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TOPIC HIGHLIGHT

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Pancreatic biomarkers: Could they be the answer?

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Abstract

Pancreatic ductal adenocarcinoma (PDA) is known for its poor prognosis. Most of the patients are diagnosed with advanced stages, when no curative treatment is available. Currently, despite extensive clinical research on PDA, the median overall survival remains short. Diagnosis delay and primary chemo-resistance due to its intrinsic biological nature may explain the challenges to improve our results. Our knowledge about the molecular biology of PDA has exponentially increased during the last decades and its use for the development of biomarkers could help to reach better results in the clinical setting. These biomarkers could be the clue for the improvement in PDA clinical research by earlier detection strategies with diagnostic biomarkers, and by an individualization of treatment approach with prognostic and predictive biomarkers. This review summarizes the current knowledge about the molecular biology of PDA and the status of the most important prognostic and predictive biomarkers.

Key words: Pancreatic adenocarcinoma; Biomarkers; Diagnosis; Prognostic; Predictive; Treatment

Core tip: Implementing the clinicopathological information with molecular characteristics for treatment individualization in pancreatic cancer seems to be one of the keys to improving survival and response to treatment. The development of new biomarkers and a better definition of the current ones are radically important. This review will summarize the most important biomarkers defined for pancreatic adenocarcinoma and their current development status.

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INTRODUCTION

Pancreatic ductal adenocarcinoma (PDA) is known for its aggressiveness and poor prognosis: it is the fourth leading cause of cancer-related death both in men and women[1]. Approximately 45220 patients are annually diagnosed with pancreatic adenocarcinoma; almost all are expected to die from the disease[2]. Five-year survival rate after the diagnosis is around 5% for all the stages, reaching 20% for the localized stages and being less than 1% for those patients diagnosed with advanced disease.

The majority of pancreatic tumours (85%) are classified as adenocarcinomas (PDA), arising from the ductal epithelium. The diagnosis is mainly made in patients in their forties and the incidence is higher in men than in women (ratio 1.3:1). Some risk factors have been suggested for the development of PDA, but no standard screening has been defined yet (Table 1). Five to ten percent of the patients diagnosed with PDA have a first degree relative with the same disease, which suggests involvement of familial aggregation and/or genetic factors[4].

Surgical resection is the only option of curative treatment. Nevertheless, because of the late presentation of

Table 1 Suggested risk factors for the development of pancreatic ductal adenocarcinoma[3]

Hereditary syndromes	Non-hereditary risk factors
Hereditary breast/ovarian cancer (BRCA2, BRCA1, PALB2)	Nonhereditary chronic pancreatitis
Familial atypical multiple mole melanoma (FAMMM) syndrome (CDKN2A)	Diabetes mellitus, glucose metabolism, and insulin resistance
Peutz-Jeghers syndrome (STK11)	Cigarette smoking
Familial adenomatous polyposis (APC)	Obesity and physical inactivity
Hereditary nonpolyposis colon cancer (Lynch Ⅱ) (DNA mismatch repair genes)	Diet (high intake of saturated fat and/or meat, particularly smoked or processed meats)
Familial pancreatic cancer (gene not identified)	Coffee and alcohol consumption
Hereditary pancreatitis (PRSS1, SPINK1)	Aspirin and nonsteroidal anti-inflammatory drug use
Ataxia telangiectasia (ATM)	History of partial gastrectomy or cholecystectomy
Li-Fraumeni syndrome (p53)	*Helicobacter pylori* infection

Table 2 Summary of the most important randomized clinical trials performed in advanced pancreatic ductal adenocarcinoma

Experimental arm treatment (number of patients included)	Median OS (mo) (Experimental arm) (95%CI)	Control arm treatment (number of patients included)	Median OS (mo) (Control arm) (95%CI)	Hazard ratio (95%CI) (*P* value)	Ref.
Gemcitabine (63 pts)	5.6 (data not shown)	5-FU (63 pts)	4.4 (data not shown)	Data not shown *P* = 0.0025	Burris *et al*[7], 1997
Gemcitabine and erlotinib (285 pts)	6.24 (data not shown)	Gemcitabine (284 pts)	5.91 (data not shown)	0.82 (0.69-0.99) *P* = 0.038	Moore *et al*[8], 2007
Gemcitabine and capecitabine (267 pts)	7.1 (6.2-7.8)	Gemcitabine (266 pts)	6.2 (5.5-7.2)	0.86 (0.72-1.02) *P* = 0.08	Cunningham *et al*[9] 2009
FOLFIRINOX (combination of 5FU, oxaliplatin and irinotecan) (171 pts)	11.1 (9.0-13.1)	Gemcitabine (171 pts)	6.8 (5.5-7.6)	0.57 (0.45-0.73) *P* < 0.001	Conroy *et al*[10], 2011
Gemcitabine and nab-paclitaxel (431 pts)	8.5 (7.9-9.5)	Gemcitabine (430 pts)	6.7 (6.0-7.2)	0.72 (0.62-0.83) *P* < 0.001	Von Hoff *et al*[11], 2013

