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Pancreatic ductal adenocarcinoma harboring microsatellite instability / DNA mismatch repair deficiency. Towards personalized medicine

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ABSTRACT

Pancreatic cancer is a major cause of cancer-associated mortality, with a dismal overall prognosis that has remained almost unchanged for many decades. Pancreatic cancer has few prevalent genetic mutations. Available data on dMMR pancreatic cancer is limited and heterogeneous with regard to its prevalence and prognostic implications. Discordant results are mainly due to differences in detection methods and sample sizes. Interest in dMMR is growing since initial reports on immune checkpoint inhibition therapy for pancreatic cancer has shown it to be effective, generating impressive and durable responses. However, it has been accompanied by several questions regarding the appropriate screening, detection tools, patient selection, timing and modality of testing. Herein, we provide an extensive literature review and outline recommendations for testing.

1. Introduction

Malignant neoplasms of the pancreas are currently classified based on the type of the neoplastic cells (ductal, acinar, neuroendocrine), combined with the macroscopic appearance (solid or cystic) of the tumor. Pancreatic ductal adenocarcinoma (PDAC) comprises about 90% of all malignant pancreatic neoplasms [1]. PDAC is a leading cause of cancer mortality worldwide with over 330,000 new cases and approximately the same number of deaths annually [2,3]. PDAC stands in stark contrast to other major cancers in that both the incidence rate and death rate are increasing [4]. Indeed, the number of PDAC deaths is projected to surpass colorectal cancer around 2020 and to become the second leading cause of cancer death in the United States [5]. Prognosis of PDAC has remained largely unchanged, 5-year survival still being around 8% [6].

Surgical resection of PDAC offers the only opportunity for cure, but less than 20% of patients are eligible for resection at the time of diagnosis [7]; even following surgery and adjuvant chemotherapy, 5-year survival remains a dismal 20% [8]. In the majority of patients who are not able to undergo surgery, palliative chemotherapy, and for locally advanced disease, radio-chemotherapy extend overall survival to a few months only [9]. In this context, new therapeutics are therefore urgently needed and current research is largely focused in identifying potential new treatment targets and subsets of PDAC patients that may benefit from personalized treatment based on specific "targeted" approach.

2. Carcinogenesis and molecular abnormalities of PDAC

PDAC most frequently arises from pancreatic intraepithelial neoplasia (PanIN), the classic pre-neoplastic lesions, but also from intraductal papillary mucinous neoplasms (IPMN) and mucinous cystic neoplasms [10]. PDAC is molecularly diverse and display important heterogeneity that might explain the poor results of systemic therapy obtained so far. Somatic alterations in PDAC are now better characterized thanks to large whole-exome and whole-genome sequencing studies [11–14]. For the most part, PDAC have 50–80 exomic non-silent mutations. The molecular alterations reported in PDAC include gene

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deletions, mutations, amplifications and rearrangements [11,12].

KRAS is activated by point mutation in more than 90% of these cancers. It is seen in almost all early pancreatic cancer precursor lesions. Other recurrent (50–80%) genetic abnormalities that play critical roles in the development of pancreatic neoplasia include *CDKN2A, TP53* and *SMAD4,* whereas other genes, including *ARID1A, MLL3* and *TGFBR2*, are mutated in approximately 10% of tumors [15,16].

PDAC is characterized by a prominent desmoplastic reaction with a dense fibrotic stroma [17], and a typical primary pancreatic cancer often demonstrates only 5%–20% neoplastic cellularity [18]. This low tumor cellularity may confound the analyses of mutational and gene expression features of the actual neoplastic cells [14]. Indeed, most genome sequencing studies have focused on tumors with neoplastic cellularity greater than 40% [12], or have employed techniques that purify tumor samples [11,19].

