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Comprehensive characterisation of pancreatic ductal adenocarcinoma with microsatellite instability: histology, molecular pathology and clinical implications

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ABSTRACT

Objective Recently, tumours with microsatellite instability (MSI)/defective DNA mismatch repair (dMMR) have gained considerable interest due to the success of immunotherapy in this molecular setting. Here, we aim to clarify clinical-pathological and/or molecular features of this tumour subgroup through a systematic review coupled with a comparative analysis with existing databases, also providing indications for a correct approach to the clinical identification of MSI/dMMR pancreatic ductal adenocarcinoma (PDAC).

Design PubMed, SCOPUS and Embase were searched for studies reporting data on MSI/dMMR in PDAC up to 30 November 2019. Histological and molecular data of MSI/dMMR PDAC were compared with non-MSI/dMMR PDAC and with PDAC reference cohorts (including SEER database and The Cancer Genome Atlas Research Network - TCGA project).

Results Overall, 34 studies with 8323 patients with PDAC were included in the systematic review. MSI/dMMR demonstrated a very low prevalence in PDAC (around 1%–2%). Compared with conventional PDAC, MSI/dMMR PDAC resulted strongly associated with medullary and mucinous/colloid histology ($p<0.01$) and with a *KRAS*/*TP53* wild-type molecular background ($p<0.01$), with more common *JAK* genes mutations. Data on survival are still unclear.

Conclusion PDAC showing typical medullary or mucinous/colloid histology should be routinely examined for MSI/dMMR status using specific tests (immunohistochemistry, followed by MSI-PCR in cases with doubtful results). Next-generation sequencing (NGS) should be adopted either where there is limited tissue or as part of NGS tumour profiling in the context of precision oncology, acknowledging that conventional histology of PDAC may rarely harbour MSI/dMMR.

Significance of this study

What is already known on this subject?

- Microsatellite instability (MSI) has recently gained considerable interest due to the success of immunotherapy in this molecular setting.
- MSI in pancreatic ductal adenocarcinoma (PDAC) is a molecular alteration with variable reported frequency.
- Tumours with MSI have perhaps a better prognosis and usually show a good response to immunotherapy.

What are the new findings?

- MSI in PDAC is very rare (around 1%–2% of cases).
- MSI PDAC are strongly associated with medullary and mucinous/colloid histology and are usually *KRAS-TP53* wild type.
- *JAK* and *KMT2* genes mutations are more common in this tumour type.
- Data on survival of MSI PDAC are still unclear.

How might it impact on clinical practice in the foreseeable future?

- The results of the present study show that MSI should be determined as part of a first-line routine analysis (immunohistochemistry; MSI-PCR in case of doubtful results; next-generation sequencing (NGS) in case of limited tissue) in PDAC with typical histology.
- In the context of precision oncology, for conventional PDAC, MSI should be assessed using NGS for analysing all potential therapeutic targets.

INTRODUCTION

Pancreatic cancer is a highly malignant disease that is projected to become the second most common cause of cancer-related death worldwide in the next decade.[1] Pancreatic ductal adenocarcinoma (PDAC) is the most common type of pancreatic malignancy, responsible for >95% of deaths from pancreatic cancer.[1] A large proportion (>75%–80%) of patients with PDAC present with locally advanced or metastatic disease, at time of diagnosis, therefore a surgical resection with curative intent is not possible. Even with radical resection and adjuvant chemotherapy, 5-year survival remains very poor

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(about 20%).[1] To improve survival of patients with PDAC, new therapeutic strategies are urgently needed. One of the main focuses of current research in this field aims at identifying new molecular targets and subgroups of PDAC that may benefit from personalised treatment, opening new landscapes for the so-called 'precision oncology'.[2]

In this context, tumours with microsatellite instability (MSI)/defective DNA mismatch repair (dMMR) represent a molecular subgroup of malignancies with novel therapeutic opportunities given the significant results of immunotherapy recently reported in this setting.[3 4] The mismatch repair system is a mechanism that recognises and repairs the erroneous insertion, deletion and misincorporation of bases that can arise during DNA replication and recombination and in some conditions of DNA damage.[3 4] Alterations affecting such a mechanism are defined as dMMR. Microsatellites are short and very repetitive sequences of 1–6 DNA base pairs that are found throughout the genome. Due to the repetitive nature, their alteration is typically present in cases of dMMR and is defined as MSI.[3 4] Tumours with MSI/dMMR usually accumulate thousands of mutations and are characterised by a hypermutated genome. Interestingly, this condition can be tested using immunohistochemistry (IHC) and molecular tests, including classic (PCR)-based microsatellite testing and novel next-generation sequencing (NGS) approaches.[4]

MSI/dMMR occurs in a respectable proportion of colorectal cancers (about 15%), is associated with distinct biological behaviour and differential response to different therapies, and thus routine screening is advocated in guidelines.[4] For PDAC, however, its frequency varies largely among different studies and a complete definition of MSI/dMMR PDACs is still lacking. Therefore, with this systematic review, coupled with a comparative analysis with existing databases, we aim at clarifying the true frequency of MSI/dMMR in PDAC, also highlighting the specific histological, immunohistochemical and molecular features of this tumour subtype.

