Molecular alterations in pancreatic tumors

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Abstract

Genetic alterations in pancreatic tumors can usually be classified in: (1) Mutational activation of oncogenes; (2) Inactivation of tumor suppressor genes; and (3) Inactivation of genome maintenance genes controlling the repair of DNA damage. Endoscopic ultrasound-guided fine-needle aspiration has improved preoperative diagnosis, but the management of patients with a pancreatic lesion is still challenging. Molecular testing could help mainly in solving these “inconclusive” specimens. The introduction of multi-gene analysis approaches, such as next-generation sequencing, has provided a lot of useful information on the molecular characterization of pancreatic tumors. Different types of pancreatic tumors (e.g., pancreatic ductal adenocarcinomas, intraductal papillary mucinous neoplasms, solid pseudopapillary tumors) are characterized by specific molecular alterations. The aim of this review is to summarize the main molecular alterations found in pancreatic tumors.
INTRODUCTION

Genetic alterations in pancreatic tumors are usually classified in: (1) Tumors with activation of oncopgenes (e.g., KRAS mutation—more than 90% of pancreatic tumors); (2) Tumors harboring inactivation of tumor suppressor genes (e.g., p16/CDKN2A, TP53, and SMAD4); and (3) Tumors with inactivation of genes controlling the repair of DNA damage (e.g., hMLH1 and MSH2) [1].

KRAS gene mutations inhibit the ability of KRAS protein to hydrolyze GTP (guanosine-5’-triphosphate), leaving the protein constitutively active, mediating cell survival and differentiation. KRAS is the most common marker used for single-gene testing. Its use is strongly limited by the identification of mutations also in low-grade pancreaticobiliary dysplasia or chronic pancreatitis (about 10%) [2-5]. To date, the “The Papanicolaou Society of Cytopathology guidelines” does not encourage testing of KRAS in bile duct strictures and solid pancreatic masses as a useful “single-gene” test.

P53 protein is involved in cell-cycle regulation, the apoptotic process, and plays a crucial role in the maintenance of genomic stability. Mutations in the TP53 gene lead to inactivation of the normal protein function. In the presence of DNA damage, the functional loss of the p53 protein enhances cellular survival, facilitating the accumulation of further genetic mutations [6]. TP53 is mostly inactivated by single-point mutations [7]. TP53 gene inactivation is a very common event in pancreatic cancer (50% to 75% of pancreatic cancers harbor TP53 mutations) [7-9].

CDKN2A/p16 maps on chromosome 9p and encodes the protein p14ARF, an activator of p53 protein, and p16INK4a protein. This protein inhibits the progression of the cell cycle at the G1-S checkpoint binding of cyclin-dependent kinases (CDKs), as CDK4 and CDK6 [10]. CDKN2A/p16 was the first tumor suppressor gene that was shown to undergo silencing and promoter hypermethylation in pancreatic cancer [11]. The Rb/p16 pathway is down-regulated in most pancreatic cancers, almost all through p16 gene inactivation [11]. Mutations in the CDKN2A gene are associated with an increased risk of cancers. Moreover, CDKN2A alterations are also frequently observed in cancer cell lines.

SMAD4/DPC4 gene is located on chromosome 18q and the protein acts in the signal transduction cascade, involving transforming growth factor β (TGF-β). A loss of SMAD4 protein leads to unregulated cellular proliferation [12].

The aim of this review is to summarize the main molecular alterations found in pancreatic tumors, both in solid (e.g., pancreatic ductal adenocarcinoma (PDAC), Table 1) and in cystic neoplasms (e.g., intraductal papillary mucinous neoplasms, IPMN).
Table 1 Main genetic alterations detectable in pancreatic ductal adenocarcinoma

| Type of pancreatic lesion | Genetic alteration | Reported frequency (%) | Type of alterations | Role in clinical practice |
|---------------------------|-------------------|------------------------|---------------------|--------------------------|
| PDAC                      | KRAS              | 70-90                  | Point mutations     | Diagnostic/prognostic    |
|                           | TP53              | 50-75                  | Point mutations/LOH | Prognostic               |
|                           | CDKN2A/p16        | 90-98                  | Point mutations/LOH | Prognostic/genetic surveillance |
|                           | SMAD4             | 40-60                  | Point mutations/LOH | Prognostic               |
|                           | BRCA1/2           | 5-10                   | Point mutations     | Predictive/genetic surveillance |
|                           | NTRK1-3           | < 1                    | Gene fusions        | Predictive               |
|                           | MSI               | < 2                    | LOF                 | Predictive               |

The percentages quoted are estimated from the literature cited in the paper. Diagnostic role appears mainly in preoperative material. In bold those markers recommended for clinical practice by National Comprehensive Cancer Network 2019 guidelines. PDAC: Pancreatic ductal adenocarcinoma; LOH: Loss of heterozygosity.

PDAC

Wide genome analysis

According to data obtained by whole-exome sequencing analysis, PDAC harbors an average of about 60 genetic alterations, and most of them are point mutations[1,8,13]. Based on these alterations, 12 cellular pathways genetically altered in pancreatic neoplasia have been identified[8]. Massive sequencing studies carried out on PDACs revealed that alterations may be found in some well-known genes (e.g., KRAS, CDKN2A, TP53, ARID1A, SMAD4) or in novel genes, that may be involved in DNA damage repair (e.g., ATM), chromatin modification (e.g., EP301 and ARID2), or in neoplastic carcinogenesis (e.g., KDM6A and PREX2)[14]. Whole-exome sequencing analysis has defined some putative therapeutic targets (e.g., RBM10) associated with longer survival in patients with pancreatic cancers, others associated with improved survival (e.g., KRAS p.Q61H mutation), and others defining sensitivity to target therapies in PDAC models (e.g., BRAF mutations as sensitivity markers for treatment with vemurafenib)[15].

An expression analysis study led to the cluster of PDAC in 4 different subtypes: (1) Squamous; (2) Pancreatic progenitor; (3) Immunogenic; and (4) Aberrantly differentiated endocrine exocrine[16]. Also analysis of the structural genomic alterations in PDACs have been classified into four different subtypes: (1) “Stable”, when PDACs contain less than 50 structural variations; (2) “Locally rearranged”, when PDACs exhibit a focal event on one or two chromosomes; (3) “Scattered subtype”, when PDACs show a fewer number of chromosomal damages and less than 200 structural variations; and (4) “Unstable”, when PDACs exhibit more than 200 structural events[17].

KRAS

KRAS is the most frequently mutated oncogene in pancreatic cancers (> 95%) and the most frequent gene mutated in PDAC (from 70% to 95%)[8,15,18,19]. The acquisition of a KRAS mutation represents an early and initiating event in PDACs. However, the low frequency of progression of precursor lesions to PDAC suggests that additional alterations are needed for neoplastic progression[20]. In PDAC, the mutations in KRAS are not located only in exon 2, but they have also been found in other exons[14,15,21]. However, the mutations harbored in exon 2 exhibited a similar association with survival, while cases mutated in exon 3 seem to have a remarkably favorable prognosis[15]. Coexistent KRAS mutations were detected in the same pancreatic neoplastic mass more frequently than in other tumors[21-23].

KRAS analysis may be of particular interest in the case of doubtful or inconclusive diagnoses (e.g., specimens with cytological atypia or acellular specimens). It is well-established that cytopathology together with KRAS analysis allows improved diagnosis of PDAC in endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) specimens[24-31]. Finding a KRAS mutation in EUS-FNA material may: (1) Indicate that a re-evaluation of the cytopathology report is needed (mainly if doubtful); (2) Indicate a second FNA or surgery[21]; and (3) Lead to a reduction in
false-negative diagnoses[32].

A worse prognosis has been observed in patients with tumors harboring coexistent KRAS mutations together with TP53 alterations and/or loss of SMAD4 protein[33,34]. KRAS p.G12C variant is a mutation that could be targeted by KRAS-G12C-specific inhibitors[35]. PDAC which also harbors the p.G12C alteration may benefit from this treatment. KRAS-wild type PDACs represent a distinct molecular subtype of pancreatic cancer that could benefit from tailored treatments, including BRAF antagonists and MAPK inhibitors[36].

**TP53**

EUS-FNA sensitivity in the diagnosis of pancreatic malignant lesions can be improved by also implementing the evaluation of the TP53 gene[37-39]. P53 protein overexpression has been detected in specimens with pancreatic cancer but not in those with chronic pancreatitis. TP53 alterations have been detected in 50%-75% of PDACs. Combining the evaluation of p53 protein expression with histological examination improves the sensitivity of diagnosis of pancreatic cancers, with a high specificity [37,38]. The sensitivity of EUS-FNA in the diagnosis of PDAC is further improved by combining p53 and Ki67 staining[38].

The combination of p53 and CA19.9 increases the sensitivity of cytology but it can negatively affect the specificity[39]. A worse patient prognosis has been correlated with loss of p53 protein, mainly if p53 loss is combined with KRAS alterations and loss of SMAD4 protein expression[33,34]. In a recent study based on next-generation sequencing (NGS) results from the CONKO-001 phase III trial, it has been demonstrated that patients with TP53 mutated PDAC tumors benefit from adjuvant gemcitabine treatment[40].

**SMAD4**

Approximately 50% of pancreatic cancers show SMAD4 protein inactivation, due to homozygous deletion and intragenic mutations[10,41-43]. Loss of SMAD4 generally occurs late in pancreatic carcinogenesis, and it has been frequently observed in pancreatic adenocarcinomas, but not in extra-pancreatic lesions[44]. In reactive and inflammatory diseases of the pancreas (e.g., chronic pancreatitis) SMAD4 activity is usually preserved. Loss of SMAD4 protein has also been linked with an increased risk of developing metastases and worse prognosis[33,34,43,45,46]. In PDAC, SMAD4 mutations lead to the prevention of the normal transduction of TGF-β signals; TGF-β inhibitor has shown efficacy in a preclinical investigation[47].

