(M)	Ductal adenocarcinoma	T2N0M0	2	IB	1+ c/m	negative	negative	1+ n	negative	negative	negative
9	44(M)	Ductal adenocarcinoma	T3N0M0	2	IIA	negative	1+ c/m	negative	negative	negative	negative	1+ c/m
10	55(F)	Ductal adenocarcinoma	T2N0M0	1	IB	1+ c/m	1+ c/m	negative	2+ c	negative	1+ c	1+ c
11	55(M)	Ductal adenocarcinoma	T3N0M0	1	IIA	negative	2+ c/ 1+ m	negative	1+ c	negative	negative	1+ c
12	55(M)	Ductal adenocarcinoma	T2N0M0	2	IB	negative	1+ c/m	negative	negative	negative	negative	1+ c/m
13	34(M)	Ductal adenocarcinoma	T3N0M0	2	IIA	negative	negative	negative	negative	negative	negative	negative
14	42(M)	Ductal adenocarcinoma	T3N0M0	1	IIA	negative	2+ c	negative	negative	negative	negative	2+ c
15	44(M)	Ductal adenocarcinoma	T3N0M0	3	IIA	1+ m	1+ c	negative	1+ n	negative	1+ c/m	1+ c
16	59(M)	Ductal adenocarcinoma	T3N0M0	2	IIA	1+ m	negative	negative	negative	negative	negative	negative
17	53(M)	Ductal adenocarcinoma	T3N0M0	3	IIA	1+ c/m	1+ c	negative	1+ n	negative	negative	2+ c
18	52(M)	Ductal adenocarcinoma	T3N0M0	2	IIA	1+ c	1+ c/m	negative	negative	negative	negative	2+ c/m
19	48(F)	Ductal adenocarcinoma	T3N0M0	2	IIA	negative	1+ c/m	negative	1+ n	negative	negative	1+ c/m

No.	Age (Sex)	Pathology diagnosis	TNM	Grade	Stage	wt-EGFR	HER2	HER3	HER4	EGFRvIII	EGFR113	CD109
20	68(F)	Ductal adenocarcinoma	T3N0M0	3	IIA	1+ c/ 2+ m	negative	negative	1+ n	negative	1+ c/m	1+ c/m
21	41(M)	Ductal adenocarcinoma	T4N1M0	3	III	negative	2+ c	negative	1+ n	negative	negative	1+ c
22	64(M)	Ductal adenocarcinoma	T3N0M0	3	IIA	negative	negative	negative	negative	negative	negative	1+ c
23	58(F)	Ductal adenocarcinoma	T3N0M0	3	IIA	1+ c/m	1+ c	negative	1+ n	negative	1+ m	2+ c/m
24	60(F)	Ductal adenocarcinoma	T4N0M0	3	III	1+ c/m	1+ c/m	negative	negative	negative	negative	negative
25	72(F)	Ductal adenocarcinoma	T3N0M0	3	IIA	1+ c/m	2+ c	negative	1+ n	negative	negative	1+ c/m
26	56(M)	Ductal adenocarcinoma	T2N1M0	3	IIB	2+ c/m	negative	negative	negative	1+ c	2+ m	negative
27	63(M)	Ductal adenocarcinoma	T2N0M0	3	IB	negative	2+ c/ 1+ m	negative	1+ c/n	negative	negative	negative
28	62(M)	Ductal adenocarcinoma	T2N0M0	3	IB	1+ c	2+ c/ 1+ m	negative	1+ c/n	negative	negative	1+ c/m
29	59(M)	Ductal adenocarcinoma	T2N0M0	3	IB	1+ m	2+ c/ 1+ m	negative	1+ c/n	negative	negative	negative
30	60(F)	Ductal adenocarcinoma	T2N1M1	3	IV	1+ c/m	1+ c/m	negative	1+ n	negative	1+ m	negative
31	53(F)	Ductal adenocarcinoma	T3N0M0	3	IIA	1+ c/m	negative	negative	negative	negative	1+ m	1+ c/m
32	50(M)	Ductal adenocarcinoma	T3N1M0	3	IIB	1+ c/ 2+ m	negative	negative	negative	negative	2+ m	negative
33	51(M)	Ductal adenocarcinoma	T3N0M0	3	IIA	1+ c/m	1+ c	negative	1+ c/n	negative	1+ c/m	1+ c/m
34	62(F)	Ductal adenocarcinoma	T3N0M0	3	IIA	1+ c/m	1+ c/m	negative	1+ c/n	negative	1+ m	1+ c
35	78(M)	Ductal adenocarcinoma	T2N0M0	3	IB	1+ c/m	negative	negative	1+ n	negative	1+ c/m	1+ c/m
36	39(F)	Ductal adenocarcinoma	T3N0M0	3	IIA	1+ c/m	1+ c	negative	negative	negative	1+ c/m	1+ c
37	55(M)	Ductal adenocarcinoma	T2N0M0	3	IB	1+ c	1+ c	negative	negative	negative	negative	negative
38	76(F)	Ductal adenocarcinoma with necrosis	T4N0M0	3	III	1+ c	2+ c	negative	1+ n	negative	negative	negative
39	76(M)	Ductal adenocarcinoma	T3N0M0	3	IIA	1+ c/m	1+ c/m	negative	1+ n	negative	1+ c/m	negative
40	57(F)	Ductal adenocarcinoma	T2N0M0	3	IB	negative	2+ c	negative	1+ n	negative	negative	negative

No.	Age (Sex)	Pathology diagnosis	TNM	Grade	Stage	wt-EGFR	HER2	HER3	HER4	EGFRvIII	EGFR113	CD109
41	40(M)	Normal pancreas tissue	-	-	-	negative	negative	negative	negative	negative	negative	negative
42	47(M)	Normal pancreas tissue	-	-	-	negative	negative	negative	negative	negative	negative	negative
43	25(M)	Normal pancreas tissue	-	-	-	negative	negative	negative	negative	negative	negative	negative
44	30(M)	Normal pancreas tissue	-	-	-	negative	negative	negative	negative	negative	negative	negative
45	30(M)	Normal pancreas tissue	-	-	-	negative	negative	negative	negative	negative	negative	negative
46	50(M)	Normal pancreas tissue	-	-	-	negative	negative	negative	negative	negative	negative	negative
47	35(M)	Normal pancreas tissue	-	-	-	negative	negative	negative	negative	negative	negative	negative
48	38(M)	Normal pancreas tissue	-	-	-	negative	negative	negative	negative	negative	negative	negative
49	35(F)	Normal pancreas tissue	-	-	-	negative	negative	negative	negative	negative	negative	negative
50	21(F)	Normal pancreas tissue	-	-	-	negative	negative	negative	negative	negative	negative	negative

C=cytoplasm, m=membranous, n=nuclear, 1+= weak intensity, 2+ = intermediate intensity, 3+= strong intensity

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