The 62nd Annual Congress of Taiwan Association of Obstetrics and Gynecology

Assessment of Mismatch Repair Deficiency and Associated Clinicopathologic Significance

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Overview

- May. 2017 Pembrolizumab (PD-1 inhibitor) received accelerated FDA approval for Solid MSI-H or dMMR tumor that has progressed following prior treatment
- Jun 2018 Cervical cancer with PD-L1 /2nd line
- Apr 2021 Dostarlimab dMMR endometrial cancer/2nd line (GARNET)
- Jul 2021 Pembrolizumab + lenvatinib endometrial cancer /2nd line (KEYNOTE-775)
- Aug 2021 Dostarlimab dMMR solid tumors /2nd line (GARNET)
- Mar 2022 Pembrolizumab dMMR endometrial cancer/2nd line (KEYNOTE-158)

- MSI-H or dMMR tumors tend to be hypermutated, release more neoantigens that stimulate an immune response, and may be more susceptible to immunotherapeutic strategies

TCGA Project

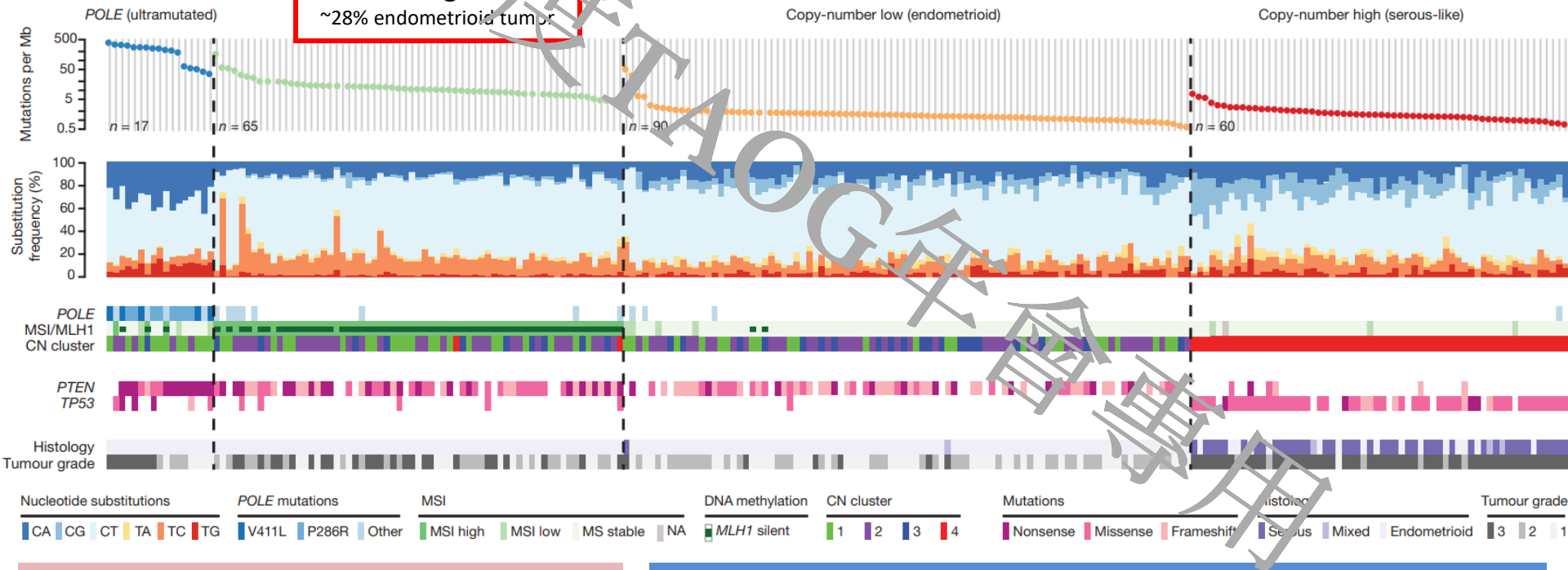
Mutation Spectra Across Endometrial Carcinomas

POLE group (ultramutated) 100-500 mut/Mb, endometrioid, *PTEN* mutation

MSI group (hypermutated) 10-20 mut/Mb, endometrioid, *PTEN* mutation, mixed grade ~28% endometrioid tumor

Copy-number low group 2-3 mut/Mb, endometrioid, *PTEN, CTNNB1* mutations, G1

Copy-number high group 2-3 mut/Mb, endometrioid, *TP53* mutations, high grade

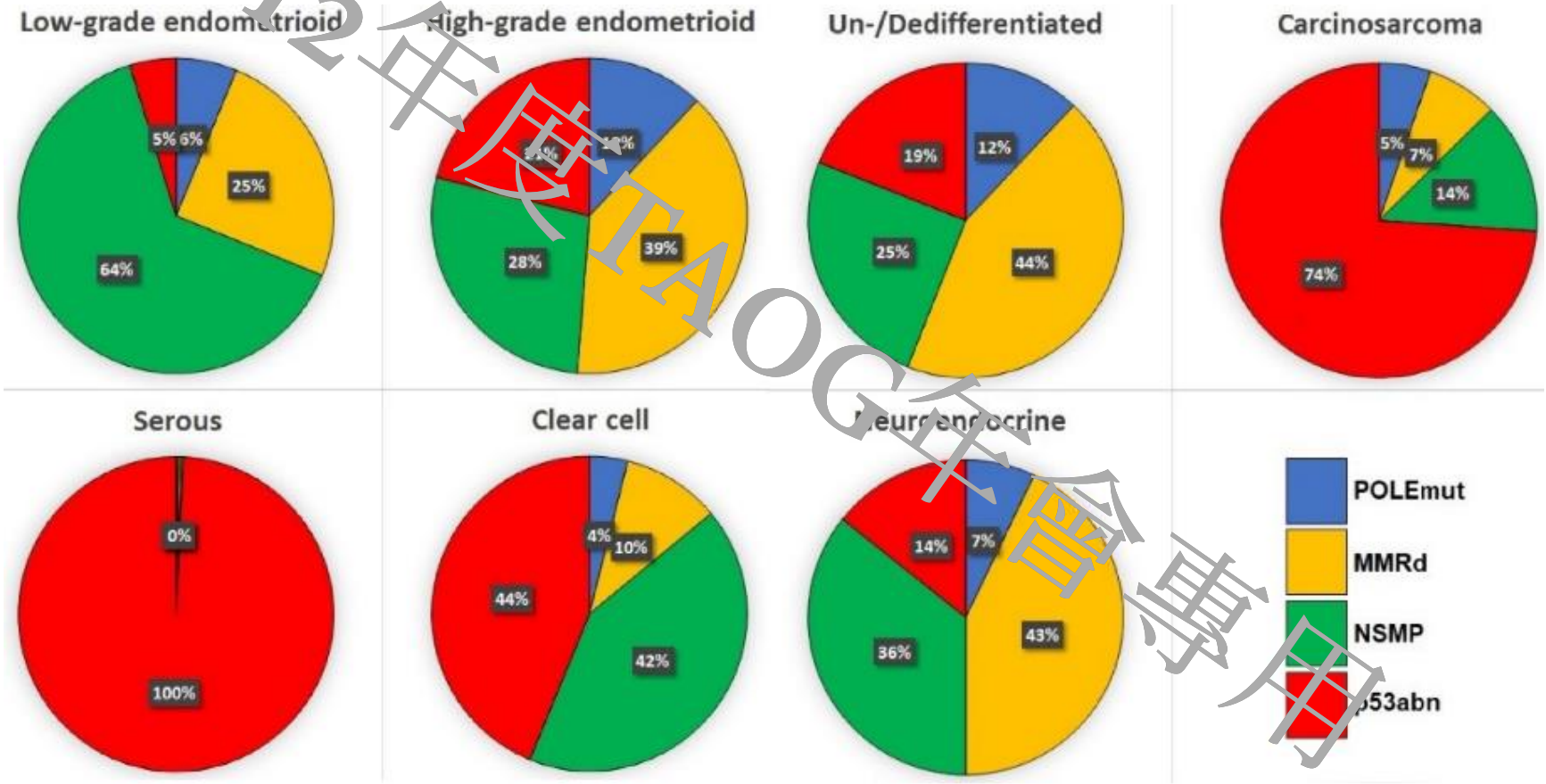


High genome instability immunogenic

Hypo-mutated, low expression of immune-related biomarkers

Nature. 2013 May 2;497(7447):67-73.

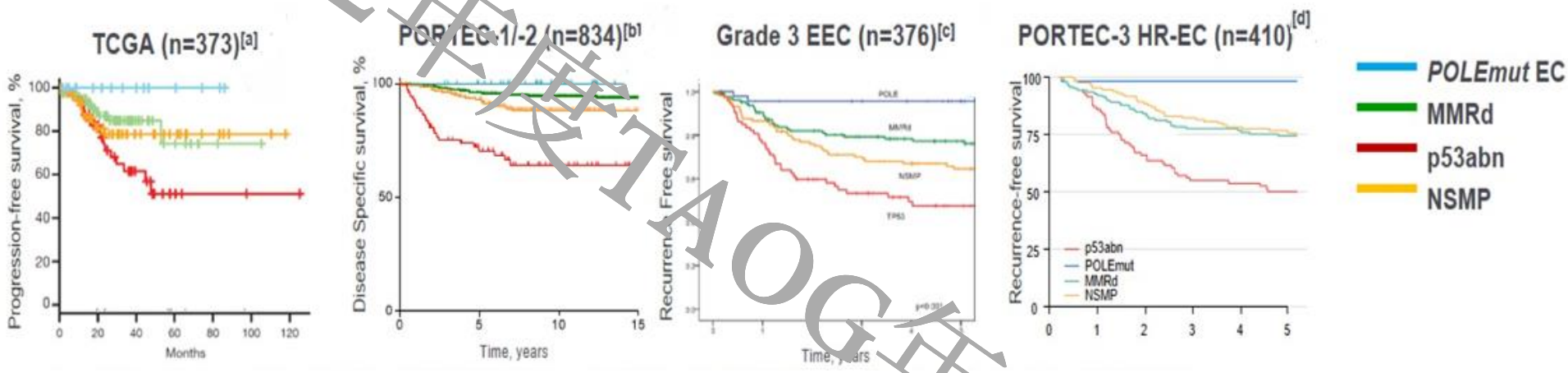
Molecular Classifications of Endometrial Carcinomas

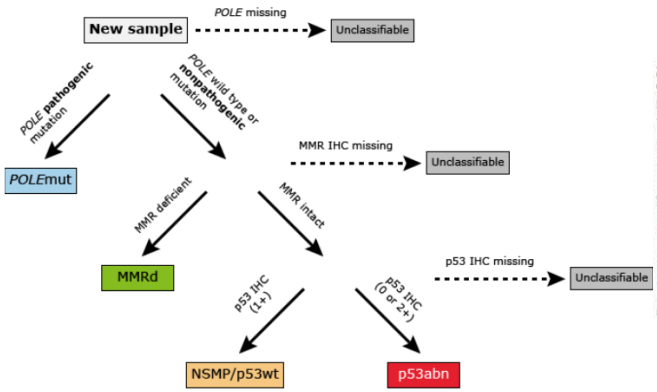


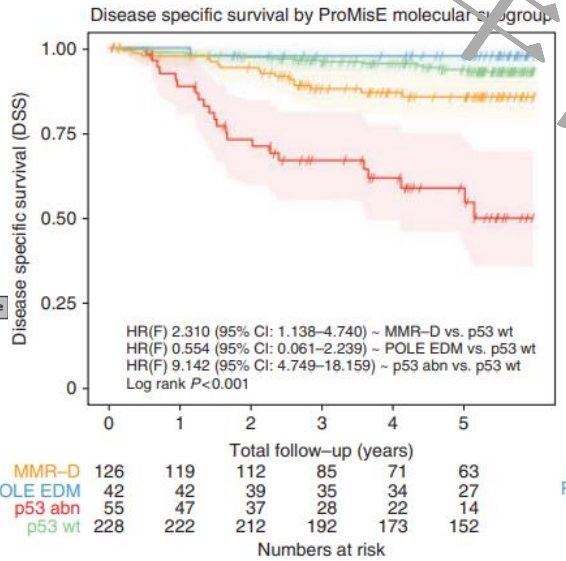
Cancers (Basel). 2021 May 26;13(11):2623. .

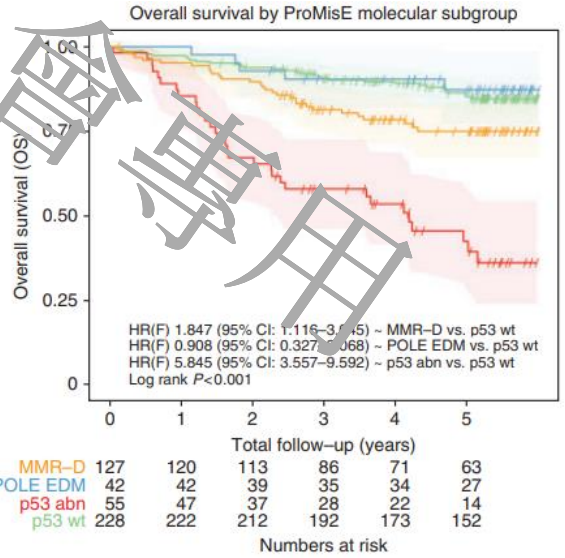
High prognostic value of molecular characterization of endometrial cancer

- Best survival for *POLE*mut (ultramutated) EC
- ProMisE (Proactive Molecular Risk Classifier for Endometrial Cancer)









a. Nature.2013;497:67-73;
b. Clin Cancer Res. 2016; 22:4215-4224;
c. Am J Surg Pathol. 2018; 42:561-568;
d. J Clin Oncol. 2020;38:3388-3397
Ann Oncol. 2018 May 1;29(5):1180-1188.

