Review Article

Future role of endoscopic ultrasound in personalized management of pancreatic cancer

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ABSTRACT

Pancreatic ductal adenocarcinoma (PDAC) is aggressive and lethal with the majority of cases presenting with advanced unresectable disease due to delayed diagnosis. Despite improvement in surgery, chemotherapies, and intensive care medicine, the outcome of PDAC remains poor, which may relate to the tumor biology. Recent data suggest that PDAC is a "systemic cancer" with complex molecular or genomics derangement with marked heterogeneity. The ability to characterize the PDAC better by detailed evaluation of tissue biomarkers or genomics allows for improved prediction of prognosis and stratification of treatment, a concept known as "personalized cancer therapy." Using tissue from resected PDAC specimens has several weaknesses and is only possible in 20% of patients with PDAC. Endoscopic ultrasound (EUS)-guided biopsy overcomes these weaknesses, and with recent advancements in needle technology, tissue can be obtained for personalized cancer therapy for all patients with PDAC. This review aims to outline our current understanding of the molecular biology of PDAC specifically focusing on how EUS-guided biopsy may play a fundamental role in tissue acquisition, allowing for assessment and stratify therapy according to the individual cancer biology as we move toward the era of precision medicine.

Key words: Endoscopic ultrasound, fine needle biopsy, pancreatic adenocarcinoma, personalized medicine, portal vein sampling, precision medicine

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer-related death in the Western population with a steady rise in its incidence.[1] PDAC is expected to be the second cause of cancer-related death in western countries by 2020, with a median survival of <1 year despite maximized therapy and a dismal 5-year survival rate of <5%.[2] One of the main reasons for such poor prognosis is because most PDAC (~80%) cases are unresectable at clinical diagnosis. While surgery remains the primary curative treatment for pancreatic cancer, the 5-year survival varies between 25% and 30% with the majority (>80%) developing local recurrence or distant metastases within 12 months of resection.[3] For the unresectable patients, treatment is mainly for palliation with chemotherapy, radiation therapy, and/or endoscopic therapy. Even

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with the improvement in chemotherapies over the past three decades, the median survival in these patients remains <12 months.[3] Recent data on focus radiotherapy, particularly the stereotactic body radiation therapy combined with chemotherapy, are promising.[4-6] In contrast to other gastrointestinal cancers, the use of a biologically targeted agent such as erlotinib, an epidermal growth factor receptor antagonist, has almost invariably negative results.[7] The reasons behind the lack of responses of PDAC to current therapies are unclear but may relate to the tumor biology, including the complex molecular or genomics derangement with marked heterogeneity between the PDAC.

The aims of this review is to outline the molecular biology of PDAC and both current and future approaches, especially with the use of endoscopic ultrasound (EUS)-guided biopsy that can be used to assess and stratify therapy according to the individual cancer biology, a concept known as "personalized cancer therapy."

BIOLOGY AND GENETICS OF PANCREATIC DUCTAL ADENOCARCINOMA

The tumor behavior is influenced by both the malignant potential of the cancer cells as well as by the host microenvironment defined by fibroblasts specifically pancreatic stellate cells, myofibroblasts, vascular cells, immune cells, and the extracellular matrix with associated cytokines.[8,9] This tumor behavior, in turn, is determined by the tumor "biology" or genetics. PDAC is well known for its aggressiveness and is considered a "systemic disease" given recent data suggest that circulating tumor cells (CTC) are found in the portal venous system of patients with resectable PDAC at the time of diagnosis.[10,11] Furthermore, PDAC is also characterized by the presence of dense desmoplastic tumor stromal devoid of functional vasculature, preventing immune evasion and the ability of chemotherapeutic agents to access the cancer; thus, the known poor response to chemotherapy over the last three decades.[12]

