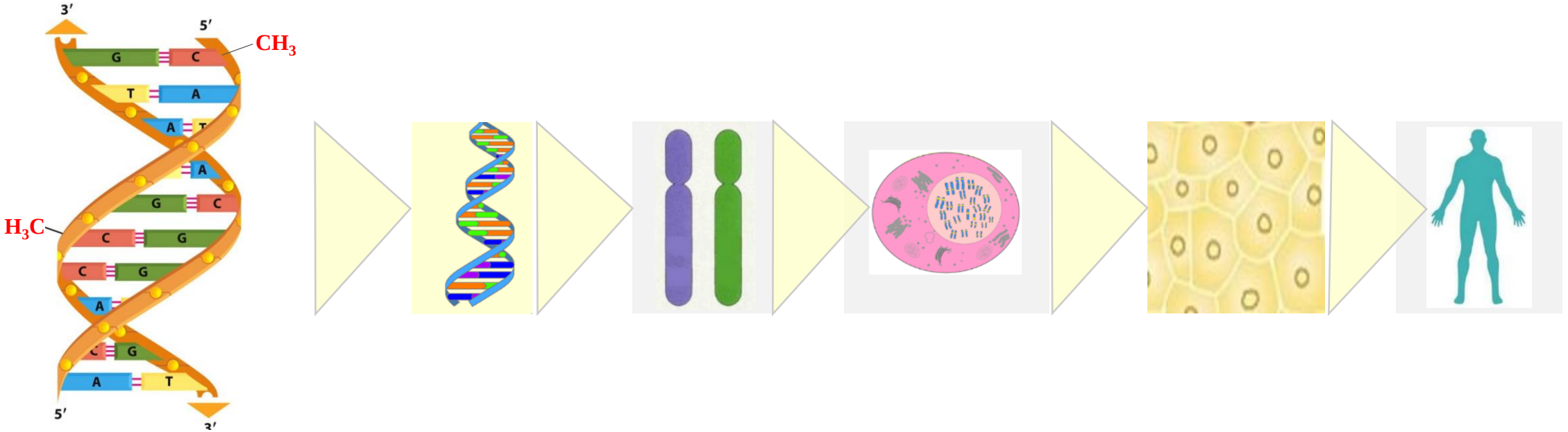
TAGMe DNA methylation detection

Female Genital Tract Cancer Theranostics
Urinary Tract Cancer Theranostics

EPIPROBE

Why receive methylation detection?



Methylation abnormalities → Genetic mutations → Cancerous Cells → Cancer cell proliferation → Cancer metastasis

Precancerous lesions | Early stages of cancer | Early to mid-stage cancer | Advanced cancer

