Review

Clinical Cancer Research

Biomarker-Based Therapy in Pancreatic Ductal Adenocarcinoma: An Emerging Reality?

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Abstract

Over the last decade, many of the major solid organ cancers have seen improvements in survival due to development of novel therapeutics and corresponding biomarkers that predict treatment efficacy or resistance. In contrast, favorable outcomes remain challenging in pancreatic ductal adenocarcinoma (PDAC), in part related to the lack of validated biomarkers for patient and treatment selection and thus optimal clinical decision-making. Increasingly, however, therapeutic development for PDAC is accompanied by bioassays to evaluate response and to study mechanism of actions with a corresponding increase in the number of trials in mid to late stage with integrated biomarkers. In addition, blood-based biomarkers that provide a measure of disease activity and allow for minimally invasive tumor analyses are emerging, including circulating tumor DNA, exosomes, and circulating tumor cells. In this article, we review potential biomarkers for currently approved therapies as well as emerging biomarkers for therapeutics under development. *Clin Cancer Res; 24(10); 2241–50. ©2017 AACR.*

Introduction

The role of biomarkers in the diagnosis and treatment of cancer is rapidly expanding. Many of the major solid organ cancers have seen improvements in survival over the last decade, in part due to development of novel therapeutics and corresponding biomarkers that predict treatment efficacy and optimize patient selection. For example, in melanoma, *BRAF V600* mutations predict response to BRAF and MEK inhibitor combinations, and in lung cancer, *EGFR*, *ROS1*, *ALK*, and *BRAF* mutations predict sensitivity to their respective inhibitors, and PD-L1 identifies patients enriched for benefit from checkpoint inhibitor therapies (Table 1).

In pancreatic ductal adenocarcinoma (PDAC), biomarkers are lacking, with treatment predominantly determined by stage of disease, performance status, and therapy dominated by cytotoxic agents. Specifically, FOLFIRINOX [5-fluorouracil (5-FU), leucovorin, oxaliplatin, irinotecan], gemcitabine/nab-paclitaxel, and liposomal irinotecan/5-FU combinations have collectively increased survival in the advanced-disease setting. Erlotinib is the only approved "targeted" agent, which was approved in a past era and was not based on biomarker selection (1–4).

Bioassays are increasingly being incorporated into PDAC therapeutic development to evaluate response and to study mechanisms of action. Given the successful development in other malignancies, arguably, an era of biomarker-selected therapy in PDAC may be emerging. Herein, we review potential biomarkers for currently approved therapies as well as emerging biomarkers for agents under development.

PDAC Pathophysiology and Biology

Biomarkers reflect underlying pathophysiology, which in PDAC is driven by characteristic mutations and epigenetic modifications that lead to aberrant signaling pathways, altered metabolism, expression of surface antigens, and remodeling of the tumor microenvironment. Ninety percent to 95% of PDAC tumors have an oncogenic *KRAS* mutation, with frequent mutations in *TP53* (75%), *SMAD4* (22%), and *CDKN2A/B* (18%; ref. 5).

Downstream from these genetic alterations, gene expression profiling has identified 12 aberrant core signaling pathways that drive PDAC tumorigenesis. These pathways, most notably KRAS signaling, G_1–S checkpoint regulation, hedgehog signaling, TGFβ signaling, and Wnt/Notch signaling, have been targeted by various therapeutics and contain numerous measurable markers of signaling activity (6).

Cell-surface carbohydrate antigen 19-9 (Ca 19-9) and carcinoembryonic antigen (CEA) overexpression is present in 94% and 71% of patients, respectively, and EGFR is overexpressed in up to 70% of patients (7–9). Other common surface antigens include mucin-1, mucin-5AC, epithelial cell adhesion molecule, mesothelin, and prostate stem cell antigen (10–12).

In the PDAC microenvironment, cancer-associated fibroblasts secrete increased amounts of hyaluronic acid (HA), increasing interstitial pressure, decreasing blood flow, impairing drug delivery, and creating a nutrient- and oxygen-deprived microenvironment (13). Multiple metabolic changes result as PDAC cells rely on nonoxidative energy production, extracellular proteins, and autophagy for metabolism (14, 15).

Therapeutic development has sought to exploit many of these characteristics, and in many cases, the assays used to study therapeutics at the bench are being incorporated as potential biomarkers clinically.