MATERIALS AND METHODS

This systematic review adhered to the Meta-analyses Of Observational Studies in Epidemiology (MOOSE) guidelines and Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement,[5 6] following a predetermined protocol.

Inclusion and exclusion criteria

Studies were eligible if they met the following criteria: (1) original and complete study on human pancreatic cancer; (2) clear description of the method(s) used for testing MSI/dMMR; (3) clear report of the total number of cases of pancreatic cancer and the number of cases of MSI/dMMR pancreatic cancer; (4) publication in a peer-review journal in English language. Exclusion criteria were: (1) cancers from organs other than pancreas; (2) no invasive cancer (eg, intraductal papillary mucinous neoplasm (IPMN)), (3) no data regarding MSI/dMMR analysis; (4) case reports, abstracts and in vitro or animal studies.

Data sources and literature search strategy

Two investigators (CL, AS) independently searched PubMed, SCOPUS and Embase up to 30 November 2019. The search terms used in PubMed included combinations of the following keywords: ('MSI' OR 'microsatellite' OR 'dMMR' OR 'mismatch') AND ('pancreatic' OR 'pancreas'). A similar search was carried out in SCOPUS and Embase. We also considered the reference lists of all included articles and of previous related reviews.

Study selection

Following the searches as outlined above, after removal of duplicates, two independent reviewers (CL, AS) screened titles and abstracts of all potentially eligible articles. The two authors applied the eligibility criteria, reviewed the full texts and a final list of selected articles was reached through consensus with a third author (RTL). In case of doubled cohort, we selected the larger cohort and the most recent paper.

Data extraction, synthesis and statistical analysis

Two authors were involved in data extraction in a standardised Microsoft Excel database. Specifically, one author (CL) extracted data from the included articles and a second independent author (AS) validated the data. For each article, information about authors, year of publication, country of origin of the analysed cohort, number of patients, number of MSI/dMMR tumours, histological and molecular data on MSI/dMMR tumours, methods for MSI/dMMR testing, presence of Lynch syndrome and survival outcomes was extracted. Finally, all extracted data were reported and summarised in table 1, and then analysed, interpreted and discussed by all authors. To assess for differences in histological features between PDAC in unselected patients versus those with dMMR/MSI, a Fisher's exact test was used to compare our results with a large published cohort, specifically reporting the histological subtypes of patients with familial and sporadic pancreatic cancers.[7] This method has also been recently used by Hruban *et al* to compare the histology of an original cohort of *ATM*-mutated PDAC with that of conventional PDAC.[8] To further corroborate our results, a comparison was also carried out considering patients from the SEER database as another validation cohort.[9] In order to assess for differences in additional molecular features between PDAC in unselected patients versus those with dMMR/MSI, a Fisher's exact test was used to compare our results with data published by The Cancer Genome Atlas Research Network (TCGA), which we used as a reference cohort.[10] Furthermore, availing the manuscripts selected for this systematic review to assess differences in terms of survival, a meta-analysis comparing the prognostic outcomes of MSI/dMMR PDAC versus non-MSI/dMMR PDAC was performed using the programmes 'Comprehensive Meta-Analysis' and 'RevMan 5' (http://www.meta-analysis.com, last access 9 March 2020). Lastly, in order to define the presence of any potentially specific driver gene in MSI/dMMR PDAC, we analysed the existing literature on genetic drivers in MSI/dMMR tumours and reviewed, using linear comparisons, all available sequencing data from our systematic review.

RESULTS

Among 1712 potential eligible studies, 54 full-text articles were retrieved. Of them, 34 studies were eligible for this systematic review (table 1).[11–44] As reported in table 1, the 34 eligible studies included a total of 8323 patients. Of these, the total number of reported MSI/dMMR PDACs was 218, which corresponds to 2.61% of all patients with PDAC. This percentage represents a slight overestimation of the real prevalence of MSI/dMMR PDAC, since some studies are focused on PDAC subtypes apparently enriched by this molecular alteration. After removing those studies, the real prevalence of MSI/dMMR tumours was 2.53%. Regarding the methodology to assess MSI/dMMR in PDAC, 23 studies used PCR, whereas 13 used IHC and 8 NGS (some

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Table 1 Summary of the main features of all studies analysed in this systematic review