Methylation detection

NGS genetic detection

CTC detection

Biochemical marker detection

Imaging detection

Discoverer of TAGMe

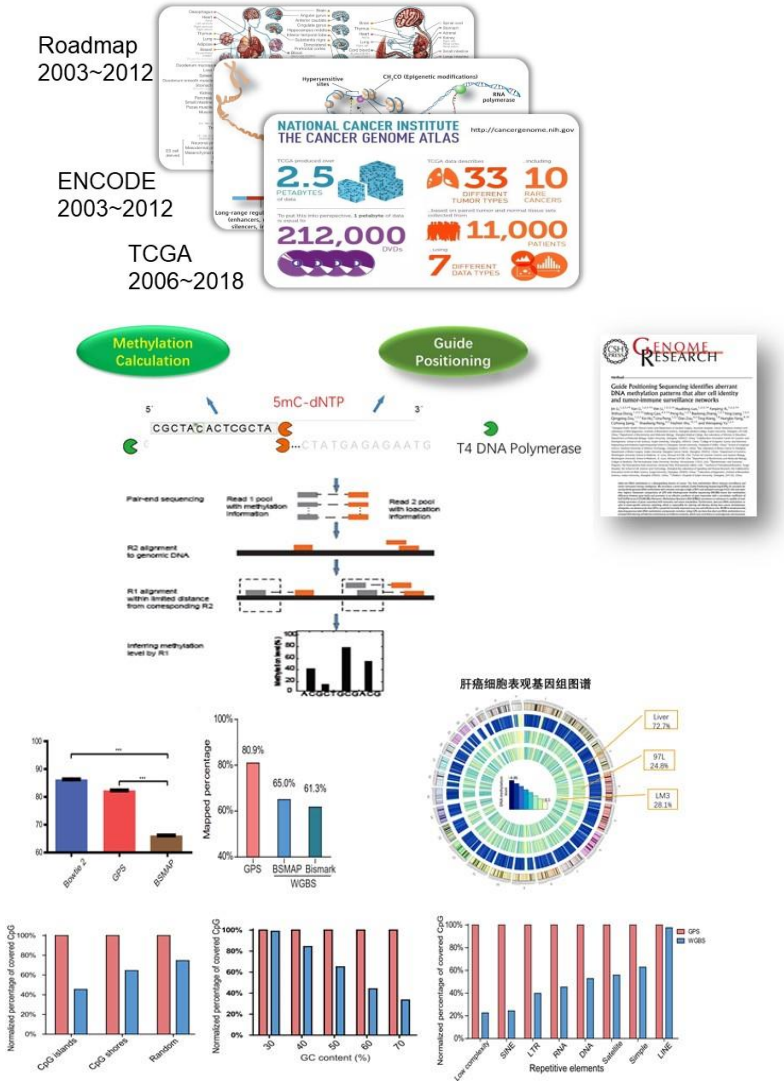
Dr. Wenqiang Yu

- Doctoral Supervisor of Fudan University, Chief Scientist of national "973" project,
- Changjiang Scholar Distinguished Professor, PI of Epigenetics Center of Fudan Biomedical Research Institute.
- 2001-2007 Postdoctoral Fellow, Uppsala University, Sweden; **Johns Hopkins University, USA (tutored by Dr. Andy)**;
- In November 2007, Faculty and Associate Research Scientist of Columbia University.

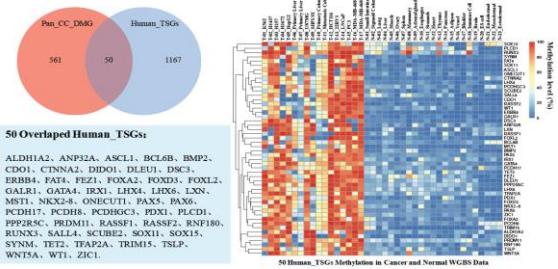
Research findings

The whole genome DNA methylation sequencing method with independent intellectual property right, **Guided Positioning Sequencing (GPS) technology**, was established and **Tumor Aligned General Methylated Epiprobe (TAGMe)** were discovered, which has been double-blind verified in **50000+** clinical samples.

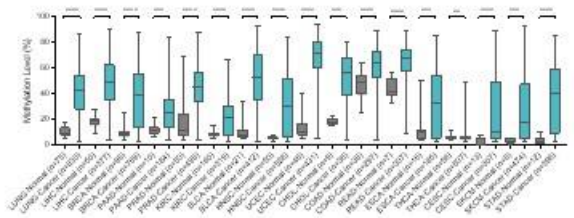
Discovery of TAGMe

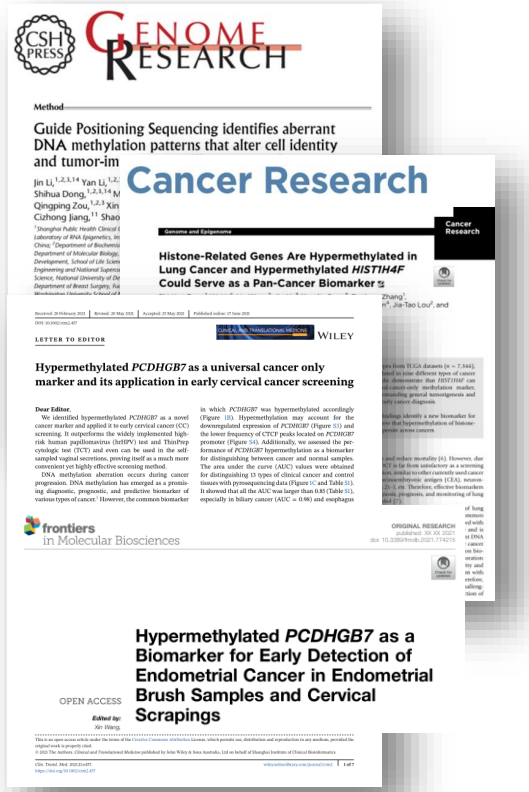


Research: From simple to complex



Application: From complex to simple





Genome Research, 2019.01 (**IF=11.1**)
Cancer Research, 2019.10 (**IF=12.7**)
Clinical and Translational Medicine, 2021.06 (**IF=11.5**)
Frontiers in Molecular Biosciences (**IF=5.2**)
Signal Transduction and Targeted Therapy（**IF=38.104**）

GPS method: covering 96% of the genome C (1.12G/1.17G);
WGBS method: covering 60 to 80% CpG of the genome.