2.1. MicroSatellite instability

MicroSatellite Instability (MSI) is one of the molecular abnormalities described in PDAC [20]. Microsatellites (MS) are short, repetitive sequences of 1–6 base pairs of DNA that are found throughout the genome, mostly in noncoding regions [21]. MSI is a tumoral phenotype due to a DNA Mismatch Repair (MMR) system deficiency, which is a system for recognizing and repairing the erroneous insertion, deletion, and misincorporation of bases that can arise during DNA replication and recombination, as well as for processing some forms of DNA damage (Fig. 1). MSI tumors develop through a distinctive molecular pathway characterized by genetic instability in numerous microsatellite DNA repeat sequences throughout the genome. The MSI phenotype was first described in the familial cancer condition known as Lynch syndrome (LS), where the MMR genes *MLH1, MSH2, MSH6* or *PMS2* harbor germline mutations. Also, germline 3′ end deletions affecting the epithelial cell adhesion molecule (EPCAM) gene located upstream of *MSH2* were identified as a novel mechanism causing LS by epigenetic inactivation of the respective *MSH2* allele [22]. MSI is also observed in approximately 10%–15% of sporadic colorectal, gastric, and endometrial cancers due to epigenetic, bi-allelic silencing of MLH1 expression by *de novo* methylation of the *MLH1* promoter [23] or acquired bi-allelic somatic mutations in MMR genes [24]. In tumor samples, the MSI phenotype can be determined by polymerase chain reaction (PCR) according to international criteria [25]. It is correlated with the loss of MMR protein expression affecting MLH1, MSH2, MSH6, or PMS2 by immunohistochemical study [26]. Very recently, we reported that the mononucleotide repeat of HSP110 (HT17), critical for correct splicing of the chaperone HSP110, might constitute a superior marker for diagnosis of the MSI phenotype in patients with CRC compared with the standard panel of markers [27].

3. PDAC and MSI

In PDAC, the MSI phenotype has been described with variable frequencies ranging from 0% to 75% (Table 1) [20,28–55]. Those contradictory results are related to the heterogeneity of used markers and selection of *at risk* subpopulations in most of published studies, and raise the question of the most appropriate approach to screening this type of tumor. In the last publications that analyzed large series of consecutive patients with PDAC, the “real” incidence rate of MSI PDAC was comprised between 1% and 2% [20,54,55].

PDAC, all types confounded, is associated with a low-moderate mutational burden, and is considered as a “cold” tumor for immunotherapy [56]. Owing to their high mutation rates, MSI is the foretype of the hypermutator phenotype which is characterized by frameshift mutations leading to truncated, functionally-impaired proteins as well as by elevated frequency of single-nucleotide variants (SNVs) [57] (Fig. 2). Although the precise link between the mutator phenotype with MSI remains to be elucidated, some recent studies have