OS: Overall survival.

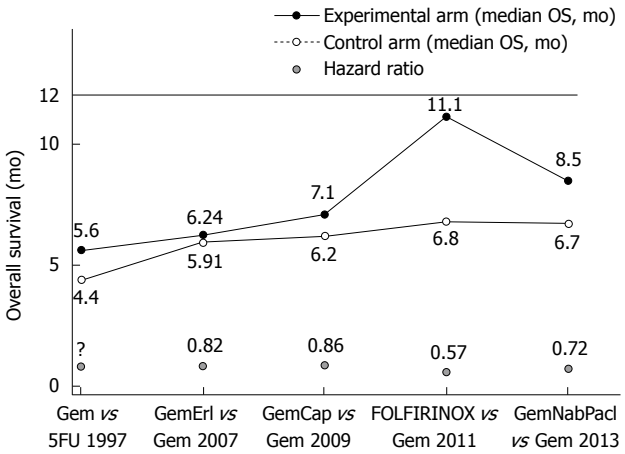

Figure 1 Multiple randomized phase III trials have been completed in the last decades; however, we have not been able to cross the barrier of 12 mo survival in advanced pancreatic cancer.

the disease, only 15%-20% of patients are diagnosed early enough to be considered for a potentially curative treatment. However, the relapse rate after surgery is high (80%-90%). Looking for a reduction in the relapse rate and an increase in the overall survival (OS), adjuvant chemotherapy is currently standard of care after resection of PDA. The most employed adjuvant chemotherapy schedules are gemcitabine or capecitabine[5,6].

Unfortunately, most of the patients (up to 80%) are diagnosed in advanced stages and palliative chemotherapy is the only option of treatment. The aim of this chemotherapy is to prolong OS and improve the quality of life. In 1997, gemcitabine was established as the drug of choice for the treatment of advanced PDA with OS of 5.6 mo compared to 4.4 mo in the arm with 5FU[7]. Since then, multiple randomized studies with combination schedules have shown improvement in OS compared to single agent gemcitabine (Table 2)[8-11]. However, as it is shown in Figure 1, the impact in the survival achieved in advanced PDA has never reached the year of median OS. This is far of being comparable to the results achieved in other malignancies such as advanced breast or colorectal cancer.

WHY THESE RESULTS? WHAT ARE THE CHALLENGES WHEN TREATING PDA?

Much effort is been employed in trying to improve the survival of our patients with PDA. The improvement in the big randomized studies with more than 1800 patients during the last decades seems to be not enough and the median OS is still less than one year after diagnosis[12]. When we compare this data with other adenocarcinomas, for example breast or colorectal adenocarcinoma with median OS longer than 24 mo, we might wonder: are we doing the right research? What makes pancreatic cancer