Study	Country	Tumour type analysed in the cohort	Number of tumours analysed for MSI	Number of MSI/dMMR (%)	Pancreatic site of MSI/dMMR tumours	Histology of MSI/dMMR tumours	Molecular data of MSI/dMMR tumours	Methodology for MSI analysis IHC PCR NGS	Lynch syndrome	Survival data of MSI/dMMR tumours
Han *et al*[11]	Japan, Korea	PC	9	6 (66.6%)	NA	NA	NA	PCR[1]	NA	NA
Seymour *et al*[12]	USA	PDAC	7	0 (0%)	NA	NA	NA	PCR[2]	NA	NA
Brentnall *et al*[13]	USA	PC	13	8 (62%)	NA	NA	NA	PCR[3]	NA	NA
Abe *et al*[14]	Japan	PC	44	7 (15.9%)	NA	NA	NA	PCR[4]	NA	NA
Venkatasubbarao *et al*[15]	USA	PDAC	14	4 (28.6%)	NA	PDAC G3	NA	PCR[5]	NA	NA
Ouyang *et al*[16]	Japan	PC	60	9 (15%)	NA	NA	NA	PCR[2]	NA	NA
Goggins *et al*[17]	USA	PDAC	82	3 (3.7%)	3 head (100%)	MED	*KRAS* wt	PCR[6]	NA	2/3 AWD at 16 and 52 months, 1/3 DOD after 4 months
Ghimenti et al[18]	Italy	PC	21	0 (0%)	NA	NA	NA	PCR[7]	NA	NA
Caligo *et al*[19]	Italy	PC	31	13 (42%)	NA	NA	NA	PCR[2]	NA	NA
Wilentz *et al*[20]		MED	18	4 (22.2%)	NA	MED; 1/4 showed also microglandular features. No associated PanIN	*KRAS* wt	IHC[a], PCR[8]	1	3/4 AWD at 13, 24 and 67 months, 1/4 DOD after 4 months
Ueki *et al*[21]	USA	PDAC	36	4 (11.1%)*	NA	MED	2/4 harboured promoter hypermethylation of *hMLH1*	PCR[2]	NA	NA
Yamamoto *et al*[22]	Japan	PDAC	103	16 (15.5%)	NA	10/13 PDAC G3, 2 G2, 1 G1	10/13 *KRAS* wt; 11/13 *TP53* wt	PCR[9]	3	MSI associated with better survival
Moriyama *et al*[23]	Japan	PDAC	18	2 (11.1%)	NA	NA	NA	PCR[10]	NA	NA
Nakata *et al*[24]	Japan	PC	46	8 (17.4%)	NA	5/8 G1, 3 G2-G3-G4	NA	PCR[11]	NA	MSI associated with better survival
Tomaszewska *et al*[25]	Poland	PDAC	30	0 (0%)	NA	NA	NA	IHC[b]	NA	NA
Lüttges *et al*[26]	Germany	11 M/C-C and 12 PDAC	23	1 (4.3%)	1 head (100%)	M/C-C, pT4 (8 cm) N1	NA	IHC[c], PCR[9a]	NA	NA
Nakata *et al*[27]	Japan	PC	55	4 (7.2%)	2 head (50%), 1 body (25%), 1 tail (25%)	2 head: G1; 1 body: G4; 1 tail: G3	NA	IHC[b]	NA	Loss of MSH2 associated with initial better survival
Maple *et al*[28]	USA	LS-PC	35	3 (8.6%)	NA	1 MED†, 2 PDAC	Germline *MLH1* mutation in 1 patient	IHC[c], PCR[12]	1	NA
Fujii *et al*[29]	Japan	PDAC	21	0 (0%)	NA	NA	NA	PCR[13]	NA	NA
Laghi *et al*[30]	272 cases Italy, 66 Germany	PDAC	338	1 (0.3%)	Head	G3, pT4N2	*KRAS*^{cod12} mutation, *BRAF* wt	IHC[d], PCR[9b]	No	NA (died of postsurgical complication)
Ottenhof *et al*[31]	The Netherlands	PDAC	78	3 (3.9%)‡	NA	NA	NA	IHC[d]	NA	NA
Mitsuhashi *et al*[32]	Japan	PDAC	283	0 (0%)	NA	NA	NA	PCR[14]	NA	NA
Riazy *et al*[33]	Canada	PDAC	265	41 (15.4%)	NA	NA	NA	IHC[d]	NA	dMMR did not correlate with survival§
Grant *et al*[34]¶	Canada	PDAC	290	4 (1.38%)	NA	NA	Germline mutations in 4 patients: 1) *MLH1*$^{c.677+3A>G}$; 2, 3) *MSH2*$^{c.942+3A>T, c.1906g>c}$; 4) *MSH6*$^{c.1707delC}$	NGS[a]	4	NA
Connor *et al*[35]	Canada	PDAC	255	4 (1.6%)	NA	NA	NA	IHC[d], PCR[9a], NGS[b]	3	NA
Humphris *et al*[36]	Australia (international cohort)	PDAC	385	4 (1%)	NA	1 case G4, 2 cases G2, 1 signet ring	All high TMB (100%), 2 *KRAS* wt (50%)	IHC[d], NGS[c]	No	NA
Salem *et al*[37]	USA	PDAC	870	12 (1.4%)	NA	NA	NA	NGS[d]	No	
Lupinacci *et al*[38]	France	PDAC	513	8 (1.6%)	NA	3pT1, 3pT2, 3pT3; 1 case medullary, 1 colloid, 6 conventional	NA	IHC[d], PCR[9]	3	dMMR did not correlate with survival
Wartenberg *et al*[39]	Greece	PDAC	110	5 (4.5%)	NA	Stroma rich in immune cells with a very high stromal CD8/FOXP3 ratio	High prevalence of *JAK3* mutations (3/5 cases vs 4/105 MSS PDAC); all *KRAS* mutated, 2/5 TP53 wt	IHC[d]	NA	Survival analysis not specific for MSI status

