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Systemic Therapies for Pancreatic Cancer - The Role of Pharmacogenetics

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Abstract

Effective systemic treatment of pancreatic cancer remains a major challenge, with progress hampered by drug resistance and treatment related toxicities. Currently available cytotoxic agents as monotherapy or in combination have provided only a modest survival benefit for patients with advanced disease. Disappointing phase III results with gemcitabine-based combinations in patients with advanced pancreatic cancer might be related to poor efficacy of systemic therapies in unselected patients. Future research strategies should prioritize identification of predictive markers through pharmacogenetic investigations. The individualization of patient treatment through pharmacogenetics may help to improve outcome by maximizing efficacy whilst lowering toxicity. This review provides an update on the pharmacogenetics of pancreatic cancer treatment and its influence on treatment benefits and toxicity.

Keywords

Pancreatic cancer; pharmacogenetics; chemotherapy

Introduction

Adenocarcinoma of the pancreas is the fourth leading cause of cancer death among both men and women in the United States. In 2008, there were about 37,680 new cases of newly diagnosed pancreatic cancer with approximately 34,300 deaths [1]. Despite improvements in imaging and surgery, long term survival remains poor with a median survival of one year for patients with resected disease, 8-10 months for locally advanced, unresectable disease and less than 6 months for patients with metastatic disease [2, 3]. The poor prognosis is attributed mainly to the high incidence of metastasis at the time of presentation, its aggressive course and limited efficacy of available systemic treatments [4].

Over the past decade gemcitabine has been considered to be the standard cytotoxic agent in advanced stage disease, with improvement in clinical benefit and survival over 5-fluorouracil (5FU) [5]. Since then, to improve treatment efficacy, numerous studies have been conducted combining gemcitabine to a second cytotoxic agent such as a platinum analog [6-8], a fluoropyrimidine [9-12], topoisomerase inhibitor [13, 14], or a multi-target

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anti-folate [15]. Whilst some of these studies yielded improvements in response rate and progression free survival, benefits in overall survival have been limited. More recently, a combination of 5FU, irinotecan and oxaliplatin (FOLFIRINOX) has been shown to improve overall survival compared with gemcitabine alone [16]. Other studies have examined the combination of gemcitabine and molecular targeted agents such as matrix metalloproteinase inhibitors [17, 18], tipifarnib [19], erlotinib [20], cetuximab [21] or bevacizumab [22, 23]. With the exception of erlotinib combined with gemcitabine [20] and FOLFIRINOX [16], these agents have failed to show a significant survival benefit compared with gemcitabine alone.

Pharmacogenetic studies have shown gene variants which can influence patient outcome after drug intervention [24, 25]. Germline and intra-tumoural genetic variation in drug transporters, metabolizing enzymes, and targets may explain differences in drug efficacy and tolerability. As only a minority of patients with pancreatic carcinoma appears to benefit from systemic therapy, the study of pharmacogenetics may help individualize the treatment of pancreatic cancer in an effort to maximize efficacy and minimize toxicity in patients. The purpose of this paper is to review tumoral and germline molecular determinants of response and toxicity to cytotoxic agents and molecular targeted therapies related to adenocarcinoma of the pancreas with an emphasis on the approved agents gemcitabine and erlotinib (Tables 1 and 2).

Cytotoxic Chemotherapy

Gemcitabine

The nucleoside analog, gemcitabine (2', 2'-difluorodeoxycytidine), is one of the most commonly used cytotoxic agents in pancreatic carcinoma. The efficacy of gemcitabine was first established in a randomized study where patients with advanced symptomatic pancreatic cancer were randomized to either gemcitabine or 5-fluorouracil. A significant improvement in benefit response (25% *versus* 5%) and one year survival (18% *versus* 2%) was reported with gemcitabine compared with 5-fluorouracil [5].

Gemcitabine is converted to its active form, gemcitabine triphosphate (dFdCTP) through a series of metabolic pathways. The intracellular uptake of gemcitabine is mediated predominantly via equilibrative nucleoside transporter hENT1 (solute carrier family 29, member 1) and, to a lesser extent, the concentrative nucleoside transporters, hCNT1 (solute carrier family 28 member 1) and hCNT3 (solute carrier family 28 member 3) [26, 27]. Intracellular gemcitabine is then phosphorylated in a rate-limiting step by deoxycytidine kinase (DCK) to gemcitabine monophosphate (dFdCMP) and subsequently by 5'-nucleotidase (5'-NT) to gemcitabine diphosphate (dFdCDP) and gemcitabine triphosphate. Gemcitabine triphosphate inhibits DNA synthesis via a process called masked chain termination where gemcitabine is incorporated into DNA via DNA polymerase α , leading to the inhibition of DNA repair and synthesis. Gemcitabine and dFdCMP are metabolised by cytidine deaminase (CDA) and deoxycytidylate deaminase (DCTD) respectively to difluorodeoxyuridine (dFdU) and difluorodeoxyuridine monophosphate (dFdUMP) respectively [28, 29]. Other key targets of gemcitabine cytotoxicity are ribonucleotide reductase (RRM1, RRM2) and thymidylate synthase (TYMS) that are inhibited by dFdCDP and dFdUMP respectively. RRM1 converts ribonucleotides to deoxyribonucleotides which are used in DNA synthesis and repair [28, 30, 31] whilst the inhibition of TYMS results in DNA damage [32].

Pancreatic cell line studies have demonstrated that hENT1 is a major gemcitabine transporter and reduced expression of hENT1 results in reduced gemcitabine sensitivity [33]. Several studies have examined the role of hENT1 expression as a potential prognostic

and predictive marker in pancreatic cancer. In an immunohistochemistry (IHC) study of patients with advanced stage pancreatic cancer, hENT1 expression was associated with longer survival compared with low or absent expression (13 months *versus* 4 months) [34]. Furthermore, in a transcriptional study of resected pancreatic cancer, high hENT-1 mRNA expression was associated with prolonged survival and disease free-survival compared with low hENT1 expression (25.7 months versus 8.5 months and 20.4 months versus 9.3 months respectively) [35]. The usefulness of hENT1 as a predictive biomarker has been reported in immunohistochemistry (IHC) studies of pancreatic cancers. In one study, tumors were retrospectively obtained from a phase III adjuvant study of comparing 5-fluorouracil with gemcitabine before and after chemoradiation for patients with resected pancreatic cancer. Using IHC tissue microarray, hENT1 overexpression was associated with increased overall survival (HR, 0.40; 95% CI, 0.22-0.75; $p=0.004$) and disease free-survival (HR, 0.39; 95% CI, 0.21-0.73; $p=0.003$) in patients who received gemcitabine whereas hENT1 expression was not associated with outcome in patients treated with 5-fluorouracil [36]. In a second study, the expression of tumoral hENT1 and hCNT3, as determined by IHC, was associated with overall and disease-free survival in patients with resected pancreatic adenocarcinoma treated with adjuvant gemcitabine-based chemotherapy. Reduced hENT1 expression was associated with increased risk of death (HR, 4.31; 95% CI, 1.95-9.52; $p=0.0001$) and increased recurrence rate (HR, 4.36; 95% CI, 2.01-9.44; $p=0.0001$) [37]. Similarly, reduced hCNT3 expression was associated with an increased risk of death (HR, 2.59; 95% CI, 1.24-5.43; $p=0.003$) and recurrence (HR, 2.27; 95% CI, 1.2-4.65; $p=0.02$). When analysed together, patients with two favourable prognostic factors (high hENT1 and high hCNT3 expression) had a longer median survival (94.8 months) compared with patients having one (18.7 months) or no favourable prognostic factor (12.2 months; $p<0.0001$) [37]. In an IHC study of patients receiving pre-operative gemcitabine and radiotherapy, positive hENT1 expression was a significant factor for overall survival [38]. In a retrospective study, hENT1 protein overexpression was significantly associated with a longer survival in patients who received adjuvant gemcitabine monotherapy after curative resection compared with low hENT1 expression (22.2 months and 11.8 months, respectively, $p=0.024$) [39].

Reduced expression of DCK has been suggested to be a mechanism for gemcitabine resistance. The association between DCK and gemcitabine sensitivity in pancreatic cancer has been reported in several studies using cell lines [40] and xenografts [41]. In a study of patients with advanced pancreatic cancer treated with gemcitabine, tumoral DCK expression on IHC was associated with clinical outcome. Patients with low DCK expression compared with high DCK expression had a lower overall survival and progression-free survival of 14.6 +/-7.3 months and 21.7 +/-13.8 months ($p<0.009$) respectively and 10.0 +/- 5.3 months and 12.0 +/- 9.3 months respectively ($p<0.04$) [42]. In a second study involving patients receiving adjuvant gemcitabine-based chemoradiation, tumor DCK protein expression was associated with improved disease free survival (HR, 3.22; 95%CI, 1.56-6.65); $p=0.002$ and overall survival (HR, 3.61; 95%CI, 1.74-7.51; $p=0.001$) [43].