Table 3 Core signalling pathways involved in pancreatic ductal adenocarcinoma

Involved pathways	PDA with pathway aberrations	Representative genes
Apoptosis	100%	*CASP10, VCP, CAD, HIP1*
DNA repair	83%	*ERCC4, ERCC6, EP300, RANBP2, TP53*
Regulation of G1/S phase	100%	*CDKN2A, FBXW7, CHD1, APC2*
Hedgehog pathway	100%	*TBX5, SOX3, LRP2, GLI1, GLI3, BOC, BMPR2, CREBBP*
Celular adhesion	79%	*CDH1, CDH10, CDH2, CDH7, FAT, PCDH15, PCDH17, PCDH18, PCDH9, PCDHB16, PCDHB2, PCDHGA1, PCDHGA11, PCDHGC4*
Integrin signaling	67%	*ITGA4, ITGA9, ITGA11, LAMA1, LAMA4, LAMA5, FN1, ILK*
c-Jun N-terminal kinase signaling	96%	*MAP4K3, TNF, ATF2, NFATC3*
KRAS signaling	100%	*KRAS, MAP2K4, RASGRP3*
Regulation of invasion	92%	*ADAM11, ADAM12, ADAM19, ADAM5220, ADAMTS15, DPP6, MEP1A, PCSK6, APG4A, PRSS23*
GTP-ase dependent signaling (not κ-ras)	79%	*AGHGEF7, ARHGEF9, CDC42BPA, DEPDC2, PLCB3, PLCB4, RP1, PLXNB1, PRKCG*
TGF-β pathway	100%	*TGFBR2, BMPR2, SMAD4, SMAD3*
Wnt/Notch pathway	100%	*MYC, PPP2R3A, WNT9A, MAP2, TSC2, GATA6, TCF4*

Addapted from Jones *et al*[19], 2008. PDA: Pancreatic ductal adenocarcinoma.

so hard to treat? Do we know enough about its molecular biology? Which is the next step?

Several reasons have been postulated for the difficulties in achieving better results in PDA[13]: (1) delay in diagnosis due to lack of symptoms until advanced stages. Most of the patients are diagnosed with distant metastases or unresectable locally advanced disease. Moreover, due to its location in the retroperitoneum, the pancreas is difficult to access and sample with traditional endoscopic techniques. This can also raise difficulties for an early diagnosis; (2) PDA is associated with several comorbidities that could affect patients' overall health with a worse impact in the OS of those who develop the PDA (Table 1); (3) limited effect of local therapies. The relapse rate is far from being acceptable, even with adjuvant chemotherapy or a combination of adjuvant chemo-radiotherapy. One possible explanation is that "field effect" mutations may affect normal appearing cells present in the residual pancreatic tissue. This, added to the high ability of spreading, even in early stages, could explain the high chances of relapse after local radical treatment[14]; and (4) PDA has been postulated to be primary (innate), rather than secondary (acquired), resistance to chemotherapy. Reasons for this could be both, related to the cancer cell itself and to the stroma surrounding the pancreatic cancer cells: (1) cancer cell characteristics. Different high penetrance genetic alterations have been described in PDA. One of the most frequent ones is activating mutations in κ-ras (present in > 90% of PDA), which is one of the most potent of all human oncogenes, and able to induce strong pro-growth, cell motility and invasion signals; and (2) a defining characteristic of PDA is the presence of a dense fibrotic proliferation surrounding the epithelial cells composed of various leukocytes, fibroblasts, endothelial cells and neuronal cells, as well as extracellular matrix components such as collagen and hyaluronan[15-17]. Moreover, in contrast to many tumours that are dependent on neo-angiogenesis, PDA is poorly vascularised and therefore, poorly perfused, making the delivery of chemotherapy more difficult into the tumour cells.

MOLECULAR BIOLOGY IN PANCREATIC CANCER: WHAT DO WE KNOW?

PDA is known to be a genetic disease, caused by inherited and acquired mutations in specific cancer-associated genes[18]. Since the sequencing of the protein-coding exons from 20661 genes in 24 advanced ductal adenocarcinomas of the pancreas was published in 2008, a better understanding of the key pathways involved in the development and maintenance of PDA was provided[19]. In 2012 the sequencing of 142 localized and resected PDAs was also published[20].

The most important genes and pathways involved in PDA biology are summarized in Table 3.

According to our current knowledge, multiple combinations of all these genetic mutations are commonly found in PDA, and can be classified as follows[21-24]: (1) mutational activation of oncogenes: predominantly K-ras; (2) inactivation of tumour suppressor genes such as *TP53, p16/CDKN2A*, and *SMAD4*; (3) inactivation of genome maintenance genes, such as *hMLH1* and *MSH2*, which control the repair of DNA damage. Most of these mutations are somatic aberrations. However, some germline aberrations were described (BRCA2, PALB2, STK11, ATM, MLH1 and MSH2) to be involved in the development of hereditary pancreatic cancer (Tables 1 and 3)[25].