A study by Hsieh et al[48] demonstrated that SMAD4 deficiency in PDAC cells led to higher sensitivity to gemcitabine. On the contrary, SMAD4 mutated cells were sensitive to gemcitabine similar to that in cells with wild-type SMAD4[48]. These data suggest that the SMAD4 copy number may be a therapeutic marker for PDAC treatment with gemcitabine[49]. In this study, it was observed that SMAD4 deficiency led to upregulation of cell cycle-related genes, such as CDKI, with consequent higher sensitivity to other agents modulating the cell cycle such as clofarabine, cytarabine, darinaparsin, and olaparib[48,49].

**CDKN2A**

More than 95% of sporadic pancreatic carcinomas harbor a CDKN2A gene inactivation due to intragenic mutation, usually coupled with the loss of the other allele, promoter hypermethylation, or homozygous deletion of both alleles[50-52]. CDKN2A is involved in familial pancreatic cancer, although CDKN2A germline mutations in patients with pancreatic cancer occur rarely (0.6%) [53,54]. Patients harboring CDKN2A mutations are more likely to report a family history of pancreatic cancer than those without CDKN2A alterations[53]. Surveillance protocols from age 40 are recommended for CDKN2A mutation carriers[55].

Decreased expression of p16 protein has been associated with a tendency for the tumor to be larger than in those with normal p16 expression levels. Pancreatic neoplasms with loss of p16 expression, due to mutations and/or promoter hypermethylation, are significantly larger. Moreover, the survival period in these patients was significantly shorter when compared to those with a pancreatic tumor characterized by intact p16 functions[33,56,57]. CDK4/6 is a potential target in CDKN2A-deficient tumors and the efficacy of CDK4 inhibitors has been confirmed in PDAC preclinical models[58].

**BRCA1-2**

Alterations in the BRCA pathway and defects in DNA maintenance (such as genomic
instability and the BRCA mutational signature) may have implications for the therapeutic selection of patients with pancreatic tumors[17]. BRCA1/2 mutation frequencies range from 5% to 10%, and BRCA alterations have been detected both in sporadic and familial PDAC[1,59]. Patients with PDAC harboring germline BRCA1 or BRCA2 mutations showed a longer progression-free survival if treated with a PARP inhibitor[60,61]. According to the recommendation from the International Cancer of the Pancreas Screening Consortium, for BRCA1 a consensus of 69.9% was reached for recommending that BRCA1 mutation carriers undergo surveillance, whereas no consensus was reached on family history criteria for BRCA1 mutation carriers[55]. For carriers of BRCA2 mutations, the consensus (agreement of 93%) was to recommend surveillance for mutation carriers who have a blood relative with pancreatic cancer[55]. For BRCA2 mutation carriers with a germline variant (deleterious), the recommended age to initiate surveillance is generally 50 years[55]. With regard to the surveillance protocols for high-risk individuals, the consensus is that pancreatic imaging with magnetic resonance imaging (MRI)/magnetic retrograde cholangiopancreatography and/or EUS should be the first-line test for pancreatic surveillance[55]. Pancreatic computed tomography is reserved for individuals unable to undergo MRI or EUS[55].

Other alterations

Even if uncommon, MSI/dMMR (Microsatellite Instability/defective DNA mismatch repair) has been described in about 1%-2% of PDAC[62,63]. PDACs with MSI are usually associated with medullary histology and are rarely mutated in KRAS or TP53 genes[62,64]. National Comprehensive Cancer Network (NCCN) guidelines recommend the MSI and MMR tests in locally advanced and metastatic pancreatic carcinomas[65]. NCCN recommends the treatment of PDAC with the PD-1 (programmed cell death-1) inhibitor, Pembrolizumab, only in those patients with MSI-H (high-MSI) or dMMR advanced/metastatic pancreatic cancer[65].

BRAF mutations are an uncommon event in PDAC[1]. Some evidence suggests that patients with pancreas tumors harboring BRAFV600E mutations may benefit from treatment with RAF-MEK-targeted therapy[66].

The MGMT promoter can be hypermethylated in PDAC[34,67]. In 1998, treatment with temozolomide in advanced pancreatic cancer has been tried in a phase II study, but no relevant clinical response was observed[68]. ARID1A oncosuppressor protein deficiency was significantly associated with poor outcome in PDAC patients[15]. Amplifications and copy-number gains of oncogenes such as ERBB2, MET, and FGFR1 may be detected in pancreatic tumors[17]. The inactivation of several genes (e.g., ROBO1, ROBO2, SLIT2, and RNF43) leads to an aberrant WNT (Wingless-related integration site) signaling[15,17]. Unresectable non-metastatic pancreatic carcinomas may also harbor mutations in GRM8 and TRIM33 genes[69], while only a small fraction (approximately 5%) of pancreatic adenocarcinomas showed Cyclin E overexpression[70]. Different to solid pseudopapillary neoplasia (SPN), CTNNB1 mutations are uncommon in PDAC[15,71]. In PDAC, the frequency of NTRK (neurotrophic tropomyosin receptor kinase) fusion is very low (about 0.3%), but clinical trials revealed that the selective TRK (tropomyosin receptor kinase) inhibitors are also effective in PDAC harboring NTRK rearrangement[58]. NCCN guidelines suggest the use of a TRK inhibitor in NTRK gene fusion-positive advanced/metastatic pancreatic cancer[61,65].

Organoids are a preclinical model that is becoming increasingly important for studying tumor behavior because they can simulate metastases, microtumors, and the tumor microenvironment better than “classical” monolayer culture systems[72]. An interesting preclinical model for the study of PDAC is the set-up of three-dimensional (3D)-tumoroids in vitro culture systems[72-78]. These organoids can also be generated from a resected PDAC and are amenable to therapeutic screening as well as genetic and biochemical perturbation[77]. In a study by Boj et al[76], organoids derived from murine and human PDAC generated lesions similar to pancreatic intraepithelial neoplasia (PanIN) and progressed to invasive PDAC. Intriguingly, the expression of mutated KRAS protein (KRASG12D) in PDAC organoids was sufficient to induce a preinvasive neoplasm[76]. PDAC tumoroid cultures retain the capacity to maintain tumor stroma and characteristics of the primary tumor including the long-term (> 44 d) production of CEA (carcinoembryonic antigen) and CA19-9 (carbohydrate antigen 19-9)[74]. This 3D cell culture model of PDAC would help the diagnostics, investigation of genetic drivers, and identification of novel therapeutic targets[72]. Moreover, this culture could also allow clarification on how the immunosuppressive mechanism affects the growth and stasis of tumors[74]. Moreover, another important aspect is that organoids are suitable for storage in biobanks and used for further
research, ensuring access to relevant sample numbers[78].

### CYSTIC PANCREATIC TUMORS

#### IPMN

KRAS mutations are harbored by over 90% of low-grade PanIN[79], and mutant KRAS is sufficient to initiate the development of PanINs and IPMNs[20,80-83] (Table 2).

The distinction between IPMNs and mucinous cystic neoplasms (MCNs) from non-neoplastic pancreatic cysts may be helped by analysis of pancreatic cyst fluid. In fact, a KRAS mutation is highly specific for mucinous differentiation, but not for identifying MCNs[84]. KRAS alterations have a very high specificity but low sensitivity for MCNs and IPMNs (approximately 15% and 70%, respectively). If KRAS analysis is combined with that of GNAS, the sensitivity increases[85,86]. The differential diagnosis of cystic mucinous lesions (IPMN and MCN), mainly when the pre-operative cytology is non-diagnostic or when the CEA cyst fluid levels are indeterminate, may be helped by the analysis of KRAS and Loss-of-Heterozygosity (LOH)[87].

IPMNs rarely harbor mutations in the TP53 gene (approximately 10%). The overexpression of TP53 was more commonly observed in IPMNs of the pancreaticobiliary type with invasion[88]. In a cohort of IPMN patients, the overexpression of TP53, together with loss of function of SMAD4, was strongly associated with patient survival[88]. In IPMNs, SMAD4 loss of function was rarely detected; SMAD4 loss is more common in IPMNs of the pancreaticobiliary type with invasion[88].

The GNAS gene encodes the α-subunit of the stimulatory G-protein (Gas). This subunit regulates the adenylate cyclase activity through Gas-coupled receptors. Alterations in GNAS may determine the characteristic IPMN phenotype[89]. GNAS activating mutations are reported prevalently in IPMN (approximately 40%-60%) [85,88,90-92] and invasive pancreatic cancers, only if arising in association with an IPMNN[79,93]. In the majority of IPMNs (approximately 90%), at least one of the KRAS or GNAS genes harbor mutations[94], and in about half of IPMN (approximately 40%), a GNAS alteration coexists with a KRAS mutation[90]. The combination of KRAS and GNAS mutations helps in distinguishing between a serous cystic neoplasm (SCN) and an IPMN with high sensitivity and specificity. In fact, if most IPMNs have a GNAS and/or a KRAS alteration, no SCNs harbor either mutation. Besides, detecting a mutation in the GNAS gene in cyst fluid may help to distinguish IPMNs from MCNs[95].

RNF43 mutation frequency in IPMN is about 25%, ranging from 10% to 75%[93,96]. These mutations are often inactivating alterations and are found in association with LOH.

CDKN2A/p16 inactivating mutations have also been found in IPMNs with high-grade dysplasia, other than in pancreatic adenocarcinoma[97].

Germline mutations of STK11/LKB1 genes have been associated with IPMNs and invasive pancreatic cancer[98,99]. Besides, somatic mutations of STK11/LKB1 are observed in about 5% of patients with sporadic IPMNs and pancreatic cancers[98,99].