The overexpression of the catabolic enzyme CDA has been detected in tumor cells resistant to nucleoside analogs [44]. In a study of patients with advanced pancreatic cancer treated with gemcitabine, patients with higher CDA expression and activity levels in peripheral blood mononuclear cells had a shorter time to progression and overall survival [45]. In a report of a Japanese patient with pancreatic cancer receiving gemcitabine and cisplatin who developed severe haematologic and non-haematologic toxicity, germline genotyping showed homozygosity for *CDA* 208A with gemcitabine levels 5-fold higher than subjects homozygous for reference sequence *CDA* 208G [46]. In a further study of Japanese cancer patients receiving gemcitabine-based chemotherapy, haplotypes containing *CDA* 208G>A were associated with reduced CDA enzyme activity and gemcitabine clearance and marked haematologic and non-haematologic toxicities [47, 48]. More recently, two studies on CDA

activity and overall survival in pancreatic cancer patients were reported in abstract form. Low plasma CDA activity and *CDA* 208G>A haplotypes were associated with prolonged survival and in the second, low plasma CDA levels but not *CDA* 208G>A haplotypes were associated longer overall survival [49]. A second germline polymorphism, *CDA* 76A>C, was reported to be associated with hematologic toxicity but not disease free survival or overall survival [50].

An increase in RRM1 and RRM2 activity has been associated with gemcitabine resistance *in vitro* [51]. In a microarray study, *RRM1* was the most overexpressed gene in gemcitabine resistant pancreatic cancer cell lines [52]. In addition, using RRM1 specific RNAi transfection, RRM1 expression was reduced leading to increased gemcitabine sensitivity. In the same study on patients with recurrent pancreatic cancer treated with gemcitabine only, high RRM1 mRNA levels were associated with a lower response and poorer overall survival at 6 months versus 14.6 months for low RRM1 levels [52]. RRM2 overexpression was associated with *in-vitro* gemcitabine resistance; and increased invasiveness of pancreatic adenocarcinoma was mediated via *RRM2* overexpression with resultant upregulation of nuclear factor-kB (NF-kappaB) transcription factor activity and MMP-9 expression [53, 54].

In an *in vitro* study of pancreatic cancer cells, the gene expression of a combination of *hENT1*, *DCK*, *RRM1* and *RRM2* was associated with acquired resistance to gemcitabine [55]. *hENT1*, *DCK*, *CDA*, *RRM1*, and *RRM2* expression levels were determined in patients with resected pancreatic tumors. High *DCK*, low RRM2 and GEM score (combined score of 4 genes) were associated with a longer disease-free survival in patients treated with gemcitabine [56]. In a study of patients with resectable pancreatic cancer treated with neoadjuvant gemcitabine-based chemotherapy plus radiation therapy, 17 germline polymorphic variants of *CDA*, *DCK*, *RRM1*, *DCTD*, *hENT1*, *hCNT1* *hCNT2*, and *hCNT3* were evaluated for an association with survival and toxicity. Individually, none of the 17 SNPs were associated with survival but a combined genotype effect of *CDA* -76A>C, *DCK* 1205C>T, *DCTD* -47T>C, *hCNT3* -69C>T, *hENT1* -549T>C and *hENT1* 913C>T on survival was reported. For patients with two to three or four to six at risk genotypes, the HR for death was 1.71 (95% CI, 1.06-2.76; p=0.028) and HR 3.16 (95% CI, 1.77-5.63; p<0.001) respectively. The genotypes *CDA* 111C>T, *DCK* -1205C>T, *DCK* 9846A>G and *hCNT3* 25A>G were associated with neutropenia. Patients with three or four variant alleles compared with those with none to two variant alleles had a higher risk of grade 3-4 neutropenia (OR, 5.88; 95% CI, 2.1-16.5; p=0.001 and OR, 3.57; 95% CI, 1.38-9.22; p=0.009, respectively) [57]. In a second study, 17 polymorphic variants of *CDA*, *DCK*, *DCTD*, *RRM1*, *hENT1* and *hCMT1-3*, were assessed in patients with locally advanced disease treated with gemcitabine based chemo-radiotherapy. The genotypes *CDA* -76 A>C, *DCK* 1205 C>T, *RRM1* 33 A>G, and *hENT1* 913 C>T were associated with neutropenia. A combined genotype effect of *CDA* -76 A>C, *RRM1* 33 A>G, *RRM1* -27 C>A, and *hENT1* -201 A>G on progression free survival was seen. For patients with two or three to four at risk variant alleles compared with none or one variant allele, the HR was 1.79 (95% CI, 1.2-2.66; p=0.004) and HR 3.25 (95% CI, 1.79-5.90; p<0.001) respectively [58]. The metabolism of gemcitabine is complex, involving multiple enzymes, transporters and targets. These findings show whilst a single SNP may have a weak effect functionally, and the frequency of a combination of adverse genotypes is uncommon, affecting about 10-13% of patients [49, 50], the combined effect of multiple markers may have clinical value in predicting outcomes.

Several studies have reported an association between clinical outcome and germline polymorphisms of genes involved in the DNA repair pathway including base excision repair, mismatch repair, and homologous recombinant repair genes in patients with pancreatic cancer.

In a study of patients with resected, locally advanced or metastatic pancreatic cancer, the base excision repair SNPs *POLB* 165A>G, *POLB* -2133T>C, *APEX1* 5T>G, *XRCC1* -22C>T and *hOGG1* G2657A were associated with survival on multivariate analysis. The hazard ratio for survival for genotypes *POLB* 165A>G AA/AG versus GG was 0.39 (95% CI, 0.17-0.90), *POLB* -2133T>C TT/TC versus CC was 0.29 (95% CI, 0.10-0.81), *APEX1* 5T>G TT/TG versus GG was 1.47 (95% CI, 1.11-1.95), and *XRCC1* -22C>T was 4.64 (95% CI, 1.43-15.0) and *hOGG1* 2657G>A AA was 1.73 (95% CI, 1.05-2.85) [59].

Pre-clinical studies have reported that homologous recombinant DNA repair may have a role in gemcitabine-mediated cell kill [60] and clinical studies have reported an association between genetic variants of homologous recombinant repair genes and survival. In a study of patients with all stages of pancreatic cancer, patients with *RecQ1* 159A>C and *RAD54L* 157C>T genotypes were associated with survival. The median survival for genotypes *RecQ1* 159 AA, AC and CC was 19.2, 14.7 and 13.2 months and *RAD54L* 157 CC, CT and TT was 16.4, 13.3 and 10.3 months respectively whereas variant alleles of *XRCC2* R188H were associated with poorer survival in nonsurgical patients [61]. In addition, a combined genotype effect of *RecQ1*, *RAD54L*, and *XRCC2* was associated with poorer survival. For patients with one or two to three adverse genotypes, the HR for survival was 1.61 (95% CI, 1.08-2.40) and 2.52 (95% CI, 1.57-4.04) respectively. In another study, an association between SNPs of DNA damage repair genes and survival was reported in patients with pancreatic cancer treated with neoadjuvant gemcitabine-based chemotherapy with radiotherapy [62]. Following multivariate analysis, patients with *RecQ1* 159A>C, *RAD54L* 157C>T and *ATM* 77T>C genotypes had a worse survival with a HR of 3.0 (95% CI, 1.4-6.3), 2.9 (95% CI, 1.4-6.0), and 1.9 (95% CI, 1.0-3.8) respectively. A strong gene-dosage effect was seen when *RecQ1* 159A>C, *RAD54L* 157C>T, *ATM* 77T>C and *XRCC1* 22C>T were analyzed in combination. Patients with none, one, two or three or more at risk alleles had a median survival of 62.1, 27.5, 14.4 and 9.9 months respectively [62]. The results of these studies suggest polymorphisms of genes in the DNA repair pathway may be prognostic in patients with pancreatic cancer. In a more recent study from the same investigators, germline SNPs of eight DNA mismatch repair genes were analysed from patients treated with neoadjuvant gemcitabine-radiotherapy. *TREX1* -460C>T and *TP73* 4G>A genotypes were associated with tumor response, *MLH1* -169C>T and *TP73* 4G>A were predictors for tumor respectability and patients with *EXO1* 20G>A, *TREX1* -460C>T and *TP73* 4G>A genotypes had a worse survival with a HR of 1.78 (95% CI, 1.05-3.0), 2.01 (95% CI, 1.26-3.19), and 2.78 (95% CI, 1.28-5.84) respectively [63]. In a more recent report, patients with advanced stage pancreatic cancer treated with cisplatin-based combination chemotherapy, patients with XPD Gln751Gln, XPD Asp312Asn + Asn312Asn or XRCC1 Arg399Gln + Gln399Gln genotypes had a worse outcome [64].