During the last two decades, a lot of effort has been done in the definition of biological pathways involved, not only in the development/maintenance of PDA cancer cells, but also in the characterisation of the stroma surrounding the PDA cells[15-17]. As we discussed above, the characteristics of this particular stroma are one of the explanations for the difficulties in the treatment of PDA[26]. Some core pathways [*e.g.*, Hedgehog, Transforming growth factor (TGF)-β and Hepatocyte growth factor (HGF)-met] have shown to be involved in its development[18,27]. Moreover, some studies are testing the effec-

Table 4 Biomarkers in pancreatic ductal adenocarcinoma

Biomarker	Prognostic biomarker	Predictive biomarker	Comments and references
MUC1	Yes		Predictive of early cancer-related death[37]
MSLN	Yes		Predictive of early cancer-related death[37]
6-gene signature	Yes		Expression of *FOSB, KLF6, NFKBIZ, ATP4A, GSG1* and *SIGLEC11* is related with metastatic spread[38]
VEGF	Yes		Worse survival in resected PDA[39]
p16	Yes		Higher expression was related to poorer prognosis[40]
TP53	Yes		Relation with tumour dedifferentiation and higher locorregional recurrence[40]
SMAD4	Yes		Higher Smad4/Dpc4 was related to bigger tumours, lymph node metastases and shorter survival[40]. Higher relapse rate (distant spread)[41]. Loss of expression correlated with resectability and better survival after surgery[42]
EGFR			No predictive/prognostic power[43,44]
K-ras	Yes		Better prognosis in Kras wild-type tumours[43,44]
RRM1	Yes	Yes	High expression of RRM1 showed significantly better overall survival[45-47] and worse response to treatment[47-49]
ERCC1	Yes		High ERCC1 expression showed significantly better overall survival[47,50,51]. No predictive power[49]
CTCs	Yes		More studies are awaited[52]
hENT1	Yes	Yes	High expression of hNENt1: worse prognosis, higher response to gemcitabine in the adjuvant setting; unclear impact in metastatic patients[50,53-57]
HuR	Yes	Yes	Low expression of HuR: worse prognosis[58] and better response to gemcitabine[59,60]
SPARC	Yes		Expression of SPARC in the peritumoural stroma is related with worse prognosis[61,62]. No predictive effect
CTGF			Preclinical data seem to suggest prognostic impact and potential predictive power for FB-3019[63-66]

VEGF: Vascular endothelial growth factor; EGFR: Epidermal growth factor receptor; CTCs: Circulating tumour cells; SPARC: Secreted protein acidic and rich in cysteine; CTGF: Connective tissue growth factor.

tiveness of anti-stroma therapies in pancreatic cancer, such as Visdemogib (Hedghog pathway inhibitor)[28,29] and nab-pacliaxel [postulated to be a secreted protein acidic and rich in cysteine (SPARC) inhibitor][30].

IMPROVING OUR RESULTS THROUGH THE DEVELOPMENT OF BIOMARKERS

A biomarker has been defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" by the Biomarkers Definitions Working Group[31]. According to this Working group, three categories of biomarkers can be defined depending on the information that they provide: diagnostic, prognostic and predictive biomarkers.

As detailed above, during the last decades, our knowledge about the PDA molecular biology is increasing exponentially and lots of pathways have been implicated in this malignancy. However, apart from CA19.9 for the diagnosis of pancreatic adenocarcinoma, no other biomarkers are currently being employed in PDA for improving the clinical management of these patients[24,32,33]. How can we apply all this new knowledge in the development of new studies looking for an improvement in OS? According to some experts, better early detection strategies with diagnostic biomarkers, treatment decisions based on prognostic biomarkers, and individualized treatment schedules based on predictive biomarkers could be the clue for the improvement in PDA research[34,35].

A lot of work has already been done in the development of a compendium of potential PDA biomarkers worth to be included in future research[36]. Nowadays, the three biomarker categories are being developed in PDA. The most important prognostic and predictors biomarkers for pancreatic cancer are summarized in Table 4.

Diagnostic biomarkers

The aim of the development of diagnostic biomarkers is to improve the rate of early diagnosis. CA19.9 is already employed as a diagnostic tool in combination with image techniques[23,30]. The definition of genetic expression and proteomic patterns could improve the diagnosis of PDA, currently based on morphological pathology studies only.

Prognostic biomarkers

The potential of classifying the patients into good and bad prognostic groups could be especially useful after surgery. We could offer more aggressive chemotherapy schedule or closer follow-up to those patients with worse prognosis or more chances of relapse. Moreover, the capability of defining the relapse pattern (local *vs* distant spread) could also improve the chosen image technique or frequency for the surveillance. See more details below.