Tumor Aligned General Methylated Epiprobe (TAGMe)

Technological breakthrough: TAGMe-DNA methylation detection method

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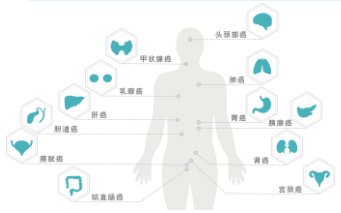
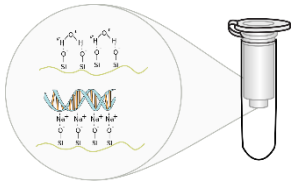
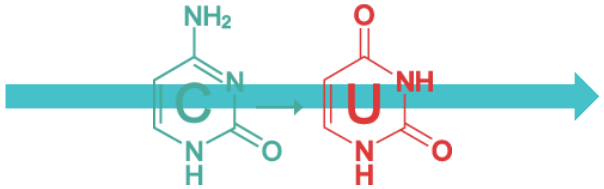
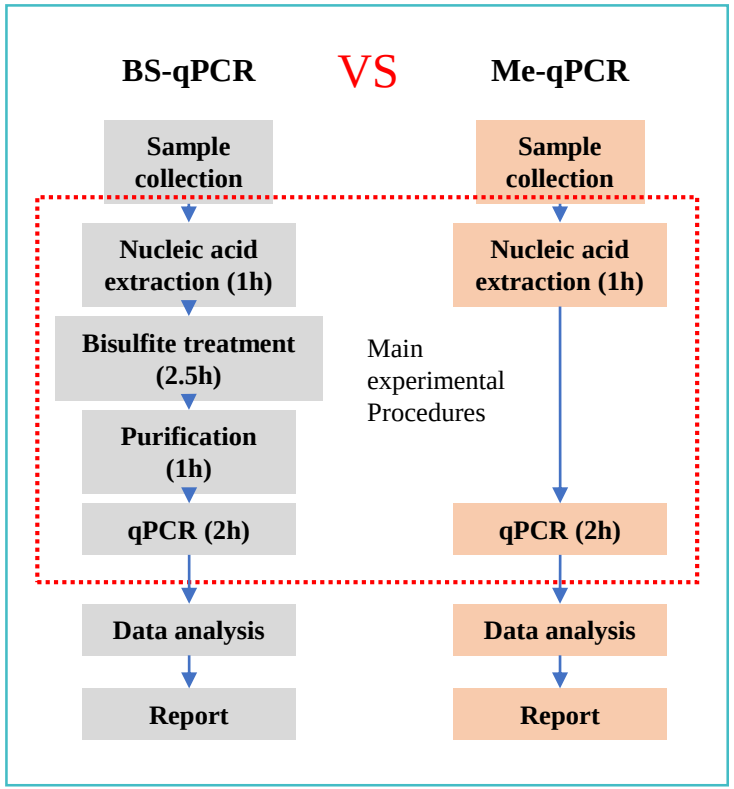
Establish a whole-process standardized system from sample collection to report generation