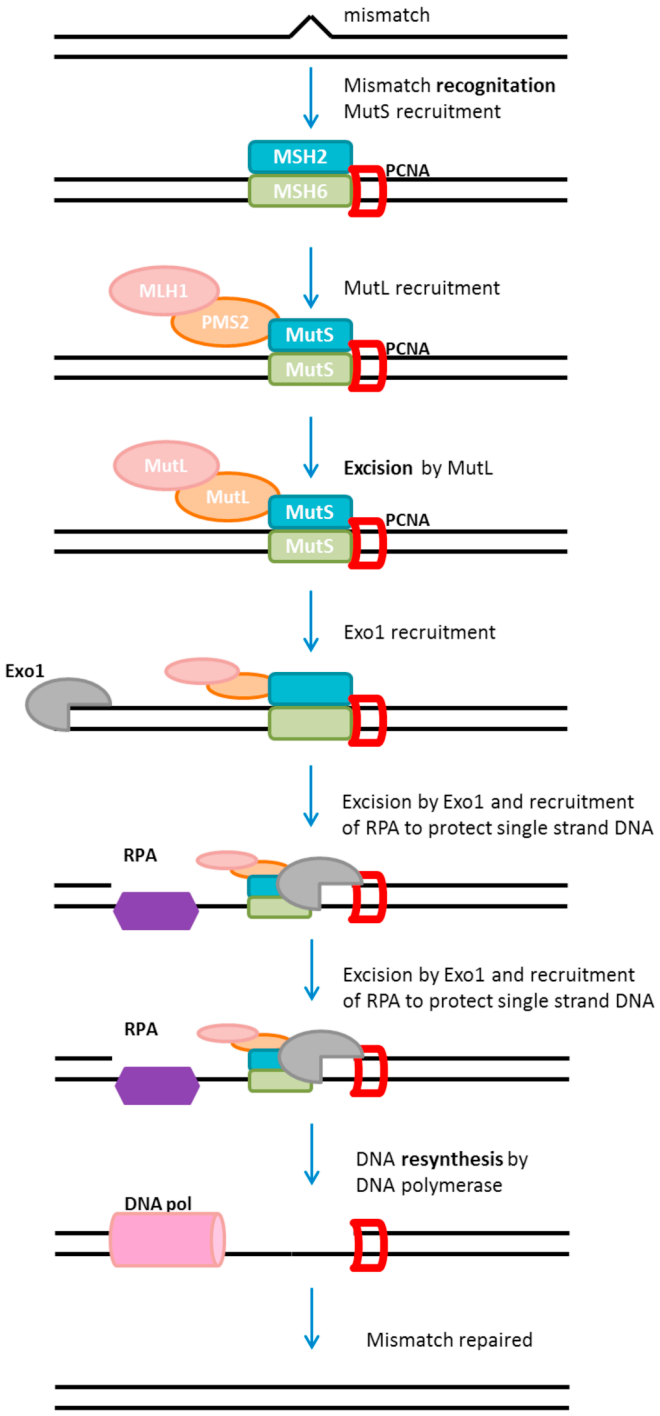

Fig. 1. The DNA MMR system functions through a series of steps. Mismatched DNA base pairs are recognized by MutS complex - MSH2–MSH6 (MutSα) recognizes single pair mismatches and small insertion/deletion loops (IDLs) whereas MSH2–MSH3 (MutSβ) complements this by also recognizing larger IDLs. Recruitment of MLH1-PMS2 (MutLα) complex following mismatch recognition. Proliferating cell nuclear antigen (PCNA) interacts with MutL. PCNA—MutL interaction mediates the endonuclease activity of PMS2. The DNA MMR protein sliding clamp interacts with exonuclease-1 and proliferating cell nuclear antigen (PCNA). This complex excises the daughter strand back to the site of the mismatch. RPA binds to the single-stranded DNA generated by excision to protect the strand, and also promotes excision termination. DNA polymerase synthesizes new DNA to fill the excised bases, and the new synthesized strand is sealed by DNA ligase.

Table 1
Literature review of the frequency of MSI in pancreatic cancers.

Author (year)	Type	Cases (n)	Number of MSI/ dMMR (%)	Methodology			Lynch Syndrome (n)	Survival analysis
				IHC[*]	PCR[§],[a]	NGS		
Han HJ (1993) [28]	pancreatic carcinoma[b]	9	6 (67%)	–	yes[c]	–	–	–
Seymour AB (1994) [29]	PDAC	7	0 (0%)	–	yes[c]	–	–	–
Brentnall TA (1995) [30]	pancreatic carcinoma[b]	8	6 (75%)[d]	–	yes[c]	–	–	–
Abe T (1996) [31]	pancreatic carcinoma[b]	44	7 (15.9%)[d]	–	yes[c]	–	–	–
Venkatasubbarao K (1998) [32]	pancreatic carcinoma[b]	14	4 (28.6%)[d]	–	yes[c]	–	–	–
Ouyang H (1997) [33] Ouyang H (1998) [34]	pancreatic carcinoma[b]	60	9 (15%)[d]	–	yes[c]	–	–	–
Goggins M (1998) [35]	PDAC	82	3 (3.7%)[d]	–	yes[c]	–	–	–
Ghimenti G (1999) [36]	pancreatic carcinoma[b]	21	0 (0%)	–	yes[c]	–	–	–
Wilentz RE (2000) [37]	medullary pancreatic carcinoma	18	4 (22.2%)	yes[e]	yes[c]	–	–	–
Ueki T (2000) [38]	pancreatic carcinoma[b]	36[f]	4 (11.1%)	–	yes[c]	–	–	–
Yamamoto H (2001) [39]	pancreatic carcinoma[b]	103	16 (15.5%)	—[g]	yes	–	3	–
Abraham SC (2002) [40]	ACC	13	1 (7.7%)	–	yes	–	–	–
Nakata B (2002) [41]	pancreatic carcinoma[b]	46	8 (17.4%)	–	yes[c]	–	–	*MSI associated with better survival*
Tomaszewska R (2003) [42]	pancreatic carcinoma[b]	30	0 (0%)	yes[e]	–	–	–	–
Lüttges J (2003) [43]	pancreatic carcinoma	23	1 (4.3%)	yes[h]	yes	–	–	–
Nakata B (2003) [44]	pancreatic carcinoma[b]	55	4 (7.2%)	yes[e]	–	–	–	*loss of MSH2 associated with longer survival*
Maple JT (2005) [45]	pancreatic carcinoma[b]	35[i]	3 (8.6%)	yes[h]	yes[c]	–	–	–
Fujii K (2009) [46]	PDAC	21	0 (0%)	–	yes[c]	–	–	–
Laghi L (2012) [47]	PDAC	338	1 (0.3%)	yes[e]	yes	–	–	–
Ottenhof NA (2012) [48]	PDAC	78	9 (12.8%)	yes[j]	–	–	–	–
Liu W (2014) [49]	ACC	36	5 (13.8%)	yes	–	–	2	*dMMR did not correlate with survival*
Mitsuhashi K (2015) [50]	PDAC	283	0 (0%)	–	yes[c]	–	–	–
Riazy M (2015) [51]	PDAC	265	41 (15.4%)	yes	–	–		*dMMR did not correlate with survival*[**]
Grant RC (2015) [52]	PDAC	290	4 (1.38%)	–	–	yes	4	–
Connor AA (2016) [53]	PDAC	255	4 (1.6%)	yes	yes	yes	3	–
Humphris JL (2017) [20]	PDAC	385	4 (1%)	yes	–	yes	–	–
Lupinacci RM (2018) [54]	PDAC	513[k]	8 (1.6%)	yes	yes	–	3[l]	*dMMR did not correlate with survival*
Hu ZI (2018) [55]	PDAC	833	7 (0.8%)	yes	yes	yes	7	*dMMR did not correlate with survival*[m]

