



HAL
open science

Prevalence of Microsatellite Instability in Intraductal Papillary Mucinous Neoplasms of the Pancreas

Renato M Lupinacci, Anastasia Goloudina, Olivier Buhard, Jean-Baptiste Bachet, Raphaël Maréchal, Pieter Demetter, Jérôme Cros, Armelle Bardier-Dupas, Ada Collura, Pascale Cervera, et al.

► **To cite this version:**

Renato M Lupinacci, Anastasia Goloudina, Olivier Buhard, Jean-Baptiste Bachet, Raphaël Maréchal, et al.. Prevalence of Microsatellite Instability in Intraductal Papillary Mucinous Neoplasms of the Pancreas. *Gastroenterology*, 2018, 154 (4), pp.1061-1065. 10.1053/j.gastro.2017.11.009 . hal-03987333

HAL Id: hal-03987333

<https://hal.science/hal-03987333v1>

Submitted on 16 Feb 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

PREVALENCE OF MICROSATELLITE INSTABILITY IN INTRADUCTAL PAPILLARY MUCINOUS NEOPLASMS OF THE PANCREAS

Short title: MSI AND PANCREATIC DUCTAL CANCER

Lupinacci RM^{1,2,3,#}, **Goloudina A**^{1,4#}, Buhard O¹, Bachet JB^{2,5}, Maréchal R⁶, Demetter P⁷, Cros J^{8,9}, Bardier-Dupas A¹⁰, Collura A^{1,2}, Cervera P^{1,2,11}, Scriva A¹¹, Dumont S², Hammel P^{9,12}, Sauvanet A^{9,13}, Louvet C¹⁴, Delpéro J-R¹⁵, Paye F^{2,16}, Vaillant J-C^{2,17}, André T^{2,18}, Closset J⁶, Emile J-F¹⁹, Van Laethem J-L⁶, Jonchère V¹, Abd Alsamad I²⁰, Antoine M^{2,21}, Rodenas A^{2,21}, Fléjou JF^{1,2,11}, Dusetti N²², Iovanna J²², **Duval A**^{1,2*£}, **Svrcek M**^{1,2,11*£}

¹ INSERM, UMR S 938 – Centre de Recherche Saint-Antoine, Equipe « Instabilité des Microsatellites et Cancers », Equipe labellisée par la Ligue Nationale contre le Cancer, F-75012, Paris, France;

² Sorbonne Université, Université Pierre et Marie Curie – Paris 6, Paris, France;

³ Groupe Hospitalier Diaconesses – Croix Saint-Simon, Service de Chirurgie Digestive, Viscérale et Endocrinienne;

⁴ Inovarion F – 75013, Paris, France;

⁵ AP-HP, Groupe Hospitalier Pitié-Salpêtrière, Service d'Hépatogastro-Entérologie, Paris, France;

⁶ Department of Gastroenterology and Digestive Oncology, Erasme Hospital, Brussels, Belgium;

⁷ Department of Pathology, Erasme Hospital, Brussels, Belgium;

⁸ AP-HP, Service d'Anatomie et Cytologie Pathologiques, , hôpital Beaujon, Clichy, France;

⁹ Université Paris Diderot – Paris 7, Paris, France;

¹⁰ AP-HP, Groupe Hospitalier Pitié-Salpêtrière, Service d'Anatomie et Cytologie Pathologiques, Paris, France;

¹¹ AP-HP, Hôpitaux Universitaires Est Parisien, Hôpital Saint-Antoine, Service d'Anatomie et Cytologie Pathologiques, Paris, France;

¹² AP-HP, Hôpital Beaujon, Department of Gastroenterology, Pôle des Maladies de l'Appareil Digestif (PMAD), Clichy, France;

¹³ AP-HP, Hôpital Beaujon, Department of Hepato-Pancreato-Biliary Surgery, Pôle des Maladies de l'Appareil Digestif (PMAD), Clichy, France;

¹⁴ Department of Oncology, Institut Mutualiste Montsouris, Paris, France;

¹⁵ Department of Digestive Surgical Oncology, Paoli Calmettes Institute, Comprehensive Cancer Centre, Marseille, France;

¹⁶ AP-HP, Hôpitaux Universitaires Est Parisien, Hôpital Saint-Antoine, Service de Chirurgie Générale et Digestive, Paris, France;

¹⁷ AP-HP, Groupe Hospitalier Pitié-Salpêtrière, Service de Chirurgie Digestive et Hépatobilio-pancréatique, Paris, France;

¹⁸ Department of Oncology, Hôpital Saint Antoine, Paris, France;

¹⁹ EA4340 and Service d'Anatomie et Cytologie Pathologiques, , hôpital Ambroise Paré, AP-HP and Versailles University, Boulogne, France;

²⁰ Hôpital Intercommunal de Créteil, Service d'Anatomie et Cytologie Pathologiques, Créteil, France ;

²¹ AP-HP, Hôpitaux Universitaires Est Parisien, Hôpital Tenon, Service d'Anatomie et Cytologie Pathologiques, Paris, France;

²² Centre de Recherche en Cancérologie de Marseille (CRCM), INSERM U1068, CNRS UMR 7258, Parc Scientifique et Technologique de Luminy, Aix-Marseille Université and Institut Paoli-Calmettes, Marseille, France

Equal contribution, co-first authors

* Equal contribution, co-last authors

Grant support: This work was supported by the Ligue Contre le Cancer. AD group has the label de « La Ligue Contre le Cancer ». This work was supported by grants from the Institut National du Cancer (to A.D; PRTK 14056).

Abbreviations: CTL, cytotoxic T-cell lymphocyte; DFS, disease-free survival; dMMR, mismatch repair deficiency; FFPE, formalin-fixed, paraffin-embedded; ICK, immune checkpoint; IPMN, intraductal papillary mucinous neoplasms; LS, Lynch syndrome; MMR, mismatch repair; MSI, microsatellite instability; MSS, microsatellite stable; OS, overall survival; PanIN, pancreatic intraepithelial neoplasms; PCR, polymerase chain reaction; PDAC, pancreatic ductal adenocarcinoma; PDX, patient-derived xenografts; TMA, tissue microarray

£ **Corresponding authors:** Magali Svrcek and Alex Duval

* To whom correspondence should be addressed. Tel: +33 1 49 28 21 79 and +33 1 49 28 66 80 ; Fax: +33 1 49 28 28 78

Email: magali.svrcek@aphp.fr

alex.duval@inserm.fr

Disclosures: The authors have no conflicts of interest to declare.

Author Contributions: **RML** (study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; approval of the final version of the manuscript); **AG** (collection, analysis and interpretation of data; revision of the manuscript; approval of the final version of the manuscript); **OB** (analysis and interpretation of data; drafting of the manuscript; approval of the final version of the manuscript); **JBB** (conception of the study; collection and assembly of data; approval of the final version of the manuscript;); **RM** (collection and assembly of data; approval of the final version of the manuscript); **PD, JC, AB-D, PC, AS, CL, JRD, FP, JCV, TA, JFE, JLVL, VJ** (revision of the manuscript; approval of

the final version of the manuscript); **PC, IS, IAA, MA** (generation, analysis and interpretation of data; approval of the final version of the manuscript); **AS** and **SD** (collection of data, approval of the final version of the manuscript); **JFF** (analysis of data; approval of the final version of the manuscript); **ND** and **JI** (generation, collection and assembly of data; approval of the final version of the manuscript); **AD** (conception and design of the study; analysis and interpretation of data; revision of the manuscript; approval of the final version of the manuscript); **MS** (conception and design of the study; generation, analysis and interpretation of data; drafting of the manuscript; approval of the final version of the manuscript).

ABSTRACT

Microsatellite instability (MSI) due to mismatch repair deficiency (dMMR) is detected in a small proportion of pancreatic ductal adenocarcinomas (PDACs). dMMR and MSI have been associated with responses of metastatic tumors, including PDACs, to immune checkpoint inhibitor therapy. We performed immunohistochemical analyses of a 445 PDAC specimens, collected from consecutive patients at multiple centers, to identify those with dMMR, based on loss of mismatch repair proteins MLH1, MSH2, MSH6, and/or PMS2. We detected dMMR in 1.6% of tumor samples; we found dMMR in a larger proportion of intraductal papillary mucinous neoplasms (IPMN)-related tumors (4/58, 6.9%) than non-IPMN PDAC (5/385, 1.3%) ($P=.02$). PDACs with dMMR contained potentially immunogenic mutations due to MSI in coding repeat sequences. PDACs with dMMR or MSI had a higher density of CD8+ T cells at the invasive front than PDACs without dMMR or MSI ($P=.08$; Fisher exact test). A higher proportion of PDACs with dMMR or MSI expressed the CD274 molecule (PD-L1, 8/9) than PDACs without dMMR or MSI (4/10) ($P=.05$). Times of disease-free survival and overall survival did not differ significantly between patients with PDACs with dMMR or MSI vs without dMMR or MSI. Studies are needed to determine whether these features of PDACs with dMMR or MSI might serve as prognostic factors.

Keywords: pancreatic cancer; marker; T-cell activation, cytokine production; anti-tumor immune response

Words count: 1247 words

Pancreatic ductal adenocarcinoma (PDAC) is a leading cause of cancer mortality worldwide ¹. PDACs are derived from two precursor lesions: pancreatic intraepithelial neoplasms (PanIN) and intraductal papillary mucinous neoplasms (IPMN) ². Approximately 30% of resected IPMN have an associated invasive carcinoma. Amongst the molecular abnormalities described in PDAC is the MicroSatellite Instability (MSI) phenotype due to a defective DNA Mismatch Repair (dMMR) system (see **Supplementary Table 1** for review). 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. It is also observed in sporadic cancers due to epigenetic methylation of the *MLH1* promoter ³. In PDAC, Humphris et al. recently identified dMMR/MSI in 1% of tumors ⁴.

The MSI-driven cancer pathway leads to the synthesis of aberrant and potentially immunogenic neo-antigens by the tumor cells. A likely consequence is that MSI tumors become highly infiltrated with CD8+ cytotoxic T-cell lymphocytes (CTLs). Recent studies have highlighted the concomitant expression of multiple active immune checkpoint (ICK) markers including PD-1 and PD-L1 ⁵. The emergence of immunotherapy with ICK inhibitors has recently constituted a source of personalized treatment for MSI cancer patients ⁶ and support the evaluation of MSI phenotype in PDAC. In the present study we examined the prevalence of dMMR/MSI in a large, retrospective and multicentric series of PDACs and compared the clinical and biological features of pancreatic tumors with or without MSI.

Overall, a total of 428 cases derived from an initial multicentric series of 602 cases of resected PDAC available (see **Supplementary Methods** for further information about this cohort) gave interpretable results by immunohistochemistry (IHC) and were considered for the study (**Supplementary Figure 1 and Supplementary Table 2**), including 41 (9.57%) IPMN-associated PDAC (**Supplementary Table 3**). Among these cases, 363 showed clear expression of all 4 MMR proteins, whereas 56 showed possible loss of expression of one or more MMR proteins. The latter were further tested using full-face sections, whereupon it was concluded that 6 samples showed a clear dMMR profile, one remained uncertain and the

other 49 were MMR proficient (**Supplementary Figure 2A**). Multiplex PCR was then performed to test for MSI in PDACs that were clearly dMMR or that displayed an uncertain dMMR status identified. This was carried out successfully in only 37 samples, with the remaining 19 samples having insufficient DNA quality and/or quantity following extraction from the paraffin-embedded tissue samples. PCR analysis identified one additional MSI case that was previously classified as having an uncertain MMR status. However, MSI was detected in only 3 of the 6 dMMR tumor samples identified by IHC, presumably due to the high degree of contamination of the samples with stromal cells (**Supplementary Figure 2A**). Thus, the dMMR/MSI phenotype was present in 7 PDACs (7/428, 1.6%). Six of these 7 cases were dMMR as revealed by IHC, but only 3/7 cases showed concordant dMMR and MSI status. This frequency was confirmed by Multiplex (pentaplex and HSP110 T17) PCR in an additional non-overlapping independent patient-derived PDAC xenografts series (1/68, 1.5%) (**Supplementary Figure 2A**).

Because a trend was observed for dMMR/MSI PDACs to arise in the setting of IPMNs (2/41 IPMNs vs. 5/385 non-IPMNs; Fisher's exact test, $P=.14$), we next further evaluate this putative association, the expression of MMR proteins being tested on full face sections of an independent, monocentric series of 17 IPMN-related tumors including 16 IPMN with an associated invasive carcinoma and 1 IPMN with high-grade dysplasia (**Supplementary Table 4**). Of these 17 IPMN-related tumors, 2 (11.7%) were shown to be dMMR (**Supplementary Figure 2A**). Due to the strong contamination of these samples with stromal cells, the MSI status was confirmed by PCR (Pentaplex and HT17 PCR) in only one case (**Supplementary Figure 2A**). Taking into account these additional results, we therefore observed a significantly higher frequency of dMMR/MSI in IPMN-related tumors (4/58, 6.9%) compared to non-IPMN PDAC (5/385, 1.3%; Fisher's exact test, $P=.02$) (**Table 1**). Three of the 4 dMMR/MSI IPMN-related tumors exhibited loss of MMR protein expression (i) both in the dysplastic and carcinomatous components in two cases of IPMN with an associated invasive carcinoma; (ii) in the dysplastic component in the unique case of IPMN with high-

grade dysplasia. Concerning the fourth patient, no dysplastic component (corresponding to IPMN) was present so that it was not possible to conclude on its MMR status by IHC (data not shown and **Table 2**).

Overall, amongst the 8 tumors that displayed dMMR by IHC, MLH1 deficiency was observed in 4 cases, MSH2 deficiency in 3 cases and MSH6 deficiency in one case. Methylation of the *MLH1* promoter was not found in any of the 4 MLH1 deficient cases. Review of the clinical data available for 8 patients was suggestive of LS in 3 cases, based on the Revised Bethesda Criteria (**Table 2**). Of notice, the DFS and OS of dMMR/MSI patients was not significantly different to that of pMMR/MSS patients ($P=.703$ and $P=.663$, respectively) (**Supplementary Table 5**). Survival analysis among IPMN patients ($n=58$) showed no differences in survival between dMMR/MSI ($n=4$) and pMMR/MSS patients ($P=.272$ and $P=.448$, for DFS and OS respectively). Sixteen target genes containing repeat sequences in their coding DNA were analyzed for somatic mutations in 4 MSI PDAC samples for which DNA was available (**Supplementary Figure 2B** and **Supplementary Methods**). Seven of these genes were altered in this small series (**Supplementary Figure 2C**) including recurrent frameshift mutations in *MBD4* that are rarely observed in other MSI primary tumors. Positive PD-L1 immunostaining in tumoral cells was observed in 8/9 MSI PDAC compared to 4/10 MSS PDAC ($P=.05$) (**Supplementary Figure 3A and 3B**; see also our analysis of PD-L1 in MSI/MSS PDA cell lines in **Supplementary Figure 3C**). In contrast, no difference was observed for PD-L1 immunostaining in immune cells between MSI and MSS PDAC ($P=0.58$, Fisher's exact test; data not shown). The pattern of PD-L1 expression was heterogeneous, both in tumour and inflammatory cells, being either focalized or diffuse in positive samples (data not shown). Finally, MSI PDAC also showed a higher density of CD8 T-cells at the invasive front compared to MSS PDAC ($P=.08$) (**Supplementary Figure 3D and 3E**). CD8 positive immune infiltrates were usually observed both at the invasive front and intra-tumoral compartments, with however a reinforcement of both these stainings at the invasive front when compared to intra-tumoral compartment (data not shown).

In this study, our results confirm the dMMR/MSI phenotype is rare in PDAC (1.6%), in line with data from a recent multi-omic platform study ⁴. In addition, we demonstrate a statistically significant positive association between dMMR/MSI and IPMNs in PDAC. This phenotype occurs in about 7% of IPMNs, thus accounting for around half of all dMMR/MSI cases in PDAC. Our results also indicate that dMMR was likely to occur early in IPMN, *e.g.* already in dysplastic lesions, in line with its initiating role in tumor development. dMMR/MSI PDACs, including IPMN-related tumors, showed Lynch-related features. In addition, the relationship between IPMN and LS has been already reported ^{7,8}. However, because we have no information concerning the presence of germline MMR defects, our results do not allow us to conclude definitively on this association. Based on our findings, we recommend systematic screening of PDACs for dMMR/MSI using IHC, and obviously in IPMN-related cases. We also propose to send patients with dMMR/MSI PDAC, especially in the setting of IPMN, for genetic counseling. Finally, in the present study we also found a positive association between PD-L1 expression and MMR status in PDAC samples, similar to observations in other primary tumor types that display dMMR/MSI ⁹. Systematic screening of PDACs for dMMR/MSI using IHC will allow to further establish whether this phenotype could be a predictive factor for positive response to ICK blockade treatment in PDAC.