A high amount of DNA and high-amplitude mutations in the pancreatic cyst fluid may be indicators of malignancy, helping to identify malignant cystic lesions[86].

The use of NGS in pancreatic cyst fluid allows high sensitivity and specificity in classifying pancreatic cancers, mainly for the diagnosis of IPMN with advanced neoplasia[100].

#### MCN

MCNs harbor alterations also commonly found in PDAC. MCNs frequently have KRAS gene mutations, mainly in MCNs with high-grade dysplasia[101]. The diagnosis of mucinous cysts may be helped by the presence of a KRAS mutation in cyst fluid[86]. p16/CDKN2A expression is altered in MCN, even if in a lower percentage (about 15%) if compared to that of PDAC[102]. P53 has been reported with aberrant expression in MCN with high-grade dysplasia[103]. TP53 alterations are often associated with aggressiveness and seem to be involved in progression to PDAC[92].

Mutations of RNF43 have been reported in MCN[92]. PIK3CA alterations were described with very low frequency in MCN and in association with high-grade dysplasia and invasive adenocarcinoma[104]. As in IPMN, no VHL mutations were detected in MCN[52], but, different to IPMN, the GNAS gene is not mutated in MCN[90]. The LOH of Dpc4/Smad4 contributes to MCN progression in mice with KRAS-G12D mutation[105], confirming that the SMAD4 gene acts as a PDAC tumor suppressor[106]. Whole-exome sequencing performed on a cohort of MCN revealed...
### OTHER PANCREATIC TUMORS

#### SPN

The CTNNB1 gene (codons from 32 to 37) encodes for a region that plays a crucial role in the regulation of the β-catenin protein[110,111]. Alterations within this CTNNB1 region usually block β-catenin phosphorylation, inhibiting degradation of the protein[112]. CTNNB1 mutations are characteristic of pancreatic SPN[71,113,114]. Different to PDAC, SPNs are not mutated in KRAS, TP53, or SMAD4 genes, and CTNNB1 mutations are the main molecular alteration detected[92]. DNA array CGH (comparative genomic hybridization) performed on a pediatric case of SPN revealed a loss in chromosome band 11p15.5, a chromosomal region encoding for the HRAS gene[115]. As suggested by Selenica et al[116], even if inhibition of the Wnt pathway may be an intuitive therapeutic option for this disease, the evidence that clinically advanced Wnt pathway inhibitors target components upstreaming β-catenin activity is a clear limitation. For this reason, future drugs should be designed to target the β-catenin protein directly[116] (Table 3).

#### Pancreatic neuroendocrine tumors

Pancreatic neuroendocrine tumors (PanNETs) show a distinct landscape of molecular alterations if compared to the other pancreatic tumors. The mutation frequency in PanNET was lower than that observed in poorly differentiated neuroendocrine carcinomas (45% and 83%, respectively)[117], and the incidence of mutations was higher in PanNET with a high risk of progression than those with low risk[117]. MEN1 alterations (both mutations and LOH) have been found in up to 70% of PanNETs and have been associated with a better prognosis[118,119]. PanNETs harbor alterations in those genes involved in the chromatin remodeling complex, such as loss of ATRX and DAXX proteins expression[118]. Mutations in the mTOR (mammalian target of rapamycin) pathway genes (e.g., PIK3CA, PTEN, and TSC2) have been detected in that the tumors harbored about 16 somatic mutations per tumor, lower than the number of mutations observed in IPMN (about 27 per tumor)[92].

### SCN

VHL mutations are frequently reported in SCN (from 40% up to 60% of cases) [100,107]. SCNs usually do not harbor alterations in genes frequently mutated in IPMN, or MCN (i.e., KRAS, GNAS, TP53), helping to distinguish SCNs from the other mucinous neoplasia[100,108]. The lack of CTNNB1 mutations allows the differentiation of SCN from SPN. Moreover, SCNs do not harbor mutations in genes frequently altered in neuroendocrine pancreatic tumors[109]. Whole exome sequencing analysis performed on a cohort of eight SCNs revealed that almost all tumors harbored a LOH on chromosome 3p[92]. An average of only 10 non-synonymous somatic mutations was detected in SCNs[92], far less than the average observed in PDAC.

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Table 2 Main genetic alterations detectable in cystic pancreatic lesions

| Type of pancreatic lesion | Genetic alteration | Reported frequency (%) | Type of alterations | Role in clinical practice |
|---------------------------|--------------------|------------------------|---------------------|--------------------------|
| IPMN                      | KRAS               | 90                     | Point mutations     | Diagnostic               |
|                           | TP53               | 10                     | Point mutations/LOH | Prognostic               |
|                           | GNAS               | 40-60                  | Point mutations     | Diagnostic               |
| MCN                       | RNF43              | 25                     | Point mutations/LOH | Diagnostic               |
|                           | KRAS               | 0-25% (LG); 50-90% (HG)| Point mutations     | Diagnostic               |
|                           | CDKN2A/p16         | 0-10 (LG); 50 (HG)     | Point mutations/LOH | Prognostic               |
|                           | TP53               | 0 (LG); 20-50 (HG)     | Point mutations/LOH | Prognostic               |
| SCN                       | VHL                | 40-60                  | Point mutations/LOH | Diagnostic               |

The percentages quoted are estimated from the literature cited in the paper. Diagnostic role appears mainly in preoperative material. IPMN: Intraductal papillary mucinous neoplasms; MCN: Mucinous cystic neoplasia; LG: Low-grade mucinous cystic neoplasia; HG: High-grade mucinous cystic neoplasia; SCN: Serous cystic neoplasm; LOH: Loss of heterozygosity.
Table 3 Main genetic alterations detectable in other pancreatic tumors

| Type of pancreatic lesion | Genetic alteration | Reported frequency (%) | Type of alterations | Role in clinical practice |
|---------------------------|--------------------|------------------------|--------------------|--------------------------|
| SPN                       | CTNNB1             | 90-100                 | Point mutations    | Diagnostic               |
| PanNET                    | MEN1               | 70                     | Point mutations/LOH| Diagnostic/prognostic    |
|                           | VHL                | 25                     | Point mutations/LOH| Diagnostic               |
| AAC                       | CTNNB1             | 5-25                   | Point mutations/LOF| Diagnostic               |
|                           | MSI                | 5-15                   | LOF                | Predictive?              |

The percentages quoted are estimated from the literature cited in the paper. Diagnostic role appears mainly in preoperative material. SPN: Solid pseudopapillary neoplasm; PanNET: Pancreatic neuroendocrine tumor; AAC: Acinic adenocarcinoma; LOH: Loss of heterozygosity; LOF: Loss of function.

PanNET[118]. For example, PTEN and TSC2 genes have been observed as inactivating in primary PanNET and their low protein levels were associated with shorter overall and disease-free survival[120]. VHL inactivation, due to deletion or methylation, was also observed in up to 25% of PanNETs[121,122].

**Acinar cell carcinomas**

Activating mutations in CTNNB1 and inactivating mutations in APC genes have been observed in up to 25% of acinar cell carcinomas (ACCs). KRAS mutations are an uncommon event in this type of neoplasia[123]. Nevertheless, TP53 mutations were also found only in a low fraction of ACCs[123-125], deletion of the TP53 region (chromosome band 17p13.1) was detected by FISH in about half of a cohort of 54 ACCs[125]. Alterations found in IPMN (e.g., GNAS and RNF43) or in PanNET (e.g., MEN1) were rarely found in ACC[126]. A genome-wide analysis performed on ACCs revealed a median number of 137 point mutations per tumor and COL12A1, FRY, FRYL, and PLB1 were the most frequently mutated genes[124]. Intriguingly, a genome-wide analysis identified no recurrent point mutations in ACCs[124]. Microsatellite instability was detected in a fraction of ACC ranging from 7% to 14% of cases[127]. ACC with MSI did not exhibit distinct morphological or clinical features[128].

**CONCLUSION**

To date, there are no targetable molecules available for personalized patient treatment of pancreatic tumors in clinical practice, as stated by the current European Society for Medical Oncology (ESMO) guidelines[129]. EUS-FNA has improved pre-operative diagnosis[130-132], but the management of patients with a pancreatic lesion is still challenging. In fact, in a subset of cases, such as lesions with atypical/suspicious cytopathologic features, the pre-operative diagnosis remains inconclusive[133]. Performing a molecular characterization could help mainly in solving these “inconclusive” specimens. The introduction of multi-gene analysis approaches, such as NGS, has provided a lot of useful information regarding the molecular characterization of pancreatic tumors[134]. EUS-FNAC (FNA cytology) is a useful diagnostic tool for pancreatic lesions[135] and molecular analysis can be successfully performed on cytological smears[21]. However, it has becoming increasingly crucial to have sufficient material for histological, immunohistochemical, and molecular characterization. Pancreatic FNAB (FNA biopsy) provides enough material to allow proper histological assessment, immunostaining, and molecular techniques [e.g., NGS, digital polymerase chain reaction (PCR)][136-138].

