Pancreatic and Biliary Neoplasms

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Pancreatic cancer and cholangiocarcinoma are aggressive diseases mostly diagnosed at an advanced and inoperable stage.

pancreatic neoplasms | endoscopic-ultrasound-guided fine needle aspiration (EUS-FNA) | pancreatic juice | circulating tumor DNA (ctDNA)

1. Introduction

Pancreatic cancer is a lethal malignancy with a very low 5-year survival rate [1][2]. Notably, it causes almost the same number of deaths and new cases, according to the GLOBOCAN 2020 estimates [3]. Pancreatic cancer is mainly detected at a locally advanced or metastatic stage; thus, most patients are unfit for surgery at diagnosis, yet a few become eligible after neoadjuvant therapy (NAT) [4][5]. Chemotherapy is the treatment of choice, and common schemes include FOLFIRINOX (folinic acid, fluorouracil, irinotecan, oxaliplatin) or gemcitabine plus nab-paclitaxel [6][7]. Pancreatic adenocarcinoma (PDAC) is the most prevalent histologic type of pancreatic cancer, whereas PDAC precursors include pancreatic intraepithelial neoplasia (PanIN) with low-grade dysplasia (LGD) or high-grade dysplasia (HGD), and also intraductal papillary mucinous neoplasm (IMPN) and mucinous cystic neoplasm (MCN) with LGD or HGD [5][8]. PanINs are microscopic lesions giving rise to most PDACs, whereas IPMNs and MCNs are macroscopic lesions [2]. Aside from PDAC, other examples of pancreatic malignancies include acinar cell carcinoma, solid pseudopapillary neoplasm (SPN), and the pancreatic neuroendocrine neoplasms (NENs), which comprise neuroendocrine tumors (NETs) and carcinomas (NECs) [9][10]. Whereas distinguishing a solid neoplasm (e.g., PDAC, another pancreatic malignancy or a metastasis) from pancreatitis is the main differential diagnosis in the evaluation of solid pancreatic lesions [11][12], cystic lesions encompass various pathologies including non-neoplastic (e.g., pseudocyst), neoplastic benign, such as serous cystadenoma (SCA), neoplastic mucinous carrying malignant potential (e.g., IPMN or MCN), and malignant entities (e.g., IPMN or MCN with associated invasive carcinoma) [12][13][14][15]. Cholangiocarcinoma, a cancer arising in the biliary tract, is a rare malignancy mainly diagnosed at an advanced and inoperable stage, resulting in dismal prognosis [16].

At the molecular level, mutations at the following genes are most often identified in PDACs: KRAS, CDKN2A/p16, TP53, and SMAD4/DPC4 [2][6][17]. According to the PDAC progression model published some years ago, KRAS mutations are detected early, whereas the inactivating TP53 and DPC4 mutations occur later during the PDAC carcinogenesis [18][19][20]. Of interest, although KRAS mutations could be detected in low- or high-grade PanINs and IPMNs/MCNs, they could also be found in non-neoplastic disorders such as in chronic pancreatitis. In contrast,

TP53 and SMAD4 alterations generally indicate the presence of HGD or cancer [21][22][23][24][25][26][27]. While evaluating a pancreatic cyst, finding a KRAS mutation favors a mucinous (e.g., IPMN or MCN) vs. a non-mucinous cyst (e.g., SCA or pseudocyst); moreover, an additional GNAS mutation indicates the presence of IPMN rather than MCN [28][29][30].

Next-generation sequencing (NGS) is an evolving modality that can simultaneously detect and quantify multiple genomic or transcriptomic targets in a single run and with a high analytical sensitivity [31][32]. In the era of precision and personalized medicine, NGS testing is often used in several clinical oncology applications [33]. Diverse sample types could be utilized, including limited tissue and cytologic samples, in addition to “liquid biopsies” such as blood, urine, pleural, and cerebrospinal fluids [34][35][36][37][38][39].