2020 ESGO/ESTRO/ESP Guidelines

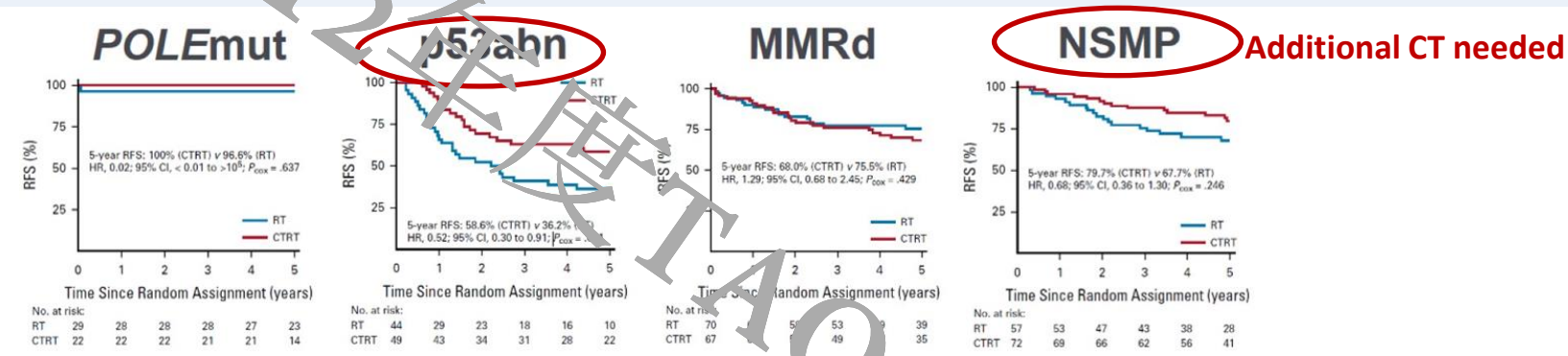
Molecular Classifications of Endometrial Carcinomas

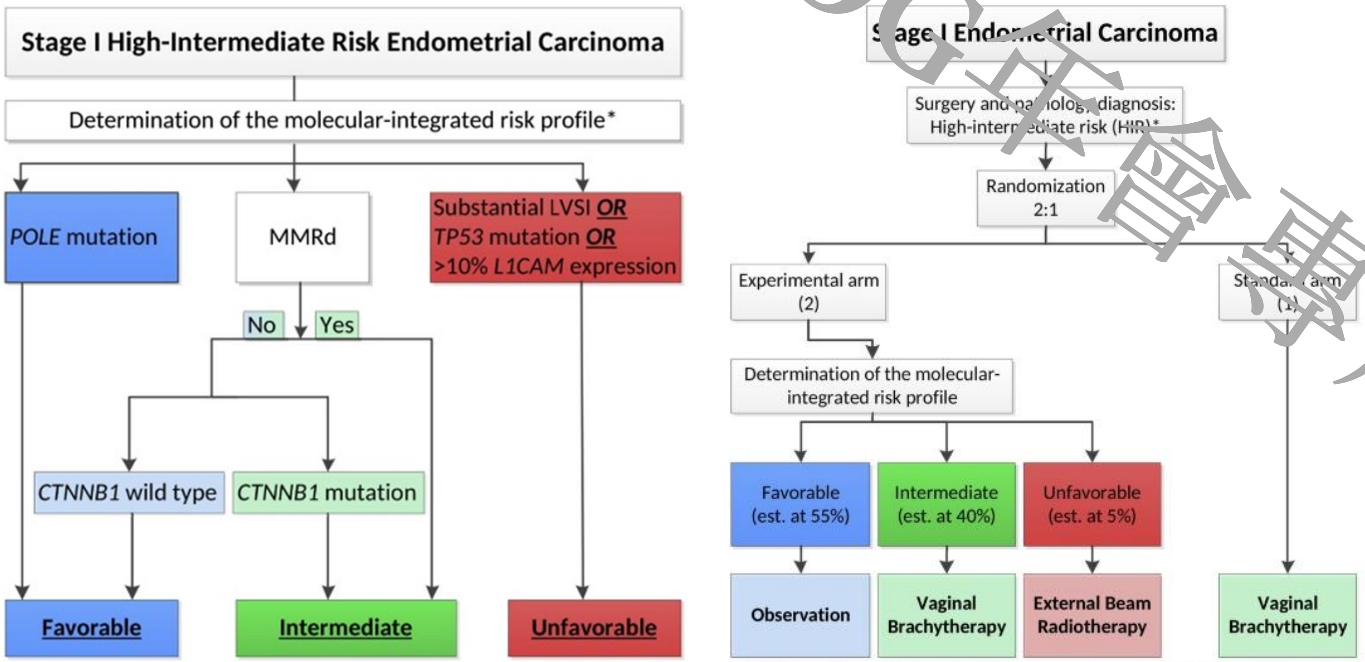
Risk Group	Molecular Classification Unknown	Molecular Classification Known
Low	➢ Stage IA endometrioid + low-grade + LVSI negative or focal	➢ Stage I-II ***POLEmut*** endometrial carcinoma, no residual disease ➢ Stage IA **dMMR/NSMP** endometrioid carcinoma + low grade + LSVI negative or focal
Intermediate	➢ Stage IB endometrioid + low-grade + LVSI negative or focal ➢ Stage IA endometrioid + high-grade + LVSI negative or focal ➢ Stage IA nonendometrioid (serous; clear cell; undifferentiated carcinoma; carcinosarcoma, mixed) without myometrial invasion	➢ Stage IB **dMMR/NSMP** endometrioid carcinoma + low grade + LVSI negative or focal ➢ Stage IA **MMRd/NSMP** endometrioid carcinoma + high grade + LSVI negative or focal ➢ Stage IA **p53abn** or nonendometrioid (serous; clear cell; undifferentiated carcinoma; carcinosarcoma, mixed) without myometrial invasion
High-intermediate	➢ Stage I endometrioid + substantial LVSI regardless of grade and depth of invasion ➢ Stage IB endometrioid high grade regardless of LVSI status ➢ Stage II	➢ Stage I **dMMR/NSMP** endometrioid carcinoma + substantial LVSI regardless of grade and depth of invasion ➢ Stage IB **dMMR/NSMP** endometrioid carcinoma high grade regardless of LVSI status ➢ Stage II **dMMR/NSMP** endometrioid carcinoma
High	➢ Stage III-IVA with no residual disease ➢ Stage I-IVA nonendometrioid (serous; clear cell; undifferentiated carcinoma; carcinosarcoma, mixed) with myometrial invasion and with no residual disease	➢ Stage III-IVA **dMMR/NSMP** endometrioid carcinoma with no residual disease ➢ Stage I-IVA **p53abn** endometrial carcinoma with myometrial invasion, with no residual disease ➢ Stage I-IVA **NSMP/dMMR** serous; undifferentiated carcinoma; carcinosarcoma with myometrial invasion, with no residual disease
Advanced metastatic	➢ Stage III-IVA with residual disease ➢ Stage IVB	➢ Stage III-IVA with residual disease of any molecular type ➢ Stage IVB of any molecular type

Int J Gynecol Cancer. 2021 Jan;31(1):12-39; Cancers (Basel). 2021 May 26;13(11):2623. .

Molecular typing-guided treatment

- Predictive potential for adjuvant platinum-based treatment in PORTEC-3
- Ongoing: PORTEC-4a NCT03469674, CANSTAMP NCT04159155, TAPER NCT04705649……

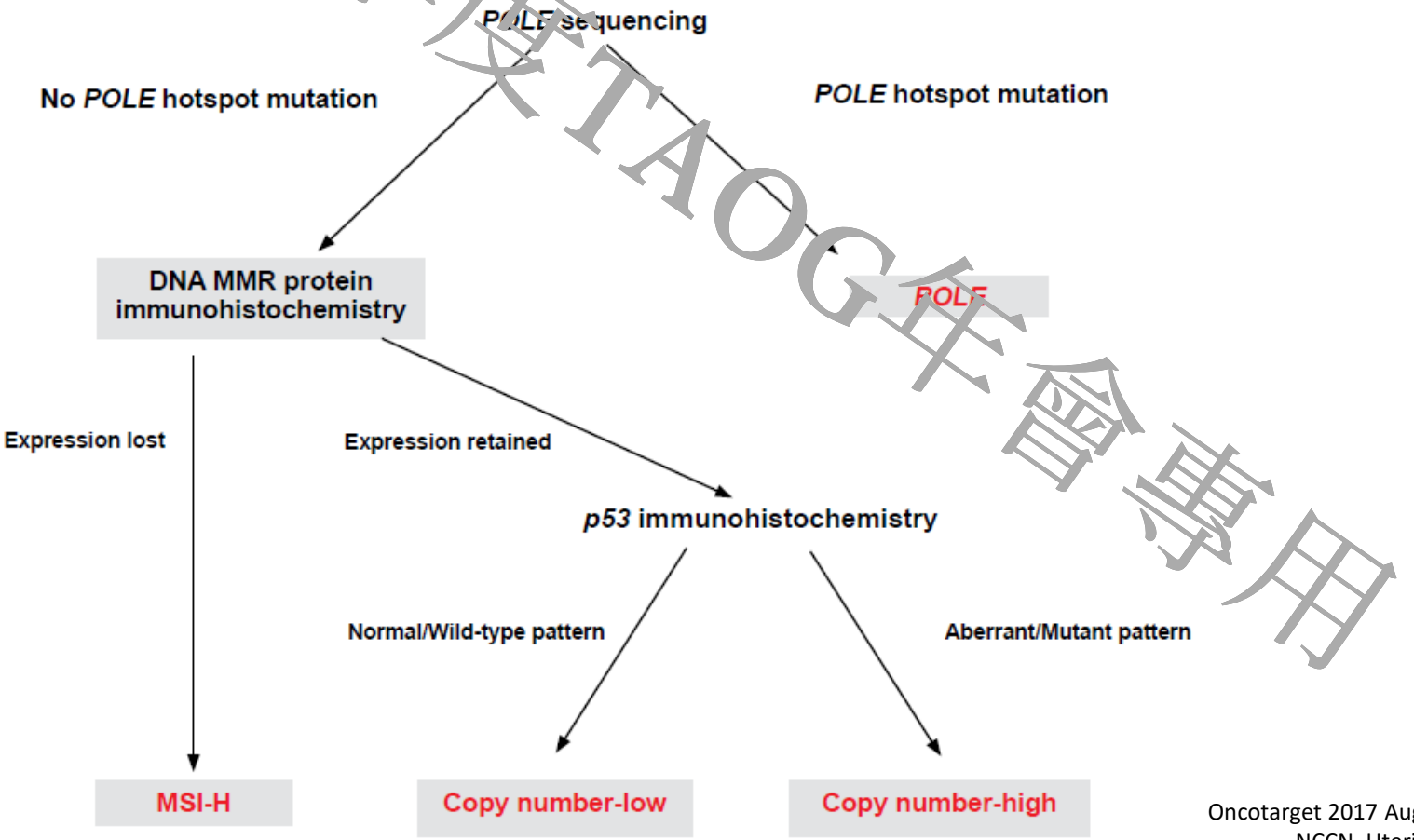




J Clin Oncol. 2020;38:3388-3397.
Curr Oncol. 2022;29(2):741-757Curr Oncol. 2022 Feb; 29(2): 741–757.

Principle of molecular analysis

- Recommend **universal testing for MMR proteins** (MSI testing if results equivocal)
- Assess promoter methylation in MLH1 loss (epigenetic mechanism)
- Consider NTRK gene fusion testing for metastatic or recurrent EC
- Consider TMB testing through validated and/or FDA-approved assays



Oncotarget 2017 Aug 3;8(48):84579-84594.
NCCN. Uterine neoplasm.V 2.2023.

Updated 2023 FIGO Staging of Endometrial Cancer

Define substages

- In all stages, grade, histological type and LVSI must be recorded. If available and feasible, molecular classification testing (POLEmut, MMRd, NSMP, p53abn) is encouraged in all patients for prognostic risk-group stratification
- addition of "m" for molecular classification and a subscript for subtype
- Non-aggressive histological types: G1/2 EECs; aggressive histological types: G3 EEC, serous, clear cell, undifferentiated, mixed, mesonephric-like, gastrointestinal mucinous type carcinomas and carcinosarcomas.
- Disease upstaging or downstaging if "m" classification of p53abn or POLEmut in stages I and II (**IIC**m_{p53abn} or **IA**$m_{POLEmut}$).

TABLE 2 FIGO endometrial cancer stage with molecular classification.[a]

Stage designation	Molecular findings in patients with early endometrial cancer (Stages I and II after surgical staging)
Stage $IAm_{POLEmut}$	*POLEmut* endometrial carcinoma, confined to the uterine corpus or with cervical extension, regardless of the degree of LVSI or histological type
Stage $IICm_{p53abn}$	p53abn endometrial carcinoma confined to the uterine corpus with any myometrial invasion, with or without cervical invasion, and regardless of the degree of LVSI or histological type

Stage II (FIGO 2009)

Stage I (FIGO 2009)

Int J Gynaecol Obstet. 2023 Aug;162(2):383-394.

Updated 2023 FIGO Staging

TABLE 1 2023 FIGO staging of cancer of the endometrium.[a,b]

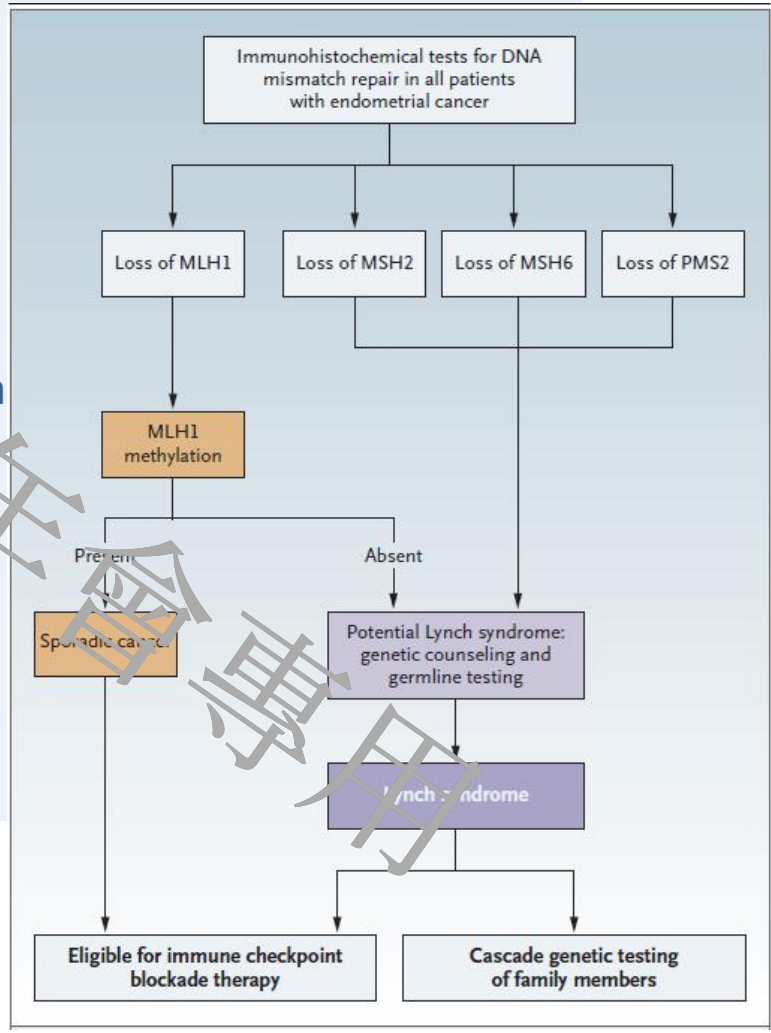
Stage	Description
Stage I	Confined to the uterine corpus and ovary[c]
IA	Disease limited to the endometrium OR non-aggressive histological type, i.e. low-grade endometroid, with invasion of less than half of myometrium with no or focal lymphovascular space involvement (LVSI) OR good prognosis disease
	IA1 Non-aggressive histological type limited to an endometrial polyp OR confined to the endometrium
	IA2 Non-aggressive histological types involving less than half of the myometrium with no or focal LVSI
	IA3 Low-grade endometrioid carcinomas limited to the uterus and ovary[c]
IB	Non-aggressive histological types with invasion of half or more of the myometrium, and with no or focal LVSI[d]
IC	Aggressive histological types limited to a polyp or confined to the endometrium
Stage II	Invasion of cervical stroma with extrauterine extension OR with substantial LVSI OR aggressive histological types with myometrial invasion
IIA	Invasion of the cervical stroma of non-aggressive histological types
IIB	Substantial LVSI[d] of non-aggressive histological types
IIC	Aggressive histological types[e] with any myometrial involvement
Stage III	Local and/or regional spread of the tumor of any histological subtype
IIIA	Invasion of uterine serosa, adnexa, or both by direct extension or metastasis
	IIIA1 Spread to ovary or fallopian tube (except when meeting stage IA3 criteria)
	IIIA2 Involvement of uterine subserosa or spread through the uterine serosa
IIIB	Metastasis or direct spread to the vagina and/or to the parametria or pelvic peritoneum
	IIIB1 Metastasis or direct spread to the vagina and/or the parametria
	IIIB2 Metastasis to the pelvic peritoneum
IIIC	Metastasis to the pelvic or para-aortic lymph nodes or both[f]
	IIIC1 Metastasis to the pelvic lymph nodes
	IIIC1i Micrometastasis
	IIIC1ii Macrometastasis
	IIIC2 Metastasis to para-aortic lymph nodes up to the renal vessels, with or without metastasis to the pelvic lymph nodes
	IIIC2i Micrometastasis
	IIIC2ii Macrometastasis
Stage IV	Spread to the bladder mucosa and/or intestinal mucosa and/or distance metastasis
IVA	Invasion of the bladder mucosa and/or the intestinal/bowel mucosa
IVB	Abdominal peritoneal metastasis beyond the pelvis
IVC	Distant metastasis, including metastasis to any extra- or intra-abdominal lymph nodes above the renal vessels, lungs, liver, brain, or bone

Int J Gynaecol Obstet. 2023 Aug;162(2):383-394.