Cell cycle checkpoints are important in cellular response to DNA damage by arresting the cell cycle to allow time for DNA repair and by inducing DNA repair genes [65]. Several studies have indicated polymorphisms in cell cycle regulating and DNA repair damage genes may be a determinant for clinical outcome in pancreatic cancer. The proteins ataxia telangiectasia and Rad3 related (ATR), ataxia telangiectasia mutated (ATM), checkpoint kinase 1 and 2 (CHEK1 and CHEK2) are regulators of cell cycle arrest and DNA repair in response to DNA damage [66]. In a study of pancreatic cancer patients receiving neoadjuvant gemcitabine-radio-therapy, *ATM* 60G>A and *CHEK1* 35G>A genotypes were reported to be associated with survival. The median survival for patients with *CHEK1* 35G>A was 14.3 months for AA genotype and 26.4 months and 21.4 months for GA and GG genotypes respectively ($p<0.039$) and for patients with *ATM* 60G>A, 16.6 months for GA genotype and 23.9 months and 36.0 months for GG and AA genotypes respectively ($P=0.048$) [67]. A combined genotype effect was seen with *ATM* 60 GA/GG, *ATR* 340CT/CC, and *CHEK1* 35AA with a median survival of 31.0, 16.2, and 10.5 months for patients

with ones or less, two or three adverse alleles respectively ($p=0.004$). In a study of patients with resectable pancreatic adenocarcinoma treated with neoadjuvant gemcitabine and radiotherapy, an association between 12 SNPs of ten cell cycle genes and clinical outcome was assessed. *MDM2* 309T>G was associated with reduced survival, with a median survival of 33.4 months and 16 months for the wild type (TT) and variants (TG/GG) respectively [68]. The tumor suppressor *p27*-109T>G was associated with increased survival (HR, 0.57; 95% CI, 0.31-1.00; $p=0.05$). A combined genotype effect of *MDM2* 309T>G and *p27*-109T>G was also associated with survival. For patients with one or two adverse genotypes, the HR for death was 2.31 (95% CI, 0.88-6.10) and 3.75 (95% CI, 1.44-9.82) respectively. The *p73* and *p16* polymorphic genotypes were associated with time to progression. The median time to progression with *p73* wild type (GG) and polymorphic genotype (GA/AA) was 17.4 and 10.2 months respectively (HR 1.79; 95% CI, 1.08-2.99; $p=0.021$) whereas for *p16* wild type (CC) and polymorphic genotype (CT/TT), time to progression was 16.2 and 10.8 months respectively (HR 1.81; 95% CI, 1.02-3.23; $p=0.039$). A combined genotype effect for time to progression was seen for polymorphisms in *p73*, *p16* and *MDM2*. For patients with one, two or three at risk genotypes, the HR for progression was 2.13 (95% CI, 1.04-4.36), 3.18 (95% CI, 1.37-7.39) and 10.09 (95% CI, 3.17-32.05) respectively [68].

The apoptotic signaling pathway is also involved in gemcitabine resistance. Phosphatidylinositol 3-kinase (PI3K)/AKT and NF-kappaB are antiapoptotic signal transduction pathways implicated in chemoresistance in pancreatic cancer [69]. A reduction in AKT expression was associated with enhanced gemcitabine-induced apoptosis and anti-tumor activity [70]. The role of AKT as a potential prognostic marker was suggested in a study of patients with resected pancreatic cancer where higher phosphorylated AKT expression by IHC was associated with increased overall survival [71]. In a preclinical study, curcumin, an agent that down-regulates NF-kappaB, potentiated the activity of gemcitabine in pancreatic cancer cell lines [72]. The dysregulation of antiapoptotic proteins (Bcl-2, Bcl-xL, and Mcl-1) and proapoptotic proteins (Bax and Bcl-xs) is commonly seen in pancreatic cancers and is associated with survival [73]. The median survival of patients with resected pancreatic cancer with Bax positive tumors on IHC compared with Bax negative tumors was 12 months and 5 months respectively ($p < 0.05$) [74]. In a study on pancreatic cancer cell lines, Bcl-2 overexpression was associated with increased gemcitabine induced apoptosis and Bax expression increased gemcitabine sensitivity [75]. The Bcl-2/adenovirus E1B interacting protein 3 (BNIP3) is associated with intrinsic gemcitabine resistance in pancreatic cancer and suppression of BNIP3 by siRNA resulted in reduced gemcitabine cytotoxicity [76].

The RNA binding protein, HuR antigen (HuR), is a stress response protein that modulates mRNA translation that encodes stress response, immune response, oncogenes, cell cycle regulatory proteins and tumor suppressor genes [77]. In a recent study, HuR has been implicated as a key player in gemcitabine efficacy [78]. In a recent study, gemcitabine treated pancreatic cancer cell lines increased cytoplasmic HuR and HuR regulated DCK mRNA, resulting in increased DCK protein levels. In the same study, cytoplasmic HuR reduced expression by IHC was associated with reduced survival (HR, 7.34; 95% CI, 2.05-26.22; $p=0.0022$) in patients with resected pancreatic carcinoma [78]. These results were confirmed in a second study where HuR over-expression by IHC was associated with increased survival compared with low cytoplasmic HuR (45 months versus 23 months, respectively, $p=0.033$) [79].

Multi-drug resistant genes such as *ABCB1* (multidrug resistance 1, MDR1), the *ABCC* (multidrug resistance-associated protein, MRP) family, and *ABCG2* (breast cancer resistance protein, BCRP) play a key role in determining chemo-resistance. In a study of patients with potentially resectable pancreatic cancer, polymorphic variants of *ABCB1*,

ABCC1-5, and ABCG2 genes were analysed. ABCC5 -2>G AA genotype was associated with survival with a HR of 1.65 (95% CI, 1.11-2.45; p=0.013). A combined genotype effect of *ABCC5*-2A>G and *ABCC2* 40G>A was associated with survival. For patients with one or two at risk genotypes the HR was 2.18 (95% CI, 1.32-3.60; p=0.002) compared with one or none at risk genotypes [80].

Other potential molecular determinants of gemcitabine sensitivity have been identified in pre-clinical studies. Focal adhesion kinase (FAK) is a cytoplasmic tyrosine kinase that is overexpressed in many types of tumors, including pancreatic cancer, and plays an important role in cell adhesion and survival signaling. Src, an intracellular tyrosine kinase, is involved in tumorigenesis, invasion and metastasis. Increased activity of Src kinase [81] and FAK [82] is associated with gemcitabine resistance in pancreatic cancer cell lines. In an IHC study of FAK and Src protein expression, Src overexpression was an independent prognostic factor for survival on multivariate analysis (p= 0.047) [83]. The transcription factor HMGA1 is overexpressed in pancreatic cancer and in a recent *in vitro* study, the over-expression of HMGA1 was reported to promote gemcitabine resistance [84]. Activation of the chemokine receptor, CXCR4 by its ligand, CXCL12, is associated with gemcitabine resistance *in vitro* [85]. MicroRNA (miRNA), a negative regulator of gene expression, is involved in cellular proliferation, differentiation, and apoptosis and may function as tumor suppressors and oncogenes [86]. In patients treated with gemcitabine, multivariate analysis showed adjuvant therapy (HR 0.3; 95% CI, 0.1–0.6; p< 0.001) and high miRNA-21 expression (HR 3.1; 95% CI, 1.2–5.3; p= 0.003) were independent predictors of survival. *In vitro*, miRNA-21 was associated with gemcitabine resistance [87]. Although promising, currently only preclinical data is available for these molecular markers, thus further validation in clinical studies is warranted prior to its application.

Fluoropyrimidines

5FU or its derivatives have been commonly used in the adjuvant treatment following resection of pancreatic cancer, either as single agent or a radiosensitizer concurrent with radiotherapy. The survival benefit of adjuvant 5-FU was demonstrated in ESPAC-1 study [88]. Recently, the results of 2 randomized controlled trials indicated that there was no survival difference between 5-FU and gemcitabine-based adjuvant therapy [89, 90]. However, the response rate of 5-FU in advanced disease is low [5]. These studies highlighted the need for predictive biomarkers to assist clinicians with treatment decision.

5-FU is a pyrimidine analog. It interferes with DNA synthesis, through its cytotoxic nucleotides, by inhibiting the target enzyme thymidylate synthase (TYMS) or incorporates into RNA and DNA. It is catabolized to its inactive form, 5,6-dihydro-5-fluorouracil, by the rate limiting enzyme dihydropyrimidine dehydrogenase (DPYD).