Such complex tumor biology of PDAC is most likely reflected by the multiple carcinogenic pathways and heterogeneity of cancer. A wide range of genetic alterations including 32 recurrent mutations aggregated into 10 pathways have been reported with the four most common oncogenic mutations are KRAS (90%), CDK2NA (90%), TP53 (75%–90%) and DPC4/SMAD4 (50%). Other lesser prevalent mutations are transforming growth factor-beta, WNT, NOTCH, ROBO/SLIT signaling, G1/S transition, SWI-SNF, chromatin modification, DNA repair, and RNA processing.[13,14] The accumulation of these early and late genetic alterations indicates that PDAC also adopts the "multi-step progression" model in its cancer pathogenesis. Recent data on integrated whole genome sequencing of 100 PDACs identified 7888 nonsilent mutations in 5427 genes, in which the subtypes cannot be characterized by a single or a cluster of mutations.[15] Instead, the PDAC can be further divided by the patterns of chromosomal structural variation into stable (18%), locally rearranged (28%), scattered (40%), and unstable (14%) subtypes. Of most clinically relevant, the unstable subtype is cosegregated with inactivation of DNA maintenance genes (BRCA1, BRCA2, PALB2, or RPA1) and is associated with good response to platinum-based therapy, defining a potential marker of therapeutic responsiveness.[15] More recently, PDAC can also be subdivided into 4 groups based on their genomics abnormalities and cell origin: squamous, pancreatic progenitor, immunogenic, and aberrantly differentiated endocrine exocrine (ADEX). This classification system has prognostic implication with the worst survival in the squamous subtype as compared to the best prognosis in ADEX (median survival of 13.3 *vs.* 30 months).[16]

The revolution in our understanding of the genetics of cancer and the exploration of gene expression on a large scale has brought hope that novel therapies can be developed specifically exploiting the genetic deletions and biochemical deficiencies present in pancreatic cancer. In addition, an increased understanding of the molecular basis of the disease has facilitated the identification of new drug targets.

CURRENT STATUS OF PERSONALIZED MEDICINE IN PANCREATIC DUCTAL ADENOCARCINOMA

The concept of personalized medicine encompasses assessment of individual tumor biology, by identifying a specific set of gene mutations or molecular profile that enables clinicians to prognosticate and stratify the most effective treatment for the individual. This would not only optimize the effectiveness of the patient's treatment but also avoid the adverse effects of unnecessary ineffective empirical therapy, which

is often adopted in the traditional "one-size-fits-all" treatment concept. Consequently, personalized medicine has been widely practiced in the treatment of a number of cancers, for example, the use of trastuzumab for HER2 positive breast cancers or cetuximab for wide-type K-ras colon cancers.[17,18] Although the field of personalized medicine for pancreatic cancer is not as well developed as other cancers, there are increasingly basic and clinical research projects in this area over the last decade. In addition to biomarkers or genomic analyses, cancer tissue can be used to create xenograft or cancer organoid, which in turn provides tissue resource for further assessment, including evaluation of drug responses. Each of these techniques has its own advantages and disadvantages, which are summarized in Table 1.

Biomarkers in pancreatic ductal adenocarcinoma

Based on surgical specimens of patients with resectable PDAC, a number of putative biomarkers have been identified to predict clinical outcome and response to certain therapies [Table 2]. Human equilibrative nucleoside transporter 1 (hENT-1) is the most commonly examined biomarker in PDAC. The high immunohistochemical expression in surgical specimen is associated with better survival and response to adjuvant gemcitabine, which is mediated by the increased intracellular uptake of gemcitabine by hENT-1.[19] Unfortunately, clinical data revealed a substantial number of patients do not have a high hENT-1 expression, thus limiting the use of this biomarker in clinical practice.[20] Another biomarker that predicts response to gemcitabine is heat shock protein 27 (HSP27), with overexpression increases sensitivity to gemcitabine and lack of expression results in gemcitabine resistance.[21] Together, the assessment of hENT-1 and/or HSP27 expression may, therefore, identify the best candidates for adjuvant chemotherapy with gemcitabine and enable optimal stratification therapy based on biomarkers assessment.

More recently, biomarkers that can predict response to surgery have been identified. Although the high expressions of HOXB2, cyclin E1, and S100A2 in PDAC were associated with poor prognosis and response to surgical resection in the discovery cohort,[22] only the overexpression of S100A2 remained predictive in the validating cohort.[22] Even in the resected cohort with clear (R0) margin, patients with high S100A2-express PDAC had significantly poorer outcome, similar to those with unresectable cancers. Hence, for the first time, we have a potential biomarker that can predict the benefit of surgery in patients with resectable PDAC.[22]

Patient-derived xenograft

Patient-derived xenograft (PDX) models are also emerging as a promising platform for translational cancer research to offset the limited ability to predict efficacy of treatment response in preclinical studies whereby tumor tissue is directly implanted *in vivo* immunodeficient mice without dissociation. This technique involves implantation of fresh cancer tissue from the patient to an immunosuppression mouse