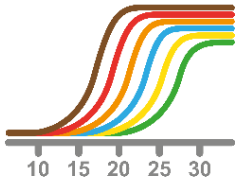






Multiple DNA methylation detection platforms





Me-qPCR platform

Without bisulfite treatment
Automate DNA methylation detection in one step

Applicable detection items

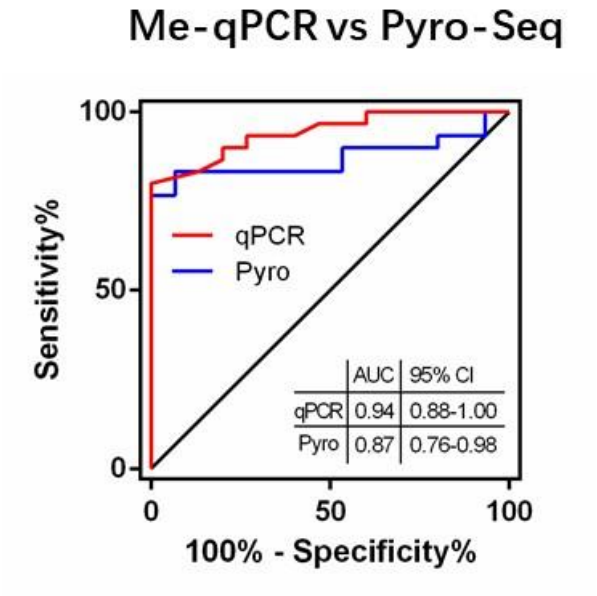
- Cervical cancer,
- Urinary tract cancer,
- Endometrial cancer

Technical advantages without bisulfite treatment

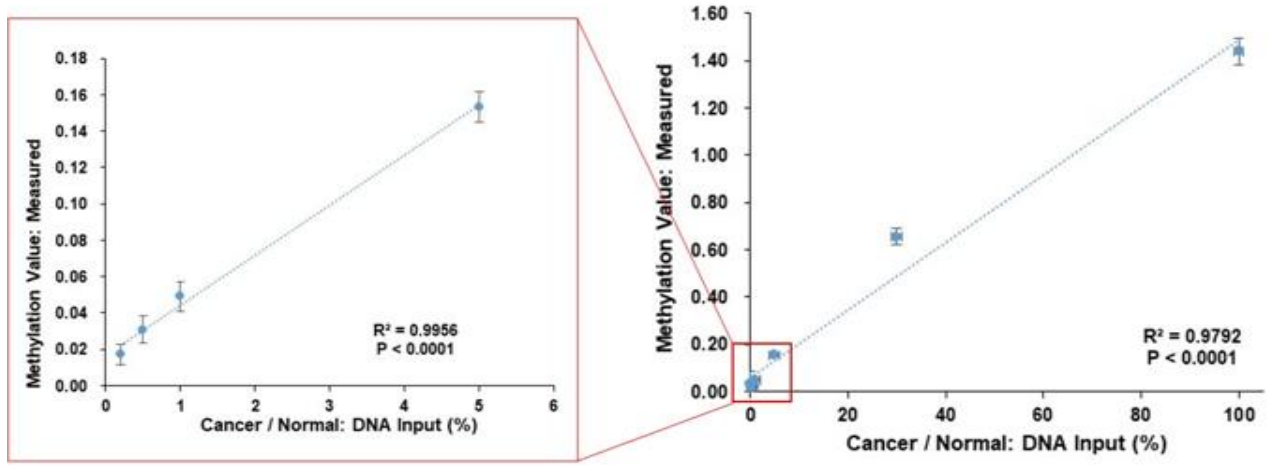
- More stable
- More sensitive
- More convenient
- Automated

Product quality: Double-blind validation

Original DNA methylation detection technology, based on qPCR platform that doesn't require bisulfite treatment -- Me-qPCR, can detect as low as 0.2% of tumor components.



Limit of Detection: 0.2%



Double-blind validation of TAGMe

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No.	Cancer type	Sample type	Sample Number	Partial research results	Performance
1	Cervical cancer	Cervical cells/vaginal secretions, etc.	36348	Clin and Trans Med (2021.06, the latest IF is 11.5) STTT（2022.07，IF 38.1）	94.3% specificity, 96% sensitivity (exfoliate cells)
2	Urinary tract carcinoma	Urine/tissue, etc.	3499	Article is scheduled for publication in 2022	92.7% specificity, 82.1% sensitivity (urine)
3	Lung cancer	Alveolar lavage fluid/pleural fluid/tissue/blood, etc.	3385	Cancer Res (2019.10, the latest IF is 12.7)	96.5% specificity, 87% sensitivity (lavage fluid/pleural fluid)
4	Endometrial cancer	Cervical/uterine cavity cells/tissues, etc.	884	Front Mol Biosci (2021.11, the latest IF is 5.2）	87.3% specificity, 90.9% sensitivity (exfoliate cells)
5	Biliary tract tumors	Bile/tissue, etc.	930	Article is scheduled for publication in 2022	100% specificity, 96.9% sensitivity (tissue)
6	Immunotherapy	blood	746	Article is scheduled for publication in 2022	85.7% specificity, 66.7% sensitivity (Blood)
7	breast cancer	Tissue/blood, etc.	150	R&D is in progress	92.5% specificity, 100% sensitivity (tissue)
8	Liver cancer	Tissue/blood, etc.	979	Article is scheduled for publication in 2022	90.1% specificity, 82.2% sensitivity (tissue)
9	gastric cancer	Tissue/blood, etc.	196	R&D is in progress	100% specificity, 90% sensitivity (tissue)
10	Colorectal cancer	Feces/tissue/blood, etc.	189	R&D is in progress	90% specificity, 100% sensitivity (tissue)
11	Thyroid cancer	Tissue	215	R&D is in progress	-
12	Other	Cerebrospinal fluid/bone marrow smear/tissue/blood, etc.	971	R&D is in progress	-

