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Expression pattern and targeting of HER family members and IGF-IR in pancreatic cancer

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1. ABSTRACT

Pancreatic cancer is still one of the most aggressive and fatal types of human cancer . Survival rates for patients with pancreatic cancer are extremely poor and one major contributing factor is the lack of specific marker(s) for the early detection of pancreatic cancer. Indeed, the great majority of pancreatic cancer cases are diagnosed at an advanced stage of the disease and these patients often have a poor response to treatment with conventional forms of therapy. In this article, we conduct a comprehensive review of the literature on the expression pattern, prognostic significance and predictive value of EGFR family members, IGF-IR and their ligands in pancreatic cancer. We also discuss recent advances in pancreatic cancer treatments and highlight the remaining challenges as well as future opportunities for more effective targeting of such receptors using a combination of growth factor receptor specific monoclonal antibodies, small molecule tyrosine kinase inhibitors and other therapeutic strategies. Such strategies could ultimately help to overcome the development of drug resistance and improve the overall survival rates for patients with pancreatic cancer.

2. INTRODUCTION

Pancreatic cancer is one of the deadliest human cancers and a major health problem worldwide. There were an estimated 277.000 new cases of pancreatic cancer and 266.000 deaths from this cancer globally in 2008 (1). Despite the introduction of new therapies in the last two decades, the collective median survival rate for patients with pancreatic cancer remains poor and is less than 6 months (2, 3). Several factors have been associated with an increased risk of developing pancreatic cancer including age (above the age of 50), male sex, smoking, presence of medical conditions like diabetes and chronic pancreatitis, family history, occupational exposure to carcinogens, race (higher incidence in black population) and high caloric consumption/obesity (4).

There are numerous histological sub-types of pancreatic cancer but the most common (90%) and aggressive type is pancreatic ductal adenocarcinoma. The majority of pancreatic lesions (60-70%) arise in the head, neck or the uncinate process of the pancreas and the remaining 5-10% and 10-15% of all cases occur in the body and tail respectively (5). The high mortality rate that accompanies this type of cancer is mainly due to the presence of metastases in the majority of patients at the time of diagnosis. Since symptoms of the disease don't usually arise until the cancer is at an advanced stage, most of the cases (85-90%) are considered to be unresectable and therefore the only therapeutic option available is palliative treatment (3). Indeed, of all pancreatic cancer cases, only 7.5% of the cases are classified as localized lesions while 29.3% are locally advanced and 47.2% are metastatic. The 5-year survival rate for patients with localized lesions is 15.2% while for locally advanced and metastatic disease is 6.3% and 1.6% respectively (6). In addition to palliative surgery, chemotherapy is also used to alleviate symptoms and, in some patients, it can also prolong survival (7). Until 1997, 5-Fluoracil (5-FU) had been the only drug with clinical benefit in pancreatic cancer. The introduction of gemcitabine prolonged median survival by approximately one month (5.65 vs 4.41 months, P=0.0025) and improved disease symptoms with a clinical benefit response rate of 23.8% compared to 4.8% of 5-FU (P=0.0022) (8). As a result, gemcitabine was approved by the United States Food and Drug Administration (FDA) for the treatment of pancreatic cancer in May 1996 and since then, despite numerous combinations of gemcitabine with other agents in clinical trials, only erlotinib has been added to the weaponry against advanced pancreatic cancer (9, 10). Erlotinib (Tarceva), an epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI), was the only agent which led to a modest, but statistically significant, improvement in median survival when used in combination with gemcitabine compared to gemcitabine monotherapy (10, 11).

The inefficiency of cytotoxic agents in the treatment of pancreatic cancer is primarily due to the intrinsic drug resistance that characterizes pancreatic cancer (12). The lethal nature of the disease, combined with the minimal improvement in prognosis and treatment during the last decades, dictates the importance of the development of new therapeutic agents, as well as the identification of more reliable tumour biomarkers for the early diagnosis of pancreatic cancer, the categorization of different prognostic groups and for predicting the response to therapy (13-15). The aim of this article is to review the role of the type-I growth factor receptor (also called HER, ErbB) family and insulin-like growth factor receptor I (IGF-IR) in the progression of pancreatic cancer and their importance as prognostic and predictive biomarkers as well as therapeutic targets in pancreatic cancer.

3. HER/ERBB FAMILY MEMBERS AND THEIR LIGANDS IN PANCREATIC CANCER.

3.1. Structure and function of HER/ErbB family members and their ligands

Growth factors are involved in the regulation of a number of cellular processes including cell growth, survival, differentiation and apoptosis (16). In contrast to hormones, growth factors are secreted by almost all tissues and mediate their effects by binding to their cell surface receptors via paracrine, autocrine and/or juxtacrine processes (17, 18). Activation of the cellular receptors by their respective ligands results in the generation of signals which are carried out by several intracellular signalling proteins, ultimately leading to the different biological responses by the activation and/or repression of specific sets of genes (18). Since the early 1990s, aberrant expression and activation of growth factor receptor signalling pathways has been reported in a wide range of human cancers and in some cases has also been associated with a poorer prognosis (17, 19). Such advances have resulted in the identification of novel therapeutic targets, shifting the interest of cancer researchers from traditional chemotherapy to targeted (14, 20).

At present, there are 58 known human receptor tyrosine kinases (RTKs) which have been divided into 20 subfamilies (18). One of the best characterised RTK subfamily is the ErbB family which consists of four members namely; EGFR (also known as ErbB1/HER-1), ErbB2 (neu/HER-2), ErbB3 (HER-3) and ErbB4 (HER-4) (21-23). All family members share a similar structure comprising of a ligand-binding extracellular domain, a transmembrane domain and a large cytoplasmic region with tyrosine kinase activity. Interestingly, there are no known ligands for HER-2, while HER-3 has been characterized as a tyrosine kinase-inactive receptor (21, 22, 24). However, recent evidence suggests that HER-3 is actually able to catalyse phosphorylation but with a much weaker efficacy compared to the other HER family members (25, 26). To date, 11 different growth factors have been identified which have the ability to bind to the extracellular domain of the ErbB family members. These growth factors are categorized into three different groups based on their receptor specificity. The first group of ligands which bind only to EGFR, consists of epidermal growth factor (EGF), transforming growth factor-α (TGF-α), amphiregulin (AR) and epigen (EPG); the second group of ligands, which binds to both EGFR and ErbB-4, includes epiregulin (EPR), betacellulin (BTC) and heparin-binding EGF (HB-EGF). Neuregulins (NRGs), the third group, is divided into two sub-groups; NRG-1 and NRG-2 which bind to both ErbB-3 and ErbB-4 while NRG-3 and NRG-4 bind only to ErbB-4 (27-29). The binding of a ligand to its respective receptor leads to conformational changes of the extracellular domain which allow the formation of homo- and/or heterodimers (30). The formation of dimers leads to the activation of the cytoplasmic kinase domains which in turn results in the auto- and transphosphorylation of specific tyrosine residues found on the C-terminal region of each receptor. These phosphorylation events allow the recruitment of several intracellular proteins which contain a Src homology 2 (SH2) or phosphotyrosine binding (PTB) domains like adaptor proteins Shc and Grb2, kinases like Src and phosphatidylinositol 3 kinase protein (PI3K) and phosphatases SHP1 and SHP2 (31). Depending on the activating ligand, different tyrosine residues on the receptor are phosphorylated (32). Both enzymes and adaptor proteins use specific phosphorylated tyrosine residues as