Table 1. Different techniques used to achieve personalize medicine in pancreatic ductal adenocarcinoma

Model	Method	Advantage	Disadvantage
Putative biomarkers and micro-RNAs	Immunohistochemistry of biomarker and mRNA testing on tumor tissue	Can be performed in small tissue sample, like FNA specimen Cheaper than xenograft or organoid creation Widely available	Diversity of biomarkers Most tumors have a lack of expression of biomarkers
PDX	Direct *in vivo* implantation of tumor tissue into mice	Retain genetic and disease specific characteristics of tumor Stable without dissociation throughout engraftment process	Expensive Require large volume of tumor tissue High failure of engraftment with FNA specimen Time delay to engraftment by at least 6 months
Cancer-derived organoid	*In vitro* generation of model from tissue	Rapid generation of model and shorter engraftment Ability to introduce transgenes for genetic manipulation for knockout studies Can be performed using small amount of tissue Retain genetic and disease-specific characteristics	Expensive Require large volume of tumor tissue Invasive and requires tumor tissue specimen

FNA: Fine needle aspiration, PDX: Patient-derived xenograft

Table 2. Putative function and associated outcomes of biomarkers and micro-RNAs in pancreatic cancer

Putative biomarkers	Function of biomarkers	Clinical significance of biomarker expression
hENT-1	↑ Intracellular uptake of gemcitabine	↑ Response to chemotherapy
HSP27	Promote cell proliferation and inhibit apoptosis signaling pathway	↑ Response to chemotherapy
S100A2/A4	Promote cell proliferation and inhibit apoptosis signaling pathway	↓ Response to surgery Associated with poor outcome
dCK	DNA damage repair to maintain normal DNA metabolism	↑ Response to chemotherapy↑Survival
SMAD4	Regulate cell proliferation and apoptosis through TGF-B signaling pathway	↑ Survival and less likely to metastasize
RRM2	Induction of oncogenes and promote tumor progression	↓ Response to gemcitabine↓Survival
Micro-RNA-10b	Control self-renewal ability of cells and promote cell invasion	↓ Response to chemotherapy
Micro-RNA-21	Negatively regulate tumor-suppressor genes and promote cell invasion	↓ Response to chemotherapy

hENT: Human equilibrative nucleoside transporter 1, HSP: Heat shock protein, dCK: Deoxycytidine kinase, RRM2: Ribonucleotide reductase subunit M2, TGF-B: Transforming growth factor beta, SMAD4: Mothers against decapentaplegic homolog 4, ↑: Increase, ↓: Decrease

and allows the cancer tissue to grow [Figure 1].[23,24] The main advantage of PDX model is the ability to retain genetic and histologic characteristics of the donor tumor.[23,24] Hence, they can be reliably used for preclinical drug evaluation, biomarker identification, biological studies, and personalized medicine strategies. Such proof-of-concept studies have been performed in patients with PDAC, and creation of PDX from resectable specimens has been successfully achieved for identification of the most optimal adjuvant chemotherapy in clinical practice.[25] The limitations to PDX models, however, are the high cost, the requirement of large volume of tissue, high failure rate, and time delay to engraftment which may take up to 6 months, limiting its applicability in real-time patient treatment.[24]

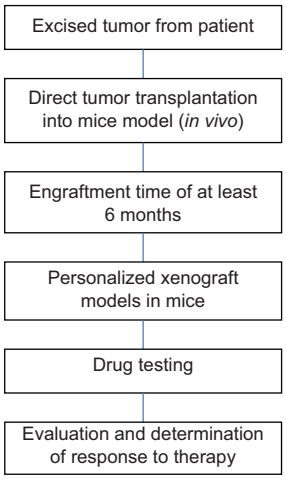

Figure 1. Steps involved in generating patient-derived cancer xenograft that allows further assessment of personalizing medicine

Cancer-derived organoid

To address the weaknesses of PDX models, cancer-derived organoid has been developed in a number of cancers such as colonic, prostatic, and breast cancers. This technique involves a three-dimensional culture method that allows *in vivo* cell growth with self-organization, differentiation, and mixed heterogeneity to exist within the culture environment [Figure 2].[26] The preliminary results appear promising given the ability to rapidly generate a three-dimensional organoid model *in vitro* from surgical specimen or biopsies, while preserving disease specific characteristics and recapitulating the full spectrum of tumor progression. It is also amenable to genetic manipulation for the introduction of transgenes or knockdown or knockout studies.[26-28] Hence, human organoids hold great potential as a tool for cancer precision medicine, with promising applications for oncogene modeling, gene discovery, and chemosensitivity studies. Most recently, cancer-derived organoids have been successfully created from resected pancreatic cancer, with adequate retention of genetic property of the original cancer that allows further evaluation, including drug testing.[29]