Summary: By the end of March 2022, total of 50552 clinical samples have been double-blind validated, and the overall consistancy rate of tissue samples is >90%.

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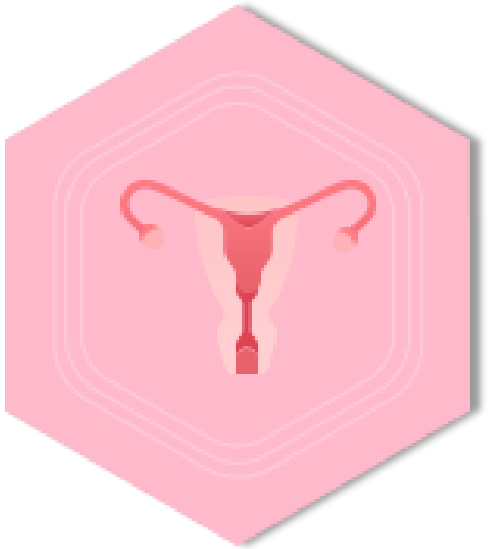


CE

Cervical Cancer(TAGMe-CeCan) & Endometrial Cancer(TAGMe-EnCan)

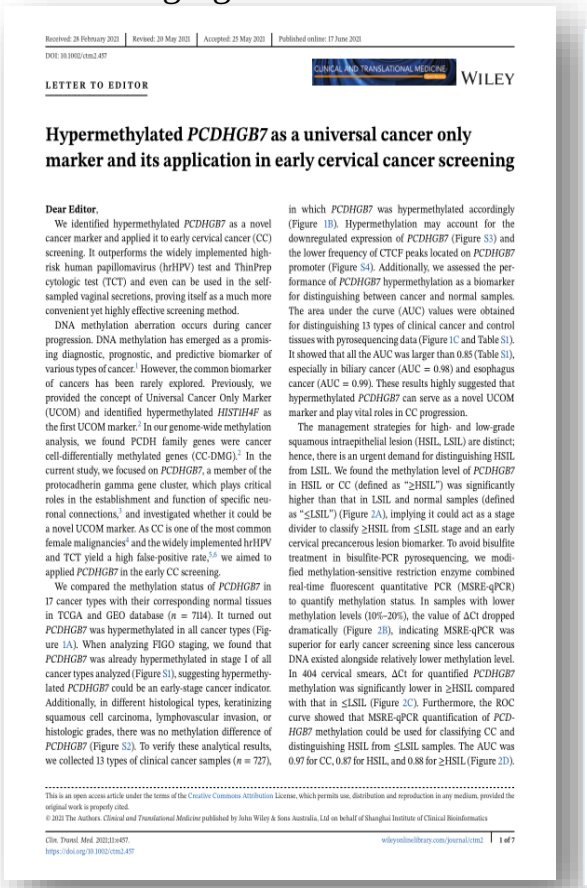
TAGMe
DNA methylation detection for female genital tract cancer

Eliminate the cancer in the precancerous stage

Product performance: better than HPV and TCT

- According to the analysis of the 3728 newly enrolled and unblinded samples in the double-blind verification, performance of DNA methylation detection is as follows:

  pathologic positive includes high-grade cervical lesions and cervical cancer patients, and pathology-negative refers to diseases that have not reached high-grade lesions or cervical cancer.