A huge amount of data needs to be properly managed to determine the information that is useful and correct[139]. The recent ESMO guidelines have outlined their indications for the use of NGS in the characterization of metastatic cancers. As regards PDAC, it is not currently recommended to perform multigene NGS in daily practice[140]. However, ESMO encourages multigene sequencing in order to get access to innovative drugs. Moreover, NGS can be an alternative technique to PCR-based assays if it is not associated with extra cost for the public health care system if the patient is informed about the putative benefits of this analysis[140]. In conclusion, a deeper knowledge of the molecular alterations characterizing pancreatic neoplasms may lead to new potential therapeutic targets for these tumors.
REFERENCES

1 Cancer Genome Atlas Research Network. Integrated Genomic Characterization of Pancreatic Ductal Adenocarcinoma. *Cancer Cell* 2017; 32: 185-203. e13 [PMID: 28810144 DOI: 10.1016/j.ccell.2017.07.007]

2 Layfield LJ, Elhay H, Filipe AC, Hruban RH, Jhala N, Joseph L, Vieth P, Pitman MB. Papanicolaou Society of Cytopathology. Utilization of ancillary studies in the cytologic diagnosis of biliary and pancreatic lesions: the Papanicolaou Society of Cytopathology guidelines for panreatobiliary cytology. *Diagn Cytopathol* 2014; 42: 351-362 [PMID: 24639388 DOI: 10.1002/dc.23093]

3 Lüttges J, Reinecke-Lüttge A, Möllmann B, Menke MA, Clemens A, Klöppel G. Duct changes and K-ras mutations in the disease-free pancreas: analysis of type, age relation and spatial distribution. *Virchows Arch* 1999; 435: 461-468 [PMID: 10592048 DOI: 10.1007/s004280050428]

4 Löhr M, Klöppel G, Maisonneuve P, Lowenfels AB, Lüttges J. Frequency of K-ras mutations in pancreatic intraductal neoplasias associated with pancreatic ductal adencarcinoma and chronic pancreatitis: a meta-analysis. *Neoplasia* 2005; 7: 17-25 [PMID: 15720814 DOI: 10.1593/neo.044445]

5 Löhr M, Maisonneuve P, Lowenfels AB. K-Ras mutations and benign pancreatic disease. *Int J Pancreatol* 2000; 27: 93-103 [PMID: 10862508 DOI: 10.1038/ijgc.27.2:093]

6 Vogelstein B, Kinzler KW. Cancer genes and the pathways they control. *Nat Med* 2004; 10: 789-799 [PMID: 15258780 DOI: 10.1038/nm1087]

7 Forbes SA, Beare D, Gunasekaran P, Leung K, Bindal N, Boutsalakis H, Ding M, Barmford S, Cole C, Ward S, Kok CY, Jia M, De T, Teague JW, Stratton MR, McDermott U, Campbell PJ. COSMIC: exploring the world's knowledge of somatic mutations in human cancer. *Nucleic Acids Res* 2015; 43: D860-D861 [PMID: 25355519 DOI: 10.1093/nar/gku1075]

8 Jones S, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Kamiyama H, Jimeno A, Hong SM, Fu B, Lin MT, Calhoun ES, Kamiyama M, Walter K, Nikoloskaya T, Nikolsky Y, Hartigan J, Smith DR, Hidalgo M, Leach SD, Klein AP, Jaffee EM, Goggins M, Maitra A, Iacobuzio-Donahue C, Kern SE, Hruban RH, Rabinovitch PS, Pappadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE, Kinzler KW. Core signaling pathways in virtually all pancreatic carcinomas. *Science* 2008; 321: 1801-1806 [PMID: 18772397 DOI: 10.1126/science.1164368]

9 Scarpà A, Capelli P, Mukai K, Zamboni G, Oda T, Iacono C, Hirohashi S. Pancreatic adenocarcinomas frequently show p53 gene mutations. *Am J Pathol* 1993; 142: 1534-1543 [PMID: 8494051]

10 Maitra A, Hruban RH. Pancreatic cancer. *Annu Rev Pathol* 2008; 3: 157-188 [PMID: 18039136 DOI: 10.1146/annurev.pathmechdis.3.121806.154305]

11 Schutte M, Hruban RH, Geradts J, Maynard R, Hilgers WR, Rabindran SK, Moskaluk CA, Hahn SA, Schwarte-Waldhoff I, Schmiegel W, Baylin SB, Klein AP, Jaffee EM, Goggins M, Maitra A, Iacobuzio-Donahue C, Kern SE, Herman JG. Abrogation of the Rb/p16 tumor-suppressive pathway in virtually all pancreatic carcinomas. *Cancer Res* 1997; 57: 3126-3130 [PMID: 9242437]

12 Siegel PM, Massagué J. Cytostatic and apoptotic actions of TGF-beta in homeostasis and cancer. *Nat Rev Cancer* 2003; 3: 807-821 [PMID: 14558717 DOI: 10.1038/nrc1208]

13 Wang L, Tsutsumi S, Kagawauchi T, Nagasaki K, Tatsuno K, Yamamoto S, Sang F, Sonoda K, Sugawara M, Sairau A, Hiroso S, Yamaue H, Miki Y, Iacono C, Hirohashi S. Pancreatic cancer. *Science* 2008; 312: 3126-3130 [PMID: 18212652 DOI: 10.1101/gr.123109.111]

14 Biankin AV, Waddell N, Kassahn KS, Gingras MC, Muthuswamy LB, Johns AL, Miller DK, Wilson PJ, Patch AM, Wu J, Chang DK, Cowley MJ, Gardiner BB, Song S, Harliwong I, Idrisoglu S, Geschwind D, Hruban RH, Jhala N, Joseph L, Vielh P, Pitman MB; Papanicolaou Society of Cytopathology guidelines for pancreatobiliary cytology. *Diagn Cytopathol* 2017; 45: 127-134 [PMID: 28343280 DOI: 10.1002/dc.23926]

15 Witkiewicz AK, McMillan EA, Balaji U, Baek G, Lin WC, Mansour J, Mollaei M, Wagner KU, Koduru P, Yopp A, Choti MA, Yeo CJ, McCue P, White MA, Knudsen ES. Whole-exome sequencing of pancreatic cancer defines genetic diversity and therapeutic targets. *Nat Commun* 2015;
Monteiro da Cunha JE, César Machado MC, Ishioka S, Forero E. Kras mutation analysis of fine needle aspiration biopsy coupled with a KRAS mutation assay using allelic discrimination improves the diagnosis of pancreatic cancer. *Nature* 2016; 531: 47-52 [PMID: 26909576 DOI: 10.1038/nature16965]

Waddell N, Pajic M, Patch AM, Chang DK, Kassahn KS, Bailey P, Johns AL, Miller D, Nones K, Quck K, Quinn MC, Robertson AJ, Fadullah MZ, Bruxner TJ, Christ AN, Harliwong I, Idrisoglu S, Manning S, Nurse C, Nourbakhsh E, Wani S, Holmes O, Holmes C, Anderson MJ, Kazakov S, Leonard C, Newell F, Waddell N, Wood S, Xu Q, Wilson PJ, Cloonan N, Kassahn KS, Taylor D, Quck K, Robertson A, Pantano L, Mincarelli L, Sanchez LN, Evers L, Wu J, Finese M, Cowley MJ, Jones MD, Colvin EK, Nagrial AM, Humphrey ES, Chantrill LA, Mawson A, Humphris J, Chou A, Pajic M, Scarlett CJ, Pinho AV, Giry-Laterriere M, Rooman I, Samra JS, Kench JG, Lovell JA, Merrett ND, Toon CW, Epari K, Nguyen NQ, Barbour A, Zeps N, Moran-Jones K, Jamieson NB, Graham JS, Duthie F, Oien K, Her J, Grützmann R, Maitra A, Iacobuzio-Donahue CA, Wolfgang CL, Morgan RA, Lawlor RT, Corbo V, Bassi C, Rusev B, Capelli P, Salvia R, Tortora G, Mukhopadhyay D, Petersen GM. Australian Pancreatic Cancer Genome Initiative, Munzy DM, Fisher WE, Karim SA, Eshleman JR, Hruban RH, Pilarsky C, Morton JP, Sansom OJ, Scarpa A, Musgrove EA, Bailey UM, Hofmann O, Sutherland RL, Wheeler DA, Gill AJ, Gibbs RA, Pearson Jv, Bionk AV, Grimmond SM. Genomic analyses identify molecular subtypes of pancreatic cancer. *Nature* 2016; 531: 47-52 [PMID: 26909576 DOI: 10.1038/nature16965]
Visani M, Leary RJ, Eshleman JR, Goggins M, Jaffee EM, Iacobuzio-Donahue CA, Maitra A, Cameron JL, Blackford A, Schutte M, Tascilar M, Castells A, Ginés A, Solé M, Mora J, Castellví-Bel S, Rodríguez-Morant F, Fernández-Esparrach G, Llach J, Bordàs JM, Navarro S, Piqué JM. Clinical usefulness of KRAS mutational analysis in the diagnosis of pancreatic adenocarcinoma by means of endosonography-guided fine-needle aspiration biopsy. *Aliment Pharmacol Ther* 2003; 17: 1299-1307 [PMID: 12755843 DOI: 10.1046/j.1365-2036.2003.01579.x]
Visani M et al. Molecular alterations in pancreatic tumors

Olino K, Schulick R, Winter J, Herman JM, Laheru D, Klein AP, Vogelstein B, Kinzler KW, Velculescu VE, Hruban RH. SMAD4 gene mutations are associated with poor prognosis in pancreatic cancer. *Clin Cancer Res* 2009; 15: 4674-4679 [PMID: 19584151 DOI: 10.1158/1078-0432.CCR-09-0227]

46 Singh P, Srinivasan R, Wig JD. SMAD4 genetic alterations predict a worse prognosis in patients with pancreatic ductal adenocarcinoma. *Pancreas* 2012; 41: 541-546 [PMID: 22504380 DOI: 10.1097/MPA.0b013e318247da6a]

47 Shi L, Sheng J, Wang M, Luo H, Zhu J, Zhang B, Liu Z, Yang X. Combination Therapy of TGF-β Blockade and Commensal-derived Probiotics Provides Enhanced Antitumor Immune Response and Tumor Suppression. *Theranostics* 2019; 9: 4115-4129 [PMID: 31281535 DOI: 10.7150/thno.35131]