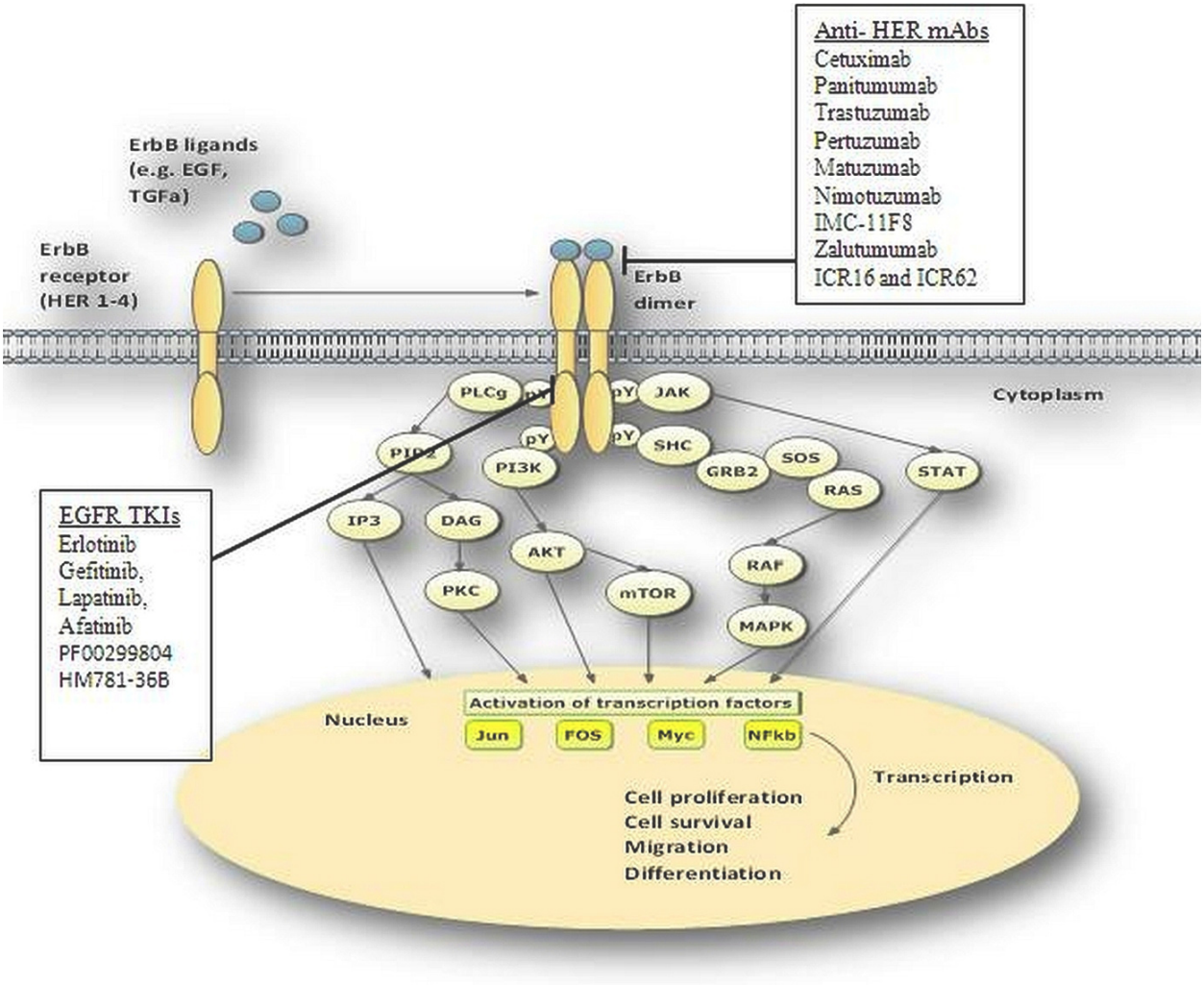

Figure 1. ErbB proteins and their downstream pathways. Ligand binding to the receptors leads to the formation of homo- or heterodimers which in turn results in the activation of tyrosine kinase activity and phosphorylation of tyrosine residues located in the C-terminal domain of the receptors. Signal transduction pathways involved in ErbB signalling include Ras-Raf-MAPK, PI3K-Akt, PLC-PKC and JAK-STAT pathways. The biological consequences of ErbB signalling activation include cell proliferation, survival, angiogenesis migration and metastasis. These can be blocked by anti-HER monoclonal antibodies (mAbs) and small molecule HER tyrosine kinase inhibitors (TKIs).

docking sites in order to bind to the activated ErbB receptors. For example, EGFR has 12 major distinct tyrosine residues which are involved in the receptor's activation and downstream signalling, including Y992, Y1045, Y1068, Y1086, Y1148 and Y1173 among others, with Grb2 adaptor protein being recruited to EGFR via Y1068 and Y1086, Shc protein via Y1148 and Y1173 and phospholipase C-γ (PLC- γ) via Y1173 and Y992 phosphorylated tyrosine residues respectively (32-34). These proteins mediate the activation of downstream signalling pathways including the Ras-Raf-mitogen activated protein kinase (MAPK), (PI3K)/Akt pathway, PLC- γ-protein kinase C (PKC) and signal transducers and activators of transcription (STAT) pathway (Figure 1; (33). The activation of the ErbB family of receptors can result in cell proliferation, differentiation, survival, angiogenesis, migration and invasion (27).

Since the early 1980s, aberrant expression and activation of the ErbB family of receptors has been reported in a wide range of human cancers and has been associated with malignant transformation (35-40). The aberrant activation of HER family members may occur in a number of ways, including receptor over-expression, receptor mutations, persistent activation by autocrine and paracrine ligands, heterodimerisation with heterologus receptors, the deficiency of specific phosphatases which are responsible for the deactivation of the phosphorylated/activated receptor and the abnormal regulation of the endocytosis of the receptors (36, 41-47).

In addition to these well-characterized traditional pathways, several studies have indicated that HER family members signalling may also be mediated by a different mechanism in which the full-length activated EGFR can be translocated to the nucleus and induce transcription of

cyclin-D1, B-Myb, COX-2 and iNOS genes by interacting with transcription factors STAT3 and E2F1 (48-50). The EGFR has been detected in the nucleus of highly proliferating tissues including breast, bladder, thyroid and pancreatic cancer and its expression has been associated with a poorer overall survival in cancer patients (51-55). The existence of the nuclear EGFR network signifies the complexity of HER signalling and the importance of clarifying the role of nuclear EGFR in tumour progression and response to therapeutics (50, 56).

3.2. Expression pattern and prognostic significance of EGFR and its ligands in pancreatic cancer

EGFR has been found to be overexpressed in several types of cancer including head and neck, breast, prostate, ovarian, oesophageal and bladder cancer among others (19, 57-61) also see Eccles *et al.* in this issue). In addition, EGFR expression was found to be a strong prognostic indicator in a small number of malignancies such as head and neck, cervical, ovarian, oesophageal and bladder cancers (19, 58).

The expression of EGFR reported for pancreatic cancer exhibits wide variation, ranging from 0 to 100% of the cases examined (Table 1). Of twenty-two studies to date, only two have reported a significant association between EGFR expression and a shorter survival. In the first study of 76 patients with pancreatic cancer, Ueda and colleagues found that cytoplasmic expression of EGFR was correlated with a shorter survival ($p=0.02$) (62). Similarly, in a more recent study, Valsecchi and colleagues investigated the expression of EGFR in 105 pancreatic cancer patients and found that high levels of expression of both cytoplasmic ($p<0.01$) and membranous ($p=0.027$) EGFR was correlated with shorter overall survival (63). Despite the small number of studies linking EGFR expression with survival in pancreatic cancer, several studies have supported the involvement of EGFR signalling pathway in tumour progression and showed a correlation between its expression and an aggressive phenotype (62-69) Table 1).

Of all the EGFR ligands, the most extensively studied and well characterized in terms of their malignant transforming ability and prognostic significance are TGFa and EGF. Both growth factors have been found to be produced at high levels in several human malignancies including pancreatic cancer (70-72). As shown in Table 1, EGF has been shown to be expressed in higher levels in metastatic lesions than non-metastatic tumours and has been correlated with the presence of local invasion and increased tumour size and stage (66, 70, 73). Similarly TGFa has been found to be over-expressed in 54% of pancreatic cancer patients and increased expression was correlated with decreased post operative survival and advanced tumour stage (70). In contrast, some studies found no correlation between EGF expression and tumour stage (74, 75). In addition to EGF and TGFa, HB-EGF expression has also been found to be higher in pancreatic cancer tissues when compared to the expression levels in normal pancreas (Table 1). However, in one study elevated levels of HB-EGF were associated with earlier tumour stage and absence of metastases (76). The expression levels of betacellulin, epiregulin and amphiregulin have also been found to be high in pancreatic cancer , but only amphiregulin and epiregulin expression were found to correlate with a more aggressive phenotype (77-79)(Table 1).

Several studies have reported an association between the coexpression of the EGFR and its ligands and a shorter overall survival (66, 70, 73, 74). For example, in 1993, Yamanaka and colleagues investigated the expression levels of EGF, EGFR and TGFa in 87 pancreatic cancers and found no correlation between EGFR overexpression (43%) and any biological parameter such as tumour stage, grade and metastasis or survival. However, coexpression of EGFR with at least one of its ligands (EGF or TGFa) was found to be associated with a more aggressive phenotype and shorter survival ($P<0.05$) (70). In another study, Dong and colleagues examined 57 patients with pancreatic cancer and found that while 68% of the cases were EGFR positive, only coexpression of the EGFR with EGF was correlated with a shorter survival (74).