MSI, microsatellite instable; MMR, mismatch repair; MN, mononucleotide marker; PDAC, pancreatic ductal adenocarcinoma; ACC, acinar cell pancreatic carcinoma. This table summarises main studies having investigated pancreatic cancer for MSI and/or dMMR.

[a] Unless otherwise indicated mononucleotide markers included any of: BAT-25, BAT-26, BAT-34C4, BAT-40, TGFβRII, NR-21, NR-22, NR- 24, NR-27, MONO-27, and MYCL. Whereas dinucleotide markers included: D2S123, D5S346, D17S250.[*] Unless otherwise indicated IHC analysis for MLH1, MSH2, MSH6 and PMS2 expression.[§] Unless otherwise indicated NCI panel (BAT-25, BAT-26, D2S123, D5S346, D17S250) or MSI PCR (with a panel containing 5 of the following 6 MN markers: BAT-25, BAT-26, NR-21, NR-22, NR-24, NR-27).

[b] Pancreatic carcinoma non specified otherwise.

[c] Non recommended panel of markers (nor NCI neither MSI PCR).

[d] Only microsatellite instability was reported (MSI-H was not specifically defined).

[e] Only IHC for MLH1 and MSH2 proteins.

[f] Thirty-five pancreatic cancer xenografts and 1 primary carcinoma; 3 of the 4 MSI tumors were previously reported by Goggins M et al.

[g] IHC not performed (immunoblotting for MLH1 expression “hardly detectable” in 5/10 sporadic MSI-H cases & negative in 3/3 LS cases).

[h] Only IHC for MLH1, MSH2 and MSH6 proteins.

[i] Defined population of long term survivors (> 3 years) of pancreatic carcinoma.

[j] Data obtained after personal contact with the senior author. dMMR were most probably 3/78 (2 MSH2/MSH6 and 1 MLH1/PMS2).

[k] 445 PDAC samples and 68 PDAC human xenografts.

[l] Based on revised Bethesda guidelines. No germline mutation analysis available.

[m] dMMR had a tendency to have localized disease at presentation and favourable natural history (mean survival of 96 months).** dMMR had no survival advantage from gemcitabine or 5-FU adjuvant chemotherapy.

used mutational burden to identify MSI PDAC in large series [20,55,58].