Why testing for MMR/MSI relevant in endometrial and ovarian carcinoma?

Recommended testing for MMR status/MSI in all EC, irrespective of age

- Diagnostic, MMRd/MSI considered a marker for endometrioid-type EC
- Pre-screening to identify patients at higher risk for Lynch syndrome
  - 3-5% of all endometrial cancer
  - About 10% of MMRd/MSI-H endometrial carcinoma
  - EC often precedes subsequent ca (sentinel cancers)
- Prognostic, as identified by TCGA classification Hypermutated MMRd/MSI category
- Predictive testing for MMRd tumors eligible for immune checkpoint inhibitors



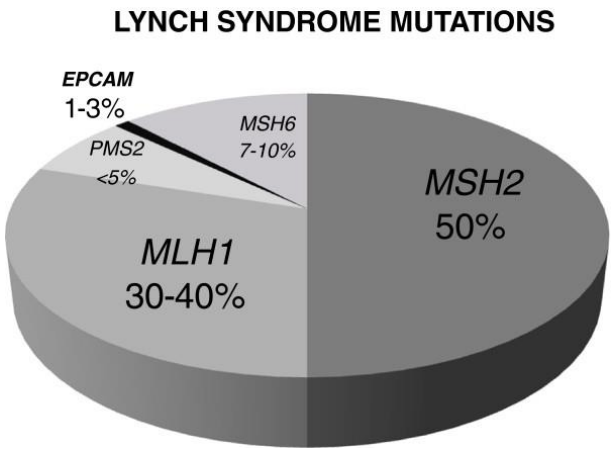
Int J Gynecol Cancer 2021;31:12–39; Genet Med 2019;21:2390–400; 10.1056/NEJMc2035378.

Lynch syndrome

- Autosomal dominant inherited, hereditary cancer syndrome

  Prevalence: 1 in 600 to 1 in 3,000 individuals
- Germline mutation in 1 of 4 MMR genes (MLH1, MSH2, MSH6, PMS2) or the epithelial cell adhesion molecule (EPCAM)
- Different risks for cancers

  Colorectum (18–61%), endometrium (16–61%), ovaries(5-10%), stomach, small bowel, bile duct, pancreas, and upper urinary tract
  - 2% to 6% (2.3 %) of endometrial cancers (5-9 % in <50 years)
  - 2.2% in women with colorectal cancer
- Screening and identification of patients by clinical characteristics Amsterdam criteria II (3-2-1 rule) and revised Bethesda criteria (2004)

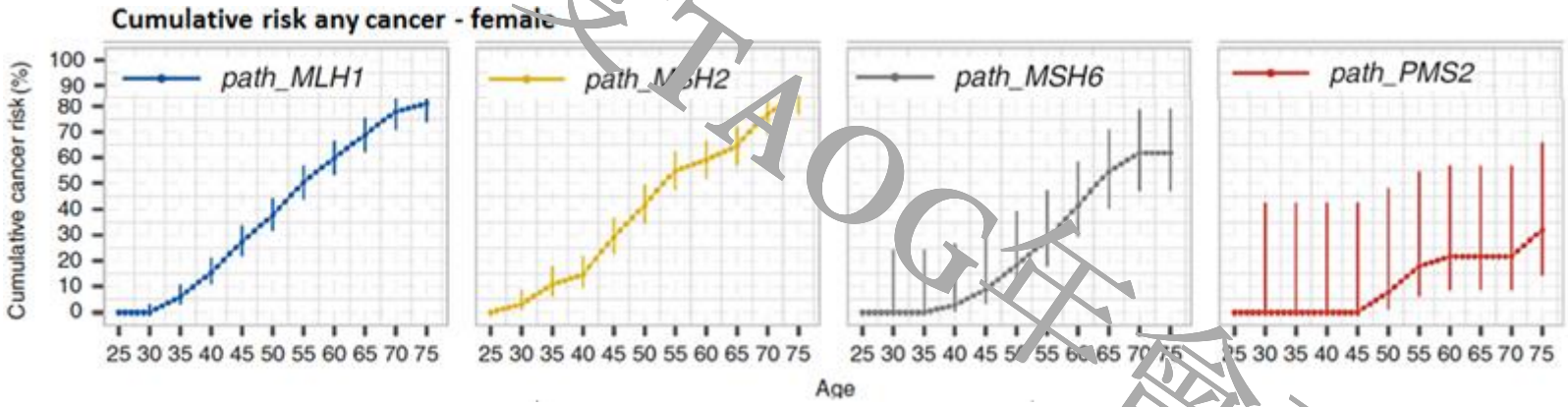


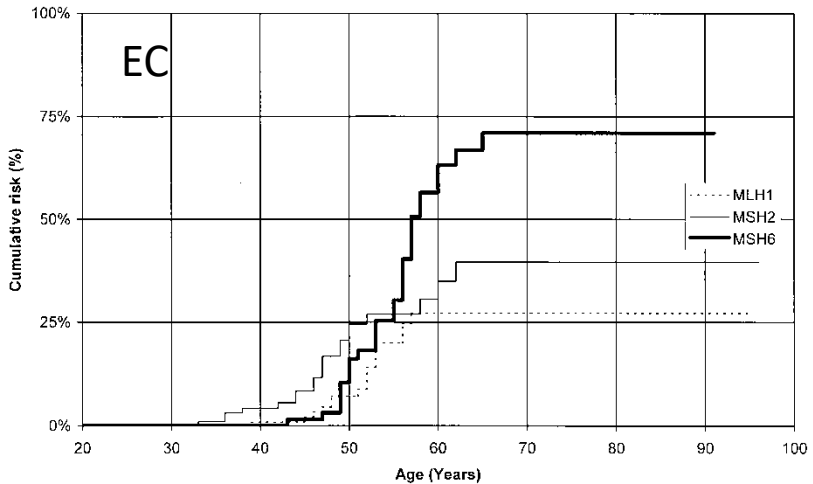
MLH1 and MSH2 mutations ~ 90% (NCBI)

International Society for Gastrointestinal Hereditary Tumours (InSiGHT) database (https://databases.lovd.nl/shared/genes)
Obstet Gynecol. 2014 Nov;124(5):1042-1054.
Hered Cancer Clin Pract. 2013; 11(1): 9.

Lynch syndrome

- Highest cancer risks in path_MLH1 and path_MSH2 carriers
- Penetrance for path_MSH6 variants lower but females had high risks for gynecological cancers
- Higher risk for EC for MSH6 mutation carriers than MLH1 and MSH2



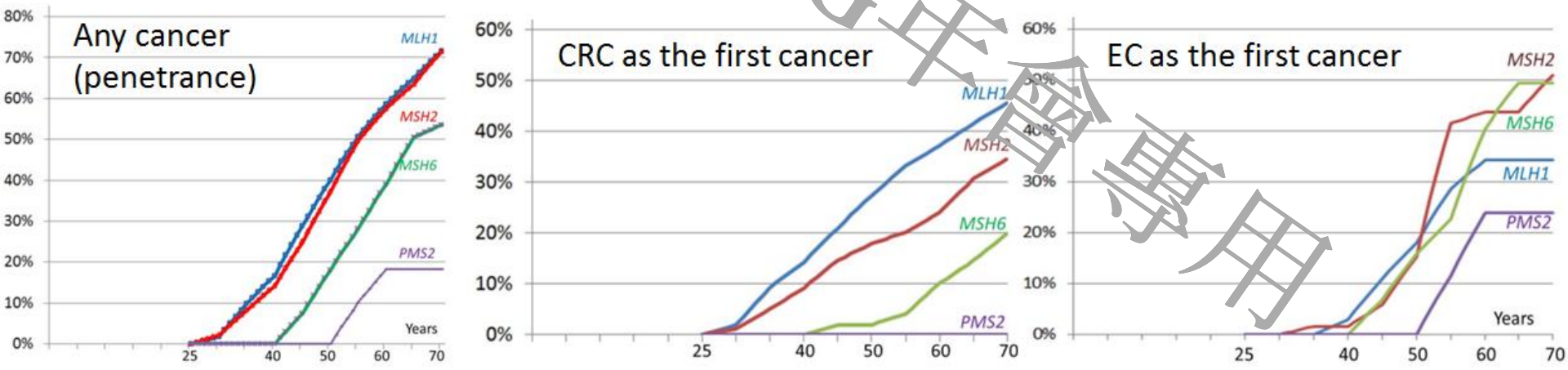


Gastroenterology. 2004 Jul;127(1):17-25
Hered Cancer Clin Pract. 2013; 11(1): 9.

Lynch syndrome

- Age of cancer onset varies among specific mutated genes, types of mutations
- Cumulative incidences for EC at 70 years:
  34%, 51%, 49%, and 24% for MLH1, MSH2, MSH6, and PMS2 mut
- Cumulative incidences for OC at 70 years:
  11%, 15%, 0%, and 0% for MLH1, MSH2, MSH6, and PMS2 mut
- Surveillance for EC in general start at the age of 35 years
  Ryan et al suggest gyn surveillance from age 30 (MSH2 mut), 35 (MLH1 mut), 40 (MSH6 mut)

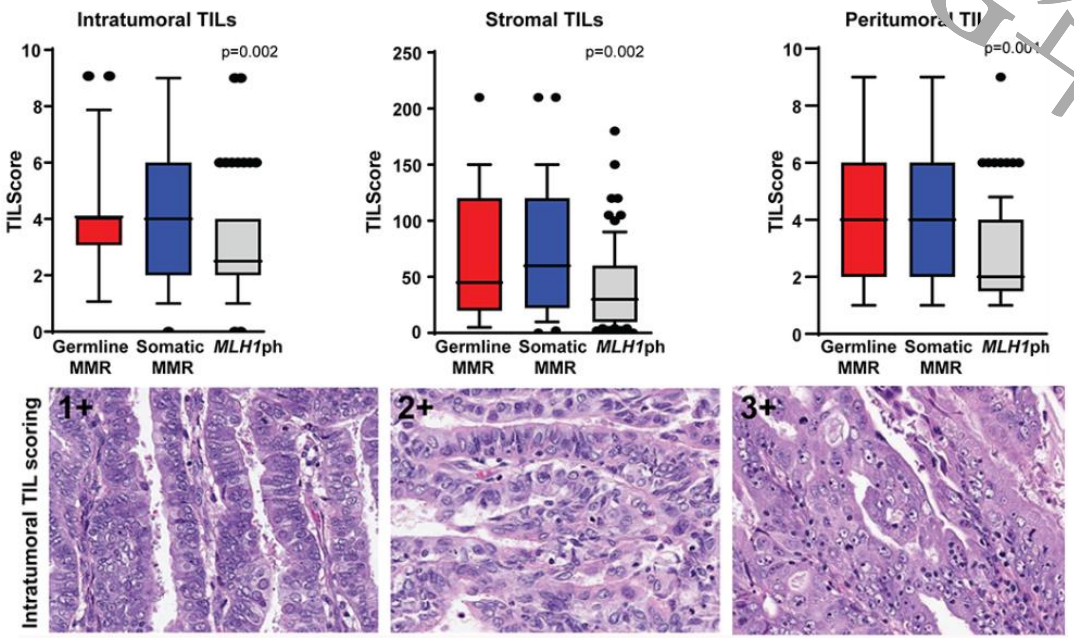
Calculated cumulative incidences by age and mutated gene

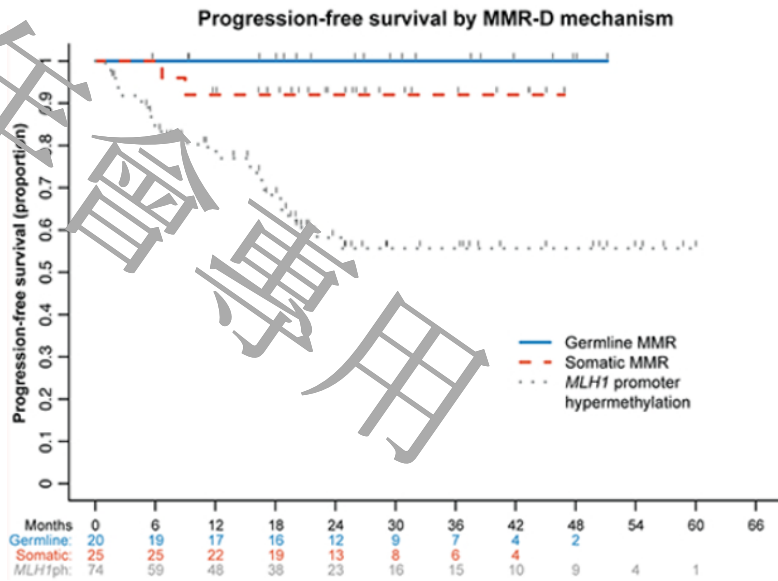


Gut 2017;66:464–72.; JAMA Oncol 2017;3:1702–6.

Hereditary or sporadic origin matters?

- Increased immune response in MSI-H tumors with increased immune cell infiltration and PD-L1 positive cells
- Equivalent immune response in MSI-H EC with sporadic or inherited Lynch syndrome origins?
  - Increased CD8+ cells and activated CTLs in stroma: LS > sporadic MSI-H cases
  - TIL score: germline/somatic MMR mutations> MLH1ph ECs
  - MLH1ph EC was associated with inferior PFS





Clin Cancer Res. 2017 Aug 1; 23(15): 4473–4481.
Clin Cancer Res. 2022 Oct 3; 28(19): 4302–4311.