Continued

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Table 1 Continued

Study	Country	Tumour type analysed in the cohort	Number of tumours analysed for MSI	Number of MSI/dMMR (%)	Pancreatic site of MSI/dMMR tumours	Histology of MSI/dMMR tumours	Molecular data of MSI/dMMR tumours	Methodology for MSI analysis IHC PCR NGS	Lynch syndrome	Survival data of MSI/dMMR tumours
Hu *et al*[40]	USA	PDAC	833	7 (0.8%)	NA	2 conventional, 4 mucinous/colloid IPMN-associated, 1 medullary**	All available for NGS (5 cases) had high TMB	IHC[d], PCR[9b], NGS[e]	7	dMMR did not correlate with survival
Mori *et al*[41]	Japan	PC	40	0 (0%)	NA	NA	NA	PCR[15]	NA	NA
Latham *et al*[42]††	USA	PDAC	824	34 (4.1%)	NA	NA	5 patients with germline mutations: 1) *MLH1*$^{c.1731G.A;\ p.Ser577Ser}$; 2) *MSH2*$^{c.1906G.C;\ p.Ala636Pro}$; 3) *MSH2*$^{c.2038C.T;\ p.Arg680*}$; 4) *PMS2*: deletion exon 11; 5) *MSH6*$^{c.3268G.T;\ p.Glu1090*}$	NGS[e]	5	NA
Kato *et al*[43]	Japan	PC	10	0 (0%)	NA	NA	NA	NGS[e]	NA	NA
Singhi *et al*[44]	USA	PDAC	2563	3 (0.1%)	NA	NA	3 *KRAS* wt, 1 TP53 wt, 1 case showed the druggable *FGFR2-POC1B* fusion; 1/3 high TMB; 3 cases harboured *KMT2* genes mutations (2 *KMT2D* and 1 *KMT2C*) and 2 a *JAK1* mutation	NGS[f]	NA	NA
Total	–	–	8323	**218 (2.61%)**	**7 head, 1 body, 1 tail**	**36 conventional PDAC, (3 G4, 16 G3, 5 G2, 8 G1, 4 NOS); 10 MED, 6 MC/C, 1 signet ring**	**Significant association of MSI/dMMR with *KRAS* and *TP53* wt status and with *JAK* and *KMT2* mutations**	–	–	**Not significant association with survival**

Microsatellite instability analysis with PCR: [1] PCR with not recommended panel of markers (nor NCI neither MSI PCR): D2S123, D2SI36 and D3S1067; [2] PCR with not recommended panel of markers (nor NCI neither MSI PCR), not further specified; [3] PCR with not recommended panel of markers (nor NCI neither MSI PCR): D2S123, D2S136, D3S1067, D5S107, D6S87, D8S255, D10SI97, D11S904, D17S261, D17S361, D17S787, D18S34; [4] PCR with not recommended panel of markers (nor NCI neither MSI PCR): D1S199, D2S123, D3S1298, TP53, D22S284; [5] PCR with not recommended panel of markers (nor NCI neither MSI PCR): D2S123, D2S136, D3S1067, D5S107, D6S87, D18S34; [6] PCR with not recommended panel of markers (nor NCI neither MSI PCR): BAT26, D10S579, D1OS541, D9S272, D9S258, D9S1809; [7] PCR with not recommended panel of markers (nor NCI neither MSI PCR): D2S313, D2S123, D5S404, D8S255, D10S197, D11S904, D17S250, THRA1, D17S579, D17S396; [8] PCR with not recommended panel of markers (nor NCI neither MSI PCR): length analysis of BAT25 and BAT26 markers; and direct sequencing of the polythymidine tract of the *TGFBR2* gene; [9] PCR with NCI / MSI PCR markers: 9[a] BAT25, BAT26, D2S123, D5S346, D17S250, or 9[b] BAT25, BAT26, NR-21, NR-24 and NR-27; [10] PCR with not recommended panel of markers (nor NCI neither MSI PCR): D2S123, D3S1067, D9S171, D9S1870, D18S58, D18S46, D18S474; [11] PCR with not recommended panel of markers (nor NCI neither MSI PCR): D2S123, D3S1611, D5S346, D7S501, NM23, TP53-Penta, TP53-Dint and D18S35; [12] PCR with not recommended panel of markers (nor NCI neither MSI PCR): Bat 26, Bat 25, Bat 40, Bat 34c4, D17s250, D5s346, ACTC, D18s55, D10s197 and mycL; [13] PCR with not recommended panel of markers (nor NCI neither MSI PCR): D2S123, D5S107, D10S197, D11S904, D13S175; [14] PCR with not recommended panel of markers (nor NCI neither MSI PCR): BAT25 and BAT26; [15] PCR with not recommended panel of markers (nor NCI neither MSI PCR): MYCL1, D9S242, D8S321, D20S82, D20S85, BAT-25, BAT-26, NR-21, NR-22, NR-25.
Microsatellite instability analysis with IHC: [a] IHC for MLH1 and MSH2: all MSI cases in this series showed MLH1 loss and MSH2 retained. This study first demonstrated the reliability of IHC in determining MSI in pancreatic cancer; [b] IHC for MLH1 and MSH2; [c] IHC for MLH1, MSH2 and MSH6; [d] IHC for MLH1, PMS2, MSH2 and MSH6.
Microsatellite instability analysis with NGS: [a] NGS using a custom panel targeting the exonic and splice site regions of 385 genes previously associated with cancer; [b] Whole-genome sequencing variant calls, RNA sequencing and microarray expression values available from the International Cancer Genome Consortium data portal; [c] NGS with 'MSI sensor' methodology; [d] MSI-NGS assessment with the reference genome hg19 from the University of California, Santa Cruz—'Genome Browser database'; [e] MSK-IMPACT panel, 'MSI sensor' methodology; [f] Illumina HiSeq technology, MSI tested using 114 loci.
*In this study, 4 out of 36 cases were MSI, but these cases were specifically and deliberately added by authors to expand the spectrum of cases to be studied for hypermethylation; in addition, 3 of the 4 MSI tumours were previously reported by Goggins *et al*.
†First reported case of medullary phenotype associated with MSH2 loss (previously reported cases were all MLH1 negative).
‡Data obtained from another manuscript (Lupinacci *et al*[48]), two cases were MSH2-MSH6 negative, and one case was PMS2-MLH1 negative.
§dMMR had no survival advantage from gemcitabine or 5-fluorouracil adjuvant chemotherapy.
¶This study investigated germline mutations.
**This case is probably a medullary PDAC (described as a poorly differentiated carcinoma with fused glands and some necrotic areas).
††This study investigated >50 cancer types, with patients with PDAC representing 5.5% of the entire cohort.
AWD, alive without disease; dMMR, defective mismatch repair; DOD, died of disease; IHC, immunohistochemistry; LS-PC, long survivors-pancreatic cancer (>36 months after surgery); M/C-C, mucinous/colloid carcinoma of the pancreas; MED, medullary pancreatic cancer;MN, mononucleotide marker; MSI, microsatellite instability; NA, not assessed (or not reported); NGS, next-generation sequencing; PC, pancreatic cancer, not otherwise specified; PCR, polymerase chain reaction; PDAC, pancreatic ductal adenocarcinoma; TMB, tumour mutation burden; wt, wild type.