Decreased DPYD activity can lead to the accumulation of 5-FU and its active metabolites. Severe thrombocytopenia, neurological symptoms, cardiotoxicity and death had been reported in pancreatic cancer patients with DPYD deficiency following administration of 5-FU or capecitabine, an oral fluoropyrimidine [91, 92]. Over 40 different germline polymorphisms have been reported, of which 17 mutations have been associated with severe 5-FU toxicity. *DPYD**2A, the most common *DPYD* polymorphism associated with 5-FU toxicity is caused by a 5' splice site mutation at intron 14 G>A resulting in the formation of an inactive enzyme [91]. Down regulation of DPYD activity by promoter methylation had recently been identified as an alternative mechanism that regulates DPYD activities [93]. Unfortunately, up to two-thirds of patients who experience treatment toxicity do not have a molecular basis for DPYD deficiency. The low frequency of *DPYD* polymorphisms as well as the low sensitivity and specificity of genotyping limit the utility of DPYD pharmacogenetics in clinical practice.

Intratumoral DPYD of pancreatic cancer was 2-3 fold higher than adjacent normal tissue and fluoropyrimidine sensitive tumors such as colorectal cancer [94, 95]. In a study examining the role of adjuvant 5FU liver perfusion chemotherapy following pancreatectomy, survival benefit was only observed in patients with low or negative intra-tumoral DPYD expression [96]. Similarly, in a small series of 28 patients with advanced pancreatic cancer treated with S-1, an oral fluoropyrimidine derivative consisting of tegafur, gimeracil (DPYD inhibitor) and oteracil potassium, intratumoral DPYD mRNA expression levels among the responders were significantly lower than the non-responders [97].

The transcriptional efficiency of *TYMS* correlates with the number of a 28-base pair tandem repeats. The number of tandem repeats varies from 2 (*TYMS* 2R) to 9 (*TYMS* 9R) copies. In Caucasian population, *TYMS* 2R and 3R are the most common alleles with the frequency of 16%, 51-55% and 29-32% for 2R/2R, 2R/3R and 3R/3R, respectively [98]. *TYMS* polymorphisms have been reported to predict for capecitabine response in colorectal cancer. Patients with *TYMS* 2R/2R genotype had superior response rate (75% vs. 8%) but had more grade 3 treatment-related toxicities when compared to patients with 3R/3R genotype [99]. The first genotype-directed study of second line capecitabine in advanced pancreatic cancer was recently reported [100]. The study was designed to evaluate the overall survival and toxicity in patients harboring *TYMS* 2R/2R allele when treated at capecitabine dose of 2000 mg/m²/day, equivalent to a 20% reduction from standard dose. Patients who underwent genetic screening but did not have *TYMS* 2R/2R received treatment according to their primary oncologists. Eighty patients were screened for *TYMS* 2R/2R genotype, however only 4 out of 16 eligible patients received capecitabine. Twelve patients did not receive capecitabine because of declining performance status. Consistent with previous report, all subjects experienced significant grade 3 or more non-haematologic toxicities despite treatment at a reduced capecitabine dose. The study was closed early due to slow accrual and treatment toxicity.

Elevated intratumoral *TYMS* expression was commonly observed in pancreatic cancers. There are several conflicting reports with regards to the prognostic value of intratumoral *TYMS* level. High *TYMS* expression was associated with a better survival outcome in patients who had resectable pancreatic cancer regardless of the status of the adjuvant treatment [101]. Conversely, the disease free survival was reported to be shorter after surgical resection of pancreatic cancer in patients with high *TYMS* and DPYD levels [102]. In another study, 132 patients who had undergone pancreatic resection for pancreatic cancer, high *TYMS* expression was associated with poorer outcome (median survival, 12 vs. 18.3 months; $p=0.026$) [103]. Significant reduction in the risk of death was observed in patients with high *TYMS*-expressing tumors treated with 5-FU-based adjuvant therapy (relative risk, 0.37; $P=0.0006$). 5-FU-based adjuvant therapy did not appear to benefit patients with low *TYMS*-expressing pancreatic cancer.

Thymidine phosphorylase (TYMP) catalyzes the conversion of oral capecitabine to 5-FU. The ratio of TYMP to DPYD correlated with the efficacy of fluoropyrimidine 5 DFUR in human cancer xenograft models. In a phase I and phase II study of capecitabine with concomitant radiotherapy in patients with unresectable locally advanced pancreatic cancer, TYMP:DPYD ratios did not correlate with treatment toxicities or treatment response. No correlation was made to overall survival because of the small sample size [104, 105].

The impact of epigenetic alternation and treatment outcome was evaluated in a large study involving 195 patients from RTOG 9704 [106]. Patients were randomized to adjuvant 5-FU or gemcitabine, 1 month before and 3 months after radiation therapy concurrent with infusional 5-FU. Exploratory analysis suggested that low histone H3 lysine 4 demethylation (H3K4me2) and H3 lysine 18 (H3K18ac) were associated with worse overall survival in

patients receiving adjuvant 5-FU but not in those receiving gemcitabine. Although it is still not clear how histone modification resulted in 5-FU resistance, the authors had postulated that acetylation of histone H3 lysine 18 (H3K18ac) led to transcriptional silencing of thymidylate synthase and hence 5-FU resistance.

Platinum Agents

The platinum agents cisplatin and oxaliplatin have been used in combination with gemcitabine with marginal survival benefit as randomized phase III trials have reported no advantage over gemcitabine alone, whilst a meta-analysis showed a small benefit (HR, 0.95; 95% CI, 0.76 – 0.96) [3]. Platinum agents binds to DNA, leading to DNA adducts that inhibit DNA synthesis. Reduced sensitivity to platinum agents is mediated through the DNA repair pathways: base excision repair, nucleotide excision repair (NER), post-replication repair, and mismatch repair (MMR) [107, 108]. Key proteins in the NER pathway are the excision repair cross complementing group 1 and 2 (ERCC 1 and 2) and overexpression of ERCC 1 confers resistance to platinum analogs [109]. In a retrospective study, low levels of ERCC1 by IHC and RT-PCR was associated with higher survival in pancreatic cancer patients treated with platinum and fluoropyrimidine as second-line chemotherapy [110]. The polymorphism in *ERCC 1* codon C118T is predictive of sensitivity with improved survival in patients treated with cisplatin or oxaliplatin in a range of solid tumors [111-114]. In a study of patients with pancreatic cancer treated with advanced or relapsed pancreatic cancer treated with S-1 with or without cisplatin, in the patients receiving cisplatin and S-1, *ERCC1* 118 CT/TT genotypes had longer progression free and overall survival compared with CC genotypes (11 months vs. 3.5 months and 25 months vs. 13.7 months respectively) [115].

Irinotecan

As a single agent, irinotecan has demonstrated a modest activity in advanced pancreatic cancer [116]. In 2 phase III studies, irinotecan/gemcitabine produced a higher tumor response rate than gemcitabine alone, but did not significantly impact on survival [13, 117]. Irinotecan is converted into its active metabolite, SN-38 by carboxylesterase-2 [118]. The movement of SN-38 across cell membrane is mediated through several influx (organic anion-transporting polypeptide; SLCO1B1) and efflux (ATP-binding cassette transporter family; ABCB1, ABCC1, ABCC2, ABCG2) transporters. SN-38 exerts its anticancer effect by stabilizing a ternary complex between the topomerase I-DNA cleavable complex, which in turn causes replication fork arrest and DNA double strand break. Several UGT1A isoenzymes mediate the conversion of SN-38 to its inactive glucuronide (SN-38G). UGT1A1 is the major UGT1A isoform responsible for glucuronidation of SN-38, whereas both UGT1A7 and UGT1A9 play a minor role.