REFERENCES

1. Ferlay J et al. *Int J Cancer* 2015; 136:E359-386.
2. Hruban RH et al. *Am J Surg Pathol* 2001; 25:579-586.
3. Veigl ML et al. *Proc Natl Acad Sci USA* 1998; 95:8698-8702.
4. Humphris JL et al. *Gastroenterology* 2017; 152:68-74.
5. Llosa NJ et al. *Cancer Discov* 2015; 5:43-51.
6. Le DT et al. *Science* 2017;357:409-413.
7. Sparr JA et al. *Am J Surg Pathol* 2009; 33:309-312.
8. Flanagan MR et al. *World J Gastroenterol* 2015; 21:2820-2825.
9. Ma C et al. *Am J Surg Pathol* 2016; 40: 1496-1506.

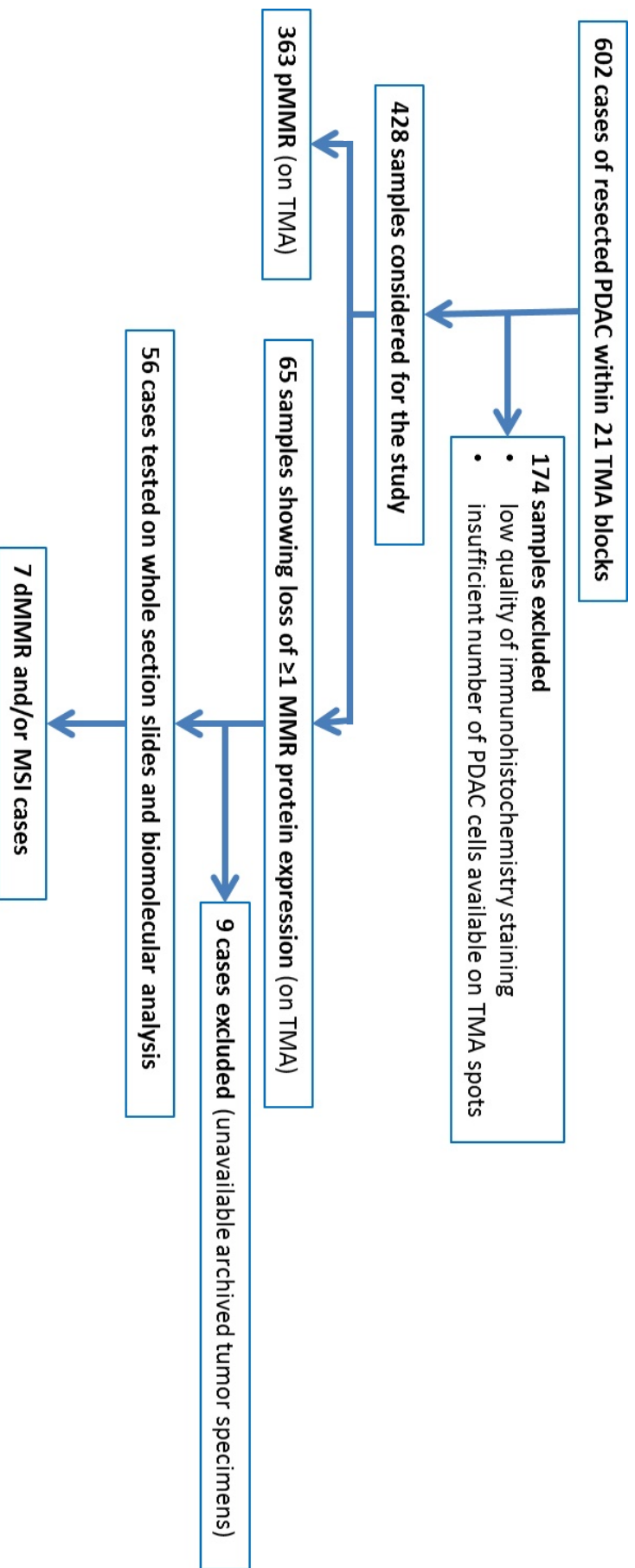
10. Maréchal R et al. *Gastroenterology* 2012; 143:664-674.
11. Delpero JR et al. *HPB (Oxford)* 2014; 16:20-33.
12. Molejon MI et al. *Oncotarget* 2015; 6:7408-7423.
13. Buhard O et al. *J Med Genet* 2016; 53: 377-384.
14. Svrcek M et al. *J Clin Oncol* 2007; 25:4231-4238.
15. Dahlin AM et al. *Mod Pathol* 2011; 24:671-682.

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1: Flowchart of patients evaluated in this study.

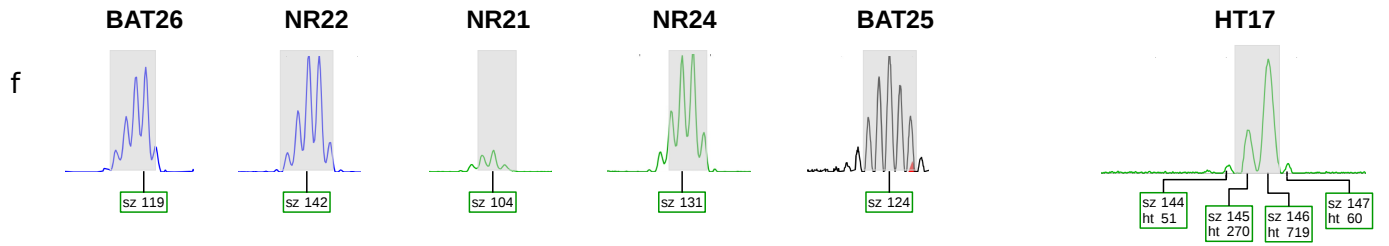
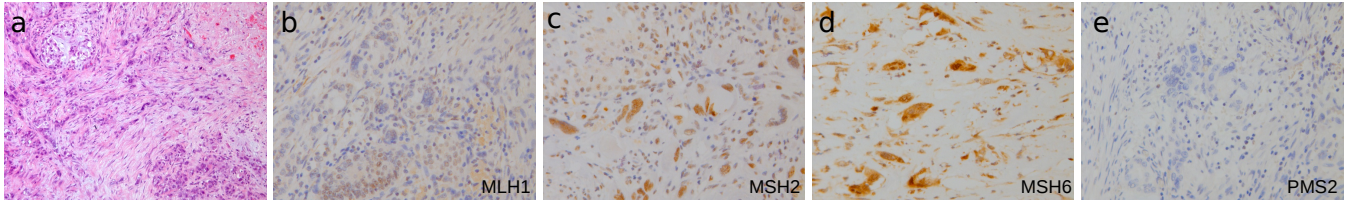
Supplementary Figure 2: (A) dMMR and/or MSI (dMMR/MSI) identified in primary PDACs, patient-derived xenograft and single centre IPMN-related tumors cohort. For patients 1, 4, 5 and PSA09, and for the xenograft, cases are referred to as MLH1/PMS2 deficient. For patients 2, 3 and 6, cases are referred to as MSH2/MSH6 deficient. For patient PSA01, the case is referred to as MSH6 deficient. For patient 7, uncertain expression of MSH2 and MSH6 in tumor cells (x200 magnification) was observed. This case displayed MSI both by Pentaplex and HSP110 T17 PCR testing; (B) Target gene frameshift mutations in dMMR/MSI PDAC, with corresponding profiles (C).

Supplementary Figure 3: (A) Schematic representation of PD-L1 immunostaining. A significant association was observed for higher levels of PD-L1 expression in MSI PDAC ($P=.05$). (B) Examples of PD-L1 positive (a) and negative (b) cases (x100 magnification); (C) Flow cytometry analysis with PD-L1, PD-L2, Galectin-3 and B7-H3 in cell lines; (D) Schematic representation of CD3⁺ and CD8⁺ cell densities at the invasive front and in the intra-tumoral compartment; (E) T-cell distribution in tumor-associated immune infiltrates: H&E stain (a and c) and a representative example of PDAC (with MSI phenotype) with highly abundant occurrence of CD8⁺ cells both at the invasive front (b); a representative example of PDAC (with MSS phenotype) with sporadic CD8⁺ cells at the invasive front (x100 magnification). T: tumor; IF: invasive front.

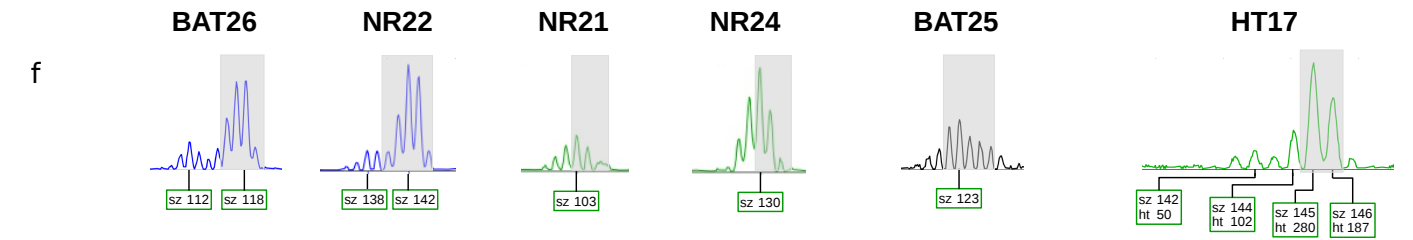
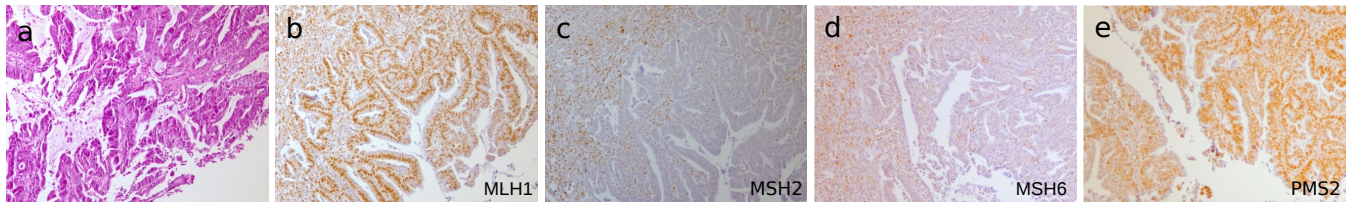


A

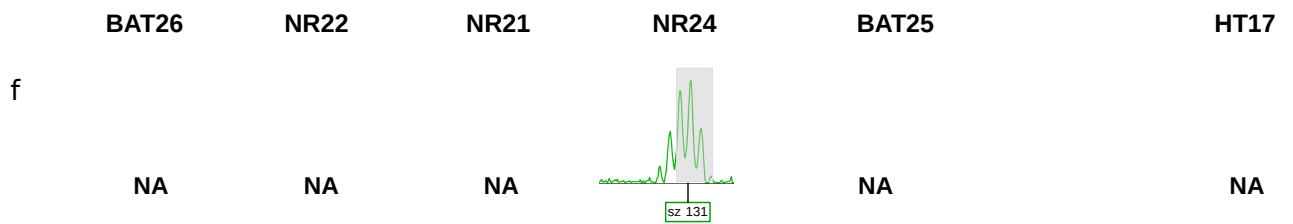
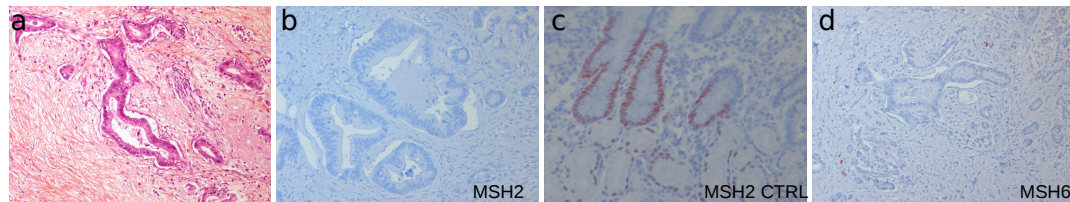
pat. 1



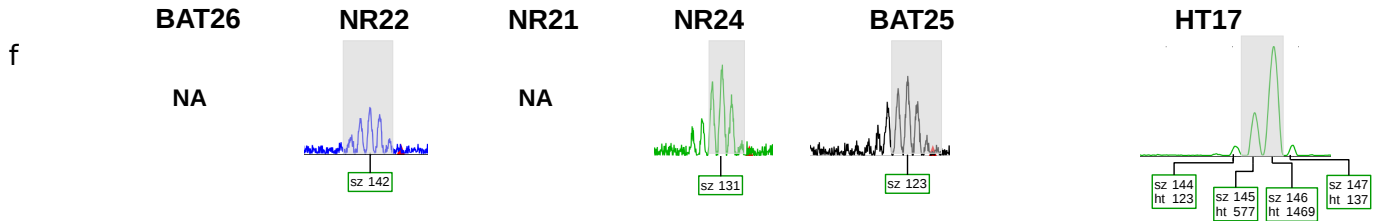
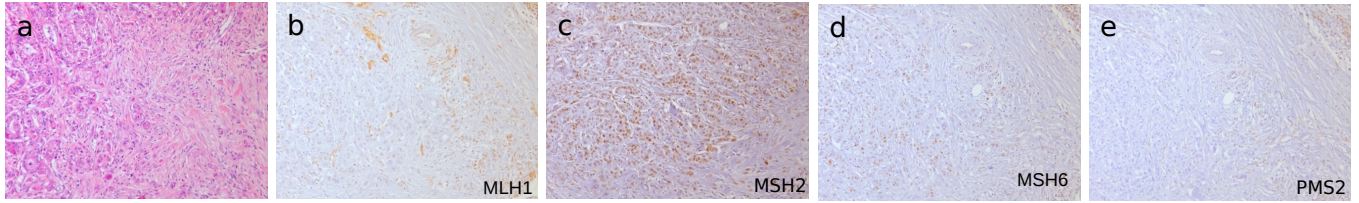
pat. 2



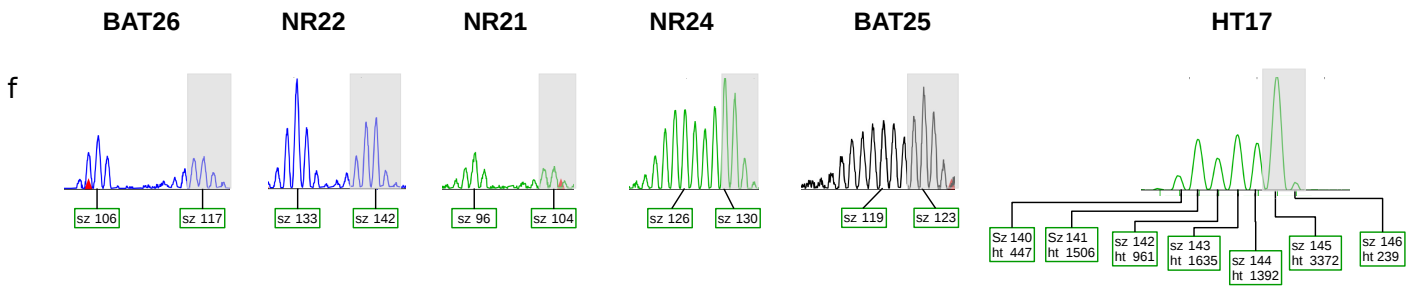
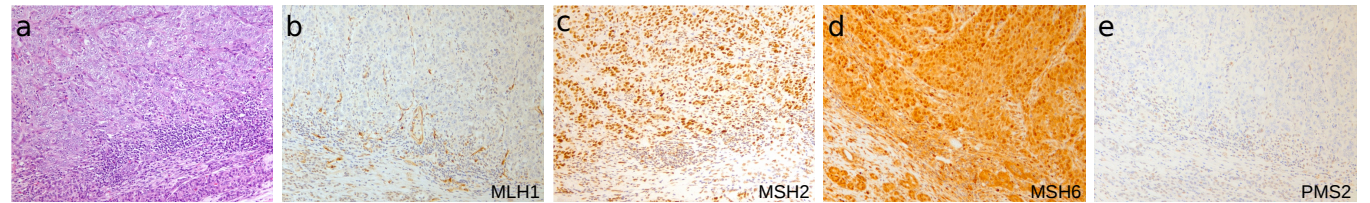
pat. 3