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studies used more than one method for MSI/dMMR assessment; table 1). However, the methods applied in different studies greatly varied, even in the case of the same category of analysis. In fact, 14 different PCRs were described, with only 6 studies (26% of all PCR-based studies) using the standardised NCI/MSI PCR markers.[4] A similar situation was observed for IHC, with four different types of analyses and only seven studies (53.8%) using the standardised antibodies.

Considering the prevalence of MSI/dMMR alterations based on the methods used for its determination, prevalence was lower in studies that used NGS (68/6030, 1.1%) alone or in combination compared with studies using PCR and/or IHC (150/2293, 6.5%), reaching a statistically significant value (Fisher's exact test; p<0.01).

The first aspect to be analysed considering histopathological data is the pancreatic site in which MSI/dMMR tumours arise. Based on reported data of tumour location in the pancreas, the vast majority of MSI/dMMR PDACs (78%) have been described in the pancreatic head. The prevalence of tumour location in MSI/dMMR tumours was not statistically significantly different from the reference cohort of familial and sporadic PDACs nor from SEER database. Next, regarding the histology of MSI/dMMR tumours, conventional PDAC represented the 67.9% of the whole cohort of this systematic review, whereas 18.9% were medullary PDAC, 11.3% were mucinous/colloid PDAC and 1.9 were of the signet ring variant. The prevalence of medullary and mucinous/colloid variant of PDAC was higher than observed in patients with familial and sporadic PDAC in the reference cohorts of Singhi *et al*[7] and in the SEER database (p<0.01),[9] indicating that these subtypes arise more typically in the MSI/dMMR molecular background.

Some studies also reported molecular data in addition to MSI/dMMR status. The vast majority of this subgroup of PDAC were wild type for *KRAS* (22/33, 66.6%) and *TP53* (14/21, 66.6%): these values were statistically significantly different from the usual molecular profile of PDAC, as resulted from a comparison with data from TCGA cohort (p<0.01). Regarding the studies that also assessed tumour mutational burden (TMB),[36 40 44] 85.7% of MSI/dMMR PDAC also showed high TMB. Singhi *et al* also reported results from NGS of a large PDAC cohort (3594 cases): interestingly, one case among the three detected MSI/dMMR PDACs harboured the druggable *FGFR2-POC1B* fusion.[44]

Regarding the presence of any potential specific driver genes in MSI/dMMR PDAC, we found a bi-univocal correspondence regarding genes belonging to the JAK/STAT pathway and those of *KMT2* family. Indeed, these have been described as frequently mutated in MSI/dMMR cancers of different extra-pancreatic sites[45 46]; the review of all molecular data of MSI/dMMR PDAC showed the involvement of the JAK/STAT pathway also in MSI/dMMR PDAC, given that the paper by Wartenberg *et al*,[39] reported a higher mutation rate of *JAK3* specifically in this genetic subgroup (3/5 MSI/dMMR cases vs 4/105 microsatellite-stable PDAC, p<0.01, Fisher's exact test; all these cases were *KRAS* mutated), and in the paper by Singhi *et al*, two of the three reported MSI/dMMR PDAC harboured a *JAK1* mutation (2/3 MSI/dMMR PDAC vs 0/608 microsatellite-stable PDAC with actionable targets, p<0.01, Fisher's exact test).[44] Furthermore, we found that alterations affecting the *KMT2* family were involved as well, since 3/3 MSI/dMMR cases described by Singhi *et al* harboured *KMT2* mutations (two cases with *KMT2D* and one case *KMT2C* mutation; 3/3 *KMT2* mutated MSI/dMMR PDAC vs 32/608 *KMT2* mutated microsatellite-stable PDAC with actionable targets, p<0.01, Fisher's exact test; the MSI/dMMR and *KMT2* mutated cases were *KRAS* wild type).