In spite of the considerable number of studies undertaken to date, the prognostic significance of EGFR and its ligands in pancreatic cancer is still unclear due to several factors. The vast majority of studies determined EGFR expression using immunohistochemistry (IHC), but even within this group of studies there was a broad variation in the reported EGFR expression levels (Table 1). A major contributing factor for these inconsistencies is the lack of a standard, universally used scoring system, the usage of different antibodies for the immunohistochemical detection of EGFR in tumour samples and differences in the definition of EGFR positive tumours in IHC. Another contributing factor is the small number of tumour specimens ($n<50$) examined in many studies (Tables 1-3). These limitations are not restricted to the assessment of EGFR but are also implicated in the evaluation of the other members of the HER family. To our knowledge, there is currently no comprehensive study of the expression pattern and prognostic significance of the EGFR and all its ligands in patients with pancreatic cancer. Such studies together with the establishment of a uniform way of determining the EGFR expression level and its cellular location are of great importance and should help to unravel the biological and clinical significance the EGFR system in pancreatic cancer as well as its more effective utilization as a therapeutic target.

3.3. Expression pattern and prognostic significance of HER-2 in pancreatic cancer

Aberrant expression and activation of HER-2 has been reported in several human malignancies and in many cases was associated with a poor prognosis (80, 81). In pancreatic cancer the expression of HER-2 reported in the literature ranges from 0-66.6% of the cases examined (Table 2). The vast majority of the studies conducted so far found no statistically significant correlation between HER-2 expression at a genetic, transcriptional or translational

Table 1. Studies investigating the expression levels and prognostic significance of EGFR and its ligands in pancreatic cancer

Study	Number of specimens	Marker assessed	Method of assessment	Percentage of specimens positive or with high expression	Other findings	Conclusions
Korc *et al.*, 1992 (204)	20 normal 22 pancreatic cancer	EGFR EGF TGF-a	IHC Northern blot analysis *In situ* hybridization	100% (all techniques) of cancer samples compared to normal tissues exhibited higher expression		Expression of EGFR and its ligands EGF and TGF-a may be involved in the carcinogenesis of pancreatic tumours.
Yamanaka , 1992 (66)	25	EGF EGFR	IHC	75% 31%		High expression of EGFR linked with local invasion, co-expression with ligand associated with shorter survival.
Yamanaka *et al.*, 1993 (70)	87	EGF TGFα EGFR	IHC, *in situ* hybridization	46% IHC 54% IHC 43% IHC	mRNA levels of proteins were elevated in carcinomas	Co-expression of the receptors with at least one of its ligands was linked with increased tumour size, advanced clinical stage and shorter survival.
Ebert *et al.*, 1994 (79)	48	Amphiregulin	IHC	-	In normal tissues localized in the nucleus while in cancerous tissues was present in cytoplasm as well.	AR is present in both normal and cancer tissues. Cytoplasmic AR was linked with advanced clinical stage.
Yokoyama *et al.*, (78)	15 normal 10 cancer samples	Betacellulin	Northern blot analysis	7.5- fold increase of Betacellulin mRNA compared to normal tissues		Betacellulin is overexpressed in pancreatic cancer
Study	Number of specimens	Marker assessed	Method of assessment	Percentage of specimens positive or with high expression	Other findings	Conclusions
Uegaki *et al.*, 1997 (73)	60 primary and 26 metastatic lesions	EGF EGFR	IHC	28% in primary and 46% in metastatic lesions 43% in primary and 46% in metastatic lesions		Coexpression of EGF and EGFR correlated with shorter survival
Gansauge *et al.*, 1998 (75)	82	EGF EGFR	IHC	46% 54%		No correlation with tumour stage or survival
Dong *et al.*, 1998 (74)	57	EGF EGFR	IHC	73.7% 68.4%		Coexpression of EGF and EGFR was associated with shorter survival
Kuniyasu *et al.*, 1999 (205)	22	EGFR	*In situ* hybridization	0%		No overexpression of EGFR mRNA
Zhu *et al.*, 2000 (77)	30	Epiregulin	Northern blot analysis	2.1-fold increase of epiregulin mRNA levels compared to normal tissues		Epiregulin is overexpressed in pancreatic cancer. No correlation with tumour size and stage.
Ito *et al.*, 2001 (76)	40	HB-EGF	IHC	55%		HB-EGF expression is correlated with well differentiated tumours without the presence of metastasis.
Thybusch-Bernhardt *et al.*, 2001 (65)	24	EGFR	IHC	33%		EGFR overexpression was correlated with a more aggressive phenotype.
Zhang *et al.*, 2002 (94)	36	EGFR	IHC	50%		Co-expression of EGFR and HER-2 was correlated with the histopathological grades and clinical stages of tumours.
Tobita *et al.*, 2003 (64)	77	EGFR	IHC	41.6%		EGFR expression correlated with tumour stage, grade and presence of metastasis.
Ueda *et al.*, 2004 (62)	76	EGFR	IHC	Membranous: 54% in intraductal and 14% in invasive components Cytoplasmic: 25% in intraductal and 62% in invasive components		Cytoplasmic EGFR was overexpressed in advanced tumours and correlated with shorter survival.
Prenzel *et al.*, 2006 (206)	31	EGFR	qPCR	68% lower expression in cancer than normal pancreas		No correlation with tumour stage and survival.
Bloomston *et al.*, 2006 (207)	71	EGFR	IHC	69%		No correlation with tumour stage and grade or survival.
Dancer *et al.*, 2007 (90)	32	EGFR	IHC, FISH	65% IHC 0% FISH		No correlation with tumour stage and grade or survival.

Tzeng *et al.*, 2007 (208)	24	EGFR	qPCR, FISH	77% qPCR 42% FISH		No correlation with tumour stage and grade or survival.
Zhang *et al.*, 2002 (94)	36	EGFR	IHC	50%		Co-expression of EGFR and HER-2 was correlated with the histopathological grades and clinical stages of tumours.
Tobita *et al.*, 2003 (64)	77	EGFR	IHC	41.6%		EGFR expression correlated with tumour stage, grade and presence of metastasis.
Ueda *et al.*, 2004 (62)	76	EGFR	IHC	Membranous: 54% in intraductal and 14% in invasive components Cytoplasmic: 25% in intraductal and 62% in invasive components		Cytoplasmic EGFR was overexpressed in advanced tumours and correlated with shorter survival.
Prenzel *et al.*, 2006 (206)	31	EGFR	qPCR	68% lower expression in cancer than normal pancreas		No correlation with tumour stage and survival.
Bloomston *et al.*, 2006 (207)	71	EGFR	IHC	69%		No correlation with tumour stage and grade or survival.
Dancer *et al.*, 2007 (90)	32	EGFR	IHC, FISH	65% IHC 0% FISH		No correlation with tumour stage and grade or survival.
Tzeng *et al.*, 2007 (208)	24	EGFR	qPCR, FISH	77% qPCR 42% FISH		No correlation with tumour stage and grade or survival.
Pryczynicz *et al.*, 2008 (67)	36	EGFR EGF	IHC	36% 50%	EGF expression was correlated with the presence of EGFR in tumours	Expression of EGF and EGFR was associated with the presence of metastasis.
te Velde *et al.*, 2009 (101)	45 (13 papilla of Vater)	EGFR	CISH, IHC	0%		No over-expression of EGFR in pancreatic cancer..
Pryczynicz *et al.*, 2009 (69)	29	EGF EGFR	IHC	N/A		No correlation with tumour stage. EGF and EGFR expression was correlated with lymph node involvement and presence of metastases.
Takikita *et al.*, 2009 (209)	154	EGFR	IHC	26%		No correlation with survival.
Bergmann *et al.*, 2010 (210)	15	EGFR	IHC	46%		No correlation with surviva.l
Mahipal *et al.*, 2011 (68)	90	EGFR	IHC	Membranous 57% Cytoplasmic 68%	Significant correlation between membranous EGFR over-expression and lymph node positivity	No association between cytoplasmic or membranous EGFR and survival. There was a trend of positive correlation of membranous EGFR and decreased survival (P=0.08).
Valsecchi *et al.* 2011 (63)	105	EGFR	IHC	32%		High EGFR expression correlated with shorter survival ($p<0.001$)

Abbreviations: IHC Immunohistochemistry, qPCR quantitative Polymerase Chain Reaction, CISH Chromogenic *in situ* hybridization, FISH fluorescence *in situ* hybridization.

level and overall survival or tumour grade and stage (75, 82-91). However, in some studies, increased expression of HER-2 was associated with shorter survival, advanced tumour stage and/or the presence of local invasion and metastasis (65, 92, 93). For example, in a study 129 patients with pancreatic cancer, Komoto and colleagues found that HER-2 overexpression determined by IHC correlated with significantly lower median survival compared with patients with normal levels of HER-2 (i.e. 14.7 vs 20.7 months, $p<0.01$) (93). Interestingly, in some studies HER-2 overexpression was found to be more common in well-differentiated than moderately and poorly differentiated tumours indicating a role of HER-2 in the early stages of carcinogenesis (82, 83). In another study, Zhang *et al.*, examined the expression levels of EGFR and HER-2 in 36 pancreatic cancers by IHC and found that only increased coexpression of both receptors was correlated with advanced tumour stage and histological grade (94). These findings indicate the important role of the interactions between different members of the HER family of receptors in carcinogenesis and tumour progression. In addition to membranous and cytoplasmic HER-2 examination in tumours, Okada and colleagues investigated the level of HER-2 extracellular domain in the serum of 100 pancreatic cancer patients. They found an association between elevated levels of HER-2 in the serum and a shorter survival and the presence of metastases (95).