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Table 1. Selected biomarker-based cancer therapies

Malignancy	Biomarker	Therapeutic
Breast	Estrogen receptor	Antihormonal therapy
	Progesterone receptor	Antihormonal therapy
	HER2	Anti-HER agents
Colorectal	KRAS	Cetuximab, panitumumab
Gastric/GEJ	HER2	Trastuzumab
GIST	c-Kit	Imatinib
Lung cancer	EGFR/KRAS	Erlotinib, afatinib
	ALK/ROS1	Crizotinib, ceritinib
	BRAF V600	Dabrafenib/trametinib
Melanoma	BRAF V600	Dabrafenib/trametinib, vemurafanib
Ovarian	BRCA	Niraparib, olaparib, rucaparib
Any	dMMR, MSI-H	Pembrolizumab

Abbreviations: dMMR, mismatch repair deficient; GEJ, gastroesophageal junction; GIST, gastrointestinal stromal tumor; MSI-H, microsatellite instability high.

Current Biomarkers

Serum CA19-9 is the only approved biomarker for PDAC, with an indication for monitoring disease status (16). CA19-9 has many limitations. It is not sufficiently sensitive or specific to be used for disease detection in asymptomatic populations and may be elevated in biliary obstruction and benign pancreatic diseases, limiting its use in high-risk populations (17). CA19-9 has shown prognostic value after surgical resection and following chemotherapy initiation, leading to its approval for disease monitoring (18). Similarly, CEA is a tumor antigen that is elevated in the serum from certain patients with PDAC and has shown prognostic value. It is used alongside CA19-9 with similar applications (19). Despite their use for disease monitoring, CA19-9 and CEA are mainly used as adjuncts to radiographic imaging and are rarely used for treatment decisions in isolation.

Frontline Cytotoxic Therapy and Pharmacokinetic Resistance

Predictive biomarkers of approved frontline cytotoxic therapy efficacy have focused on variability in drug delivery and metabolism with mixed results. For example, human nucleoside transporter 1 (hENT1) plays a key role in gemcitabine cellular uptake. Supporting evidence comes from retrospective analyses of phase III adjuvant studies in which high hENT expressers demonstrated improved survival relative to low expressers (20). This led to prospective study in the LEAP trial, which stratified patients by hENT1 status and compared gemcitabine with a gemcitabine–lipid conjugate designed for hENT1-independent cell entry. Unfortunately, LEAP failed to show a difference in therapeutic response by agent or hENT1 status (21). Data remain conflicting, however, with a recent systematic review showing hENT1 as a prognostic marker in patients receiving adjuvant gemcitabine-based therapy (22).

Countering gemcitabine effects, ribonucleotide reductase catalyzes the rate-limiting step in the production of deoxyribonucleotides and is essential for DNA synthesis and repair. Increased ribonucleotide reductase activity, determined by ribonucleotide reductase catalytic subunit M1 (RRM1) expression, is a potential marker of gemcitabine resistance, with supportive data from lung cancer and preclinical study in PDAC (23). In human trials, Valsecchi and colleagues and Farrel and colleagues found no relationship between RRM1 expression and survival (24, 25).

Nab-paclitaxel, a nanoparticle albumin-bound paclitaxel, achieves increased tumor levels relative to free paclitaxel by albumin-mediated transcytosis and enhanced vascular permeability and possibly albumin binding by proteins in the tumor microenvironment (26). Secreted protein acidic and rich in cysteine (SPARC) is an albumin-binding protein that is overexpressed in PDAC tissue. Thirty-six patients in a phase I/II study of gemcitabine/nab-paclitaxel were evaluated for SPARC expression and demonstrated a correlation with improved overall survival (OS, 17.8 vs. 8.1 months; ref. 27). The follow-up MPACT study, which led to gemcitabine/nab-paclitaxel FDA approval, however, did not confirm an association between SPARC level and survival (28). SPARC measurement has subsequently not been incorporated into clinical practice.

With respect to FOLFIRINOX, 5-FU is degraded by dihydropyrimidine dehydrogenase (DPD) and targets the enzyme thymidylate synthase (TS). It follows that the study of pancreatic cancer cell lines demonstrated an association between DPD and TS levels and 5-FU sensitivity. Retrospective analysis of the RTOG-9704 study showed a correlation between DPD expression and survival, but overall clinical implication has been limited, and further study is needed (20).

Excision repair cross-complementation group 1 (ERCC1) is an endonuclease that has shown promise as a biomarker for platinum resistance. ERCC1 is involved in repair of interstrand crosslinks and double-stranded DNA breaks caused by platinum agents. High ERCC1 levels by IHC staining and RT-PCR gene expression have been associated with lower response rates and decreased survival in single-center, retrospective analyses of patients treated with platinum-containing regimens (29, 30).

