Published in final edited form as:

Clin Cancer Res. 2018 May 15; 24(10): 2241–2250. doi:10.1158/1078-0432.CCR-16-3169.

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, in pancreatic ductal adenocarcinoma (PDAC) favorable outcomes remain challenging, in part related to the lack of validated biomarkers for patient and treatment selection and thus optimal clinical decision-making. Nonetheless, increasingly therapeutic development for PDAC is accompanied by bioassays to evaluate response and study mechanism of actions with a corresponding increase in the number of trials in mid to late-stage with integrated biomarkers. Additionally, 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 will review potential biomarkers for currently approved therapies as well as emerging biomarkers for therapeutics under development.

Keywords

Biomarkers; Pancreatic Ductal Adenocarcinoma; Therapeutics; Predictive biomarkers

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,

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Disclosure

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.

EGFR, ROS1, ALK, BRAF mutations predict sensitivity to their respective inhibitors and PD-L1 identifies patients enriched for benefit from checkpoint inhibitor therapies (Table 1).

In PDAC, biomarkers are lacking with treatment predominantly determined by stage of disease and performance status and therapy dominated by cytotoxic agents. Specifically, FOLFIRINOX (5-fluorouracil, leucovorin, oxaliplatin, irinotecan), gemcitabine/nab-paclitaxel and liposomal irinotecan/5-fluorouracil 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 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 to 95% of PDAC tumors have an oncogenic KRAS mutation with frequent mutations in TP53 (75%), SMAD4 (22%) and CDKN2A/B (18%) (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, G1/S checkpoint regulation, hedgehog signaling, transforming growth factor beta 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 epithelial growth factor receptor (EGFR) is overexpressed in up to 70% (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 hyaluronic acid, 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 non-oxidative 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.

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 use in high-risk populations (17). CA19-9 has shown prognostic value post-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 PDAC patients 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.

Front-line 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 to 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 remains conflicting, however, with a recent systematic review showing hENT1 as 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 pre-clinical study in PDAC (23). In human trials, Valsecchi et al and Farrel et al 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, enhanced vascular permeability and possibly albumin binding by proteins in the tumor microenvironment (26). SPARC, secreted protein acidic and rich in cysteine, 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 OS (17.8 vs 8.1 months) (27). The follow up MPACT study, which led to gemcitabine/nab-paclitaxel Federal Drug Authority 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 inter-strand 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 the RRM1, ERCC1, and TS status. The study demonstrated 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 median OS of 10.4 months (31).

Targeted Therapy Biomarkers

Despite strong pre-clinical 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 vs placebo did not identify EGFR protein expression, EGFR copy number/mutations/polymorphisms or KRAS mutation status to correlate with progression-free survival (PFS) (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 PDAC's. 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 immunohistochemical assays and gene expression profiling by PT-PCR.

Targeted therapeutics in development are increasingly being studied in biomarker-selected populations or with biomarker correlatives during clinical trials (Tables 2, 3). For example, cabozantinib with erlotinib are being studied in patients with EGFR and c-MET expressing tumors (NCT03213626, enzalutamide with gemcitabine/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 monoclonal 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 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, NCT03118349).

Biomarkers for Immunotherapy

The first PDAC biomarker based therapy, pembrolizumab, was recently approved for patients with high microsatellite instability (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 5 studies including 149 patients with multiple malignancies. Published data for PDAC has included 4 patients with dMMR of which two 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 non-colorectal cancers with median duration of response not reached (37). These abnormalities are rare, occurring in < 1% of PDAC patients, 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 e.g., MSISensor, mSINGs, etc (38).

Outside of MSI-H and dMMR populations, checkpoint inhibitors are intensively being studied 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 multi-arm study evaluating anti-PD-L1 monoclonal antibody, atezolizumab in combination with cobimetinib, PEGPH20 or BL-8040 vs 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 IL-2 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, NCT02744287).

Biomarkers for Stromal Targeting Agents

High interstitial pressure caused by hyaluronic acid in the PDAC stroma impairs drug delivery (13). PEGPH20 is a recombinant pegylated hyaluronidase enzyme developed to break down stromal hyaluronic acid (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 2 study of PEGPH20 in combination with gemcitabine/nab-paclitaxel vs gemcitabine/ nab-paclitaxel alone, demonstrated an improved median PFS in patients with high HA level tumors (9.2 vs 5.2 months; $p=0.048$) (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 data set is being retrospectively analyzed. A registration trial evaluating PEGPH20 in combination with gemcitabine/nab-paclitaxel vs 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 poly ADP-ribose polymerase (PARP) inhibitors in PDAC. BRCA1/2 is the most common mutation with approximately 3.6–7% of PDAC patients having germline BRCA 1/2 mutations and up to 12.1% of PDACs in Ashkenazi Jews (42, 43). Homologous recombination is required for repair of double stranded DNA breaks caused by platinum agent mediated DNA crosslinks. Additionally, 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 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 BRCA mutant PDAC patients, and is currently being studied as maintenance therapy in a phase III

trial for patients with metastatic PDAC and germline BRCA who have had at least 16 weeks of stable disease with platinum treatment (NCT02184195) (45). Olaparib is also under study in a phase II trial for 'BRCAness' phenotype (NCT02677038) for patients without germline BRCA1/2 mutations with family history of BRCA related malignancies or other DNA damage repair deficiencies in absence of family history. Veliparib, on the other hand, is being studied in a phase II of patients with BRCA1/2 or PALB2 mutations in combination with first line gemcitabine/cisplatin vs gemcitabine/cisplatin/veliparib vs 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-asparaginase 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 improved PFS and OS improved as well. The role of ASNS as a biomarker is being further investigated (47).