Weaknesses of current approaches

The major weakness of the current approach in personalized medicine of PDAC is that the evaluation is mostly performed on surgical specimens, which would not be applicable to the majority of PDAC that are unresectable (80% of all cases); thus, the concept is not useful for most patients with PDAC. Furthermore, in cases where the biomarker's predictive response to surgery is not known until the surgery had been performed, this is not ideal given pancreatic cancer surgery carries significant morbidities and mortality. Together, these weaknesses demand a better, noninvasive, preoperative method of tissue acquisition that allows either the assessments for biomarkers or

genomic profile or creation of PDX or cancer-derived organoids.

ENDOSCOPIC ULTRASOUND-GUIDED BIOPSY: AN IDEAL, NONSURGICAL APPROACH FOR PERSONALIZED MEDICINE

Tissue acquisition from the pancreas is best achieved by EUS-guided approach, which has higher diagnostic yield and less complication than percutaneous approach.[30,31] Conventionally, this is performed by using a fine hollow sharp tip needle, and most often, cytological specimen is obtained, which is mostly not sufficient for biomarkers assessment or creation of PDX/organoid. Even with recent techniques of mRNA or DNA assessment, the contamination from the needle pass makes the analysis unreliable or uninterpretable. For whole genome analysis, at least 50 μg or 1 mm^3 of diagnostic tissue is needed.[32] While Tru-cut or larger caliber (i.e., 19G) needles can provide a larger tissue specimen, these needles are very stiff and technically difficult to use, thus, the lack of success of obtaining diagnostic tissue for assessment. Recognizing these weaknesses, recent needle technology has created a "hybrid needle" that allows for acquisition of mini tissue core but highly flexible and ensuring the success of the biopsy. These needles range from modified needle tip (bevel, folk like, and tripod like) to highly flexible nitinol needle material. Such improvements in needle technology allow a larger amount of diagnostic tissue to be acquired for biomarkers and/or genomic sequencing, making personalized medicine a reality in clinical practice [Figure 3]. The "hybrid needle" that is most evaluated is the Procore needles (Cook, USA). Available data suggest that the Procore needles are able to acquire more histological samples than the regular hollow tip needles.[33] In our biomarkers study of S100A2 and S100A4, histological evaluation and IHC staining were possible in 92% of cases when the 22G and 20G Procore needles were used [Figure 4], supporting the concept of fine needle "biopsy" (FNB) for achieving personalized medicine.[34]

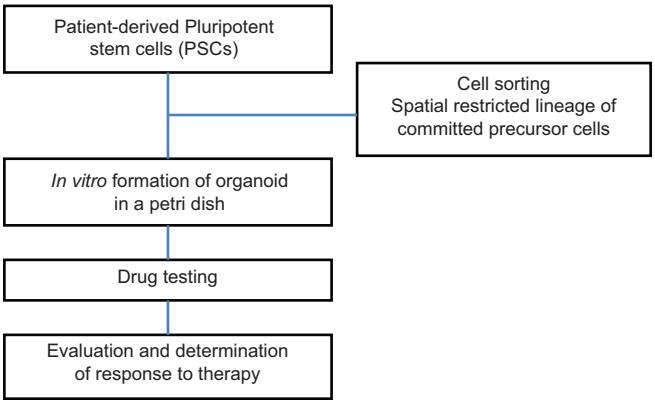

Figure 2. Steps involved in creating patient-derived cancer organoid to allow further testing and drug response evaluations

Biomarkers from endoscopic ultrasound-guided biopsy

Although it is possible to perform microRNA analysis from EUS fine needle aspiration (FNA) sample (22G needle), the "cancer or diagnostic tissue" needed to be isolated with laser microdissection to ensure the

