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Available technologies and clinical applications of targeted chemotherapy in pancreatic cancer

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The incidence of pancreatic cancer, the fourth leading cause of cancer death in United States, is increasing worldwide. Even though the cure rate has doubled in 40 years, it is abysmally poor at 6–7%. As surgical resection remains the only curative treatment and less than 20% of the newly diagnosed cancers are resectable, the major burden of disease management lies in early diagnosis, good prognostication, and proper neo-adjuvant and/or adjuvant therapy. With advancing technologies and their ease of availability, researchers have better tools to understand pancreatic cancer. In the post-genetic era, proteomic, phosphoproteomic, metabolomic, and more have brought us to a multi-omics era. These newer avenues bring promises of better screening modalities, less invasive diagnostics and monitoring, subtyping of pancreatic cancer, and fine tuning the treatment modalities not only to the right stage of tumor but also to the right tumor biology. As the multitudes of technologies are generating extensive amounts of incongruous data, they are giving clinicians a lot of non-actionable information. In this paper, we wish to encompass the newer technologies, sub-classifications, and future treatment modalities in personalized care of patients with pancreatic cancer.

Keywords Molecular profiling, targeted therapy, pancreatic cancer

Introduction

The first connection between genetic inheritance and susceptibility to a disease was made in 1902 by Sir Archibald Garrod for alkaptonuria. In 1956, the discovery of selective toxicity for drugs primaquine on genetic basis was made. It wasn't until 2003 with the complete sequencing of the human genome that more personalized and targeted therapies were popularized. Tests are now moving beyond the gene into the entire spectrum of molecular profiling, including the epigenomic, transcriptomic, proteomic, and metabolomic profiling.

Pancreatic cancer, the fourth leading cause of cancer death in the United States, is often diagnosed at a very late stage, when curative treatments are no longer as good. The incidence of this cancer is increasing worldwide as well as its rank among cancer causing mortality. More than 80% of these cancers are known to be locally advanced or metastatic at the time of diagnosis. The only curative treatment remains to be resection, but is only possible in less than 20% of patients.

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Pancreatic duct adenocarcinoma, or PDAC, is the most common form of pancreatic cancer. The causes and risk factors of PDAC are largely unknown. The risk factors of tobacco, obesity, exposure to chemicals, diabetes, chronic pancreatitis, and other conditions are up for debate.

Genetics

The fundamental essence in PDAC, like in all cancers, lies in genetic mutations. The inherited genetic mutations are much less common than sporadic mutations. Some genetic mutations like mutations in p16 and TP53 genes can be found in both inherited and sporadic occurrences. Others, such as KRAS, BRAF, and DPC4 (Smad4), are usually found in sporadic PDAC. These genetic mutation signatures give each PDAC a different prognosis and possibly different therapeutic options. The p53 gene encodes for a nuclear phosphoprotein which activates apoptosis. It is mutated in over 95% of pancreatic cancer cells. Loss of p53 has been proposed to be a negative prognostic factor, even though no conclusive studies have shown a correlation between p53 mutation and a poorer clinical outcome (1). Germline mutation of p16 is a characteristic

genetic alteration observed in over 80% of pancreatic cancer cells. This locus encodes for suppressor genes. Multiple studies have shown p16 expression as a positive prognostic factor and any mutations as negative (2). In the Smad pathway, deletion or mutations of Smad4 have been reported in over 55% of PDAC (3). The loss of Smad4 triggers the increase of cellular proliferation and was associated with improved survival after resection (4). In the Bcl-2 gene family, Bax gene has been reported to promote apoptosis, whereas Bcl-2 seems to inhibit it. The expression of Bcl-2 gene is remarkably correlated with a better survival. The mitochondrial pro-apoptotic protein BNIP3, a member of the Bcl-2 family, plays a role in hypoxia-induced apoptosis. Its expression is increased in hypoxic regions of tumors and correlates with a worsened prognosis (5,6).

Proteomics

Proteomics help identify the functional units of cellular processes, proteins, and their intricate interaction networks and signaling pathways. The advances of proteomic technologies, especially quantitative proteomics, have stimulated a great interest in applying this technology for pancreatic cancer studies.

PDAC has been well characterized at the genetic level; however, these advances have failed to improve the understanding of disease progression and thus its clinical management. The process as a whole—as we understand—is a collection of multiple gene mutations causing activation and inactivation of various cellular processes. Pharmacologic targeting of genetic mutations has not been a viable option in PDAC, because the common genes mutated in pancreatic cancer are also the key regulators in normal cell expression. It has become evident that genetic change alone is not sufficient enough information to understand most PDAC. Thus, proteomic based approaches have served to complement the genomic data and provide critical information about the active molecules driving PDAC. As such, it is believed that proteomic based approaches are the next frontier. Harsha et al. made a compendium of potential biomarkers of pancreatic cancer (7). Identifying a novel biomarker opens avenues for earlier diagnosis, targeted therapy, and monitoring of disease.

The most commonly used proteomic tools are two-dimensional gel electrophoresis mass spectrometry (2D GE-MS) and liquid chromatography mass spectrometry (LC-MS). Improvements on gel-based techniques have been made with better protein separation and quantification, but liquid chromatography (LC) is still currently the most-used technology for protein separation prior to mass spectrometry analysis, as it allows for the collection of both quantitative and structural information at very high sensitivity (8). Multiple kits, like subcellular fractionation, protein depletion, and post translational modification are available commercially to increase the specificity and sensitivity of these tests.

By using a combination of the techniques, Theodoridis et al. in 2012 identified over 3900 proteins in four pancreatic cancer cell lines with 134 proteins significantly and differentially expressed between primary and metastatic cell populations (8). Britton et al. identified 152 significantly different proteins among 2101 proteins, all between the tumor and non-tumor tissues of 12 PDAC (9).

Quantitative proteomics has advanced with the use of labeled techniques, such as stable isotope labeling by amino acids in cell culture (SILAC), isotope-coded affinity tags (ICAT), isobaric tags for relative and absolute quantitation, (iTRAQ) and tandem mass tags (TMT). Using these techniques, Kosanam et al. identified laminin gamma 2 (LAMC2) as a potential PDAC biomarker (10).

On the other hand, quantitative label-free proteomics is achieved through techniques like selected-reaction monitoring (SRM) or multiple reactions monitoring (MRM). Using these techniques, Ansari et al. considered it similar to protein deep-mining and aggregated candidate biomarkers such as asporin, CD9, CXC chemokine ligand 7, fibronectin 1, galectin-1, gelsolin, intercellular adhesion molecule 1, insulin-like growth factor binding protein 2, metalloproteinase inhibitor 1, stromal cell derived factor 4, and transforming growth factor beta-induced protein, among others (11).

Phosphoproteomics

Phosphorylation is a key event modulating protein activity, therefore measuring protein phosphorylation is a useful indicator of the activation status of a protein and of the gene itself. In a study by Britton et al. (9), 12 PDAC tumor and peritumoral pancreatic tissues were studied for proteomic and phosphoproteomic data. Using techniques of Tandem Mass Tag™ (TMT) Systems, liquid chromatography, tandem mass spectrometry, immobilized metal affinity chromatography (IMAC), and titanium dioxide (TiO_2), Britton et al. found 2101 proteins and identified 6543 unique phosphopeptide sequences. Among those identified, 152 proteins and 635 phospho-peptides demonstrated a significant difference in abundance between tumor and nontumor tissues. These include the known and new up-regulated proteins in pancreatic cancer: Mucin-1, HIPK1, and MLCK. Among the phosphopeptides that showed significant regulation, proteins involved in cell migration (Rho guanine nucleotide exchange factors & MRCKα) and formation of focal adhesions were identified. Also found during the study were phosphorylation events that indicate activation status of drug-targets like FYN, ERK2, AKT1, RAF1, BRAF, GSK3a and others were found to be highly modulated.