48 Hsieh YY, Liu TP, Chou CJ, Chen HY, Lee KH, Yang PM. Integration of Bioinformatics Resources Reveals the Therapeutic Benefits of Gemcitabine and Cell Cycle Intervention in SMAD4-Deleted Pancreatic Ductal Adenocarcinoma. *Genes* (Basel) 2019; 10 [PMID: 31569425 DOI: 10.3390/genes10100766]

49 Dardare J, Witz A, Merlin JL, Gilson P, Harlé A. SMAD4 and the TGFβ Pathway in Patients with Pancreatic Ductal Adenocarcinoma. *Int J Mol Sci* 2020; 21 [PMID: 32429474 DOI: 10.3390/ijms21103554]

50 Bartsch D, Shevlin DW, Tung WS, Kisker O, Wells SA Jr, Goodfellow PJ. Frequent mutations of CDKN2 in primary pancreatic adenocarcinomas. *Genes Chromosomes Cancer* 1995; 14: 189-195 [PMID: 8589035 DOI: 10.1002/gcc.2780140306]

51 Caldas C, Hahn SA, da Costa LT, Redston MS, Schutte M, Seymour AB, Weinstein CL, Hruban RH, Yeo CJ, Kern SE. Frequent somatic mutations and homozygous deletions of the p16 (MTS1) gene in pancreatic adenocarcinoma. *Nat Genet* 1994; 8: 27-32 [PMID: 7726912 DOI: 10.1038/ng0994-27]

52 Rozenblum E, Schutte M, Goggins M, Hahn SA, Panzer S, Zahurak M, Goodman SN, Sohn TA, Hruban RH, Yeo CJ, Kern SE. Tumor-suppressive pathways in pancreatic carcinoma. *Cancer Res* 1997; 57: 1731-1734 [PMID: 9135016]

53 Bartsch DK, Sina-Frey M, Lang S, Wild A, Gerdes B, Barth P, Kress R, Grützmacher R, Colombo-Benkmann M, Ziegler A, Hahn SA, Rothmund M, Rieder H. CDKN2A germline mutations in familial pancreatic cancer. *Ann Surg* 2002; 236: 730-737 [PMID: 12454511 DOI: 10.1097/00000658-200212000-00005]

54 McWilliams RR, Wieben ED, Rabe KG, Pedersen KS, Wu Y, Sicotte H, Petersen GM. Prevalence of CDKN2A mutations in pancreatic cancer patients: implications for genetic counseling. *Eur J Hum Genet* 2011; 19: 472-478 [PMID: 21150883 DOI: 10.1038/ejhg.2010.198]

55 Goggins M, Overbeek KA, Dikk V, Brand R, Syngal S, Del Chiaro M, Bartsch DK, Bassi C, Carrato A, Farrell J, Fishman EK, Fockens P, Gress TM, van Hooff JE, Hruban RH, Kastrinos F, Klein A, Lennom AM, Lucas A, Park W, Rustgi A, Simeone D, Stoffel E, Venet HFA, Cahen DL, Canto MI, Bruno M. International Cancer of the Pancreas Screening (CPS) consortium. Management of patients with increased risk for familial pancreatic cancer: updated recommendations from the International Cancer of the Pancreas Screening (CPS) Consortium. *Gut* 2020; 69: 7-17 [PMID: 31672839 DOI: 10.1136/gutjnl-2019-319352]

56 Ohtsubo K, Watanabe H, Yamaguchi Y, Hu YX, Motoo Y, Okai T, Sawabu N. Abnormalities of tumor suppressor gene p16 in pancreatic carcinoma: immunohistochemical and genetic findings compared with clinicopathological parameters. *J Gastroenterol* 2003; 38: 663-671 [PMID: 12898359 DOI: 10.1007/s00535-003-1119-6]

57 Gerdes B, Ramaswamy A, Ziegler A, Lang SA, Kersting M, Baumann R, Wild A, Moll R, Rothmund M, Bartsch DK. p16INK4a is a prognostic marker in resected ductal pancreatic cancer: an analysis of p16INK4a, p53, MDM2, and an Rb. *Ann Surg* 2002; 235: 51-59 [PMID: 11753042 DOI: 10.1097/00000658-200201000-00007]

58 Qian Y, Gong Y, Fan Z, Luo G, Huang Q, Deng S, Cheng H, Jin K, Ni Q, Yu X, Liu C. Molecular alterations and targeted therapy in pancreatic ductal adenocarcinoma. *J Hematol Oncol* 2020; 13 [PMID: 33004326 DOI: 10.1186/s13045-020-00958-3]

59 Wong W, Raufl AG, Safyan RA, Bates SE, Manji GA. BRCA Mutations in Pancreas Cancer: Spectrum, Current Management, Challenges and Future Prospects. *Cancer Manag Res* 2020; 12: 2731-2742 [PMID: 32368150 DOI: 10.2147/CMAR.S211151]

60 Golan T, Hammel P, Renni M, Van Cutsem E, Macarulla T, Hall MJ, Park JO, Hochhauser D, Arnold D, Oh DY, Reinaicher-Schick A, Tortora G, Algil H, O'Reilly EM, McGuinness D, Cui KY, Schlienger K, Locker GY, Kindler HL. Maintenance Olaparib for Germline BRCA Mutations in Pancreatic Cancer. *Clin Cancer Res* 2020; 26: 3179-3188 [PMID: 32755482 DOI: 10.1158/1078-0432.CCR-20-01364]

61 Sohal DPS, Kennedy EB, Cinar P, Conroy T, Copray MS, Crane CH, Garrido-Laguna I, Lau MW, Johnson T, Krishnamurthi S, Moravek C, O'Reilly EM, Philip PA, Pant S, Shah MA, Sahai V, Urenos HE, Zaidi N, Laheru D. Metastatic Pancreatic Cancer: ASCO Guideline Update. *J Clin Oncol* 2020; 38: 2301-2314 [PMID: 32157965 DOI: 10.1200/JCO.20.01387]

62 Lucchini C, Brosens LA, Wood LD, Chatterjee D, Shin JI, Sciammarella C, Fiadone G, Malleo G, Salvia R, Kryklyva V, Piredda ML, Cheng L, Lawlor RT, Adsay V, Scarpa A. Comprehensive characterisation of pancreatic ductal adenocarcinoma with microsatellite instability: histology, molecular pathology and clinical implications. *Gut* 2021; 70: 148-156 [PMID: 32350089 DOI: 10.1136/gutjnl-2020-320726]
Molecular alterations in pancreatic tumors

Visani M et al.

63 Hu ZI, Shia J, Stadler ZK, Varghese AM, Capano M, Salo-Mullen E, Lowery MA, Diaz LA Jr, Mandelker D, Yu KH, Zervoudakis A, Kelsen DP, Iacobuzio-Donahue CA, Klimstra DS, Saltz LB, Sahin IH, O'Reilly EM. Evaluating Mismatch Repair Deficiency in Pancreatic Adenocarcinoma: Challenges and Recommendations. *Clin Cancer Res* 2018; 24: 1326-1336 [PMID: 29367431 DOI: 10.1158/1078-0432.CCR-17-3099]

64 Bazzichetto C, Luchini C, Consentori F, Vaccaro V, Di Cello I, Mattioli P, Falcone I, Ferretti G, Scarpa A, Cognetti F, Milella M. Morphologic and Molecular Landscape of Pancreatic Cancer Variants as the Basis of New Therapeutic Strategies for Precision Oncology. *Int J Mol Sci* 2020; 21 [PMID: 33266496 DOI: 10.3390/ijms21228841]

65 National Comprehensive Cancer Network®. NCCN guidelines for patients: Pancreatic Cancer. National Comprehensive Cancer Network®, 2019

66 Pishvaian MJ, Blais EM, Brody JR, Lyons E, DeArbeloa P, Hendifar A, Mikhail S, Chung V, Sahai V, Sohal DPS, Bellakisha S, Thach D, Rahib L, Madhavan S, Matrisian LM, Petricoin EF 3rd. Overall survival in patients with pancreatic cancer receiving matched therapies following molecular profiling: a retrospective analysis of the Know Your Tumor registry trial. *Lancet Oncol* 2020; 21: 508-518 [PMID: 32135508 DOI: 10.1016/S1470-2045(20)30074-7]

67 Peng DF, Kanai Y, Sawada M, Ushijima S, Hiraoka N, Kitaizawa S, Hirohashi S. DNA methylation of multiple tumor-related genes in association with overexpression of DNA methyltransferase 1 (DNMT1) during multistage carcinogenesis of the pancreas. *Carcinogenesis* 2006; 27: 1160-1168 [PMID: 16535762 DOI: 10.1093/carcin/bgi361]

68 Moore MJ, Feld R, Hedley D, Oza A, Siu LL. A phase II study of temozolomide in advanced untreated pancreatic cancer. *Invest New Drugs* 1998; 16: 77-79 [PMID: 9740547 DOI: 10.1023/a:1006904333265]

69 Valero V 3rd, Saunders TJ, He J, Weiss MI, Cameron JL, Dholakia A, Wild AT, Shin EJ, Khshab MA, O'Brien-Lennon AM, Ali SZ, Laheru D, Hruban RH, Iacobuzio-Donahue CA, Herman JM, Wolfgang CL. Reliable Detection of Somatic Mutations in Fine Needle Aspirates of Pancreatic Cancer With Next-generation Sequencing: Implications for Multicenter Management. *Ann Surg* 2016; 263: 153-161 [PMID: 26020105 DOI: 10.1097/SLA.0000000000001156]

70 Calhoune ES, Jones JB, Ashfaq R, Adsay V, Baker SJ, Valentine V, Hempen PM, Hilgers W, Yeo CJ, Hruban RH, Kern SE. BRAF and FBXW7 (CDC4, FBW7, AGO, SEL11) mutations in distinct subsets of pancreatic cancer: potential therapeutic targets. *Am J Pathol* 2003; 163: 1255-1260 [PMID: 14507635 DOI: 10.1016/S0002-9440(10)34852-2]