Irinotecan is one of the models for the utility of pharmacogenetics in personalized therapy. In 2005, the US FDA had recommended a revision of the irinotecan label to include a warning on the risk of severe neutropenia in subjects homozygous for *UGT1A1**28 alleles. *UGT1A1**28 is caused by a insertion of a dinucleotide (TA) repeat in the TATA box of the *UGT1A1* promoter (TA)₆ resulting in the (TA)₇ repeat and a reduction of transcriptional efficiency. Decreased level of UGT1A1 leads to reduction in SN-38 clearance and increased systemic exposure to this enzyme. Several clinical studies, mostly involving colorectal cancer patients, had demonstrated the association between *UGT1A1**28 and severe neutropenia [119-126]. It has been prospectively demonstrated that in patients homozygous for *UGT1A1**28, the risk of developing grade 4 neutropenia was increased by 9.3 folds. Grade 4 neutropenia was seen in 50%, 12.5% and 0% of patients who were homozygous, heterozygous for *UGT1A1**28 and those lacking this allele, respectively, when treated with irinotecan at 350 mg/m² [122]. In the largest published series to date, common polymorphisms of UGT1A1, UGT1A7, UGT1A9 and their haplotypes were investigated for

their role in predicting for irinotecan toxicity and response [123]. Although the risk of developing severe neutropenia in the first treatment cycle was higher in patients homozygous for *UGT1A1**28, *UGT1A1**60 (3279T>G) and *UGT1A7**3, only *UGT1A7**3 remain significant in multivariate analysis. *UGT1A1**28 was associated with improved response rate and time to disease progression. In addition, female gender and the absence of UGT1A haplotype I (reference sequence alleles with the exception of *UGT1A9**22) was associated with severe hematological toxicity during the entire course of treatment. The association between UGT1A polymorphisms and diarrhea is less consistent. At least 3 studies had demonstrated increase treatment related diarrhea with irinotecan base chemotherapy in subjects homozygous for *UGT1A1**28 [125, 127, 128]. However, the relationship was not demonstrated in a meta-analysis of 9 studies [129]. In the same study, the association of *UGT1A1**28 with neutropenia was only observed in patients treated with medium to high dose irinotecan (>150 mg/m²). In an attempt to establish the optimal dosing of irinotecan-based chemotherapy (FOLFIRI), patients with *1/*1 and *1/*28 genotype were treated at escalating doses of 2-weekly irinotecan from 215 mg/m². The maximal tolerated doses for *1/*1 and *1/*28 genotypes were 370 mg/m² and 310 mg/m², respectively and were considerably higher than the standard recommended dose 180 mg/m² [130]. To date, the relationship of UGT1A1 polymorphisms and treatment outcome has not been validated in clinical trial involving pancreatic cancer patients.

In East Asian population, the frequency of the low activity allele *UGT1A1**6 (13 to 23%) is higher than *UGT1A1**28 (1 to 5%) [131-134]. *UGT1A1**6 homozygosity was recently associated with lower tumor response and higher incidence of severe neutropenia in Korean patients treated with irinotecan and cisplatin for lung cancer [135]. Similarly, in 2 Japanese studies, patients with haplotype containing *UGT1A1**6 and *UGT1A1**28 had reduced SN-38G/SN-38 ratio and were most susceptible to severe neutropenia [136, 137]. In Japan, the label of irinotecan was updated to include information treatment toxicity and *UGT1A1**6/*UGT1A1**28 genotypes (<http://www.pmda.go.jp/english/service/pdf/precautions/PMDSI-248.pdf>).

CYP3A4 enzyme is involved in the conversion of irinotecan to the inactive metabolites APC, 7-ethyl-10-(4-N-(5-aminopentanoic)-1-piperidino)-carbonyloxy-camptothecin, NPC, 7-ethyl-10-(4-amino-1-piperidino)-carbonyl-oxy-camptothecin and M4. It was previously demonstrated that midazolam clearance, a surrogate for CYP3A4 activity, correlated with irinotecan clearance [138]. The role of CYP3A4 phenotyping to individualized therapy was evaluated in a study where patients were randomized to conventional dosing of 350 mg/m² or model-based dosing using CYP3A4 phenotyping with midazolam clearance, height and - glutamyltransferase [139]. The use of model-based dosing had led to a trend towards a reduction of inter-individual variability in the systemic exposure of irinotecan and SN-38 by 19% and 25%, respectively (p>0.22). The incidence of grade 3 and 4 neutropenia was 4.5 times lower in patients dosed by model-based approach (p=0.013).

Several polymorphisms in genes encode for transporters involved in irinotecan pathways had been reported to influence irinotecan pharmacokinetics and toxicity [126, 140-143]. *ABCB1**2 haplotype with variants 1236C>T, 2677G>T and 3435C>T were associated with reduced renal clearance of irinotecan and SN-38 [144]. However, there were conflicting reports on *ABCB1* variants with irinotecan exposure and toxicity [141, 145]. In a comprehensive analysis of over forty polymorphisms in 12 genes relevant to irinotecan pathways, *ABCC1* IVS11 -48C>T and *SLCO1B1**1b 338A>G, baseline neutrophil counts, sex, and race could account for almost half of the variation in nadir neutrophil counts [146]. The functional significance of these polymorphisms had yet to be determined. *SLCO1B1**1b 338A>G, on the other hand, was demonstrated to affect transport activity due to reduced membrane expression [147], and patients with variant alleles *SLCO1B1**15 (338A>G,

521T>C) were associated with lower irinotecan clearance and increased systemic exposure to irinotecan and SN-38 [143]. Taken together, interindividual variation in irinotecan disposition and toxicity can partially be accounted for by *UGT1A* haplotypes and CYP3A4 phenotyping. ABC and SLCO transporters may influence irinotecan disposition but further validation is needed to assess their effect on treatment toxicity, as conflicting results had been reported. In addition, non-genetic variables such as irinotecan dose, gender and race may contribute to variation in treatment toxicity.

Molecular Targeted Agents

Epidermal Growth Factor Inhibitors

The epidermal growth factor receptor (EGFR) is over-expressed in pancreatic cancer and is associated with poorer survival [148]. Activation of the EGFR results in the downstream activation signaling pathways such as the ras-raf-MAPK and the phosphatidylinositol 3-kinase/protein (PI3K) - Akt pathways, leading to the inhibition of apoptosis, promotion of cellular proliferation, angiogenesis, and invasion. Inhibitors of EGFR include erlotinib, a small molecule that competes for the ATP binding site within the tyrosine kinase domain of the EGFR; and cetuximab, a recombinant humanized anti-EGFR monoclonal antibody. Studies of combination gemcitabine with either erlotinib or cetuximab, in advanced stage pancreatic cancer have reported marginal benefit over gemcitabine alone. A slight survival benefit was seen with erlotinib combined with gemcitabine (6.2 months vs. 5.9 months) [20] vs. gemcitabine alone whilst no survival benefit was seen with cetuximab/gemcitabine [21]. The limited benefit of the addition EGFR inhibitors to gemcitabine warrants pharmacogenetic studies in patients with pancreatic cancer.

A potential surrogate marker for efficacy is skin rash. Retrospective analysis suggests the intensity of erlotinib-related skin rash was associated with improved survival and progression free survival [20, 149]. The median survival and one year survival for patients with grade 0, 1, and 2 rash were 5.3, 5.8 and 10.5 months respectively and 16%, 9% and 43% respectively (HR, 0.47, $P < 0.001$). Similarly, grade 2 rash was strongly correlated with improvements in PFS (HR, 0.43 for grade 2 versus grade 0, $P < 0.001$) [20, 149]. In a recent study by Rudin *et al.*, erlotinib trough levels and *EGFR* intron 1 polymorphism (discussed further below) was associated with skin rash [150]. Further prospective studies are warranted to determine the role of erlotinib dose-escalation leading to rash to improve the efficacy of EGFR tyrosine kinase inhibitors (TKIs) [151].

An association between *ABCG2*, the gene encoding an efflux transporter, and erlotinib pharmacokinetics has been reported. The genotypes *ABCG2* 1143 C/T or TT and -15622 C/T or T/T conferred lower *ABCG2* levels and the higher erlotinib area under the curve and maximum concentration respectively [150]. Polymorphism of genes involved in erlotinib metabolism may play a role in the development of rash and treatment efficacy.

Somatic activating *EGFR* mutations and *EGFR* gene copy number are determinants of response to EGFR TKIs in patients with non-small cell lung cancer [152, 153]. *EGFR* mutations in human pancreatic cancers however, are rare, with a frequency of 0-3.5% [154-156]. There are no studies on the prognostic and predictive role of *EGFR* mutations in pancreatic cancer patients treated with an EGFR TKI. *EGFR* gene copy number is increased in human pancreatic carcinoma in over 40% of cases [155, 157] but was not associated with survival [155]. In a retrospective analysis of samples from patients with pancreatic cancer receiving erlotinib and gemcitabine, no association between gene copy number and survival was reported [158]. In the same study, *Kras* mutation status was also not associated with survival. Further studies are required to determine the predictive role of *Kras* mutations and *EGFR* gene copy number in patients with pancreatic cancer.

Another member of the EGFR family, ErbB3, may be implicated in erlotinib sensitivity. Pancreatic cancer cell lines lacking ErbB3 were reported to be less dependent on EGFR signaling and were resistant to erlotinib [159] whilst in another *in vitro* study, ErbB3 was activated by EGFR through AKT and was a determinant of erlotinib sensitivity [160].

