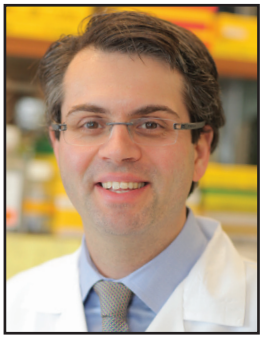
Douglas G. Adler MD, FACG, AGAF, FASGE, Series Editor

Prognostic Biomarkers in Pancreatic Ductal Adenocarcinoma

Valerie Gausman



Tamas Gonda

Pancreatic ductal adenocarcinoma (PDAC) carries significant morbidity and mortality and remains one of the most difficult malignancies to treat. When deciding upon a management strategy, the benefits of surgery (only available to a minority of patients) and chemotherapy need to be weighed against the morbidity of these treatments. Individual patient and tumor factors need to be taken into account to provide an optimal, personalized approach. In this review, we highlight established and novel biomarkers that have the potential to be used as prognostic biomarkers in PDAC and some that may be used to guide therapeutic decisions. We briefly review some blood based biomarkers but focus on those that are tissue based and may be identified and characterized in pancreatic cancer biopsies.

INTRODUCTION

Despite major advances in the therapies of many solid tumors, survival in pancreatic ductal adenocarcinoma (PDAC) has not improved.[1] Delayed diagnosis, aggressive biology and marked chemoresistance have all contributed to this disappointing trend. Prognostic biomarkers inform of a likely cancer outcome (disease recurrence, progression or death) independent of type of treatment. A biomarker is predictive if there is a difference in treatment effect for biomarker positive patients compared to biomarker negative patients. General prognostic markers, not specific to a defined therapeutic regimen, can be useful in distinguishing which patients are at higher risk of a poor outcome and should therefore be managed more aggressively. While large gene expression panels have been identified for use in prognostication of other malignancies and some have been linked to therapeutic response, few such markers have been well characterized in pancreatic cancer and even fewer have been used in clinical practice. We provide an overview of the most promising markers and those that may be closest to clinical use.

Valerie Gausman, MD, Resident, Department of Medicine, NYU School of Medicine, New York, NY
Tamas Gonda, MD, Assistant Professor of Medicine, Division of Digestive and Liver Diseases, Columbia University Medical Center, New York, NY

BLOOD OR SERUM BASED BIOMARKERS

Established Serum Based Biomarkers

There are few established and widely clinically used biomarkers for PDAC compared to other malignancies. Carbohydrate antigen 19-9 (CA 19-9), or sialyl Lewis antigen, is the only biomarker recommended by the National Comprehensive Cancer Network guidelines in the evaluation of PDAC. Its role in carcinogenesis may be related to its association with an increased adherence of cancer cells to endothelial cells through E-selectin,[2] In addition to its well-known role in the diagnosis of PDAC, higher levels of pre-operative CA 19-9 have been shown to be correlated with advancing stage,[3] less resectability,[4,5] and decreased survival.[6-8] However, its sensitivity is limited due to 10% of the population being non-secretors of CA 19-9 and its specificity limited due to its secretion by normal biliary epithelium.[7]

Other commonly used pre-operative laboratory markers include carcinoembryonic antigen (CEA), an intercellular adhesion glycoprotein normally present in very low levels in the blood, lactate dehydrogenase (LDH), an enzyme involved in sugar metabolism and C-reactive protein (CRP), an acute phase reactant. Compared to normal levels, elevated LDH,[9] CEA,[10,11] and CRP[12,13] have all been shown to be independent unfavorable prognostic factors of survival. However, similar to CA 19-9, these laboratory markers are limited in their specificity. Table 1 highlights the accuracy of these markers in distinguishing resectability and prognosticating survival.

Blood or Serum Based Biomarkers in Development

The value of serum-based markers is their less invasive approach and ability to collect multiple samples for various analyses. The most notable serum-based markers currently under investigation include circulating tumor cells, circulating tumor DNA and microRNAs and are summarized in Table 2.

Metastatic spread is commonly perceived to be one of the latest steps in the progression of cancer; however, the presence of circulating tumor cells (CTCs) in early stages as well as in pre-invasive lesions have challenged this. CTCs are cancer cells that are shed off the primary or metastatic tumor and can travel through the blood stream, potentially leading to new metastasis. A meta-analysis has revealed that the presence of CTCs in PDAC corresponds with worse progression-free and overall survival.[41] A recent study found that

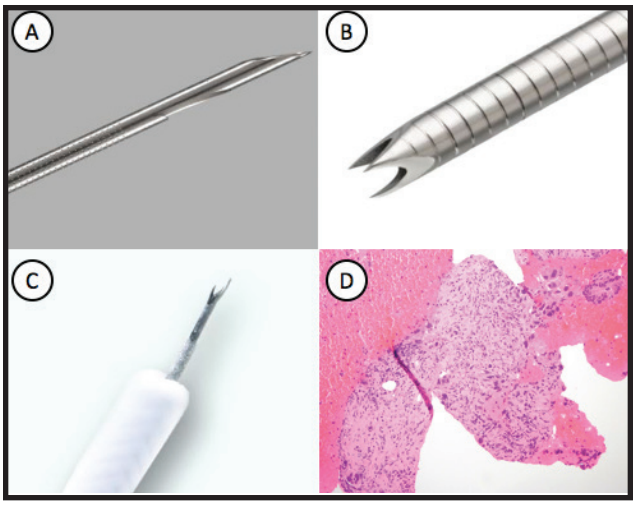


Figure 1. (A) ProCore Needle (Cook Endoscopy) (B) Acquire FNB needle (Boston Scientific) (C) SharkCore FNB needle (Medtronic) (D) Pancreatic cancer core biopsy specimen

cytokeratin-expressing CTCs, but not mesenchymal-like CTCs, had prognostic utility, highlighting the importance of phenotypic identification of CTCs.[42] The CellSearch system is the only FDA-approved CDC detection technology (since 2004), but it is expensive, requires a complicated enrichment step, has a long detection time, low sensitivity and does not allow for isolation of CTCs for further molecular analyses. Thus, new detection modalities are being investigated including line confocal microscopy, surface-enhanced Raman spectroscopy (SERS), new enrichment technologies and fluorescence in situ hybridization (FISH).[43,44]