71 Zeng G, Germinario M, Micsenyi A, Monga NK, Bell A, Sood A, Malhotra V, Sood N, Midda V, Monga DK, Kokkinakis DM, Monga SP. Aberrant Wnt/beta-catenin signaling in pancreatic adenocarcinoma. *Neoplasia* 2006; 8: 279-289 [PMID: 16756720 DOI: 10.1593/neo.050607]

72 Doctor A, Seifert V, Ullrich M, Hauser S, Pietzsch J. Three-Dimensional Cell Culture Systems in Radiopharmaceutical Cancer Research. *Cancers (Basel)* 2020; 12 [PMID: 32993034 DOI: 10.3390/cancers12102762]

73 Lee JH, Kim SK, Khawar IA, Jeong SY, Chung S, Kuh JJ. Microfluidic co-culture of pancreatic tumor spheroids with stellate cells as a novel 3D model for investigation of stroma-mediated cell motility and drug resistance. *J Exp Clin Cancer Res* 2018; 37: 4 [PMID: 29329547 DOI: 10.1186/s13046-017-0654-6]

74 Finnberg NK, Gokare P, Lev A, Grivennikov SI, MacFarlane AW 4th, Campbell KS, Winters RM, Kaputa K, Farma JM, Abbas AE, Grasso L, Nicolaides NC, El-Deiry WS. Application of 3D tumor spheroids with stellate cells as a novel 3D model for investigation of stroma-mediated cell motility and drug resistance. *J Exp Clin Cancer Res* 2018; 37: 4 [PMID: 29329547 DOI: 10.1186/s13046-017-0654-6]

75 Ehlen L, Arndt J, Treue D, Bischoff P, Loch FN, Hahn EM, Kotsch K, Klauschen F, Beyer K, Tschilgen S, Klimstra D, Kuhn R. 3D cell culture modeling of pancreatic cancer. *Clin Cancer Res* 2018; 24: 407-418 [PMID: 29692415 DOI: 10.1158/1078-0432.CCR-17-3099]

76 Boster J, Bester M, Hamburger A, Hwang CI, Baker LA, Chio II, Engle DD, Corbo V, Jager M, Ponz-Sarvise M, Tiriac H, Tuveson DA. Generation and Culture of Human Pancreatic Ductal Adenocarcinoma Organoids from Resected Tumor Specimens. *Methods Mol Biol* 2019; 1882: 97-115 [PMID: 30370407 DOI: 10.1007/978-1-4939-8879-2_9]

77 Drost J, Clevers H. Organoids in cancer research. *Nat Rev Cancer* 2018; 18: 407-418 [PMID: 29692415 DOI: 10.1038/s41568-018-0007-6]

78 Kanda M, Matthaei H, Wu J, Hong SM, Yu J, Borges M, Hruban RH, Maitra A, Kinzler K, Vogelstein B, Goggins M. Presence of somatic mutations in most early-stage pancreatic intraepithelial neoplasia. *Gastroenterology* 2012; 142: 730-733. e9 [PMID: 22226782 DOI: 10.1053/j.gastro.2011.12.042]

79 Guerra C, Schuhmacher AJ, Cañamero M, Grippo PJ, Verdaguer L, Pérez-Gallego L, Dubus P,
Visani M et al. Molecular alterations in pancreatic tumors

Sandgren EP, Barbacid M. Chronic pancreatitis is essential for induction of pancreatic ductal adenocarcinoma by K-Ras oncopgenes in adult mice. Cancer Cell 2007; 11: 291-302 [PMID: 17349585 DOI: 10.1016/j.ccr.2007.01.012]

81 Hingorani SR, Petricoin EF, Maitra A, Rajapakse V, King C, Jacobetz MA, Ross S, Conrads TP, Veenstra TD, Hitt BA, Kawaguchi Y, Johann D, Liotta LA, Crawford HC, Putt ME, Jacobs T, Wright CV, Hruban RH, Lowy AM, Tuveson DA. Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse. Cancer Cell 2003; 4: 437-450 [PMID: 14706336 DOI: 10.1016/s1535-6108(03)00309-x]

82 Pilyavea-Gupta Y, Grabocka E, Bar-Sagi D. RAS oncopgenes: weaving a tumorigenic web. Nat Rev Cancer 2011; 11: 761-774 [PMID: 21993244 DOI: 10.1038/nrc3106]

83 Seidler B, Schmidt A, Mayr U, Nakhai H, Schmid RM, Schneider G, Saur D. A Cre-loxP-based mouse model for conditional somatic gene expression and knockdown in vivo by using avian retroviral vectors. Proc Natl Acad Sci USA 2008; 105: 10137-10142 [PMID: 18621715 DOI: 10.1073/pnas.0710848105]

84 Nikiforova MN, Khalid A, Fasanella KE, McGrath KM, Brand RE, Chennat JS, Lu X, Papachristou GI, Slivka A, Zeh HJ, Zureikat AH, Krasingikas AM, Ohori NP, Schooedel KE, Navina S, Mantha GS, Pai RK, Singhii AD. Integration of KRAS testing in the diagnosis of pancreatic cystic lesions: a clinical experience of 618 pancreatic cysts. Mod Pathol 2013; 26: 1478-1487 [PMID: 23743931 DOI: 10.1038/modpathol.2013.91]

85 Molin MD, Matthaei H, Wu J, Blackford A, Debeljuk M, Rezaee N, Wolfgang CL, Butturini G, Salvia B, Bassi C, Goggins MG, Kinzler KW, Vogelstein B, Eshleman JR, Hruban RH, Maitra A. Clinicopathological correlates of activating GNAS mutations in intraductal papillary mucinous neoplasm (IPMN) of the pancreas. Ann Surg Oncol 2013; 20: 3802-3808 [PMID: 23846778 DOI: 10.1245/s10434-013-3096-1]

86 Khalid A, Zahid M, Finkelstein SD, LeBlanc JK, Kaushik N, Ahmad N, Brugge WR, Edmundowicz SA, Hawes RH, McGrath KM. Pancreatic cyst fluid DNA analysis in evaluating pancreatic cysts: a report of the PANDA study. Gastrointest Endosc 2009; 69: 1095-1102 [PMID: 19152896 DOI: 10.1016/j.gie.2008.07.033]

87 Al-Haddad M, DeWitt J, Sherman S, Schmidt CM, LeBlanc JK, McHenry L, Cote G, El Chafic AH, Luz L, Stuart JS, Johnson CS, Klocichan C, Imperiale TF. Performance characteristics of molecular (DNA) analysis for the diagnosis of mucinous pancreatic cysts. Gastroenterology 2014; 79: 79-87 [PMID: 23845445 DOI: 10.1016/j.gie.2013.05.026]

88 Kuboki Y, Shimizu K, Hatori T, Yamamoto M, Shibata N, Shiratori K, Furukawa T. Molecular biomarkers for progression of intraductal papillary mucinous neoplasm of the pancreas. Pancreas 2015; 44: 227-235 [PMID: 25423559 DOI: 10.1097/MPA.0000000000000253]

89 Komatsu H, Tanji E, Sakata N, Aoki T, Motoi F, Naitoh T, Katayose Y, Egawa S, Unno M, Furukawa T. A GNAS mutation found in pancreatic intraductal papillary mucinous neoplasms induces drastic alterations of gene expression profiles with upregulation of mucin genes. PLoS One 2014; 9: e87875 [PMID: 24498386 DOI: 10.1371/journal.pone.0087875]

90 Amato E, Molin MD, Mafficini A, Yu J, Malleo G, Rusev B, Fassan M, Antonello D, Sadakari Y, Castelli P, Zamboni G, Maitra A, Salvia R, Hruban RH, Bassi C, Capelli P, Lawlor RT, Goggins M, Scapar A. Targeted next-generation sequencing of cancer genes dissect the molecular profiles of intraductal papillary neoplasms of the pancreas. J Pathol 2014; 233: 217-227 [PMID: 24604757 DOI: 10.1002/path.4344]

91 Rosenbaum MW, Jones M, Dudley JC, Le LP, Iafraite AJ, Pitman MB. Next-generation sequencing adds value to the preoperative diagnosis of pancreatic cysts. Cancer Cytopathol 2017; 125: 41-47 [PMID: 27647802 DOI: 10.1002/ency.217775]

92 Wu J, Jiao Y, Dal Molin M, Maitra A, de Wilde RF, Wood LD, Esheleman JR, Goggins MG, Wolfgang CL, Canto MI, Schulick RD, Edil BH, Choti MA, Adsay V, Klimstra DS, Offerhaus GJ, Klein AP, Kopelowich L, Carter H, Karchin R, Allen PJ, Schmidt CM, Naoy T, Diaz LA Jr, Kinzler KW, Papadopoulous N, Hruban RH, Vogelstein B. Whole-exome sequencing of neoplastic cysts of the pancreas reveals recurrent mutations in components of ubiquitin-dependent pathways. Proc Natl Acad Sci USA 2011; 108: 21188-21193 [PMID: 22158988 DOI: 10.1073/pnas.1110846108]

93 Furukawa T, Kuboki Y, Tanji E, Yoshida S, Hatori T, Yamamoto M, Shibata N, Shimizu K, Kamatani N, Shiratori K. Whole-exome sequencing uncovers frequent GNAS mutations in intraductal papillary mucinous neoplasms of the pancreas. Sci Rep 2011; 1: 161 [PMID: 22355676 DOI: 10.1038/srep00161]

94 Singh AD, Nikiforova MN, Fasanella KE, McGrath KM, Pai RK, Ohori NP, Barthowoloul TL, Brand RE, Chennt JS, Lu X, Papachristou GI, Slivka A, Zeh HJ, Zureikat AH, Lee KK, Tsung A, Mantha GS, Khalid A. Preoperative GNAS and KRAS testing in the diagnosis of pancreatic mucinous cysts. Clin Cancer Res 2014; 20: 4381-4389 [PMID: 24938521 DOI: 11.1182/1078-0432.CCR-14-0515]

95 Wu J, Matthaei H, Maitra A, Dal Molin M, Wood LD, Esheleman JR, Goggins M, Canto MI, Schulick RD, Edil BH, Wolfgang CL, Klein AP, Diaz LA Jr, Allen PJ, Schmidt CM, Kinzler KW, Papadopoulous N, Hruban RH, Vogelstein B. Recurrent GNAS mutations define an unexpected pathway for pancreatic cyst development. Sci Transl Med 2011; 3: 92ra66 [PMID: 21775669 DOI: 10.1126/scitranslmed.3002548]

96 Lee JH, Kim Y, Choi JW, Kim YS. KRAS, GNAS, and RNF43 mutations in intraductal papillary mucinous neoplasm of the pancreas: a meta-analysis. Springerplus 2016; 5: 1172 [PMID: 27512631 DOI: 10.1186/s40064-016-2847-4]
Kuwatani M, Hatanaka Y, Mitsuhashi T, Matsuno Y, Sakamoto N. CTNNB1 mutational analysis of ductal adenocarcinomas and almost always harbor beta-catenin mutations.