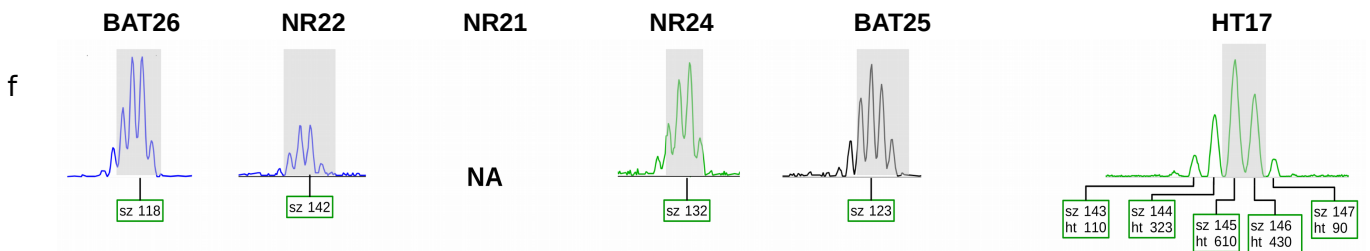
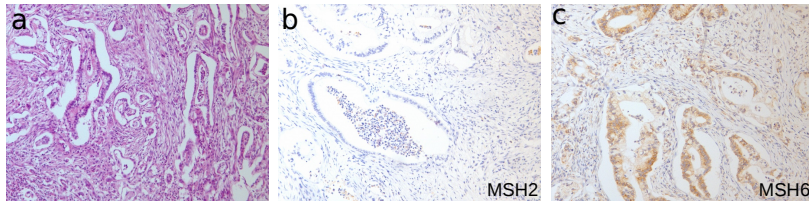
pat. 4



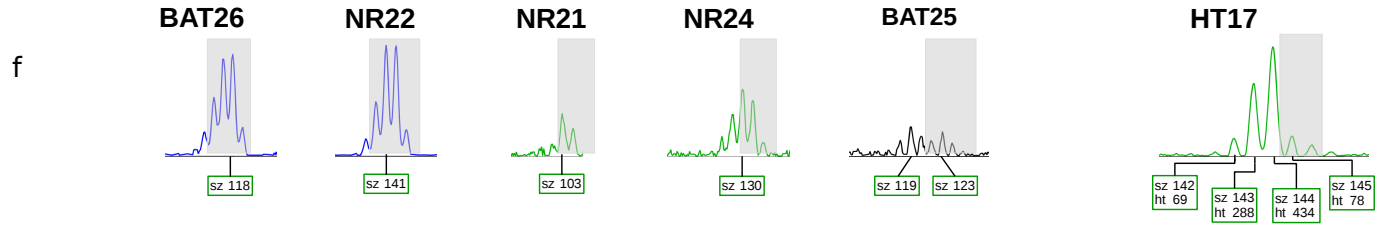
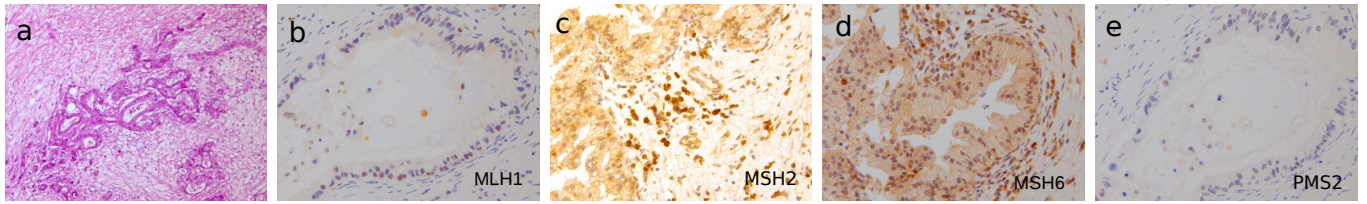
pat. 5



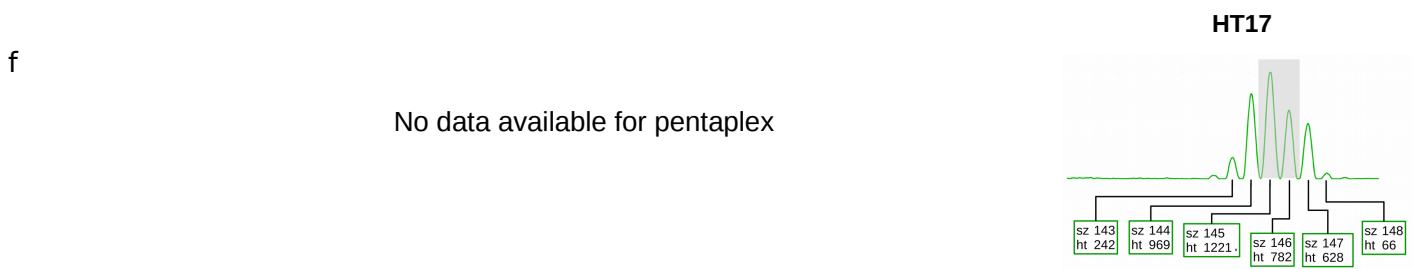
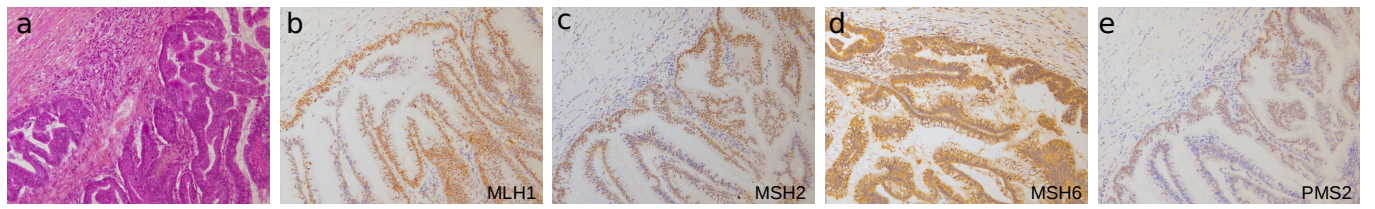
pat. 6



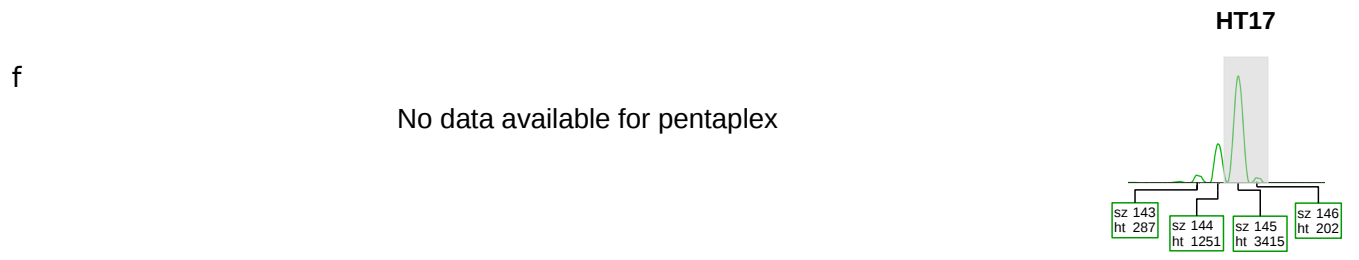
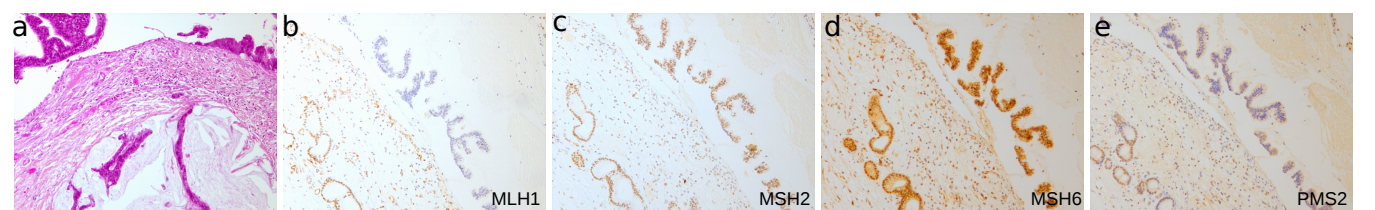
pat. 7



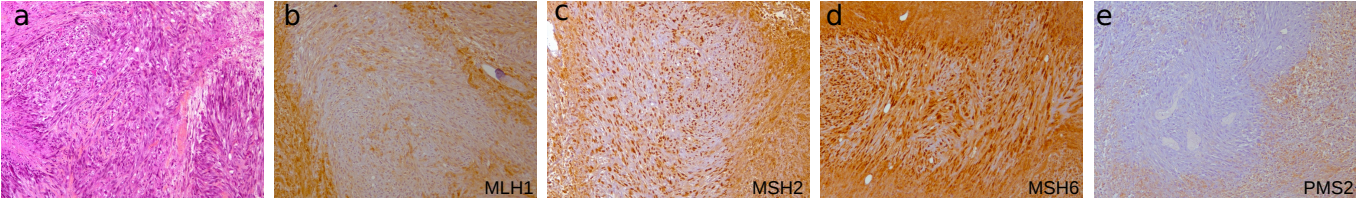
PSA01



PSA09

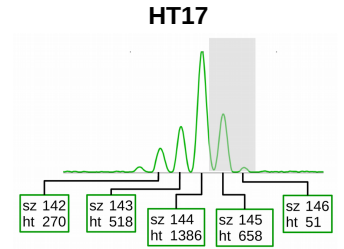


PX 1

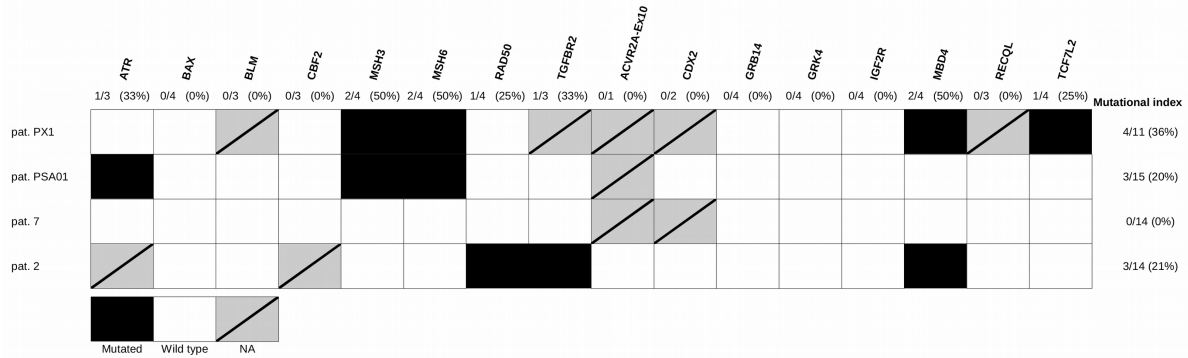


f

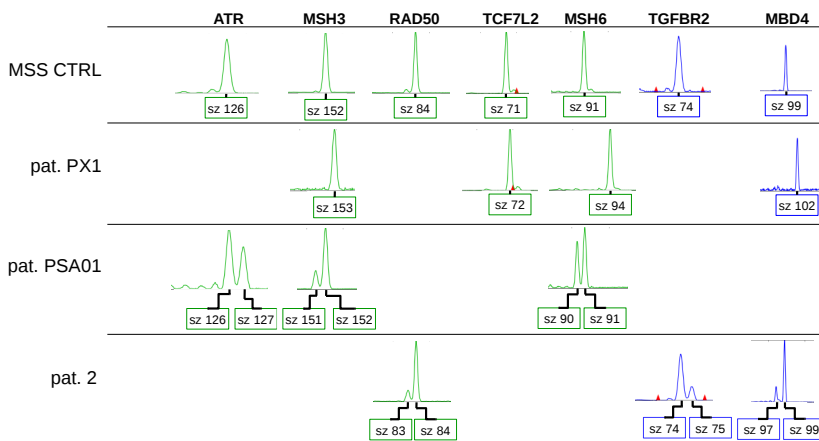
No data available for pentaplex

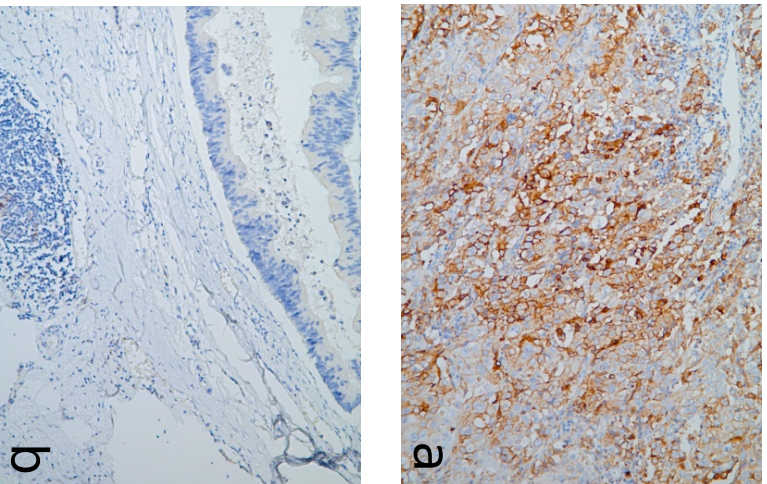
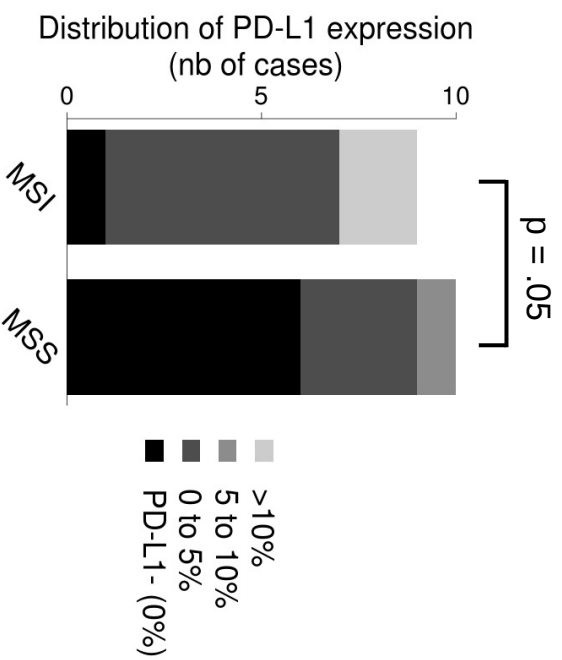
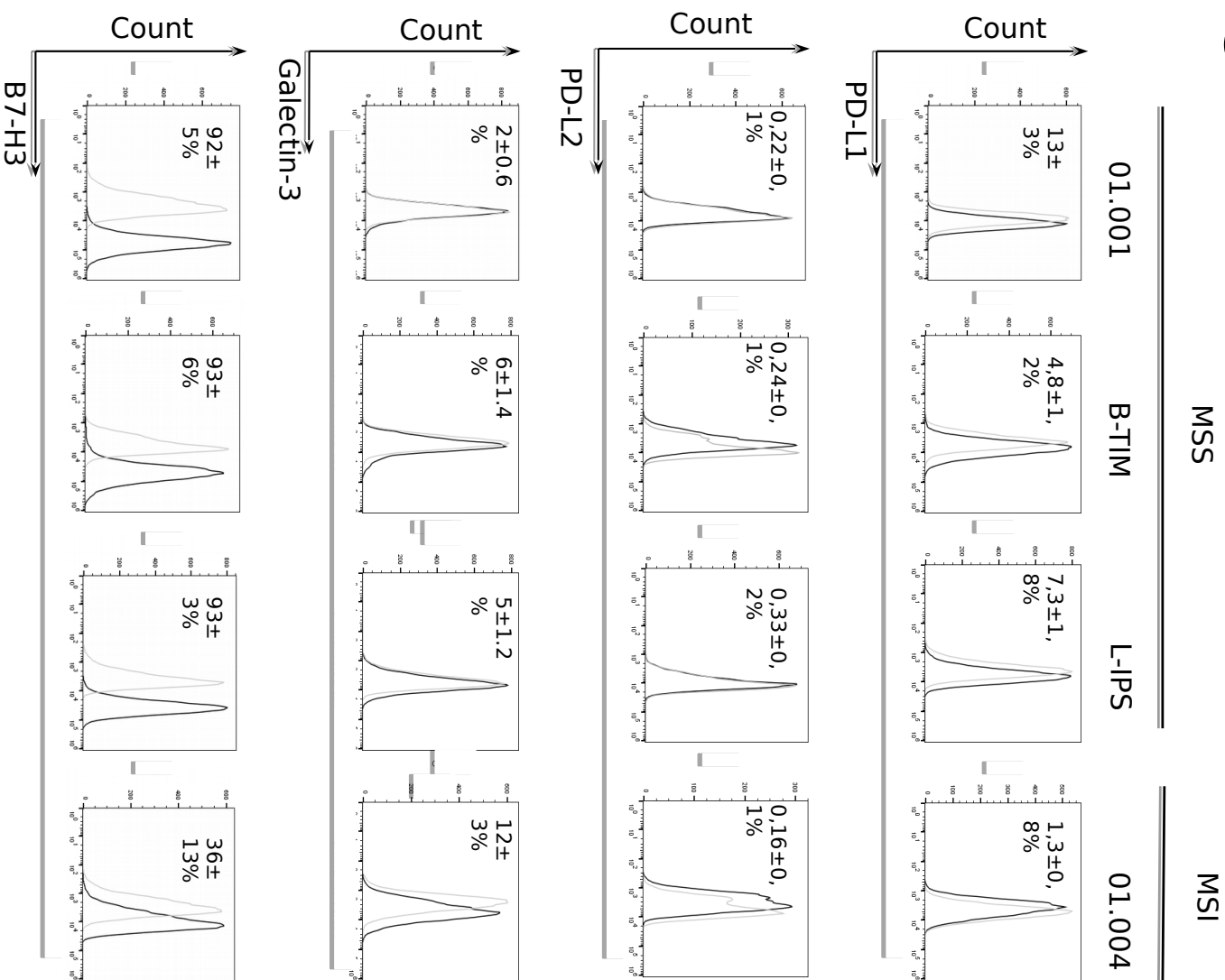