Microsatellite Instability (MSI)

- **Microsatellites, short tandem repeats**

  Repetitive DNA sequences, 1-6 bases, in both coding and noncoding regions along the genome, particularly sensitive to DNA mismatching errors during DNA replication or damage

- **MSI** A condition of genetic hypermutability

- Clustering of mutations in microsatellites consisting of repeat length alterations, phenotypic evidence of defective DNA mismatch repair
- A marker of dMMR, characterizes a hypermutable state
- Assessed with :

(I) Defective expression of MMR proteins as determined by IHC

(ii) Molecular tests, including PCR-based tests and NGS approaches

Annals of Oncology 30: 1232–1243, 2019

DNA Mismatch Repair, MMR

- **DNA MMR**

Restore DNA mismatching errors, single base mismatches or short indels

- Critical genes include MLH1, MSH2, MSH6 and PMS2
- Function in heterodimers, MLH1-PMS2 and MSH2-MSH6.
- Germline, somatic mutations or epigenetic silencing of one of these genes results in a defective MMR (dMMR)

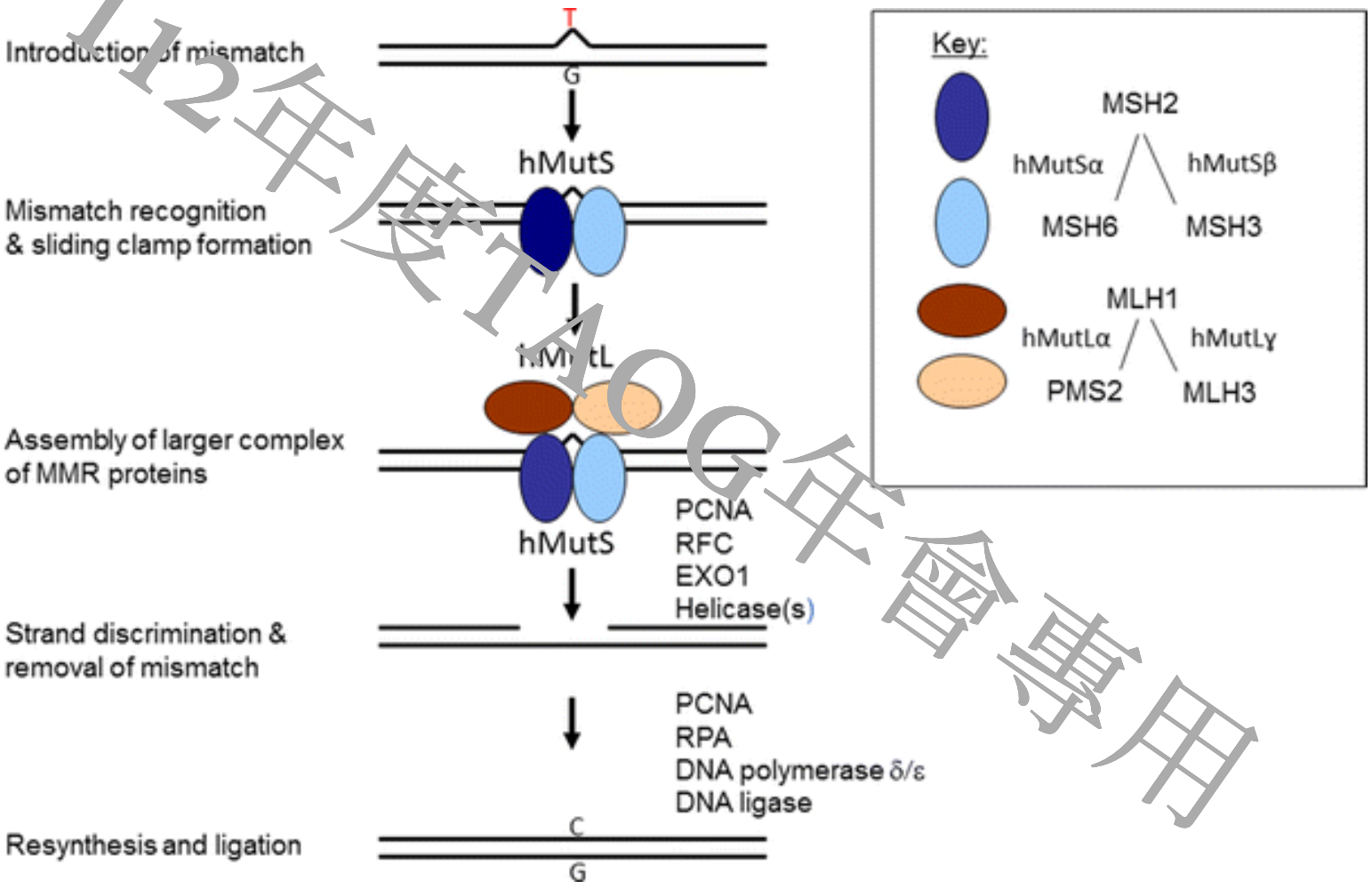
- **MSI-H/MMRd tumor**

A tumor that accumulates thousand of mutations, particularly clustered in microsatellites, consisting of repeat length alterations, result in MSI

Heterodimer	Components	Type of mismatches repaired	Predominant type of MSI (defective gene)
hMutSα	MSH2 + MSH6	Single-base mismatches, ins/del loops	MSI-H (*MSH2* or *MSH6*)
hMutSβ	MSH2 + MSH3	Ins/del loops	MSI-L/EMAST (*MSH3*)
hMutLα	MLH1 + PMS2	Single-base mismatches, ins/del loops	MSI-H (*MLH1* or *PMS2*)
hMutLβ	MLH1 + PMS1	?	?
hMutLγ	MLH1 + MLH3	Single-base mismatches, small loops	MSI-L/EMAST or MSI-H (*MLH3*)

Encyclopedia of Cancer (Third Edition)2019, 374-388
Ann Oncol. 2019;30(8):1232-1243.

DNA Mismatch Repair, MMR



PCNA, proliferating cell nuclear antigen; RFC, replication factor C;
EXO1, exonuclease 1; RPA, replication protein A
Familial Cancer 15, 385–393 (2016).

MSI status defined by IHC

- IHC for 4 MMR proteins required in cancer type belonging to spectrum of Lynch syndrome [colorectal, endometrial, small intestine, urothelial, gliomas/glioblastomas and sebaceous gland]

MMR genes mutation interfere with dimerization→ Heterodimers degradation

→ Loss of both obligatory and secondary proteins

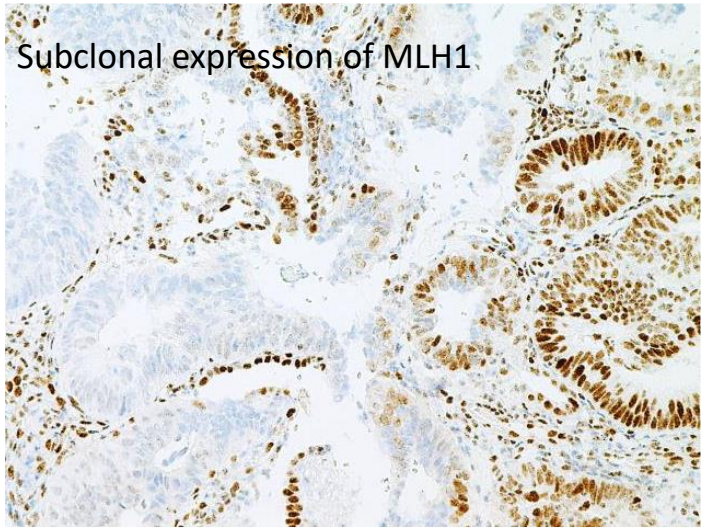
- Mutations in MLH1→ IHC loss of both MLH1 and PMS2
- Mutations in MSH2 →IHC loss of both MSH2 and MSH6
- PMS2 antibody detects MLH1 or PMS2 abnormalities
- MSH6 antibody detects MSH2 or MSH6 abnormality

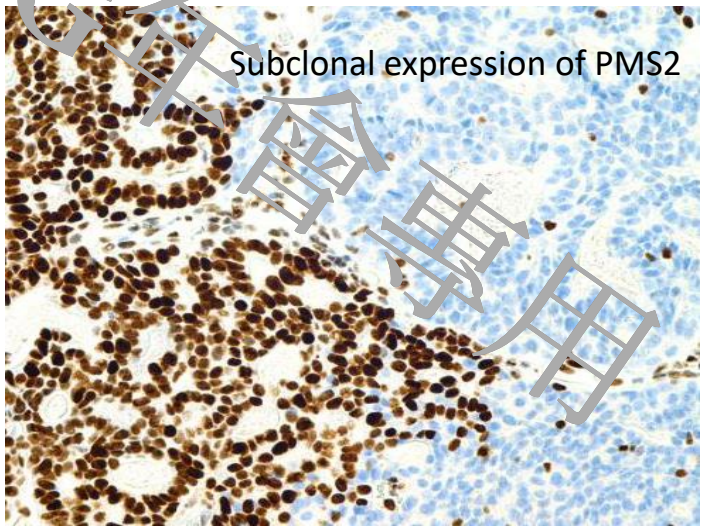
- Advantages
- Perform IHC on biopsies or surgical specimens?

- Pitfalls
- False negative due to tissue fixation, aberrant staining patterns
- Missense mutation with catalytically inactive but antigenically intact mutant protein
- Lack of PMS2 or MSH6 substituted by other secondary proteins (MSH3, MLH3, PMS1)

Common problems in MMR IHC interpretation

- Suboptimal fixation
- Defective or equivocal staining: very weak or focal expression seen in the presence of MMRd, unknown diagnostic, and clinical implications of this pattern
- Subclonal expression (focal expression loss): normal staining must be seen as internal control; occur as acquired defect during tumor progression, such as MLH1 promoter methylation; "passenger mutation" in MSH6 gene



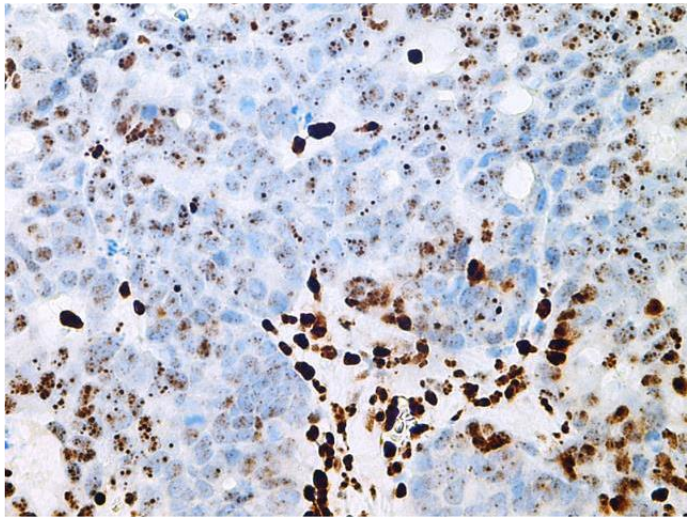


BAGP Guidance Document: MMR Immunohistochemistry interpretation and terminology. 2020

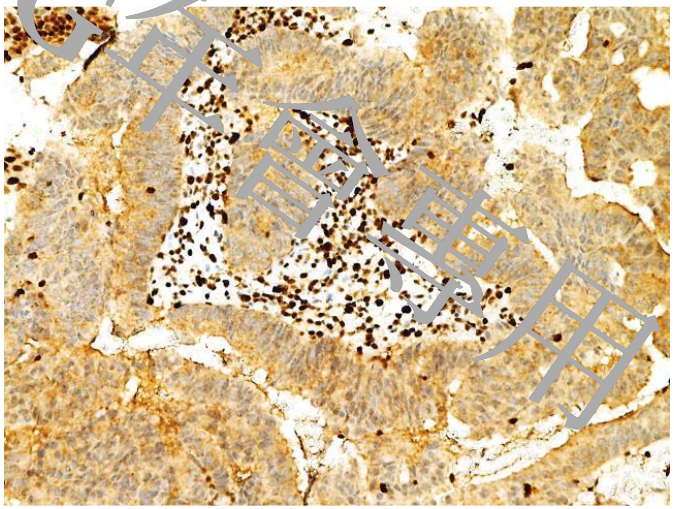
Common problems in MMR IHC interpretation

- Punctate nuclear expression pattern in some case of MLH1 loss: erroneously interpreted as retained/normal expression
- Cytoplasmic/membranous staining should be reported as abnormal
- Others: 3 or more proteins loss, MMR IHC/MSI or MMR IHC/genetic testing discordancy…

Punctate nuclear staining for MLH1



Cytoplasmic staining for PMS2



BAGP Guidance Document: MMR Immunohistochemistry interpretation and terminology. 2020

MSI status defined by IHC

- ESMO recommendations
- The first test of choice is IHC
- Use all 4 MMR proteins. Whether testing all 4 antibodies simultaneously or in a sequential manner, i.e. using two-antibody screening followed by reflex IHC for the appropriate partner protein
- Screen for MMRd by testing for PMS2 and MSH6: cost-effective but equivalent accuracy to testing for all 4 proteins ?
- Move to MSI-PCR whenever there is any doubt in IHC interpretation

Table 5 Selected literature reports on patterns of immunohistochemical staining for MLH1, MSH2, MSH6, and PMS2 in extraintestinal neoplasms

Tumor site	*Reference*	*Total no.*	*Abnormal mismatch repair protein immunohistochemical staining pattern*							*All intact*
			MLH1/ PMS2	*MLH1-only*	*PMS2-only*	*MSH2/ MSH6*	*MSH2-only*	*MSH6-only*	*Other patterns*	
Skin sebaceous tumor	Orta *et al*[19]	27	2	0	1	8	0	1	0	15
Gynecologic tract	Modica *et al*[39]	85	23	0	6	6	1	9	1 with MSH2/ MSH6, 2 with MLH1/ PMS2/MSH6	37
Gynecologic tract	Garg *et al*[40]	71	19	0	0	9	0	4	0	39
Gynecologic tract	Backes *et al*[41]	140	24	0	0	4	0	2	0	110
Ampulla of Vater	Agaram *et al*[42]	54	1	0	0	0	0	2	0	51

Mod Pathol 2011;24:1004–14.; Am J Surg Pathol 2009;33:1639–45.; Ann Oncol. 30: 1232–1243, 2019