Cell-free DNA (cfDNA) is another minimally invasive potential sample source that is also referred to as a liquid biopsy. These are small fragments of DNA that are released after cellular necrosis or apoptosis and circulate in the bloodstream; when they are released from tumor cells, they are called circulating tumor DNA (ctDNA). ctDNA is, on its own, a negative prognostic marker,[45] and it can also be used to identify genetic mutations which can further prognosticate survival in PDAC.[46] One study looking at ctDNA in a variety of early and late stage malignancies found that ctDNA was detectable in some patients without detectable CTCs (but not vice versa), suggesting that these biomarkers are separate entities.[47]

The majority of the human genome is made up of non-coding RNA molecules, which are not transcribed into proteins, but have been shown to play a major role in regulating gene expression. MicroRNAs (miRNAs)

are small (18-25 nucleotide) single stranded transcripts of non-coding RNA, which are highly stable and can act as tumor suppressors or oncogenes depending on their dysregulation. Nearly 100 miRNAs are differentially expressed in pancreatic cancer and they have been analyzed in human blood, bile, pancreatic juice, pancreatic cysts and stool. miR-21 is commonly considered an oncogene as many of its targets are tumor suppressors such as programmed cell death 4 (PDCD4) and PTEN. A large number of studies have identified miR-21 as a strong negative prognostic marker of survival in PDAC. A meta-analysis of 11 studies

Table 1. Accuracy of Established Prognostic Biomarkers in PDAC

Marker	Countries	Resectability, median serum levels		Overall Survival Based on Median Serum Levels (months)	
CA 19-9	Turkey[4]	Resectable: 68.8 Unresectable: 622 Cut off = 256.4	**p<.05** Sen: 82.4 Sp: 92.3		
	USA[5]	Resectable: 73.5 Unresectable: 374 Cut off = 150	**p<.001** Sen: 71 Sp: 68		
	Italy[61]	Resectable: 94* Unresectable: 563*	**p>.05**		
	China[62]	Resectable: 130 Unresectable: 656	**p<.01**		
	China[63]	Resectable: 774 Unresectable: 1311	p=.56	<1000: 9 >1000: 6	p=.26
	USA[64]	Cut off = 149	Sen: 54 Sp: 55	<37: 52.8 >37: 21.2	**p=.02**
	Finland[65]			<370: 9.5 >370: 4.4	**p<.05**
	Germany[66]			<400: 17.3 >400: 7.1	**p=.0001**
CEA	China[63]	Resectable: 14.6 Unresectable: 32.6	p=.64	<5: 9 >5: 6	p=.052
	Finland[67]			<5: 11.6%** >5: 6.5%**	**p=.02**
	Finland[65]			Cut off =15	**p<.001**
	USA[5]	Resectable: 5.8 Unresectable: 18.1	p=.66		
LDH	Turkey[9]			<470: 39 >470: 10	**p=.0001**
CRP	United Kingdom[13]			<5: 10.3 >5: 2.5	**p=.027**
	Japan[12]			<.5: 7.9 .5-2: 5.9 >2: 3.4	**p=.01**

PDAC, pancreatic ductal adenocarcinoma; Sen, sensitivity; Sp, specificity
CA 19-9 values are in U/mL. CEA values are in ng/mL. LDH values are in U/L. CRP values are in mg/L.
*Mean serum levels **2 year survival

found tissue miR-21 levels to be strongly associated with reduced survival.[48] In the serum, high levels of miR-21 have also been shown to be correlated with low survival and decreased time to recurrence.[49,50] Conveniently, miR-21 has been found to be elevated early in pancreatic carcinogenesis. Up-regulation of miR-21 in precursor lesions such as intraductal papillary mucinous neoplasms (IPMN) and pancreatic intraepithelial neoplasia (PanIN) is also associated with a worse prognosis.[48] A large supportive study analyzing miRNA levels in PDAC revealed high expression of miR-21 and miR-31 with low expression of miR-375 were associated with poor overall survival following surgical resection.[51]

HOX Transcript Antisense RNA (HOTAIR) is a powerful predictor of metastasis and poor prognosis in multiple cancers. It is a non-coding RNA that works via histone modifications to decrease the expression of multiple genes. In PDAC, high HOTAIR expression has been shown to be associated with decreased survival and more aggressive tumors (those that extend to lymph nodes and beyond the pancreas).[52] HOTAIR has also shown potential to be quantified in salivary samples.[53]

TISSUE BASED BIOMARKERS

High Quality Pancreatic Tumor Biopsies

There have been marked recent advances in the ability to obtain high quality histologically intact core biopsies from pancreatic cancer and this shift will likely allow a far greater use of tissue based biomarkers. Although few studies have evaluated different biopsy techniques and needle designs side-by-side, it is apparent that the newer generation "core" fine needle biopsies (FNB) will provide far better quality and quantity specimens than the first-generation fine needle aspirations (FNA). Several studies have demonstrated that the overall diagnostic accuracy of FNA and FNB is similar (92.5-94.8% vs. 90-98.3%, respectively).[35-37] In some studies, FNB required a significantly lower number of needle passes and was associated with greater accuracy at onsite cytology.[37] However, more recent studies have found similar overall diagnostic accuracy and per path diagnostic accuracy.[38]