B



C



A**B****C**

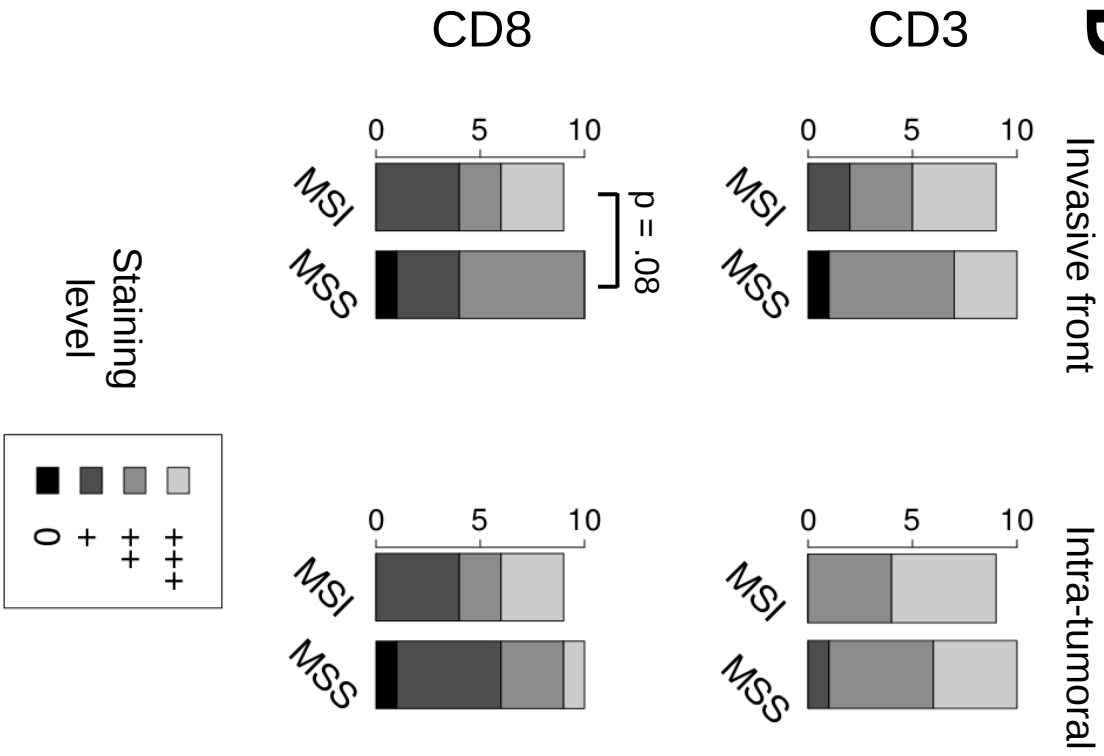
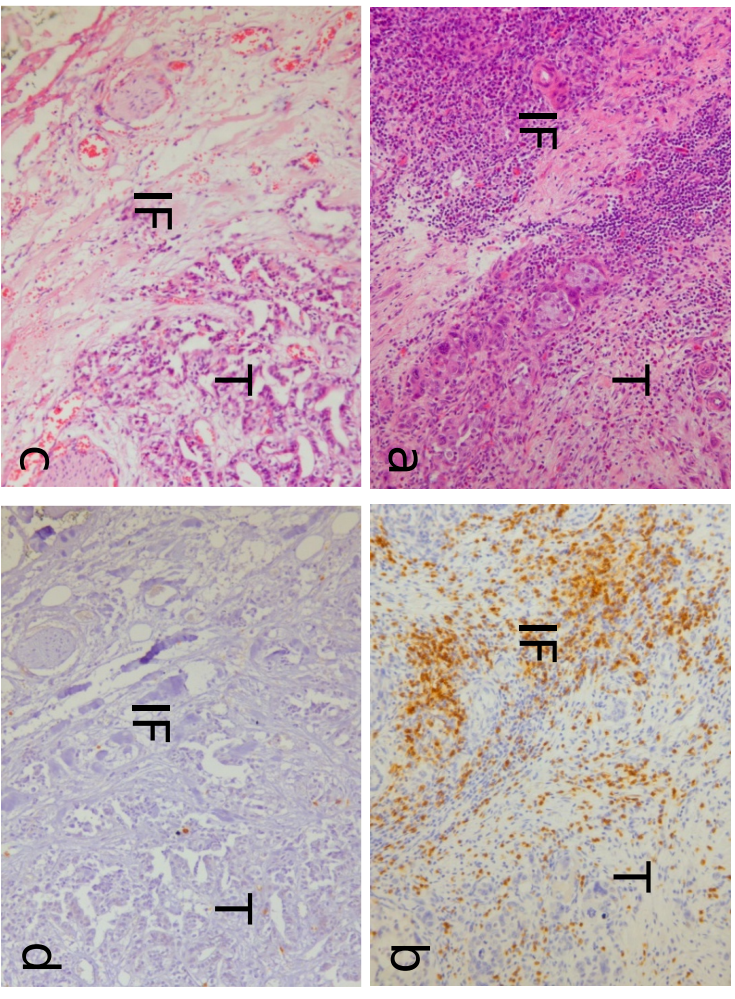
D**E**

Table 1. Patient's Clinical Characteristics Comparison in Function of dMMR/MSI Status (pooled multicentric retrospective cohort and Saint-Antoine's IPMN-related tumors cohort)

Characteristics (n)	pMMR/MSS	dMMR/MSI	P value*
Age (445) median, range	63,04 (34-85,6)	69,94 (50,4-87,6)	.059
Sex (444)			.180
male	251	3	
female	184	6	
Tumor size (mm) (430)			.494
≥30mm	253	4	
<30mm	168	5	
Lymph nodes (440)			.056
Negative, N0	320	4	
Positive, N+	111	5	
Recurrence (444)			.743
yes	284	5	
no	151	4	
IPMN (443)			.020
yes	54	4	
no	380	5	

* T-test, Chi² or Fisher's exact test when appropriate

Table 2. Patients' Characteristics (dMMR/MSI pancreatic tumors)

Patient	Age	Sex	IPMN-related tumors	Revised Bethesda Guidelines	Size (mm)	UICC (6th ed.)	Surgical Margins	Recurrence	Dead	DFS (months)	OS (months)	Histology	Lost MMR protein	MLH1	Biomolecular analysis
1	74	female	no	no	60	pT3N1	R0	local	yes	11	15	PDAC	MLH1/PMMS2	U	MSS
2	68	female	yes	yes ^a	10	pT2N0	R0	no	no	26	26	PDAC	MSH2/MSH6*	ND	MSI (pentaplex and HT17)
3	68	male	no	no	35	pT3N0	R0	local	yes	21	21	PDAC	MSH2/MSH6	ND	unsuccessful amplification
4	70	female	no	yes ^b	25	pT2N1	R0	distal	yes	9	16	PDAC	MLH1/PMMS2	U	MSS
5	88	male	no	no	90	pT3N0	R0	no	no	8	35	Medullary carcinoma	MLH1/PMMS2	U	MSI (pentaplex and HT17)
6	72	female	yes	NA	20	pT1N1	R0	no	no	22	22	PDAC	MSH2/MSH6	ND	MSI (HT17)
7	50	male	no	no	30	pT3N1	R0	local	no	25	25	PDAC	doubtful (MSH2/MSH6)	ND	MSI (pentaplex and HT17)
PSA01	83	female	yes	yes ^c	is	pTisN0	R0	no	no	26	26	High-grade dysplasia	MSH6*	ND	MSI (HT17)
PSA09	56	female	yes	no	10	pT3N0	R0	local + distal	yes	9	27	Mucinous adenocarcinoma	MLH1/PMMS2*	U	MSS

NA: not available; ND: not done ; U: unmethylated; is: in situ

^a personal history of urothelial cancer, breast cancer, and endometrial cancer (<50 years)

^b family history: grand-father: rectal cancer; father : pancreatic cancer; sister: breast cancer; brother: several colonic adenomatous polyps (<50 years)

^c personal history of colonic polyps, and family history: grand-father: colon cancer; uncle: colon cancer; aunt: colon cancer; brother1: colon cancer; brother2: colon cancer; sister: endometrial cancer.

* For these three dMMR IPMN-related tumors, loss of MMR protein expression was observed both in the dysplastic and carcinomatous components (for the 2 cases of IPMN with an associated invasive carcinoma) and in the dysplastic component (for the case of IPMN with high-grade dysplasia).

Reference	Country	Type	n	MSI-H and/or MIMRd n(%)	NCI panel included ^a	Markers used MIM ^b total	MSI-H cutoff	MIMR protein IHC (markers used)	MIMR protein IHC n	Survival analysis
Abe T, 1996 ¹	Japan	pancreatic carcinoma ^c	44	7 (15.9%) ^d	No	0 5	≥2 ^d	No	---	Not performed
Venkatasubbarao K, 1998 ²	USA	pancreatic carcinoma ^c	14	4 (28.6%) ^d	No	0 6	≥2 ^d	No	---	Not performed
Ouyang H, 1997 ³ Ouyang H, 1998 ⁴	Japan	pancreatic carcinoma ^c	60	9 (15%) ^d	No	0 5	≥2 ^d	No	---	Not performed
Goggins M, 1998 ⁵	USA	PDAC	82	3 (3.7%) ^d	No	1 6	≥2 ^d	No	---	Not performed
Ghimentì C, 1999 ⁶	Italy	pancreatic carcinoma ^c	21	0 (0%) ^e	No	0 10	≥40%	No	---	---
Wientz RE, 2000 ⁷	USA & Netherlands	medullary pancreatic carcinoma ^{f,g}	18 ^g	4 (22.2%) ^g	No	3 3	≥2 ^d	MLH1 ^{g,h} MSH2	4/4 MLH1 ⁱ	Not performed
Ueki T, 2000 ⁸	USA	pancreatic adenocarcinoma ^c	36 ⁱ	4 (11.1%) ⁱ	No	Not stated	Not stated	No	No	Not performed
Yamamoto H, 2001 ⁹	Japan	pancreatic carcinoma ^c	10 0 & 3/3 (100%) 3/5	13/100 (13%) 3/3 (100%)	Yes	2 5	≥2	No ^k	No	Not performed
Abraham SC, 2002 ¹⁰	USA	ACC	13	1 (7.7%) ^l	Yes	2 5	≥2	No	No	Not performed
Nakata B, 2002 ¹¹	Japan	pancreatic carcinoma ^c	46	8 (17.4%)	No	0 8	≥3	No	No	<i>MSI patients had significantly longer survival</i>
Tomaszewska R, 2003 ¹²	Poland	pancreatic carcinoma ^c	30	0 (0%)	---	---	---	MLH1 ^h MSH2	0	---
Lüttges J, 2003 ¹³	Germany	pancreatic carcinoma ^m	23	1 (4.3%)	Yes	2 5	≥2	MLH1 ^h MSH2, MSH6	1/23 MLH1 ^m	Not performed

Reference	Country	Type	n	MSI-H and/or MIMRd n(%)	NCI panel included ^a	Markers used MIM ^b total	MSI-H cutoff	MIMR protein IHC (markers used)	MIMR protein IHC n	Survival analysis
Nakata B, 2003 ¹⁴	Japan	pancreatic carcinoma ^c	55	4 (7.2%)	---	---	---	MLH1 ^h MSH2	4/55 MSH2 ⁿ	median survival time of hMSH2-neg patients was longer
Maple JT, 2005 ¹⁵	USA	pancreatic carcinoma ^{c,o}	35 ^o	3 (8.6%) ^o	No	5 10	≥30%	MLH1 ^h MSH2 MSH6	3/35 2/3 MLH1 1/3 MSH2	Not performed
Fujii K, 2009 ¹⁶	Japan	PDAC ^b	21	0 (0%)	No	0 5	Not stated	No	---	---
Laghi L, 2012 ¹⁷	Italy & Germany	PDAC ^b	33 8	1 (0.3%)	No	5 5	Not stated ^g	MLH1 ^{h,r} MSH2	1/182	Not performed
Ottenhof NA, 2012 ¹⁸	Netherlands	PDAC	78	9 (12.8%) if we consider loss of expression ≥ 1 MMR protein ^s	---	---	---	MLH1 ^h MSH2 MSH6 PMS2	2/78 MSH2 2/78 MSH2/MSH6 1/78 MLH/PMS2 5/78 PMS2	Not performed
Liu W, 2014 ¹⁹	USA	ACC and mixed-ACC	36 ^v	5 (13.8%) ^t	---	---	---	MLH1 ^h MSH2 MSH6 PMS2	2/36 MSH2/MSH6 2/36 MLH1/PMS2 ^t 1/36 MSH6 ^t	Loss of MMR proteins did not correlate with survival
Mitsunashi K, 2015 ²⁰	Japan	PDAC	28 3	0 (0%)	No	2 2	Not stated	---	---	---

Reference	Country	Type	n	MSI-H and/or MMRd n(%)	NCI panel included ^a	Markers used MMR ^b total	MSI-H cutoff	MIMR protein IHC (markers used)	MIMR protein IHC n	Survival analysis
Riazy M, 2015 ²¹	Canada	PDAC ^u	26 5 ^x	41 (15.4%) ^u	---	---	---	MLH1 ^h MSH2 MSH6 PMS2	64% MSH2/MSH6 29% MLH1/PMS2 5% MSH2 2% MLH1/MSH2/ PMS2	No survival difference but MMRd had no survival advantage from gemcitabine or 5-FU adjuvant chemotherapy

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

This table summarises main and recent studies with a focus on larger series (>10 patients) that were investigated for MSI and/or MMRd. This table is not intended to be a comprehensive list of all available literature on MSI or MMR in pancreatic carcinoma

^a Studies that included the NCI panel used at least the following microsatellite markers: BAT-25, BAT-26, D2S123, D5S346, D17S250.

^b Unless otherwise indicated mononucleotide markers included any of : BAT-25, BAT-26, BAT-34C4, BAT-40, TGFBR1I, NR-21, NR-22, NR- 24, NR-27, MONO-27, and MYCL.

^c Pancreatic carcinoma non specified otherwise.

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

^e 14/21 with low unstable phenotype (≤40% markers).

^f Series of medullary pancreatic carcinomas, 3 of the 4 MSI tumors were previously reported by Goggins M, *et al.*

^g Also included 77 pancreatic carcinomas. None (0/77) of them were MSI nor MMRd.

^h Loss of expression was defined as total absence of staining in tumor cells.

ⁱ MSH2 (0.0%).

^j Thirty-five pancreatic cancer xenografts and 1 primary carcinoma; 3 of the 4 MSI tumors were previously reported by Goggins M, *et al.*

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

^l Series of acinar cell pancreatic carcinoma; 2/13 were MSI-low with allelic shifts only in one marker.

^m Series of 12 PDAC, 3 IPMN-associated adenocarcinoma and 8 mucinous noncystic carcinomas; the MSI case was unstable at all NCI markers and showed loss of hMLH1 expression on IHC (MSH2 and MSH6 status unclear).