MSI status defined by PCR-based testing

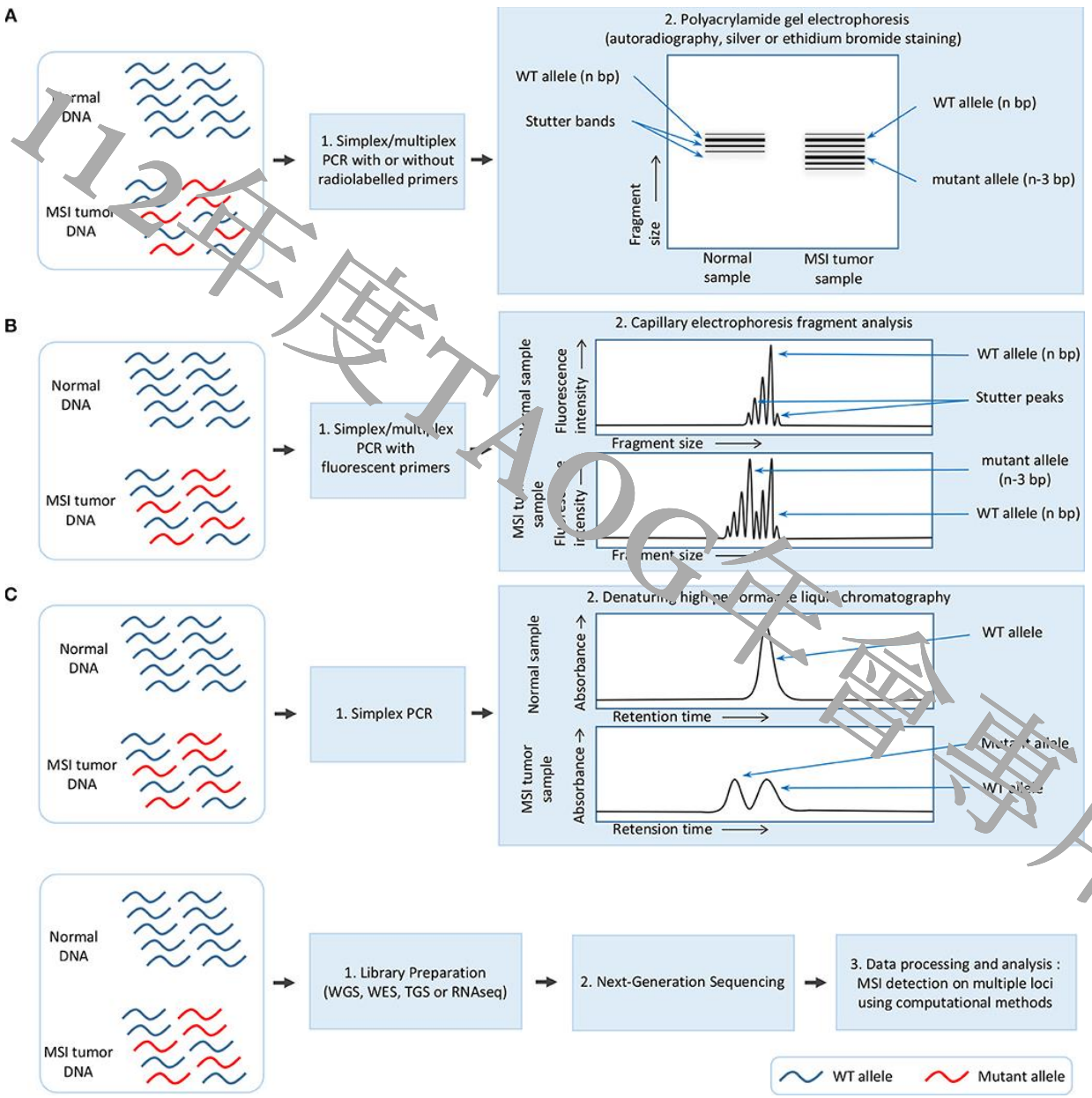
- MSI-PCR molecular testing indicated in indeterminate IHC results or in case of loss of only one heterodimer subunit
- PCR amplification of microsatellite markers
  - Panel 1 (Bethesda/NCI): BAT-25, BAT-26, D5S346, D2S123 and D17S250
  - Panel 2: BAT-25, BAT-26, NR-21, NR-24, NR-27 (Sen 95.6%, Spe 100%).
- MSI: 2+ mononucleotide markers show repeats length alteration

  The terms MSI-H (>=2) or MSI-L (1 marker) should be abandoned and MSI-L should be included with MSS tumors
- NGS-based MSI testing
  - Couple MSI analysis with the determination of TMB
  - Larger set of microsatellites
  - Identify other targetable alterations other than immunotherapy

    MSIplus panel, ColonCore Panel, smMIP panel

J Natl Cancer Inst. (2004) 96(4): 261–268.
Mol Diagn. (2006) 8:305–11.
Clin Chem. (2018) 64:950–8.
Sci Rep. (2021) Jun 18;11(1):12880.

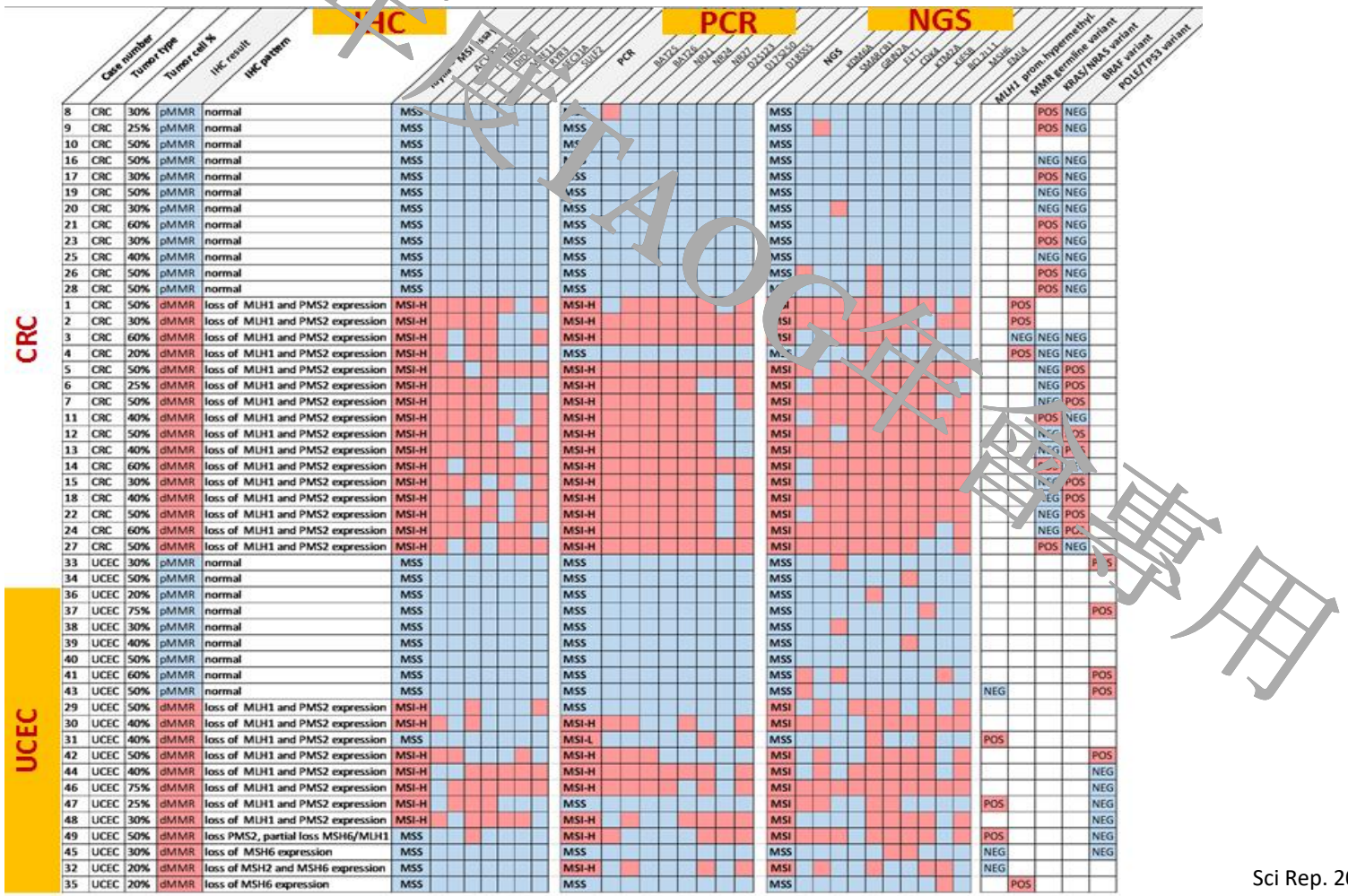
MSI status defined by PCR-based testing



Front Oncol. 2018 Dec 12;8:621.

Testing Algorithm for MSI

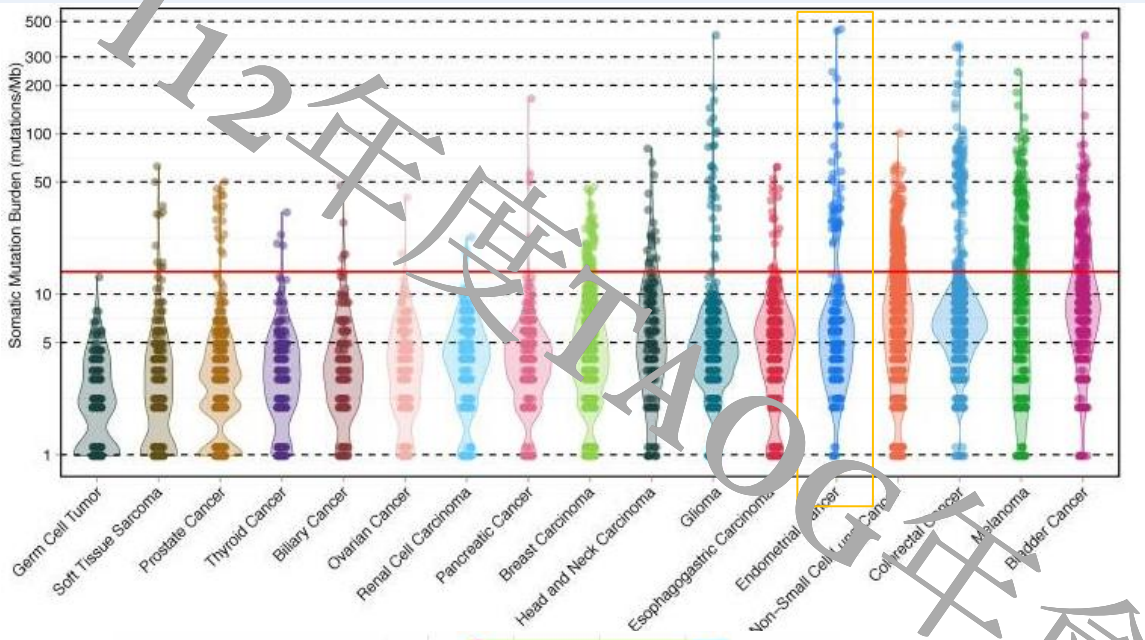
- Better diagnostic performance of molecular assays in CRC than in EC
- In EC, lower sensitivity for PCR assay (67%) and NGS (75%)
- Discrepant IHC and molecular MSI in samples with loss of MSH6 expression

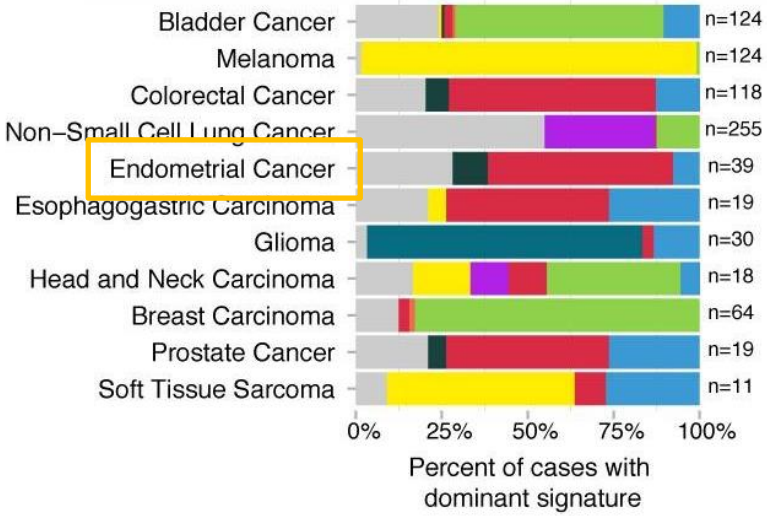


Sci Rep. 2021 Jun 18;11(1):12880.

Significant mutational burden in endometrial cancer

- Microsatellite stable (MSS) in 70-75%; microsatellite instability high (MSI-H) in 25-30%

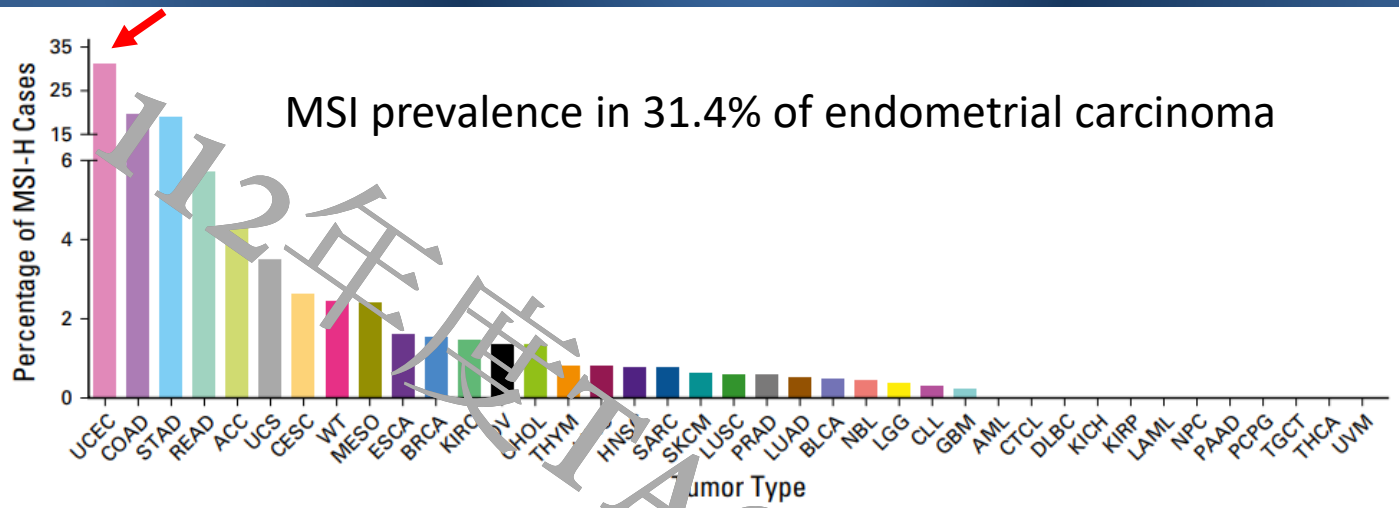




Mutational signatures (MSK-IMPACT)

Nat Med. 2017 Jun;23(6):703-713
JCO Precis Oncol. 2017;2017:PO.17.00073

Significant mutational burden in endometrial cancer



MSI prevalence in 31.4% of endometrial carcinoma

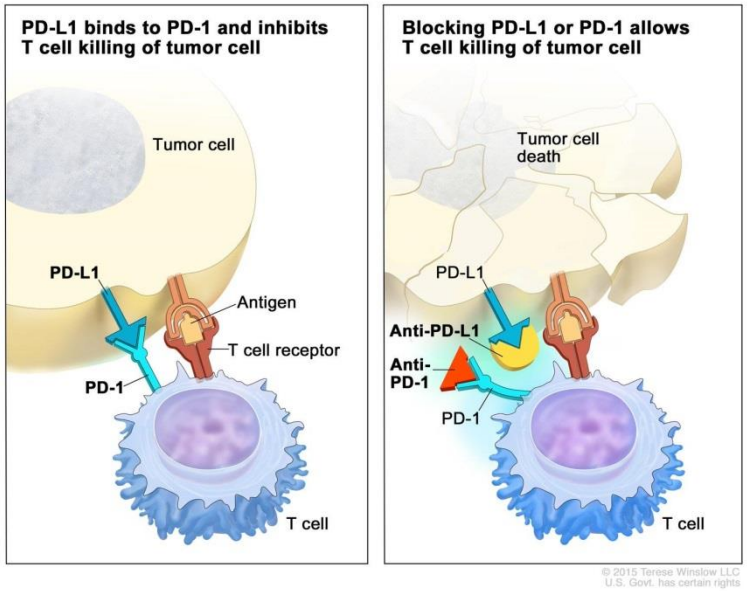
Table 2. Recommendation for MSI testing in different cancer types and in the framework of immunotherapy