Predictive biomarkers

The definition of predictive biomarkers, both for already employed drugs and for new therapies, could enrich our prospective studies. We need to improve our ability for selecting those patients that, according to the tumour expression of predictive biomarkers in PDA, may have better response to the chosen treatment and individual-

ize the chemotherapy according to this information. See more details below.

CURRENT DEVELOPMENT OF BIOMARKERS IN PANCREATIC CANCER

The most important prognostic and predictors biomarkers for pancreatic cancer are summarized in Table 4.

PROGNOSTIC BIOMARKERS IN PDA

To look for effective biomarkers able to stratify PDA based on biologic behaviour, a survival tissue microarray of 137 resected PDAs was analysed[37]. In a multivariate model, MUC1 (OR = 28.95, 3+ *vs* negative expression, $P = 0.004$) and MSLN (OR = 12.47, 3+ *vs* negative expression, $P = 0.01$) were highly predictive of early cancer-related death. In this study, MUC1 and MSLN were superior to pathologic features (tumour size, lymph node metastases, and nuclear grade) in predicting survival.

Stratford *et al*[38] identified a six-gene signature (*FOSB*, *KLF6*, *NFKBIZ*, *ATP4A*, *GSG1* and *SIGLEC11*) associated with metastatic disease. The results from the training set of 34 patients were validated in an independent series of 67 patients. The six-gene signature was independently predictive of survival and superior to established clinical prognostic factors such as grade, tumour size and nodal status (HR = 4.1, 95%CI: 1.7-10.0). Patients defined to be "high-risk" had a 1-year survival rate of 55% compared to 91% in the "low-risk" group.

In 2011 a meta-analysis of immunohistochemical markers in resected pancreatic cancer was published[39]. The aim of the study was to conduct a systematic review of the literature evaluating p53, p16, SMAD4, bcl-2, bax, vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR) expression as prognostic factors in resected PDA. VEGF emerged as the most potentially informative prognostic marker (11 eligible studies, 767 patients, HR = 1.51, 95%CI: 1.18-1.92). Bcl-2, bax and p16 were also related to OS. Neither p53, SMAD4 or EGFR were found to have significant prognostic value.

The expression of hENT1, involved in the internalization of gemcitabine into the cancer cell, has been widely explored in PDA (See below, "predictive factors"). The prognostic value of the expression of hENT1 has been shown in several studies. Kim *et al*[32] reported in 2011 a series of eighty-four resected PDAs. Total RNA was isolated from paraffin-embedded tumours and the multivariate analysis confirmed the association of low expression of hENT-1 ($P = 0.007$) with worse OS and progression free survival ($P = 0.016$).

The genes *p16*, *TP53* and *SMAD4/DPC4* were included in a study by Jones *et al*[19] in 2008 as core pathways of PDA development and have been widely studied in PDA for its potential prognostic prediction.

A retrospective study published in 2013 aimed to clarify the implications of 3 major genes (*CDKN2A/p16*, *p53*, and *SMAD4/DPC4*) with clinico-pathological findings, including survival and patterns of disease progression, in 106 patients with resected PDA[40]. The expression of protein products of these genes was determined immunohistochemically. Genetic aberrations of these 3 genes were associated with malignant behaviour of PDA: a significant correlation was found between SMAD4/DPC4 immunolabeling and tumour size ($P = 0.006$), lymphatic invasion ($P = 0.033$) and lymph node metastasis ($P = 0.006$); loss of p16 immunolabeling ($P = 0.029$) and loss of SMAD4/DPC4 immunolabeling ($P < 0.001$) were significantly associated with shorter OS; and abnormal immunolabeling of p53 was significantly associated with tumour dedifferentiation ($P = 0.022$) and the presence of locoregional recurrence ($P = 0.020$).

Moreover, the expression of *SMAD4* has been analysed to define higher relapse rate and therefore worse prognosis, in several studies[40-42,67]. A study including patients with resected PDA analysed the expression of cell-cycle and cell-signaling involved key proteins using immunohistochemistry in a subgroup of 129 patients[42]. While aberrant expression of *p21*(WAF1/CIP1), cyclinD1, *p53* or *p16*(INK4A) was not associated with a difference in survival; loss of *SMAD4/DPC4* expression correlated with resectability ($P < 0.0001$) and was associated with improvement in survival after resection ($P < 0.0001$). In contrast, resection did not improve survival in patients whose tumour expressed *SMAD4/DPC4* ($P = 0.5$). The authors concluded that preoperative assessment of *SMAD4/DPC4* expression could be useful in the selection of patients that may benefit from surgical resection.