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CLINICAL AND TRANSLATIONAL MEDICINE WILEY

LETTER TO EDITOR

Hypermethylated *PCDHGB7* as a universal cancer only marker and its application in early cervical cancer screening

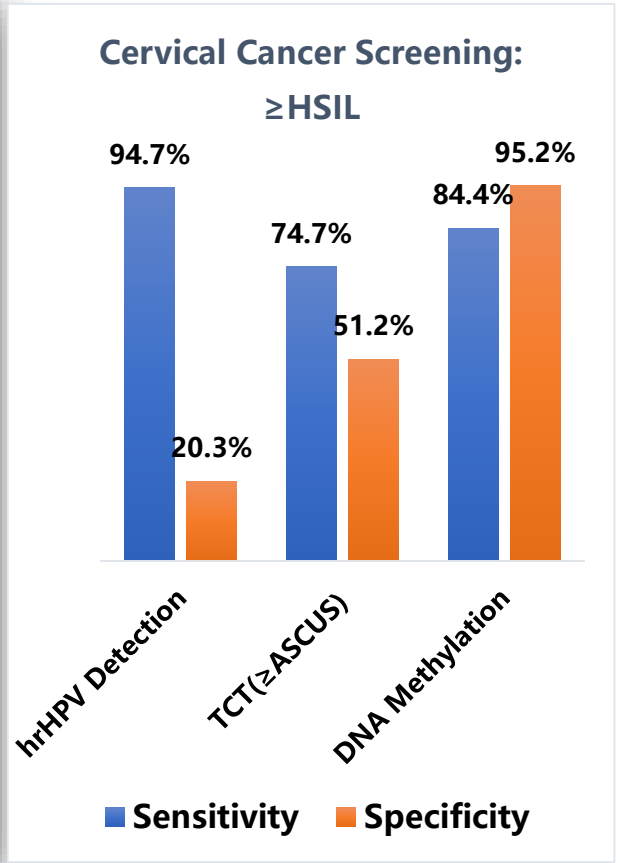
Dear Editor,

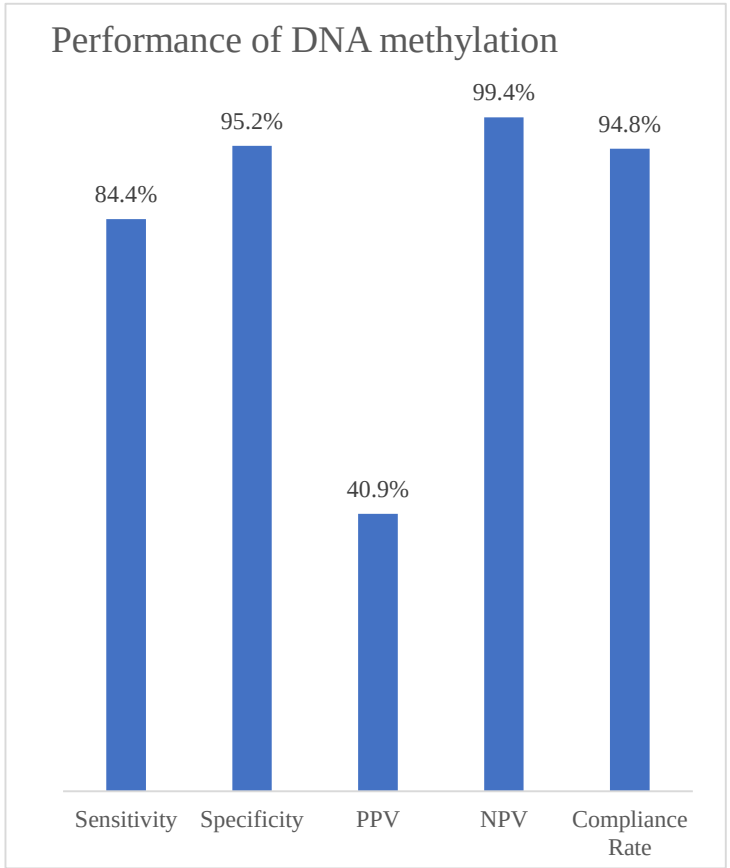
We identified hypermethylated *PCDHGB7* as a novel cancer marker and applied it to early cervical cancer (CC) screening. It outperforms the widely implemented high-risk human papillomavirus (hrHPV) test and ThinPrep cytologic test (TCT) and even can be used in the self-sampled vaginal secretions, proving itself as a much more convenient yet highly effective screening method.