Genetic subtypes and prognosis

As technology has helped us to better understand pancreatic adenocarcinoma, more literature has been able to discern different sub-categories of PDAC. Although no classification system is universally accepted, it is in a stage of evolution. The first real classification was attempted in 2011 by Collisson et al. (12), and has been improved by Moffitt et al. (13), and more recently by Bailey (14).

Collisson et al. (12) have used Global gene-expression analysis on micro dissected PDAC tissues to subtype pancreatic adenocarcinoma into classical, quasi-mesenchyma(QM), and exocrine-like. They had 62 designated gene signatures. The classical subtype had high expression of adhesion-associated and epithelial genes. The QM subtype showed high expression of mesenchyme-associated genes. The exocrine-like subtype showed relatively high expression of tumor cell-derived digestive enzyme genes. Collisson et al. also followed

the gene-signatures for clinical outcome and therapeutic responses. They found that the classical subtypes had longer survival periods after resection than cancers of the QM type. QM subtype cell-lines were also found to be more sensitive to Gemcitabine than the classical subtype, while Erlotinib was found to be more effective against the classical subtype. They suggested that KRAS mutation status is an imperfect predictor of sensitivity to EGFR-targeted therapy (12).

Moffitt et al. (13) used digital microdissection for tumor, stromal, and normal gene expression. They identified and validated two tumor subtypes: classical and basal. The latter was found to have a worse outcome in resected PDAC, with a median survival time of 11 months compared to the 19 months for classical subtype. They also found "normal" and "activated" stromal subtypes. Patients with PDAC, with activated stromal subtype, had a worse median survival time of 15 months compared to 24 months for normal stromal subtype. In multivariate Cox analysis, they found both classifications to be independently and significantly associated with survival (stroma subtypes, $P = 0.037$; tumor subtypes, $P = 0.003$).

More recently Bailey et al. (14) identified 32 genes from 10 genetic pathways (KRAS, TGF-B, WNT, NOTCH, ROBO/SLIT signaling, G1/S transition, SWI-SNF, chromatin modification, DNA repair, RNA processing) that are consistently mutated in pancreatic adenocarcinoma. Further expression analysis of gene activity revealed four distinct subtypes of PDAC: (1) squamous, (2) pancreatic progenitor, (3) immunogenic, and (4) aberrantly differentiated endocrine exocrine (ADEX), that correlated with the histopathology of the tumor. Squamous tumors were found to be enriched for TP53 and KDM6A mutations, up-regulation of theTP63N transcriptional network, hypermethylation of pancreatic endodermal cell-fate determining genes, and also have a poor prognosis. Pancreatic progenitor tumors preferentially express genes involved in early pancreatic development (FOXA2/3, PDX1 and MNX1). ADEX tumors displayed up-regulation of genes that regulate networks involved in KRAS activation, exocrine (NR5A2 and RBPJL), and endocrine differentiation (NEUROD1 and NKX2-2). Immunogenic tumors contained up-regulated immune networks including the pathways involved in B-cell signaling pathways, antigen presentation, CD4+ T cell, CD8+ T cell and Toll-like receptor signaling pathways.

Treatment predictive markers in PDAC

i. Systemic chemotherapy for PDAC delivers a very low response rate compared to other solid malignancies, and the treatment has not drastically altered the longevity of patients diagnosed with pancreatic adenocarcinoma. Therapies that improve on the status quo tend to cause debilitating systemic toxicity. Therefore, there is a need to carefully select therapy on the basis of specific prognostic factors in order to allocate proper resources to realistic goals. The predictive biomarkers hope to be helpful for selecting treatments, in neoadjuvant, adjuvant, and palliative settings.
ii. Gemcitabine is a nucleoside pyrimidine analogue, which has become the standard single agent treatment of PDAC, as it was proven to improve clinical symptoms, quality of life, and survival (15). Variability of key proteins in gemcitabine transport and metabolism probably impact its treatment response and toxicity. Gemcitabine needs the presence of transporter mechanisms to enter cells. Two processes of nucleoside transport have been identified, hENT-1 being the major route for transporting gemcitabine. It has been postulated that cells with lower hENT-1 expression have reduced intracellular penetration, and therefore are relatively resistant. Studies have shown a positive correlation between hENT-1 gene expression and chemosensitivity, as well as resistance with its pharmacologic inhibition. Increased hENT-1 expression has also shown longer survival after Gemcitabine chemotherapy. (16–19). There is a lot of data suggesting routine testing for hENT-1 expression, and giving Gemcitabine to those with high hENT-1 expression.
iii. FOLFIRINOX is a newer regimen for PDAC with a combination of folinic acid, 5-fluorouracil (5-FU), irinotecan, and oxaliplatin. Multiple preclinical and clinical trials have shown efficacy of FOLFIRINOX over other therapies including Gemcitabine. Unfortunately, the combination of irinotecan and oxaliplatin comes with severe systemic toxicity and neurotoxicity. Rarely is it given to our geriatric population. A percentage of PDAC patients have defective DNA maintenance. This may predict chemosensitivity to platinum-based therapy, and if proven, oxaliplatin may be used preferentially (20).
iv. Nab-PACLITAXEL is a nanoparticle albumin-bound paclitaxel with an average size of 130 nm. It was developed to penetrate the thick stromal cells in the desmoplastic stromal cells around PDAC, thereby increasing tumoral paclitaxel concentration. It has been postulated that its uptake into cells may be dependent on SPARC expression (21). The role of SPARC in carcinogenesis is not well established, as there are many conflicting reports. We do know that as cells transform from PAN-INs to IPMN and finally to PDAC they increasingly under express SPARC. Therefore, the increased expression of SPARC would mean more efficacious delivery of drug to the tumor but with resistance as it is not taken up by the PDAC cells. SPARC expression has not been proven to show any clinical significance yet, but it is involved in numerous mechanisms in cancer (22). As of now, its role is still underexplored.

Targeted therapy in PDAC

Apart from surgery and cytotoxic therapies for PDAC, actual advances have been made in targeted therapies. Targeted therapies are defined as drugs that target specific genes or proteins, thereby preventing cancer from growing and spreading. More than 40% of PDAC has such targets for which we have or can design medications. Some of these targets are as follows:

EGFR pathway inhibitors

EGFR is a transmembrane receptor member of the ErbB family with a tyrosine kinase domain. It is involved in cell cycle regulation, cell survival, adhesion and differentiation. It is overexpressed in up to 90% of pancreatic cancer samples. Therefore, inhibitors targeting EGFR have been considered as a promising therapeutic agent. Erlotinib is a tyrosine kinase inhibitor which blocks downstream signal transduction. In a large phase III trial of 569 patients with advanced pancreatic cancer who received gemcitabine plus placebo or gemcitabine

plus Erlotinib, the median survival length was increased in the Erlotinib limb of the study (6.24 months versus 5.91 months, P = .038) (23). Surprisingly, when EGFR or KRAS status was analyzed in this subgroup, it was not shown to be predictive of survival benefit. Erlotinib has been approved by the FDA in combination with gemcitabine as a first-line treatment for advanced PDAC. Cetuximab is a monoclonal antibody against EGFR. After initial promise, it has not shown much survival benefit when given with gemcitabine in advanced PDAC (24). Facial rash was also a negative side effect of administered Cetuximab, and an increase of the dose to achieve results did not help. Gefitinib is a competitive inhibitor of ATP binding to the intracellular kinase domain of EGFR. Of the 53 patients who were treated with gemcitabine and Gefitinib, responses were seen in six, and stabilization of the disease in twelve. The median progression free survival was 4.1 months and median survival was 7.3 months, with a 1-year survival rate of 27% (25).