Despite promising preliminary data, these biomarkers have yet to demonstrate validity in large-scale clinical trials. A pilot study evaluated the ability to treat metastatic patients with one of seven different regimens based on RRM1, ERCC1, and TS status. The study demonstrated the feasibility of incorporating biomarker-selected therapy into practice, although challenges in delaying frontline treatment were noted, and results did not show clear alterations of disease course. Response rate was only 9%, but disease control rate was more optimistically 82%, with a median OS of 10.4 months (31).

Targeted-Therapy Biomarkers

Despite strong preclinical data and sound physiologic rationale, targeted therapy has met with significant challenges in PDAC. Various agents targeting PDAC core signaling pathways have been studied, including mitogen-activated protein kinase kinase, AKT, hedgehog, janus kinase, and notch inhibitors, with negative results in predominantly unselected populations.

Erlotinib is the only targeted agent that has been approved for PDAC. Its approval, in combination with gemcitabine, was based on a modest survival benefit in an unselected population. Retrospective analysis of the PA.3 trial found that 49% of patients had increased EGFR expression; however, there was no correlation between EGFR expression and OS (32). KRAS wild-type patients had improved OS, but subsequent prospective study of patients treated with second-line erlotinib versus placebo did not identify EGFR protein expression, *EGFR* copy number/mutations/polymorphisms, or *KRAS* mutation status to correlate with progression-free survival (PFS; refs. 33, 34).

With next-generation sequencing of tumors becoming increasingly common practice, targeted therapy selection based on genetic analysis is an attractive concept. To date, most of these analyses come from evaluation of primary PDACs. Analysis of both primary and metastatic tumor specimens by our group, however, suggests that currently application remains relatively limited. We analyzed 335 PDAC tumor specimens with our institutional sequencing panel (MSK-IMPACT). Although 26% of samples had potentially actionable mutations defined by OncoKB, only 5.5% contained an alteration that is currently an FDA-approved biomarker in another cancer indication. Three (1%) patients had matched systemic therapy based on their molecular profiling, and neither of the two patients evaluable for response had benefit (5). Beyond genetic analysis, common targeted therapeutic bioassays include IHC assays and gene expression profiling by RT-PCR.

Targeted therapeutics in development are increasingly being studied in biomarker-selected populations or with biomarker correlatives during clinical trials (Tables 2 and 3). For example, cabozantinib with erlotinib is being studied in patients with EGFR- and c-MET–expressing tumors (NCT03213626), enzalutamide with gemcintabine/nab-paclitaxel is being evaluated in patients with androgen receptor expression (NCT02138383), and a phase I of dinaciclib/MK2206 has completed, with results pending and planned pretreatment RAS pathway signaling analysis (NCT01783171).

Targeted monocolonal antibodies are also being studied in biomarker-selected populations, including a portfolio of CA19-9–directed therapeutics and diagnostics. MVT-5873 is an anti–CA19-9 monoclonal antibody (mAb) with an 89Zr-labeled version being developed as a PET imaging agent (MVT-2163) and a 177Lu-labeled version as a radioimmunotherapeutic (MVT-1075). All agents are currently in phase I study in patients selected for CA19-9 expression (NCT02672917, NCT02687230, and NCT03118349).

Biomarkers for Immunotherapy

The first PDAC biomarker-based therapy, pembrolizumab, was recently approved for patients with microsatellite instability–high (MSI-H) and mismatch repair–deficient (dMMR) tumors agnostic to organ of origin that have progressed following prior treatment and who have no satisfactory alternative treatment options (35). Approval was based on data from five studies including 149 patients with multiple malignancies. Published data for PDAC have included four patients with dMMR tumors—two of whom demonstrated partial response and two stable disease (36). Nine PDAC patients with MSI-H tumors were included in KEYNOTE158, which demonstrated an overall response rate of 37.7% across all 77 noncolorectal cancers, with median duration of response not reached (37). These abnormalities are rare, occurring in <1% of patients with PDAC, but are important to identify. Methodologies for identification of mismatch repair deficiency include immunohistochemical analysis for loss of mismatch repair protein expression, PCR for microsatellites, and increasingly the use of next-generation sequencing bioinformatics analyses, for example, MSISensor and mSINGs (38).

Outside of MSI-H and dMMR populations, checkpoint inhibitors are being studied intensively in PDAC. Initial monotherapy studies have not demonstrated benefit, likely due to variable expression of checkpoint signaling molecules, modulation of tumor antigens, and immunosuppressive cytokines inhibiting T-cell migration and activation (39). Combinations of agents aiming to unlock tumor immunogenicity are being studied with planned biomarker analyses. The ALPS trial is a phase II study of durvalumab ± tremelimumab (NCT02558894), which recently completed, with results pending. Morpheus pancreatic cancer is a multiarm study evaluating the anti–PD-L1 mAb atezolizumab in combination with cobimetinib, PEGPH20, or BL-8040 versus standard-of-care cytotoxics (NCT03193190). Correlatives including PD-L1 status are being explored in both.