Two subtypes of pancreatic cancer are considered to be associated with a MSI phenotype. Medullary carcinomas of the pancreas are histologically distinct subset of poorly differentiated adenocarcinomas characterized by a distinctive “medullary” histological appearance (in which the cells form solid sheets and have abundant eosinophilic cytoplasm and large, vesicular nuclei with prominent nucleoli), usually associated with loss of hMLH1 expression and wild-type *KRAS*[37]. Acinar cell carcinomas of the pancreas (ACC) are highly cellular with a lobular architecture, scant fibrous stroma whereas necrosis is frequent and prominent in about 1/3 of cases. The diagnostic hallmark of ACC is the immunohistochemical demonstration of acinar-specific products such as trypsin, lipase, amylase, and carboxyl ester lipase [59]. We recently published our results on MSI screening of 445 resected PDAC (Table 1), where we showed a significantly higher frequency of MSI in IPMN-associated PDAC (6.9%) compared to non-IPMN PDAC (1.3%) [54]. Also, Hu et al. found MMR-deficient (dMMR) PDAC compared to MMR-proficient (pMMR) PDAC to have a tendency to be associated with IPMN and localized disease at presentation as well as a favorable natural history [55].

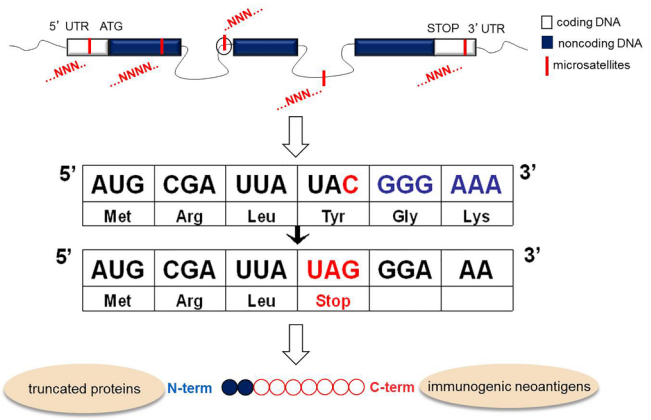

Fig. 2. Specific genomic regions, including microsatellites, are more prone for DNA polymerase slippage that can result in mismatch or insertion–deletion loop (IDL) in DNA during replication. Such DNA mismatches or IDLs not corrected by MMR can generate mis-sense or frameshift mutations in coding genes during subsequent rounds of DNA replication, resulting in dysfunctional proteins during translation. Also, insertions and deletions may potentially result in the transcription and translation of frameshift peptides with c-terminally altered amino acid sequences. These frameshift peptides are called neoantigens and are highly immunogenic, which explains the enhanced immunogenicity of MSI cancers.

3.1. Diagnosis of dMMR/MSI in PDAC

Unlike to colorectal cancer (CRC), where screening for LS is universally recommended, PDACs are not routinely tested for dMMR. Moreover, evaluation for dMMR by IHC and MSI by PCR were originally optimized for detecting dMMR/MSI in CRC and not PDAC.

IHC evaluation assesses the absence of MMR protein expression on the tumor tissue [60] (Fig. 3). It's widely available, inexpensive, and considered to be an extremely sensitive technique, usable even if very little tumor material is present within the core of a given PDAC sample. However, IHC interpretation can be limited by variations in tissue fixation and staining. The extent of IHC measurement is also dependent on the specific panel of antibodies used for staining. IHC testing in extra-colonic tissues have been reported to have a weaker internal control compared to colonic tissues [61]. Accordingly, even if IHC is usually considered to be reproducible, we do think that this method in PDAC should be done in a specialized environment and by experienced pathologists [54,62].