In addition to the comparable and possibly superior accuracy, the main objective when using a core biopsy is to obtain histologically intact tumor architecture and greater tumor volume. Figure 1A-C demonstrates three of the new FNB needles and an example of a pancreatic cancer specimen obtained through FNB. There are multiple ways to perform tissue acquisition and no certain needle size has shown clear superiority. It appears that both the tumor biology and architecture, as well as the endoscopic position may determine the most successful method. Therefore, we recommend individualizing this approach. Negative suction in the needle may be created by withdrawing the stylet during tissue acquisition ("slow pull technique" associated with lowest suction force), by dry suction (attaching the needle to a syringe that contains a 10-50 ml of vacuum column) or by wet suction (preloading the needle with saline prior to attaching the syringe to create negative suction).[39,40] We recommend examining the specimen obtained by a low suction force method and if the material is minimal, attempt a higher suction method as second line. However, in hard fibrotic tumors (either based on expected histology such as sarcomas or NETs or by feel during the procedures), a higher suction force method would be reasonable to try first and only switch to a lower suction method if the aspirate is overwhelmingly bloody.

Handling of core biopsy specimens is possibly as important to acquiring high quality and quantity tissue as needle design. Perhaps the most reliable way of examining the specimen is by expelling the needle content on a glass slide. In our practice, we expel the tissue in a serpentine manner to allow visual examination of the entire content. Often, white materials can be seen interspersed with the red coagulum and these may be highest yield for diagnostic tissue. We use one of these suspected microcores to generate a smear for on site evaluation and for cytologic evaluation. Given that cytologic details such as nuclear and cytoplasmic details are often better preserved in CytoLyt, it remains important to have some material in this preservative. In addition, the quality of nucleic acid isolated from tissue material is somewhat better from CytoLyt than from formalin fixed material. The remainder, and the majority, of the microcores are placed in formalin. As shown in Figure 1D, the formalin-processed cell blocks often yield significant areas of intact tissue.

Prognostic Markers

Tissue-based markers harbor the benefit of being more specific to the tumor tissue, but at the expense of requiring more invasive collection techniques. Immunohistochemical (IHC) analysis is a widely-used process utilized to visualize specific molecular markers and identify their distribution in clinical tissue

Table 2. Prognostic Blood-Based Markers in PDAC

Marker	Country	Total Patients, n	Marker Dysregulated, n	Type of Dysregulation	Prognostic Value	HR (95% CI) or p-value
CTCs	Japan[71]	26	11	Positive count	**OS** +CTC: 3.7 -CTC: 12.5	**<.001**
	France[72]	79	9		**OS***	**2.5 (1.2-5.4)**
	Germany[73]	172	81	CK+ CTCs	**OS** +CTC: 17.9 -CTC: 26.1	**.05**
	USA[42]	50	39		**OS** +CTC: 13.7 -CTC: Not reached	**.008**
ctDNA	Japan[45]	105	33	Positive count	**RFS** +ctDNA: 6.1 -ctDNA: 16.1 **OS** +ctDNA: 13.6 -ctDNA: 27.6	**<.001** **3.2 (1.8–5.4)**
	Norway, USA[74]	14	10		**RFS*** **OS***	**.020** **.047**
miR-21	China[49]	177	89	High expression	**TTP*** **OS** +miR-21: 12 -miR-21: 32	**1.92 (1.27-2.90)** **1.71 (1.15-2.54)**
	China[50]	38	19		**OS***	**.01**
HOTAIR	USA[52]	102	14	High expression	**OS*** **Metastasis***	**.011** **<.0001**

PDAC, pancreatic ductal adenocarcinoma; HR, hazard ratio; OS, overall survival; TTP, time to progression; RFS, recurrence-free survival; CTCs, circulating tumor cells; CK, cytokeratin; HOTAIR, HOX Transcript Antisense RNA; ctDNA, circulating tumor DNA
*Reported only in graph form
TTP, RFS and OS reported in months
Bolded values are statistically significant.
These studies include patients with and without adjuvant chemotherapy.

specimens. Though these markers may be useful in patients who undergo surgical resection, investigations are still needed to discern if there is prognostic value to these biomarkers in pre-operative brush or biopsy specimens. Perhaps the best characterized treatment predictive biomarker is human equilibrative nucleoside transporter 1 (hENT1). hENT1, ribonucleotide reductase subunit 1 and 2 (RRM1, RRM2), and excision repair cross-complementing gene-1 (ERCC1) are vital for cellular transport and DNA synthesis and are frequently implicated as poor prognostic factors in various malignancies.[14] In one multivariate analysis, high expression of RRM2 and ERCC1, but not the others, were associated with worse recurrence-free survival (RFS) and overall survival (OS).[14] Another study found low hENT1 expression to be associated with poor RFS and OS.[15] As hENT1 plays a major role in the internalization of Gemcitabine by pancreatic cancer cells, the primary role of hENT1 is as a predictive marker to Gemcitabine chemotherapy, for which there is more data available. Table 3 summarizes the data for hENT1 and other tissue-based markers as prognostic

markers for survival in PDAC.

Secreted protein acidic and rich in cysteine (SPARC) is a matricellular glycoprotein which undergoes epigenetic silencing in pancreatic adenocarcinoma, but is often strongly expressed at the interface between the tumor and stroma by stromal fibroblasts.[16] Supporting data suggest this interaction is important for tumor progression, metastasis and chemoresistance. Stromal SPARC expression is observed in all disease stages suggesting early expression is critical for tumor progression.[17] Strong stromal SPARC expression in patients with well to moderately differentiated cancer who underwent surgical resection was associated with decreased overall survival when compared to patients with no SPARC expression.[17,18] Furthermore, patients with diffuse stromal SPARC expression extending beyond the peri-tumoral region had a significantly worse prognosis.[19] Most reports of cytoplasmic SPARC expression by malignant pancreatic cells have shown no prognostication value.[17] Some studies have found no prognostic benefit in observational cohorts, but only a strong predictive association in patients who were treated with gemcitabine.[20] Elevated SPARC mRNA expression has similarly been found to be a negative prognostic marker for PDAC survival and can be beneficial in that this analysis can be run on samples that are too small for IHC, such as from pre-operative fine needle aspiration.[21] Vascular endothelial growth factor (VEGF) is a potent stimulator of angiogenesis, thus facilitating tumor growth and progression. In IHC analysis, staining for VEGF is mainly demonstrated within the cytoplasm and cell membrane of cancer cells. Increased VEGF expression has been associated with a poor prognosis, including lower survival and increased lymphatic vessel invasion and lymph node metastasis.[22-24] Similar to SPARC and hENT1, there are therapies targeted against VEGF, so it also has potential as a predictive marker.