Cergutuzumab amunaleukin is a hybrid targeted immunotherapeutic consisting of a CEA-specific antibody fused to an IL2 variant designed to increase local immune activity. Cergutuzumab is being studied in combination with atezolizumab in patients with CEA-positive malignancies (NCT02350673).

Chimeric antigen receptor T cells (CART) are designed to engage specific tumor antigens, and biomarker selection is inherent in their use. Various CARTs are in early-stage clinical trials targeting CEA, mesothelin, MUC1, and prostate stem cell antigen in populations selected for their respective antigens (NCT03267173, NCT03323944, NCT03267173, and NCT02744287).

Biomarkers for Stromal-Targeting Agents

High interstitial pressure caused by HA in the PDAC stroma impairs drug delivery (13). PEGPH20 is a recombinant pegylated hyaluronidase enzyme developed to break down stromal HA to increase delivery of chemotherapy. PEGPH20 is being developed with a companion immunohistochemical based assay to determine HA levels under the premise that high HA tumors are more likely to benefit from PEGPH20. Consistent with this hypothesis, a phase II study of PEGPH20 in combination with gemcitabine/nab-paclitaxel versus gemcitabine/nab-paclitaxel alone demonstrated an improved median PFS in patients with high HA level tumors (9.2 vs. 5.2 months; $P = 0.048$; ref. 40). In a non–biomarker-selected study evaluating FOLFIRINOX ± PEGHP20, however, interim analysis demonstrated futility (41). It is not yet known if the inclusion of low HA patients contributed to the negative result of this study or if the partnering cytotoxic regimen influenced the negative results; however, the dataset is being retrospectively analyzed. A registration trial evaluating PEGPH20 in combination with gemcitabine/nab-paclitaxel versus gemcitabine/nab-paclitaxel/placebo in high HA–expressing patients is recruiting (NCT02715804).

DNA Damage Repair as a Biomarker

DNA damage repair deficits, specifically homologous recombination deficits secondary to *BRCA1/2*, *PALB2*, *ATM*, and *RAD51* mutations, may be efficacious biomarkers for enhanced sensitivity to platinum and PARP inhibitors in PDAC. BRCA1/2 is the most common mutation, with approximately 3.6% to 7% of patients with PDAC having germline BRCA1/2 mutations and up to 12.1% of PDACs in Ashkenazi Jews (42, 43). Homologous recombination is required for repair of double-strand DNA breaks caused by platinum agent–mediated DNA cross-links. In addition, PARP is required for the repair of single-strand breaks, which if not repaired, leads to double-strand breaks, which are strong signals for cell-cycle arrest and