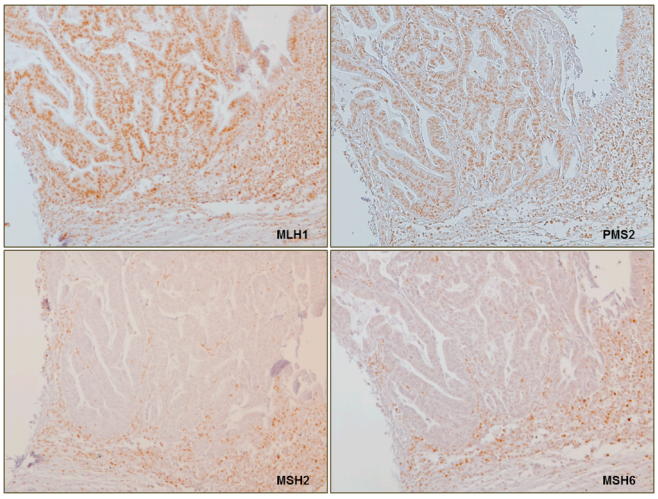

Fig. 3. Normal expression of MMR proteins consists of neoplastic cells with strong nuclear immunoreactivity for all four markers. Loss of protein expression is defined as complete absence of nuclear staining within tumor cells with concurrent positive labelling in internal non-neoplastic tissue (stromal and lymphoid cells). Here, we show a PDAC with complete loss of MSH2 and MSH6, and intact staining of MLH1 and PMS2.

The MSI phenotype is a consequence of an increased incidence of insertions or deletions within microsatellite sequences caused by dMMR. MSI PCR testing is used to detect MSI tumors by PCR amplification of a panel of five microsatellite markers. The first consensual panel proposed in 1998, "the Bethesda panel", consisted of two mononucleotide (BAT-25 and BAT-26) and three dinucleotide (D5S346, D2S123, and D17S250) repeats [25]. However, dinucleotide markers are highly polymorphic and need non-tumoral ADN comparative analysis [63]. Also, *MSH6* mutated tumors may not show variations in dinucleotide markers [64]. An alternative panel was proposed by our group in 2002, which replaces the dinucleotide markers with mononucleotide markers (NR-21, NR-24 (or NR-22), and MONO-27) [65]. Tumors with instability in two or more of these markers are listed as MSI-H, whereas those with one unstable marker are described as MSI-L [66]. Its main advantages are the high specificity and sensibility, and reproducibility. Moreover, it doesn't need constitutional and comparative analysis [67]. PCR MSI testing also has the potential to identify tumors with defective MMR but intact staining secondary to a non-truncating missense mutation [37,68].

Eventually, some recent studies have used mutational burden to identify MSI PDAC in large series. Cortes-Ciriano et al. analyzed the extent and characteristics of MSI in ~8000 exomes and ~1000 whole genomes spanning 23 tumour types, utilizing data from The Cancer Genome Atlas (TCGA) [58]. They found MSI in 2 out of 177 (1.1%) tumor samples. The monocentric series of Hu et al. using NGS assay designed to perform targeted deep sequencing of cancer-associated genes found a 0.8% frequency (7/833)[55]. This frequency is pretty the same found by Humphris et al. who interrogated 385 pancreatic cancer genomes for mutational signatures inferring defects in DNA repair. dMMR was identified in 1% of tumors [20]. Noteworthy, they state that immunohistochemistry was the most accurate method in defining MMR due to multiple genomic mechanisms of MMR gene inactivation. Our previously published results also found that IHC analysis using antibodies against the four MMR proteins was the most sensitive method for the assessment of MSI status in PDAC [54]. Moreover, in contrast to PCR MSI testing, IHC can help identify the affected gene, therefore directing germline mutation analysis to one gene [26].

3.2. PDAC and Lynch syndrome

It is estimated that a hereditary component may be implicated in nearly 10% of all PDAC cases, but currently in less than 20% of them a defined hereditary cancer predisposition syndrome with increased risk of PDAC development can be identified [69,70]. The more remarkable hereditary cancer predisposition syndromes with increased risk of PDAC are: hereditary breast and ovarian cancer syndrome, familial melanoma, Lynch syndrome, familial adenomatous polyposis, Peutz-Jeghers syndrome and Li-Fraumeni syndrome.

LS, previously known as hereditary non-polyposis colorectal cancer, is a cancer predisposing syndrome characterized by the autosomal dominant inheritance of a heterozygous germline mutation in one of the MMR genes (mutations in *MLH1*, *MSH2*, *MSH6* or *PMS2*) [71]. The proportion of mutations in LS is *MLH1* (40%), *MSH2* (34%), *MSH6* (18%) and *PMS2* (8%), although the PMS2 mutation frequency may be underdiagnosed due to technical issues and low penetrance of the mutation in affected families. Deletion of the last exons of EPCAM, located immediately upstream of the *MSH2* gene, is also involved in LS [72].