In 2011, Crane *et al*[41] reported the results of a phase Ⅱ clinical trial to assess the efficacy and safety of cetuximab, gemcitabine and oxaliplatin followed by cetuximab, capecitabine, and radiation therapy in locally advanced pancreatic cancer. Diagnostic cytology specimens were analysed for SMAD4/DPC4 protein expression (by immunohistochemistry). In this study, SMAD4/DPC4 protein expression correlated with local rather than distant disease progression ($P = 0.016$).

Finally, Iacobuzio-Donahue *et al*[67] performed rapid autopsies on 76 patients with documented pancreatic cancer. The histological features, the status of the *KRAS2*, *p53* and *SMAD4/DPC4* genes were correlated to the stage at initial diagnosis and patterns of failure (locally destructive *vs* metastatic disease). *SMAD4/DPC4* genetic status was highly correlated with the presence of widespread metastasis but not with locally destructive tumours ($P = 0.007$).

SPARC is expressed in the cell matrix and it is involved in cell matrix interactions, wound repair, cell migration and cancer growth regulation. The high expression of *SPARC* in the peritumoural stroma was defined as a worse prognostic factor both in localized and locally advanced patients[61,62]. The expression patterns of *SPARC* were characterized by immunohistochemistry in 299 resected pancreatic adenocarcinomas to evaluate the

Table 5 "Classic" predictive biomarkers for "classic" chemotherapies with potential interest in pancreatic cancer

Predictive biomarker	Drug	Theoretical impact[1]	Studies performed in pancreatic cancer (predictive outcome)?	Impact confirmed in pancreatic cancer?	Notes	Ref.
Thymidylate synthase	5FU	When negative, better response to 5FU	Yes	No	Predictive value in PDA not validated	[55,72-74]
DPD	5FU	When mutation DPD, more 5FU related toxicity	Yes	No	Survival benefit with S1 and DPD mutation	[73]
Topoisomerase I	Irinotecan	When positive, better response to Irinotecan	No	No	No data in pancreatic cancer	-
RRM1	Gemcitabine	When positive, better response to gemcitabine	Yes	Yes	Low expression correlates with better response	[47-49]
ERCC1	Oxaliplatin	When negative, better response to Oxaliplatin	Yes	No	No predictive effect	[49,51]
XRCC1	Oxaliplatin	When negative, better response to Oxaliplatin	No	No	No data in pancreatic cancer	-
EGFR/kras	Erlotinib	Erlotinib effective when EGFR mutation/kras wild type present	Yes	No	No predictive effect	[43,44]
PALB2	Mitomycin C	Mitomicin C effective when PALB2 mutation present	No	Yes	Case report	[75]
BRCA2	PARP inhibitors	PARP inhibitors effective when BRCA2 mutation present	Yes	Yes	Phase I trial	[76]

[1]This impact is suggested in other malignancies.

prognostic significance of tumoural and peritumoural *SPARC* expression[61]. In the multivariate analysis, *SPARC* expression in the surrounding stroma was a biomarker of worse prognosis (HR = 1.89, 95%CI: 1.31-2.74), while the expression of *SPARC* in pancreatic cancer cells remained unrelated to prognosis (HR = 1.02, 95%CI: 0.73-1.42). These data have been validated in further studies[62]. However, in animal models this prognostic impact of *SPARC* was not verified: the prognosis was worse in those *SPARC* knock-out mice[68].

Other prognostic biomarkers are: Kras[43,44] (better prognosis in Kras wild type tumours), HuR[59,60] (higher expression of HuR related to worse prognosis; See below "Predictive biomarkers"), RRM1[45-47] and ERCC1[47,50] (high RRM1 and high ERCC1 showed significantly better OS).

The analysis of circulating tumour cells is also being developed in PDA patients. However, further studies are awaited for a better understanding of its impact[52].

Collisson *et al*[69] reported in 2011 a study identifying three PDA molecular subtypes (classical, quasimesenchymal and exocrine-like) both in human tumours and cell lines, with different profiles of survival and response to treatment. The subtypes were defined according to the gene expression profile. These data need to be further validated but could be useful in the improvement of the individualize management of PDA[70].