Regarding the association of MSI/dMMR pancreatic cancers with Lynch syndrome, a total of 27 cases were reported in the background of this genetic condition. Integrating histological data when available (18 cases) from the original papers, 9/18 (50%) had conventional histology, whereas 4/18 (22.2%) were medullary and 5/18 (27.7%) were mucinous/colloid. Comparing this prevalence with that of all the non-hereditary MSI/dMMR PDACs, there were no statistically significant differences between the two cohorts.

The final important aspect to analyse is regarding the survival of patients with MSI/dMMR PDAC. We performed a meta-analysis for calculating the relative risks for overall survival (OS), disease-specific survival (DSS) and also for 'all-types' of survival (ATS, putting together OS and disease-free survival), to find any potential association between MSI/dMMR and prognosis in PDAC. No data for calculating the HRs were present. The results on risk ratios showed that there is not a significant impact on the survival for MSI/dMMR in PDAC (OS: p=0.36; DSS: p=0.50; ATS: p=0.16 ; online supplementary figures 1–3). At the same time, it is also of importance to highlight the high heterogeneity of the results (I^2=86%, 88% and 63% for OS, DSS and ATS, respectively) and that there are too few data (only five manuscripts) to draw any definitive conclusion.

DISCUSSION

With this systematic review-based study, we have definitively clarified that MSI/dMMR in PDAC: i) has a very low prevalence (1%–2%); ii) is strongly associated with medullary and mucinous/colloid histology; iii) is associated with a *KRAS/TP53* wild-type molecular background, and more common *JAK* (*JAK1* and *JAK3*) and *KMT2* (*KMT2C* and *KMT2D*) genes mutations and iv) does not show a clear survival benefit, as for example in colorectal cancer.

Regarding the prevalence of MSI/dMMR in PDAC, it is around 2.5% considering all published data, but this value goes down significantly to 1.1% when considering only studies that use more recently developed, standardised and validated NGS techniques. Thus, the percentage of 2.5% appears as an overestimation of the real MSI/dMMR prevalence in PDAC. This may be due, at least in part, to the different and not validated methods used in the past for MSI/dMMR assessment. Indeed, 15 different PCR tests and 4 different IHC panels have been used considering all the studies selected for this systematic review. However, only six studies based on IHC/PCR used the suggested and standardised IHC antibodies and/or NCI/MSI PCR markers.[9 47 48] It is also important to acknowledge that the NCI guidelines regarding MSI testing were first published in 1998,[47] thus papers published up to this time could not have adopted an NCI panel. The most important MSI marker in the initial NCI guidelines was BAT26, which is a highly sensitive and specific marker of MSI. Some early manuscripts reported high levels of MSI-likely but these were potentially due to inappropriate microsatellite markers. Contrary to this situation, all studies based on NGS appeared more reliable: they used NGS coupled with validation tools, analysed larger cohorts and gave more homogeneous results, with a range of MSI/dMMR prevalence from 0% to 1.6% (mean value of 1.1%). Based on these considerations, the real prevalence of MSI/dMMR in PDAC could be reasonably considered to be around 1%–2%, or even less (<1%). Furthermore, along these lines, it is evident that the use of reliable and standardised procedures is mandatory.

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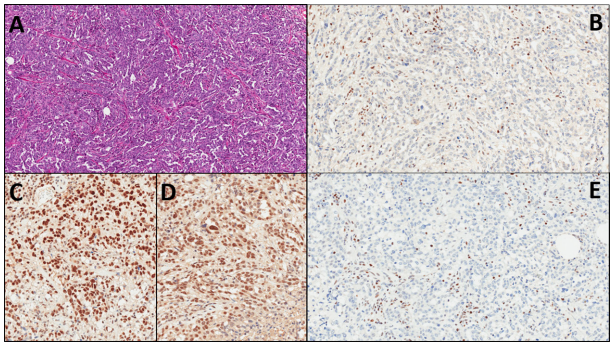


Figure 1 A classical example of a MSI/dMMR medullary pancreatic ductal adenocarcinoma. The medullary variant is a typical histological aspect associated with MSI/dMMR in pancreatic ductal adenocarcinoma. At the immunohistochemical level, the loss of expression of one heterodimer of the mismatch repair proteins (MSH2 with MSH6, MLH1 with PMS2) is a reliable surrogate of MSI. In this representative case, there is the loss of the expression of MSH2-MSH6 proteins. (A) Medullary histology: this pattern is characterised by a syncytial growth with marked lymphocytes infiltration (H&E staining, original magnification: 4×). (B) Immunohistochemical analysis for MSH2 shows the loss of the protein in tumour cells. The positive cells inside the tumour area are lymphocytes, endothelial and stromal cells (original magnification: 10×). (C, D) Immunohistochemical analysis for MLH1 (C) and PMS2 (D) shows positive staining also in tumour cells (expression of the protein; original magnification: 20×). (E) Immunohistochemical analysis for MSH6 shows the loss of expression of the protein in tumour cells. The positive cells inside the tumour area are lymphocytes, endothelial and stromal cells (original magnification: 10×). dMMR, defective mismatch repair; MSI, microsatellite instability.