Sampling of pancreatic lesions is most often performed with endoscopic ultrasound fine-needle aspiration (EUS-FNA), fine-needle biopsy (FNB), or a combination of the two [40][41]. However, due to various reasons such as inadequate material or low cellularity, such sampling could result in non-diagnostic or indeterminate interpretations [42]. Pancreatic juice, collected in the duodenum following secretin stimulation, has been studied in high-risk individuals undergoing surveillance [23]. Blood-based liquid biopsies could be used to assess prognosis, select for targeted therapy, and dynamically monitor cancer progression, most often at an advanced stage; these are mainly composed of circulating tumor cells (CTCs), cell-free DNA (cfDNA—a component of which is the circulating tumor DNA (ctDNA), and exosomes [43]. Assessing biliary strictures is a challenging task, whereas tissue sampling includes brush cytology and/or biopsy [44]. Notably, as most PDACs and cholangiocarcinomas are inoperable at diagnosis, the surgical specimen is mainly unavailable for further molecular testing. Additionally, chemotherapy is often ineffective with short median survival [4][5][7][16]. Thus, checking for potentially targetable molecular alterations in FNAs, FNBs, blood, or any other “small biopsy” would be valuable for PDAC or cholangiocarcinoma patients [16][45][46][47].

2. The Role of NGS Performed on Pancreatic Small Biopsies

The summary of the published studies reporting on the role of small biopsy-based NGS in the evaluation and management of pancreatic lesions is shown in **Table 1**. Most studies highlighted its value in diagnosis (e.g., indeterminate case clarification or pre-operative stratification) or targeted therapy selection.

Table 1. The role of NGS performed on pancreatic small biopsies (EUS-FNA and FNB, brushings, and pancreatic juice): doing more with less.

First Author, Year	Small Biopsy Type Clinical Setting	NGS Strategy	Main Findings
Ren, 2021 [48]	EUS-FNA Pancreatic mucinous cystic lesions	48 gene panel	KRAS and/or GNAS mutations were detected in 59/68 cases tested; NGS was more sensitive to detect a neoplastic mucinous cyst than cytologic examination or elevated CEA cystic fluid levels, whereas their

First Author, Year	Small Biopsy Type Clinical Setting	NGS Strategy	Main Findings
			combination showed a sensitivity of 94.1% and a specificity of 100%; in 6/10 mucinous cysts without a KRAS mutation, a combination of BRAF and GNAS mutations were detected
Haeberle, 2021 [49]	EUS-FNA Pancreatic mucinous cystic lesions	50 gene panel	NGS enhanced the diagnostic accuracy of EUS-FNA cytology to detect neoplastic mucinous cysts
Takano, 2021 [50]	EUS-FNA/FNB PDACs	50 gene panel	Mutations in KRAS, TP53, SMAD4, and PTEN genes were the most common ones detected; 22.4% of the cases exhibited potentially targetable alterations
Perez, 2021 [51]	EUS-FNA Pancreatic cystic lesions	39 gene panel	KRAS and/or GNAS mutations were 83.3% sensitive and 60% specific to detect a neoplastic mucinous cyst
Schmitz, 2021 [52]	EUS-FNA Pancreatic mucinous cystic lesions	14 gene panel	KRAS or GNAS mutations were found in 43/47 patients tested; NGS exhibited higher sensitivity to detect a neoplastic mucinous cyst than cytology or elevated CEA levels
Kuratomi, 2021 [53]	Pancreatic juice IPMNs with and without invasion	miRNA sequencing	The miR-10a-5p was upregulated at a significant level in invasive, compared with noninvasive IPMNs
Sekita-Hatakeyama, 2021 [22]	FNA Pancreatic and periampullary lesions suspicious for malignancy	6 gene panel	Mutations in KRAS, TP53, CDKN2A, and SMAD4 genes were the most common ones detected; 18/33 PDACs were identified as carrying at least HGD (KRAS and CDKN2A/PIK3CA/TP53/SMAD4 mutations) with NGS performed on residual LBC specimens, whereas 10/11 benign cases showed no mutations
Habib, 2021 [54]	FNA; plasma cfDNA Lesions suspicious for PDAC	9 gene panel	FNA-based NGS identified 16/16 of the KRAS mutations found in their paired histological specimens, in contrast to 6/8 identified by the plasma-based molecular analysis; mutations in the KRAS and TP53 genes were the most common ones detected
Dupain, 2020 [55]	CT or EUS-FNA and EUS-FNB Pancreatic cancer metastases	87 gene panel	Among the metastatic tumors (e.g., from pancreas, breast, and colon) prospectively tested, FNA-based was highly concordant with the CNB-based NGS; potentially actionable alterations were also identified
De Biase, 2020 [56]	FNAs and direct fluid samples	22 gene panel	KRAS p.G12V and p.G12D were the most common mutations detected in the 42 pancreatic lesions tested