Table 2. Studies investigating the expression levels and prognostic significance of HER-2 in pancreatic cancer

Study	Number of specimens	Marker assessed	Method of assessment	Percentage of specimens positive or with high expression	Other findings	Conclusions
Yamanaka , 1992 (66)	25	HER-2	IHC	28%		High expression linked with local invasion and shorter survival.
Yamanaka *et al.*, 1993 (82)	76	HER-2	IHC, Northern blot analysis	45% 34/76 IHC 52% 13/25 NB	HER-2 expression correlated with well differentiated tumours	HER-2 is frequently over-expressed in pancreatic cancer.
Okada *et al.*, 1995 (95)	100	Serum and tissue HER-2	IHC	34% in serum 28% in tissue		High levels of serum or tissue HER-2 are associated with presence of metastasis and shorter survival.
Lei *et al.*, 1995 (92)	21	HER-2	IHC	47.6%		High expression correlated with shorter survival.
Dugan *et al.*, 1997 (83)	79	HER-2	IHC	58%	Expression levels of HER-2 were higher in well differentiated tumours compared with moderately and poorly differentiated lesions.	No correlation with survival
Gansauge *et al.*, 1998 (75)	82	HER-2	IHC	56%		No correlation with tumour stage or survival
Kawesha *et al.*, 2000 (84)	157	HER-2	IHC	32.6%		No correlation with tumour stage or survival
Novotny *et al.*, 2001 (85)	51	HER-2	IHC	19.6%		No correlation with tumour stage or survival
Safran *et al.*, 2001 (86)	154	HER-2	IHC	21%		No correlation with tumour stage or survival
Thybusch-Bernhardt *et al.*, 2001 (65)	24	HER-2	FACS analysis	25%		HER-2 over-expression is correlated with a more aggressive phenotype.
Koka *et al.*, 2002 (87)	308	HER-2	IHC	5.2%		No correlation with tumour stage or survival.
Zhang *et al.*, 2002 (94)	36	HER-2	IHC	41.7%		Co-expression of EGFR and HER-2 is correlated with the histopathological grades and clinical stages of tumours.
Tamiolakis *et al.*, 2004 (88)	100	HER-2	IHC	21%		No correlation with tumour stage or survival.
Ueda *et al.*, 2004 (62)	76	HER-2	IHC	Membranous: 20% in intraductal and 3% in invasive components Cytoplasmic: 16% in intraductal and 11% in invasive components		No correlation with tumour stage or survival.
Stoecklein *et al.*, 2004 (89)	50	HER-2	CISH, IHC	24% CISH 10% IHC	Tumour ploidy levels correlated with prognosis	No correlation with tumour stage and survival.
Saxby *et al.*, 2005 (211)	30	HER-2	IHC, qPCR, FISH	17% IHC 23% qPCR 3% FISH		Expression of HER-2 mRNA and protein associated with tumour stage. Over-expression of HER-2 at any level (genetic, transcriptional or translational) was linked with poor survival (P<0.01 IHC, P=0.05 qPCR, P=0.02 FISH).
Tsiambas *et al.*, 2006 (212)	50	HER-2	IHC, CISH	20% IHC 16% CISH	HER-2 gene amplification was not correlated with any biological parameter	HER-2 protein over expression correlated with tumour grade.

Dancer *et al.*, 2007 (90)	32	HER-2	IHC, FISH	17% IHC 11% FISH		No correlation with tumour stage and grade or survival.
Pryczynicz *et al.*, 2008 (67)	36	HER-2	IHC	66.6%		No correlation with tumour stage and grade or survival
Sharif *et al.*, 2008 (91)	63	HER-2	FISH	25%		No correlation with tumour stage and grade or survival.
Komoto *et al.*, 2009 (93)	129	HER-2	IHC	61.2%		Over-expression correlated with shorter survival.
te Velde *et al.*, 2009 (101)	45 (13 papilla of Vater)	HER-2	CISH, IHC	0%IHC 0% CISH		No over-expression of HER-2 in pancreatic cancer.
Pryczynicz *et al.*, 2009 (69)	29	HER-2	IHC	N/A		No correlation with tumour stage.
Takikita *et al.*, 2009 (209)	154	HER-2	IHC	62%		No correlation with survival.

Abbreviations: IHC Immunohistochemistry, qPCR quantitative Polymerase Chain Reaction, CISH Chromogenic *in situ* hybridization, FISH fluorescence *in situ* hybridization.

3.4. Expression pattern and prognostic significance of HER-3 and HER-4 in pancreatic cancer

In contrast to the extensively investigated EGFR and HER-2, there is a limited number of studies on the expression levels and prognostic significance of HER-3 and HER-4 in pancreatic cancer. HER-3 overexpression reported in the literature ranges between 0-56% of pancreatic cancer cases examined (Table 3).

Interestingly, two studies showed a correlation between HER-3 over-expression and malignant characteristics and/or poor prognosis (Table 3). Friess *et al.* in 1995 investigated the expression levels of HER-3 in 58 pancreatic cancer patients and found that 47% (27/58) of specimens overexpressed HER-3 concluding that HER-3 expression at a protein level is correlated with shorter survival and advanced tumour stage (96). Similarly in a recent study, Hirakawa and colleagues determined the expression level of HER-3 in 126 pancreatic cancer patients using IHC. They found HER-2 expression in 41.3% of the cases examined and this in turn was associated with shorter survival rate (p=0.008) (97) However, other studies did not find a similar relationship between HER-3 expression and tumour stage, aggressive phenotype or survival (65, 84).

HER-4 is normally expressed in skeletal muscle, brain, heart and the pituitary gland and the pancreas (98). In a few studies, the assessment of the level of HER-4 expression in pancreatic cancer at mRNA level by PCR and at a protein level by IHC led to no clear association with tumour malignancy and survival. Graber *et al.* in 1999 showed that HER-4 protein is equally expressed in normal and cancer tissue while only high levels of HER-4 mRNA were associated with increased malignancy (presence of metastasis) (99). The expression levels of HER-3 and HER-4 ligands (Neuregulins) have also been assessed at mRNA level in a study undertaken by Kolb *et al.* Neuregulins were elevated in cancer tissues when compared to normal tissues however, only NRG 2 over-expression was associated with decreased survival (100).

To date, only two studies examined the expression of all HER family members in pancreatic cancer with contradictory results. In the first study, te Velde and colleagues compared the expression levels of all HER family members in normal pancreatic tissue and resected pancreatic cancer specimens and did not find overexpression of the HER family members in any of the 45 pancreatic cancer specimens examined (101). In contrast, Thybusch-Bernhardt *et al.*, showed that the expression of both EGFR and HER-2 was correlated with a more aggressive phenotype, while HER-4 expression was linked to a more favourable tumour stage (65), Table 4).

4. HER FAMILY OF RECEPTORS AS THERAPEUTIC TARGETS IN PANCREATIC CANCER

Since the discovery of the HER family members in the early 1980s and their association with human malignancy, they have emerged as an attractive target for cancer therapy. To date, several approaches have been developed for the targeting of the HER family members. These include: small interference RNA, antisense oligonucleotides, gene therapy, small molecule inhibitors and monoclonal antibodies (mAbs) (19, 45, 102). Of these approaches, only mAbs and low molecular weight TKIs have been used clinically for the treatment of human cancer and will be discussed in the following sections. MAbs bind to the extracellular domain of the receptor and by blocking the binding of the ligands they prevent the activation of the receptor and downstream cell signalling pathways. In contrast, TKIs target the cytoplasmic tyrosine kinase domain of the receptor and by competing with adenosine-5'-triphosphate (ATP) for the ATP docking site of the receptor they block the activation of the downstream signalling pathways (103). TKIs can be specific for one member of the EGFR family (e.g. the EGFR TKIs gefitinb and erlotinb and gefitinib) or more members of HER family (e.g. dual EGFR and HER-2 TKIs lapatinib or pan HER inhibitor afatinib). In addition, their binding to the ATP docking site of the receptor may be reversible (e.g. erlotinib) or irreversible (e.g. afatinib) (104).