Table 2. Selected biomarker-based studies with results

ClinicalTrials.gov ID	Phase	Biomarker/ therapeutic	Mechanism of action	Study drugs	Results
NCT00203892	I/II	CEA/CAP1-6D	CEA vaccine	CEA peptide (CAP1-6D)/ montanide/GM-CSF vaccine	Increased ELISPOT T-cell responses in 20%/60%/100% for patients at 10 μg/100 μg/ 1,000-μg doses (57)
NCT00674973	II	EGFR and KRAS status/erlotinib	Anti-EGFR mAb	Erlotinib	mPFS erlotinib vs. placebo: 6.1 vs. 5.9 weeks; HR, 0.83 ($P = 0.1909$) EGFR expression and KRAS status did not predict response to erlotinib (34)
NCT00769483	I/II	Blood IGF-1, tissue IGF-1 gene expression/MK-0646	IGF-1 mAb	MK-0646 with gemcitabine ± erlotinib	High tissue IGF-1: 76% reduction in risk of disease progression ($P = 0.16$; ref. 58)
NCT00837876	II	Veristrat multivariate protein test/ sorafenib and erlotinib	PDGFR/EGFR mAbs	Sorafenib/erlotinib	Veristrat good vs. poor: PFS 62 vs. 48 days, HR, 0.18 ($P = 0.001$), OS 128 vs. 47 days, HR, 0.31 ($P = 0.008$; ref. 59)
NCT01040000	I/II	MUC5AC staining/ NEO-102	Anti-MUC5AC mAb	NEO-102	59% of PDAC patients expressed MUC5AC mOS 20 weeks (60)
NCT01098344	I	Hair follicle notch pathway gene expression and tumor IHC/MK-0752	Gamma-secretase inhibitor	MK-0752 with gemcitabine	11/18 with SD, 1/18 with PR Notch pathway signature in 16/18 hair follicles (61)
NCT01124786	II	hENT level/CO-101	Gemcitabine–lipid conjugate with hENT1-independent cellular uptake	CO-1.01 vs. gemcitabine	No difference in mOS in the hENT1-low subgroup or overall (HR, 0.994 and 1.072, respectively) Gemcitabine arm, no difference in survival between the hENT1-high and -low subgroups (HR, 1.147; ref. 21)
NCT01647828	II	Notch 3 expression/ tarextumab	Anti-notch 2/3 antibody	Gemcitabine/nab-paclitaxel ± tarextumab	mOS tarextumab vs. placebo: 6.4 vs. 7.9 months (HR, 1.3; $P = 0.119$) mPFS Notch 3 expression <25th percentile 3.5 vs. 6.9 (HR 3.2; $P = 0.009$; ref. 62)
NCT01839487	II	HA expression/ PEGPH20	Pegylated hyaluronidase enzyme	Gemcitabine/nab-paclitaxel ± PEGPH20	High HA group: mPFS 9.2 vs. 5.2 months ($P = 0.048$; ref. 40)
NCT01844817	II	HSP27/OGX-427	Anti-sense mRNA	Gemcitabine/nab-paclitaxel ± OGX-427	ORR 18%, mOS 5.3 vs. 6.9 (HR 1.2) High HSP27 mPFS 3.3 vs. 0.9 months (HR, 0.4); OS 3.3 vs. 1.0 months (HR, 0.6; ref. 63)
NCT01888978	II	RRM1/gemcitabine, ERCC1/oxaliplatin, TS/5-FU	Various: antimetabolite, alkylating agents, microtubule inhibitor, topoisomerase inhibitor	Gemcitabine/oxaliplatin, gemcitabine/5-FU, gemcitabine/docetaxel, modified FOLFOX-6, oxaliplatin/docetaxel, FOLFIRI, docetaxel/irinotecan	ORR 9%, DCR 82%, mPFS 5.9, mOS 10.4 months (31)
NCT02005315	I	TGF3, IGF2, SMO gene signature/ vantictumab	WNT inhibitor	Vantictumab with gemcitabine/ nab-paclitaxel	At interim analysis, 7/8 biomarker-positive patients had PR, 1/8 SD (64)
NCT02042378	II	Deleterious BRCA1/2 germline or somatic/rucaparib	PARPi	Rucaparib	ORR 11%, DCR 32% (65)
NCT02050178	I	WNT pathway gene expression/ ipfricept	WNT trap	Ipfricept with gemcitabine/nab-paclitaxel	High baseline WNT pathway had 40% greater tumor reduction than low (66)
NCT02138383	I	Androgen receptor/ enzalutimide	Antiandrogen receptor	Enzalutimide with gemcitabine/ nab-paclitaxel	Phase Ia: 1/10 with PR, 9/10 SD (67)

Abbreviations: DCR, disease control rate; mAb, monoclonal antibody; mOS, median overall survival; mPFS, median progression-free survival; ORR, overall response rate; PARPi, PARP inhibitor; PR, partial response; SD, stable disease.

Table 3. Biomarker-selected studies currently recruiting

ClinicalTrials.gov ID	Phase	Biomarker	Therapeutics	Mechanism of action
NCT03213626	II	EGFR and c-MET overexpresssion by IHC	Cabozantinib and erlotinib	c-Met/VEGFR2
NCT01489865	I/II	BRCA or BRCAness (*BRCA1*, *BRCA2*, *PALBB2* or one of the FANC genes, personal history of BRCA-related malignancy, multiplex family)	ABT888 and modified FOLFOX6	PARPi
NCT01506973	I/II	JNK1	Hydroxychloroquine + gemcitabine/nab-paclitaxel	Autophagy inhibitor
NCT01585805	II	Germline *BRCA1/2* or *PALB2*	Gemcitabin/cisplatin ± veliparib, veliparib alone	PARPi
NCT02184195	III	Germline *BRCA1/2*	Olaparib after 16 weeks of platinum without progression	PARPi
NCT02350673	I	CEA	Cergutuzumab and atezolizumab	CEA-targeted IL2 variant + PD-L1 inhibitor
NCT02395016	III	*KRAS* WT	Nimotuzumab	EGFR antagonist
NCT02672917	I	CA19-9	MVT-5873	Anti–CA19-9 mAb
NCT02715804	III	HA	Gemcitabine/nab-paclitaxel ± PEGPH20	Pegylated hyaluronidase
NCT02744287	I	Prostate stem cell antigen	BPX-601	Prostate stem cell antigen–directed CART
NCT03023722	II	Mesothelin	Anetumab ravtansine	Mesothelin mAb conjugated to DM4
NCT03040986	II	*KRAS* G12R mutation	Selumetinib	MEK inhibitor
NCT03118349	I	CA19-9	MVT-1075	177Lu-labeled anti–CA19-9 mAb
NCT03140670	II	Deleterious *BRCA1/2* or *PALB2* mutation	Rucaparib after 16 weeks of platinum without progression	PARPi
NCT03323944	I	Mesothelin	huCART-meso cells	Mesothelin-directed CART