- ⁿ One tumor (1.8%) was considered hMLH1 negative and also demonstrated hMSH2 negative staining.
- ^o Defined population of long term survivors (>3 years) of pancreatic carcinoma; 2/3 MMRd were MSI-high, the third could not be tested.
- ^p All tumors were PDAC with the exception of one mucinous noncystic carcinoma.
- ^q Only 1/338 was a medullary pancreatic carcinoma that was the single MSI case; it was unstable at all markers tested, remaining 337 cases were stable at all 5 markers tests.
- ^r IHC for MLH1 and MSH2 were tested in 182 patient. The MSI patient also showed loss of MLH1 expression.
- ^s Data obtained after personal contact with the senior author. MMRd were most probably 3/78 (2 MSH2/MSH6 and 1 MLH1/PMS2).
- ^t The MSH6 deficient case and 1/2 MSH2/MSH6 deficient case had a known history of LS.
- ^u 2/41 MMRd patients presented medullary-like features on histology.
- MSI, microsatellite instable; MMR, mismatch repair; MN, mononucleotide marker; NCI, National Cancer Institute, PDAC, pancreatic ductal adenocarcinoma; ACC, acinar cell pancreatic carcinoma
1. Abe T, Ouyang H, Migita T, et al. The somatic mutation frequency of the transforming growth factor beta receptor type II gene varies widely among different cancers with microsatellite instability. *Eur J Surg Oncol* 1996; 22(5):474-477.
 2. Venkatasubbarao K, Ahmed MM, Swiderski C, et al. Novel mutations in the polyadenine tract of the transforming growth factor beta type II receptor gene are found in a subpopulation of human pancreatic adenocarcinomas. *Genes Chromosomes Cancer* 1998; 22(2):138-144.
 3. Ouyang H, Shiwaku HO, Hagiwara H, et al. The insulin-like growth factor II receptor gene is mutated in genetically unstable cancers of the endometrium, stomach, and colorectum. *Cancer Res* 1997; 57(10):1851-1854.
 4. Ouyang H, Furukawa T, Abe T, et al. The BAX gene, the promoter of apoptosis, is mutated in genetically unstable cancers of the colorectum, stomach, and endometrium. *Clin Cancer Res* 1998; 4(4):1071-1074.
 5. Goggins M, Offerhaus GJ, Hilgers W, et al. Pancreatic adenocarcinomas with DNA replication errors (RER+) are associated with wild-type K-ras and characteristic histopathology. Poor differentiation, a syncytial growth pattern, and pushing borders suggest RER+. *Am J Pathol* 1998; 152(6):1501-1507.
 6. Ghimenti C, Tannergård P, Wahlgren S, et al. Microsatellite instability and mismatch repair gene inactivation in sporadic pancreatic and colon tumours. *Br J Cancer* 1999; 80(1-2):11-16.
 7. Wilentz RE, Goggins M, Redston M, et al. Genetic, immunohistochemical, and clinical features of medullary carcinoma of the pancreas: A newly described and characterized entity. *Am J Pathol* 2000; 156(5):1641-1651.
 8. Ueki T, Toyota M, Sohn T, et al. Hypermethylation of multiple genes in pancreatic adenocarcinoma. *Cancer Res* 2000; 60(7):1835-1839.
 9. Yamamoto H, Itoh F, Nakamura H, et al. Genetic and clinical features of human pancreatic ductal adenocarcinomas with widespread microsatellite instability. *Cancer Res* 2001; 61(7):3139-3144.
 10. Abraham SC, Wu TT, Hruban RH, et al. Genetic and immunohistochemical analysis of pancreatic acinar cell carcinoma: frequent allelic loss on chromosome 11p and alterations in the APC/beta-catenin pathway. *Am J Pathol* 2002; 160(3):953-962.
 11. Nakata B, Wang YQ, Yashiro M, et al. Prognostic value of microsatellite instability in resectable pancreatic cancer. *Clin Cancer Res* 2002; 8(8):2536-2540.
 12. Tomaszewska R, Okoń K, Stachura J. Expression of the DNA mismatch repair proteins (hMLH1 and hMSH2) in infiltrating pancreatic cancer and its relation to some phenotypic features. *Pol J Pathol* 2003; 54(1):31-37.
 13. Lütjens J, Beyser K, Pust S, et al. Pancreatic mucinous noncystic (colloid) carcinomas and intraductal papillary mucinous carcinomas are usually microsatellite stable. *Mod Pathol* 2003; 16(6):537-542.

14. Nakata B, Wang YQ, Yashiro M, et al. Negative hMSH2 protein expression in pancreatic carcinoma may predict a better prognosis of patients. *Oncol Rep* 2003; 10(4):997-1000.
15. Maple JT, Smyrk TC, Boardman LA, et al. Defective DNA mismatch repair in long-term (> or =3 years) survivors with pancreatic cancer. *Pancreatology* 2005; 5(2-3):220-227.
16. Fujii K, Miyashita K, Yamada Y, et al. Simulation-based analyses reveal stable microsatellite sequences in human pancreatic cancer. *Cancer Genet Cytogenet* 2009; 189(1):5-14.
17. Laghi L, Beghelli S, Spinelli A, et al. Irrelevance of microsatellite instability in the epidemiology of sporadic pancreatic ductal adenocarcinoma. *PLoS One* 2012; 7(9):e46002.
18. Ottenhof NA, Morsink FH, Ten Kate F, et al. Multivariate analysis of immunohistochemical evaluation of protein expression in pancreatic ductal adenocarcinoma reveals prognostic significance for persistent Smad4 expression only. *Cell Oncol (Dordr)* 2012; 35(2):119-126.
19. Liu W, Shia J, Gönen M, et al. DNA mismatch repair abnormalities in acinar cell carcinoma of the pancreas: frequency and clinical significance. *Pancreas* 2014; 43(8):1264-1270.
20. Mitsuhashi K, Noshō K, Sukawa Y, et al. Association of Fusobacterium species in pancreatic cancer tissues with molecular features and prognosis. *Oncotarget* 2015; 6(9):7209-7220.
21. Riazzy M, Kalloger SE, Sheffield BS, et al. Mismatch repair status may predict response to adjuvant chemotherapy in resectable pancreatic ductal adenocarcinoma. *Mod Pathol* 2015; 28(10):1383-1389.

Patient	Cohort	Age	Sex	IPMN-associated invasive PDAC
2	Maréchal R, 2012	68	woman	yes
1	Maréchal R, 2012	74	woman	no
4	Maréchal R, 2012	70	woman	no
3	Maréchal R, 2012	68	man	no
5	Maréchal R, 2012	88	man	no
6	Delpero JR, 2013	72	woman	yes
7	Maréchal R, 2012	50	man	no
388	Delpero JR, 2013	63	woman	no
379	Maréchal R, 2012	67	man	no
380	Maréchal R, 2012	71	woman	no
382	Maréchal R, 2012	47	man	no
383	Maréchal R, 2012	75	man	no
384	Maréchal R, 2012	73	man	no
385	Maréchal R, 2012	60	man	no
387	Delpero JR, 2013	56	woman	no
389	Delpero JR, 2013	69	woman	no
390	Delpero JR, 2013	60	man	no
381	Maréchal R, 2012	69	man	yes
386	Maréchal R, 2012	69	woman	yes
79	Maréchal R, 2012	49	man	no
393	Maréchal R, 2012	57	man	no
403	Maréchal R, 2012	54	man	no
391	Maréchal R, 2012	55	woman	yes
392	Maréchal R, 2012	61	woman	no
394	Maréchal R, 2012	75	woman	no
395	Maréchal R, 2012	70	man	no
396	Maréchal R, 2012	52	man	no
397	Maréchal R, 2012	74	woman	no
398	Maréchal R, 2012	68	woman	no
399	Maréchal R, 2012	61	man	no
400	Maréchal R, 2012	56	woman	no
401	Maréchal R, 2012	73	man	no
402	Maréchal R, 2012	69	woman	no
404	Maréchal R, 2012	59	woman	no
405	Maréchal R, 2012	77	woman	no
406	Maréchal R, 2012	56	man	no
407	Maréchal R, 2012	56	man	no
408	Maréchal R, 2012	61	woman	no
409	Maréchal R, 2012	71	man	no
410	Maréchal R, 2012	59	man	no
418	Delpero JR, 2013	63	man	no
419	Delpero JR, 2013	74	woman	no
427	Delpero JR, 2013	67	man	no
415	Maréchal R, 2012	75	man	yes
411	Maréchal R, 2012	74	woman	no
412	Maréchal R, 2012	75	man	no
413	Maréchal R, 2012	53	man	no
414	Maréchal R, 2012	75	man	no
417	Delpero JR, 2013	71	man	no

420	Delpero JR, 2013	58	man	no
421	Delpero JR, 2013	65	woman	no
422	Delpero JR, 2013	54	woman	no
423	Delpero JR, 2013	76	man	no
424	Delpero JR, 2013	49	woman	no
425	Delpero JR, 2013	66	man	no
426	Delpero JR, 2013	70	man	no
428	Delpero JR, 2013	73	man	no
416	Maréchal R, 2012	55	man	yes
17	Maréchal R, 2012	76	man	no
18	Maréchal R, 2012	47	woman	no
19	Maréchal R, 2012	66	man	no
20	Maréchal R, 2012	73	woman	no
21	Maréchal R, 2012	66	man	no
22	Maréchal R, 2012	67	man	no
23	Maréchal R, 2012	74	man	no
24	Maréchal R, 2012	53	man	no
25	Maréchal R, 2012	49	woman	no
26	Maréchal R, 2012	65	man	no
27	Maréchal R, 2012	61	woman	no
28	Maréchal R, 2012	78	man	no
29	Maréchal R, 2012	62	woman	no
30	Maréchal R, 2012	57	man	no
31	Maréchal R, 2012	50	man	no
32	Maréchal R, 2012	68	woman	no
33	Maréchal R, 2012	72	man	no
34	Maréchal R, 2012	57	woman	no
35	Maréchal R, 2012	57	man	no
36	Maréchal R, 2012	72	woman	no
37	Maréchal R, 2012	63	woman	no
38	Maréchal R, 2012	55	woman	no
39	Maréchal R, 2012	57	woman	no
40	Maréchal R, 2012	55	woman	no
41	Maréchal R, 2012	71	man	no
42	Maréchal R, 2012	69	woman	no
43	Maréchal R, 2012	56	man	no
44	Maréchal R, 2012	58	woman	no
45	Maréchal R, 2012	34	woman	no
46	Maréchal R, 2012	54	man	no
47	Maréchal R, 2012	75	man	no
48	Maréchal R, 2012	55	man	no
49	Maréchal R, 2012	76	woman	no
50	Maréchal R, 2012	59	woman	no
51	Maréchal R, 2012	52	woman	no
52	Maréchal R, 2012	61	woman	no
53	Maréchal R, 2012	63	woman	no
54	Maréchal R, 2012	60	woman	no
55	Maréchal R, 2012	75	woman	no
56	Maréchal R, 2012	54	woman	no
57	Maréchal R, 2012	52	man	no

58	Maréchal R, 2012	60	woman	no
59	Maréchal R, 2012	67	man	no
60	Maréchal R, 2012	52	man	no
61	Maréchal R, 2012	45	woman	no
62	Maréchal R, 2012	55	woman	no
63	Maréchal R, 2012	65	woman	no
64	Maréchal R, 2012	75	man	no
65	Maréchal R, 2012	74	woman	no
66	Maréchal R, 2012	52	woman	no
67	Maréchal R, 2012	79	woman	no
68	Maréchal R, 2012	64	man	no
69	Maréchal R, 2012	67	woman	no
70	Maréchal R, 2012	64	woman	no
71	Maréchal R, 2012	57	man	no
72	Maréchal R, 2012	55	man	no
73	Maréchal R, 2012	64	woman	no
74	Maréchal R, 2012	65	man	no
80	Maréchal R, 2012	67	woman	no
81	Maréchal R, 2012	71	woman	no
82	Maréchal R, 2012	48	man	no
83	Maréchal R, 2012	44	woman	no
84	Maréchal R, 2012	69	woman	no
85	Maréchal R, 2012	60	man	no
86	Maréchal R, 2012	53	man	no
87	Maréchal R, 2012	71	woman	no
88	Maréchal R, 2012	58	woman	no
89	Maréchal R, 2012	64	man	no
90	Maréchal R, 2012	58	man	no
91	Maréchal R, 2012	73	man	no
92	Maréchal R, 2012	74	woman	no
93	Maréchal R, 2012	52	man	no
94	Maréchal R, 2012	48	woman	no
95	Maréchal R, 2012	80	woman	no
96	Maréchal R, 2012	71	man	no
97	Maréchal R, 2012	71	man	no
98	Maréchal R, 2012	51	man	no
99	Maréchal R, 2012	63	woman	no
100	Maréchal R, 2012	53	woman	no
101	Maréchal R, 2012	67	man	no
102	Maréchal R, 2012	58	man	no
103	Maréchal R, 2012	79	woman	no
104	Maréchal R, 2012	67	man	no
105	Maréchal R, 2012	59	man	no
106	Maréchal R, 2012	43	man	no
107	Maréchal R, 2012	65	woman	no
108	Maréchal R, 2012	61	woman	no
109	Maréchal R, 2012	71	woman	no
110	Maréchal R, 2012	62	man	no
111	Maréchal R, 2012	68	woman	no
112	Maréchal R, 2012	61	man	no

113	Maréchal R, 2012	48	man	no
114	Maréchal R, 2012	57	woman	no
115	Maréchal R, 2012	75	man	no
116	Maréchal R, 2012	58	man	no
117	Maréchal R, 2012	65	man	no
118	Maréchal R, 2012	79	man	no
119	Maréchal R, 2012	59	man	no
120	Maréchal R, 2012	60	man	no
121	Maréchal R, 2012	86	man	no
122	Maréchal R, 2012	53	man	no
123	Maréchal R, 2012	65	man	no
124	Maréchal R, 2012	58	man	no
125	Maréchal R, 2012	62	man	no
126	Maréchal R, 2012	79	woman	no
127	Maréchal R, 2012	46	man	no
128	Maréchal R, 2012	68	woman	no
135	Maréchal R, 2012	43	man	no
136	Maréchal R, 2012	79	woman	no
137	Maréchal R, 2012	60	man	no
138	Maréchal R, 2012	66	woman	no
139	Maréchal R, 2012	53	woman	no
140	Maréchal R, 2012	67	man	no
141	Maréchal R, 2012	68	man	no
142	Maréchal R, 2012	50	woman	no
143	Maréchal R, 2012	70	woman	no
144	Maréchal R, 2012	58	woman	no
145	Maréchal R, 2012	49	man	no
146	Maréchal R, 2012	71	woman	no
147	Maréchal R, 2012	58	man	no
148	Maréchal R, 2012	85	woman	no
149	Maréchal R, 2012	78	man	no
150	Maréchal R, 2012	77	woman	no
151	Maréchal R, 2012	54	woman	no
152	Maréchal R, 2012	43	man	no
153	Maréchal R, 2012	83	woman	no
154	Maréchal R, 2012	67	woman	no
155	Maréchal R, 2012	46	woman	no
156	Maréchal R, 2012	65	woman	no
157	Maréchal R, 2012	64	man	no
158	Maréchal R, 2012	61	woman	no
159	Maréchal R, 2012	37	woman	no
160	Maréchal R, 2012	52	man	no
161	Maréchal R, 2012	62	woman	no
162	Maréchal R, 2012	68	man	no
163	Maréchal R, 2012	78	woman	no
164	Maréchal R, 2012	50	man	no
165	Maréchal R, 2012	79	woman	no
166	Maréchal R, 2012	81	man	no
167	Maréchal R, 2012	57	man	no
168	Maréchal R, 2012	51	man	no

169	Maréchal R, 2012	55	woman	no
170	Maréchal R, 2012	55	man	no
171	Maréchal R, 2012	79	woman	no
172	Maréchal R, 2012	58	woman	no
173	Maréchal R, 2012	60	man	no
174	Maréchal R, 2012	65	woman	no
175	Maréchal R, 2012	73	man	no
176	Maréchal R, 2012	47	man	no
177	Maréchal R, 2012	69	man	no
178	Maréchal R, 2012	73	woman	no
179	Maréchal R, 2012	46	woman	no
180	Maréchal R, 2012	69	man	no
181	Maréchal R, 2012	67	man	no
182	Maréchal R, 2012	73	woman	no
183	Maréchal R, 2012	63	man	no
184	Maréchal R, 2012	60	man	no
185	Maréchal R, 2012	73	man	no
186	Maréchal R, 2012	64	man	no
187	Maréchal R, 2012	75	man	no
188	Maréchal R, 2012	51	man	no
189	Maréchal R, 2012	75	woman	no
190	Maréchal R, 2012	73	man	no
191	Maréchal R, 2012	70	man	no
192	Maréchal R, 2012	73	man	no
193	Maréchal R, 2012	59	woman	no
194	Maréchal R, 2012	64	woman	no
195	Maréchal R, 2012	55	man	no
196	Maréchal R, 2012	75	man	no
197	Maréchal R, 2012	57	man	no
198	Maréchal R, 2012	70	man	no
199	Maréchal R, 2012	59	man	no
200	Maréchal R, 2012	54	woman	no
201	Maréchal R, 2012	52	man	no
202	Maréchal R, 2012	62	man	no
203	Maréchal R, 2012	69	woman	no
204	Maréchal R, 2012	58	man	no
205	Maréchal R, 2012	69	man	no
206	Maréchal R, 2012	74	woman	no
207	Maréchal R, 2012	55	woman	no
208	Maréchal R, 2012	58	man	no
209	Maréchal R, 2012	77	man	no
210	Maréchal R, 2012	50	woman	no
211	Maréchal R, 2012	72	man	no
214	Maréchal R, 2012	72	woman	no
215	Maréchal R, 2012	60	woman	no
216	Maréchal R, 2012	62	man	no
217	Maréchal R, 2012	69	man	no
218	Maréchal R, 2012	62	man	no
219	Maréchal R, 2012	50	man	no
220	Maréchal R, 2012	80	woman	no