HER2 is another ErbB family of transmembrane tyrosine kinase receptors, which is overexpressed in 11% of pancreatic adenocarcinoma cases, correlating with poor survival (26). Many studies have been done with HER2, but none has shown significant improvement over standard chemotherapy, at least for its inhibition with Trastuzumab in metastatic disease (27). Another HER-2 inhibitor, Lapatinib, is being used in combination with Capecitabine with mixed to favorable results. Nimotuzumab, another anti-EGFR monoclonal antibody, and Afatinib, an inhibitor of EGFR, HER2 and HER4, are among some newer drugs being evaluated (28).

KRAS pathway and secondary signaling inhibitors

The KRAS pathway and downstream signaling cascade inhibitors created mutations that are present in over 70% cases of pancreatic cancer. It is a GTPase protein with oncogenic activity, and gain-of-function mutations result in proliferation and inhibition of apoptosis through the RAF/MEK/ERK and PIK3/AKT pathways. It has no inhibitors in clinical practice at present. Farnesylation is an important post-translational modification required for Ras activation. Farnesyl-transferase inhibitors like Tipifarnib have failed to improve overall survival (29). Targets downstream of KRAS, such as the protein kinase MEK, are being targeted by inhibitors like Selumetinib. In a trial as a second-line therapy in combination with Capecitabine, showed a minimal survival benefit (median overall survival of 5.4 mo vs 5.0 mo) (30). Trametinib, another MEK1/2 inhibitor, and Rigosertib, a first-in-class Ras mimetic are few drugs that are being trialed.

IGFR pathway inhibitors

Insulin like growth-factor 1 receptor is also overexpressed in pancreatic adenocarcinoma cells. Upon binding, there is activation of several pathways involved in cell proliferation and cell survival, such as the PIK3/AKT pathway. Monoclonal antibodies like Cixutumumab and Ganitumab have been evaluated in PDAC treatment, but failed to show a statically significant survival benefit.

Angiogenesis pathway inhibitors

Neo-angiogenesis is essential for tumor progression and metastasis. Vascular endothelial growth factor (VEGF) has been is shown to overexpress in PDAC. VEGF inhibitors like Bevacizumab have failed to improve overall survival in combination with Gemcitabine in advanced pancreatic cancer. Aflibercept, a recombinant fusion, binds VEGF-A, VEGF-B and placental growth factors 1 and 2 thereby inhibiting VEGF-ligand-dependent signaling processes, suppresses tumor growth in pancreatic cell lines and xenografts. Sorafenib, an oral multikinase inhibitor of Raf-kinase, VEGF-R2/-R3 and PDGFR-β, Axitinib, an anti-angiogenesis and Necuparanib, a re-engineered drug from Heparin with possible anti-tumor activity, are being studied as well.

Embryonic pathway inhibitors

Hedgehog signaling has a critical role in cell proliferation. Normal pancreatic cells silence this pathway, but pathological activation is observed in PDAC. Sonic Hedgehog (SHH) and other pathway proteins detected in precursor lesions and tumors contribute to desmoplastic reaction (31). Genetically engineered mouse models demonstrated a depletion of the tumor matrix from SHH pathway inhibition, which could be a promising strategy in pancreatic cancer therapy (32). Two drugs being trialed are smoothened inhibitors Vismodegib and Saridegib. Notch signaling is up-regulated in PDAC and promotes tumorigenesis, and also proteolytic cleavages. It regulates transcription of several genes involved in proliferation and differentiation of cells, interacting with other pathways such as Hedgehog, KRAS and NF-κB signaling (33). RO4929097 and Demcizumab are also drugs being trialed.

Poly ADP-Ribose Polymerase (PARP) inhibitors

Poly ADP-ribose polymerase (PARP) is a nuclear enzyme recruited to repair cell DNA damage, and as recent evidence showed, patients with defects in the homologous DNA recombination pathway may benefit from the use of PARP inhibitors. Clinical trials testing those new agents like Olaparib are ongoing (34,35). Mutations affecting BRCA genes promote deficiency in DNA damage repair mechanisms, and may be targeted by these agents.

mTOR and PI3K/Akt pathway inhibitors

RAS phosphorylates PI3K, thus activating Akt, a serine/threonine kinase. Signal transduction by activated PI3K/Akt plays a role in tumor cell proliferation, survival, and metabolism, usually through several downstream targets, including the mammalian target of rapamycin (mTOR). BKM120 (a PI3K inhibitor), RX-0201 (an Akt antisense oligonucleotides), BEZ235 (a combined inhibitor of PI3K and mTOR), and everolimus (an oral mTOR inhibitor) are all being trialed.

Tumor stroma inhibitors

The stroma is a critical compartment of PDAC formation, progression, metastasis, and resistance to therapy. Targeting the stromal micro-environment is a strategy being studied. PEGPH20, a PEGylated formulation of recombinant Hyaluronidase in Nab-Paclitaxel and Gemcitabine, is being studied. Inhibition of PDGFR with Gleevec and TKI258 is being tested. Matrix metalloproteinase inhibitors such as Marimastat are also an option.

Test samples

Blood

Tumor marker: CA19-9

CA19-9 is a sialylated Lewis (a) antigen, which is an epitope produced by epithelial cells. It is present on the surface of erythrocytes and in mucin secreted by pancreatic cancer cells. Lewis (a) antigen is formed after fucosylation with the Le enzyme, while the Lewis (b) antigen is formed by both the Le and Se enzymes. Humans who do not have a functional Le enzyme (le-/le- genotype), approximately 5–10% of the population, do not produce detectable CA19-9 (36). False elevations of CA19-9 are commonly encountered in patients with benign pancreatobiliary conditions like pancreatitis and biliary obstruction (37). It is not recommended for routine screening of PDAC as specificity of the test and prevalence in PDAC is low (38).

CA19-9 has a significant value as a prognostic factor. It has been used as a predictor of unresectability—especially in the absence of pancreatitis or biliary obstruction (39). It can also be used post-operatively to prognosticate survival. Berger et al. (40) demonstrated that patients with CA 19-9 ≥ 90 U/mL after resection had a significantly worse overall survival. In that study, patients with CA 19-9 < 90 U/mL, had a median survival of 21 months compared to 10 months for patients with CA 19-9 ≥ 90 U/mL. A rising CA19-9 in a known patient with PDAC suggests progression—prompting further investigation—and when confirmed with other radiographic data, could be used clinically to alter management protocol.

Circulating tumor cells

A major cause of pancreatic cancer mortality is tumor metastasis, prompting a search for biomarkers for cancer diagnosis, staging, prognosis and therapeutic monitoring. Thomas Ashworth in 1869 first reported tumor cells circulating in the blood of cancer patients. These Circulating Tumor Cells (CTCs) are becoming widely recognized as one of these novel biomarkers. These cells have acquired the ability to invade and disseminate in the circulatory system. It is hypothesized that the presence of CTCs in blood correlates with advanced disease and metastasis, with varying degrees of evidence in other solid cancers like breast, colorectal, and prostate cancer.

CTCs are usually very rare—in the range of one CTC per 10 million leukocytes (41). Pancreatic cancer patients are reported to show one of the lowest CTC levels among cancer patients (42). All tests usually include a two-step process: enrichment and detection. The enrichment portion is usually done using density gradient centrifugation. Some labs use varied techniques like membrane filtration, buoyant density, and also tetrameric antibody complex that increase the specific cell's density. For more specific enrichment immunological capture techniques have been developed. For detection of metastatic malignancies, epithelial-specific proteins have been employed for positive selection of CTCs. The epithelial cell adhesion molecule (EpCAM) is the most frequently used antigen for this application. This concept has been developed into immunomagnetic bead separation systems and is commercially available in magnetic-activated cell sorting systems (Miltenyi Biotec GmbH), EasySep cell separation (StemCell Technologies), cell isolation by Dynabeads (Invitrogen), and the Cell-Search system (Veridex) (43).