Abbreviations: CART, chimeric antigen receptor T cell; huCART-meso, human CAR T mesothelin; mAb, monoclonal antibody; PARPi, PARP inhibitor; WT, wild type.

apoptosis. DNA damage repair signatures result from compensatory DNA damage repair mechanisms including large structural deletions from single-strand annealing and short deletions from end joining creating another potential biomarker (44).

Olaparib demonstrated promising results in a phase II study, which included 23 patients with *BRCA*-mutant PDAC, and is currently being studied as maintenance therapy in a phase III trial for patients with metastatic PDAC and germline BRCA mutations who have had at least 16 weeks of stable disease with platinum treatment (NCT02184195; ref. 45). Olaparib is also under study in a phase II trial for "BRCAness" phenotype (NCT02677038) for patients without germline *BRCA1/2* mutations with a family history of BRCA-related malignancies or other DNA damage repair deficiencies in the absence of family history. Veliparib, on the other hand, is being studied in a phase II study of patients with *BRCA1/2* or *PALB2* mutations in combination with first-line gemcitabine/cisplatin versus gemcitabine/cisplatin/veliparib versus veliparib alone (NCT01585805).

Biomarkers for Metabolic Pathways

PDAC tumor metabolic pathways that support survival in a hypoxic, nutrient-poor tumor microenvironment are actively being targeted with multiple agents in clinical study with predictive biomarker correlatives. Eryaspase is a red blood cell–encapsulated formulation of L-asparaginase that is being developed to treat tumors with low asparagine synthetase levels. Asparagine is synthesized by the enzyme asparagine synthetase (ASNS), which has low levels in some PDACs. It is predicted that depletion of asparagine by L-asaparaginase in tumors with impaired asparagine synthesis will deplete the asparagine pool impairing protein synthesis, leading to cell-cycle arrest and apoptosis (46). A phase II study randomized patients to receive standard second-line chemotherapy of gemcitabine or FOLFOX with or without eryaspase. The primary endpoint of improvement in survival in patients with no or low ASNS was met, and interestingly, the entire population, of which 30% were ASNS high, had both improved PFS and OS. The role of ASNS as a biomarker is being further investigated (47).

The autophagy inhibitor hydroxychloroquine is being studied with gemcitabine in a phase I/II trial with a robust correlative design. JNK1 will be evaluated as a potential marker of autophagy along with expression of various autophagy-related proteins in pre- and posttreatment biopsies (NCT01506973).

Blood-Based Biomarkers and Therapy Selection

Tissue biopsies are invasive and can be obtained only in selected patients at selected time points, and specimens do not account for tumor heterogeneity. Blood-based bioassays including circulating tumor DNA (ctDNA), tumor-derived exosomes, and circulating tumor cells (CTC) offer a number of advantages, as they are minimally invasive, repeatable over time, and theoretically reflect the entire malignant cell population. Exosomes, in particular, offer the potential to study an array of biomarkers including surface proteins, intracellular proteins, DNA, and RNA. Early data supporting roles as diagnostic, prognostic, and predictive markers are emerging. Preliminary evidence has demonstrated that ctDNA, exosomes, and CTCs can be detected in blood and correlated with disease stage, survival measures, and therapeutic response (48). Blood-based biomarkers could ultimately influence therapeutic selection in multiple ways. For example, ctDNA increases 2 to 4 weeks after treatment initiation are correlated with worse disease-free survival and OS, and ctDNA increases may precede radiographic progression (49, 50). Therefore, ctDNA