Smad4 is a tumor suppressor gene involved in mediating transforming growth factor beta (TGF-B). As evidenced by its alternative name, DPC4 (deleted in pancreatic carcinoma, locus 4), loss or inactivation of Smad4 is seen in ~50% of PDAC[25] and leads to increased cellular proliferation by upregulating the progression from G1-S in the cell cycle. Loss of Smad4 expression has frequently been shown to be associated with reduced survival in PDAC.[8,26] Interestingly, one study discordantly demonstrated that low expression of Smad4 was associated with improved overall survival and importantly, pancreatic resection only benefited (via longer survival) tumors who had lost Smad4 expression.[27]

KRAS (V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog) is a GTPase that activates proteins required for propagation of growth factors and other cell signaling receptors. Overall, KRAS mutations have correlated with a reduction in survival.[8,28,29] Interestingly, the different mutational subtypes show varying duration of survival, with wild type GGT (glycine) converted to GAT (aspartate) being the most common and the only one to be prognostic of poor survival.[8,28] Additionally, mutational analysis performed for these KRAS mutations can be performed with quantitative polymerase chain reaction (PCR) which is cheaper and faster than other sequencing methods and uses less DNA data, making it easier to perform multiple molecular analyses on the same sample.[28]

Perhaps the most exciting development of cancer therapy in the last few years has been the remarkable progress of the use of immunotherapy. Despite the success seen in several solid malignancies (melanoma, lung cancer, urological cancers), response rates have been minimal in pancreatic cancer. However, an immune response is present in pancreatic cancer and emerging strategies to turn on this immune response or identify tumors with an immune sensitive phenotype are promising. Parallel to these efforts, there is increasing evidence that the native immune response in pancreatic cancer is predictive of treatment outcomes. Immunohistochemical analyses that identify T cell populations and myeloid cells in pancreatic cancer,[30] or the level of expression of negative checkpoint regulators (NCR)[31,32] have already demonstrated prognostic value. These markers may also serve as important predictors of response to immunotherapies in the future.

Smad4, hENT1 and SPARC possess another benefit as biomarkers, in that they have been shown to be effectively assessed on pre-operative biopsy specimens.[19,27,33,34] Since quantitative PCR of VEGF and KRAS has also been shown to be accurate, there may be a role for these biomarkers during the pre-operative assessment with the smaller samples associated with biopsies.

Treatment Predictive Markers

There are various imaging modalities involved in the diagnosis and staging of PDAC including computed

(continued on page 28)

(continued from page 24)

Table 3. Prognostic Tissue-Based Markers in PDAC

(Table 3. continued on page 30)

Marker	Country	Total Patients, n	Marker Dysregulated, n	Type of Dysregulation	Prognostic Value	HR (95% CI) or p-value
					OS	
SPARC	USA[17]	299	200	High stromal expression	+SPARC: 15 -SPARC: 30	**1.89 (1.31-2.74)**
	Sweden[18]	88	68		+SPARC: 11.5 -SPARC: 25.3	**2.12 (1.19-3.98)**
	Germany[19]	58	58		+SPARC: 7.6 -SPARC: 10.2	**2.23 (1.05-4.72)**
	Germany[20]	160	95		+SPARC: 21.5 -SPARC: 18.2	.765
	Japan[21]	104	104	High mRNA expression	**5 year survival** +SPARC: 0% -SPARC: 22.5%	**2.92 (1.63-5.50)**
hENT1	Belgium, France, Canada[68]	142	54	High expression	**Death (%)** +hENT1: 76 -hENT1: 64	.92 (.57-1.50)
	USA[14]	95	81		**RFS** +hENT1: 9.5 -hENT1: 44.5 **OS** +hENT1: 15.2 -hENT1: 19.5	**.029** .175
	USA[15]	84	61		**RFS** +hENT1: 11.2 -hENT1: 4.5 **OS** +hENT1: 20 -hENT1: 14.8	**2.14 (1.14-4.02)** **1.97 (1.19-3.24)**
RRM2	USA[14]	95	16	High expression	**RFS** +RRM2: 6.9 -RRM2: 16.0 **OS** +RRM2: 9.1 -RRM2: 18.4	**<.0001** **<.0001**
ERCC1	USA[14]	95	15	High expression	**RFS** +ERCC1: 6.1 -ERCC1: 14.9 **OS** +ERCC1: 8.9 -ERCC1: 18.1	**.037** **.032**

PDAC, pancreatic ductal adenocarcinoma; HR, hazard ratio; OS, overall survival; RFS, recurrence-free survival; SPARC, Secreted protein acidic and rich in cysteine; hENT1, human equilibrative nucleoside transporter 1; RRM2, ribonucleotide reductase subunit 2; ERCC1, excision repair cross-complementing gene-1; VEGF, vascular endothelial growth factor; KRAS, Kirsten rat sarcoma viral oncogene homolog

*Reported only in graph form

RFS and OS reported in months.

Bolded values are statistically significant.

These studies include patients with and without adjuvant chemotherapy.