4.1. EGFR targeting

4..1.1. Anti-EGFR mAbs

To date, the anti-cancer efficacy of several anti-EGFR mAbs has been investigated in numerous studies and for several types of human malignancies. Some of these mAbs (e.g. cetuximab and panitumumab) have been approved for cancer therapy while others (e.g. matuzumab,

Table 3. Studies investigating the expression levels and prognostic significance of HER-3 and HER-4 in pancreatic cancer

Study	Number of specimens	Marker assessed	Method of assessment	Percentage of specimens positive or with high expression	Other findings	Conclusions
Friess *et al.*, 1995 (96)	58	HER-3	IHC, qPCR, Northern blot analysis	47% IHC	In 17 of 27 pancreatic cancers there was a 6.7-fold increase in HER-3 mRNA levels	High expression of HER-3 was correlated with advanced tumour stage and shorter survival.
Graber *et al.*, 1999 (99)	75	HER-4	IHC, qPCR	-	HER-4 protein was equally expressed in pancreatic cancer and normal tissue	High expression of HER-4 mRNA is correlated with the presence of metastasis.
Kawesha *et al.*, 2000 (84)	157	HER-3	IHC	56.6%		No correlation with tumour stage or survival
Thybusch-Bernhardt *et al.*, 2001 (65)	24	EGFR HER-2 HER-3 HER-4	IHC	33% 25% 50% 4%	EGFR overexpression was correlated with a more aggressive phenotype.	No correlation with tumour stage and grade or survival
te Velde *et al.*, 2009 (101)	45 (13 papilla of Vater)	EGFR HER-2 HER-3 HER-4	CISH, IHC	0% 0% 73% loss of expression in cancer tissues 18% loss of expression in cancer		HER-3 and HER-4 expression found in normal tissue is lost in tumours.
Hirakawa *et al.*, 2011 (97)	126	HER-3	IHC	41.3%		Significant correlation between HER-3 expression and a shorter survival (p=0.008).

Abbreviations: IHC Immunohistochemistry, qPCR quantitative Polymerase Chain Reaction, CISH Chromogenic *in situ* hybridization, FISH fluorescence *in situ* hybridization.

nimotuzumab and zalutumumab) are currently under clinical investigation (104, 105). At present, clinical data is available for the use anti-EGFR mAbs Cetuximab, Matuzumab, Panitumumab and Nimotuzumab in the treatment of pancreatic cancer and these will be discussed in the following sections.

4.1.1.1. Cetuximab

Cetuximab (Erbitux), an anti-EGFR chimeric (mouse/human) mAb, is one of the most commonly used mAbs in cancer therapy and has been approved for the treatment of patients with head and neck and metastatic colorectal cancer (104), also see Fan *et al.*, in this issue). Both *in vitro* and *in vivo* studies supported its utilization for the treatment of pancreatic cancer, however, the results of clinical trials undertaken so far are rather disappointing.

In a phase II trial conducted by Xiong *et al.*, cetuximab was used in combination with gemcitabine in 41 patients with EGFR-positive tumours and it was found that in 12.5% of patients there was a partial response and 63.4% had a stable disease. The median overall survival (OS) was 7.1 months accompanied by a median progression free survival (PFS) of 3.8 months and the 1-year OS was 31.7% (106). However, the rest of the clinical trials exhibited no benefit on survival from the combination of cetuximab with chemotherapy. The combination of cytotoxic agents gemcitabine and oxaliplatin with cetuximab was evaluated in two recent phase II studies. In the first study by Merchan *et al.*,(n=41) the combination of Gemcitabine, Oxaliplatin, and Cetuximab led to no improvement in PFS. In the second study by Crane and colleagues, 69 patients with locally advanced pancreatic cancer received cetuximab, gemcitabine, and oxaliplatin followed by cetuximab, capecitabine, and radiation therapy. The 1-year survival rate was 66% meeting the study's primary end point which was a 1-year survival rate of >45% (107, 108) Another phase II trial (randomised) by the Italian Group for the Study of Digestive Tract Cancer (GISCAD) investigated the possible synergistic effect of cetuximab with gemcitabine and cisplatin compared with use of gemcitabine/cisplatin alone in 84 patients. The median OS in the cetuximab group was 7.5 months compared to 7.8 months of the non-cetuximab group showing that addition of cetuximab did not lead to a survival benefit (109). A randomized phase II trial by the Eastern Cooperative Oncology Group (ECOG) investigated the effect of docetaxel and irinocetan with or without cetuximab in patients with advanced pancreatic cancer. The initial results were presented in 2007 and were updated in 2008. Despite a small improvement in median PFS (4.5 vs. 3.9 months) in the cetuximab arm, interestingly, the median OS was shorter (5.3 months for the combination of cetuximab with chemotherapy versus 6.5 months of the docetaxel and irinocetan arm). In addition to that, the toxicity was high in both arms of the study including grade 3-4 neutropenia and

Table 4. Studies investigating the expression levels of components of IGF signalling system in pancreatic cancer

Study	Number of specimens	Marker assessed	Method of assessment	Percentage of specimens with high expression	Other findings	Conclusions
Bergmann *et al.*, 1995(183)	12	IGF-I IGF-IR	Northern Blot analysis	66.6% 50%	32-fold increase of IGF-I mRNA levels in cancer compared to normal tissues	Only over-expression of IGF-I was statistically significant
Ishiwata *et al.*, 1997(188)	12	IGF-IIR	qPCR	58%	5.6-fold increase in IGF-IIR mRNA levels. IGF-IIR localized in the nucleus in cancer tissues.	IGF-IIR may contribute to the pathophysiology of pancreatic cancer.
Ouban *et al.*, 2003(184)	7	IGF-IR	IHC	57.1%	No detectable levels of IGF-IR in normal pancreatic tissues	IGF-IR is over-expressed in pancreatic cancer.
Stoeltzing *et al.*, 2003(186)	11	IGF-IR	IHC	-	Phosphorylated IGF-IR was detectable only in adenocarcinomas	IGF-IR was detectable in both normal and cancer tissues. No over-expression of IGF-IR.
Hakam *et al.*, 2003(187)	47	IGF-IR	IHC	64%		IGF-IR implicated in pancreatic carcinogenesis.
Lin *et al.*, 2004(179)	69 patients 207 controls	sIGF-I sIGFBP-3	Immunoradiometry	-		Expression of IGFBP-3 was marginally significantly higher in cancer patients
Stolzenberg-Solomon *et al.*, 2004(182)	93 patients 400 controls	sIGF-I sIGFBP-3	ELISA	-		No correlation between IGF-I and IGFBP-3 levels and pancreatic risk.
Wolpin *et al.*, 2007(181)	212 patients 635 controls	sIGF-I sIGF-II sIGFBP-3	ELISA	-		No association between prediagnostic levels of IGF-I, IGF-II or IGFBP-3 with risk of pancreatic cancer
Valsecchi *et al.* 2011 (63)	105	IGF-IR	IHC	56%		High IGF-IR expression correlated with shorter survival

Abbreviations: IHC Immunohistochemistry, qPCR quantitative Polymerase Chain Reaction, ELISA Enzyme-linked immunosorbent assay

diarrhoea (110). A phase III clinical trial by the Southwest Oncology Group (SWOG) examined the effect of the combination of cetuximab with gemcitabine compared with gemcitabine monotherapy in more than 700 patients. The results showed no statistically significant improvement in survival. The median OS was 6 months for gemcitabine monotherapy versus 6.5 months for the combination of gemcitabine and cetuximab (P=0.14) while the PFS was improved by 0.5 months (3 vs. 3.5) for the combination arm (P=0.058) (111). The combination of cetuximab with gemcitabine/oxaliplatin was evaluated in a Phase III trial in 64 patients with metastatic pancreatic cancer. Patients were infused 400mg/m2 cetuximab at first dose followed by weekly doses of 250mg/m2 combined with 1000mg/m2 infusion of gemcitabine on day 1 and 100mg/m2 of oxaliplatin on day 2, every 2 weeks. Results showed a 33% overall response rate and a clinical benefit of 39%. The time to PFS was 3.4 months in the cetuximab group and 4.2 months in the non-cetuximab group while the mean OS was 7.5 and 7.8 months respectively. The investigators concluded that addition of cetuximab to the combination of gemcitabine/oxaliplatin does not increase overall survival in patients with metastatic pancreatic cancer (112). The efficacy of cetuximab has also been investigated in combination with bevacizumab, a fully human mAb which targets vascular endothelial growth factor-A (VEGF-A), with or without gemcitabine in patients with metastatic pancreatic cancer. Results showed no promising activity for either arm (113).

Currently, cetuximab is being investigated in numerous ongoing trials in combination with cytotoxic agents and other targeted agents. The results of such investigations should unravel the full potential of cetuximab for the treatment of pancreatic cancer.

4.1.1.2. Panitumumab

Panitumumab is an anti-EGFR fully human IgG2 mAb (114). Preclinical and clinical data have shown that it's both efficacious and well tolerated when used both as monotherapy and in combination with other agents (115). The effects of panitumumab monotherapy were investigated in a phase I study by Weiner *et al.*, in 2008. In total, 96 patients participated including different types of

Table 5. Monoclonal antibodies (mAb) and Tyrosine kinase inhibitors (TKIs) targeting the IGF-IR in development.