CellSearch™ CTC test (Veridex, Raritan, NJ) is the first FDA cleared test for capturing and enumerating CTCs. Their kit is intended for the enumeration of CTCs of epithelial origin (CD45–, EpCAM+, and cytokeratins 8, 18+ , and/or 19+) in whole blood. It has been approved as a prognostic test in breast, colorectal, and prostate cancers.

CellSearch and other labs have been used to detect CTCs in pancreatic cancer as well. Among the 16 patients studied by Allard et al. (42) with metastatic pancreatic cancer, six (37.5%) had CTCs in their blood. Bidard et al. (44) in 2013 presented a case series of 79 patients with locally advanced pancreatic cancer and showed detection of one or more CTCs per 7.5 ml of blood in 5% of patients at onset, and 9% of patients after two months of chemotherapy. CTC positivity was associated with poor tumor differentiation ($P = 0.04$), and with shorter overall survival in multivariable analysis (RR = 2.5, $P = 0.01$). In a meta-analysis by Han et al. (45) that included nine studies with 268 CTC-positive markers among 623 PDAC patients, there was a significantly worse overall survival and progress-free survival than those in the CTC-negative group.

In a study by Ren et al. (46) in 2011 of 41 patients with advanced PDAC, CTCs were detected in 33 (80.5%) patients before any therapy. This number decreased to 12 (29.3%) patients a week after the first cycle of 5-fluorouracil chemotherapy. Apoptotic CTCs were also detected after the cycle of chemotherapy, thus showing that detection of CTCs may be able to validate the efficacy of chemotherapy.

Cell-free DNA (cfDNA)

Advanced-stage tumors often shed DNA into the bloodstream, which can be isolated from a serum from peripheral blood draws. They can be detected by polymerase chain reaction (PCR) or next-generation sequencing (NGS) based testing. Cell-free DNA testing could make tissue biopsy unnecessary and thus reduce complications, delays, and costs associated with invasive tissue biopsy in PDAC patients. This technique also has a potential benefit of being able to serially monitor the quantity and identities of genetic matter over time by peripheral blood draws. This technique could help monitor therapy response and relapse.

cfDNA is more abundant in PDAC than healthy controls. More than 75% of metastatic PDAs have tumor-derived cfDNA detectable by PCR-based single-gene methods (47). The concordance between mutations observed in the primary or metastatic PDAC tumor tissue and the cfDNA has yet to be established. In a recent study by Zill et al. (48) they analyzed 54 genes in tumors for cfDNA, and 26 patients suggested that cfDNA sequencing should be considered in pancreatic-biliary cancer trials where tissue sampling is unsafe, infeasible, or otherwise unsuccessful. The sequencing failed in nine patients (35%) but in the remaining 17, 90.3% (95% CI: 73.1–97.5%) of mutations detected in tumor biopsies were also detected in cfDNA, giving knowledge of tumor genotype or the abundance of circulating tumor DNA. Many more such studies are being designed as "Liquid biopsies" and are available commercially.

Urine

Metabolites of pancreatic cancer are excreted in urine. Many have found multiple microRNAs (miRNA) to discriminate

between resectable and non-resectable pancreatic cancer, and benign pathology. Debernardi et al. showed significant overexpression for a subset of miRNAs (miR-143, miR-223, miR-30e) to differentiate between PDAC Stage I versus healthy individuals, and another a subset of miRNAs (miR-204, miR-143, miR-223) to differentiate between Stage I and Stages II–IV PDAC (49). Schonemeier et al., using immunoassay and IHC testing, did urine proteome analysis and found that it outperformed CA 19-9 by a 15% increase in sensitivity. They found that Fetuin-A was the most prominent peptide marker for PDAC (50). Radon et al. identified LYVE-1, REG1A, and TFF1 as protein biomarkers to detect patients with early-stage pancreatic cancer via SDS-PAGE-Liquid Chromatography-Tandem Mass Spectrometry with eighteen urine samples from healthy patients with chronic pancreatitis and patients with PDAC (51).

Feces

Cologuard® (Exact Sciences Corporation, Madison, WI, USA) is an FDA approved multitarget stool DNA test in CRC screening (52). It includes assays for aberrantly methylated BMP3 and NDRG4 genes, mutant KRAS and β-actin, as well as an immunochemical assay for human hemoglobin. The test showed a sensitivity of 92% with the DNA testing (53). The process includes sending the stool sample where laboratory-based processing entails amplification and detection with the use of Quantitative Allele-specific Real-time Target and Signal Amplification (QuARTS™) technology (54). The results of assays are given a composite score which is used to determine a positive or negative result. If positive, the patient is to proceed with a colonoscopy.

This principle has been used by Kisiel et al. They published their data in 2012 with 90% specificity, methylated BMP3 detected 51% of PDACs, mutant KRAS detected 50%, and combination detected 67%. This study demonstrates that stool assay of a methylated BMP3 and mutant KRAS can detect PDAC (55). Its feasibility is being studied by Chung et al. ClinicalTrials.gov Identifier: NCT01104129 (56).

Microbiome

Our understanding of the biological mechanisms leading to carcinogens has been evolving, and the concept of parasitic origin of cancer is an older concept. With advancing technology, we have shed greater light in this field. This parasitic effect is probably because of the immunological response to such infections, most often bacterial or viral (57). Many cancer inducing viruses have been documented, including Hepatitis C, Human Papilloma Virus, and the Human Immunodeficiency Virus. They either interfere at the genetic level or affect the cells immunologically. Two bacteria, *Porphyromonas gingivalis* in the oral flora and *Helicobacter pylori* in the gastric flora, have been studied extensively in pancreatic cancer.

P. gingivalis has been stated to cause periodontal disease, and periodontal disease has been linked with pancreatic cancer, meaning *P. gingivalis* may have some causality to pancreatic cancer (58). In some studies, a 4-fold increase in risk of pancreatic cancer was seen in people with severe periodontitis (59). This could be because of the direct effects of inflammation, but immunomodulatory effects of *P. gingivalis* is also postulated (59). Its diagnosis with culture and antibody testing may be helpful in screening for high risk patients.

H. pylori's epidemiological relationship with gastric cancer has been well studied. Its association with pancreatic cancer has been hypothesized, but in the few studies of the last few decades, no consistent relationship has been shown.

Saliva

Similar to stool testing, testing saliva is a non-invasive procedure. Apart from detecting *P. gingivalis*, which was discussed in the previous section, it has been postulated that metabolites of the pancreatic cancer itself might be identified in the saliva. Many circulatory molecules are present in saliva, which can be discriminatory for diseases including cancer screening and detection. Many studies have reported that salivary constituents can distinguish between oral diseases and systemic diseases (lung cancer, breast cancer, pancreatic cancer, and ovarian cancer). RNAs, both coding RNAs (mRNAs) and noncoding RNAs (ncRNAs), have been studied extensively. ncRNAs have recently been interesting for researchers as their short size makes them more stable and less susceptible to degradation as compared to RNAs. Their diverse roles in physiology and cancer biology are being studied extensively. RNAs, metabolites, and other biochemical compounds are being reviewed for translational clinical application and may have huge implications with regard to both costs and benefits for pancreatic cancer care.

mRNA

Zhang et al. identified 12 messenger RNA biomarkers to diagnose pancreatic cancer, of which 4 mRNAs identified are discriminatory for the detection of resectable and borderline resectable pancreatic cancer with high sensitivity and specificity (60). The logistic regression model with the combination of the 4 mRNA biomarkers (KRAS, MBD3L2, ACRV1, and DPM1) could differentiate pancreatic cancer patients from unaffected patients.

miRNA

MicroRNAs have been reported to be aberrantly expressed in patients with pancreatic cancer (61). Many salivary miRNAs have been studied. According to one study, miR-3679-5p and miR-940 had discriminatory power to detect resectable pancreatic cancer with sensitivities of 62.5–72.5% and specificities of 70.0–80.0% (62).