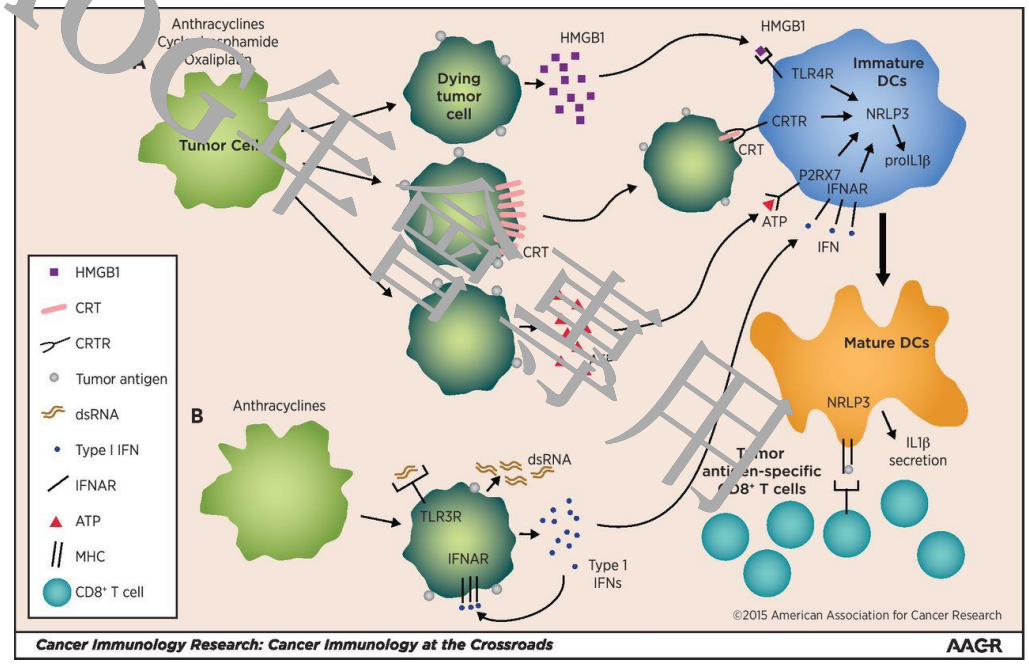
Cancer type	MSI prevalence (all stages)	Specific MSI-associated histotype, if any
Sporadic tumour types belonging to the spectrum of Lynch syndrome that can be tested using IHC and MSI-PCR or NGS (testing is indicated for stage IV cancers, whose MSI prevalence is lower than that of earlier stages)		
Colorectal	17%	Medullary, mucinous, poorly differentiated, neuroendocrine
Endometrial	20%	Lower uterine segment-located, undifferentiated/dedifferentiated, mixed morphology, tumours showing high levels of tumour-infiltrating lymphocytes/lymphoid stroma
Gastric-oesophageal	13%	Adenocarcinoma (MSI up to 39% in case of carcinoma with lymphoid stroma, and absent in oesophageal squamous cell carcinoma)
Small intestine	8.3%	Including duodenum and ampulla of Vater
Ovarian	3.5%–10%	Endometrioid, clear cell
Glioblastoma	6%–13%	
All common or rare tumour types not belonging to the spectrum of Lynch syndrome with low prevalence of MSI and little data available on the reliability of IHC and MSI-PCR, to be tested using NGS		
Unknown primary	1.8%	
Cervical	4%	
Extrahepatic bile duct	3.4%	
Pancreatic	1%–7%	Medullary, IPMN-associated, periampullary (when the origin from ampulla, terminal bile duct or pancreatic duct is uncertain)
Prostate	3%	
Non-small-cell lung cancer	< 1%	
Head and neck	<1%	
Melanoma	NS	1% uveal melanoma
Sarcomas	2%	Uterine, peritoneal and retroperitoneal
Anal	NS	
Kidney	NS	

Nat Med. 2017 Jun;23(6):703-713
JCO Precis Oncol. 2017;2017:PO.17.00073
Annals of Oncology 30: 1232–1243, 2019

Rationale for Immunotherapy-Based Combinations

- Checkpoint immunotherapy mechanism of action
  - Immune checkpoint inhibitors (ICIs) block interaction of PD-L1 with PD-1; allow T cells to kill tumor cells
- Immunogenic effects of chemotherapy
  - Taxanes: T-cell priming, DC activation, MDSCs depletion
  - Platinum-based agents: Downregulation of PD-L1/L2 on DC, Induction of immunogenic cancer cell death

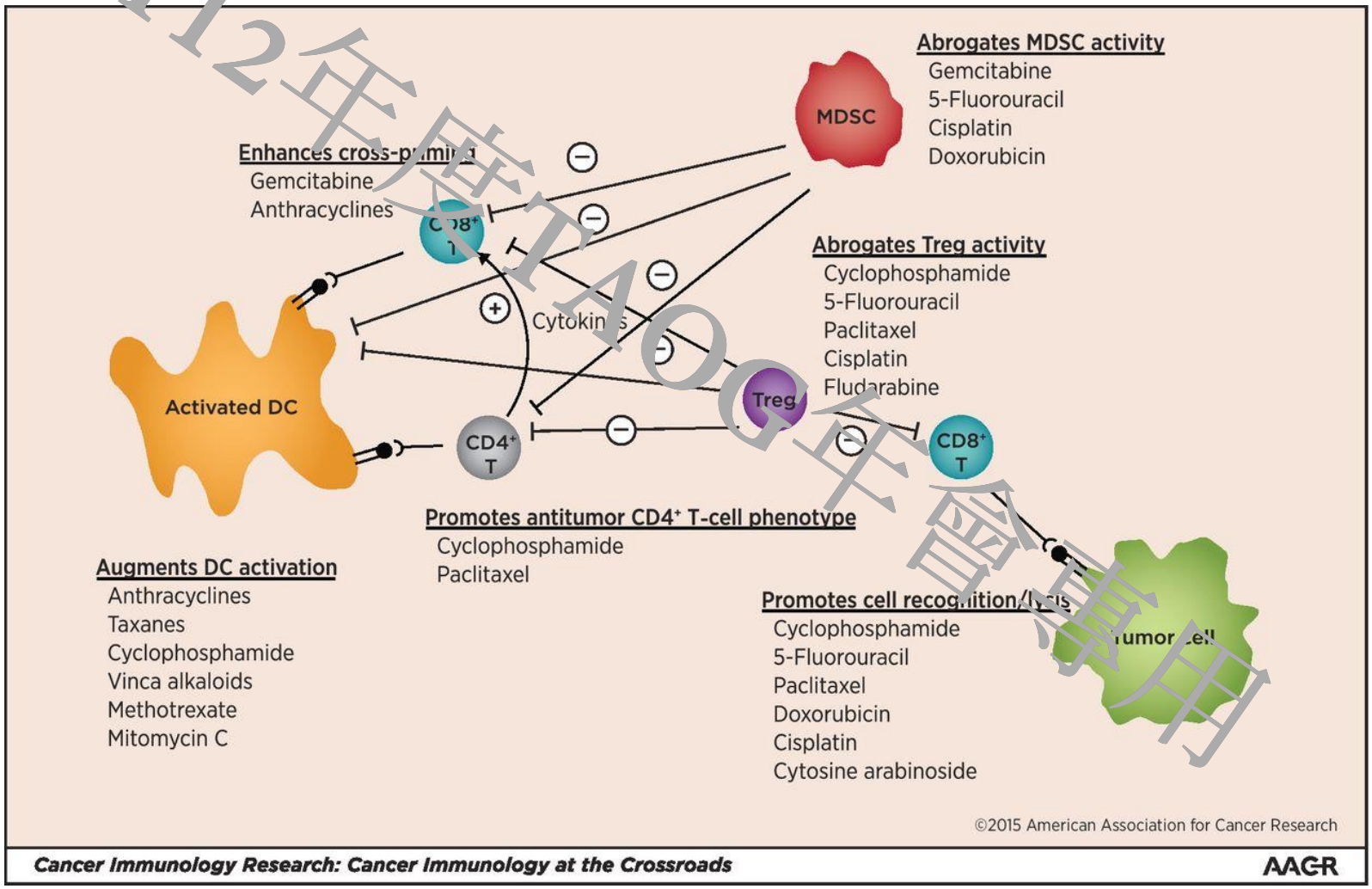




NCI. Accessed Aug 10.2023.https://www.cancer.gov/about-cancer/treatment/types/immunotherapy/checkpoint-inhibitors; Clin Cancer Res . 2014 Jun 1;20(11):2831-7; Cancer Immunol Res. 2015 May;3(5):436-43; Cell Immunol. 2010;263(1):79-87

Rationale for Immunotherapy-Based Combinations

Immunogenic effects of chemotherapy



Cancer Immunol Res. 2015 May;3(5):436-43

NCCN Recommended Systemic Treatments for Endometrial Carcinoma

Primary or Adjuvant Therapy (Stage I-IV)	
Chemoradiation Therapy	**Systemic Therapy**
Preferred Regimens • Cisplatin plus RT followed by carboplatin/paclitaxel[1,2]	**Preferred Regimens** • Carboplatin/paclitaxel[3] • Carboplatin/paclitaxel/pembrolizumab (for stage III-IV tumors, except for carcinosarcoma) (category 1)[a,b,4] NRG-GY018 • Carboplatin/paclitaxel/dostarlimab-gxly (for stage III-IV tumors) (category 1)[b,c,5] RUBY (ENGOT-EN6; GOG-3031) • Carboplatin/paclitaxel/trastuzumab (for stage III/IV HER2-positive uterine serous carcinoma)[d,e,6] • Carboplatin/paclitaxel/trastuzumab (for stage III/IV HER2-positive carcinosarcoma) (category 2B)[d,e,6]

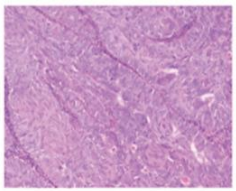
NCCN Clinical Practice Guidelines in Oncology. Uterine Neoplasm. V 2.2023

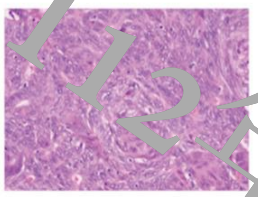
NCCN Recommended Systemic Treatments for Endometrial Carcinoma

RECURRENT DISEASE[f,g]	
First-Line Therapy for Recurrent Disease[h]	Second-Line or Subsequent Therapy
Preferred • Carboplatin/paclitaxel (category 1 for carcinosarcoma)[i,3] • Carboplatin/paclitaxel/pembrolizumab (except for carcinosarcoma) (category 1)[b,4] • Carboplatin/paclitaxel/dostarlimab-gxly (category 1)[b,5] • Carboplatin/paclitaxel/trastuzumab[e] (for HER2-positive uterine serous carcinoma)[d,6] • Carboplatin/paclitaxel/trastuzumab[e] (for HER2-positive carcinosarcoma) (category 2B)[d,6] **Other Recommended Regimens** • Carboplatin/docetaxel[j] • Carboplatin/paclitaxel/bevacizumab[k,7,8] **Useful in Certain Circumstances (Biomarker directed: after prior platinum-based therapy including neoadjuvant and adjuvant)** • Lenvatinib/pembrolizumab (category 1) for mismatch repair proficient (pMMR) tumors[b,9] • Pembrolizumab[b] for TMB-H[l,10] or MSI-H/dMMR[m] tumors[11] • Dostarlimab-gxly for dMMR/MSI-H tumors[b,12]	**Other Recommended Regimens** • Cisplatin/doxorubicin[13] • Cisplatin/doxorubicin/paclitaxel[n,13] • Cisplatin • Carboplatin • Doxorubicin • Liposomal doxorubicin • Paclitaxel[14] • Albumin-bound paclitaxel[o] • Topotecan • Bevacizumab[k,p,15] • Temsirolimus[16] • Cabozantinib • Docetaxel[f] (category 2B) • Ifosfamide (for carcinosarcoma) • Ifosfamide/paclitaxel (for carcinosarcoma)[17] • Cisplatin/ifosfamide (for carcinosarcoma) **Useful in Certain Circumstances (Biomarker directed therapy)** • Lenvatinib/pembrolizumab (category 1) for mismatch repair proficient (pMMR) tumors[b,9] • Pembrolizumab[b] for TMB-H[l,8] or MSI-H/dMMR tumors[m,11] • Dostarlimab-gxly for dMMR/MSI-H tumors[b,12] • Larotrectinib or entrectinib for NTRK gene fusion-positive tumors (category 2B) • Avelumab for dMMR/MSI-H tumors[b] • Nivolumab for dMMR/MSI-H tumors[b,18]

NCCN Clinical Practice Guidelines in Oncology. Uterine Neoplasm. V 2.2023

Molecular subtype–specific adjuvant therapy





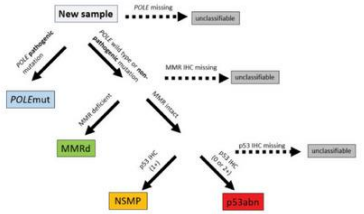
Endometrial Biopsy or Curettage

↓

Pathology + Molecular Classification

↓

Impact surgical decision /staging



POLEmut

- Early ~90% → De-escalation e.g., surgery only
- Advanced ~10% → ? De-escalation ? Radiation only

Clinicopathologic and molecular parameters; e.g., LVI, grade, myoinvasion, L1CAM do not add prognostic or predictive stratification

Adjuvant Clincal Trials

PORTEC-4a (de-escalation)
RAINBO Blue (de-escalation)
TAPER (de-escalation)

MMRd

Adjuvant Radiation

? No additional benefit from chemotherapy

- Loss of MSH6, MSH2, PMS2 → Hereditary Cancer Referral → Consider ICB
- Loss of MLH1 → Not Methylated → Hereditary Cancer Referral → Consider ICB
- Loss of MLH1 → Methylated → ? Less response to ICB ? Pembro + Lenvat

Further stratified by MLH1 and immune profile?

Adjuvant Clincal Trials

NRG GY 020 (+ICB; pembrolizumab)
RAINBO Green (+ICB; durvalumab)
ADELE Trial (+ICB; tislelizumab)

NSMP

Additional Stratification

- LVI
- Grade
- ER/PR
- L1CAM?
- CTNNB1?
- Immune e.g., CD8?