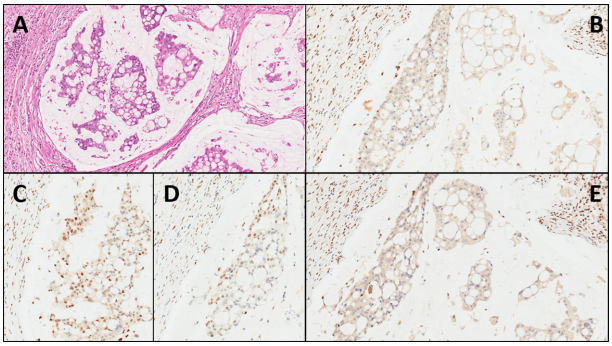


Figure 2 A classical example of a MSI/dMMR mucinous/colloid pancreatic ductal adenocarcinoma. The mucinous/collolid variant is a typical histological aspect associated with MSI/dMMR in pancreatic ductal adenocarcinoma. At the immunohistochemical level, the loss of the expression of one heterodimer of the mismatch repair proteins (MSH2 with MSH6, MLH1 with PMS2) is a reliable surrogate of MSI. In this representative case, there is the loss of expression of MLH1-PMS2 proteins. (A) Mucinous/colloid histology: this pattern is characterised by large mucin pools with floating tumour cells/clusters (H&E staining, original magnification: 10×). (B) Immunohistochemical analysis for MLH1 shows the loss of the protein in tumour cells. The positive cells in the periphery are lymphocytes, endothelial and stromal cells (original magnification: 20×). (C, D) Immunohistochemical analysis for MSH2 (C) and MSH6 (D) shows positive staining also in tumour cells (expression of the protein; original magnification: 20×). (E) Immunohistochemical analysis for PMS2 shows the loss of expression of the protein in tumour cells. The positive cells in the periphery are lymphocytes, endothelial and stromal cells (original magnification: 20×). dMMR, defective mismatch repair; MSI, microsatellite instability.

An important point concerns tumour site within the pancreas. There were no statistically significant differences between conventional PDAC and MSI/dMMR PDAC, with pancreatic head confirmed as the elective location also for this PDAC subgroup. However, this tumour location may also be responsible for the overestimation of the true prevalence of MSI/dMMR in PDAC. Indeed, large ampullary/periampullary-duodenal cancers with pancreatic infiltration may be misdiagnosed as PDAC (in these cases it could be very difficult to establish the real site of origin) and MSI/dMMR is a molecular alteration more typical of neoplasms with intestinal differentiation.[9 49]

Regarding the histology of MSI/dMMR PDAC, medullary and mucinous/colloid variants of PDAC resulted significantly more common in this PDAC subgroup (two representative cases, including the immunohistochemical pattern, are illustrated in figures 1 and 2). However, these histological subtypes are not always associated with MSI/dMMR. Indeed, medullary histology can be found in microsatellite stable PDAC, for example, in association with Epstein-Barr virus (EBV) infection.[20] Similarly, mucinous/colloid features can be found in microsatellite stable PDAC, for example, in association with *GNAS* (also in association with pre-existing IPMN) or germline-*ATM* mutations.[8 38 50] It should be reiterated here that medullary and colloid carcinoma are significantly more common in the ampulla than in the pancreas, and considering the well-known proneness of ampullary cancers to be mistaken as pancreatic origin,[51] in a case with this diagnosis, the possibility of a secondary invasion from the ampulla, or even a metastasis from the colon ought to be carefully excluded.[52] However, due to the strong association of these two PDAC variants with MSI/dMMR, for cases of medullary and mucinous/colloid histology, the final pathology report should be integrated with the assessment of MSI/dMMR status. This should be performed using IHC as first-line analysis, also following existing guidelines,[4] and, only in the case of doubtful or not reliable IHC results, MSI-based PCR should be executed. Considering the different advantages and limitations of the methods of MSI testing in PDAC (which have been summarised in table 2), NGS is recommended as first-line analysis in the case of limited tissue, and in the context of precision oncology.

Taking into account the genetic profile of MSI/dMMR PDAC, this systematic review highlighted that the vast majority of this molecular subgroup is *KRAS* and *TP53* wild type. This is a very unusual profile for PDAC, which calls for further genetic analysis for the selection of therapeutic strategies. Indeed, *KRAS* wild-type PDAC, although unusual, include a heterogeneous group of neoplasms that may have potential targets for precision medicine. These comprise MSI/dMMR, and other important genetic alterations, such as those involving *BRAF* gene, and kinase fusion genes (eg, *FGFR2* and *NTRK* fusions).[44 53] Notably, one case with *FGFR2* fusion has been described in the context of MSI/dMMR.[44] Moreover, TMB resulted high in the majority of MSI/dMMR PDAC, and this represents another variable strictly associated with benefits from immunotherapy. Further studies in PDAC should also address whether better response to immunotherapy could be reached where there is co-existence of MSI/dMMR and high TMB, such as in colorectal cancer.[54] We also found additional potential driver genes typically involved in MSI/dMMR PDAC: *JAK* (*JAK1* and *JAK3*) and *KMT2* (*KMT2C* and *KMT2D*). *JAK* genes code for a homonymous family of kinases, which are required for the signalling of a host of immune modulators in tumour, stromal and immune cells; alterations in this family have been associated with an immune evasion by tumour