Hruban RH. Solid-pseudopapillary tumors of the pancreas are genetically distinct from pancreatic pancreatic solid-pseudopapillary neoplasm.

Nakatani Y, Kobayashi Y. Frequent beta-catenin mutation and cytoplasmic/nuclear accumulation in genetic defects in melanoma cell lines.

Rubinfeld B, Nelson WJ, Lab Med 2018; 492-501 [PMID: 2870292 DOI: 10.1158/gutnl-2016-313586]

Yoshizawa K, Nagai H, Sakurai S, Hironaka M, Morinaga S, Saitoh K, Fukayama M. Clonality and K-ras mutation analyses of epithelia in intraductal papillary mucinous tumor and mucinous cystic tumor of the pancreas. Virchows Arch 2002; 437-443 [PMID: 12447672 DOI: 10.1007/s00428-002-0645-6]

Kim SG, Wu TT, Lee JH, Yun VK, Issa JP, Hamilton SR, Rashid A. Comparison of epigenetic and genetic alterations in mucinous cystic neoplasm and serous microacinar adenocarcinoma of the pancreas. Mod Pathol 2003; 16: 1086-1094 [PMID: 14614047 DOI: 10.1097/01.mp.0000094088.37888.a6]

Jimenez RE, Warshaw AL, Zgraggen K, Hartwig W, Taylor DZ, Compton CC, Fernandez-del Castillo C. Sequential accumulation of K-ras mutations and p53 overexpression in the progression of pancreatic mucinous cystic neoplasms to malignancy. Ann Surg 1999; 230: 501-9; discussion 509 [PMID: 10522720 DOI: 10.1097/00000658-199910000-00006]

Garcia-Carracedo D, Chen ZM, Qiu W, Huang AS, Tang SM, Hruban RH, Su GH. PIK3CA mutations in mucinous neoplasms of the pancreas. Pancreas 2014; 43: 245-249 [PMID: 24518503 DOI: 10.1097/MPA.0000000000000434]

Iz eradjen e K, Combs C, Best M, Gopinathan A, Wagner A, Grady WM, Deng CX, Hruban RH, Adsay NV, Tuveson DA, Hingorani SR. Kras(G12D) and Smad4/Dpc4 haploinsufficiency cooperate to induce mucinous cystic neoplasms and invasive adenocarcinoma of the pancreas. Cancer Cell 2007; 11: 229-243 [PMID: 17349581 DOI: 10.1016/j.ccr.2007.01.017]

Bardeesy N, Cheng KH, Berger JH, Chu GC, Pahler J, Olson P, Hezel AF, Horner J, Lauwers GY, Hanahan D, DePinho RA. Smad4 is dispensable for normal pancreas development yet critical in progression and tumor biology of pancreas cancer. Genes Dev 2006; 20: 3130-3146 [PMID: 17114584 DOI: 10.1101/gad.147870c]

Springer S, Wang Y, Dal Molin M, Masica DL, Jao Y, Kinde I, Blackford A, Raman SP, Wolfgang CL, Tomita T, Niknafs N, Douville C, Puak J, Dobbyn L, Allen PJ, Klimstra DS, Schattner MA, Schmidt CM, Vip-Schneider M, Cummings OW, Brand RE, Zeh HJ, Singh AD, Scarp a A, Salvia R, Malleo G, Zamboni G, Falconi M, Jang JY, Kim SW, Kwon W, Hong SM, Song KB, Kim SC, Swan N, Murphy J, Geoghegan J, Brugge W, Fernandez-Del Castillo C, Mino-Kenudson M, Schulick R, Edl BH, Adsay V, Paulino J, van Hooft J, Yachida S, Nara S, Hiraoka N, Yamano K, Hijikoa S, van der Merwe S, Goggi ns M, Canto MI, Ahuja N, Hirose K, Makary M, Weiss MJ, Cameron J, Pittman B, der Merwe S, Goggins M, Yeo CJ, Kern SE. Germline and somatic mutations of the STK11/LKB1 Peutz-Jeghers gene in pancreatic ductal adenocarcinomas and almost always harbor beta-catenin mutations. Am J Pathol 2002; 160: 1361-1369 [PMID: 11943721 DOI: 10.1016/s0002-9440(10)65263-1]

Tanaka Y, Kato K, Notohara K, Hojo H, Ijiri R, Miyake T, Nagahara N, Sasaki F, Kitagawa N, Nakatani Y, Kobayashi Y. Frequent beta-catenin mutation and cytoplasmic/nuclear accumulation in pancreatic solid-pseudopapillary neoplasm. Cancer Res 2001; 61: 8401-8404 [PMID: 11731417]

Abraham SC, Klimstra DS, Wilzent RE, Yeo CJ, Conlon K, Brennan M, Cameron JL, Wu TT, Hruban RH. Solid-pseudopapillary tumors of the pancreas are genetically distinct from pancreatic ductal adenocarcinomas and almost always harbor beta-catenin mutations. Am J Pathol 2002; 160: 1361-1369 [PMID: 11943721 DOI: 10.1016/s0002-9440(10)65263-1]

Kubota Y, Kawakami H, Natsuiizaka M, Kawakubo K, Marukawa K, Kudo T, Abe Y, Kubo K, Kuwatan i M, Hatana ka Y, Mitsuhashi T, Masuno Y, Sakamoto N. CTNNB1 mutational analysis of solid-pseudopapillary neoplasms of the pancreas using endoscopic ultrasound-guided fine-needle
Visani M et al. Molecular alterations in pancreatic tumors

aspiration and next-generation deep sequencing. J Gastroenterol 2015; 50: 203-210 [PMID: 24700283 DOI: 10.1007/s00353-014-0954-y]

115 Kempski HM, Austin N, Chatters SJ, Toomey SM, Chalker J, Anderson J, Sebire NJ. Previously unidentified complex cytogenetic changes found in a pediatric case of solid-pseudopapillary neoplasm of the pancreas. Cancer Genet Cytoenet 2006; 164: 54-60 [PMID: 16364763 DOI: 10.1016/j.cancergenet.2005.06.017]

116 Selenica P, Raj N, Kumar R, Brown DN, Arqués O, Reidy D, Klimstra D, Sznuder M, Serrano J, Palmer HG, Weigelt B, Reis-Filho JS, Scalfatti M. Solid pseudopapillary neoplasms of the pancreas are dependent on the Wnt pathway. Mol Oncol 2019; 13: 1684-1692 [PMID: 30972907 DOI: 10.1002/1878-0261.12490]

117 Vijayvergia N, Boland PM, Handorf E, Gustafsson KS, Gong Y, Cooper HS, Sheriff F, Assaturov I, Cohen SJ, Engstrom PF. Molecular profiling of neuroendocrine malignancies to identify prognostic and therapeutic markers: a Fox Chase Cancer Center Pilot Study. Br J Cancer 2016; 115: 564-570 [PMID: 27482646 DOI: 10.1038/bjc.2016.229]

118 Jiao Y, Shi C, Edil BH, de Wilde RF, Klimstra DS, Maitra A, Schulick RD, Tang LH, Wolfgang CL, Choti MA, Velculescu VE, Diaz LA Jr, Vogelstein B, Kinzler KW, Hruban RH, Papadopoulos N. DAXX/ATRX, MEN1, and mTOR pathway genes are frequently altered in pancreatic neuroendocrine tumors. Science 2011; 331: 1199-1203 [PMID: 21252315 DOI: 10.1126/science.1200609]

119 Corbo V, Dalai I, Scardoni M, Barbi S, Beghelli S, Bersani S, Alibello L, Doglioni C, Schott C, Capelli P, Chilosi M, Boninsegna L, Becker KF, Falconi M, Scarpa A. MEN1 in pancreatic endocrine tumors: analysis of gene and protein status in 169 sporadic neoplasms reveals alterations in the vast majority of cases. Endocr Relat Cancer 2010; 17: 771-783 [PMID: 20566584 DOI: 10.1677/ERC-10-0028]

120 Missiaglia E, Dalai I, Barbi S, Beghelli S, Falconi M, della Peruta M, Piemonti L, Capurso G, Di Florio A, delle Fave G, Pederzoli P, Croce CM, Scarpa A. Pancreatic endocrine tumors: expression profiling evidences a role for AKT-mTOR pathway. J Clin Oncol 2010; 28: 245-255 [PMID: 19917848 DOI: 10.1200/JCO.2008.21.5988]