Table 4. Selected blood-based biomarker studies

Blood-based biomarker	Reference	Disease stage (number)	Biomarker collection time point	Assay	Key results and application
cfDNA	Takai et al. 2015 (52)	Resectable (108) LA (44) Metastatic (107)	Pretreatment	*KRAS* ddPCR, NGS	Detection: ● Resectable: 8.3% ● LA: 18.2% ● Metastatic: 58.9% Predictive: ● 29.2% (14/48) with targeted sequencing had potential therapeutic target gene
	Sausen et al. 2015 (50)	Resectable (51; 9 longitudinal)	Preresection, multiple postresection time points	*KRAS* dPCR, NGS	Detection: ● Resectable: 43% Monitoring: ● mDFS by ctDNA 3.1 vs. 9.6 months by CT imaging Predictive: ● 38% with clinically applicable mutations ● 6% with FDA-approved agent
	Zill et al. 2015 (68)	26 hepatobiliary (18 PDAC) 23/26 metastatic		54-gene NGS sequencing panel—tumor and cfDNA	Tumor genome surrogate: ● 90% of tumor mutations in cfDNA ● One of 7 most common mutations identified in 89% of samples ● 7/9 tumor biopsies with insufficient sample for analysis had ctDNA mutations found
	Lee et al. 2017 (48)	Stage I (7), stage II (99), stage III (8), stage IV (5)	Diagnosis, postresection	*KRAS* NGS	Detection: ● Stage I: 42.9%, stage II: 54.5%, stage III: 50%, stage IV: 100% Prognosis: ● Postresection ctDNA associated with poorer OS (mOS 8 months, HR, 6.93, $P = 0.006$)
	Del Re et al. 2017 (69)	LA (4), metastatic (23)	Day 0, 14, CT	*KRAS* ddPCR	Detection: 70.3% Predictive: ● ctDNA increase at day 14 vs. stable/decrease (mPFS: 2.5 vs. 7.5 months, $P = 0.03$; mOS: 9 vs. 11.5 months, $P = 0.009$) ● All increased ctDNA progressed on next imaging
Exosomes	Madhavan et al. 2015 (70)	Pancreas cancer (131), CP (25), BPT (22), non-PC tumor (12), HC (30)		Immunoaffinity: anti-CD44v6, anti-Tspan8, anti-EpCAM, anti-CD104 qRT-PCR: miR-1246, miR-4644, miR-3976, miR-4306	Detection: ● Sensitivity 100% ● Specificity 80% (93% when non-PC malignancies removed)
	San Lucas et al. 2015 (71)	Metastatic (2; 1 blood, 1 pleural fluid)	1 pretreatment, 1 POD	ddPCR, WGS	Preclinical: ● 56%–82% tumor fraction in exosomal DNA by ddPCR ● 95%–99% of targeted genome covered in exosomal DNA
	Melo et al. 2015 (72)	Discovery: stage I (2), IIa (19), IIb (117), III (11), IV (41) Validation: stage I (2), IIa (15), IIb (35), IV (3)	Pre-/postresection, prechemotherapy	Glypican-1	Detection: ● Sensitivity: 100% ● Specificity: 100% ● AUC 1.0 Prognosis: ● Mean bead bound–GPC1 level: ○ Metastatic 58.5% ○ Nodal 50.5% ○ Local 39.9% ● Postresection GPC1 reduction: ○ Low reduction OS 15.5 months ○ Greater reduction OS 26.2 months

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Table 4. Selected blood-based biomarker studies (Cont'd)

Blood-based biomarker	Reference	Disease stage (number)	Biomarker collection time point	Assay	Key results and application
	Allenson et al. 2017 (73)	Discovery: Local (33), LA (15), metastatic (20), HC (54) Control: Validation: Local (39), HC (82)	Pre-/postresection	exoDNA and cfDNA *KRAS* ddPCR	Detection: ● Discovery: exoDNA vs. cfDNA ○ Local: 66.7% vs. 45.5% ○ LA: 80% vs. 30.8% ○ HC: 7.4% vs. 14.8% ○ Postresection 5% vs. 0% ● Validation: ○ Local: 43.6% HC: 20.7%
CTCs	de Albuquerque et al. 2012 (74)	Stage II (4), III (2), IV (28), HC (40)	Pretreatment	Anti-MUC1 and anti-EPCAM immunocapture followed by RT-PCR of KRT19, MUC1, EPCAM, CEACAM5, BIRC5	Detection: ● 47.1% Prognosis: ● CTC positive vs. negative mPFS 66 vs. 138 days ($P = 0.01$)
	Hong et al. 2012 (75)	Multiple solid malignancies treated with dasatinib (30, 17% PDAC)	Pretreatment, day 8, day 28	CellSearch	Predictive: ● SD $\geq$6 months/PR vs. all others: days 1 to 28 mean CTC count change, −0.92 vs. 1.61 ($P = 0.123$) Mean CTC count/7.5 mL at day 28, 0.5 vs. 3.85, $P = 0.052$)
	Yu et al. 2014 (53)	Metastatic (50)	Pretreatment	Collagen adhesion matrix cell invasion assay, gene expression–based pharmacogenomic model	Predictive: ● Predicted sensitive/intermediate/resistant mPFS 10.4/7.8/3.6 months ($P < 0.0001$) mOS 17.2/13.8/8.3 months ($P < 0.0304$)
	Okubo et al. 2017 (76)	Borderline resectable (9), metastatic (56)	Pretreatment, under treatment (mean 3 months)	CellSearch	Detection: ● 32.3% Predictive: ● 45.4% with POD had CTC detected at 3 months vs. 24.1% with SD or PR detected at 3 months ● 2 POD, 4 SD in increased CTC count patients vs. 4 SD, 1 PR in patients with decreased CTCs