(continued on page 30)

(continued from page 28)

Table 3. Prognostic Tissue-Based Markers in PDAC ***(Table 3. continued from page 28)***

Marker	Country	Total Patients, n	Marker Dysregulated, n	Type of Dysregulation	Prognostic Value	HR (95% CI) or p-value
VEGF	China, USA[22]	58	22	High expression	**OS***	**2.60 (1.21-5.60)**
	China[23]	62	37		**OS** +VEGF: 7.7 -VEGF: 17.9	**2.27 (1.17-4.43)**
	Japan[69]	40	27		**OS** +VEGF: 7.8 -VEGF: 23.3	**.048**
	Japan[70]	55	39		**OS***	**.021**
Smad4	USA, Netherlands[26]	249	138	Loss of expression	**OS** +Smad4: 19.2 -Smad4: 14.7	**1.36 (1.01–1.83)**
	Korea[8]	272	222		**OS** +Smad4: 26.2 -Smad4: 17.4	**2.17 (1.24-3.79)**
	Australia[27]	119	63		**OS** +Smad4: 6.0 -Smad4: 9.2	**.60 (.41-.89)**
KRAS	Korea[8]	234	126	Codon mutation (Wild type = GGT)	**OS** Mutation, any: 26.4 Wild type: 14.3	**1.63 (1.12-2.38)**
	France[28]	219	147		**OS** Mutation, GAD: 6 Wild type: 9	**1.47 (1.19-2.20)**
	Germany[29]	153	105		**OS** Mutation, any: 12.7 Wild type: 20.7	**1.68 (1.07-2.62)**
miR-21	Multiple (meta-analysis)[48]	541	Not reported	High expression	**OS***	**2.66 (2.06-3.43)**

PDAC, pancreatic ductal adenocarcinoma; HR, hazard ratio; OS, overall survival; RFS, recurrence-free survival; SPARC, Secreted protein acidic and rich in cysteine; hENT1, human equilibrative nucleoside transporter 1; RRM2, ribonucleotide reductase subunit 2; ERCC1, excision repair cross-complementing gene-1; VEGF, vascular endothelial growth factor; KRAS, Kirsten rat sarcoma viral oncogene homolog
*Reported only in graph form
RFS and OS reported in months.
Bolded values are statistically significant.
These studies include patients with and without adjuvant chemotherapy.

tomography (CT), magnetic resonance imaging (MRI), endoscopic ultrasound (EUS), and endoscopic retrograde cholangiopancreatography (ERCP). The roles of these modalities have changed over time, but the relative importance of EUS greatly increased with the advent of EUS-guided fine needle aspiration (EUS-FNA) over 20 years ago. Though limited by relative invasiveness and operator variability, it has been demonstrated to have excellent sensitivity (91-100%) and specificity (94-100%) for the diagnosis of PDAC.[54]

While many tissue-based biomarkers show promise in the evaluation of PDAC, most of these studies have relied on surgically resected material, which comprise less than 20% of all patients diagnosed with this disease. As EUS-FNA technology continues to improve, including the ability to obtain more histologically intact core biopsies with new needles and improved visibility with the use of contrast-enhancement and elastography, the clinical utility of these biopsies broadens. Though not yet in widespread use, we can reliably perform immunohistochemistry analyses to identify prognostic and predictive markers such as hENT1, VEGF and microRNAs on these tissue samples pre-operatively.[33,55-57] Unfortunately, truly targeted therapies in pancreatic cancer are not yet available, but as our understanding of the biology of cancer evolves, it is important that our tissue acquisition methods improve and are ready for "prime time."

One could imagine that a combination of currently available and previously discussed biomarkers may help in the selection of therapies, but only if our ability to evaluate them in pancreatic cancer biopsies can be validated. There a few emerging examples of clinical trials that require specific markers in biopsies. Pegylated recombinant human hyaluronidase (PEGPH20) is an enzyme that has been shown to potentiate chemotherapy in PDAC by removing excess hyaluronic acid from the tumor microenvironment. A phase 1b trial of PEGPH20 in combination with Gemcitabine as first line therapy in metastatic disease demonstrated a good safety profile and promising therapeutic benefit.[58] There are several ongoing clinical trials of PEGPH20; participation not only requires histologically confirmed PDAC, but frequently also evaluation of PEGPH20 expression in biopsies.

In addition, from many other cancer treatment algorithms, we know that understanding changes in tumor biology following a first line treatment regimen is critical. Recent phase II trials of a VEGF inhibitor, Vatalinib[59] and nab-paclitaxel[60] as individual second line therapies in advanced PDAC have demonstrated a favorable survival outcome in some patients; however, serum-based biomarkers did not correlate with response and the predictive value of tissue-based biomarkers (SPARC) were inconclusive due to small sample size. With high quality biopsies, we can expect that most trials, and increasingly, the standard of practice, will emphasize the need for post-treatment biopsies.

CONCLUSION

This review focused on biomarkers that can be used to prognosticate outcome in PDAC, independent of treatment strategy. There are a multitude of potential biomarkers in the literature, but many are limited by specificity, heterogeneity of disease, difficulty in obtaining adequate samples and conflicting results. Also, the majority of the tissue-based biomarkers have been studied in resection specimens, and these patients only account for a minority of most PDAC cohorts. The most promising tissue biomarkers include hENT1, SPARC, Smad4, and VEGF as they may be valuable in the pre-operative period and may additionally have predictive value in guiding individualized pancreatic cancer therapy. The novel serum-based markers are also valuable due to their minimally invasive approach and foundation for genetic analysis. As both endoscopic methods to obtain high quality biopsy specimens and the molecular understanding of pancreatic cancer advances, it is likely that these and many other biomarkers will enter into routine clinical practice. It is increasingly important to obtain the highest quality tumor biopsies at the time of diagnostic procedures and assure that sufficient tumor tissue material is available for molecular and immunohistochemical analysis. ■