221	Maréchal R, 2012	66	man	no
222	Maréchal R, 2012	67	man	no
223	Maréchal R, 2012	69	man	no
224	Maréchal R, 2012	61	woman	no
225	Maréchal R, 2012	60	man	no
226	Maréchal R, 2012	65	man	no
227	Maréchal R, 2012	56	man	no
228	Maréchal R, 2012	59	man	no
229	Maréchal R, 2012	75	man	no
230	Maréchal R, 2012	70	woman	no
231	Maréchal R, 2012	72	man	no
232	Maréchal R, 2012	70	woman	no
233	Maréchal R, 2012	59	woman	no
234	Maréchal R, 2012	69	man	no
235	Maréchal R, 2012	77	man	no
236	Maréchal R, 2012	40	man	no
237	Maréchal R, 2012	66	man	no
238	Maréchal R, 2012	56	man	no
239	Maréchal R, 2012	83	man	no
240	Maréchal R, 2012	82	woman	no
241	Maréchal R, 2012	60	man	no
242	Maréchal R, 2012	72	woman	no
243	Maréchal R, 2012	60	woman	no
244	Maréchal R, 2012	53	woman	no
245	Maréchal R, 2012	57	man	no
246	Maréchal R, 2012	62	woman	no
247	Maréchal R, 2012	72	man	no
248	Maréchal R, 2012	54	man	no
249	Maréchal R, 2012	50	woman	no
250	Maréchal R, 2012	58	man	no
251	Maréchal R, 2012	63	man	no
252	Maréchal R, 2012	73	woman	no
253	Maréchal R, 2012	57	man	no
254	Maréchal R, 2012	75	man	no
255	Maréchal R, 2012	60	man	no
256	Maréchal R, 2012	75	woman	no
257	Maréchal R, 2012	56	man	no
258	Maréchal R, 2012	79	man	no
259	Maréchal R, 2012	79	woman	no
260	Maréchal R, 2012	64	woman	no
261	Maréchal R, 2012	79	woman	no
262	Maréchal R, 2012	66	woman	no
263	Maréchal R, 2012	81	woman	no
264	Maréchal R, 2012	62	man	no
265	Maréchal R, 2012	54	man	no
266	Maréchal R, 2012	83	woman	no
267	Maréchal R, 2012	59	man	no
268	Maréchal R, 2012	74	man	no
269	Maréchal R, 2012	62	woman	no
270	Maréchal R, 2012	52	woman	no

271	Maréchal R, 2012	78	woman	no
281	Delpero JR, 2013	65	man	no
282	Delpero JR, 2013	68	woman	no
283	Delpero JR, 2013	75	man	no
284	Delpero JR, 2013	74	man	no
285	Delpero JR, 2013	63	man	no
286	Delpero JR, 2013	55	man	no
287	Delpero JR, 2013	62	man	no
288	Delpero JR, 2013	54	man	no
289	Delpero JR, 2013	62	man	no
290	Delpero JR, 2013	60	man	no
291	Delpero JR, 2013	61	man	no
292	Delpero JR, 2013	61	woman	no
293	Delpero JR, 2013	62	woman	no
294	Delpero JR, 2013	54	woman	no
295	Delpero JR, 2013	65	man	no
296	Delpero JR, 2013	71	man	no
297	Delpero JR, 2013	63	woman	no
298	Delpero JR, 2013	68	woman	no
299	Delpero JR, 2013	62	man	no
300	Delpero JR, 2013	79	woman	no
301	Delpero JR, 2013	63	man	no
302	Delpero JR, 2013	60	woman	no
303	Delpero JR, 2013	75	woman	no
304	Delpero JR, 2013	52	man	no
305	Delpero JR, 2013	57	man	no
306	Delpero JR, 2013	55	man	no
307	Delpero JR, 2013	62	man	no
308	Delpero JR, 2013	76	man	no
309	Delpero JR, 2013	48	woman	no
310	Delpero JR, 2013	57	woman	no
311	Delpero JR, 2013	51	woman	no
312	Delpero JR, 2013	72	man	no
313	Delpero JR, 2013	72	man	no
314	Delpero JR, 2013	59	man	no
315	Delpero JR, 2013	57	woman	no
316	Delpero JR, 2013	60	man	no
317	Delpero JR, 2013	76	man	no
318	Delpero JR, 2013	53	man	no
319	Delpero JR, 2013	78	man	no
320	Delpero JR, 2013	62	woman	no
321	Delpero JR, 2013	41	woman	no
322	Delpero JR, 2013	76	man	no
323	Delpero JR, 2013	59	woman	no
324	Delpero JR, 2013	74	woman	no
325	Delpero JR, 2013	76	man	no
326	Delpero JR, 2013	56	man	no
327	Delpero JR, 2013	53	man	no
328	Delpero JR, 2013	79	man	no
329	Delpero JR, 2013	71	woman	no

330	Delpero JR, 2013	67	woman	no
331	Delpero JR, 2013	64	woman	no
332	Delpero JR, 2013	77	man	no
333	Delpero JR, 2013	74	man	no
334	Delpero JR, 2013	55	woman	no
335	Delpero JR, 2013	76	woman	no
336	Delpero JR, 2013	59	man	no
337	Delpero JR, 2013	57	woman	no
338	Delpero JR, 2013	64	woman	no
339	Delpero JR, 2013	60	woman	no
340	Delpero JR, 2013	67	man	no
341	Delpero JR, 2013	67	woman	no
342	Delpero JR, 2013	73	woman	no
343	Delpero JR, 2013	60	man	no
344	Delpero JR, 2013	77	man	no
345	Delpero JR, 2013	65	woman	no
346	Delpero JR, 2013	52	man	no
347	Delpero JR, 2013	60	man	no
348	Delpero JR, 2013	43	woman	no
349	Delpero JR, 2013	67	man	no
350	Delpero JR, 2013	67	man	no
351	Delpero JR, 2013	59	man	no
352	Delpero JR, 2013	63	man	no
353	Delpero JR, 2013	67	woman	no
354	Delpero JR, 2013	56	man	no
355	Delpero JR, 2013	76	man	no
356	Delpero JR, 2013	63	man	no
357	Delpero JR, 2013	54	man	no
358	Delpero JR, 2013	74	woman	no
359	Delpero JR, 2013	71	man	no
360	Delpero JR, 2013	63	woman	no
361	Delpero JR, 2013	51	woman	no
362	Delpero JR, 2013	46	man	no
363	Delpero JR, 2013	68	man	no
364	Delpero JR, 2013	66	man	no
365	Delpero JR, 2013	56	man	no
16	Delpero JR, 2013	75	woman	yes
75	Maréchal R, 2012	44	man	yes
76	Maréchal R, 2012	53	man	yes
77	Maréchal R, 2012	50	woman	yes
78	Maréchal R, 2012	43	man	yes
129	Maréchal R, 2012	76	man	yes
130	Maréchal R, 2012	74	man	yes
131	Maréchal R, 2012	60	man	yes
132	Maréchal R, 2012	42	man	yes
133	Maréchal R, 2012	62	man	yes
134	Maréchal R, 2012	75	woman	yes
212	Maréchal R, 2012	63	woman	yes
213	Maréchal R, 2012	70	man	yes
272	Maréchal R, 2012	72	man	yes

273	Maréchal R, 2012	83	woman	yes
274	Maréchal R, 2012	58	woman	yes
275	Maréchal R, 2012	71	man	yes
276	Maréchal R, 2012	78	woman	yes
277	Maréchal R, 2012	78	woman	yes
278	Maréchal R, 2012	80	woman	yes
279	Maréchal R, 2012	62	woman	yes
280	Maréchal R, 2012	50	man	yes
366	Delpero JR, 2013	66	man	yes
367	Delpero JR, 2013	64	woman	yes
368	Delpero JR, 2013	63	man	yes
369	Delpero JR, 2013	64	man	yes
370	Delpero JR, 2013	61	man	yes
371	Delpero JR, 2013	60	man	yes
372	Delpero JR, 2013	60	woman	yes
373	Delpero JR, 2013	68	man	yes
374	Delpero JR, 2013	75	woman	yes
375	Delpero JR, 2013	77	woman	yes
376	Delpero JR, 2013	74	woman	yes
377	Delpero JR, 2013	80	man	yes
378	Delpero JR, 2013	85	man	
8	Maréchal R, 2012	46	man	no
9	Maréchal R, 2012	71	man	no
10	Maréchal R, 2012	64	man	no
11	Maréchal R, 2012	70	man	no
12	Maréchal R, 2012	60	woman	no
13	Maréchal R, 2012	58	man	no
14	Maréchal R, 2012	69	man	no
15	Maréchal R, 2012	57	man	no

Size (mm)	pN+	pM+	TNM (AJCC 6th)	Surgical Margins
10	no	no	IB	R0
60	yes	no	IIB	R0
25	yes	no	IIB	R0
35	no	no	IIA	R0
90	no	no	IIA	R0
20	yes	no		R0
30	yes	no	IIB	R0
35	no	no		R0
20	yes	no	IIB	R0
35	no	no	IB	R0
20	yes	no	IIB	R0
30	yes	no	IIB	R0
25	yes	no	IIB	R0
30	yes	no	IIB	R1
4	yes	no		R0
30	no	no		R0
40	yes	no		R1
20	no	no	IB	R0
35	yes	no	IIB	R1
38	yes	no	IIB	R0
30	yes	no	IIB	R0
22	yes	no	IIB	R0
11	yes	no	IIB	R0
10	yes	no	IIB	R0
40	yes	no	IIB	R1
	no	no	IIA	R0
20	yes	no	IIB	R0
30	yes	no	IIB	R0
30	yes	no	IIB	R0
100	yes	no	IIB	R0
50	yes	no	IIB	R0
30	yes	no	IIB	R0
20	yes	no	IIB	R1
30	yes	no	IIB	R0
20	no	no	IA	R0
60	yes	no	IIB	R1
20	yes	no	IIB	R1
20	yes	no	IIB	R0
50	yes	no	IIB	R0
45	yes	no	IIB	R1
70	yes	no		R1
20	yes	no		R0
40	yes	no		R1
35	yes	no	IIB	R0
40	yes	no	IIB	R0
30	yes	no	IIB	R0
35	yes	no	IIB	R1
22	yes	no	IIB	R0
40	yes	no		R0

25	yes	no		R0
50	yes	no		R0
13	no	no		R0
65	yes	no		R1
40	yes	no		R1
25	yes			
40	yes	no		R1
60	yes	no	IIB	R0
15	yes	no	IIB	R0
20	yes	no	IIB	R0
40	yes	no	IIB	R0
15	no	no	IB	R0
36	no	no	IIA	R0
35	no	no	IIA	R1
14	yes	no	IIB	R0
30	no	no	IIA	R0
25	no	no	IIA	R0
20	no	no	IIA	R0
25	no	no	IIA	R0
12	yes	no	IIB	R0
30	no	no	IB	R1
13	yes	no	IIB	R0
30	yes	no	IIB	R0
50	yes	no	IIB	R1
20	yes	no	IIB	R0
40	yes	no	IIB	R0
30	yes	no	IIB	R1
40	yes	no	IIB	R0
20	yes	no	IIB	R1
30	no	no	IIA	R0
40	yes	no	IIB	R0
20	no	no	IB	R0
30	yes	no	IIB	R1
20	no	no	IIA	R0
30	yes	no	IIB	R0
25	yes	no	IIB	R0
35	yes	no	IIB	R0
24	yes	no	IIB	R0
35	yes	no	IIB	R0
30	yes	no	IIB	R1
30	yes	no	IIB	R1
40	yes	no	IIB	R0
30	yes	no	IIB	R0
50	yes	no	IIB	R1
17	no	no	IA	R0
15	no	no	IIA	R0
15	yes	no	IIB	R0
20	yes	no	IIB	R1
100	yes	no	IIB	R0

40	yes	no	IIB	R0
25	yes	no	IIB	R0
40	yes	no	IIB	R1
40	no	no	IIA	R0
45	no	no	IB	R0
35	yes	no	IIB	R1
40	yes	no	IIB	R1
30	no	no	IIA	R1
30	yes	no	IIB	R0
28	yes	no	IIB	R0
40	yes	no	IIB	R1
35	yes	no	IIB	R0
35	yes	no	IIB	R1
35	yes	no	IIB	R0
30	yes	no	IIB	R0
35	yes	no	IIB	R1
25	no	no	IIA	R1
15	yes	no	IIB	R1
35	yes	no	IIB	R0
20	no	no	IIA	R0
20	yes	no	IIB	R0
20	yes	no	IIB	R0
40	yes	no	IIB	R1
22	no	no	IIA	R0
25	yes	no	IIB	R0
22	yes	no	IIB	R1
40	yes	no	IIB	R1
25	yes	no	IIB	R0
30	yes	no	IIB	R0
25	yes	no	IIB	R0
23	yes	no	IIB	R0
28	no	no	IB	R0
25	yes	no	IIB	R0
15	yes	no	IIB	R0
20	yes	no	IIB	R0
34	no	no	IIA	R0
7	no	no	IIA	R0
16	yes	no	IIB	R1
24	yes	no	IIB	R0
	yes	no	IIB	R0
30	yes	no	IIB	R0
50	yes	no	IIB	R0
	yes	no	IIB	R0
50	no	no	IIA	R0
40	yes	no	IIB	R0
25	no	no	IIA	R0
50	yes	no	IIB	R0
50	no	no	IIA	R0
15	yes	no	IIB	R0
15	yes	no	IIB	R0

20	no	no	IIA	R0
30	no	no	IIA	R0
20	yes	no	IIB	R0
27	yes	no	IIB	R0
30	yes	no	IIB	R0
60	yes	no	IIB	R0
40	no	no	IIA	R0
50	yes	no	IIB	R0
25	yes	no	IIB	R0
35	no	no	IIA	R0
35	yes	no	IIB	R0
16	yes	no	IIB	R0
20	no	no	IIA	R0
70	no	no	IB	R0
40	yes	no	IIB	R1
20	yes	no	IIB	R0
30	yes	no	IIB	R0
45	yes	no	IIB	R0
55	yes	no	IIB	R0
20	yes	no	IIB	R0
45	yes	no	IIB	R1
20	no	no	IIA	R0
30	yes	no	IIB	R1
18	yes	no	IIB	R1
	yes	no	IIB	R0
12	no	no	IB	R0
18	yes	no	IIB	R0
50	no	no	IIA	R0
35	yes	no	IIB	R1
40	yes	no	IIB	R0
25	no	no	IIA	R0
25	yes	no	IIB	R0
15	yes	no	IIB	R0
35	yes	no	IIB	R1
20	yes	no	IIB	R1
25	no	no	IB	R0
25	yes	no	IIB	R1
45	yes	no	IIB	R1
30	yes	no	IIB	R1
	yes	no	IIB	R0
35	yes	no	IIB	R1
35	no	no	IIA	R1
20	yes	no	IIB	R1
25	yes	no	IIB	R1
60	yes	no	IIB	R0
63	yes	no	IIB	R1
40	yes	no	IIB	R1
30	yes	no	IIB	R1
40	yes	no	IIB	R1
	yes	no	IIB	R1

25	no	no	IIA	R0
25	yes	no	IIB	R0
25	yes	no	IIB	R0
25	yes	no	IIB	R0
35	yes	no	IIB	R1
30	yes	no	IIB	R0
10	yes	no	IIB	R0
30	yes	no	IIB	R0
20	no	no	IB	R0
30	yes	no	IIB	R0
	yes	no	IIB	R1
50	yes	no	IIB	R1
40	yes	no	IIB	R0
40	no	no	IIA	R0
25	yes	no	IIB	R0
40	yes	no	IIB	R0
30	no	no	IIA	R0
30	yes	no	IIB	R0
20	yes	no	IIB	R0
20	yes	no	IIB	R0
22	yes	no	IIB	R0
30	yes	no	IIB	R1
30	yes	no	IIB	R0
50	yes	no	IIB	R0
40	yes	no	IIB	R0
35	yes	no	IIB	R0
20	yes	no	IIB	R0
20	no	no	IB	R0
35	yes	no	IIB	R0
20	no	no	IIA	R0
30	yes	no	IIB	R1
25	yes	no	IIB	R0
20	no	no	IIA	R0
30	yes	no	IIB	R0
30	yes	no	IIB	R1
20	yes	no	IIB	R0
35	no	no	IB	R0
40	yes	no	IIB	R0
35	yes	no	IIB	R0
35	yes	no	IIB	R0
20	yes	no	IIB	R0
30	no	no	IB	R0
50	yes	no	IIB	R0
50	yes	no	IIB	R0
25	yes	no	IIB	R0
45	no	no	IIA	R0
30	yes	no	IIB	R0
30	no	no	IB	R0
30	no	no	IIA	R0
25	no	no	IIA	R0