First Author, Year	Small Biopsy Type Clinical Setting	NGS Strategy	Main Findings
	Solid and cystic pancreatic lesions		
Carrara, 2020 [57]	EUS-FNA and EUS-FNB PDACs	161 gene panel	In this clinical trial, NGS was successful in almost all samples tested and exhibited higher diagnostic yield (94%) than histology (91%) or cytology (88%); at least two mutations were found in the majority of PDAC cases, whereas KRAS mutations were the most common ones detected
Fulmer, 2020 [58]	EUS-FNA Solid and cystic pancreatic lesions	143 gene panel	DNA of high quality was retrieved from most samples; NGS revealed clinically significant mutations in 10/14 mucinous cysts (e.g., KRAS, GNAS, TP53 mutations) and 13/15 PDACs (KRAS mutations in 10 and TP53 in 9 samples), whereas it did not exhibit any mutation in the 4 PanNETs tested
Plougmann, 2020 [59]	EUS-FNA Solid pancreatic lesions	19 gene panel	Mutations in KRAS and TP53 were only detected in the malignant and indeterminate cases; NGS could aid in the stratification of imaging and cytology indeterminate cases
Ishisawa, 2020 [60]	EUS-FNA Pancreatic cancers	409 gene panel	In addition to improving the diagnostic accuracy of EUS-FNA, ROSE facilitated the acquisition of material for subsequent NGS testing, sparing patients from additional invasive procedures; mutations in KRAS, TP53, SMAD4, and CDKN2A genes were the most common ones detected
Laquiere, 2020 [61]	EUS-FNA Pancreatic cystic lesions	526 gene panel	Cystic fluid-based NGS was concordant with its paired post-surgical NGS testing in 15/17 matched samples, whereas it also identified additional molecular alterations; mutations in KRAS and GNAS genes were the most common ones detected
Paziewska, 2020 [27]	EUS-FNA Pancreatic cystic lesions	409 gene panel	Mutations were mostly found in the TP53, KRAS, PI3CA, and GNAS genes; except for IPMNs, MCNs, and malignant cysts, 13% of SCAs and 14% of pseudocysts also exhibited KRAS mutations
Yamaguchi, 2020 [62]	Pancreatic juice PDACs	28 gene panel	SMAD4, CDKN2A, and TP53 mutations were identified by performing NGS on residual LBC specimens
Sugimori, 2020 [63]	EUS-FNA PDACs	50 gene panel	NGS was performed in two PDACs and was concordant to digital PCR concerning the absence of KRAS G12/13 mutations; NGS additionally detected KRAS Q61K and TP53 mutations in one of the cases tested

First Author, Year	Small Biopsy Type Clinical Setting	NGS Strategy	Main Findings
Park JK, 2019 [64]	EUS-FNA and FNB PDACs	83 gene panel	Larger gauge needles were more likely to result in successful NGS results (OR = 2.19; 95% CI: 1.08 to 4.47; p = 0.031)
Volckmar, 2019 [65]	EUS-FNA Pancreatic cystic lesions	14 gene panel	Mutations were found in all tested IPMNs (n = 12), most often in the KRAS and GNAS genes, whereas none of the tested pseudocysts (n = 3) showed any KRAS/GNAS mutations; cellular fraction exhibited superior results than the liquid fraction molecular analysis
Vestrup Rift, 2019 [66]	EUS-FNB Pancreatic cystic lesions	50 gene panel	Mutations in KRAS and GNAS genes were the most common ones detected in IPMNs (11/19 and 13/19 cases, respectively), whereas the three SCAs tested did not show any mutations
Takano, 2019 [67]	Pancreatic juice IPMNs with and without invasive component	2 panels, targeting 50 and 6 genes	TP53 or multiple KRAS mutations were associated with invasive IPMN
Sakhdari, 2019 [68]	EUS-FNA Pancreatic cystic lesions	50 gene panel	NGS was more sensitive than cytology, whereas their combination improved the diagnostic sensitivity; KRAS and GNAS mutations were the ones most often detected, whereas SMAD4 and VHL mutations were found in PDACs and SCAs, respectively
Choi, 2019 [69]	Pancreatic juice PDACs	15 gene panel	Most pancreatic juice samples revealed KRAS mutations, even when these were not found in the resected primary tissue molecular analysis; six juice samples (29%) also revealed TP53 mutations, whereas the cases with a concurrent KRAS and TP53 mutational profile were concordant between the paired tissue and pancreatic juice molecular analysis
Elhanafi, 2019 [70]	EUS-FNA and FNB PDACs	47 gene panel	FNB was more likely to result in adequate material for subsequent NGS testing than FNA (OR = 4.95; 95% CI: 1.11–22.05; p = 0.04), especially in PDACs ≤ 3 cm or PDACs located in the head or neck of the pancreas; KRAS, TP53, and SMAD4 mutations were the most frequent mutations found, whereas actionable alterations (e.g., in BRAF, MET, ERBB2, ARID1A, and BRCA1 genes) were identified in several PDACs
Larson, 2018 [71]	EUS-FNA and FNB, forceps biopsies, percutaneous CNBs	324 gene panel	Adequacy for subsequent NGS analysis was significantly associated with larger-gauge needles and sampling of the metastatic lesions

