

TUMOR MSI

Turn Complexity into Simplicity

Chinese medical device registration number (NMPA registration number): 20213400936

Microreader™ Microsatellite Instability Detection Kit

(Fluorescence PCR-capillary electrophoresis method)

Domestic
initiative

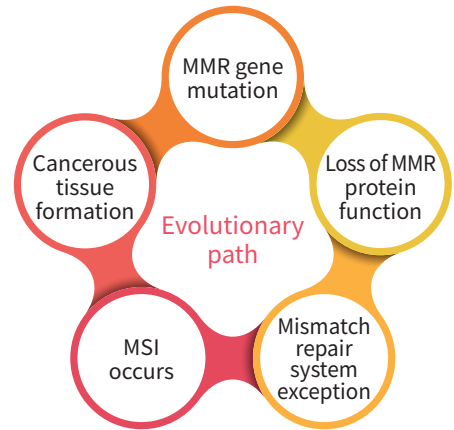
Patented compound amplification system

Patent No.: ZL 2011 1 0152226. X

Basic Information

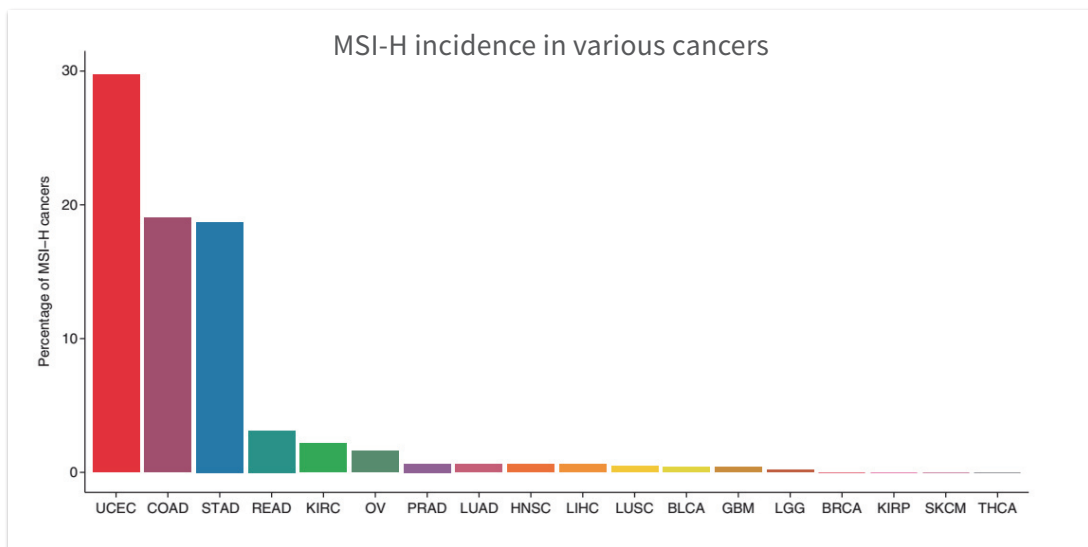
Gene profile

Microsatellites are short tandem repeats throughout the human genome. Compared with normal tissue, the length of microsatellites in tumor tissue is changed due to the insertion or deletion of repeating units, which is called microsatellite instability (MSI). A large number of studies have shown that MSI is caused by a defect in the mismatch repair (MMR) gene and is closely related to the occurrence of tumors.



Microsatellite instability evolutionary path

Multiple cancers have MSI-H features^[1]



Clinical application

NCCN, ASCO, ESMO, CSCO, "Chinese Expert Consensus on Molecular Markers of Colorectal Cancer" and other guidelines jointly recommend that microsatellite instability detection is widely used in the screening of Lynch syndrome, the judgment of cancer prognosis, the prediction of the efficacy of adjuvant chemotherapy and the Immunotherapy Guidelines.