16	no	no	IA	R0
20	no	no	IIA	R0
10	no	no	IA	R0
20	yes	no	IIB	R0
25	yes	no	IIB	R0
70	yes	no	IIB	R1
22	yes	no	IIB	R0
20	yes	no	IIB	R0
38	yes	no	IIB	R1
25	yes	no	IIB	R0
25	yes	no	IIB	R0
27	no	no	IIA	R0
40	yes	no	IIB	R1
40	yes	no	IIB	R0
50	yes	no	IIB	R1
30	yes	no	IIB	R0
60	yes	no	IIB	R1
25	yes	no	IIB	R1
40	yes	no	IIB	R0
35	yes	no	IIB	R0
60	yes	no	IIB	R1
25	yes	no	IIB	R0
30	yes	no	IIB	R0
	yes	no	IIB	R0
30	yes	no	IIB	R0
40	yes	no	IIB	R0
20	no	no	IIA	R1
35	no	no	IB	R0
35	yes	no	IIB	R0
30	yes	no	IIB	R0
20	yes	no	IIB	R0
60	no	no	IIA	R0
20	yes	no	IIB	R0
45	yes	no	IIB	R0
40	yes	no	IIB	R0
25	yes	no	IIB	R1
100	no	no	IIA	R0
40	yes	no	IIB	R0
35	no	no	IB	R0
30	yes	no	IIB	R0
15	no	no	IA	R0
25	no	no	IIA	R0
20	no	no	IIA	R0
28	yes	no	IIB	R0
30	yes	no	IIB	R0
30	no	no	IIA	R0
35	yes	no	IIB	R0
>30	no	no	IIA	R0
35	yes	no	IIB	R0
20	yes	no	IIB	R0

30	yes	no	IIB	R0
20	yes	no		R0
20	yes	no		R1
35	yes	no		R1
35	no	no		R0
65	yes	yes		R1
90	yes	no		R1
35	yes	no		R0
15	no	no		R0
15	yes	no		R1
30	yes	no		R1
35	yes	no		R0
15	yes	no		R1
60	yes	no		R0
36	yes	no		R0
40	yes	no		R0
40	yes	no		R0
40	yes	no		R0
13	no	no		R0
36	no	no		R0
43	no	no		R0
30	yes	no		R0
35	no	no		R0
33	yes	no		R0
35	yes	no		R0
	yes	no		R1
20	yes	no		R0
20	yes	no		R1
40	yes	no		R1
25	yes	no		R0
30	yes	no		R0
22	yes	no		R1
31	yes	no		R0
35	yes	no		R1
20	no	no		R0
15	no	no		R0
30	no	no		R0
40		no		R1
50	yes	no		R1
30	yes	no		R1
40	no	no		R1
25	yes	no		R0
50	no	no		R0
15	yes	no		R0
20	yes	no		R1
20	yes	no		R0
30	no	no		R0
20	yes	no		R1
23	yes	no		R0
20	yes	no		R0

30	yes	no		R0
35	yes	no		R1
35	no	no		R0
40	yes	no		R0
35	yes	no		R0
35	yes	no		R0
2	yes	no		R0
15	yes	no		R1
30	yes	no		R0
40	yes	no		R0
35	yes	yes		R0
40	yes	no		R0
35	yes	no		R1
40	yes	no		R0
30	yes	no		R1
	yes	no		R0
50	yes	yes		R0
50	yes	yes		R1
120	yes	no		R1
80	yes	yes		R1
50	no	no		R0
25	no	no		R0
60	yes	no		R0
60	yes	no		R1
15	no	no		R0
30	yes	no		R1
40	yes	no		R0
35	yes	no		R0
45	yes	no		R0
45	yes	no		R1
30	yes	no		R1
30	yes	no		R0
35	yes	no		R0
45	yes	no		R1
34	yes	no		R1
21	yes	no		R0
55	yes	no		R0
30	yes	no	IIB	R0
10	yes	no	IIB	R1
10	no	no	IA	R0
30	no	no	IIA	R0
35	no	no	IIA	R0
28	no	no	IIA	R0
50	yes	no	IIB	R0
50	no	no	IIA	R0
35	no	no	IIA	R0
100	no	no	IA	R0
30	yes	no	IIB	R0
20	yes	no	IIB	R0
30	no	no	IIA	R0

22	yes	no	IIB	R0
12	no	no	IA	R0
45	yes	no	IIB	R0
35	yes	no	IIB	R0
35	yes	no	IIB	R0
35	yes	no	IIB	R1
25	yes	no	IIB	R0
70	yes	no	IIB	R0
35	yes	no		R0
30	no	no		R0
	no	no		R0
40	yes	no		R1
23	no	no		R0
25	no	no		R0
32	no	no		R0
35	yes	no		R0
20	no	no		R0
13	yes	no		R0
45	yes	no		R0
40	yes	no	IIB	R0
40	yes	no	IIB	R0
30	yes	no	IIB	R0
40	yes	no	IIB	R0
25	yes	no	IIB	R0
70	yes	no	IIB	R0
25	yes	no	IIB	R0
30	yes	no	IIB	R0

Preoperative treatment	Postoperative treatment	Local recurrence	Distal recurrence
no	yes	no	no
no	yes	yes	no
no	no	no	yes
no	yes	yes	no
no	no	no	no
no	no	no	no
no	yes	yes	no
yes		no	no
no	yes	yes	no
no	yes	no	no
no	yes	yes	yes
no	yes	yes	yes
no	yes	no	no
no	yes	yes	yes
no		no	no
no		no	no
yes		yes	no
no	no	no	no
no	yes	no	no
no	yes	no	no
no	yes	no	no
no	yes	no	yes
no	yes	yes	no
no	yes	no	yes
no	yes	yes	no
no	yes	yes	no
no	yes	no	yes
no	yes	no	no
no	no	yes	no
no	no	no	yes
no	no	yes	yes
no	yes	yes	yes
no	yes	no	yes
no	yes	no	no
no	yes	no	no
no	no	no	no
no	yes	no	yes
no	yes	no	yes
no	no	no	yes
no	yes	yes	yes
no	yes	yes	yes
yes		yes	yes
yes		no	yes
no		no	yes
no	yes	no	yes
no	no	no	no
no	no	no	no
no	yes	yes	yes
no	yes	yes	no
no			

yes		no	yes
yes		yes	yes
no			
no		no	yes
no			
K		yes	no
yes			
no	yes	no	no
no	yes	yes	no
no	yes	yes	no
no	yes	yes	yes
no	no	no	no
no	no	yes	yes
no	no	yes	yes
no	no	yes	no
no	no	yes	yes
no	yes	yes	yes
no	no	no	no
no	no	yes	yes
no	no	no	no
no	no	yes	yes
no	yes	no	no
no	yes	yes	no
no	no	yes	yes
no	no	yes	yes
no	no	no	no
no	no	no	no
no	yes	no	yes
no	yes	no	yes
no	yes	yes	yes
no	yes	no	yes
no	yes	yes	no
no	no	no	yes
no	yes	yes	no
no	yes	no	no
no	yes	no	no
no	yes	yes	yes
no	yes	yes	yes
no	yes	yes	yes

no	yes	no	yes
no	yes	yes	yes
no	yes	no	yes
no	yes	yes	yes
no	yes	yes	yes
no	yes	yes	yes
no	yes	no	no
no	yes	no	yes
no	no	no	yes
no	yes	no	no
no	yes	no	no
no	yes	no	yes
no	yes	no	yes
no	yes	yes	no
no	no	no	no
no	yes	yes	yes
no	yes	no	yes
no	yes	no	no
no	yes	yes	no
no	no	yes	no
no	yes	no	yes
no	yes	no	no
no	yes	yes	no
no	no	yes	no
no	no	no	no
no	2	no	no
no	yes	no	yes
no	yes	yes	yes
no	yes	yes	yes
no	yes	no	no
no	yes	yes	no
no	yes	yes	yes
no	yes	yes	yes
no	yes	yes	no
no	yes	yes	yes
no	no	yes	yes
no	yes	yes	yes
no	yes	no	no

no	yes	yes	yes
no	no	yes	no
no	2	yes	yes
no	yes	yes	no
no	yes	no	yes
no	2	yes	yes
no	no	no	no
no	yes	yes	yes
no	no	yes	yes
no	no	yes	no
no	yes	yes	yes
no	no	yes	yes
no	no	no	no
no	no	no	no
no	yes	no	no
no	yes	yes	no
no	yes	no	yes
no	2	yes	yes
no	no	no	yes
no	no	no	no
no	yes	no	no
no	2	yes	yes
no	no	no	no
no	no	no	no
no	no	yes	yes
no	no	yes	yes
no	no	yes	no
no	no	yes	yes
no	no	no	no
no	2	no	no
no	no	no	no
no	no	no	yes
no	no	yes	yes
no	no	yes	yes
no	no	yes	no
no	no	no	yes
no	no	yes	yes
no	no	yes	no
no	no	no	yes

no	yes	no	yes
no	no	yes	no
no	no	no	no
no	no	no	no
no	no	yes	yes
no	yes	no	yes
no	yes	no	no
no	no	no	yes
no	no	yes	no
no	no	no	yes
no	no	yes	no
no	no	no	yes
no	yes	no	yes
no	no	yes	no
no	yes	yes	yes
no	yes	yes	no
no	no	yes	no
no	yes	yes	yes
no	yes	yes	no
no	no	yes	no
no	yes	yes	yes
no	yes	yes	no
no	no	no	yes
no	yes	yes	no
no	yes	yes	no
no	no	no	no
no	no	no	no
no	yes	yes	no
no	no	no	no
no	no	no	no
no	yes	no	no
no	yes	no	no
no	no	no	no
no	yes	no	no
no	yes	no	no
no	yes	no	no
no	no	yes	yes
no	no	no	no
no	yes	no	no
no	yes	no	no
no	yes	no	no
no	no	yes	yes

no	yes	yes	no
yes		no	yes
no			yes
no			yes
yes		no	no
no		no	yes
no		no	yes
no		no	no
yes		no	no
no		no	yes
no		no	yes
yes		no	yes
no		no	no
no		no	no
no		yes	no
no		yes	no
no		no	no
no		no	yes
no		no	no
no		no	no
no		no	no
no		yes	yes
no		yes	yes
no		no	yes
no		no	no
no		no	no
no		no	no
no		yes	no
no		no	no
no		yes	yes
no		no	yes
no		yes	yes
no		yes	yes
no			
no		no	yes

yes		no	yes
no		no	yes
yes		no	yes
no		yes	A
no			
no			
no			
no		yes	yes
yes		no	yes
no		no	yes
no		no	yes
no		no	yes
no		yes	yes
no		no	yes
no		no	yes
no		no	no
no			
no		no	no
no			
no		no	no
yes		no	yes
no			
yes		no	yes
no			
no		no	yes
no		no	yes
no		no	yes
yes		no	yes
yes			
no		yes	no
no		no	yes
no		yes	yes
no	no	no	yes
no	yes	yes	no
no	yes	no	no
no	yes	no	no
no	yes	no	no
no	no	yes	no
no	yes	yes	yes
no	yes	yes	no
no	no	no	no
no	no	no	no
no	yes	no	no
no	no	yes	yes
no	no	yes	yes

Recurrence	Dead	TMA status
no	no	dMMR
yes	yes	dMMR
yes	yes	dMMR
yes	yes	dMMR
no	no	dMMR
no	no	indeterminate
yes	no	indeterminate
no	no	dMMR
yes	yes	dMMR
no	yes	dMMR
yes	yes	dMMR
yes	yes	dMMR
no	no	dMMR
yes	yes	dMMR
no	no	dMMR
no	no	dMMR
yes	no	dMMR
no	no	dMMR
no	no	indeterminate
yes	yes	indeterminate
yes	yes	indeterminate
yes	yes	indeterminate
yes	yes	indeterminate
yes	yes	indeterminate
yes	yes	indeterminate
yes	yes	indeterminate
no	no	indeterminate
no	no	indeterminate
no	no	indeterminate
yes	yes	indeterminate
yes	yes	indeterminate
yes	yes	indeterminate
yes	no	indeterminate
yes	yes	indeterminate
yes	yes	indeterminate
yes	yes	indeterminate
yes	yes	indeterminate
yes	no	indeterminate
yes	yes	indeterminate
no	no	indeterminate
no	no	indeterminate
yes	yes	indeterminate
yes	yes	indeterminate
no	no	indeterminate

yes	yes	indeterminate
yes	no	indeterminate
no	no	indeterminate
yes	yes	indeterminate
yes	yes	indeterminate
yes	yes	indeterminate
no	no	indeterminate
no	no	indeterminate
no	no	indeterminate
yes	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
no	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
no	no	pMMR
yes	yes	pMMR
no	yes	pMMR
yes	yes	pMMR
no	no	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
no	yes	pMMR
yes	yes	pMMR
no	no	pMMR
no	no	pMMR
yes	yes	pMMR
yes	no	pMMR
yes	no	pMMR

yes	yes	pMMR
no	no	pMMR
yes	yes	pMMR
yes	no	pMMR
yes	no	pMMR
yes	no	pMMR
yes	yes	pMMR
no	no	pMMR
no	no	pMMR
no	no	pMMR
yes	yes	pMMR
no	no	pMMR
no	no	pMMR
yes	no	pMMR
yes	no	pMMR
no	no	pMMR
no	no	pMMR
yes	yes	pMMR
yes	yes	pMMR
no	no	pMMR
no	no	pMMR
no	yes	pMMR
yes	no	pMMR
no	no	pMMR
yes	yes	pMMR
no	no	pMMR
yes	yes	pMMR
yes	no	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	no	pMMR
yes	yes	pMMR
no	no	pMMR
yes	yes	pMMR
yes	yes	pMMR
no	no	pMMR
yes	yes	pMMR
yes	yes	pMMR
no	no	pMMR
no	no	pMMR

yes	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	no	pMMR
yes	no	pMMR
yes	yes	pMMR
no	no	pMMR
yes	yes	pMMR
yes	yes	pMMR
no	no	pMMR
no	no	pMMR
yes	no	pMMR
yes	yes	pMMR
no	no	pMMR
yes	yes	pMMR
no	no	pMMR
no	no	pMMR
yes	yes	pMMR
no	no	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
no	no	pMMR
yes	yes	pMMR
yes	no	pMMR
no	no	pMMR
no	no	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	no	pMMR
yes	no	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	no	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	no	pMMR
no	no	pMMR

yes	yes	pMMR
yes	no	pMMR
yes	yes	pMMR
yes	yes	pMMR
no	no	pMMR
yes	yes	pMMR
yes	yes	pMMR
no	no	pMMR
no	no	pMMR
yes	no	pMMR
yes	no	pMMR
yes	yes	pMMR
no	no	pMMR
no	no	pMMR
yes	yes	pMMR
yes	no	pMMR
no	yes	pMMR
yes	yes	pMMR
no	no	pMMR
no	yes	pMMR
yes	no	pMMR
yes	yes	pMMR
yes	no	pMMR
yes	yes	pMMR
no	no	pMMR
yes	no	pMMR
yes	yes	pMMR
no	yes	pMMR
yes	no	pMMR
no	no	pMMR
no	no	pMMR
yes	yes	pMMR
no	no	pMMR
no	no	pMMR
yes	yes	pMMR
yes	no	pMMR
yes	yes	pMMR
no	no	pMMR
no	no	pMMR
yes	yes	pMMR
yes	yes	pMMR

yes	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
no	no	pMMR
no	no	pMMR
no	yes	pMMR
no	no	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	no	pMMR
yes	yes	pMMR
yes	no	pMMR
yes	yes	pMMR
no	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
no	no	pMMR
no	no	pMMR
no	yes	pMMR
no	no	pMMR
yes	yes	pMMR
no	no	pMMR
yes	yes	pMMR
no	no	pMMR
yes	yes	pMMR
yes	no	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
no	no	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
yes	yes	pMMR
no	no	pMMR
no	no	pMMR
no	no	pMMR
yes	no	pMMR
yes	no	pMMR
yes	yes	pMMR
no	no	pMMR
no	no	pMMR
no	no	pMMR
yes	yes	pMMR
yes	yes	pMMR

yes	yes	pMMR
no	no	pMMR
yes	yes	pMMR
yes	no	pMMR
no	no	pMMR
yes	no	pMMR
no	no	pMMR
no	no	pMMR
no	no	pMMR
no	no	pMMR
no	no	pMMR
no	no	pMMR
yes	yes	pMMR
no	no	pMMR
yes	no	pMMR
no	no	pMMR
yes	no	pMMR
no	no	pMMR
yes	yes	pMMR
yes	yes	pMMR
no	no	pMMR
no	no	indeterminate
yes	yes	indeterminate
yes	yes	indeterminate
yes	yes	indeterminate
yes	yes	indeterminate
no	no	indeterminate
no	no	indeterminate
yes	yes	indeterminate