References

1. Yadav D, Lowenfels AB. The epidemiology of pancreatitis and pancreatic cancer. Gastroenterology. 2013;144(6):1252-1261.
2. Swords DS, Firpo MA, Scaife CL, Mulvihill SJ. Biomarkers in pancreatic adenocarcinoma: current perspectives. Onco Targets Ther. 2016;9:7459-7467.
3. Ferrone CR, Finkelstein DM, Thayer SP, Muzikansky A, Fernandez-delCastillo C, Warshaw AL. Perioperative CA19-9 levels can predict stage and survival in patients with resectable pancreatic adenocarcinoma. J Clin Oncol. 2006;24(18):2897-2902.
4. Kilic M, Gocmen E, Tez M, Ertan T, Keskek M, Koc M. Value

of preoperative serum CA 19-9 levels in predicting resectability for pancreatic cancer. Can J Surg. 2006;49(4):241-244.
5. Schlieman MG, Ho HS, Bold RJ. Utility of tumor markers in determining resectability of pancreatic cancer. Arch Surg. 2003;138(9):951-955; discussion 955-956.
6. Kondo N, Murakami Y, Uemura K, et al. Prognostic impact of perioperative serum CA 19-9 levels in patients with resectable pancreatic cancer. Ann Surg Oncol. 2010;17(9):2321-2329.
7. Bergquist JR, Puig CA, Shubert CR, et al. Carbohydrate Antigen 19-9 Elevation in Anatomically Resectable, Early Stage Pancreatic Cancer Is Independently Associated with Decreased Overall Survival and an Indication for Neoadjuvant Therapy: A National Cancer Database Study. J Am Coll Surg. 2016;223(1):52-65.
8. Shin SH, Kim SC, Hong SM, et al. Genetic alterations of K-ras, p53, c-erbB-2, and DPC4 in pancreatic ductal adenocarcinoma and their correlation with patient survival. Pancreas. 2013;42(2):216-222.
9. Tas F, Aykan F, Alici S, Kaytan E, Aydiner A, Topuz E. Prognostic factors in pancreatic carcinoma: serum LDH levels predict survival in metastatic disease. Am J Clin Oncol. 2001;24(6):547-550.
10. Wu L, Huang P, Wang F, et al. Relationship between serum CA19-9 and CEA levels and prognosis of pancreatic cancer. Ann Transl Med. 2015;3(21):328.
11. Lee KJ, Yi SW, Chung MJ, et al. Serum CA 19-9 and CEA levels as a prognostic factor in pancreatic adenocarcinoma. Yonsei Med J. 2013;54(3):643-649.
12. Mitsunaga S, Ikeda M, Shimizu S, et al. C-Reactive Protein Level Is an Indicator of the Aggressiveness of Advanced Pancreatic Cancer. Pancreas. 2016;45(1):110-116.
13. Pine JK, Fusai KG, Young R, et al. Serum C-reactive protein concentration and the prognosis of ductal adenocarcinoma of the head of pancreas. Eur J Surg Oncol. 2009;35(6):605-610.
14. Fisher SB, Patel SH, Bagci P, et al. An analysis of human equilibrative nucleoside transporter-1, ribonucleoside reductase subunit M1, ribonucleoside reductase subunit M2, and excision repair cross-complementing gene-1 expression in patients with resected pancreas adenocarcinoma: implications for adjuvant treatment. Cancer. 2013;119(2):445-453.
15. Kim R, Tan A, Lai KK, et al. Prognostic roles of human equilibrative transporter 1 (hENT-1) and ribonucleoside reductase subunit M1 (RRM1) in resected pancreatic cancer. Cancer. 2011;117(14):3126-3134.
16. Sato N, Fukushima N, Maehara N, et al. SPARC/osteonectin is a frequent target for aberrant methylation in pancreatic adenocarcinoma and a mediator of tumor-stromal interactions. Oncogene. 2003;22(32):5021-5030.
17. Infante JR, Matsubayashi H, Sato N, et al. Peritumoral fibroblast SPARC expression and patient outcome with resectable pancreatic adenocarcinoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2007;25(3):319-325.
18. Gundewar C, Sasor A, Hilmersson KS, Andersson R, Ansari D. The role of SPARC expression in pancreatic cancer progression and patient survival. Scand J Gastroenterol. 2015:1-5.
19. Mantoni TS, Schendel RR, Rodel F, et al. Stromal SPARC expression and patient survival after chemoradiation for non-resectable pancreatic adenocarcinoma. Cancer Biol Ther. 2008;7(11):1806-1815.