Agent	Type/Class	Company
CP-751, 871/Figitumumab (195)	Fully human IgG2 mAb	Pfizer
IMC-A12/ Cixutumumab (194)	Fully human IgG1 mAb	ImClone
MK-0646/Dalotuzumab (213)	Humanized IgG1 mAb	Merck
R1507 (214)	Fully human IgG1 mAb	Roche
AMG-479/Ganitumab (196)	Fully human mAb	Amgen
AVE-1642 (215)	Humanized mAb	Sanofi-Aventis
BIIB022 (216)	Fully human IgG4 mAb	Biogen Idec.
19D12 (217)	Fully human mAb	ScheringPlough
NVP-AEW541(218)	Reversible TKI	Novartis
NVP-ADW742 (219)	Reversible TKI	Novartis
OSI-906(220)	TKI	OSI
BMS-554417(221)	Reversible TKI	BMS
INSM-18 (222)	Reversible TKI	Insmed
AG-1024(223)	TKI	Merck

malignancies mainly colorectal and renal cancer as well as pancreatic cancer (3%). Results showed that panitumumab monotherapy exhibited antitumour activity particularly in colorectal cancer patients (116). The clinical efficacy of panitumumab in the treatment of pancreatic cancer has been investigated in a phase II trial comparing erlotinib plus gemcitabine with or without panitumumab. The preliminary results were presented in ASCO 2011 Gastrointestinal Cancers Symposium showing a significant difference in median PFS between the two arms (2.0 months for GE and 3.3 months for PGE arm) (117).

4.1.1.3. Matuzumab

Matuzumab is a fully human anti-EGFR mAb which has been evaluated in a number of studies for its anti-tumour efficacy and safety in human cancers (118). In preclinical studies, matuzumab exhibited significant inhibition of cell proliferation and migration in pancreatic cancer (119). A phase I trial which was conducted by Graeven *et al.* in 2006, investigated the effect of the combination of matuzumab with gemcitabine in chemotherapy-naive advanced pancreatic cancer patients. The primary goal of this study was to evaluate the safety of the combination of these two agents. Results showed that not only matuzumab and gemcitabine can be used together, but also that this combination may have enhanced activity since 8 out of 12 (66.7%) patients exhibited partial response or stable disease (120).

4.1.1.4. Nimotuzumab and other anti-EGFR antibodies

Nimotuzumab is a humanized anti-EGFR mAb (121). Evidence regarding the efficacy of nimotuzumab in pancreatic cancer comes from one phase II study by Strumberg *et al.* in 2010. Nimotuzumab was given intravenously at 200 mg once weekly for 6 weeks in 56 pts. Results verified that nimotuzumab is safe and very well tolerated in pancreatic cancer patients. A placebo-controlled trial with gemcitabine is currently in progress (122). Currently, there are several anti-EGFR antibodies under preclinical or clinical development in pancreatic cancer in combination with chemotherapy and/or other targeted form of therapies (Table 6).

4.1.2. EGFR Tyrosine kinase inhibitors

4.1.2.1. Gefitinib

Gefitinib (Iressa), an anti-EGFR TKI was approved by the US FDA as a first line therapy for NSCLC in 2003 (123). Preclinical studies demonstrated that gefitinib inhibits cell growth and invasiveness of pancreatic cancer cells (124). Gefitinib has been evaluated in many clinical trials so far, regarding its effectiveness against several types of cancer including pancreatic cancer. Two phase I trials examined the effect of gefitinib in combination with other therapeutic strategies. The first trial by Czito *et al.* evaluated the combination of gefitinib with capecitabine and radiotherapy and the second study by Maurel *et al.* investigated the effect of the combination of gefitinib with gemcitabine and radiotherapy. The results of both studies were rather disappointing; the first study concluded that the combination of gefitinib with capecitabine and radiotherapy is accompanied by high toxicity and should be approached with caution and the second study showed that the effectiveness of the combination of gefitinib with gemcitabine and radiotherapy was low (125, 126). Another phase I trial by Carneiro *et al.* investigated the toxicity of gemcitabine in combination with gefitinib with the purpose of defining the maximum tolerated dose of these agents. Thirteen patients with metastatic disease were enrolled and results showed that this combination is feasible with a recommended dose for gefitinib of 250 mg/m2 daily (127). A phase II study was conducted by the Hellenic Cooperative Oncology Group, investigating the effectiveness of the combination of gemcitabine and gefitinib in 54 patients with locally advanced or metastatic pancreatic cancer. Results showed a promising activity of this combination with a median survival of 7.4 months while the 1-year survival rate was 23% accompanied by a median PFS of 4.1 months (128). In another phase II trial, the effectiveness of the combination of gefitinib with docetaxel as a second line therapy was investigated in 41 patients pre-treated with gemcitabine. The median survival was 4.5 months while 19 patients had stable disease indicating the limited efficiency of this combination (129).

4.1.2.2. Erlotinib

Erlotinib (Tarceva, Genentech Inc), another reversible EGFR TKI is the most extensively studied small molecule EGFR inhibitor and has been approved by the US FDA for the treatment of both NSCLC and advanced pancreatic cancer (130). Preclinical studies involving pancreatic cancer cell lines such as HPAC cells, as well as *in vivo* experiments in orthotopic mice, showed that erlotinib can lead to significant growth inhibition and promote gemcitabine driven apoptosis (131, 132). These promising experimental results led to several clinical trials,

Table 6. Examples of anti-ErbB Monoclonal antibodies (mAb) and Tyrosine kinase inhibitors (TKIs) approved or in development

Agent	Type/Class	Target	Status	Company
Cetuximab (224)	Chimeric mouse–human IgGl	EGFR	Approved	Bristol-Myers Squibb/ Merck
Panitumumab (118)	human IgG2	EGFR	Approved	Amgen
Trastuzumab (225)	Humanized IgG1	HER-2	Approved	Roche
Nimotuzumab (121)	Humanized IgG1 mAb	EGFR	Phase II/III	YM Biosciences
Matuzumab (120)	Humanized IgG1 mAb	EGFR	Phase II/III	EMD Pharms/Merck
MDX-447 (226)	Humanized mAb	EGFR, CD64 (FcRg1)	Phase I	Medarex/Merck
Mab-806/ ABT-806 (227)	Chimeric mouse–human IgGl/ ABT humanized	EGFR	Phase I	Ludwig Institute
Erlotinib (228)	Reversible TKI	EGFR	Approved	OSI pharmaceuticals
Lapatinib (229)	Reversible TKI	EGFR/HER-2	Approved	GlaxoSmithKline
Canertinib (230)	Irreversible TKI	Pan-HER	Phase II/III	Pfizer
BMS-599626 (231)	Reversible TKI	EGFR, HER-2	Phase I	Bristol Myers Squibb
BIBW-2992 (232)	Irreversible TKI	Pan-HER	Phase II/III	Boehringer Ingelheim
AEE-788 (233)	Reversible TKI	EGFR, HER-2, VEGF-2	Phase I/II	Novartis

the most important of which, was a phase III trial of the National Cancer Institute of Canada Clinical trials Group conducted by Moore and colleagues. The results of this study showed that the combination of erlotinib with gemcitabine improved median survival from 5.91 months for gemcitabine monotherapy to 6,24 months. In addition to that the 1-year survival rate was 23% for patients receiving erlotinib plus gemcitabine compared with 17% for the gemcitabine arm. (11). The positive results of this study led to the approval of erlotinib as a first line therapy for locally advanced or metastatic pancreatic cancer in combination with gemcitabine by the US FDA (133). However since then, due to the survival benefit of short duration and the increased cost, the clinical significance of erlotinib has been challenged (134). This indicates the need for the identification of more reliable markers for predicting response to treatment with erlotinib and other EGFR inhibitors.

Several studies evaluated the therapeutic advantage of erlotinib in combination with bevacizumab, an anti-VEGFR mAb, and/or gemcitabine. A phase II study investigated the efficacy of the combination of erlotinib with bevacizumab in 36 patients with gemcitabine-refractory metastatic pancreatic cancer. In another phase III study, by Van Cutsem and colleagues the effect of the combination of erlotinib with gemcitabine and bevacizumab in 306 patients with metastatic pancreatic cancer was investigated. Both studies concluded that there was no statistically significant improvement in overall survival even though the PFS was significantly longer in the second study (135, 136). Erlotinib has also been evaluated in patients with metastatic/recurrent disease in combination with capecitabine and gemcitabine. In a phase II trial involving 47 patients, the combination of erlotinib with capecitabine/gemcitabine led to promising results with a 32.6% response rate and an overall survival of 12 months (137). In a phase II study in 2005 erlotinib was used in combination with capecitabine in 30 patients with metastatic pancreatic cancer after failure of first-line treatment with gemcitabine. Results showed a median survival of 6.5 months and 10% partial response (138). Numerous ongoing clinical trials are investigating the efficacy of erlotinib in combination with different cytotoxic and biological agents, the results of which are awaited.