Metabolites

Sugimoto et al. (63) had studied 215 patients for salivary metabolites using capillary electrophoresis time-of-flight mass spectrometry (CE-TOF-MS). The cohort included oral cancer patients (N = 69), pancreatic cancer patients (N = 18), breast cancer patients (N = 30), periodontal disease patients (N = 11), and healthy controls (N = 87). They identified 48 metabolites for pancreatic cancer, of which eight metabolites (leucine with isoleucine, tryptophan, valine, glutamic acid, phenylalanine, glutamine, and aspartic acid) were markers specific to pancreatic cancer in the cohort.

Exosomes

In 2010, it was found that saliva had proteins and functional mRNA containing exosomes. These are cell specific lipid micro vesicles (30–100 nm) that are able to migrate in the body promoting intercellular communication. It has been suggested that tumor-derived exosomes could function as the shuttle between the distal tumor and the oral cavity leading to the development of discriminatory salivary biomarkers.

Common commercial pathology laboratories doing analysis of tissue

FoundationOne® (Cambridge, MA)

FoundationOne is a comprehensive genomic profile that applies Next Generation Sequencing (NGS) to identify genomic alterations across genes known to be drivers of solid tumors. They simultaneously sequence the coding region of 315 cancer related genes including introns from 28 genes. Each reading represents a unique DNA fragment to enable the highly sensitive and specific detection of genomic alterations that occur at low frequencies due to tumor heterogeneity, low tumor purity, and small tissue samples. It detects all classes of genomic alterations, including base substitutions, insertions, deletions (indels), copy number alterations (CNAs), and rearrangements, using a small, routine FFPE sample (with core or fine needle biopsies).

The test reporting is provided in an interpretive report both in hard copy and via a web portal. The report identifies the gene alterations and provides an interpretation that is specific to the patient's tumor (e.g. PDAC vs. gastric adenocarcinoma). It stresses on the more clinically relevant alterations. In some cases, pertinent normal genes are also reported. It also categorizes the variations into variants of unknown significance, equivocal, and subclonal. Variants of unknown significance (VUS) are deemed alterations detected at their lab without any published characterization. It is considered equivocal when there is some, not unambiguous evidence of amplification or homozygous loss of a gene; it is regarded as subclonal if the presence of the alteration can be identified in less than 10% of the estimated tumor DNA.

At present, the genomic alteration list that FoundationOne tests for includes: ABL1, ABL2, ACVR1B, AKT1, AKT2, AKT3, ALK, AMER1(FAM123B), APC, AR, ARAF, ARFRP1, ARID1A, ARID1B, ARID2, ASXL1, ATM, ATR, ATRX, AURKA, AURKB, AXIN1, AXL, BAP1, BARD1, BCL2, BCL2L1, BCL2L2, BCOR, BCORL1, BLM, BRAF, BRCA1, BRCA2, BRD4, BRIP1, BTG1, BTK, C11 or f30(EMSY), CARD11, CBFB, CBL, CCND1, CCND2, CCND3, CCNE1, CD274, CD79A, CD79B, CDC73Ê, CDH1, CDK12, CDK4, CDK6, CDK8, CDKN1A, CDKN1B, CDKN2A, CDKN2B, CDKN2C, CEBPA, CHD2, CHD4, CHEK1, CHEK2, CIC, CREBBP, CRKL, CRLF2, CSF1R, CTCF, CTNNA1, CTNNB1, CUL3, CYLD, DAXX, DDR2, DICER1, DNMT3A, DOT1L, EGFR, EP300, EPHA3, EPHA5, EPHA7, EPHB1, ERBB2, ERBB3, ERBB4, ERG, ERRFI1, ESR1, EZH2, FAM46C, FANCA, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCL, FAS, FAT1, FBXW7, FGF10, FGF14, FGF19, FGF23, FGF3, FGF4, FGF6, FGFR1, FGFR2, FGFR3, FGFR4, FH, FLCN, FLT1, FLT1, FLT4, FOXL2, FOXP1, FRS2, FUBP1, GABRA6, GATA1, GATA2, GATA3, GATA4, GATA6, GID4(C17orf39), GLI1, GNA11, GNA13, GNAQ, GNAS, GPR124, GRIN2A, GRM3, GSK3B, H3F3A, HGF, HNF1A, HRAS, HSD3B1, HSP90AA1, IDH1, IDH2, IGF1R, IGF2, IKBKE, IKZF1, IL7R, INHBA, INPP4B, IRF2, IRF4, IRS2, JAK1, JAK2, JAK3, JUN, KAT6A(MYST3), KDM5A, KDM5C, KDM6A, KDR, KEAP1, KEL, KIT, KLHL6, KMT2A(MLL), KMT2C(MLL3), KMT2D(MLL2), KRAS, LMO1, LRP1B, LYN, LZTR1, MAGI2, MAP2K1, MAP2K2, MAP2K4, MAP3K1, MCL1, MDM2, MDM4, MED12, MEF2B, MEN1, MET, MITF, MLH1, MPL, MRE11A, MSH2, MSH6, MTOR, MUTYH, MYC, MYCL(MYCL1), MYCN, MYD88, NF1, NF2, NFE2L2, NFKBIA, NKX2-1, NOTCH1, NOTCH2, NOTCH3, NPM1, NRAS, NSD1, NTRK1, NTRK2, NTRK3, NUP93, PAK3, PALB2, PARK2, PAX5, PBRM1, PDCD1LG2, PDGFRA, PDGFRB, PDK1, PIK3C2B, PIK3CA, PIK3CB, PIK3CG, PIK3R1, PIK3R2, PLCG2, PMS2, POLD1, POLE, PPP2R1A, PRDM1, PREX2, PRKAR1A, PRKCI, PRKDC, PRSS8, PTCH1, PTEN, PTPN11, QKI, RAC1, RAD50, RAD51, RAF1, RANBP2, RARA, RB1, RBM10, RET, RICTORÊ, RNF43, ROS1, RPTOR, RUNX1, RUNX1T1, SDHA, SDHB, SDHC, SDHD, SETD2, SF3B1, SLIT2, SMAD2, SMAD3, SMAD4, SMARCA4, SMARCB1, SMO, SNCAIP, SOCS1, SOX10, SOX2, SOX9, SPEN, SPOP, SPTA1, SRC, STAG2, STAT3, STAT4, STK11, SUFU, SYK, TAF1, TBX3Ê, TERCÊ, ÊTERT(promoter only), ÊTET2, TGFBR2, TNFAIP3, TNFRSF14, TOP1, TOP2A, TP53, TSC1, TSC2, TSHR, U2AF1, VEGFA, VHL, WISP3, WT1, XPO1, ZBTB2, ZNF217, ZNF703.

The rearrangements searched for include:

ALK, BCL2, BCR, BRAF, BRCA1, BRCA2, BRD4, EGFR, ETV1, ETV4, ETV5, ETV6, ETV6, FGFR2, FGFR3, KIT, MSH2, MYB, MYC, NOTCH2, NTRK1, NTRK2, PDGFRA, RAF1, RARA, RET, ROS1, TMPRSS2.

Guardant Health, Inc. (Redwood City, CA)

The Lab uses 10 mL blood to isolate and purify the cell-free DNA (cfDNA). They then apply digital Next Generation Sequencing technology to identify genomic alterations in more than 150,000 base-pairs across 70 oncogenes. They report the amount of cfDNA mutant allele frequency (MAF) as a percentage as it relates to the germ line.