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Table 2 Advantages and limitations of the different methods for assessing MSI/dMMR status in pancreatic ductal adenocarcinoma

Advantages	Limitations
Immunohistochemistry	
Widely available and reliable in PDAC using the staining for the four classical MMR proteins MLH1, PMS2, MSH2, MSH6 (above all for surgical specimens—'large' amount of tissue)	Suboptimal tissue fixation may impact its reliability.
Economical	Limited by antibodies available.
Reproducible	Limited by the amount of tissue. Limited/inadequate tissue can lead to false loss of MMR proteins in PDAC.
Rapid turn-around time	Can give false results (eg, loss of expression of one MMR protein) in case of the presence of a different partner of MMR proteins in the usual MLH1-PMS2 and MSH2-MSH6 heterodimers (eg, MLH1-PMS1, MSH2-MSH3).
More sensitive than MSI-PCR testing in detecting absence of MSH6	
MSI-PCR	
Reproducible	Not able to detect the specific mutated gene.
Can detect MSI/dMMR tumours that have intact MMR protein staining on IHC	Less sensitive than MSI-PCR testing in detecting absence of MSH6.
Rapid turnaround time	
NGS	
Reliable also in case of limited tissue/biopsy (also for EUS-FNB)	Expensive.
Can detect simultaneously specific somatic and germline mutations of different genes	Still not widely available.
Can also be used to assess MSI and TMB	Longer turnaround time.
Can identify targetable mutations	

dMMR, defective mismatch repair; EUS-FNB, endoscopic ultrasound-guided fine-needle biopsy; IHC, immunohistochemistry; MMR, mismatch repair; MSI, microsatellite instability; NGS, next-generation sequencing; PDAC, pancreatic ductal adenocarcinoma; TMB, tumour mutational burden.

cells.[45] *KMT2* genes code for a homonymous family of methyltransferases, which are the effectors of histone H3 methylation, one of the epigenetic mechanisms regulating gene transcription.[46] In case of mutations, both *JAK* and *KMT2* genes have been already described as potential drivers in MSI/dMMR tumours of other sites,[45 46] and we highlighted their potential involvement also in MSI/dMMR PDAC, further refining the knowledge on the genetic landscape of this tumour entity.

Regarding survival of MSI/dMMR patients with PDAC, this systematic review revealed that there are no significant improvements in survival outcomes for this subgroup of patients. However, regarding this point, the results of our meta-analysis cannot be considered definitive, because available data on this aspect are still limited and also because of their high heterogeneity; further studies are needed to address this important point. Indeed, although MSI/dMMR is a well-recognised prognostic moderator of some cancers, with a strong association to better prognosis such as in colorectal, gastric, duodenal and ampullary cancers,[4] in PDAC such survival improvement is not so clear. The morphological and genetic complexity of this tumour type and its high aggressiveness may explain only in part these findings, indicating the probable presence of other still unknown but important factors along this line. However, the new opportunities of immunotherapy against MSI/dMMR tumours may open new important horizons for the prognosis also of patients with PDAC with this molecular alteration.

Regarding the clinical/therapeutic aspects related to MSI/dMMR PDAC, it is important to note that the US Food and Drug Administration (FDA) has recently approved the PD-1 immune checkpoint inhibitor pembrolizumab for the 'site-agnostic' treatment of MSI/dMMR tumours.[55] This decision was no doubt based on scientific evidence from the initial observations in a cohort mostly including colorectal cancers,[3] after further confirmation in the findings of KEYNOTE-158, a phase II basket trial on non-intestinal MSI tumours.[56] Initially, among eight patients with MSI/dMMR PDAC, five of them showed objective responses (two complete and three partial). However, an update of the trial including a total of 22 MSI/dMMR patients with PDAC, showed only 4 out of 22 patients with objective responses (1 complete and 3 partial), which represented the lowest objective response among the different investigated cancers.[57] These findings pointed out the potential differences, based on cancer site, of the response rate to immunotherapy of MSI/dMMR tumours and confirmed the complex biological and clinical nature of PDAC.

In conclusion, with this systematic review coupled with a comparative analysis with existing databases, we have definitively clarified the very low prevalence of MSI/dMMR in PDAC; this type of molecular alteration is strongly associated with medullary and mucinous/colloid histology, arises in a *KRAS/TP53*-wild type molecular background, with more common *JAK* and *KMT2* genes mutations, and its association with a longer survival is controversial. Due to its very low prevalence and also on the basis of this systematic review, MSI/dMMR should be determined as first-line analysis and with specific tests (IHC, then MSI-based PCR only in case of doubtful results; NGS in case of limited tissue) during PDAC routine diagnostic activity only in case of typical histology (medullary or mucinous/colloid). Conversely, to search for new potential targets for precision oncology (eg, the *FGFR-POC1B* fusion described in a MSI/dMMR PDAC or other targets in non-MSI/dMMR PDAC), MSI should be assessed as second-line action ideally using NGS, to permit additional simultaneous analysis and potentially provide more options for treatment.

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Patient consent for publication Not required.

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