Abbreviations: AUC, area under the curve; cfDNA, cell-free DNA; CP, chronic pancreatitis; ddPCR, digital droplet PCR; dPCR, digital PCR; HC, healthy control; LA, locally advanced; mDFS, mean disease-free survival; mOS, median overall survival; mPFS, median progression-free survival; NGS, next-generation sequencing; POD, progression of disease; PR, partial response; SD, stable disease; WGS, whole-genome sequencing.

could be used to guide early therapeutic changes. In addition, ctDNA sequencing has identified potentially actionable mutations in 29% to 38% of patients. As previously noted, utilizing genetic analyses to guide therapy selection is currently limited for PDAC, and in particular, it remains unknown as to whether treating potentially actionable mutations identified in PDAC translates into clinical benefit (51, 52). Overall, circulating biomarkers have immense potential but require significant prospective study to define their applications (Table 4).

Pharmacogenomic modeling using CTC gene expression to predict treatment response is one of the most exciting applications for blood-based biomarkers with increasing supportive evidence. Yu and colleagues applied this technique to predict effective and ineffective chemotherapeutic agents typically used in PDAC. From 10 mL of blood, CTCs were captured and sufficient RNA isolated for analysis in all participants. Patients were classified as "sensitive," "intermediate," or "resistant." As predicted, PFS was longest in the "sensitive" group (10.4 months), shortest in the resistant (3.6 months), and in between in the intermediate (7.5 months; $P = 0.0001$; ref. 53). We are currently recruiting patients for a follow-up study to predict response to frontline therapy with FOLFIRINOX- and gemcitabine/nab-paclitaxel–based regimens (NCT03033927).

Conclusions

Multiple biomarkers are emerging in PDAC with the potential to influence therapy selection. Currently in the clinic, pembrolizumab's approval for MSI-H and dMMR malignancies is the first approval for a biomarker-based therapeutic for PDAC, although the overall indication is disease agnostic. Deleterious mutations in BRCA and other homologous repair genes appear to predict benefit to platinum and PARP inhibitors, and PEGPH20 and eryaspase have shown positive results in mid-stage biomarker-based studies.

The importance of biomarker-based therapeutic selection is becoming increasingly recognized. Jardim and colleagues compared anticancer drug development programs that failed in phase III with programs that reached approval. Only 16% of the failed programs used biomarker-driven patient selection compared with 57% of successful programs ($P < 0.001$; ref. 54).

Novel trial platforms that integrate biomarker-based therapies are being designed. In the Pancreatic Cancer Network's Precision Promise initiative and a parallel program in the United Kingdom entitled Precision Panc, all patients will have pathologic evaluation, detailed genomic, immune sequencing, and transcriptome analysis performed on their tumors to subsequently determine assignment into substudies focused on DNA damage repair defects, stromal disruption, and immunotherapy. Patients will then be able to move between studies to help determine the most efficacious therapeutics for that individual and the biomarkers that predict response (55, 56).

Speaking to constraints, biomarker-based trials and clinical application are not without significant challenge in PDAC. Most biomarkers are tissue based and reflect a small sample from a heterogeneous tumor, often with rare epithelial cells in low cellularity specimens. In addition, cost issues, validation, and reproducibility related to sequencing and biomarker assays are concerns that remain to be fully addressed.

So, to answer the question posed by the article title, Biomarker-based therapies in PDAC—an emerging reality? To these authors, there is little doubt that the identification of reproducible and validated biomarkers that reliably identify subsets of patients and predict treatment response will be a major step toward improving outcomes in selected patient subgroups with PDAC, and we anticipate routine use of such biomarkers in the proximate future.

Disclosure of Potential Conflicts of Interest

E.M. O'Reilly is a consultant/advisory board member for Celgene and Halozyme. No potential conflicts of interest were disclosed by the other author.

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