The lab reports a diagnostic accuracy of 97% with concurrent tumor tissue biopsies. In a study published in 2015, they analyzed 54 genes in the tumors and cfDNA of 26 cancer patients. Tumor sequencing failed in nine patients (35%). Of the 31 mutations detected by tumor-biopsy NGS, 28 were also detected by the cfDNA test (90.3% overlap with 95% CI). KRAS, TP53, APC, SMAD4, GNAS, FBXW7, and BRAF were the commonly mutated genes. On serial blood draws the direction of change in tumor marker CA 19-9 and cfDNA-percentage agreed significantly suggesting that cfDNA mutant allele fraction changes reflect changes in disease burden over time and treatment (48).

At present, the genomic alteration list that Guardant Health, Inc. tests for includes:

a. 70 Point Mutations (SNVs) Genes: AKT1, ALK, APC, AR, ARAF, AR1D1A, ATM, BRAF, BRCA1, BRCA2, CCND1, CCND2, CCNEI, CDH1, CDK4, CDK6, CDKN2A, CDKN2B, CTNNB1, EGFR, ERBB2, ESR1, EZH2, FBXW7, FGFR1, FGFR2, FGFR3, GATA3, GNA11, GNAQ, G NAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, K IT, KRAS, MAP210, MAP2K2, MET, MLH1, MPL, MYC, NF1, NFE2L2, NOTCH1, NPM1, NRAS, NTRK1, PDGFRA, PIK3CA,

PTEN, PTPNI1, RAF1, RB1, RET, RHEB, RHOA, R1T1, ROS1, SMAD4, SMO, SRC, STK11, TERT, TP53, TSC1, and VHL.
b. 18 CNV Genes: AR, BRAF, CCND1, CCND2, CCNE1, CDK4, CDK6, EGFR, ERBB2, FGFR1, FGFR2, KIT, KRAS, MET, MYC, PDGFRA, PIK3CA, RAF1.
c. 6 Fusion Genes: ALK, FGFR2, FGFR3, NTRK1, RET, ROS1.
d. 3 Indel Genes: EGFR, ERBB2, MET.

Personal Genome Diagnostics (Baltimore, MD)

This company offers tissue and cfDNA analysis. For tissue analysis it has three products: CancerSelect-88, CancerSELECT-203 and CancerComplete. In CancerSelect-88, 88 well-characterized cancer genetic alterations are screened using Next Generation Sequencing to identify point mutations, copy number alterations, and rearrangements. They also do in-depth computational analyses, such as Digital Karyotyping, PARE, and other approaches.

The genetic alteration list that CancerSelect-88 tests for includes:

a. sequence analysis for76 well-characterized cancer genes: ABL1, ERBB4, GNAQ, MTOR, RET, AKT1, EZH2, GNAS, NF1, ROS1, ALK, FANCA, HNF1A, NF2, SMAD4, APC, FANCC, HRAS, NOTCH1, SMARCB1, ATM, FANCD2, IDH1, NPM1, SMO, BRAF, FANCE, IDH2, NRAS, SRC, BRCA1, FANCF, JAK2, NTRK1, STK11, BRCA2, FANCG, JAK3, PALB2, TERT, BRIP1, FANCL, KDR, PDGFRA, TP53, CDH1, FBXW7, KIT, PDGFRB, TSC1, CDKN2A, FGFR1, KRAS, PIK3CA, TSC2, CSF1R, FGFR2, MET, PMS2, VHL, CTNNB1, FGFR3, MLH1, PTCH1, DDR2, FLT3, MPL, PTEN, EGFR, FOXL2, MSH2, PTPN11, ERBB2, GNA11, MSH6 and RB1.
b. Copy number analyses for 13 well-characterized cancer genes: ALK, ERBB3, FGFR3, MYC, RET, EGFR, FGFR1, KIT, MYCN, ERBB2, FGFR2, MET and PDGFRA.
c. Rearrangement analyses for 14 well-characterized cancer genes: ALK, EGFR, ETV6, PDGFRA, ROS1, BCL2, ETV1, EWSR1, PDGFRB, TMPRSS2, BCR, ETV4, MLL and RARA.
d. Microsatellite analyses, for five well-characterized cancer genes: BAT-25, BAT-26, MONO-27, NR-21 and NR-24.

In CancerSELECT-203, 203 well-characterized cancer genes are screened in both PDAC and normal samples. They claim to be able to accommodate low abundance and poor quality sample DNA. It also includes a high-quality integrated analysis report.

The genes evaluated in CANCERSELECT-R™ 203 are:

a. Rearrangement analyses for selected regions of 24 well-characterized cancer genes. ALK, EGFR, EWSR1, PDGFRB, ROS1, BCL2, ETV1, MLL, PRKACA, TMPRSS2, BCR, ETV4, MYC, RAF1, FGFR3, BRAF, ETV5, NTRK1, RARA, TACC3, DNAJB1, ETV6, PDGFRA and RET.
b. Sequence and copy number analyses for the coding regions of 195 well-characterized cancer genes: ABL1*, CBL*, ERBB3*, FGFR2*, KDR*, ACVR1, CCND1*, ERBB4*, FGFR3*, KIT*, AKT1*, CCNE1*, ERCC1, FGFR4*, KRAS*, AKT2*, CDC73, ERCC2, FH, MAML1*, ALK*, CDH1, ERCC3, FLCN, MAP2K1*, APC, CDK4*, ERCC4, FLT3*, MAP2K4, AR*, CDK6*, ERCC5, FLT4, MDM2*, ARID1A, CDKN1B, ESR1, FOXL2*, MDM4*, ARID1B, CDKN2A, ETV1, GATA1, MED12*, ASXL1, CDKN2B, ETV5, GATA2*, MEN1, ATM, CDKN2C, EWSR1, GNA11*, MET*, ATRX, CEBPA, EXT1, GNAQ*, MLH1, AURKA, CHEK2, EXT2, GNAS*, MLL*, AXIN2, CIC, EZH2*, GPC3, MPL*, BAP1, CREBBP, FANCA, H3F3A*, MSH2, BCL2*, CSF1R*, FANCB, H3F3B, MSH6, BCR, CTNNB1*, FANCC, HNF1A, MTOR, BLM, CYLD, FANCD2, HRAS*, MUTYH, BMPR1A, DAXX, FANCE, IDH1*, MYC*, BRAF*, DDB2, FANCF, IDH2*, MYCL1*, BRCA1, DDR2, FANCG, IGF1R*, MYCN*, BRCA2, DICER1, FANCI, IGF2R*, MYD88*, BRIP1, DNMT3A*, FANCL, IKZF1, NBN, BTK, EGFR*, FANCM, JAK1*, NCOA3*, BUB1B, EP300, FBXW7, JAK2*, NF1, CALR, ERBB2*, FGFR1, JAK3*, NF2, NKX2-1*, PIK3CA*, RAD51C, SF3B1*, TNFAIP3, NOTCH1*, PIK3R1, RAF1, SMAD2, TOP1, NOTCH2*, PMS1, RB1, SMAD3, TP53, NOTCH3*, PMS2, RECQL4, SMAD4, TSC1, NOTCH4*, POLD1, RET*, SMARCB1, TSC2, NPM1, POLE, RNF43, SMO*, TSHR*, NRAS*, POLH, ROS1, SRC, VHL, NTRK1, POT1, RUNX1*, STAG2, WAS, PALB2, PRKAR1A, SBDS, STK11, WRN, PAX5*, PRSS1, SDHAF2, SUFU, WT1, PBRM1, PTCH1, SDHB, TERT, XPA, PDGFRA*, PTEN, SDHC, TET2, XPC, PHOX2B, PTPN11*, SDHD, TGFBR2, XRCC1.
c. Microsatellite instability analyses for five markers: BAT-25, BAT-26, NR-21, NR-24 and MONO-27.

In CancerComplete they utilize exome capture to selectively analyze only the coding regions (exome) of the genome. Additionally, the CancerSelect-88 panel genes are also analyzed, analyzing regions over >20,000 genes.