Highly Favourable
- De-escalation
- Vault brachy
- Endocrine therapy

Unfavourable
- ? ChemoRT
- ? Pembro + Lenvat

Clinicopathologic parameters d add prognostic and possible predictive stratification

Adjuvant Clincal Trials

RAINBO Orange (+endocrine therapy)
TAPER (de-escalation)

p53abn

- Stage IA No myoinvasion → ?Surgery only ?Vault brachy ?ChemoRT
- Stage IA with myoinvasion/Stage IB+ → Significant benefit from chemotherapy

HRD (20-25%) PARPi
HER2 (20-25%) Anti-HER2 Rx
CCNE1 (20-30%) Wee1-i
Pembro + Lenvat (50% response in serous)

Aggressive tumors regardless of grade and histotype

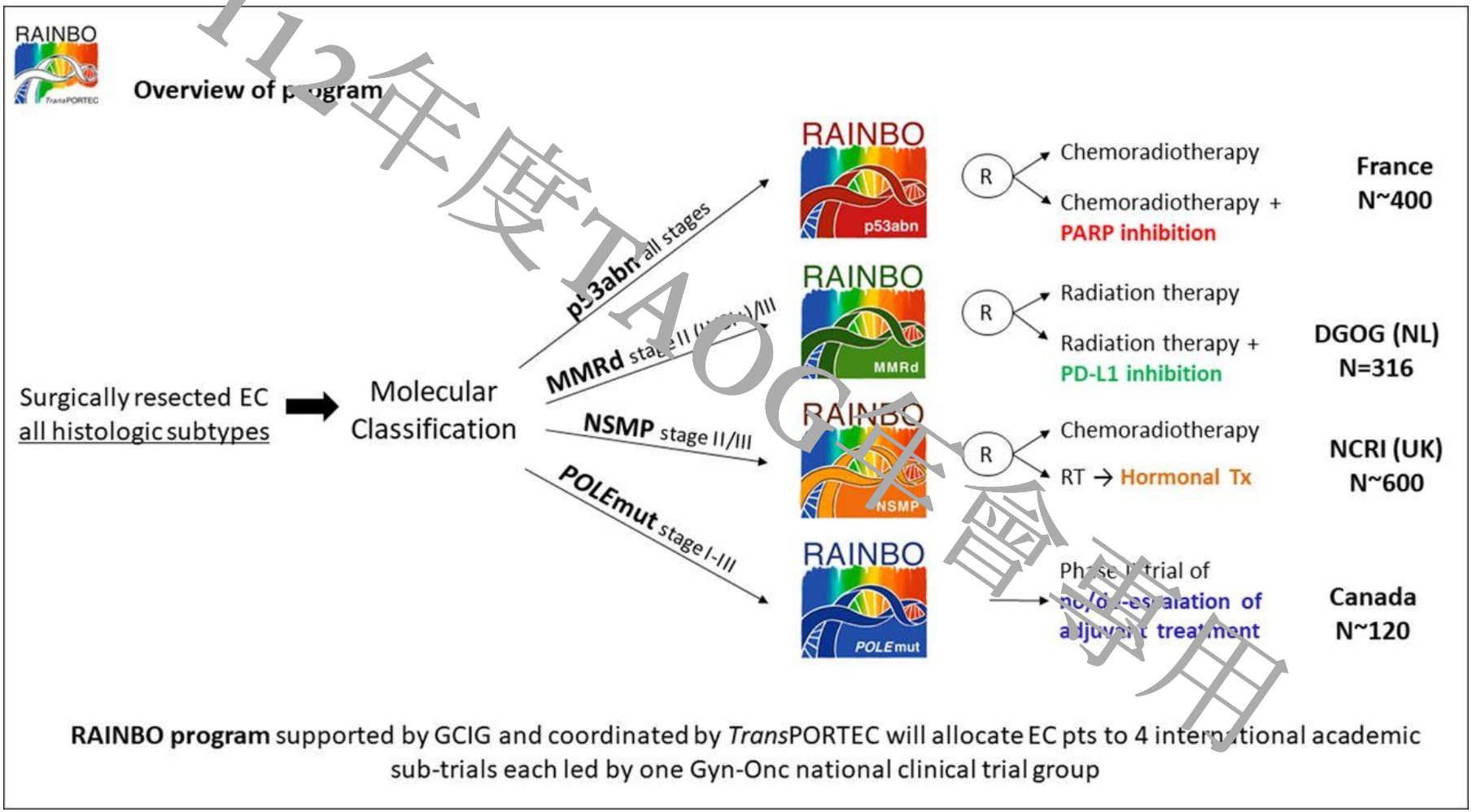
Molecular parameters for predictive stratification

Adjuvant Clincal Trials

RAINBO Red (+PARPi; olaparib)
CANSTAMP (+PARPi; niraparib)
NRG GY 026 (+HER2; trastuzumab/pertuzumab)

Cancer. 2022 Aug 1;128(15):2853-2857

TransPORTEC RAINBO Umbrella Trial



Int J Gynecol Cancer 2021;31(Suppl 3):A1–A395

MMR protein loss by IHC, PCR-based or NGS based MSI analysis?

- MSI-NGS discrepancies in non-CRC cancers may due to other involved loci not measured by MSI-PCR
- Different MSI levels between EC and CRC patients with Lynch syndrome
  - MSI lower in EC patients
  - MSI lower in EC patients from MSH6 mutation carriers
  - More MSH6 mutations in EC patients (x5)
  - MSI-L or MSS status in EC with MSH6 deficiency range from 29% to 50%

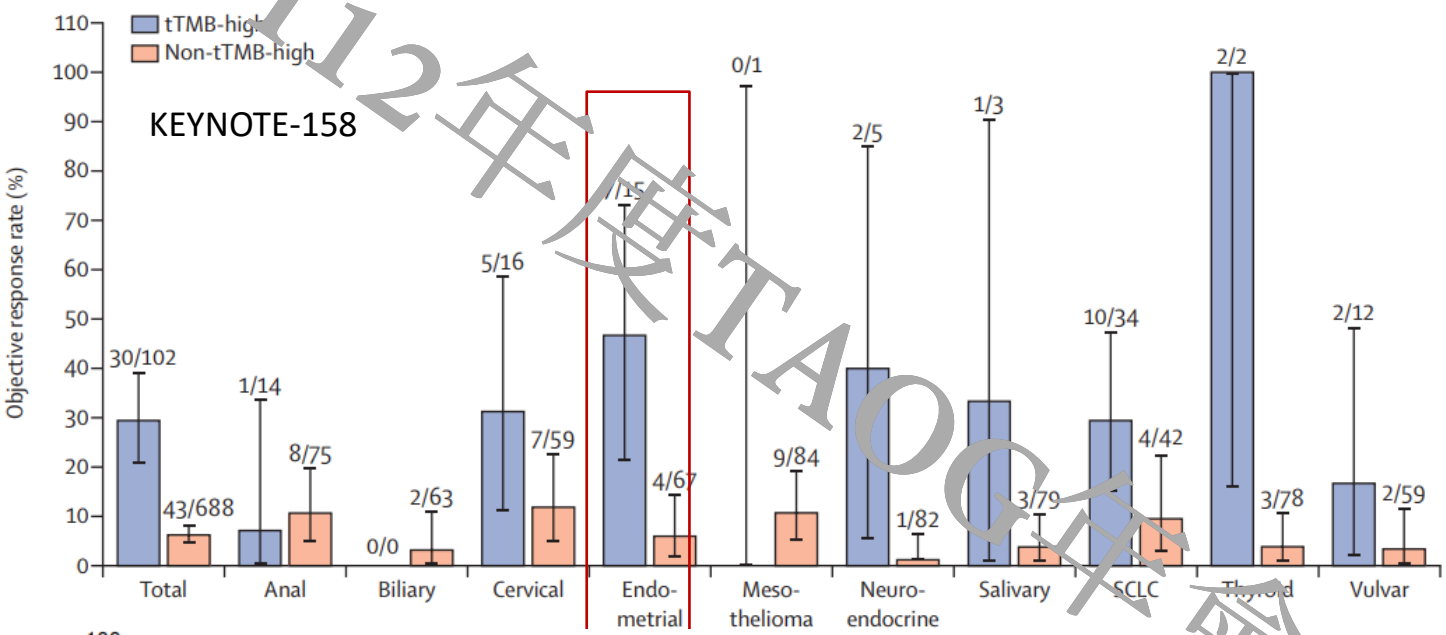
Table 3. Results of MSI Analyses in Tumors of *MSH6* Mutation Carriers

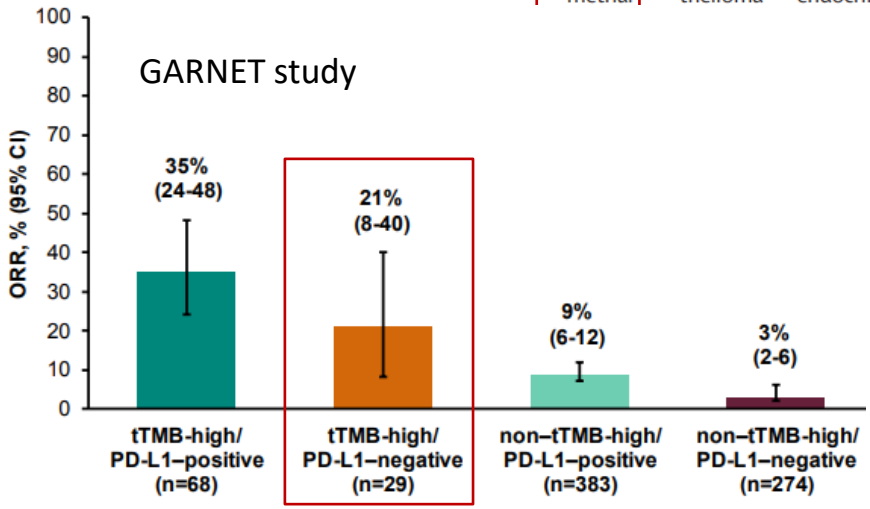
Tumor	MSI high	MSI low	Microsatellite stable	Total
Colorectal carcinoma (%)	18 (86)	3 (14)	0	21
Endometrial carcinoma (%)	11 (69)	4 (25)	1 (6)	16
Transitional cell carcinoma (%)	5 (71)	2 (29)	0 (0)	7
Ovarian carcinoma	2	0	0	2
Breast carcinoma	1	0	0	1
Stomach carcinoma	0	0	1	1
Adenocarcinoma of the cervix	0	1	0	1
Total	35	9	5	49

J Pathol 2000; 192: 328–35.
Am J Pathol 2002; 160: 1953–8.
Gastroenterology. 2004 Jul;127(1):17-25

TMB as biomarker beyond dMMR/MSI-H

- TMB predicted outcomes with pembrolizumab irrespective of PD-L1 expression

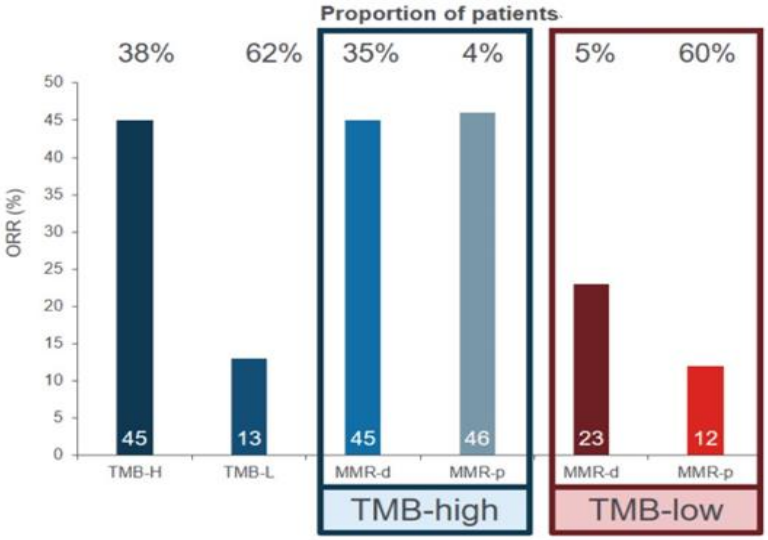


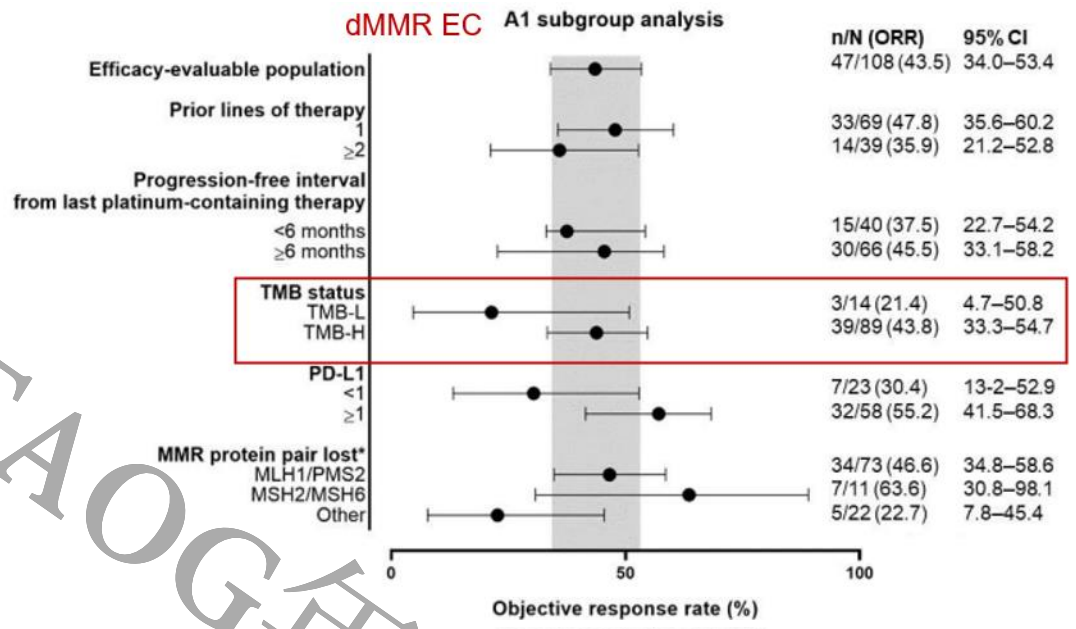


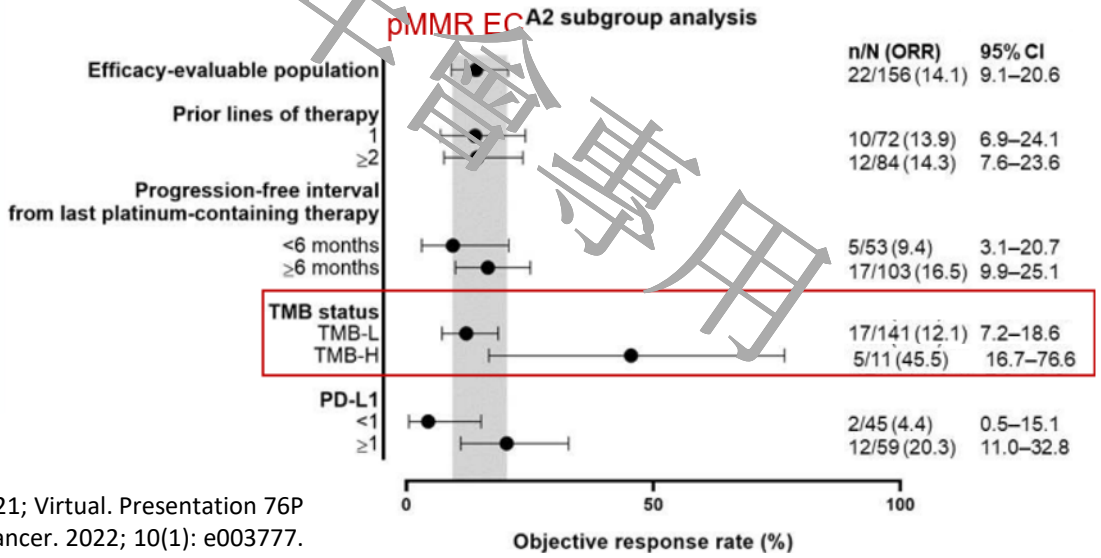
Lancet Oncol 2020; 21: 1353–65
Oaknin et al. ESMO 2021. Abstract 76P

TMB as biomarker beyond dMMR/MSI-H

- ***GARNET* study**
- Patients with MMR-d had high response rates
- But TMB-high tumors were associated with high responses irrespective of MMR status