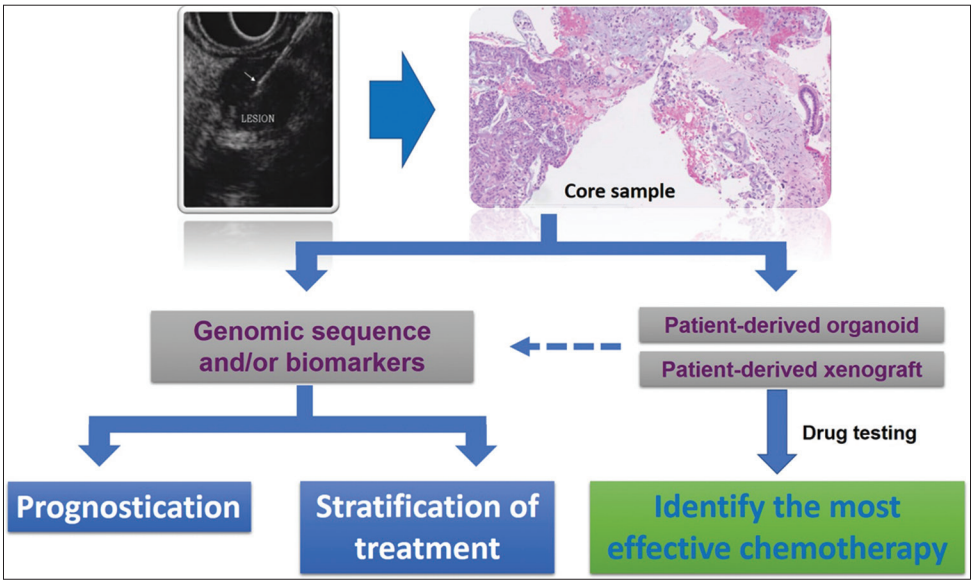

Figure 3. Outline of the potential role of endoscopic ultrasound-guided biopsy in achieving personalized management of pancreatic ductal adenocarcinoma

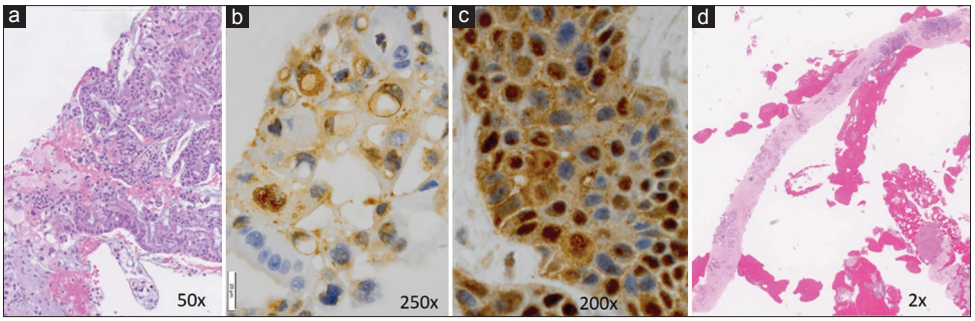

Figure 4. Core samples of endoscopic ultrasound-guided biopsies of pancreatic ductal adenocarcinoma using a 22G fine needle biopsy (a-c) and the new 20G fine needle biopsy (d) needles. The specimens allow immunohistochemical staining of S100A2 biomarkers (b), which correlated well with the surgical specimen (c) from the same patient

adequate quality for the mRNA evaluation.[25] Fujita *et al.* found that high levels of deoxycytidine kinase and low levels of ribonucleotide reductase subunit M2 mRNA from on EUS FNA specimens are associated with significantly longer disease-free survival in the gemcitabine-treated group.[35] Since then, several other microRNAs have been identified as a prognostic tool in predicting response to therapy and survival outcomes. Overexpression of miR-10b and miR-21 have been shown to associate with reductions in response to multimodality neoadjuvant therapy, surgical resection, time to metastasis, which in turn, resulted in a decreased survival.[36,37] Unfortunately, the technique of laser dissection of diagnostic tissue from EUS-FNA specimens is not widely available and technically challenging.

Our group had recently completed a prospective study that examined the impact of S100A2 and S100A4 biomarkers on EUS-guided biopsies of patients with both resectable and unresectable PDACs. Using of either the 22G and 20G Procore needles (Cook, USA), there were 83%–86% correlations between the surgical specimens and the EUS-guided biopsied specimen in patients who underwent surgery. Similar to our previous surgical cohort, the expression of S100A2/A4 biomarkers in EUS-guided biopsies was associated with poorer prognosis and can predict outcomes of surgery and chemotherapy with gemcitabine.[34] Similarly, the loss of SMAD4 staining on preoperative cell blocks was strongly correlated with poorer outcome and higher chance of metastases.[38] Therefore, the advances in EUS-guided biopsy have enabled assessment of biomarkers direct from the slide without the needle for microdissection of cancer tissue. Such integration of these biomarkers into preoperative strategies has great potential in assisting the clinicians to identify patients who will ultimately benefit from surgery or chemotherapy.