Population prevalence of LS is estimated at 1:440 [73]. In the hallmark study of Kastrinos et al. there was an 8.6-fold increase in risk of developing PDAC among families with pathogenic MMR gene mutations compared to the general population [74]. The estimated relative risk of PDAC was higher before age 50 years (HR 30.5 for ages 20–49

years, 95% CI: 14.2, 65.7). The absolute cumulative risk of developing PDAC in MMR gene mutation carriers at age 50 years was 1.31% and 3.68% at age 70 years that are significantly higher than the general population.

Reports have varied on whether dMMR/MSI PDAC arise largely from germline or sporadic mutations. In the study of Humphris et al. the authors identified private somatic events as the underlying cause of dMMR/MSI in all 4 cases [20]. In contrast, Connor et al. reported that of their 4 dMMR/MSI cases, 3 had germline and 1 had a somatic mutation [53]. *MLH1* promoter hypermethylation has also been reported as a cause of sporadic MSI PDAC [39]. Recently, all of the dMMR PDAC analyzed in the study of Hu et al. were found to have arisen in the context of LS, from germline mutations in MMR genes [55].

Given the relative rarity of dMMR PDAC, one shall not entirely exclude the possibility that dMMR PDAC can also arise from somatic mutations. However, it seems clear to us that dMMR PDAC patients should have germline mutation analyses in order to confirm or exclude LS.

3.3. Survival analysis of dMMR/MSI PDAC

Some authors reported dMMR/MSI PDAC patients to have a significantly prolonged survival time [39,41]. Nakata et al. reported that MSI PDAC compared favorably to MSS PDAC with survival times of 62 months vs. 10 months, respectively; p = 0.011[41]. Recently, Cloyd et al. reported a retrospective study of 10 MSI-PDAC patients from their familial and high-risk cancer clinic [75]. Six patients with localized disease who underwent surgery with curative intent had remarkably good outcomes with a 5-year overall survival rate of 100%, which is significantly better than the 25% reported in the largest randomized clinical trial of surgical resection and adjuvant chemotherapy [76]. Also, the 4 patients who presented with metastatic disease experienced better-than-expected outcomes. Hu et al. found dMMR/MSI PDAC to have a tendency to be associated with localized disease at presentation as well as a favorable natural history, with a mean survival time of 96.6 months (range 2–320 months) [55]. However, we and others didn't find prognostic differences between dMMR/MSI and pMMR/MSS PDAC [20,49,54]. The small number of dMMR/MSI PDAC patients in each series may explain these contradictory results.

4. MSI status and systemic treatment of PDAC

It is now well established that dMMR is not in itself a direct transforming event and that MSI tumors develop through a distinctive molecular pathway characterized by the genetic instability of numerous MS repeated sequences throughout the genome. These mutations accumulate in tumor cells together with other somatic alterations at non-repetitive DNA sequences [77]. dMMR may affect the natural history of malignancies through immunologic mechanisms, but this phenotype has also been associated with drug resistance [78].

4.1. Standard chemotherapy

In vitro studies have shown that dMMR cells are resistant to certain alkylating, methylating and platinum-containing agents as well as select antimetabolites [79]. The mechanisms of drug resistance in dMMR malignancies include increased tolerance to DNA damage, which allows for accumulation of (and selection for) critical mutations, inability to induce cell-cycle arrest, and/or defective apoptotic signalling [57,79,80].

To the best of our knowledge, only two studies demonstrated MMR status as a potential predictive factor in PDAC classic chemotherapy regimen [51,75]. Riazy et al. found that MMR status was able to separate resected PDAC patients into two groups with a significantly different efficacy of adjuvant chemotherapy with a pyrimidine analog [51]. Actually, while their pMMR cohort showed a 10-month increase in disease-specific survival with gemcitabine or 5-FU treatment, no statistically significant survival advantage was observed in the treated dMMR cohort. As mentioned above, Cloyd et al. reported a retrospective study of 10 MSI-PDAC patients from their familial and high-risk cancer clinic [75]. The 4 patients with metastatic disease received conventional systemic chemotherapy and experienced better-than-expected outcomes, achieving a median of 16.5 months' survival with one 5-year survivor (95%CI, 7.2–24.0) compared with a median of 11.1 months in patients treated with FOLFIRINOX (fluorouracil, leucovorin, irinotecan and oxaliplatin) and 6.8 months in patients treated with gemcitabine [81]. Both studies, however, have several limitations, mainly related to the small sample size, the single institution design, and their retrospective nature.