20. Sinn M, Sinn BV, Striefler JK, et al. SPARC expression in resected pancreatic cancer patients treated with gemcitabine: results from the CONKO-001 study. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO. 2014;25(5):1025-1032.
21. Miyoshi K, Sato N, Ohuchida K, Mizumoto K, Tanaka M. SPARC mRNA expression as a prognostic marker for pancreatic adenocarcinoma patients. Anticancer Res. 2010;30(3):867-871.
22. Sun HC, Qiu ZJ, Liu J, et al. Expression of hypoxia-inducible factor-1 alpha and associated proteins in pancreatic ductal adenocarcinoma and their impact on prognosis. Int J Oncol. 2007;30(6):1359-1367.
23. Ai KX, Lu LY, Huang XY, Chen W, Zhang HZ. Prognostic significance of S100A4 and vascular endothelial growth factor expression in pancreatic cancer. World J Gastroenterol. 2008;14(12):1931-1935.
24. Tang RF, Itakura J, Aikawa T, et al. Overexpression of lymphangiogenic growth factor VEGF-C in human pancreatic cancer. Pancreas. 2001;22(3):285-292.
25. Ahmed S, Bradshaw AD, Gera S, Dewan MZ, Xu R. The TGF-beta/Smad4 Signaling Pathway in Pancreatic Carcinogenesis and Its Clinical Significance. J Clin Med. 2017;6(1).
26. Tascilar M, Skinner HG, Rosty C, et al. The SMAD4 protein and prognosis of pancreatic ductal adenocarcinoma. Clin Cancer Res. 2001;7(12):4115-4121.
27. Biankin AV, Morey AL, Lee CS, et al. DPC4/Smad4 expression and outcome in pancreatic ductal adenocarcinoma. J Clin Oncol. 2002;20(23):4531-4542.
28. Bournet B, Muscari F, Buscail C, et al. KRAS G12D Mutation Subtype Is A Prognostic Factor for Advanced Pancreatic Adenocarcinoma. Clin Transl Gastroenterol. 2016;7:e157.
29. Sinn BV, Striefler JK, Rudl MA, et al. KRAS mutations in codon 12 or 13 are associated with worse prognosis in pancreatic ductal adenocarcinoma. Pancreas. 2014;43(4):578-583.
30. Ino Y, Yamazaki-Itoh R, Shimada K, et al. Immune cell infiltration as an indicator of the immune microenvironment of pancreatic cancer. Br J Cancer. 2013;108(4):914-923.
31. Nomi T, Sho M, Akahori T, et al. Clinical significance and therapeutic potential of the programmed death-1 ligand/programmed death-1 pathway in human pancreatic cancer. Clin Cancer Res. 2007;13(7):2151-2157.
32. Shibuya KC, Goel VK, Xiong W, et al. Pancreatic ductal adenocarcinoma contains an effector and regulatory immune cell infiltrate that is altered by multimodal neoadjuvant treatment. PLoS One. 2014;9(5):e96565.
33. Yamada R, Mizuno S, Uchida K, et al. Human Equilibrative Nucleoside Transporter 1 Expression in Endoscopic Ultrasonography-Guided Fine-Needle Aspiration Biopsy Samples Is a Strong Predictor of Clinical Response and Survival in the Patients With Pancreatic Ductal Adenocarcinoma Undergoing Gemcitabine-Based Chemoradiotherapy. Pancreas. 2016;45(5):761-771.
34. Zapata M, Cohen C, Siddiqui MT. Immunohistochemical expression of SMAD4, CK19, and CA19-9 in fine needle aspiration samples of pancreatic adenocarcinoma: Utility and potential role. Cytojournal. 2007;4:13.
35. Vanbiervliet G, Napoleon B, Saint Paul MC, et al. Core needle versus standard needle for endoscopic ultrasound-guided biopsy of solid pancreatic masses: a randomized crossover study. Endoscopy. 2014;46(12):1063-1070.
36. Lee YN, Moon JH, Kim HK, et al. Core biopsy needle versus standard aspiration needle for endoscopic ultrasound-guided sampling of solid pancreatic masses: a randomized parallel-group study. Endoscopy. 2014;46(12):1056-1062.
37. Khan MA, Grimm IS, Ali B, et al. A meta-analysis of endoscopic ultrasound-fine-needle aspiration compared to endoscopic ultrasound-fine-needle biopsy: diagnostic yield and the value of onsite cytopathological assessment. Endosc Int Open. 2017;5(5):E363-E375.
38. Nagula S, Pourmand K, Aslanian H, et al. Comparing EUS-Fine Needle Aspiration and EUS-Fine Needle Biopsy for Solid Lesions: A Multicenter, Randomized Trial. Clin Gastroenterol Hepatol. 2017.