Xenograft derived from endoscopic ultrasound-guided biopsy

Successful cases of PDX from EUS-FNA specimen have been reported. However, with the amount of fresh tissue from the FNA needles, the engraftment is between 20% and 30%, which is clearly not ideal.[39,40] The use of fresh tissue from the FNB needles has not yet been evaluated. Given the time taken for successful engraftment, which is around 2–3 months, such delay in appropriate treatment while waiting for the PDX can lead to adverse outcome in these patients.

Organoid derived from endoscopic ultrasound-guided biopsy

As the creation of cancer-derived organoid can be much shorter (usually within 10 days), the concept of creating cancer-derived organoid from EUS-guided biopsy specimen in patients with PDAC is promising. Huang *et al.* have successfully generated cancer-derived organoid from FNA of PDAC-derived xenograft model, with good correlation of genetic profile as the original cancer.[41] Ideally, the future of personalized medicine in PDAC is to generate cancer-derived organoid from EUS-guided biopsies, which can be used for further prognostication and drug testing.

PORTAL VEIN SAMPLING FOR MOLECULAR STAGING AND BIOMARKERS ASSESSMENT

Despite the advances in imaging, the rate of metastasis detected after 12 months of surgery in patients who were deemed to have "resectable" PDAC is over 50%. This indicates that our current technique of staging based on cross-sectional imaging is clearly inadequate. As PDAC is a "systemic disease" with the significant proportion with detectable CTCs in the portal vein, a more effective staging approach is to detect CTCs or cancer mutations in the portal vein.[10] While

evaluation of CTCs or cancer mutations in peripheral blood is much more simple, the detection is quite low as most CTCs will be filtered by the liver. Even in advanced disease, the density of circulating numbers is very low, with an estimation of 1 tumor cell per billion circulating blood cells.[10] Detecting CTCs or cancer mutations in portal vein but not in the peripheral blood indicates the need for neoadjuvant chemotherapy. On the contrary, regardless of the absence of macroscopic disease on imaging, patients who have both CTCs or mutations in both portal and peripheral blood will not benefit surgery, and aggressive chemotherapy would be required. As a proof of concept, others and our group have successfully and safely sampled the portal vein by EUS-guided approach in patients with PDAC for the assessment of mutations from free circulating DNA [Figure 5].

CONCLUSIONS

The advances in EUS-guided needle biopsy have allowed the concept of a novel "molecular staging" approach for PDAC by combining EUS-guided biopsy and portal vein sampling for whole genome sequencing and the detection of free circulating DNA. By integration of adequate tissue specimen, tissue biomarkers, genomics characterization, and drug testing, we will have a better understanding of the individual tumor behavior and will be a step closer to precision cancer treatment.

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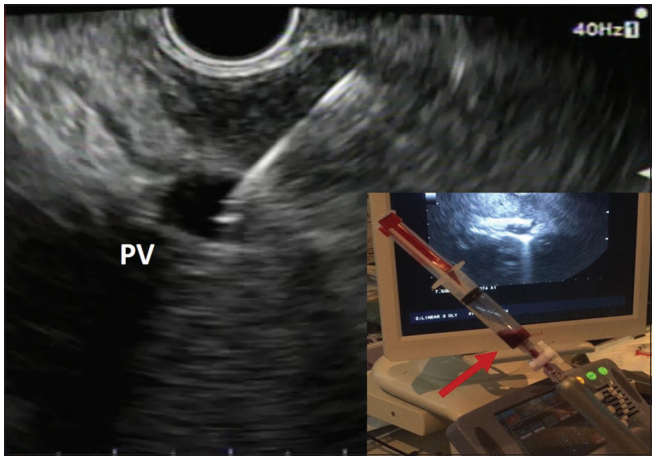

Figure 5. Endoscopic ultrasound-guided portal vein sampling, during the initial Endoscopic ultrasound fine needle aspiration for the pancreatic mass, as a novel "molecular staging approach" for patients with pancreatic ductal adenocarcinoma

Conflicts of interest
There are no conflicts of interest.

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