121 Schmitt AM, Schmid S, Rudolph T, Anlauf M, Prinz C, Klöppel G, Moch H, Heitz PU, Kimmnitho P, Perren A. VHL inactivation is an important pathway for the development of malignant sporadic pancreatic endocrine tumors. Endocr Relat Cancer 2009; 16: 1219-1227 [PMID: 19690016 DOI: 10.1677/ERC-08-0209]

122 Scarpa A, Chang DK, Nones K, Corbo V, Patch AM, Bailey P, Lawlor RT, Johns AL, Miller DK, Mafficini A, Rusey B, Scardoni M, Antonello D, Barbi S, Sikora KO, Cingarlini S, Vicentini C, McKay S, Quinn MC, Bruzzer TJ, Christ AN, Hariiow HG, Idsirou S, McLean S, Nourse C, Nourjahok ES, Wilson PI, Anderson MJ, Fink JL, Newell F, Waddell N, Holmes O, Kassakoff SH, Leonard C, Wood S, Xu Q, Nagaraj SH, Amato E, Dalai I, Bersani S, Cataldo I, Dei Tos AP, Capelli P, Davi MV, Landoni L, Malpaga A, Miotto M, Whitehall VL, Leggett BA, Harris JL, Harris J, Jones MD, Humphris J, Chandraper L, Chin V, Nagrial AG, Pacic M, Scarlett CJ, Pinho A, Roomen I, Toon C, Wu J, Pinse M, Cowley M, Barbour A, Mawson A, Humphrey ES, Colvin EB, Choti MA, Velculescu VE, Diaz LA Jr, Vogelstein B, Kinzler KW, Hruban RH, Papadopoulos N, DAXX/ATRX, MEN1, and mTOR pathway genes are frequently altered in pancreatic neuroendocrine tumors. Science 2011; 331: 1199-1203 [PMID: 21252315 DOI: 10.1126/science.1200609]

123 Rigaud G, Moore PS, Zamboni V, Alarandi L, Eganlini DP, Tarusicio D, Paraisi S, Lemoine NR, Klöppel G, Scarpa A. Allelotype of pancreatic acinar cell carcinoma. Int J Cancer 2000; 88: 772-777 [PMID: 11072247 DOI: 10.1002/1097-0215(20001201)88:5<772::aid-ijc14>3.0.co;2-w]

124 Jäkel C, Bergmann F, Toth R, Assenov Y, van der Duijn D, Strobel O, Hank T, Klöppel G, Durrell C, Grompe M, Moss L, Dan Y, Schirmer C, Plass C, Popanda O, Schmezer P. Genome-wide genetic and epigenetic analyses of pancreatic acinar cell carcinomas reveal alterations in genome stability. Nat Commun 2017; 8: 1323 [PMID: 29109526 DOI: 10.1038/s41467-017-01118-x]

125 La Rosa S, Bernascioni B, Frattini M, Tibilite MG, Molinari F, Furlan D, Sahnane N, Vanoli A, Alibello L, Zhang L, Notohara K, Casneda S, Chennard MP, Adsay V, Asielo S, Capella C, Sessa F. Tp53 alterations in pancreatic acinar cell carcinoma: new insights into the molecular pathology of this rare cancer. Virchows Arch 2016; 468: 289-296 [PMID: 26586531 DOI: 10.1007/s00428-015-1882-9]

126 Jiao Y, Yonescu R, Offerhaus GJ, Klimstra DS, Maitra A, Eshleman JR, Herman JG, Poh W, Pelosof L, Wolfgang CL, Vogelstein B, Kinzler KW, Hruban RH, Papadopoulos N, Wood LD. Whole-exome sequencing of pancreatic neoplasms with acinar differentiation. J Pathol 2014; 232: 428-435 [PMID: 2429293 DOI: 10.1002/path.4310]

127 Thompson ED, Wood LD. Pancreatic Neoplasms Wth Acinar Differentiation: A Review of Pathologic and Molecular Features. Arch Pathol Lab Med 2020; 144: 808-815 [PMID: 31869246 DOI: 10.5858/arpa.2019-0472-RA]

128 Liu W, Shiia J, Gonen M, Lowery MA, O'Reilly EM, Klimstra DS. DNA mismatch repair abnormalities in acinar cell carcinoma of the pancreas: frequency and clinical significance. Pancreas 2014; 43: 1264-1270 [PMID: 25058881 DOI: 10.1097/MPA.0000000000000190]

129 Ducreux M, Cuna AS, Caramella C, Hollebecque A, Burtin P, Gőreré D, Seufferlein T,
Haustermans K, Van Laethem JL, Conroy T, Arnold D; ESMO Guidelines Committee. Cancer of the pancreas: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2015; 26 Suppl 5: v6-v68 [PMID: 26314780 DOI: 10.1093/annonc/mdv295]

130 **Hong SK**, Loren DE, Rogart JN, Siddiqui AA, Sendecki JA, Bibbo M, Coben RM, Meckes DP, Kowalski TE. Targeted cyst wall puncture and aspiration during EUS-FNA increases the diagnostic yield of premalignant and malignant pancreatic cysts. *Gastrointest Endosc* 2012; 75: 775-782 [PMID: 22317883 DOI: 10.1016/j.gie.2011.12.015]

131 **Dumonceau JM**, Polkowski M, Larghi A, Vilmann P, Giovannini M, Frossard JL, Heresbach D, Pujol B, Fernández-Esparrrach G, Vazquez-Sequeiros E, Ginés A; European Society of Gastrointestinal Endoscopy. Indications, results, and clinical impact of endoscopic ultrasound (EUS)-guided sampling in gastroenterology: European Society of Gastrointestinal Endoscopy (ESGE) Clinical Guideline. *Endoscopy* 2011; 43: E33-E76 [PMID: 21851966 DOI: 10.1055/s-0035-1553754]

132 **Jenssen C**, Hocke M, Fusaroli P, Gilja OH, Buscarini E, Havre RF, Ignee A, Saitou A, Vilmann P, Burmester E, Nolsoe CP, Nürnberg D, Dietrich CF. EFSUMB Guidelines on Interventional Ultrasound (INVUS), Part IV - EUS-guided Interventions: General aspects and EUS-guided sampling (Long Version). *Ultraschall Med* 2016; 37: E33-E76 [PMID: 26515966 DOI: 10.1055/s-0035-1553785]

133 **Varadarajulu S**, Fockens P, Hawes RH. Best practices in endoscopic ultrasound-guided fine-needle aspiration. *Clin Gastroenterol Hepatol* 2012; 10: 697-703 [PMID: 22475740 DOI: 10.1016/j.cgh.2012.03.017]

134 **Pishvaian MJ**, Bender RJ, Halverson D, Rahib L, Hendifar AE, Mikhail S, Chung V, Picozzi VI, Sohal D, Blais EM, Mason K, Lyons EE, Matrisian LM, Brody JR, Madhavan S, Petricoin EF 3rd. Molecular Profiling of Patients with Pancreatic Cancer: Initial Results from the Know Your Tumor Initiative. *Clin Cancer Res* 2018; 24: 5018-5027 [PMID: 29954777 DOI: 10.1158/1078-0432.CCR-18-0531]

135 **Ko SH**, Pyo JS, Son BK, Lee HY, Oh IW, Chung KH. Comparison between Conventional Smear and Liquid-Based Preparation in Endoscopic Ultrasonography-Fine Needle Aspiration Cytology of Pancreatic Lesions. *Diagnostics (Basel)* 2020; 10 [PMID: 32397572 DOI: 10.3390/diagnostics10050293]

136 **Fabbri C**, Fornelli A, Fuccio L, Giovannelli S, Tarantino I, Antonini F, Liotta R, Frazzoni L, Gusella P, La Marca M, Barresi L, Macarri G, Traina M, De Biase D, Fiorino S, Jovine L, Larghi A, Centeno VH. High diagnostic adequacy and accuracy of the new 20G procore needle for EUS-guided tissue acquisition: Results of a large multicentre retrospective study. *Endosc Ultrasound* 2019; 8: 261-268 [PMID: 31115386 DOI: 10.4103/eus.eus_14_19]

137 **Sho S**, Court CM, Kim S, Braxton DR, Hou S, Muthusamy VR, Watson RR, Sedarat A, Tseng HR, Tomlinson JS. Digital PCR Improves Mutation Analysis in Pancreas Fine Needle Aspiration Biopsy Specimens. *PLoS One* 2017; 12: e0170897 [PMID: 28125707 DOI: 10.1371/journal.pone.0170897]

138 **de Biase D**, Visani M, Acquaviva G, Fornelli A, Masetti M, Fabbri C, Pession A, Tallini G. The Role of Next-Generation Sequencing in the Cytologic Diagnosis of Pancreatic Lesions. *Arch Pathol Lab Med* 2018; 142: 458-464 [PMID: 29565213 DOI: 10.5858/arpa.2017-0215-RA]

139 **de Biase D**, Fassan M, Malapelle U. Next-Generation Sequencing in Tumor Diagnosis and Treatment. *Diagnostics (Basel)* 2020; 10 [PMID: 33212911 DOI: 10.3390/diagnostics10110962]

140 **Mosele F**, Remon J, Mateo J, Westphalen CB, Barlesi F, Lolkema MP, Normanno N, Scarpa A, Robson M, Meric-Bernstam F, Wagle N, Stenzinger A, Bonastre J, Bayle A, Michiels S, Bieche I, Rouleau E, Jezdic S, Douillard JY, Reis-Filho JS, Dienstmann R, André F. Recommendations for the use of next-generation sequencing (NGS) for patients with metastatic cancers: a report from the ESMO Precision Medicine Working Group. *Ann Oncol* 2020; 31: 1491-1505 [PMID: 32853681 DOI: 10.1016/j.annonc.2020.07.014]