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 post-treatment biopsies (NCT01506973).

Blood-Based Biomarkers and Therapy Selection

Tissue biopsies are invasive, can only be obtained 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 (CTCs) 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 is emerging. Preliminary evidence has demonstrated ctDNA, exosomes and CTCs can be detected in blood and correlate with disease stage, survival measures and therapeutic response (48). Blood based biomarkers could ultimately influence therapeutic selection in multiple ways. For example, ctDNA increases 2–4 weeks after treatment initiation are correlated with worse disease-free survival and OS and ctDNA increases may precede radiographic progression (49, 50). Therefore, ctDNA could be used to guide early therapeutic changes. Additionally, ctDNA sequencing has identified potentially actionable

mutations in 29–38% 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 translate to 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 et al applied this technique to predict effective and ineffective chemotherapeutic agents typically used in PDAC. From 10mL 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) ($p=0.0001$) (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).

Conclusion

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 et al compared anticancer drug development programs that failed in phase III to programs that reached approval. Only 16% of the failed programs used biomarker-driven patient selection compared to 57% of successful programs ($p<0.001$) (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 UK 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 sub-studies 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. Additionally, cost issues, validation and reproducibility related to sequencing and biomarker assays remain concerns 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.

Acknowledgments

Financial Support:

Eileen M. O'Reilly:

David M. Rubenstein Center for Pancreatic Cancer Research

P30 CA008748 Cancer Center Support Grant

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Table 1

Selected Biomarker-Based Cancer Therapies

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

dMMR: mismatch repair deficient, MSI-H: microsatellite instability high, PARP: poly adp-ribose polymerase

Table 2

Selected Biomarker Based Studies with Results

NCT	Phase	Biomarker/Therapeutic	MOA	Study drugs	Results
NCT00203892	I/II	CEA/CAP1-6D	CEA vaccine	carcinoembryonic antigen (CEA) peptide (CAP1-6D)/montanide/GM-CSF-vaccine	Increased ELISPOT T-cell responses in 20%/60%/100% for patients at 10ug/100ug/1000ug 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)(58)
NCT00837876	II	Veristat multivariate protein test/sorafenib and erlotinib	PDGFR/EGFR Mabs	sorafenib/erlotinib	Veristat 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)(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)(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)

NCT	Phase	Biomarker/Therapeutic	MOA	Study drugs	Results
					mPFS N3 expression <25% ile: 3.5 vs 6.9 (HR 3.2; p=0.009)(62)
NCT01839487	II	Hyaluronic acid expression/PEGPH20	Pegylated hyaluronidase enzyme	gemcitabine/nab-paclitaxel +/- PEGPH20	High hyaluronic acid group: mPFS 9.2 vs 5.2 mo (p=0.048) (40)
NCT01844817	II	HSP27/OGX-427	Anti-sense mRNA	gemcitabine/nab-paclitaxel +/- OGX-427	Overall RR 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)(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	Anti-androgen receptor	Enzalutimide with gemcitabine/nab-paclitaxel	Phase Ia: 1/10 with PR, 9/10 SD(67)

DCR: disease control rate, ERCC1: excision repair cross-complementation group 1, hENT: human equilibrative nucleoside transporter, HR: hazard ratio, Mab: monoclonal antibody, mOS: median overall survival, ORR: overall response rate, PR: partial response, RRM1: Ribonucleotide reductase catalytic subunit M1, SD: stable disease, TS: thymidylate synthase

Table 3**Biomarker Selected Studies Currently Recruiting**

NCT	Phase	Biomarker	Therapeutics	MOA
NCT03213626	II	EGFR and c-MET overexpression by IHC	cabozantinib and erlotinib	c-Met/VEGFR2
NCT01489865	I/II	BRCA or BRCAness (BRCA1, BRCA2, PALB2, or one of the FANC genes, personal history of BRCA related malignancy, multiplex family)	ABT888 and mFOLFOX6	PARPi
NCT01506973	I/II	JNK1	Hydroxychloroquine + gemcitabine/nab-paclitaxel	autophagy inhibitor
NCT01585805	II	Germline BRCA 1 or 2 or PALB2	Gemcitabin/Cisplatin +/- veliparib, veliparib alone	PARPi
NCT02184195	III	Germline BRCA 1/2	Olaparib after 16 weeks of platinum without progression	PARPi
NCT02350673	I	CEA	Cergutuzumab and Atezolizumab	CEA targeted IL-2 variant + PDL1 inhibitor
NCT02395016	III	KRAS WT	nimotuzumab	EGFR antagonist
NCT02672917	I	CA19-9	MVT-5873	anti-CA19-9 monoclonal antibody
NCT02715804	III	Hyaluronic Acid	Gemcitabine/nab-paclitaxel +/- PEGPH20	Pegylated hyaluronidase
NCT02744287	I	PSCA	BPX-601	Prostate stem cell antigen directed CART
NCT03023722	II	Mesothelin	Anetumab ravtansine	mesothelin monoclonal antibody 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