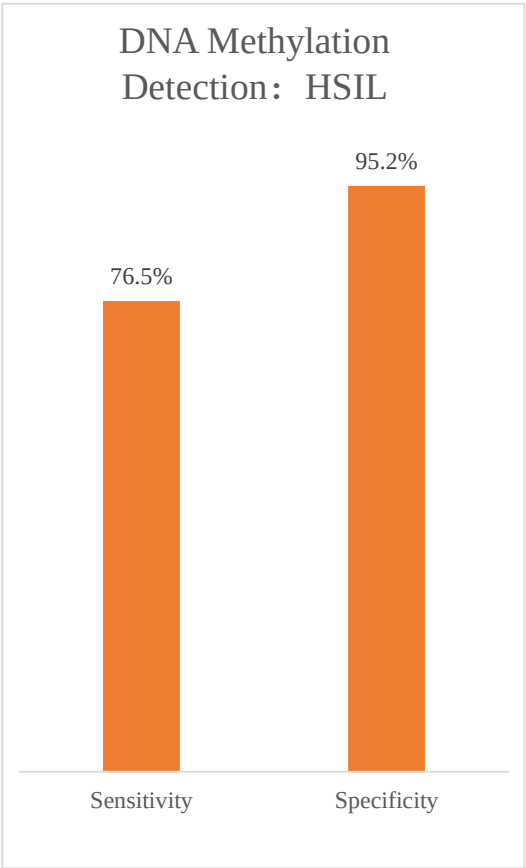
DNA methylation aberration occurs during cancer progression. DNA methylation has emerged as a promising diagnostic, prognostic, and predictive biomarker of various types of cancer.[1] However, the common biomarker of cancers has been rarely explored. Previously, we provided the concept of Universal Cancer Only Marker (UCOM) and identified hypermethylated *HIST1H4F* as the first UCOM marker.[2] In our genome-wide methylation analysis, we found PCDH family genes were cancer cell-differentially methylated genes (CC-DMG).[2] In the current study, we focused on *PCDHGB7*, a member of the protocadherin gamma gene cluster, which plays critical roles in the establishment and function of specific neuronal connections,[3] and investigated whether it could be a novel UCOM marker. As CC is one of the most common female malignancies[4] and the widely implemented hrHPV and TCT yield a high false-positive rate,[5,6] we aimed to applied *PCDHGB7* in the early CC screening.

We compared the methylation status of *PCDHGB7* in 17 cancer types with their corresponding normal tissues in TCGA and GEO database (n = 7114). It turned out *PCDHGB7* was hypermethylated in all cancer types (Figure 1A). When analyzing FIGO staging, we found that *PCDHGB7* was already hypermethylated in stage 1 of all cancer types analyzed (Figure S1), suggesting hypermethylated *PCDHGB7* could be an early-stage cancer indicator. Additionally, in different histological types, keratinizing squamous cell carcinoma, lymphovascular invasion, or histologic grades, there was no methylation difference of *PCDHGB7* (Figure S2). To verify these analytical results, we collected 13 types of clinical cancer samples (n = 727), in which *PCDHGB7* was hypermethylated accordingly (Figure 1B). Hypermethylation may account for the downregulated expression of *PCDHGB7* (Figure S3) and the lower frequency of CTCF peaks located on *PCDHGB7* promoter (Figure S4). Additionally, we assessed the performance of *PCDHGB7* hypermethylation as a biomarker for distinguishing between cancer and normal samples. The area under the curve (AUC) values were obtained for distinguishing 13 types of clinical cancer and control tissues with pyrosequencing data (Figure 1C and Table S1). It showed that all the AUC was larger than 0.85 (Table S1), especially in biliary cancer (AUC = 0.98) and esophagus cancer (AUC = 0.99). These results highly suggested that hypermethylated *PCDHGB7* can serve as a novel UCOM marker and play vital roles in CC progression.

The management strategies for high- and low-grade squamous intraepithelial lesion (HSIL, LSIL) are distinct; hence, there is an urgent demand for distinguishing HSIL from LSIL. We found the methylation level of *PCDHGB7* in HSIL or CC (defined as "≥HSIL") was significantly higher than that in LSIL and normal samples (defined as "≤LSIL") (Figure 2A), implying it could act as a stage divider to classify ≥HSIL from ≤LSIL stage and an early cervical precancerous lesion biomarker. To avoid bisulfite treatment in bisulfite-PCR pyrosequencing, we modified methylation-sensitive restriction enzyme combined real-time fluorescent quantitative PCR (MSRE-qPCR) to quantify methylation status. In samples with lower methylation levels (10%–20%), the value of ΔCt dropped dramatically (Figure 2B), indicating MSRE-qPCR was superior for early cancer screening since less cancerous DNA existed alongside relatively lower methylation level. In 404 cervical smears, ΔCt for quantified *PCDHGB7* methylation was significantly lower in ≥HSIL compared with that in ≤LSIL (Figure 2C). Furthermore, the ROC curve showed that MSRE-qPCR quantification of *PCDHGB7* methylation could be used for classifying CC and distinguishing HSIL from ≤LSIL samples. The AUC was 0.97 for CC, 0.87 for HSIL, and 0.88 for ≥HSIL (Figure 2D).