4. 2. HER-2 targeting

The most common and well characterized HER2 targeting agent is Trastuzumab (Herceptin), a recombinant humanized mAb (102). Currently, trastuzumab is licensed for the treatment of HER-2 overexpressing breast cancer as well as advanced gastric cancer as monotherapy or in combination with chemotherapy (139). In one preclinical study, Büchler and colleagues examined the effect of trastuzumab on growth of five human pancreatic tumour cell lines (Panc-1, Capan-1, MiaPaca-2, AsPc-1 and Hpaf-2). Of these, they found that the HER-2 overexpressing pancreatic tumour cell lines were sensitive to treatment with trastuzumab both *in vitro* and *in vivo* (in an orthotropic mouse model (140). In their subsequent study in 2005, where trastuzumab was used in combination with gemcitabine and docetaxel in orthotopically xenografted mice, a dramatic improvement in tumour growth inhibition was exhibited (141). These data were supported by other studies demonstrating a link between high levels of HER-2 and growth inhibition by trastuzumab in mice xenograft model. However, trastuzumab demonstrated no inhibitory effects *in vitro* suggesting that the anti-tumour activity of trastuzumab *in vivo* could be due to it ability to induce antibody dependent cellular cytotoxicty (ADCC) and complement dependent cytotoxicity (CDC) of tumour cells (142).

The first clinical trial investigating the effect of trastuzumab, was undertaken by Safran *et al,*. the results of which, were first presented at the ASCO meeting in 2001. In this study, 34 patients with HER-2 overexpressing metastatic pancreatic cancer, received gemcitabine in combination with trastuzumab. The median OS was 7 months while the median 1-year survival was 19%. The response rate to the combination was no different when compared with gemcitabine monotherapy. However the 7 months median survival was an indication that some patients might have benefited by this combination (143). Since then, several phase II clinical trials followed investigating the effect of trastuzumab when used against pancreatic cancer in combination with other agents like cetuximab and gemcitabine. Some of them have been completed but no results have been published yet.

4. 3. HER-3 targeting

Until recently targeted therapy of HER family was focused primarily on the first two HER family members, namely EGFR and HER-2. Due to the lack of tyrosine kinase activity in HER-3, its utilization as a target for HER signalling inhibition, was not considered to be as promising as EGFR and HER-2 targeted therapy. However,

recent studies have demonstrated a key role of HER-3 in the activation of PI3K/AKT signalling pathway and subsequently its great importance in HER addicted cancers and in the development of resistance against HER targeted therapies (144, 145). MM-121 (Merrimack Pharmaceuticals) is a fully human mAb that targets HER-3 and is currently under clinical development. Pre-clinical data have shown a significant efficacy of this agent in a wide range of tumours including pancreatic cancer both *in vitro* and *in vivo*, especially in cancers which are dependent on ligand-induced activation of the receptor (145, 146).

4.4. Dual and Pan-HER inhibitors

In addition to therapeutic agents which target only a specific member of the HER family, several mAbs and TKIs have been developed, that concurrently target more than one receptors. These agents include dual TKIs like Lapatinib and Pelitinib which target EGFR and HER-2, Afatinib and Canertinib which are Pan-HER inhibitors or bi- specific mAbs like MM-111 which targets HER-2 and HER-3 (Table 6). The *in vitro* efficacy of some of these TKIs has been investigated in preclinical studies and was accompanied by some promising results. For example, in a recent study we showed that afatinib was superior in inhibiting the growth of pancreatic cancer cell lines compared to first generation TKI erlotinib (147). Most of these agents are in preclinical or early stages of clinical development. Currently, clinical data regarding the efficacy of such agents in pancreatic cancer has only been reported for Lapatinib. Lapatinib (Tykerb, Glaxosmith Kline), a dual reversible EGFR/HER-2 tyrosine kinase inhibitor has been evaluated in both phase I and phase II clinical trials, in combination with gemcitabine (148, 149). In the phase II study, 125 patients with metastatic pancreatic cancer were treated with 1500mg of Lapatinib per day and 1.000 mg/m^2/wk of gemcitabine for 3 weeks until the disease progression. However, there was no therapeutic benefit for such combination (149). It will be interesting to determine the therapeutic advantages of the irreversible HER inhibitor such a Pan HER blocker afatinib in the treatment of pancreatic cancer (147). Preclinical studies and clinical trials with several HER TKIs are currently underway and the results are awaited.

5. PREDICTIVE VALUE OF EGFR FOR RESPONSE TO EGFR INHIBITORS

The predictive value of EGFR expression for response to therapy with gemcitabine and/or the EGFR inhibitors has been investigated in several studies. In a study by Philip *et al.*, in 2010, EGFR expression was assessed in 595 pancreatic cancer patients who underwent therapy with either gemcitabine alone or a combination of gemcitabine and anti-EGFR mAb cetuximab. Results showed that 92% of patients evaluated were positive for EGFR expression, however, there was no difference in overall survival between the two arms (111). In another study, the expression status of EGFR was evaluated in 100 Caucasian patients who underwent surgical resection of the tumour and were treated with gemcitabine. Membranous EGFR was expressed in 84% of patients while cytoplasmic EGFR was detectable in 82% of patients. Despite the positive correlation between EGFR expression and tumour grade there was no significant association with overall survival (150). In contrast, Fujita *et al.*, in 2011 found that increased EGFR mRNA expression was correlated with decreased survival in patients who received gemcitabine chemotherapy (151). In a recent phase II study by Oh *et al.*, investigating the efficacy of the combination of erlotinib with capecitabine/gemcitabine in 47 patients with pancreatic cancer, EGFR expression was found to be correlated with decreased OS (137). EGFR expression was also examined in 162 patients who received gemcitabine or gemcitabine plus erlotinib treatment. However, there was no correlation between EGFR status and response to treatment and disease stability. Interestingly in this study, the development of a $\geq$ grade 2 rash was associated with an increased PFS and OS (11).

6. Mechanisms of resistance to EGFR targeted therapy in pancreatic cancer

While the rationale of targeting the HER signalling pathway for cancer treatment seemed justified, the clinical benefit of this approach has been limited. The efficacy of several HER inhibitors which were developed based on this rationale has been extensively investigated in many clinical trials but was accompanied by moderate success. The majority of cancer patients simply do not respond to this treatment or eventually acquire resistance to it (152, 153). In the last few years, several mechanisms involved in the development of resistance to EGFR-targeted therapy have been identified. The key mechanisms which are believed to be implicated in the acquisition of resistance to anti-EGFR agents include i) mutations in the EGFR gene which leads to alteration of the target and consequently affects the sensitivity to an agent (loss/modification of target), ii) activation of alternative signalling pathways which are responsible for cell survival or cell proliferation and iii) activation of downstream mediators within the EGFR signalling pathway by EGFR independent mechanisms and several other factors such as the cellular location of EGFR (154).

6. 1. Mutations in EGFR gene

The best example of EGFR mutations which can affect the sensitivity of cancer cells to EGFR inhibitors is the Epidermal Growth Factor Receptor variant III (EGFRvIII) mutant. EGFRvIII is one of the most extensively investigated and well characterized mutations of the EGFR gene and is very common in lung, brain and breast cancer among others (155, 156). EGFRvIII contains a deletion (exons 2-7) which leads to the production of a truncate receptor which lacks the ligand binding domain and is constitutively active, leading to increased malignancy (157). Studies have shown that this type of mutation is involved in resistance to TKI gefitinib in glioblastoma patients (158). On the contrary, some mutations have been shown to lead to increased sensitivity to EGFR therapy in lung cancer (159). However, it has been shown that EGFR mutations in pancreatic cancer is a rather rare event (160, 161). In a study by Kwak *et al*, in 2 of 55 pancreatic cancer patients a deletion in exon 19 was identified but its clinical significance was not determined,

while Tzeng *et al.* showed that a shorter EGFR intron 1 in pancreatic cancer was correlated with increased sensitivity to erlotinib even though the presence of this polymorphism was not correlated with increased activation of the EGFR pathway (162, 163). Similarly in a recent study, Lozano-Leon *et al.* examined the EGFR status of 52 pancreatic tumours and found only 3 mutations (R841R, T571T, R831C) in the EGFR gene with no association being observed with any clinical characteristic (164). More recently, acquired mutation of the EGFR extracellular domain (S429) was associated with resistance to cetuximab in colorectal cancer (165). However, the biological and clinical significance and predictive value of such mutations for response to therapy with the EGFR inhibitor in pancreatic cancer remains unclear and warrants further investigation.