Type of cancer	Clinical significance			
	Lynch Syndrome Screening	Prognosis	Predicting the efficacy of adjuvant chemotherapy	immunotherapy guidance
Colorectal cancer	✓	✓	✓	✓
Stomach cancer	✓	✓	✓	✓
Endometrial cancer	✓	✓		✓
Small bowel adenocarcinoma	✓			✓
Prostate cancer	✓			✓
Ovarian cancer	✓			✓
Hepatobiliary tumors	✓			✓
Upper urinary tract urothelial carcinoma	✓	✓		✓
Pancreatic cancer	✓			✓
Other solid tumors				✓

Screening of Lynch syndrome [2]

Lynch syndrome, formerly known as hereditary non-polyposis colorectal cancer (HNPCC), is an autosomal dominant genetic tumor syndrome in which patients suffer from colorectal cancer or other multiple The risk of malignant tumors in various parts (including endometrium, ovary, stomach, small intestine, liver and gallbladder, upper urinary tract, brain and skin) was significantly increased.

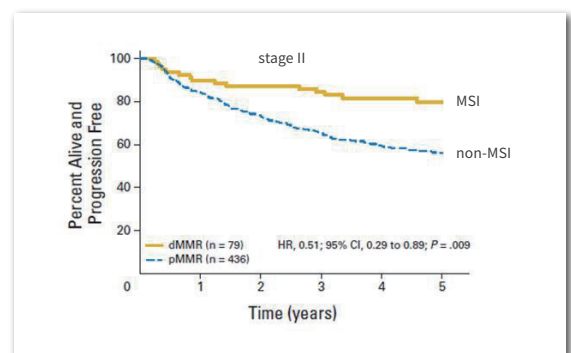
More than 90% of Lynch syndrome have MSI features, while only about 15% of sporadic colon cancer, therefore, MSI detection can be clinically used to screen for Lynch syndrome.

Prognosis

Colorectal cancer

At present, there is a large amount of evidence that MSI-H is a marker of good prognosis in patients with stage II colorectal cancer.

The 5-year survival rate of MSI-H stage II colorectal cancer patients is significantly higher than that of MSI-L/MSS, and the risk of recurrence is lower. [3]

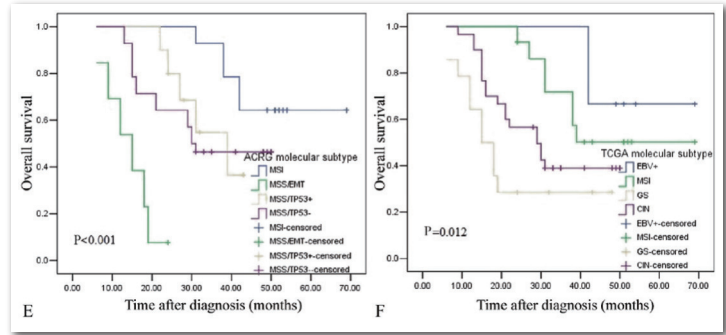


Comparison of five-year survival rates between MSI and non-MSI patients with stage II colorectal cancer

2 Stomach cancer

According to the Asian Cancer Research Group (ACRG) molecular classification of gastric cancer in 2015, MSI-H gastric cancer has the best prognosis.

In 2020, the research team from Wang Qiang et al. [4], based on the research data of the Chinese population, showed that regardless of ACRG or TCGA classification, MSI-H/dMMR gastric cancer patients had the best prognosis. Most other domestic and foreign studies have suggested that MSI-H is a good predictor of prognosis in resectable gastric cancer.