EGFR intron 1 has a highly polymorphic (CA) repeat region, typically consisting of 14–21 repeats with allele 16 (alleles named according to the number of repeats) having the highest frequency (42%) in Caucasians [161]. Increased CA repeat length is associated with decreasing transcription efficiency with allele 21 showed 80% reduction of gene expression compared with the shorter allele 16 [162]. In patients with colorectal cancer, repeats <20 per allele was associated with more aggressive disease and decreased survival [163, 164]. *EGFR* CA repeats is also associated with anti-EGFR drug sensitivity in colorectal, breast and head and neck cancer [165–167] with decreased repeat length associated with increased anti-EGFR response. In a retrospective clinical study of patients with resected pancreatic carcinoma, shorter CA repeats with a median sum of <36 was associated with a poorer prognosis with a median survival of 13.7 months compared with 30.6 months in patients with median sum of ≥36 repeats [168], whilst shorter *EGFR* intron length was associated with *in vitro* response to erlotinib. In a prospective pharmacogenetic and pharmacokinetic study of patients with non-small cell lung, head and neck, and ovarian cancers treated with erlotinib, an association between *EGFR* intron 1 polymorphism and skin rash was reported [150]. However, no clinical studies have been reported on the predictive role of EGFR intron length and anti-EGFR therapy in pancreatic cancer.

In a retrospective study of patients with advanced pancreatic cancer treated with chemotherapy combined with erlotinib, molecular analysis on *Kras* mutation status, EGFR expression by IHC and FISH, PTEN expression, *EGFR* intron 1 polymorphism and *EGFR* exon 13 R497K polymorphism, only *Kras* mutation status in codon 12 was associated with survival on univariate analysis with a HR 1.68 (95% CI 1.17–2.41; *p*=0.005) [169].

Angiogenesis Inhibitors

Vascular epithelial growth factor (VEGF) and VEGF-receptors (VEGFR) are frequently overexpressed in pancreatic cancer. Overexpression of VEGF was associated with liver metastasis and poorer outcome [170, 171]. Bevacizumab is a humanized monoclonal antibody against VEGF. Several phase II studies of bevacizumab combinations yielded promising results with median survival ranging from 8.2 to 9.8 months [172–174]. Despite the biological rationale of combining bevacizumab with gemcitabine-based chemotherapy, it has not been translated into improved survival in 2 phase III studies [22, 23]. The addition of bevacizumab to gemcitabine and erlotinib led to a modest improvement of progression free survival but without significant impact on overall survival [23]. With these disappointing results, it unlikely that bevacizumab will have a role in the treatment of pancreatic cancer patients unless predictive biomarkers are available to identify subjects that may potentially benefit from bevacizumab.

The US FDA had approved the first line use of bevacizumab in combination with paclitaxel in patients with HER2 negative metastatic breast cancer based on the result of E2100 study [175]. In the phase III study, the addition of bevacizumab almost doubled the PFS from 5.9 to 11.8 months, although it did not improve the overall survival. Subsequent analysis of VEGF polymorphisms demonstrated that patients homozygous for promoter variants *VEGF* -2578C>A and -1154G>A had improved treatment outcome when treated with paclitaxel and bevacizumab with median overall survival of 37 and 46.5 months, respectively compared with 25.2 months in the control arm. This finding is potentially relevant to a large number of patients as the variant alleles were fairly common in Caucasians with frequencies of 42% for *VEGF* -2578C>A and 29% for *VEGF* -1154G>A. In the same study, it was

observed that individuals with promoter genotypes *VEGF*-634CC and -1498TT had fewer incidence of grade 3 and 4 hypertension. In addition, patients who experienced severe hypertension had improved median overall survival. These polymorphisms were not found to correlate with clinical response when prospectively analysed in 137 women with locally recurrent or metastatic breast cancer treated with bevacizumab containing therapy. However, patients homozygous for *VEGF*936C had shorter time to progression compared to those with 936T alleles [176]. The combination of *TYMS* enhancer region and *VEGF*405G>C polymorphisms was predictive for progression free survival in metastatic colorectal cancer patients treated with bevacizumab, capecitabine and oxaliplatin [177]. Several studies, unfortunately, had failed to validate these findings [178, 179].

It was recently reported in the AVITA trial that patients with advanced pancreatic cancer did not benefit from the addition of bevacizumab to gemcitabine and erlotinib. However, an exploratory analysis of *VEGFR-1* polymorphisms in a small subset of patients had demonstrated superior median survival (309 days vs. 226 days) in those homozygous for the rs9582036AA genotype compared with CC genotype patients. Further validation is needed to evaluate if these VEGF/VEGFR polymorphisms may be used to identify subset of pancreatic cancer patients that may benefit from bevacizumab and those who experience severe toxicity so that closer monitoring or aggressive prophylactic treatment may be administered to reduce the incidence of severe hypertension.

Although VEGF is probably the most prominent activator of tumor angiogenesis, there are alternative proangiogenic factors, upstream regulators and molecules that stimulate endothelial cells migration. In an exploratory study, 33 polymorphisms in 30 candidate genes involved in the angiogenic pathway were evaluated for their association with treatment response to bevacizumab and metronomic cyclophosphamide in patients with ovarian cancer [180]. Significant poorer PFS was observed in patients homozygous for *CXCR2*785C>T (3.7 vs. 7.4 months) and <9 adrenomedullin 3 CA dinucleotide repeats (3.4 vs. 7.2 months) when compared to those homozygous for wild type alleles. In addition, patients with at least 1 copy of *IL-8* -251T>A (increased IL-8 production) were associated with poorer response (0 vs. 19%). Increased IL-8 production was previously observed in subjects with *IL-8* -251T>A and supports the hypothesis that overexpression of IL-8 may be implicated in resistance to bevacizumab therapy [181]. The angiogenic effect of IL-8 is mediated by combined activation of CXCR1 and CXCR2 receptors, although the functional significance of *CXCR2*785C>T is unclear. Adrenomedullin is a vasodilatory peptide that can induce tumor angiogenesis independent of VEGF [182]. The adrenomedullin 3 CA dinucleotide repeats polymorphism has not been demonstrated to influence the transcriptional efficiency of the adrenomedullin gene, but higher number of dinucleotide repeats were associated with increased risk of hypertension [183]. Further study will be required to validate the predictive value of these markers.

Clinical Genome-Wide Investigations in Pancreatic Cancer

The studies described above were focused on single candidate genes. The completion of the Human Genome Project has opened the era of genomic medicine. New genomic methodologies are now available to interrogate the entire genome of patients for hundreds of thousands of heritable genetic variants, leading to the identification of new genes, additional to those already known to play a role in the outcome of chemotherapy. These new strategies are expected to improve the treatment outcome of cancer patients.

A genome-wide association study (GWAS) was conducted in advanced pancreatic cancer patients treated with chemotherapy in CALGB 80303. Patients receiving gemcitabine were randomized to either placebo or bevacizumab. The study did not show a survival advantage by bevacizumab [22]. As part of the study, we prospectively collected germline DNA for

pharmacogenetic studies. We interrogated >500,000 SNPs in 294 genetically European patients, and established an association with an amino acid changing SNP in the IL17F gene conferring reduced survival in patients in both arms. There was no evidence of an interaction with bevacizumab, suggesting that this SNP is prognostic rather than predictive of survival [184]. Wildtype IL17F is a pro-inflammatory, anti-angiogenic cytokine. The variant IL17F antagonizes wildtype IL17F [185], potentially resulting in a proangiogenic effect. Replication studies in pancreatic cancer and other solid tumors are ongoing.

This study led to the discovery of novel genetic markers of survival in patients treated with chemotherapy, and provides the foundation for individualizing therapy of advanced pancreatic cancer patients by identifying subgroup of patients with a more favorable prognosis according to their heritable genetic make-up. Similarly, tumor DNA sequencing efforts in pancreatic cancer [186] could identify novel targets and pathway to be exploited for therapeutic intervention.

Conclusions and Look at the Role of Personalized Medicine in Advanced Pancreatic Cancer

As the benefits of systemic therapies in the treatment of pancreatic cancer remain limited, it is crucial to identify patients who will benefit from therapy whilst minimizing toxicity. Whilst tumor and germline determinants of resistance and toxicity have been identified, many of the markers described are either in the pre-clinical stage or require further validation in appropriately powered prospective clinical studies prior to their application in clinical practice. Novel strategies for more efficient clinical validation of biomarkers are needed [187]. In addition, an improvement in the understanding of the complexity of the molecular pathways involved in pancreatic carcinogenesis, invasion and metastasis may allow identification of new targets therefore facilitating drug development. The National Cancer Institute Gastrointestinal Cancer Steering Committee has provided recommendations to improve outcomes in patients with pancreatic cancer. This includes greater understanding of tumor biology, optimal use of preclinical models, establishment of tissue repositories, biomarker development, and closer collaboration between clinical and basic researchers and pharmaceutical industry to develop rationally designed clinical trials [188].