(continued on page 35)

(continued from page 32)

39. Villa NA, Berzosa M, Wallace MB, Raijman I. Endoscopic ultrasound-guided fine needle aspiration: The wet suction technique. Endosc Ultrasound. 2016;5(1):17-20.
40. Nakai Y, Isayama H, Chang KJ, et al. Slow pull versus suction in endoscopic ultrasound-guided fine-needle aspiration of pancreatic solid masses. Dig Dis Sci. 2014;59(7):1578-1585.
41. Han L, Chen W, Zhao Q. Prognostic value of circulating tumor cells in patients with pancreatic cancer: a meta-analysis. Tumour Biol. 2014;35(3):2473-2480.
42. Poruk KE, Valero V, 3rd, Saunders T, et al. Circulating Tumor Cell Phenotype Predicts Recurrence and Survival in Pancreatic Adenocarcinoma. Ann Surg. 2016;264(6):1073-1081.
43. Shen Z, Wu A, Chen X. Current detection technologies for circulating tumor cells. Chem Soc Rev. 2017;46(8):2038-2056.
44. Xu Y, Qin T, Li J, et al. Detection of Circulating Tumor Cells Using Negative Enrichment Immunofluorescence and an In Situ Hybridization System in Pancreatic Cancer. Int J Mol Sci. 2017;18(4).
45. Hadano N, Murakami Y, Uemura K, et al. Prognostic value of circulating tumour DNA in patients undergoing curative resection for pancreatic cancer. Br J Cancer. 2016;115(1):59-65.
46. Cheng H, Liu C, Jiang J, et al. Analysis of ctDNA to predict prognosis and monitor treatment responses in metastatic pancreatic cancer patients. Int J Cancer. 2017;140(10):2344-2350.
47. Bettegowda C, Sausen M, Leary RJ, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. Sci Transl Med. 2014;6(224):224ra224.
48. Frampton AE, Krell J, Jamieson NB, et al. microRNAs with prognostic significance in pancreatic ductal adenocarcinoma: A meta-analysis. European journal of cancer. 2015;51(11):1389-1404.
49. Wang P, Zhuang L, Zhang J, et al. The serum miR-21 level serves as a predictor for the chemosensitivity of advanced pancreatic cancer, and miR-21 expression confers chemoresistance by targeting FasL. Molecular oncology. 2013;7(3):334-345.
50. Liu R, Chen X, Du Y, et al. Serum microRNA expression profile as a biomarker in the diagnosis and prognosis of pancreatic cancer. Clin Chem. 2012;58(3):610-618.
51. Ma MZ, Kong X, Weng MZ, et al. Candidate microRNA biomarkers of pancreatic ductal adenocarcinoma: meta-analysis, experimental validation and clinical significance. J Exp Clin Cancer Res. 2013;32:71.
52. Kim K, Jutooru I, Chadalapaka G, et al. HOTAIR is a negative prognostic factor and exhibits pro-oncogenic activity in pancreatic cancer. Oncogene. 2013;32(13):1616-1625.
53. Xie Z, Chen X, Li J, et al. Salivary HOTAIR and PVT1 as novel biomarkers for early pancreatic cancer. Oncotarget. 2016;7(18):25408-25419.
54. Chang DK, Nguyen NQ, Merrett ND, Dixson H, Leong RW, Biankin AV. Role of endoscopic ultrasound in pancreatic cancer. Expert Rev Gastroenterol Hepatol. 2009;3(3):293-303.
55. Puleo F, Demetter P, Eisendrath P, et al. Feasibility of Immunohistochemistry on Endoscopic Ultrasound Fine-Needle Aspiration Samples for Evaluating Predictive Biomarkers in Pancreatic Cancer Management. Pancreas. 2016;45(9):e50-52.
56. Costache MI, Iordache S, Costache CA, Dragos E, Dragos A, Saftoiu A. Molecular Analysis of Vascular Endothelial Growth Factor (VEGF) Receptors in EUS-guided Samples Obtained from Patients with Pancreatic Adenocarcinoma. J Gastrointestin Liver Dis. 2017;26(1):51-57.
57. Frampton AE, Krell J, Prado MM, et al. Prospective validation of microRNA signatures for detecting pancreatic malignant transformation in endoscopic-ultrasound guided fine-needle aspiration biopsies. Oncotarget. 2016;7(19):28556-28569.
58. Hingorani SR, Harris WP, Beck JT, et al. Phase Ib Study of PEGylated Recombinant Human Hyaluronidase and Gemcitabine in Patients with Advanced Pancreatic Cancer. Clin Cancer Res. 2016;22(12):2848-2854.
59. Dragovich T, Laheru D, Dayyani F, et al. Phase II trial of vatalanib in patients with advanced or metastatic pancreatic adenocarcinoma after first-line gemcitabine therapy (PCRT O4-001). Cancer Chemother Pharmacol. 2014;74(2):379-387.
60. Hosein PJ, de Lima Lopes G, Jr., Pastorini VH, et al. A phase II trial of nab-Paclitaxel as second-line therapy in patients with advanced pancreatic cancer. Am J Clin Oncol. 2013;36(2):151-156.
61. Paganuzzi M, Onetto M, Marroni P, et al. CA 19-9 and CA 50 in benign and malignant pancreatic and biliary diseases. Cancer. 1988;61(10):2100-2108.
62. Zhang S, Wang YM, Sun CD, Lu Y, Wu LQ. Clinical value of serum CA19-9 levels in evaluating resectability of pancreatic carcinoma. World J Gastroenterol. 2008;14(23):3750-3753.
63. Ni XG, Bai XF, Mao YL, et al. The clinical value of serum CEA, CA19-9, and CA242 in the diagnosis and prognosis of pancreatic cancer. Eur J Surg Oncol. 2005;31(2):164-169.
64. Katz MH, Varadhachary GR, Fleming JB, et al. Serum CA 19-9 as a marker of resectability and survival in patients with potentially resectable pancreatic cancer treated with neoadjuvant chemoradiation. Ann Surg Oncol. 2010;17(7):1794-1801.
65. Lundin J, Roberts PJ, Kuusela P, Haglund C. The prognostic value of preoperative serum levels of CA 19-9 and CEA in patients with pancreatic cancer. Br J Cancer. 1994;69(3):515-519.
66. Safi F, Schlosser W, Kolb G, Beger HG. Diagnostic value of CA 19-9 in patients with pancreatic cancer and nonspecific gastrointestinal symptoms. J Gastrointest Surg. 1997;1(2):106-112.
67. Louhimo J, Alfthan H, Stenman UH, Haglund C. Serum HCG beta and CA 72-4 are stronger prognostic factors than CEA, CA 19-9 and CA 242 in pancreatic cancer. Oncology. 2004;66(2):126-131.
68. Marechal R, Bachet JB, Mackey JR, et al. Levels of gemcitabine transport and metabolism proteins predict survival times of patients treated with gemcitabine for pancreatic adenocarcinoma. Gastroenterology. 2012;143(3):664-674 e661-666.
69. Ikeda N, Adachi M, Taki T, et al. Prognostic significance of angiogenesis in human pancreatic cancer. Br J Cancer. 1999;79(9-10):1553-1563.
70. Kuwahara K, Sasaki T, Kuwada Y, Murakami M, Yamasaki S, Chayama K. Expressions of angiogenic factors in pancreatic ductal carcinoma: a correlative study with clinicopathologic parameters and patient survival. Pancreas. 2003;26(4):344-349.
71. Kurihara T, Itoi T, Sofuni A, et al. Detection of circulating tumor cells in patients with pancreatic cancer: a preliminary result. J Hepatobiliary Pancreat Surg. 2008;15(2):189-195.
72. Bidard FC, Huguet F, Louvet C, et al. Circulating tumor cells in locally advanced pancreatic adenocarcinoma: the ancillary CirCe 07 study to the LAP 07 trial. Ann Oncol. 2013;24(8):2057-2061.
73. Soeth E, Grigoleit U, Moellmann B, et al. Detection of tumor cell dissemination in pancreatic ductal carcinoma patients by CK 20 RT-PCR indicates poor survival. J Cancer Res Clin Oncol. 2005;131(10):669-676.
74. Tjensvoll K, Lapin M, Buhl T, et al. Clinical relevance of circulating KRAS mutated DNA in plasma from patients with advanced pancreatic cancer. Molecular oncology. 2016;10(4):635-643.