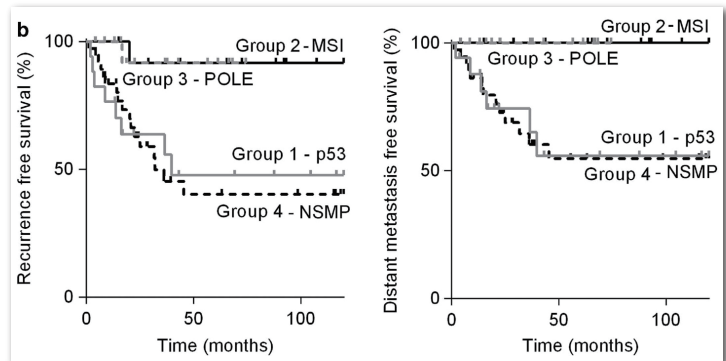


Prognostic significance of TCGA and ACRG classification in gastric cancer in Chinese population

3 Endometrial cancer

In 2015, the research team of Ellen Stelloo [5] based on the TransPORTEC typing study

of 116 cases of high-risk EC, divided EC into four groups with different prognosis: POLE mutation, MSI-H and TP53 mutation, and NSMP (no mutation) type. Among them, POLE mutant type and MSI-H type have the best prognosis.



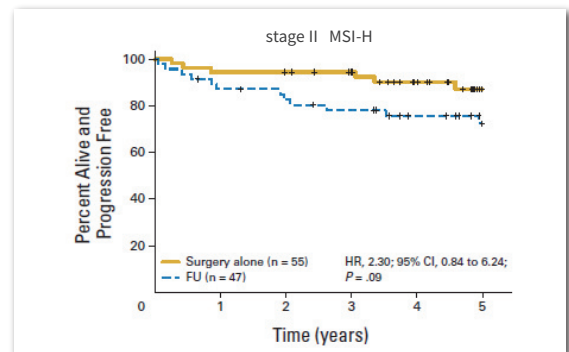
Recurrence-free and distant metastasis-free survival in high-risk endometrial cancer patients

Predicting response to adjuvant chemotherapy

1 Colorectal cancer

Patients with MSI-H stage II colorectal cancer cannot benefit from fluorouracil single-agent adjuvant chemotherapy, and chemotherapy has greater side effects. Therefore, such patients are not recommended to use fluorouracil (such as 5-FU, capecitabine) single drug adjuvant chemotherapy.

NCCN specifically pointed out that for stage II colon cancer with MSI-H features, poor tissue differentiation is no longer considered a high-risk factor. [3]

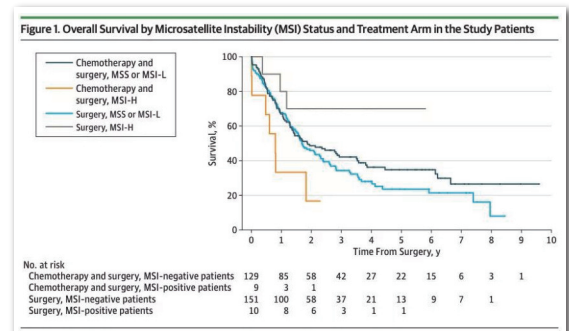


MSI-H patients with stage II colorectal cancer do not benefit from fluorouracil-based chemotherapy

2 Stomach cancer

The results of the MAGIC study [6] showed that among patients who only received surgical treatment, the OS of dMMR/MSI-H patients was not reached, while the OS of pMMR/MSI-L/MSS patients was 20.5 months (P value=0.09). Among patients who received surgery and chemotherapy, the OS of dMMR/MSI-H patients was 9.6 months, and the OS of pMMR/MSI-L/MSS patients was 19.5 months (P value = 0.03).

In conclusion, dMMR/MSI-H conferred a positive predictive effect in patients treated with surgery alone, whereas it was associated with a negative predictive effect in patients treated with chemotherapy, therefore, operable dMMR/MSI-H patients do not benefit from perioperative chemotherapy.



The relationship between MSI status and overall survival in patients with gastric cancer who received different treatments

Immunotherapy Guidelines (PD-1)

FDA approves Keytruda to treat all solid tumors with MSI-H

Based on the clinical data of 149 MSI-H/dMMR cancer patients, a total of 15 types of cancer, the objective response rate after Keytruda treatment was 39.6%, of which 11 were in complete remission, 48 were in partial remission, and 78% of the patients who responded the curative effect lasts for more than 6 months.