Given the promising list of candidate biomarkers together with advances in the field of pharmacogenetics and pharmacogenomics, and improved collaboration among pancreatic cancer researchers, it is hoped that an individualized approach to treatment selection for patient with pancreatic cancer will be achieved in the near future.

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Table 1
Intra-Tumoral Molecular Determinants of Clinical Outcome in Patients with Pancreatic Cancer

Molecular Determinant	General Function	Expression	Clinical Outcome
Equilibrative nucleoside transporter 1 (hENT1)	Cellular uptake of nucleosides, and cytotoxic agents such as gemcitabine	Overexpression by IHC	Longer survival in advanced stage pancreatic cancer [33]
		High mRNA levels	Longer survival in resected pancreatic cancer [34]
		Overexpression by IHC	Longer survival in patients treated with adjuvant gemcitabine before and after chemo-irradiation [35]
Concentrative nucleoside transporter 1 (hCNT1)	Sodium-dependent and pyrimidine-selective nucleoside transporter	Reduced expression by IHC	Reduced survival, increased recurrence rate in patients treated with adjuvant gemcitabine [36]
deoxycytidine kinase (DCK)	Phosphorylation of deoxyribonucleosides and their nucleoside analogs.	Reduced expression	Reduced survival, reduced progression-free survival in patients with advanced stage treated with gemcitabine [39]
Ribonucleotide reductase M1 (RRM1)	Production of deoxyribonucleotides prior to DNA synthesis	High mRNA levels	Reduced response and survival in patients with recurrent disease [46]
BCL2-associated $\times$ protein (Bax)	Apoptotic activator	Positive by IHC	Increased survival in patients with resected pancreatic cancer [65]
Hu antigen R (HuR)	Modulates mRNA translation	Reduced expression by IHC	Reduced survival in patients with resected pancreatic cancer [69]
Dihydropyrimidine dehydrogenase (DPYD)	Catabolism of thymidine and uracil	Increased expression	Reduced progression free survival in patients with resected pancreatic cancer [87]
		Reduced or no expression	Increased survival in patients treated with adjuvant 5FU [81]
		Increased mRNA	Increased response in patients receiving palliative S-1 [82]
Thymidylate synthetase (TYMS)	DNA replication and repair	Increased expression	Increased survival in patients with resected pancreatic cancer [86]
		Increased expression	Reduced progression free survival in patients with resected pancreatic cancer [87]
		Increased expression	Reduced survival in patients with resected pancreatic cancer [88]
		Increased expression	Increased survival in patients treated with adjuvant 5FU [88]
Thymidine phosphorylase (TYMP)	Involved in nucleotide synthesis	TP: DPYD ratio	No association with response/toxicity in patients with unresectable

Molecular Determinant	General Function	Expression	Clinical Outcome
			locally advanced pancreatic cancer treated with radiotherapy [89, 90]
Epidermal growth factor receptor (EGFR)	Inhibition of apoptosis, promotion of cellular proliferation, angiogenesis, and invasion.	Gene copy number	No association with survival in patients with unresectable or metastatic cancer treated with gemcitabine based chemotherapy [139] or with erlotinib and gemcitabine [132]

Table 2
Genetic Polymorphisms Associated with Clinical Outcome in Patients with Pancreatic Cancer

Gene	General Function	Polymorphism	SNP Reference ID	Clinical Outcome
Equilibrative nucleoside transporter 1 (<i>hCNT1</i>)	Sodium-independent nucleosides	-201A>G -549T>C 913C>T	760370 324148 9394992	No association with outcome in patients treated with neoadjuvant gemcitabine- based chemotherapy plus radiation therapy [50]
Concentrative nucleoside transporter 1 (<i>hCNT1</i>)	Sodium-dependent and pyrimidine-selective nucleoside transporter	-16A>G -9C>A	2242048 8187758	No association with outcome in patients treated with neoadjuvant gemcitabine- based chemotherapy plus radiation therapy [50]
Concentrative nucleoside transporter 2 (<i>hCNT2</i>)	Sodium-dependent and purine-selective transporter	-38C>A -17C>T	1060896 11854484	No association with outcome in patients treated with neoadjuvant gemcitabine- based chemotherapy plus radiation therapy [50]
Concentrative nucleoside transporter 3 (<i>hCNT3</i>)	Sodium-dependent, pyrimidine- and purine-selective transporter	-69C>T 25A>G	7853758 7867504	No association with outcome in patients treated with neoadjuvant gemcitabine- based chemotherapy plus radiation therapy [50] Increased risk of neutropenia with AA genotypes in patients treated with neoadjuvant gemcitabine-based chemotherapy plus radiation therapy [50]
Cytidine deaminase (<i>CDA</i>)	Converts cytosine and deoxycytosine to uridine and deoxyuridine respectively. Metabolises gemcitabine to difluorodeoxyuridine	208G>A	60369023	Reduced gemcitabine clearance, increased haematologic toxicities with homozygote variant in patients treated with palliative gemcitabine [42-44]
		-76A>C	2072671	No association with survival in patients treated with neoadjuvant gemcitabine- based chemotherapy plus radiation therapy [50]
		111C>T	1048977	Increased risk of neutropenia with T allele in patients treated with neoadjuvant gemcitabine-based chemotherapy plus radiation therapy [50]
Deoxycytidine kinase (<i>DCK</i>)	Phosphorylation of deoxyribonucleosides and their nucleoside analogs.	-1205C>T	4694362	No association with outcome. Increased risk of neutropenia with T allele in patients treated

Gene	General Function	Polymorphism	SNP Reference ID	Clinical Outcome
				with neoadjuvant gemcitabine- based chemotherapy plus radiation therapy [50]
		9846A>G	12648166	Increased risk of neutropenia with G allele in patients treated with neoadjuvant gemcitabine-based chemotherapy plus radiation therapy [50]
dCMP deaminase (<i>DCTD</i>)	Involved in the deamination of dCMP to dUMP. Involved in the metabolism of gemcitabine monophosphate to difluorodeoxyuridine monophosphate	-47T>C	7663494	No association with outcomes in patients treated with neoadjuvant gemcitabine- based chemotherapy plus radiation therapy [50]
Ribonucleotide reductase M1 (RRM1)	Production of deoxyribonucleotides prior to DNA synthesis	42G>A 33A>G 27C>A	1042858 3177016 183484	No association with outcomes in patients treated with neoadjuvant gemcitabine- based chemotherapy plus radiation therapy [50]
RecQ protein-like (DNA helicase Q1-like) (<i>RecQ1</i>)	DNA repair including mismatch repair, nucleotide excision repair and direct repair	159A>C	13035	Reduced survival with in patients with CC genotypes in all stages of pancreatic cancer, resected or treated with chemotherapy/radiation therapy [53] and reduced survival in patients with AC/CC genotypes treated with neoadjuvant gemcitabine-based chemotherapy plus radiation therapy [54]
		-86A>G	4987216	No association with survival in all stages of pancreatic cancer, resected or treated with chemotherapy/radiation therapy [53] or in patients treated with neoadjuvant gemcitabine-based chemotherapy plus radiation therapy [54]
RAD54-like (<i>S. cerevisiae</i>) (<i>RAD54L</i>)	Homologous recombination related repair of DNA double-strand breaks	157C>T	1048771	Reduced survival in patients with TT genotypes in all stages of pancreatic cancer, resected or treated with chemotherapy/radiation therapy [53] and reduced survival in patients with CT/TT genotypes treated with neoadjuvant gemcitabine-based chemotherapy plus radiation therapy [54]
Excision repair cross-complementing rodent repair deficiency, complementation group 1 (<i>ERCC1</i>)	Nucleotide excision repair pathway	118C>T	11615	Increased progression free and overall survival in patients with inoperable or relapsed