First Author, Year	Small Biopsy Type Clinical Setting	NGS Strategy	Main Findings
	PDACs (also one ACC and one AAC)		
Sibinga Mulder, 2018 [72]	EUS-FNA and brushings Pancreatic or periampullary lesions	50 gene panel	KRAS, TP53, SMAD4, and CDKN2A mutations were the ones most often detected; NGS exhibited high diagnostic accuracy and facilitated preoperative risk stratification, leading to management change in 10% of the patients
Suenaga, 2018 [23]	Pancreatic juice PDACs and precursors; non-neoplastic controls	12 gene panel	Patients with HGD or cancer showed higher number and concentration of mutations other than KRAS/GNAS (also higher overall mutation concentration) in their pancreatic juice; mutations in TP53 and/or SMAD4 or a high SMAD4/TP53 mutation score were associated with HGD or cancer, whereas they were not detected in the controls; NGS could facilitate the stratification of high-risk patients under pancreatic surveillance, by identifying patients harboring HGD or cancer
Takano, 2017 [24]	Pancreatic juice IPMNs	2 panels, targeting 50 and 6 genes	Mutations in the KRAS and GNAS genes were the most common ones detected, whereas TP53 mutations were associated with malignant IPMNs, both in the pancreatic juice and tumor resection specimens tested
Rosenbaum, 2017 [25]	EUS-FNA Pancreatic cystic lesions	39 gene panel	Mutations in the KRAS and GNAS genes supported the diagnosis of an IPMN over a non-mucinous cyst; additional non-KRAS/GNAS aberrations (SMAD4, TP53, CDKN2A, or NOTCH1 mutations) indicated the presence of IPMN with HGD or invasion; NGS improved the overall diagnostic accuracy when added to cytology for both the detection of mucinous vs. non-mucinous cysts and the presence of at least HGD (high-risk cysts)
Sibinga Mulder, 2017 [73]	EUS-FNA PDAC	50 gene panel	Mutations in KRAS, TP53, and CDKN2A were detected in both the EUS-FNA and matched tumor resection specimen tested (SMAD4 mutation was found only in the former); NGS modified the management plan of this patient
Yu, 2017 [26]	Pancreatic juice Pancreatic solid and cystic lesions, also non-neoplastic controls	9 gene panel	PDAC patients showed higher mutation concentrations than IPMNs or controls; mutations in the TP53 and SMAD4 genes were found most often in PDACs, whereas they were also detected in 15/57 and 1/57 of the IPMNs tested, respectively, albeit in none of the controls; KRAS mutations were also found in 10/24 of the controls; two high-risk patients under surveillance showed TP53 or SMAD4 mutations in the pancreatic