Type of cancer	Enrollment	Objective response rate
Colorectal cancer	90	36%
14 non-colorectal cancers	59	46%

A wide range of tumor types responded, including: colorectal cancer (32 patients), endometrial cancer (5 patients), gastric cancer (5 patients), bile duct cancer (3 patients), pancreatic cancer (5 patients), small bowel cancer (3), breast cancer (2), prostate cancer (1), esophageal cancer (1), small cell lung cancer (1). That is to say, even if the cancer has different onset sites, PD-1 therapy can be tried because of the common characteristics of MSI-H/dMMR.

Genetic loci selection and Chinese population data

Site selection

For MSI detection sites, the NCI recommended sites in 1997 were 2 single nucleotide sites + 3 double nucleotide detection sites^[7], subsequent studies have proved that single nucleotide sites have high sensitivity and specificity Sex, and the dinucleotide site is not sensitive to MSI caused by hMSH6 mutation^[8]. In view of this, the 2004 revised NCI guidelines recommend single nucleotide sites as MSI detection sites^[9].

In August 2019, ESMO released recommendations for MSI detection in cancer immunotherapy^[10]. In view of the high sensitivity and specificity of single nucleotide repeat sequence panels, the consensus recommended the use of single nucleotide repeat sequence panels for MSI detection.

The 2019 "Chinese Expert Consensus on Detection of Microsatellite Instability in Colorectal Cancer and Other Related Solid Tumors"^[11] pointed out that the combination of 5-7 marker sites based on the Pentaplex panel in the PCR method BAT25, BAT26, NR21, NR22, NR24, NR27 and MONO27 are recognized as the gold standard for PCR.

It can be seen that it is the general trend to use single nucleotide sites as the standard detection of MSI detection.

Chinese population data

Test items		MR panel				NCI panel			
		MSS	MSI-L	MSI-H	总计	MSS	MSI-L	MSI-H	Total
IHC	pMMR	125	1	0	126	118	6	2	126
	dMMR	5	0	61	66	5	2	59	66
Total		130	1	61	192	123	8	61	192
Sensitivity		61/66=92.4%				59/66=89.3%			
Specificity		125/126=99.2%				124/126=98.4%			

Compared with the results of multiple fluorescent PCR-capillary electrophoresis and immunohistochemistry, the result consistency rate is higher; while the single nucleotide site panel selected by Microread Genetics is superior in both sensitivity and specificity analysis. In 1997 NCI panel, consistent with literature reports.

Product Design

At the same time, the DNA of normal tissue and tumor tissue samples of the same individual is extracted, and the detection sites are amplified by multiplex fluorescent PCR; The amplified products are detected by capillary electrophoresis using a gene analyzer, and the results are analyzed by professional software.

Marker category	Marker name	Repeat sequence	illustrate
Single nucleotide repeat marker	NR-21	(A) ₂₁	Detect MSI
	NR-24	(A) ₂₄	
	NR-27	(A) ₂₇	
	BAT-25	(A) ₂₅	
	BAT-26	(A) ₂₆	
	MONO-27	(A) ₂₇	
Pentanucleotide repeat marker	Penta C	(AAAAG) ₃₋₁₅	Identify potential sample mix-up or contamination
	Penta D	(AAAAG) ₂₋₁₇	
Sex locus markers	Amel		
<p>*MSI stable (microsatellite stable, MSS): no changes in single nucleotide repeat markers MSI low-frequency type (low-frequency MSI, MSI-L): only one of the single nucleotide repeat markers is changed MSI high-frequency type (high-frequency MSI, MSI-H): 2 of the single-nucleotide repeat markers are changed</p>			

- **Detection method:** Fluorescent PCR-capillary electrophoresis
- **Sample type:** Paraffin-embedded tissue section (tumor tissue + paracancerous tissue)
- **Applicable instruments:** PCR instrument: Applied Biosystems Veriti/ Veriti Dx/9700/2720, Bio-Rad T100.
Gene analyzer: Applied Biosystems 3500 Dx /3500xl Dx, 3500/3500xl, 3130xl; Seqstudio.