4.2. Immunotherapy

The MSI-driven pathway to cancer leads to the synthesis of aberrant and potentially immunogenic neo-antigens by the tumor cells, typically 10 to 50 times those of MSS tumors [82] (Fig. 2). A likely consequence is that MSI tumors are highly infiltrated with $CD8^+$ cytotoxic T-cell lymphocytes (CTLs) expressing activation markers, as well as activated Th1 cells with IFNγ production [82]. Recent studies have highlighted the concomitant expression of multiple active immune checkpoint (ICK) markers like PD-1, PD-L1, LAG-3, and TIM-3, which cause T cells functional exhaustion and unresponsiveness, and counterbalance the anti-tumoral TH1/CTL immune response, notably in MSI colorectal cancers [83]. Based on these findings, Le et al. evaluated in 2015 the clinical activity of an anti-PD-1 immune checkpoint inhibitor (pembrolizumab) in a cohort of metastatic colorectal and non-colorectal carcinoma patients with or without MSI [84]. None patient with PDAC was included in this study. The results of this phase 2 study convincingly showed that MSI status was able to predict clinical benefit from immune checkpoint blockade therapy with pembrolizumab, whereas anti-PD-L1 therapy in PDAC globally failed to demonstrate anti-tumor activity [85,86]. In 2017, the same team published another trial on PD-1 blockade efficacy in patients with advanced dMMR cancers across 12 different tumor types (including PDAC) [87]. Eighty-six consecutive patients were enrolled. Objective radiographic responses were noted in 53% of patients, with 21% achieving a complete radiographic response. Disease control was achieved in 77%. In the subgroup of 8 patients with PDAC, 2 patients had a complete response, 3 a partial response, 1 a stability and 2 were not evaluable for RECIST criteria. The objective response rate was similar between CRC versus other cancer subtypes. There was also no significant difference in the objective response rate between LS and non LS-associated tumors.

5. Conclusion

The incidence rate of MSI PDAC is inferior to 2%, and these tumors are frequently associated with specific histological features (medullary carcinoma, ACC, IPMN). Most MSI PDAC seems related to LS. This tumor phenotype could be associated with a better prognosis, in adjuvant as in metastatic stage, but also to resistance to some cytotoxics and a high sensibility to inhibitors of immune checkpoint. The advent of the last as an effective therapy for dMMR/MSI PDAC has been accompanied by several questions regarding the appropriate screening, detection tools, patient selection, timing and modality of testing. In our point of view, the recent medical literature data argues for a systematic screening of dMMR/MSI phenotype in PDAC, at least in the subgroups with specific histological/clinical characteristics including history of IPMN. We recommend that all PDAC patients be considered for dMMR/MSI screening considering the clinical implications of LS identification for the patients and its family relatives, as well as its effect on therapy choice. Because of the abundant stroma in PDAC and multiple genomic mechanisms of MMR gene inactivation, IHC appears today to be the most accurate method to screen MSI phenotype in PDACs, also allowing

to identify the MMR protein involved in each case. Nevertheless, we expect that molecular diagnosis of MSI would become feasible in the next few years because of the great improvement of MSI PCR through next generation sequencing. We thus recommend that both approaches could be used synergistically to detect dMMR/MSI in PDAC in the near future.

Disclosures

T. André has acted as a consultant or advisory roles for Bristol-Myers Squibb, MSD Oncology and Roche. JB Bachet has acted as a consultant or advisory roles for Amgen, Bayer, Merck Serono, and Servier.

Author contributions

RML (acquisition of data; analysis and interpretation of data; drafting of the manuscript; approval of the final version of the manuscript); **JBB** (conception of the study; drafting of the manuscript; approval of the final version of the manuscript); **TA** (revision of the manuscript; approval of the final version of the manuscript); **AD** (conception of the study; revision of the manuscript; approval of the final version of the manuscript); **MS** (acquisition of data; analysis and interpretation of data; approval of the final version of the manuscript).

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