PREDICTIVE BIOMARKERS IN PDA

Use of "classic" biomarkers to predict response to "classic" chemotherapies in pancreatic cancer

Based on data from other subtypes of adenocarcinoma such as colorectal tumour or breast cancer, some studies tried to employed "classic" predictive biomarkers to improve the results achieved with standard chemotherapy in advanced PDA. Although most of these biomarkers have not been prospectively validated in pancreatic adenocarcinoma (Table 5), the rationale for this design is the individualization of first line chemotherapy according to the molecular expression profile of each tumour.

This idea has been executed in some studies. Von Hoff *et al*[71] published in 2012 the results of a clinical trial, where patients with PDA were treated according to molecular profiles of their tumour. The molecular analysis included immunohistochemistry, fluorescent in situ hybridization assays and immediately frozen tissue for oligonucleotide microarray gene expression assays. From the 86 patients included, there was a molecular target detected in 84 (98%) and 66 were treated according to the molecular profiling results. This was a pilot study, and the authors confirmed the feasibility of this rationale. However, prospective studies are ongoing and data are awaited for its wide use (NCT01726582, NCT01394120).

Gemcitabine response predictive biomarkers: RRM1, hENT1 and HuR

Apart from *RRM1*[47-49], whose lack of expression is predictive of response to gemcitabine (Tables 4 and 5), other biomarkers were suggested as predictors of response to gemcitabine in PDA: hENT1[50,53-55] and HuR[59,60].

Human equilibrative nucleoside transporter-1 (hENT1) was found to be the major gemcitabine transporter into the cell. Therefore, those cells with low expression of hENT1 will not transport the gemcitabine into the cancer cells, avoiding its activity (inhibition of the cell growth). In contrast, increased hENT1 abundance facilitates efficient cellular entry of gemcitabine and confers increased cytotoxicity. Nakano *et al*[77] reported in 2007 a preclinical study with pancreatic cancer cell lines where expression of hENT1 changed in the development of gemcitabine resistance.

However, interpreting these results in human samples is challenging. In patients receiving adjuvant treatment, the expression of hENT1 showed to be predictive biomarker for response to gemcitabine. However, this was not validated in the metastatic setting.

The multicentre ESPAC-3 trial randomized patients to adjuvant gemcitabine or 5FU after pancreatic adenocarcinoma resection[78]. According to the safety profile, gemcitabine was chosen as the preferred agent when compared with monthly bolus (Mayo Clinic) 5-FU/LV for the adjuvant setting. The samples collected from the adjuvant ESPAC1/3 randomized trials were employed in a translational project to define the predictive value of hENT1[54]. One-hundred and seventy-five gemcitabine treated and 176 5-FU treated patients were included in the analysis. In the gemcitabine group a significantly lower survival (P = 0.002) was noted with low hENT1 [median survival 17.1 (95%CI: 14.3-23.8) mo *vs* 26.2 (95%CI: 21.2-31.4) mo]. Multivariate analysis confirmed hENT1 expression as a predictive biomarker of response to gemcitabine in the adjuvant setting.

However, the findings in metastatic patients are different. During the 2013 ASCO congress, data of a new gemcitabine-like drug (CO-101) were presented[57]. CO-101 (also known as CP-4126), a lipid-drug conjugate of gemcitabine, was rationally designed to enter cells independently of hENT1. The authors presented the results of a randomized trial comparing CO-101 and gemcitabine in the metastatic setting. The aim of the study was to determine whether CO-101 improved survival compared to gemcitabine in patients with low hENT1 tumours and to test prospectively the hypothesis that hENT1 was a predictive marker of response to gemcitabine. Unfortunately, CO-101 was not superior to gemcitabine in patients with low tumour hENT1 expression and, moreover, hENT1 expression did not predict gemcitabine treatment outcome in this study.

From these data, we conclude that while hENT1 seems to be a predictor of response in the adjuvant setting, this was not reproducible in metastatic patients. The molecular biology of the metastatic PDA may differ from the localized tumours, explaining the differences in the results.

The ubiquitous RNA-binding protein (RBP) HuR is involved in the control of gene expression, mRNA stability and translation and cellular response to internal and external signals[79]. Through its post-transcriptional effect by targeting mRNAs, HuR can alter the cellular response to proliferative, stress, apoptotic, differentiation, senescence, inflammatory and immune signals. The high expression of HuR has already been defined as a prognostic factor in PDA and some studies postulated HuR as a predictive biomarker for response to gemcitabine in cancer cell lines[59,60].