OncoPlex Diagnostics (now Nantomics) (Rockville, MD)

The company OncoPlex Diagnostics provides quantitative analysis of proteins and genomic targets in cancer diagnostics. With the patented Liquid Tissue technology, coupled with mass spectrometry and NGS, the OncoPlex Diagnostics test measures the amount of functional proteins and identifies genetic mutations. Using laser microdissection, researchers are able to isolate and sample only the tumor cells of interest, thereby avoiding analysis of stroma. They also offer information on treatment options that target the identified gene alteration, as well as curated information enabling oncologists to aid on patient management strategies.

The specimens required are usually Formalin-Fixed, Paraffin-embedded (FFPE) tissue. Their standard protocol entails one 5 μm H&E section and two or three 10 μm sections for laser microdissection.

Testing offered by OncoPlex Diagnostics includes:

a. Protein Expression Panel: ALK, AR, AXL, EGFR, ERCC1, FGFR2, FR-α, hENT1, HER2, HER3, IGF1R, MET, MGMT, MSLN, PD-L1, RON, ROS1, RRM1, SPARC, TOPO1, TOPO2A and TUBb3.
b. Focus areas of the Protein Expression Panel (Chemotherapy protein biomarkers): AR, ERCC1, FR-α, hENT1, MGMT, RRM1, SPARC, TOPO1, TOPO2A, and TUBb3.
c. Protein biomarkers and targets by cancer type:
    i. Breast cancer: AXL, EGFR, HER2, HER3, IGF1R, MET, PD-L1, and ROS1.
    ii. Gastrointestinal cancer: EGFR, FGFR2, HER2, HER3, IGF1R, MET, MSLN, PD-L1, and RON.

iii. Lung cancer: ALK, AXL, EGFR, HER2, HER3, IGF1R, MET, MSLN,4 PD-L1, ROS1.

d. NSCLC differentiation: CK5, CK7, TP63, TTF-1.
e. Gene Mutation Panel: BRAF, EGFR, KRAS, NRAS, ABL1, AKT1, ALK, APC, ATM, CDH1, CDKN2A, CSF1R, CTNNB1, ERBB2, ERBB4, EZH2, FBXW7, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAS, GNAQ, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, MET, MLH1, MPL, NOTCH1, NPM1, PDGFRA, PIK3CA, PTEN, PTPN11, RB1, RET, SMAD4, SMARCB1, SMO, SRC, STK11, TP53, and VHL.
f. Supplemental Testing Offered: HPV-infection associated protein p16 (For head and neck cancers), ALK translocation by FISH (For NSCLC) and NSCLC differentiation: CK5, CK7, TP63, and TTF-1.

Conclusion

In this post-genetic and data mining era with the availability of rapidly advancing and varied levels of patient and tumor data, academicians and clinicians are at a flexion point. The surmounting data and inability to fully understand its capabilities greatly humble even the best clinicians. As the data gathering in multi-omic silos are being gradually structured and paired with clinical data, researchers will be better equipped to understand PDAC, and clinicians will be able to better manage PDAC with more personalized care.

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References

1. Le N, Sund M, Vinci A. Prognostic and predictive markers in pancreatic adenocarcinoma. Dig Liver Dis 2015;48:223–230.
2. Jeong J, Park YN, Park JS, et al. Clinical significance of p16 protein expression loss and aberrant p53 protein expression in pancreatic cancer. Yonsei Med J 2005;46:519–525.
3. Tang ZH, Zou SQ, Hao YH, et al. The relationship between loss expression of DPC4/Smad4 gene and carcinogenesis of pancreatobiliary carcinoma. Hepatobiliary Pancreat Dis Int 2002;1:624–629.
4. Biankin AV, Morey AL, Lee CS, et al. DPC4/Smad4 expression and outcome in pancreatic ductal adenocarcinoma. J Clin Oncol 2002;20:4531–4542.
5. Erkan M, Kleeff J, Esposito I, et al. Loss of BNIP3 expression is a late event in pancreatic cancer contributing to chemoresistance and worsened prognosis. Oncogene 2005;24:4421–4432.
6. LaCasse EC, Baird S, Korneluk RG, et al. The inhibitors of apoptosis (IAPs) and their emerging role in cancer. Oncogene 1998;17:3247–3259.
7. Harsha HC, Kandasamy K, Ranganathan P, et al. A compendium of potential biomarkers of pancreatic cancer. PLoS Med 2009;6:e1000046.
8. Theodoridis GA, Gika HG, Want EJ, et al. Liquid chromatography-mass spectrometry based global metabolite profiling: a review. Anal Chim Acta 2012;711:7–16.
9. Britton D, Zen Y, Quaglia A, et al. Quantification of pancreatic cancer proteome and phosphorylome: indicates molecular events likely contributing to cancer and activity of drug targets. PLoS ONE 2014;9:e90948.
10. Kosanam H, Prassas I, Chrystoja CC, et al. Laminin, gamma 2 (LAMC2): a promising new putative pancreatic cancer biomarker identified by proteomic analysis of pancreatic adenocarcinoma tissues. Mol Cell Proteomics 2013;12:2820–2832.
11. Ansari D, Aronsson L, Sasor A, et al. The role of quantitative mass spectrometry in the discovery of pancreatic cancer biomarkers for translational science. J Transl Med 2014;12:87.
12. Collisson EA, Sadanandam A, Olson P, et al. Subtypes of pancreatic ductal adenocarcinoma and their differing responses to therapy. Nat Med 2011;17:500–503.
13. Moffitt RA, Marayati R, Flate EL, et al. Virtual microdissection identifies distinct tumor- and stroma-specific subtypes of pancreatic ductal adenocarcinoma. Nat Genet 2015;47:1168–1178.
14. Bailey P, Chang DK, Nones K, et al. Genomic analyses identify molecular subtypes of pancreatic cancer. Nature 2016;531:47–52.
15. Burris HA 3rd, Moore MJ, Andersen J, et al. Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. J Clin Oncol 1997;15:2403–2413.
16. Baldwin SA, Mackey JR, Cass CE, et al. Nucleoside transporters: molecular biology and implications for therapeutic development. Mol Med Today 1999;5:216–224.
17. Nakano Y, Tanno S, Koizumi K, et al. Gemcitabine chemoresistance and molecular markers associated with gemcitabine transport and metabolism in human pancreatic cancer cells. Br J Cancer 2007;96:457–463.
18. Spratlin J, Sangha R, Glubrecht D, et al. The absence of human equilibrative nucleoside transporter 1 is associated with reduced survival in patients with gemcitabine-treated pancreas adenocarcinoma. Clin Cancer Res 2004;10:6956–6961.
19. Greenhalf W, Ghaneh P, Neoptolemos JP, et al. Pancreatic cancer hENT1 expression and survival from gemcitabine in patients from the ESPAC-3 trial. J Natl Cancer Inst 2014;106:djt347.
20. Waddell N, Pajic M, Patch AM, et al. Whole genomes redefine the mutational landscape of pancreatic cancer. Nature 2015;518:495–501.
21. Desai N, Trieu V, Damascelli B, et al. SPARC expression correlates with tumor response to albumin-bound paclitaxel in head and neck cancer patients. Transl Oncol 2009;2:59–64.
22. Hidalgo M, Plaza C, Musteanu M, et al. SPARC expression did not predict efficacy of nab-paclitaxel plus gemcitabine or gemcitabine alone for metastatic pancreatic cancer in an exploratory analysis of the phase III MPACT trial. Clin Cancer Res 2015;21:4811–4818.
23. Moore MJ, Goldstein D, Hamm J, et al. Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. J Clin Oncol 2007;25:1960–1966.
24. Philip PA, Benedetti J, Corless CL, et al. Phase III study comparing gemcitabine plus cetuximab versus gemcitabine in patients with advanced pancreatic adenocarcinoma: Southwest Oncology Group-directed intergroup trial S0205. J Clin Oncol 2010;28:3605–3610.
25. Fountzilas G, Bobos M, Kalogera-Fountzila A, et al. Gemcitabine combined with gefitinib in patients with inoperable or metastatic pancreatic cancer: a phase II Study of the Hellenic Cooperative Oncology Group with biomarker evaluation. Cancer Invest 2008;26:784–793.
26. Kimura K, Sawada T, Komatsu M, et al. Antitumor effect of trastuzumab for pancreatic cancer with high HER-2 expression and enhancement of effect by combined therapy with gemcitabine. Clin Cancer Res 2006;12:4925–4932.
27. Harder J, Ihorst G, Heinemann V, et al. Multicentre phase II trial of trastuzumab and capecitabine in patients with HER2 overexpressing metastatic pancreatic cancer. Br J Cancer 2012;106:1033–1038.