Product Features

Domestic initiative

Developed the first microsatellite instability gene detection kit in China, and obtained the invention patent in 2012.

Patent No.: ZL 2011 1 0152226. X

Guideline recommendations

The revised Bethesda guideline/NCI guideline recommends the use of single nucleotide sites.

Clear meaning

In clinical studies of keytruda companion diagnostics, MSI detection uses a single nucleotide site.

01

Multiple quality control

- UDG anti-pollution system
- Quality control loci
- Molecular weight standard
- Normal tissue control of the sample

02

Automatic analysis

Self-developed analysis software can automatically analyze the results and generate reports

03

Accurate interpretation

Based on the analysis experience of many clinical samples, the kit can accurately interpret complex continuous unstable peaks

04

Overall solution

Instrument + Reagent + Software
MSI overall solution

Testing process and results

Detection process

01

Sample collection



02

Multiple fluorescence
PCR amplification



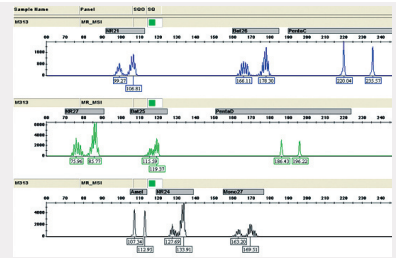
03

Capillary
electrophoresis
detection



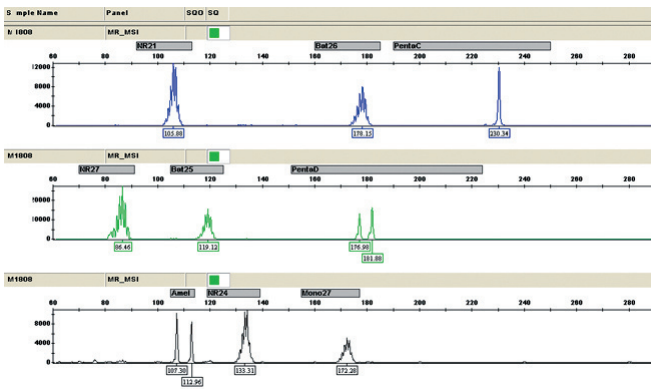
04

Results
interpretation

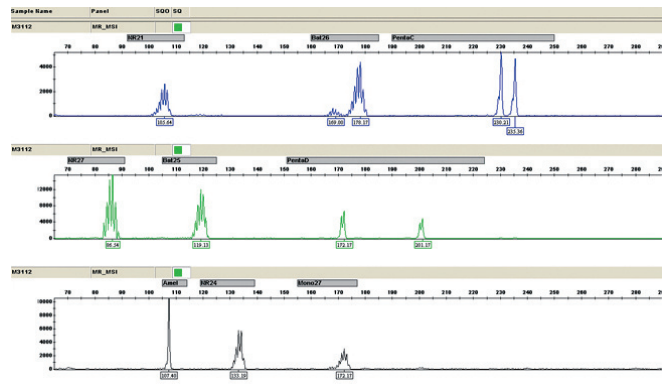


Test results

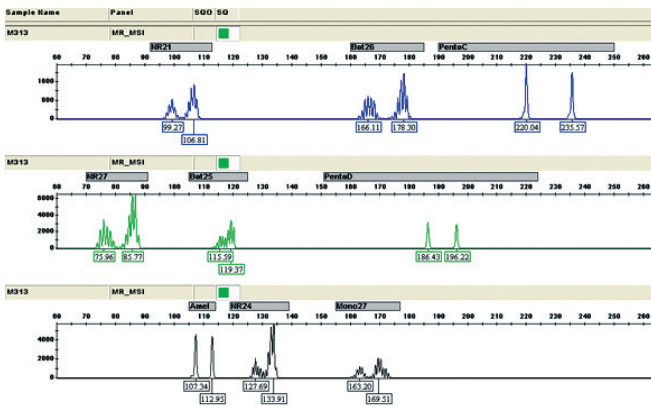
1 MSS type



2 MSI-L type



3 MSI-H type



Diagnostic methods for microsatellite instability (MSI)