These results were confirmed in a series of 29 localized PDA patients in whom correlation between HuR expression levels and OS was evaluated[58]. The results indicated an increase in risk of death in patients with low HuR levels compared to high HuR levels among patients receiving gemcitabine. Authors concluded that HuR was regulating the key metabolic enzyme for gemcitabine activation (deoxycytidine kinase) and could be a marker for therapeutic efficacy of gemcitabine based regimens: better response in patients with high HuR expression.

SPARC

As we detailed above, SPARC has prognostic impact in PDA[61,62]. Nab-paclitaxel is a 130-nm albumin-bound formulation of paclitaxel particles. Data from the phase I / II trial with nab-paclitaxel postulated SPARC as a predictive factor of anti-stromal therapies[30]. SPARC status was evaluated in 36 patients and a significant increase in OS was observed in high-SPARC expression subgroup compared with patients in the low-SPARC subgroup (median OS, 17.8 mo *vs* 8.1 mo, respectively; P = 0.0431). Moreover, some studies in animal models postulated that the addition of nab-paclitaxel could increase the intratumoural gemcitabine delivery due to anti-stromal effect of nab-paclitaxel[30,80]. However, the predictive impact of the expression of SPARC has not been clarified in the prospective studies with combination chemotherapy with gemcitabine and nab-paclitaxel[11,30].

Connective tissue growth factor/CCN2

Also focused in the stroma and the importance in PDA, connective tissue growth factor (CTGF) expression was analysed in pancreatic cancer. CTGF is a cysteine-rich matricellular secreted protein, which regulates diverse cell functions including adhesion, migration, proliferation, differentiation, survival, senescence and apoptosis[81,82].

Due to the hypoxic conditions surrounding the PDA, Eguchi *et al*[82] analyzed the tumour-stroma interaction signalling in cell lines of pancreatic cancer in hypoxia and normoxia using RNA interference techniques. The results showed that cell invasion was more enhanced under hypoxia than under normoxia ($P < 0.05$) and that CTGF was one of the overexpressed molecules in hypoxic conditions. Moreover, cell invasiveness was reduced by CTGF knockdown in hypoxic cancer cells ($P < 0.05$). The authors concluded that hypoxia induced CTGF expression could be a prognostic factor related to higher aggressiveness in PDA. This results match with those from other studies[63-65].

Therefore, the data available shows that CTGF is overexpressed in PDA and facilitates local desmoplasia, tumour survival and metastasis. FG-3019 is a human monoclonal antibody to CTGF, able to control the tumour growth in cancer cell lines[83] and tumour xenografts[65], without damage to the healthy tissue. Neesse *et al*[84] reported data from animal model research to clarify the antitumour effect of FB-3019. The authors concluded that FG-3019 may have antitumour effect itself, more than improving the delivery of gemcitabine into the tumour. First data in humans were presented in ASCO-GI 2013 by Picozzi *et al*[66], showing that the combination with gemcitabine, erlotinib and FG-3019 was safe in ad-

vanced pancreatic cancer patients. The authors showed that baseline CTGF plasma level was related to worse survival. Further clinical data for the prognostic and predictor relevance of CTGF in humans are awaited.

FUTURE, HOW TO IMPLEMENT THE ACTUAL DATA?

There is no doubt that the knowledge in molecular biology will continue to improve in the following years. New generation techniques are being employed in PDA research and will give much more data. However, it is crucial to incorporate this knowledge in a rational way, and this could be challenging. Moreover, the huge economic cost of this research needs to be analysed. Some panels of experts have defined the most suitable way for biomarker development and its addition to the clinical research in pancreatic cancer[12,85,86].

In conclusion, a lot of work needs to be done in the improvement of our understanding in pancreatic adenocarcinoma. Treatment individualization seems to be one of the keys, implementing the clinicopathological information with molecular characteristics. In order to achieve this, the development of new biomarkers and a better definition of the current ones are radically important. Most of the detailed biomarkers in this review are available just for research purposes; only Ca19.9 (with diagnostic and follow-up aim) is employed in the clinical practice. The results of the ongoing clinical trials with new biomarker research and the selection of the therapies according to these molecular characteristics are awaited.

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