First Author, Year	Small Biopsy Type Clinical Setting	NGS Strategy	Main Findings
			juice-based molecular analysis, more than a year before their cancer diagnosis
Gleeson, 2017 [74]	EUS-FNA PanNETs (primary and liver metastases)	15 gene panel	Alterations in the MEN1, DAXX, ATRX, and TSC2 genes were the most common ones detected in primary PanNETs; TSC2, KRAS, and TP53 alterations were associated with poor prognosis; potentially actionable alterations in members of the mTOR pathway (PTEN, TSC2, and PIK3CA) were identified in 10% of the primary and 12.5% metastatic PanNETs tested
Gleeson, 2016 [75]	EUS-FNA PDACs, IPMNs with invasion, AACs	160 gene panel	Mutations in the KRAS, TP53, SMAD4, and GNAS genes were the most common ones detected; SMAD4 mutations were detected in nine patients, yet in none of the four AAC patients tested; FNA-based NGS was highly concordant with the matched tumor resection-based NGS analysis
Jones, 2016 [76]	EUS-FNA Pancreatic cystic lesions	39 gene panel	Mutations in the KRAS, GNAS, and CDKN2A genes were the most common ones detected; KRAS and GNAS mutations supported the diagnosis of IPMN, even when the CEA levels were low; additional non-KRAS/GNAS aberrations (SMAD4, TP53, or CDKN2A) indicated the presence of IPMN with HGD or cancer; VHL mutations supported the diagnosis of SCA
Valero, 2016 [46]	EUS-FNA Unresectable PDACs	409 gene panel	NGS revealed at least one mutation in 17/19 PDAC patients tested; mutations in KRAS, TP53, SMAD4, and ARID1A genes were the most common ones detected; actionable mutations (e.g., in the ATM or mTOR genes) were also detected in a few cases
Kameta, 2016 [77]	EUS-FNA Solid and cystic pancreatic lesions	50 gene panel	KRAS mutations were found in 26/27 PDAC albeit none of the non-PDAC cases; KRAS, TP53, CDKN2A, and SMAD4 mutations were the most common ones detected
Dudley, 2016 [78]	Main pancreatic and bile duct brushings Pancreatobiliary duct strictures	39 gene panel	Mutations in the KRAS, TP53, SMAD4, and CDKN2A genes were the most common ones detected; a KRAS mutation was also found in a non-neoplastic case (cholecystitis); NGS was more sensitive, specific, and accurate than FISH, whereas it improved the overall sensitivity and diagnostic accuracy when combined with cytology
Springer, 2015 [79]	EUS-FNA or direct collection from the resected tissue specimens	11 gene panel	KRAS and GNAS mutations were the most common ones found in IPMNs (78% and 58% of the cases, respectively); KRAS mutations were the most common ones found in MCNs (6/12 cases tested); CTNNB1

First Author, Year	Small Biopsy Type Clinical Setting	NGS Strategy	Main Findings
	Pancreatic cystic lesions		mutations were found in SPNs, whereas VHL mutations were found in SCAs
Wang, 2015 [80]	EUS-FNA Pancreatic cystic lesions	Non-coding RNA sequencing	miRNA expression profiling was used to distinguish low-grade from high-grade/malignant pancreatic cystic lesions; the latter showed enrichment of 13 and depletion of two miRNAs
Kubota, 2015 [81]	EUS-FNA Pancreatic solid and cystic lesions	WES (CTNNB1 gene)	A CTNNB1 mutation in exon 3 was found in all seven SPNs tested 1/11 NETs but none of the PDACs, ACC, or non-neoplastic cases tested displayed a CTNNB1 mutation
Di Marco, 2015 [82]	EUS-FNB PDACs	WTS	KRAS, TP53, SMAD4, and CDKNA mutations were the most common ones found in PDACs; ARID1A alterations were found in 6/16 of the PDACs tested, whereas PTEN inactivation was identified only in advanced PDACs
De Biase, 2014 [83]	EUS-FNA Pancreatic solid and cystic lesions	KRAS (exons 2 and 3)	KRAS mutations were found in most of the PDACs and IPMNs, but in none of the PanNET cases tested; NGS exhibited superior sensitivity than PCR or Sanger sequencing, whereas it maintained a high specificity; sensitivity was higher when cytology slide scraping of selected areas (rather than fresh aliquots) was used for NGS analysis
Amato, 2014 [84]	Direct cystic fluid collection from surgical specimens IPMNs	50 gene panel	GNAS, KRAS, and TP53 mutations were the most common ones found in PDACs
Takano, 2014 [29]	Pancreatic juice Pancreatic solid and cystic lesions	46 gene panel	GNAS mutations were found in 41.5% of the IPMNs tested; all PDAC cases with GNAS mutations had concurrent IPMN; GNAS mutations were associated with main duct IPMNs exhibiting dilatation ≥6 mm
Young, 2013 [85]	FNA PDACs (also one PanNET)	Exons of 287 and introns of 19 genes	Mutations in KRAS, TP53, CDKN2A/B, SMAD4, and PTEN were the most common ones found; FNA-based NGS was 100% concordant with its matched tissue-based NGS analysis for the aberrations discovered