Compare content	PCR + capillary electrophoresis	MMR-IHC	MSI-NGS
Test content	Microsatellite loci	MMR protein expression of MLH-1, MSH-2, MSH-6, PMS-2	Microsatellite loci (There is no unified standard for the location)
Sample quality	Tumor proportion >20%	Lower	Tumor proportion >10%
Interpretation of results	30% and more of the sites are unstable as MSI-H	Loss of expression of any protein is dMMR	Developed thresholds according to different sites and analysis methods
Accuracy	Gold standard	Medium	Standards are not uniform
Advantage	Accurate, simple and fast High sensitivity and specificity Easy to standardize and automate	Developed method Can directly prompt MMR gene	Simultaneous capture of multiple genome sequences covering dozens of microsatellite sites up to thousands
Shortcoming	Tumor cell content >20% It is not possible to directly determine the abnormality of the protein	Requires standardized tissue pretreatment tissue heterogeneity affected by interpretation factors, possible misjudgment	Technical complexity Validation with a large sample is required Expensive
Clinical application	Suitable for clinical department automation, batch detection	Basic recommendations for clinical MSI/MMR detection in China	Standards are not yet unified

※References:

- [1] Hause, R. J. , et al. "Classification and characterization of microsatellite instability across 18 cancer types." *Nature Medicine* 22.11(2016):1241.
- [2] "Identification of Lynch syndrome among patients with colorectal cancer." *Jama* (2012).
- [3] Sargent, D. J. , et al. "Defective Mismatch Repair As a Predictive Marker for Lack of Efficacy of Fluorouracil-Based Adjuvant Therapy in Colon Cancer." *Journal of Clinical Oncology* 28.20(2010):3219-3226.
- [4] Wang, Qiang , et al. "Clinical characteristics and prognostic significance of TCGA and ACRG classification in gastric cancer among the Chinese population." *Molecular Medicine Reports* 22.2(2020).
- [5] Stelloo, E. , et al. "Refining prognosis and identifying targetable pathways for high-risk endometrial cancer; a TransPORTEC initiative." *Modern Pathology*.
- [6] Smyth, Elizabeth C , et al. "Mismatch Repair Deficiency, Microsatellite Instability, and Survival: An Exploratory Analysis of the Medical Research Council Adjuvant Gastric Infusional Chemotherapy (MAGIC) Trial." *Jama Oncology* 3.9(2017).
- [7] Boland, C. R. . "A National Cancer Institute work-shop on microsatellite instability for cancer detection and familial predisposition : Development of international criteria for the determination of microsatellite instability in colorectal cancer." *Cancer Res* 58(1998).
- [8] Suraweera, Nirosha , et al. "Evaluation of tumor microsatellite instability using five quasimonomorphic mononucleotide repeats and pentaplex PCR." *Gastroenterology* 123. 6(2002):1804-1811.
- [9] Asad, U. , et al. "Revised Bethesda Guidelines for Hereditary Nonpolyposis Colorectal Cancer (Lynch Syndrome) and Microsatellite Instability." *Journal of the National Cancer Institute* 4(2004):936.
- [10] Luchini, C. , et al. "ESMO recommendations on microsatellite instability testing for immunotherapy in cancer, and its relationship with PD-1/PD-L1 expression and tumour mutational burden: a systematic review-based approach." *Annals of Oncology* 30.8(2019):1232-1243.
- [11] Colorectal Cancer Expert Committee of Chinese Society of Clinical Oncology, Chinese Anticancer Cancer Society Colorectal Cancer Professional Committee Genetics Group, and Chinese Medical Doctor Association Colorectal Cancer Professional Committee Genetics Committee." Chinese Expert Consensus on Microsatellite Instability Detection of Colorectal Cancer and Other Related Entities." *Chinese Journal of Tumor* 041.010(2019):734-741.

※ See the manual for details.

WeChat public account



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