whole-section slides Status

MMR

dMMR

MSH2/MSH6

dMMR

MLH1/PMS2

dMMR

MLH1/PMS2

dMMR

MSH2/MSH6

dMMR

MLH1/PMS2

dMMR

MSH2/MSH6

dMMR

MSH2/MSH6

pMMR

unsuccessful amplification

pMMR

NA

pMMR

NA

pMMR

NA

pMMR

NA

pMMR

NA

pMMR

NA

pMMR

NA

pMMR

NA

pMMR

NA

pMMR

NA

pMMR

NA

lack of available tumor

Lack of available tumor

pMMR

NA

pMMR

NA

pMMR

NA

pMMR

NA

pMMR

NA

pMMR

NA

pMMR

NA

pMMR

NA

pMMR

NA

pMMR

NA

pMMR

NA

pMMR

NA

pMMR

NA

pMMR

NA

pMMR

NA

pMMR

NA

pMMR

NA

pMMR

NA

pMMR

NA

pMMR

NA

pMMR

NA

pMMR

NA

pMMR

NA

pMMR

NA

pMMR

NA

pMMR

NA

pMMR

NA

pMMR

NA

pMMR

NA

PentaPlex	Hsp110 T17	dMMR/MSI
MSI	MSI	yes
MSS	MSS	yes
MSS	MSS	yes
unsuccessful amplification	unsuccessful amplification	yes
MSI	MSI	yes
MSS	MSI	yes
MSS	MSI	yes
unsuccessful amplification	no	no
unsuccessful amplification	MSS	no
MSS	MSS	no
MSS	MSS	no
unsuccessful amplification	MSS	no
unsuccessful amplification	MSS	no
MSS	MSS	no
MSS	unsuccessful amplification	no
unsuccessful amplification	MSS	no
unsuccessful amplification	MSS	no
unsuccessful amplification	MSS	no
unsuccessful amplification	unsuccessful amplification	no
Lack of available tumor	Lack of available tumor	no
unsuccessful amplification	unsuccessful amplification	no
unsuccessful amplification	unsuccessful amplification	no
unsuccessful amplification	MSI	no
MSS	unsuccessful amplification	no
unsuccessful amplification	MSS	no
MSS	MSS	no
MSS	MSS	no
MSS	MSS	no
MSS	MSS	no
unsuccessful amplification	MSS	no
unsuccessful amplification	MSS	no
unsuccessful amplification	MSS	no
unsuccessful amplification	unsuccessful amplification	no
unsuccessful amplification	MSS	no
unsuccessful amplification	MSS	no
MSS	MSS	no
unsuccessful amplification	MSS	no
unsuccessful amplification	MSS	no
unsuccessful amplification	MSS	no
MSS	MSS	no
MSS	MSS	no
MSS	MSS	no
unsuccessful amplification	MSS	no
MSS	MSS	no
unsuccessful amplification	unsuccessful amplification	no
unsuccessful amplification	unsuccessful amplification	no
unsuccessful amplification	unsuccessful amplification	no
unsuccessful amplification	unsuccessful amplification	no
unsuccessful amplification	unsuccessful amplification	no

Supplementary Table 3. Patients' Characteristics (multicentric retrospective cohort)

Variables	N (%)
Sex (427)	
male	244 (57%)
female	183 (43%)
Age (median, range)	63.8 years (34-87)
IPMN-associated PDAC (426)	
yes	41 (9%)
no	385 (91%)
Tumor size (mm) (413)	
≥30	253 (61%)
<30	160 (39%)
Lymph nodes (423)	
Negative, N0	105 (25%)
Positive, N1	318 (75%)
Resection Margins (423)	
R0	318 (76%)
R1	105 (24%)
Preoperative treatment (424)	
yes	26 (6%)
no	398 (94%)
Postoperative treatment (302)	
yes	102 (33%)
no	210 (67%)
Local recurrence (397)	
yes	172 (43%)
no	225 (57%)
Distal recurrence (397)	
yes	195 (49%)
no	202 (51%)
Recurrence (427)	
yes	285 (66%)
no	142 (34%)

Supplementary Table 4. Patient's Clinical and Pathological Characteristics of the Saint-Antoine's IPMN-related tumors cohort

Patient	Sex	Age	UICC (7th ed.)	Recurrence	Dead	DFS (months)	OS (months)	IPMN location	Size of the invasive component (mm)
PSA01	female	83	TisN0	no	no	26	26	main duct	NA
PSA02	male	59	T2N0	no	no	15	15	main duct	>20
PSA03	female	55	T2N0	no	no	155	155	main duct	>20
PSA04	male	55	T3N1	no	no	95	95	mixed	60
PSA05	female	60	T2N0	no	no	96	96	side branch	10-20
PSA06	male	55	T3N0	no	no	2	2	main duct	60
PSA07	male	73	T3N1	no	no	2	2	main duct	20
PSA08	female	69	T3N1	yes	yes	16	40	mixed	20
PSA09	female	56	T3N0	yes	no	9	27	mixed	10
PSA10	female	80	T1N0	no	no	34	34	mixed	10
PSA11	male	47	T3N1	yes	yes	19	34	Main duct	40
PSA12	male	58	T3N1	no	no	71	71	side branch	25
PSA13	male	63	T3N0	yes	yes	28	32	mixed	20
PSA14	male	76	T3N0	no	no	107	107	side branch	22
PSA15	female	53	T3N1	no	no	77	77	side branch	30
PSA16	male	73	T1N0	no	no	3	3	mixed	6 mm
PSA17	male	58	T1N0	no ^d	no	223	223	mixed	<1

Supplementary Table 5. Survival Analysis (pooled multicentric retrospective cohort and Saint Antoine's IPMN-related tumors cohort)

Variables	DFS				OS			
	Univariate		Multivariate		Univariate		Multivariate	
	median survival (months)	<i>P</i> value	95% CI	<i>P</i> value	median survival (months)	<i>P</i> value	95% CI	<i>P</i> value
Sex		.1	0,93-1,54	.16		.237		
female	17,6±1,9				33,2±3			
male	14,2±1,2				26,4±3,2			
Tumor size		<.0001	1,15-1,96	.002		<.0001	1,33-2,33	<.0001
≥30mm	13,4±0,7				21,8±1,6			
<30mm	24±4				36,9±3,3			
Margins		<.0001	1,22-2,16	.001		<.0001	1,12-2,04	.007
R0	17,6±2,2				33,2±2,2			
R1	11,0±1,1				18,4±2,3			
Lymph Nodes		<.0001	1,47-2,90	<.0001		<.0001	1,36-2,78	<.0001
positive, N+	13±0,7				22,9±1,9			
negative, N0	42,7±7,8				57,7±13			
IPMN		.039	0,48-1,25	.301		.025	0,35-1,10	.105
yes	38,5±15,8				44,9±11,3			
no	14,7±1				27,4±2,6			
dMMR/MSI		.703				.663		
yes	21,4±10,5				35,1±0,1			
no	15,6±1,1				29,9±2,4			

Supplementary methods

Patient Cohorts and Tumor Samples

This study was approved by the institutional review boards/ethics committees of the participating centers. Informed consent was recorded in each case. Patient selection, clinicopathological data and demographic information have been described previously ¹⁰. Briefly, we included patients from three different cohorts. The first cohort comprised a retrospective series of patients with primary PDAC who underwent curative surgical resection between September 1996 and August 2009 in five European university centers with high volume and expertise in pancreatic cancer management ¹⁰. Subjects were excluded if they had undergone preoperative treatment (chemotherapy or radiotherapy), macroscopically incomplete resection (R2), or had tumor histology other than ductal adenocarcinoma. The second cohort included patients from a prospective French multicenter study (NCT00918853) which determined the impact of a standardized pathological protocol on resection margin status after pancreatoduodenectomy for PDAC ¹¹. Patients were enrolled between August 2008 and May 2010. Exclusion criteria included R2 resections, non-ductal adenocarcinoma, and margin analysis performed without using the predefined criteria. An aggregated clinical database (referred to as the “Multicentric Retrospective Cohort”) was created with standardized clinicopathological variables including sex, age at diagnosis, tumor site, type of operative procedure, tumor stage according to the Union for International Cancer Control, TNM classification, and maximal tumor size. The third cohort was comprised of 17 consecutive patients operated at the Saint-Antoine Hospital for IPMN-associated PDAC from 1998 to 2015 and is referred to as the “Monocentric IPMN Cohort”.

Patient-derived xenograft (PDX) mouse model

In addition to clinical tumor samples, DNA samples from 68 human PDAC xenografts were available for dMMR/MSI testing. These xenografted tumors were all derived from samples that were not included in the formalin-fixed, paraffin-embedded tumor specimens (FFPE) cohorts. In brief, two types of samples were included in the PDX cohort as previously

described ¹²: endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) biopsies from patients with unresectable tumors and tumor tissue samples from patients undergoing surgery. All the samples were anonymized, and postsurgical anatomopathology reports were provided. Histopathologic evaluation was performed on 5- μ m hematoxylin and eosin stained sections of patient tumors and xenografts and examined under a light microscope. These sections were compared with the original human tumor when available. Tumor specimens (100 mm³) from resected PDAC patients were then mixed with Matrigel (BD Biosciences) and implanted subcutaneously on the upper right flank of 5- to 6-week-old nude mice (Swiss Nude Mouse Crl: NU(lco)-Foxn1nu, Charles River Laboratories). Tumor size and body weights of all animals were measured weekly. When tumors reached 1 cm³, the mice were sacrificed and tumors removed. Mice were cared for and manipulated in accordance with National and European legislation on animal experimentation. The experimental design was approved by the Aix-Marseille University Institutional Animal Care and Use Committee (CE2A-14).

Construction and Processing of Tissue Microarrays and Determination of Tumor MMR status Using Immunohistochemistry

Tissue microarrays (TMA) were constructed from 0.6-mm diameter tissue cores obtained from (FFPE tumor specimens. A total of 602 cases of resected PDAC (collected from the Multicentric Retrospective Cohort) were placed into 21 TMA blocks, with each tumor represented by 3–5 cores. Hematoxylin and Eosin (H&E) staining was performed on each TMA slide to confirm the presence of tumor tissue.

PDACs are usually inflamed and highly contaminated with stromal cells. MSI testing by PCR has poor sensitivity in this context and hence we first screened for dMMR in these tumors using IHC. Assessment for loss of expression of the 4 MMR proteins (MLH1, MSH2, MSH6 and PMS2) in tumor tissue is a more appropriate method in these conditions ¹³.

The expression of MLH1, MSH2, MSH6, and PMS2 was assessed as described previously¹⁴. The cores were independently examined by at least three observers (RML, MS, J-FF) who were blinded to both clinicopathological and outcome data for the patients. Samples showing clear loss of MMR protein expression or uncertain MMR expression (defined by less intense expression in tumor cells compared to normal epithelial cells or controls) in the TMAs were further tested by IHC using full face sections. These slides were reviewed by two experienced pathologists (MS and J-FF).

MSI Testing

PCR was used to screen for MSI only in samples that were suspected to be MMR-deficient by IHC or that displayed an uncertain dMMR status. PCR was also used to screen the additional series of PDACs derived from PDXs, since these were clear of any human contaminating inflammatory cells.

MSI Using Pentaplex PCR

The same paraffin blocks were used for DNA extractions and for IHC analyses. DNA was extracted from formalin-fixed, paraffin-embedded neoplastic tissue that had been macrodissected with reference to the H&E-stained section. DNA was extracted after deparaffinization by treatment with sodium dodecyl sulfate-proteinase K and phenol-chloroform-isoamyl alcohol. To evaluate MSI, 5 quasimonomorphic mononucleotide markers (BAT-25, BAT-26, NR-21, NR-22, and NR-24) were coamplified in a single pentaplex PCR mix (mononucleotide repeat pentaplex [MRP] assay) and then analyzed for amplicon size in an automated DNA sequencer¹³. Because the date of fixation and the treatment and conservation of tissues were highly variable due to the different origins of paraffin-embedded tissues, it was not always possible to amplify all markers, particularly the longer ones. Samples in which results were available for only one or two of the 5 markers were considered as uninterpretable. For all other samples, tumors showing instability at two or more markers were defined as MSI, whereas those with instability at only one marker or showing no instability were defined as microsatellite stable (MSS).

MSI Testing Using HSP110 T17 PCR

HT17 status was determined as previously published¹³. Briefly, PCR products were run on an ABI 3100 Genetic Analyzer with GS400HD ROX size standard and POP-7 polymer (Applied Biosystems). GeneMapper (V4.0) software (Applied Biosystems) was used to analyze the HT17 traces. HT17 traces were considered to be acceptable when the amplitudes of the major peaks were between 100 and 6000 fluorescence units. Height ratios between peaks were also calculated, especially between T14 and T16 (R1) and between T15 and T16 (R2). The HT17 DNA repeat is remarkably monomorphic, with the size of normal alleles ranging from 145 bp (T16) to 147 bp (T18). Tumors were classified as MSI when the extent of stuttering by Taq polymerase observed for HT17 was ≥ 2 bp, or when the height ratios between the T14 and T15 stutter peaks and the T16 peak in germline DNA exceeded 0.15 (R1) and/or 0.5 (R2).

Determination of MLH1 promoter methylation

The DNA methylation pattern of the *MLH1* promoter region was determined by methylation-specific PCR of bisulfite-treated DNA (1 μ g)¹⁴. This was performed only for cases that showed loss of MLH1 protein expression by IHC.

Mutation analysis at target genes for MSI

Sixteen genes containing repeat sequences were chosen for study because they are either frequent targets for MSI-driven mutations in MSI tumors, or because they play key roles in the maintenance of genome integrity (*ACVR2A*, *ATR*, *BAX*, *BLM*, *CBF2*, *CDX2*, *GRB14*, *GRK4*, *IGF2R*, *MBD4*, *MSH3*, *MSH6*, *RAD50*, *RECQL*, *TCF7L2* and *TGFBR2*). PCR was performed with primers specific for each selected target gene (sequences available upon request).

Characterization of PD-L1 staining and characterization of T cells in immune infiltrates in MSI PDAC and controls

An IHC study using antibodies to CD3, CD8 and PD-L1 was performed on MSI PDAC (n=9) and on a series of MSS PDAC controls (n=10). For PD-L1, slides were incubated with

the clone SP263 (Ventana) on unstained tissue sections (4 μ m) using the Ventana Benchmark Ultra platform automated stainer according to the manufacturers' recommendations. Positive PD-L1 expression was defined as any membranous staining, in either tumor cells or infiltrating inflammatory cells. The percentage of tumor cells demonstrating membranous PD-L1 staining was also scored in 5% increments⁸.

T cells in the tumor-associated immune infiltrates were assessed by semi-quantitative estimation of CD3-positive (CD3: Thermo Scientific, clone SP7, 1:300 dilution) and CD8-positive (CD8: DAKO, clone C8/144B, 1:30 dilution) cell densities. They were scored as follows: 0, no positive cells; 1, scattered positive cells; 2, moderate number of positive cells; 3, abundant occurrence of positive cells. In each tumor, the density of T-cell infiltration was examined at the invasive front (cells localized in stroma adjacent to the invasive tumor margin) and in the intra-tumoral compartment¹⁵.

Cell lines

Primary PDAC cell lines (3 MSS cell lines: 01.001, B-TIM and L-IPS; and 1 MSI cell line: 01.004) were cultured in DMEM/F12 medium supplemented with 5% NuSerum, ITS+ (Corning), PSA, BPE, EGF, nicotinamide, glucose, gentamicin, T3, dexamethasone and cholera toxin (Sigma).

For flow cytometry analysis, cells were blocked in 5% BSA in PBS, then stained with corresponding antibodies and analyzed by a Gallios flow cytometer (Beckman Coulter). FlowJo Software (TreeStar, Ashland, USA) was used for data analysis.

For flow cytometry analysis the following antibodies were used: Gal-3 FITC mouse anti-human clone 9M1-3, PD-L2 PE mouse anti-human clone 24F.10C12 and B7-H3 PE mouse anti-human clone DCN.70 (all from BioLegend). PD-L1 FITC mouse anti-human clone MIH1 was purchased from BD Pharmingen.

Statistical Analysis

Descriptive statistical analysis used χ^2 tests and *t* tests. Two-by-two table contingency analyses were performed using a two-tailed Fisher's exact test because some numerical

values were less than five. A *P* value of <0.05 was considered significant. Overall survival was measured from the date of surgery to the date of death (all causes). Survival curves were estimated using the Kaplan–Meier technique and compared using the log-rank test. For each test, statistical significance was set at a two-sided *P* value of <0.05. Statistical analyses were performed using SPSS 20 software.