Abbreviations: EUS-FNA, endoscopic ultrasound-guided fine-needle aspiration; EUS-FNB, endoscopic ultrasound-guided fine-needle biopsy; PDAC, pancreatic adenocarcinoma; IPMN, intraductal papillary mucinous neoplasm; MCN, mucinous cystic neoplasm; SCA, serous cystadenoma; SPN, solid pseudopapillary neoplasm; cfDNA, cell-free DNA; CNB, core needle biopsy; AAC, ampullary adenocarcinoma; ACC, acinar cell carcinoma; PanNET,

pancreatic neuroendocrine tumor; WES, whole exome sequencing; WTS, whole transcriptome sequencing; NGS, next-generation sequencing; HGD, high-grade dysplasia; LBC, liquid-based cytology; ROSE, rapid on-site evaluation; FISH, fluorescence in in situ hybridization.

2.1. Most Common Mutations Detected in PDACs

NGS was performed on distinct small biopsy types, including EUS-FNAs or FNBs, brushings, and pancreatic juice, whereas several NGS panels were utilized. The initial material used for nucleic acid extraction was either fresh, directly collected for further NGS testing [25][55][61], frozen [23][57], also derived from formalin-fixed, paraffin-embedded tissue or cell blocks [70][71][85], residual liquid-based cytology (LBC) samples [58][62], or cytology slide scraping [59][60]. Mutations in the KRAS, TP53, CDKN2A, and SMAD4 genes were the most common ones detected in the PDAC patients tested [22][60][72][77]. Although KRAS mutations, as an early carcinogenic step, were also found in non-malignant cases, TP53 and SMAD4 alterations indicated HGD or carcinoma, triaging surgically fit patients for surgery [22][23][24][25][26][27].

2.2. Preoperative Evaluation of Pancreatic Cysts

A few of the published studies aimed to unravel the value of NGS in the preoperative evaluation of pancreatic cysts, in order to reduce unnecessary surgical procedures. This challenging task emerges more often in recent years, as more incidental cysts are detected, following the prevalent use of enhanced imaging technology [86]. To manage pancreatic cysts effectively, physicians should generally decipher if: (a) the cysts are mucinous or non-mucinous (the latter can be either non-neoplastic or benign with minimal malignant potential which can safely be managed conservatively), and (b) there is presence of at least HGD within the cysts; these would be classified as high-risk cysts, which are triaged for surgery [42]. In accordance with the literature, this review also found that the presence of KRAS mutations supported the diagnosis of a mucinous (IPMN or MCN) over a non-mucinous cyst (e.g., pseudocyst or SCA), whereas GNAS mutations favored IPMN over MCN [25][29][65][66][76][79]. NGS enhanced the diagnostic accuracy of EUS-FNA cytology to detect neoplastic mucinous cysts and differentiate them from the non-mucinous ones [49]. Notably, evidence indicated that NGS was more sensitive than the cytologic examination or elevated CEA cystic fluid levels (≥192 ng/mL), which are the two modalities traditionally used to evaluate pancreatic cysts [25][48][52][68]. For instance, Ren et al. showed the combination of cytologic examination, elevated CEA cystic fluid levels, and NGS reached a sensitivity of 94.1% and a specificity of 100% for the detection of neoplastic mucinous cysts [48]. Apart from discriminating between mucinous and non-mucinous pancreatic cysts, NGS was able to robustly identify high-risk cysts. A few studies indicated that specific mutations detected with NGS were associated with cystic neoplasms exhibiting HGD or invasion [25][76][87]. Rosenbaum et al. examined 113 pancreatic cystic fluid lesions from 105 patients and reported that SMAD4, TP53, CDKN2A, or NOTCH1 mutations indicated the presence of IPMN with high-grade dysplasia or cancer. Of interest, NGS combined with cytology improved the overall diagnostic accuracy to detect IPMNs and identify the high-risk IPMNs [25]. Similarly, Jones et al. also found that the presence of SMAD4, TP53, or CDKN2A alterations, discovered with NGS, indicate IPMNs with high-grade dysplasia or invasion [76].