CART: chimeric antigen receptor T-cell

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)	Pre-treatment	KRAS ddPCR, NGS	Detection: <ul style="list-style-type: none"> Resectable: 8.3% LA: 18.2% Metastatic: 58.9% Predictive: <ul style="list-style-type: none"> 29.2% (14/48) with targeted sequencing had potential therapeutic target gene
	Sausen et al., 2015(50)	Resectable (51; 9 longitudinal)	Pre-resection, multiple post-resection timepoints	KRAS dPCR, NGS	Detection: <ul style="list-style-type: none"> Resectable 43% Monitoring: <ul style="list-style-type: none"> mDFS by cfDNA 3.1 months vs 9.6 by CT imaging Predictive: <ul style="list-style-type: none"> 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: <ul style="list-style-type: none"> 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 cfDNA mutations found
	Lee et al., 2017(48)	Stage 1 (7), Stage 2 (99), Stage 3 (8), Stage 4 (5)	Diagnosis, post-resection	NGS	Detection: <ul style="list-style-type: none"> Stage 1: 42.9%, Stage 2: 54.5%, Stage 3: 50%, Stage 4: 100% Prognosis:

Blood based biomarker	Reference	Disease Stage (Number)	Biomarker Collection Time Point	Assay	Key Results and Application
					<ul style="list-style-type: none"> Post-resection 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: <ul style="list-style-type: none"> ctDNA increase at d14 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)		Immunofluorescence (anti-CD44v6, anti-Tspan8, anti-EpCAM and anti-CD104) qRT-PCR: miR-1246, miR-4644, miR-3976 and miR-4306	Detection: <ul style="list-style-type: none"> Sensitivity 100% Specificity 80% (93% when non-PC malignancies removed)
	San Lucas et al. (2015)(71)	Metastatic (2; 1 blood, 1 pleural fluid)	1 pre-treatment, 1 POD	ddPCR, WGS	Pre-clinical: <ul style="list-style-type: none"> 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-/post-resection, pre-chemotherapy	Glypican-1	Detection: <ul style="list-style-type: none"> Sensitivity: 100% Specificity: 100% AUC 1.0 Prognosis: <ul style="list-style-type: none"> Mean bead bound GPC1 level: <ul style="list-style-type: none"> – Metastatic 58.5% – Nodal 50.5% – Local 39.9% Post-resection GPC-1 reduction: <ul style="list-style-type: none"> – Low reduction OS 15.5 months

Blood based biomarker	Reference	Disease Stage (Number)	Biomarker Collection Time Point	Assay	Key Results and Application
					– Greater reduction OS 26.2 months
	Allenson et al, 2017(73)	Discovery: Local (33), LA (15), Metastatic (20), HC (54) Control: Validation: Local (39), HC (82)	Pre-/post-resection,	exoDNA and cDNA KRAS ddPCR	Detection: • Discovery: exoDNA vs cDNA – Local: 66.7% vs 45.5% – LA: 80% vs 30.8% – HC: 7.4% vs 14.8% – Post-resection 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)	Pre-treatment	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 days vs 138 (p=0.01)
	Hong et al, 2012(75)	Multiple solid malignancies treated with dasatinib (30, 17% PDAC)	Pre-treatment, day 8, day 28	CellSearch	Predictive: • SD >=6 months/PR vs all others: • Day 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)	Pre-treatment	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)	Pre-treatment, under treatment (mean 3 months)	CellSearch	Detection: • 32.3% Predictive:

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Blood based biomarker	Reference	Disease Stage (Number)	Biomarker Collection Time Point	Assay	Key Results and Application
					<ul style="list-style-type: none">• 45.4% with PD had CTC detected at 3 months vs 24.1% with SD or PR detected at 3 months• 2 PD, 4 SD in increased CTC count patients vs 4 SD, 1PR in decreased CTC patients

AUC: area under the curve, CP: chronic pancreatitis, CT: computed tomography, ddPCR: digital droplet PCR, HC: healthy control, IPMN: intrapapillary mucinous neoplasm, LA: locally advanced, mDFS: mean disease-free survival, NED: no evidence of disease, NET: neuroendocrine tumor, NGS: next generation sequencing, OS: overall survival, PD: progression of disease, PR: partial response, PV: portal venous, SD, stable disease, SV: systemic venous, WES: whole exome sequencing