6.2. Activation of downstream pathways in an EGFR-independent manner

Several studies have shown that acquired resistance to EGFR inhibitors could be mediated by the constitutive activation of oncogenic pathways downstream of the receptor. Both PI3K/Akt and RAs/Raf/MAPK pathways, which are the main signalling cascades activated by EGFR, can become constitutively activated by *de novo* mutations independently of EGFR. A very good example is the common activation of MAPK pathway by mutations in the K-ras gene. K-ras mutation status has been indicated as a predictive marker for response to therapy with anti-EGFR mAbs and specifically cetuximab in colorectal cancer (CRC). In 2009 it was suggested by the American Society of Clinical Oncology that EGFR therapy (EGFR mAbs) candidates have to be tested for K-ras mutation before receiving treatment (166). In regards to pancreatic cancer, it has been shown that approximately 90% of pancreatic tumours have a K-ras mutation; consequently, this mechanism could be one of the most important factors limiting the effectiveness of anti-HER inhibitors in pancreatic adenocarcinoma and warrants further investigation (167, 168).

6.3. Activation of alternative pathways

The activation of alternative signalling pathways is another mechanism by which resistance to anti-HER treatment can occur (169). Cellular processes such as proliferation, survival, differentiation and angiogenesis are under regulation by a great number of growth factors and their receptor families, with each family being able to control multiple cell functions. This overlapping activity of different growth factors and their receptors supports the notion that EGFR inhibition can be overcome by hyper-activation of other pathways such as VEGFR, PDGFR and IGF-IR pathways (170). Both EGFR and IGF-IR pathways exert their biological actions mainly via the same downstream signalling pathways; namely PI3K/Akt and Ras/Raf/MAPK, thus IGF-IR pathway is highly likely to be involved in EGFR-targeted therapy resistance (Figure 1). In agreement with this hypothesis, several studies demonstrated that over-expression and activation of IGF-IR can eliminate the inhibitory effects of anti-HER agents (41, 171-173). This is achieved by maintaining the activity of Akt, as shown in several types of cancer including hepatocellular carcinomas, breast cancer and NSCLC. In addition, it has been shown that inhibition of EGFR in NSCLC cell lines by gefitinib leads to EGFR/IGF-IR heteredimerization and subsequent activation of IGF-IR and its downstream mediators (174), see also Corvaia *et al* in this issue).

7. IGF-IR SIGNALLING SYSTEM IN PANCREATIC CANCER

7.1. Expression and prognostic significance of IGF signalling system in pancreatic cancer

The Insulin Growth Factor (IGF) system plays an important role in the development and growth of multiple human tissues and is involved in the regulation of overall growth (175). The IGF signalling network includes the IGF ligands IGF-I, IGF-II and Insulin, the cellular receptors IGF-IR, IGF-IIR and the Insulin receptor (IR) as well as a group of regulatory IGF binding proteins (IGFBPs) (176). Increased expression of IGF-I and IGF-II has been reported in several human cancers, suggesting a role for these growth factors in carcinogenesis (177, 178). There is a limited number of studies on the expression levels and prognostic significance of the IGF-system components in pancreatic cancer. Serum levels of IGF ligands and IGFBPs have been shown to be correlated with increased risk of certain types of cancer. In pancreatic cancer, a study of 69s subjects and 207 controls found an association between high pre-diagnostic serum levels of IGFBP-3 and IGF-I and the risk of pancreatic cancer. However, such an association was not statistically significant. (179). More recently, Douglas *et al.*, showed that while the pre-diagnostic levels of IGF-I, IGF-II or IGFBP3 were not associated with pancreatic cancer, a high molar ratio of IGF-I/IGFBP3 was correlated with a high risk for this malignancy (n=187) (180). However, other studies found no correlation between elevated plasma levels of IGF I/II or IGFBP-3 levels and the risk of pancreatic cancer (181, 182).

IGF-IR has been shown to be commonly overexpressed in pancreatic cancer (Table 4). In 1995 Bergmann *et al.* showed that 50% of pancreatic cancer tissues had increased IGF-IR mRNA levels (n=12) (183). In another study Ouban *et al.* determined the expression level of IGF-IR by IHC and found that 57.1% (n=7) of pancreatic cancer specimens overexpressed the receptor compared to normal tissues (184). Similarly, Karna and colleagues demonstrated elevated levels of IGF-IR as well as IGF-I, IGFBP1 and IGFBP3 in pancreatic tumours when compared with normal tissues (185). On the contrary, Stoeltzing *et al.*, found that IGF-IR was detectable in both cancer specimens (n=11) and normal ductal epithelium (n=10) even though the activated form of the receptor was only detectable in adenocarcinomas (10/10) (186). In the largest study to date, the expression level of IGF-IR was determined in 47 human pancreatic adenocarcinomas by IHC, and strong IGF-IR staining was found in 64% of tumours (187). The expression levels of IGF-IIR have also been investigated by Ishiwata *et al.* in 1997 and showed that 7 out of 12 pancreatic cancer specimens exhibited a 5.6-fold increase in IGF-IIR mRNA levels (188).

7. 2. Targeting of IGF signalling system in pancreatic cancer

The implication of the IGF signalling system in the development and progression of cancer, as well as, in drug resistance makes it an attractive target for drug development. The IGF pathway can be targeted at several levels including the IGF receptor, its ligands and downstream binding proteins (189). A major concern regarding this type of targeted therapy is the possible interaction of anti-IGF-IR agents with the insulin receptor due to the great homology shared by these two receptors since such interference could affect normal physiological processes.

There are several strategies for targeting of the IGF-IR including IGF-IR blocking mAbs and small molecules IGF-IR TKIs, disruption of IGF-IR translation with antisense oligonucleotides (AS) and small interference RNA (siRNA), synthetic IGF peptides which, by mimicking the natural ligands, can compete with the latter for binding to the receptor and recombinant IGFBPs which can down-regulate active ligands (190-193), for details please see Corvaia *et al* in this issue).

To date, only two of these therapeutic approaches have been evaluated in clinical trials; anti-IGF-IR Mabs and TKIs. There are numerous mAbs which are currently under clinical development such as Figitumumab (CP-751, 871 by Pfizer), Cixutumumab (IMC-A12 by Imclone), AMG-479 (by Amgen) among others and TKIs such as NVP-AEW541 by Novartis (194-197), Table 5). Positive initial results from several phase I trials led to the initiation of larger studies for several of these agents and currently a great number of anti-IGF-IR mAbs and TKIs are being evaluated in phase II/III clinical trials, used alone or in combination with chemotherapeutic and other targeted agents (198). In regards to the effectiveness of anti-IGF-IR agents in pancreatic cancer, no clinical data are available. In addition, while the number of preclinical studies undertaken so far investigating the potential of IGF-IR targeting in pancreatic cancer is limited, initial results are very promising (197, 199, 200). The vast majority of the studies investigating the status of IGF-IR in pancreatic cancer have demonstrated that its overexpression is a common phenomenon. These findings in combination with the established role of IGF-IR in tumour progression and angiogenesis as well as its implication in acquired resistance to HER targeted therapy support the incorporation of anti IGF-IR strategies into the current therapeutic approaches (186, 201-203).

8. PERSPECTIVE

The dreadful nature of pancreatic cancer indicates the need for the development of new therapeutic strategies to overcome the intrinsic and acquired resistance of pancreatic cancer cells to conventional forms of therapy (152, 153). Currently, gemcitabine remains the standard approach for treatment of patient with metastatic pancreatic cancer and despite the numerous studies of gemcitabine in combination with other agents, only erlotinib has been added to the weaponry against advanced pancreatic cancer. Therefore, to improve on the modest survival benefit with erlotinib in pancreatic cancer, it is essential to conduct detailed studies of the expression pattern (e.g. receptor mutational status as well as quantity and cellular location) and prognostic significance of all HER family members and their ligands for response to therapy with the HER inhibitors (i.e. HER dependent tumours) as well as evaluating their predictive value. In addition, to resolve some of the contradictory results in the literature, such studies should be conducted in a larger group of pancreatic cancer patients. Finally, due to the heterogeneous nature of human cancers, it is imperative to investigate the therapeutic benefit of new agents which block multiple HER family members (i.e. dual, pan, reversible or irreversible TKIs) in combination with agents targeting other molecular pathways such as the IGF-IR and C-Met inhibitors (see Corvaia *et al.,* Goetsch *et al.,* in this issue). These studies together with the identification and co-targeting of new molecular pathways that are implicated in this malignancy could help to overcome drug resistance, prolong the duration of response and ultimately improve the overall survival rates for patients diagnosed with this deadly disease.

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10. REFERENCES

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