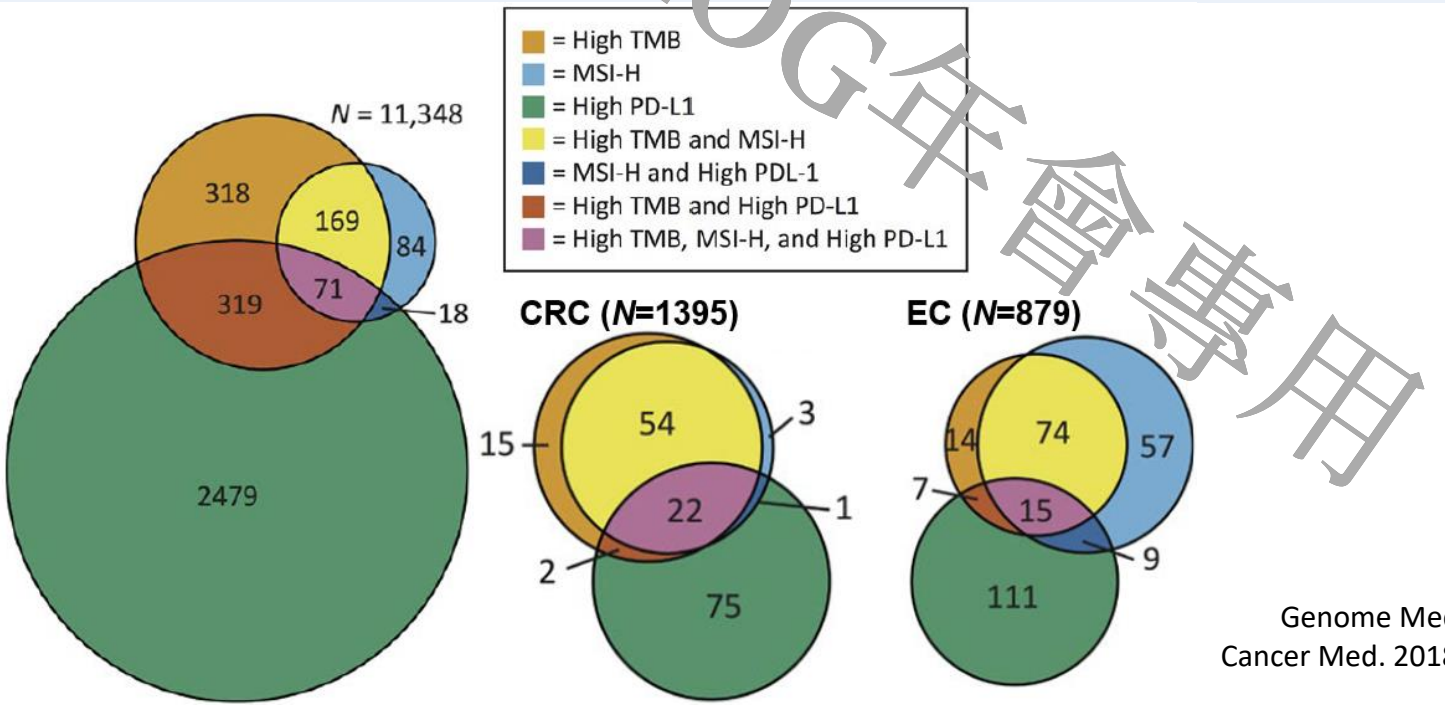




Oaknin A, et al. Presented at ESMO Annual Meeting; Sep 16-21, 2021; Virtual. Presentation 76P
PJ Immunother Cancer. 2022; 10(1): e003777.

TMB as a surrogate for PCR- and NGS based MSI assays?

- Elevated TMB associated with other etiologies e.g. POLE exonuclease-domain mutations
- 30% of MSI-H tumors were TMB-Low (<17 mut/Mb)
- Higher discrepant TMB and MSI-H rates in non-CRC
- 95% concordance in CRCs but only 57% MSI-H EC were TMB-H
- Discrepancy observed in OV (24%), NE (57%), and CC (33%)



Genome Med. 2017;9(1):34.
Cancer Med. 2018;7(3):746–756.

FDA Approved in vitro diagnostic companion diagnostic device

Diagnostic Name (Manufacturer)	Indication - Sample Type	Drug Trade Name (Generic) NDA / BLA	Biomarker(s)	Biomarker(s) (Details)	Clearance / Grant Date)
PD-L1 IHC 22C3 pharmDx (Dako North America, Inc.)	Cervical Cancer - Tissue	Keytruda (pembrolizumab) BLA 125514	PD-L1	PD-L1 protein expression	P150013/S009 (06/12/2018)
Ventana MMR RxDx Panel (Ventana Medical Systems, Inc.)	Endometrial Carcinoma (EC) - Tissue	Keytruda (pembrolizumab) BLA 125514 in combination with Lenvima (lenvatinib) NDA 20694	proficient mismatch repair (pMMR) proteins	MLH1, PMS2, MSH2 and MSH6	P210001/S002 (06/16/2022)
PD-L1 IHC 22C3 pharmDx (Dako North America, Inc.)	Esophageal Squamous Cell Carcinoma (ESCC) - Tissue	Keytruda (pembrolizumab) BLA 125514	PD-L1	PD-L1 protein expression	P150013/S016 (07/30/2019)
PD-L1 IHC 22C3 pharmDx (Dako North America, Inc.)	Head and Neck Squamous Cell Carcinoma (HNSCC) - Tissue	Keytruda (pembrolizumab) BLA 125514	PD-L1	PD-L1 protein expression	P150013/S014 (06/10/2019)
PD-L1 IHC 22C3 pharmDx (Dako North America, Inc.)	Non-Small Cell Lung Cancer (NSCLC) - Tissue	Keytruda (pembrolizumab) BLA 125514	PD-L1	PD-L1 protein expression	P150013 (10/02/2015)
Ventana MMR RxDx Panel (Ventana Medical Systems, Inc.)	Solid Tumors	Keytruda (pembrolizumab) BLA 125514	deficient mismatch repair (dMMR) proteins	MLH1, PMS2, MSH2 and MSH6	P210001/S001 (03/21/2022)
FoundationOne CDx (Foundation Medicine, Inc.)	Solid Tumors - Tissue	Keytruda (pembrolizumab) BLA 125514	TMB	TMB ≥ 10 mutations per megabase	P170019/S016 (06/16/2020)
FoundationOne CDx (Foundation Medicine, Inc.)	Solid Tumors - Tissue	Keytruda (pembrolizumab) BLA 125514	MSI-High	Microsatellite instability-High (MSI-H)	P170019/S029 (02/18/2022)

FDA Approved in vitro diagnostic companion diagnostic device

Diagnostic Name (Manufacturer)	Indication - Sample Type	Drug Trade Name (Generic) NDA / BLA	Biomarker(s)	Biomarker(s) (Details)	(Approval / Clearance / Grant Date)
Ventana MMR RxDx Panel (Ventana Medical Systems, Inc.)	Endometrial Carcinoma (EC) - Tissue	Jemperli (dostarlimag-gxly) NDA 761174	deficient mismatch repair (dMMR) proteins	MLH1, PMS2, MSH2 and MSH6	P200019 (04/22/2021)
Ventana MMR RxDx Panel (Ventana Medical Systems, Inc.)	Solid Tumors	Jemperli (dostarlimag-gxly) NDA 761174	deficient mismatch repair (dMMR) proteins	MLH1, PMS2, MSH2 and MSH6	P210001 (08/17/2021)

Summary of guidelines for patients considered immune checkpoint inhibitors

ESMO recommendations for MSI testing

Table 1. Summary table of recommendations for MSI testing in the framework of immunotherapy and comments from the ESMO TR and PM WG consensus panel

Recommendation A: immunohistochemistry	The first test of choice is IHC, using antibodies recognising the four MMR proteins: MLH1, MSH2, MSH6 and PMS2.
Coefficient of agreement: strong (8.7)	
Main comment: MMR proteins form heterodimers; for a correct IHC interpretation, the consensus panel highlights that mutations in MLH1 are associated with IHC loss of both MLH1 and PMS2, while mutations in MSH2 are associated with IHC loss of both MSH2 and MSH6. There exist isolated losses of PMS2, MSH2 or MSH6, this strengthening the recommendation to use all four antibodies.	
Recommendation B: polymerase chain reaction	In case of doubt of IHC, confirmatory molecular analysis is mandatory. The first-line of molecular analysis is represented by PCR. It can be carried out using two possible panels: (i) a panel with two mononucleotide (BAT-25 and BAT-26) and three dinucleotide (D5S346, D2S123 and D17S250) repeats and (ii) a panel with five poly-A mononucleotide repeats (BAT-25, BAT-26, NR-21, NR-24, NR-27). The five poly-A panel is the recommended panel given its higher sensitivity and specificity.
Coefficient of agreement: strong (8.6)	
Main comment: both the suggested panels have been and are being used to assess MSI in clinical trials. Molecular tests guarantee the highest values of specificity and sensitivity in MSI testing.	
Recommendation C: next-generation sequencing	NGS represents another type of molecular tests to assess MSI. Its main advantages are represented by the possibilities of coupling MSI analysis with the determination of tumour mutational burden (TMB).
Coefficient of agreement: very strong (9.0)	
Main comment: NGS should be carried out only in selected centres devoted to these techniques.	

Annals of Oncology 30: 1232–1243, 2019

2022 CAP (College of American Pathologists) guidelines

Guideline Statement	Strength of Recommendation
1. For patients with CRC being considered for immune checkpoint inhibitor therapy, pathologists should use MMR-IHC and/or MSI by PCR for the detection of DNA mismatch repair defects. Although MMR-IHC or MSI by PCR are preferred, pathologists may use a validated MSI by NGS assay for the detection of DNA mismatch repair defects	Strong Recommendation
Note: MSI by NGS assay must be validated against MMR-IHC or MSI by PCR and must show equivalency	
2. For patients with gastroesophageal and small bowel cancer being considered for immune checkpoint inhibitor therapy, pathologists should use MMR-IHC and/or MSI by PCR over MSI by NGS for the detection of DNA mismatch repair defects	Strong Recommendation
Note: This recommendation does not include esophageal squamous cell carcinoma	
3. For patients with endometrial cancer being considered for immune checkpoint inhibitor therapy, pathologists should use MMR-IHC over MSI by PCR or NGS for the detection of DNA mismatch repair defects	Strong Recommendation ***Certainty of Evidence: Low***
4. For patients with cancer types other than CRC, GEA, small bowel, and endometrial being considered for immune checkpoint inhibitor therapy, pathologists should test for DNA mismatch repair, although the optimal approach for the detection of mismatch repair defects has not been established	Conditional Recommendation
Note: Assays must be adequately validated for the specific cancer type being tested with careful consideration of performance characteristics of MMR-IHC and MSI by NGS or PCR for the detection of DNA mismatch repair defects	
5. For all cancer patients being considered for immune checkpoint inhibitor therapy based on defective mismatch repair, pathologists should not use TMB as a surrogate for the detection of DNA mismatch repair defects. If a tumor is identified as TMB-High, pathologists may perform IHC and/or MSI by PCR to determine if high TMB is secondary to mismatch repair deficiency	Strong Recommendation ***Certainty of Evidence: Low***
6. For cancer patients being considered for immune checkpoint inhibitor therapy, if a mismatch repair deficiency consistent with Lynch syndrome is identified in the tumor, pathologists should communicate this finding to the treating physician	Strong Recommendation

Table 4. Number of Studies by Outcome

Test	Rec 1 CRC	Rec 2 GEA and SI	Rec 3 EC	Rec 4 Other Cancer	Rec 5 TMB	Rec 6 LS
MMR-IHC diagnostic test characteristics	2 PCS 8 RCS	2 RCS	3 RCS	2 PCS 2 RCS	0	0
MMR-IHC status concordance with germline testing	1 PCS 8 RCS	0	2 RCS	0	0	1 PCS 9 RCS
MSI-PCR diagnostic test characteristics	2 PCS 7 RCS	0	3 RCS	1 RCS	0	0
MSI-PCR status concordance with germline testing	1 PCS 2 RCS	0	1 RCS	0	0	3 PCS 10 RCS
MMR-IHC and MSI-PCR status concordance	6 PCS 16 RCS	4 RCS	2 PCS 7 RCS	2 RCS	0	0
MSI-NGS diagnostic test characteristics	1 PCS 5 RCS	0	1 PCS 2 RCS	5 RCS	0	0
MSI-NGS and MMR-IHC status concordance	1 PCS 1 RCS	1 PCS	2 PCS	1 PCS 1 RCS	0	0
MSI-NGS and MSI-PCR status concordance	0	0	0	1 RCS	0	0
TMB diagnostic test characteristics	0	0	0	0	1 RCS	0
TMB and MMR-IHC status concordance	0	0	0	0	2 RCS	0
TMB and MSI-NGS status concordance	0	0	0	0	1 PCS 2 RCS	0
Association between LS prevalence and MMR MSI status	0	0	0	0	0	5 PCS 10 RCS

Pathol Lab Med 146:1194-1210,2022

Endorsement of CAP guidelines by ASCO

- Consider other potential information via NGS testing in decision making.

  Beyond MSI detection,

- Detection of HER2 amplification, high TMB due to non-MSI mechanisms

Recommendation 1. For patients with CRC, being considered for immune checkpoint inhibitor therapy, pathologists should use MMR-immunohistochemistry (IHC) and/or microsatellite instability (MSI) by polymerase chain reaction (PCR) for the detection of DNA MMR defects. Although MMR-IHC or MSI by PCR is preferred, pathologists may use a validated MSI by next-generation sequencing (NGS) assay for the detection of DNA MMR defects. *Note: MSI by NGS assay must be validated against MMR-IHC or MSI by PCR and must show equivalency.* (Strong recommendation)

Recommendation 2. For patients with gastroesophageal and small bowel cancer, being considered for immune checkpoint inhibitor therapy, pathologists should use MMR-IHC and/or MSI by PCR over MSI by NGS for the detection of DNA MMR defects. *Note: This recommendation does not include esophageal squamous cell carcinoma.* (Strong recommendation)

Recommendation 3. For patients with endometrial cancer, being considered for immune checkpoint inhibitor therapy, pathologists should use MMR-IHC over MSI by PCR or NGS for the detection of DNA MMR defects. (Strong recommendation)

Recommendation 4. For patients with cancer types other than CRC, gastroesophageal adenocarcinoma, small bowel, and endometrial being considered for immune checkpoint inhibitor therapy, pathologists should test for DNA MMR although the optimal approach for the detection of MMR defects has not been established. *Note: Assays must be adequately validated for the specific cancer type being tested with careful consideration of performance characteristics of MMR-IHC and MSI by NGS or PCR for the detection of DNA MMR defects.* (Conditional recommendation)

Recommendation 5. For all cancer patients being considered for immune checkpoint inhibitor therapy based on defective MMR, pathologists should not use tumor mutation burden (TMB) as a surrogate for the detection of DNA MMR defects. If a tumor is identified as TMB-high, pathologists may perform IHC and/or MSI by PCR to determine if high TMB is secondary to MMR deficiency. (Strong recommendation)

Recommendation 6. For cancer patients being considered for immune checkpoint inhibitor therapy, if a MMR deficiency consistent with Lynch syndrome is identified in the tumor, pathologists should communicate this finding to the treating physician. (Strong recommendation)

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Thank you for your attention