28. Di Marco M, Grassi E, Durante S, et al. State of the art biological therapies in pancreatic cancer. World J Gastrointest Oncol 2016;8:55–66.
29. Van Cutsem E, van de Velde H, Karasek P, et al. Phase III trial of gemcitabine plus tipifarnib compared with gemcitabine plus placebo in advanced pancreatic cancer. J Clin Oncol 2004; 22:1430–1438.
30. Bodoky G, Timcheva C, Spigel DR, et al. A phase II open-label randomized study to assess the efficacy and safety of selumetinib (AZD6244 [ARRY-142886]) versus capecitabine in patients with advanced or metastatic pancreatic cancer who have failed first-line gemcitabine therapy. Invest New Drugs 2012;30:1216–1223.
31. Bailey JM, Swanson BJ, Hamada T, et al. Sonic hedgehog promotes desmoplasia in pancreatic cancer. Clin Cancer Res 2008;14:5995–6004.
32. Olive KP, Jacobetz MA, Davidson CJ, et al. Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. Science 2009;324:1457–1461.
33. Ristorcelli E, Lombardo D. Targeting Notch signaling in pancreatic cancer. Expert Opin Ther Targets 2010;14:541–552.
34. Samol J, Ranson M, Scott E, et al. Safety and tolerability of the poly(ADP-ribose) polymerase (PARP) inhibitor, olaparib (AZD2281) in combination with topotecan for the treatment of patients with advanced solid tumors: a phase I study. Invest New Drugs 2012;30:1493–1500.
35. Tacket CO, Davis BR, Carter GP, et al. Yersinia enterocolitica pharyngitis. Ann Intern Med 1983;99:40–42.
36. Narimatsu H, Iwasaki H, Nakayama F, et al. Lewis and secretor gene dosages affect CA19-9 and DU-PAN-2 serum levels in normal individuals and colorectal cancer patients. Cancer Res 1998;58:512–518.
37. Mann DV, Edwards R, Ho S, et al. Elevated tumour marker CA19-9: clinical interpretation and influence of obstructive jaundice. Eur J Surg Oncol 2000;26:474–479.
38. Homma T, Tsuchiya R. The study of the mass screening of persons without symptoms and of the screening of outpatients with gastrointestinal complaints or icterus for pancreatic cancer in Japan, using CA19-9 and elastase-1 or ultrasonography. Int J Pancreatol 1991;9:119–124.
39. Maithel SK, Maloney S, Winston C, et al. Preoperative CA 19-9 and the yield of staging laparoscopy in patients with radiographically resectable pancreatic adenocarcinoma. Ann Surg Oncol 2008;15:3512–3520.
40. Berger AC, Winter K, Hoffman JP, et al. Five year results of US intergroup/RTOG 9704 with postoperative CA 19-9 </=90 U/mL and comparison to the CONKO-001 trial. Int J Radiat Oncol Biol Phys 2012;84:e291–e297.
41. Tibbe AG, Miller MC, Terstappen LW. Statistical considerations for enumeration of circulating tumor cells. Cytometry A 2007;71:154–162.
42. Allard WJ, Matera J, Miller MC, et al. Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with nonmalignant diseases. Clin Cancer Res 2004;10:6897–6904.
43. Tjensvoll K, Nordgard O, Smaaland R. Circulating tumor cells in pancreatic cancer patients: methods of detection and clinical implications. Int J Cancer 2014;134:1–8.
44. Bidard FC, Hueguet 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;24:2057–2061.
45. Han L, Chen W, Zhao Q. Prognostic value of circulating tumor cells in patients with pancreatic cancer: a meta-analysis. Tumour Biol 2014;35:2473–2480.
46. Ren C, Han C, Zhang J, et al. Detection of apoptotic circulating tumor cells in advanced pancreatic cancer following 5-fluorouracil chemotherapy. Cancer Biol Ther 2011;12:700–706.
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;6:224ra24.
48. Zill OA, Greene C, Sebisanovic D, et al. Cell-free DNA next-generation sequencing in pancreatobiliary carcinomas. Cancer Discov 2015;5:1040–1048.
49. Debernardi S, Massat NJ, Radon TP, et al. Noninvasive urinary miRNA biomarkers for early detection of pancreatic adenocarcinoma. Am J Cancer Res 2015;5:3455–3466.
50. Schonemeier B, Metzger J, Klein J, et al. Urinary peptide analysis differentiates pancreatic cancer from chronic pancreatitis. Pancreas 2016;45:1018–1026.
51. Radon TP, Massat NJ, Jones R, et al. Identification of a three-biomarker panel in urine for early detection of pancreatic adenocarcinoma. Clin Cancer Res 2015;21:3512–3521.
52. Anon. A stool DNA test (Cologuard) for colorectal cancer screening. JAMA 2014;312:2566.
53. Imperiale TF, Ransohoff DF, Itzkowitz SH, et al. Multitarget stool DNA testing for colorectal-cancer screening. N Engl J Med 2014;370:1287–1297.
54. Huddy JR, Ni MZ, Markar SR, et al. Point-of-care testing in the diagnosis of gastrointestinal cancers: current technology and future directions. World J Gastroenterol 2015;21:4111–4120.
55. Kisiel JB, Yab TC, Taylor WR, et al. Stool DNA testing for the detection of pancreatic cancer: assessment of methylation marker candidates. Cancer 2012;118:2623–2631.
56. Columbia University, Mayo Clinic, Stool Testing for Pancreatic Cancer. 2015.
57. Greer JB, Whitcomb DC. Inflammation and pancreatic cancer: an evidence-based review. Curr Opin Pharmacol 2009;9:411–418.
58. Michaud DS, Joshipura K, Giovannucci E, et al. A prospective study of periodontal disease and pancreatic cancer in US male health professionals. J Natl Cancer Inst 2007;99:171–175.
59. Ahn J, Segers S, Hayes RB. Periodontal disease, *Porphyromonas gingivalis* serum antibody levels and orodigestive cancer mortality. Carcinogenesis 2012;33:1055–1058.
60. Zhang L, Farrell JJ, Zhou H, et al. Salivary transcriptomic biomarkers for detection of resectable pancreatic cancer. Gastroenterology 2010;138:949–957, e1-e7.
61. Wan C, Shen Y, Yang T, et al. Diagnostic value of microRNA for pancreatic cancer: a meta-analysis. Arch Med Sci 2012;8:749–755.
62. Xie Z, Yin X, Gong B, et al. Salivary microRNAs show potential as a noninvasive biomarker for detecting resectable pancreatic cancer. Cancer Prev Res (Phila) 2015;8:165–173.
63. Sugimoto M, Wong DT, Hirayama A, et al. Capillary electrophoresis mass spectrometry-based saliva metabolomics identified oral, breast and pancreatic cancer-specific profiles. Metabolomics 2010;6:78–95.