2.3. Evaluation of High-Risk Patients under Surveillance with Pancreatic Juice-Based NGS

Likewise, some teams utilized pancreatic juice-based NGS to recognize HGD or cancer while evaluating solid or cystic pancreatic lesions. For instance, TP53 or multiple KRAS mutations were associated with invasive IPMNs [24][67]. Furthermore, Suenaga et al. tested the pancreatic juice from a mixture of pancreatic cancer and precursors (with both LGD and HGD) under surveillance, in addition to normal controls. They found that patients with HGD or cancer exhibited higher numbers and concentration of mutations other than KRAS/GNAS (also a higher overall mutation concentration) in their pancreatic juice. Mutations in TP53 and/or SMAD4 or a high SMAD4/TP53 mutation score were associated with HGD or cancer, whereas none of them were detected in the controls. Thus, NGS facilitated the stratification of high-risk patients under pancreatic surveillance, by identifying the patients harboring at least HGD [23]. Yu et al. applied pancreatic juice-based NGS in a cohort of 115 pancreatic solid and cystic lesions (34 PDACs, 57 IPMNs, and 24 non-neoplastic controls). They reported that PDAC patients showed higher mutation concentrations than IPMNs or controls. Although TP53 and SMAD4 mutations were associated with PDACs, they were also detected in 15/57 and 1/47 of IPMNs, respectively, but in none of the controls. Notably, two high-risk patients of the cohort under surveillance showed TP53/SMAD4 mutations more than a year before their cancer diagnosis [26].

2.4. Identification of Potentially Actionable Mutations in PDAC Patients

Apart from its use in diagnosis and preoperative risk stratification of pancreatic solid and cystic lesions, small biopsy-based NGS also showed potential in identifying potentially actionable alterations in PDAC patients. Takano et al. found such alterations in 22.4% of the cases tested [50], whereas Elhanafi et al. identified actionable mutations in the BRAF, MET, ERBB2, ARID1A, and BRCA1 genes in a few of the PDACs tested [70]. Lastly, Valero et al. reported at least one mutation in 17/19 of PDAC patients of their cohort, whereas KRAS, TP53, SMAD4, and ARID1A mutations were the ones most commonly detected. Notably, actionable alterations (e.g., in ATM or mTOR genes) were also found in some samples [46].

2.5. Evaluation of Neoplasms Other Than PDAC and Its Precursors

Whereas most studies focused on PDAC and its precursors, small biopsy-based NGS was also used to evaluate the molecular profile of other pancreatic neoplasms, pointing to a specific diagnosis or providing additional prognostic and therapeutic information. Gleeson et al. tested 90 primary and 32 metastatic PanNETs from the liver and reported that the former most often harbored MEN1, DAXX, ATRX, and TSC2 mutations. In addition, they found that alterations in TSC2, KRAS, and TP53 genes were associated with poor prognosis, whereas they also identified potentially actionable alterations in some members of the mTOR pathway (PTEN, TSC2, and PIK3CA) in 10% of primary and 12.5% metastatic NETs tested [74]. Whereas KRAS mutations were often in PDACs and IPMNs, they were not detected in the PanNET cases tested in two studies [58][83]. VHL mutations indicated a diagnosis of SCA in some studies. Of interest, Vestrup Rift et al. found that although mutations in KRAS and GNAS genes were the most common ones found in IPMNs, they were not detected in the three SCAs tested [66][68][76].

Furthermore, the presence of a CTNNB1 mutation indicated SPN; Kubota et al. found a CTNNB1 mutation in all seven SPNs, yet in just 1/11 NETs and in none of the PDACs, acinar cell carcinomas and pancreatitis cases of their cohort [81].

2.6. NGS Performed on FNA vs. Tissue Biopsy Samples

Evidence has shown that FNA-based NGS was highly concordant with its matched tissue-based molecular analysis, where it often revealed additional alterations, modifying the management plan of the patients [54][61][73][75][85]. In addition, it exhibited superior sensitivity than PCR or Sanger sequencing [83]. Rapid on-site evaluation (ROSE), besides improving the diagnostic accuracy of EUS-FNA, facilitated the acquisition of material for subsequent NGS testing, sparing the patients from additional invasive procedures [60]. Of interest, FNB was more likely to result in adequate material for subsequent NGS testing than FNA (OR: 4.95; 95% CI: 1.11–22.05; p = 0.04) [70], whereas larger gauge biopsy needles were more likely to result in successful NGS findings [64].

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