Gene	General Function	Polymorphism	SNP Reference ID	Clinical Outcome
				pancreatic cancer with CT/TT genotypes treated with cisplatin and S-1 [99]
X-ray repair complementing defective repair in Chinese hamster cells 1 (<i>XRCC1</i>)	Repair of DNA single-strand breaks	22C>T	1799782	No association with survival in patients treated with neoadjuvant gemcitabine- based chemo-therapy plus radiation therapy [54]. Reduced survival in patients with resected, locally advanced or metastatic pancreatic cancer with TT genotypes treated with chemotherapy/radiation therapy [51]
		-4A>G	25487	No association with survival in patients treated with neoadjuvant gemcitabine- based chemotherapy plus radiation therapy [54] in patients with resected, locally advanced or metastatic pancreatic cancer with TT genotypes treated with chemotherapy/radiation therapy [51]
X-ray repair complementing defective repair in Chinese hamster cells 2 (<i>XRCC2</i>)	Transcription-coupled nucleotide excision repair	442G>A	3218536	Reduced survival with AA genotypes in patients treated with surgery [53]. No association with survival in patients treated with neoadjuvant gemcitabine- based chemotherapy plus radiation therapy [54]
X-ray repair complementing defective repair in Chinese hamster cells 3 (<i>XRCC3</i>)	Homologous recombination to maintain chromosome stability and repair DNA damage	IVS7-14 A>G	1799796	No association with survival in patients with all stages of pancreatic cancer resected or treated with chemotherapy/radiation therapy [53]. No association with survival in patients treated with neoadjuvant gemcitabine- based chemotherapy plus radiation therapy [54]
		-53C>T	861539	No association with survival in patients with all stages of pancreatic cancer resected or treated with chemotherapy/radiation therapy [53]. No association with survival in patients treated with neoadjuvant gemcitabine- based

Gene	General Function	Polymorphism	SNP Reference ID	Clinical Outcome
				chemotherapy plus radiation therapy [54]
Three prime repair exonuclease 1 (<i>TREX1</i>)	Exonuclease that may serve a proofreading function for DNA polymerase.	-460C>T	11797	Reduced response and reduced survival in patients with CC genotypes treated with neoadjuvant gemcitabine- based chemotherapy plus radiation therapy [55]
		-9A>C	11925638	No association with survival in patients treated with neoadjuvant gemcitabine- based chemotherapy plus radiation therapy [55]
Tumor protein p73 (<i>TP73</i>)	Transcription factor involved in cellular responses to stress and development	4G>A	2273953	In patients treated with neoadjuvant gemcitabine-based chemotherapy plus radiation therapy, a reduced response [55] and reduced time to progression [59] was seen with GA/AA genotypes and reduced overall survival [55] with AA genotypes
		14C>T	1801173	No association with outcomes in patients treated with neoadjuvant gemcitabine- based chemotherapy plus radiation therapy [59]
Polymerase (DNA directed), beta (<i>POLB</i>)	Base excision repair	165A>G	2272615	Increased survival in patients with GG genotypes resected or locally advanced or metastatic pancreatic cancer treated with chemotherapy/radiation therapy [51]
		-2133T>C	1799782	Increased survival in patients with CC genotypes resected or locally advanced or metastatic pancreatic cancer treated with chemotherapy/radiation therapy [51]
APEX nuclease (multifunctional DNA repair enzyme) 1 (<i>APEX1</i>)	Multifunctional DNA repair enzyme	5T>G	3136820	Increased survival in patients with GG genotypes resected or locally advanced or metastatic pancreatic cancer treated with chemotherapy/radiation therapy [51]
8-oxoguanine DNA glycosylase (<i>hOGG1</i>)	DNA repair enzyme	2657G>A	293794	Increased survival in patients with AA genotypes resected or locally advanced or metastatic pancreatic cancer treated with

Gene	General Function	Polymorphism	SNP Reference ID	Clinical Outcome
				chemotherapy/radiation therapy [51]
		-315C>G	1052133	No association with outcome in patients resected or locally advanced or metastatic pancreatic cancer treated with chemotherapy/radiation therapy [51]
Exonuclease 1 (<i>EXO1</i>)	5 to 3 exonuclease involved in mismatch repair and recombination	20G>A	735943	Reduced survival with GA/AA genotypes in patients treated with neoadjuvant gemcitabine-based chemotherapy plus radiation therapy [55]
		105G>A 59C>T	4149965 9350	No association with outcome in patients treated with neoadjuvant gemcitabine- based chemotherapy plus radiation therapy [55]
mutL homolog 1, colon cancer, nonpolyposis type 2 (<i>MLH1</i>)	DNA mismatch repair	-92G>A 41G>A	1800734 2308317	No association with outcome in patients treated with neoadjuvant gemcitabine- based chemotherapy plus radiation therapy [55]
		-169C>T	2286940	Reduced tumor respectability in patients with CC genotypes treated with neoadjuvant gemcitabine-based chemotherapy plus radiation therapy [55]
mutS homolog 2, colon type 1 (<i>MSH2</i>)	DNA mismatch repair	23G>A -6T>C	4987188 2303428	No association with outcome in patients treated chemotherapy plus radiation therapy [55]
mutS homolog 3 (<i>MSH3</i>)	DNA mismatch repair	-100G>A 3A>G	1805355 26279	No association with outcome in patients treated with neoadjuvant gemcitabine- based chemotherapy plus radiation therapy [55]
mutS homolog 6 (<i>MSH6</i>)	DNA mismatch repair	-145G>A	1042821	No association with outcome in patients treated with neoadjuvant gemcitabine- based chemotherapy plus radiation therapy [55]
postmeiotic segregation increased 1 (<i>PMS1</i>)	Repair of DNA mismatches	-4G>C	5742933	No association with outcome in patients treated with neoadjuvant gemcitabine- based chemotherapy plus radiation therapy [55]

Gene	General Function	Polymorphism	SNP Reference ID	Clinical Outcome
ligase III (<i>LIG3</i>)	Excision repair of DNA	IVS18-39G>A	2074522	No association with survival in patients treated with neoadjuvant gemcitabine- based chemotherapy plus radiation therapy [54] or patients with resected or locally advanced or metastatic pancreatic cancer treated with chemotherapy/radiation therapy [51]
ligase IV (<i>LIG4</i>)	Double-strand break repair	54C>T	1805388	No association with survival in patients treated with neoadjuvant gemcitabine- based chemotherapy plus radiation therapy [54] or patients with resected or locally advanced or metastatic pancreatic cancer treated with chemotherapy/radiation therapy [51]
thymidylate synthetase (<i>TYMS</i>)	DNA replication and repair	5' UTR of <i>TYMS</i> enhancer region.		Increased non-haematological toxicities in patients with advanced stage pancreatic cancer with 2R/2R genotypes treated with capecitabine [85]
ataxia telangiectasia mutated (<i>ATM</i>)	Cell cycle checkpoint kinase that controls cell cycle checkpoint signaling pathways	-77T>C	664677	Reduced survival with TC genotypes in patients treated with neoadjuvant gemcitabine- based chemotherapy plus radiation therapy [54]
		60G>A	66143	Increased survival with AA genotypes in patients treated with neoadjuvant gemcitabine- based chemotherapy plus radiation therapy [58]
		61A>G 47A>T	1801516 2234997	No association with survival in patients treated with neoadjuvant gemcitabine- based chemotherapy plus radiation therapy [54]
ataxia telangiectasia and Rad3 related (<i>ATR</i>)	Cell cycle checkpoint gene	340C>T -76G>A	2227928 2229032	No association with survival in patients treated with neoadjuvant gemcitabine- based chemotherapy plus radiation therapy [58]
CHK1 checkpoint homolog (<i>CHEK1</i>)	Involvement in checkpoint regulation and DNA repair and apoptotic events	35G>A	521102	Increased survival with AA genotypes in patients treated with neoadjuvant gemcitabine- based

Gene	General Function	Polymorphism	SNP Reference ID	Clinical Outcome
				chemotherapy plus radiation therapy [58]
CHK2 checkpoint homolog (<i>CHEK2</i>)	Cell cycle checkpoint regulator and putative tumor suppressor	-200T>C	2267130	No association with survival in patients treated with neoadjuvant gemcitabine- based chemotherapy plus radiation therapy [58]
cyclin-dependent kinase inhibitor 1B (<i>p27</i>)	Cyclin-dependent kinase inhibitor, tumor suppressor that induces cell cycle arrest	109T>G	2066827	Increased survival with TG/GG genotypes in patients treated with neoadjuvant gemcitabine-based chemotherapy plus radiation therapy [59]
cyclin-dependent kinase	Cyclin-dependent kinase inhibitor, tumor suppressor that blocks cell cycle progression	580C>T	3088440	Reduced time to progression with CT/TT genotypes in patients treated with neoadjuvant gemcitabine- based chemotherapy plus radiation therapy [59]
		540C>G	11515	No association with outcome in patients treated with neoadjuvant gemcitabine- based chemotherapy plus radiation therapy [59]
Mdm2 p53 binding protein homolog (<i>MDM2</i>)	Negative regulator of p53	309T>G	2279744	Reduced survival with TG/GG genotypes in patients treated with neoadjuvant gemcitabine-based chemotherapy plus radiation therapy [59]
Epidermal growth factor receptor (EGFR)	Inhibition of apoptosis, promotion of cellular proliferation, angiogenesis, and invasion.	intron 1 (CA) _n repeat		Reduced survival with <36 repeats in patients with resected pancreatic cancer [151]