Clinical and Translational Medicine.

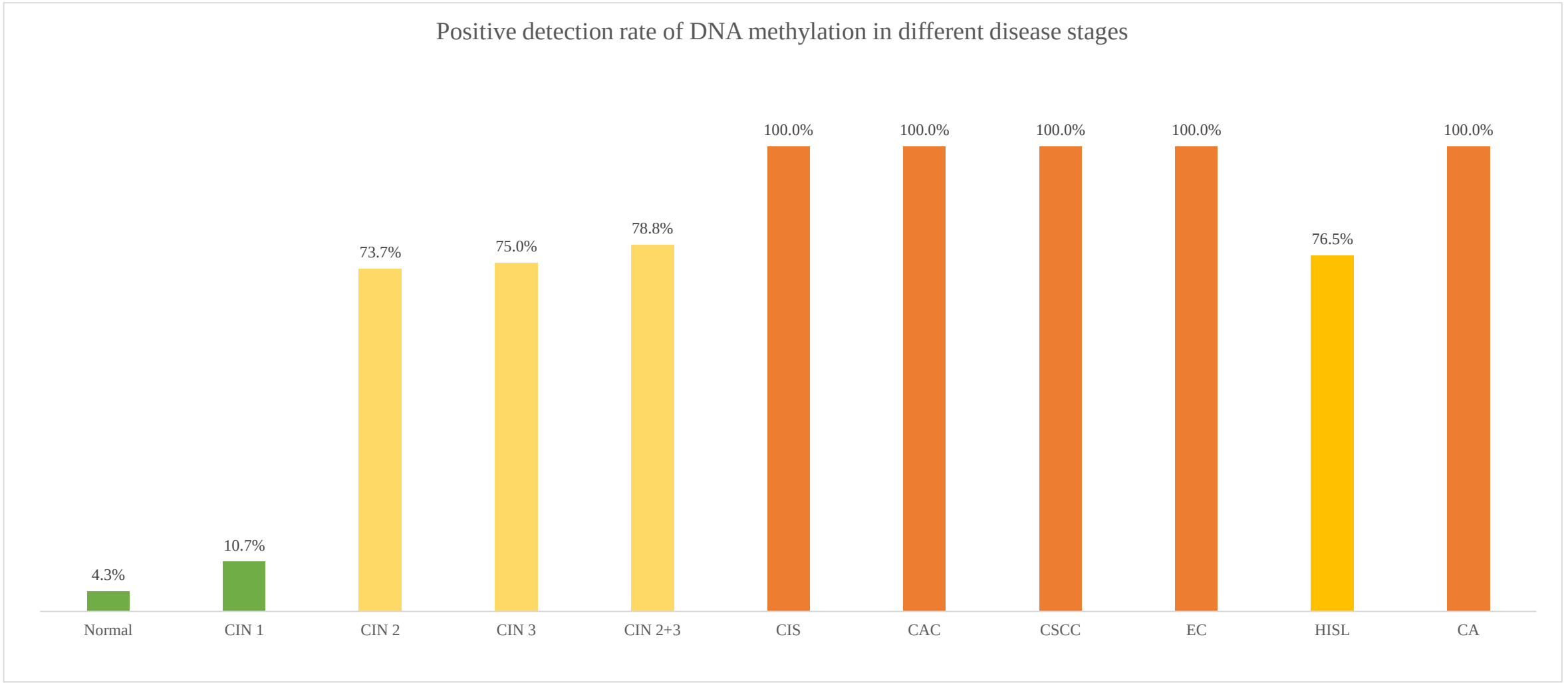
2021.06 (IF=11.5)

- DNA methylation in cervical high-grade lesions (HSIL) screening has a specificity of **95.2%**, and sensitivity of **76.5%**;
- Overall (≥HSIL) screening specificity is **95.2%**, and sensitivity is **84.4%**.

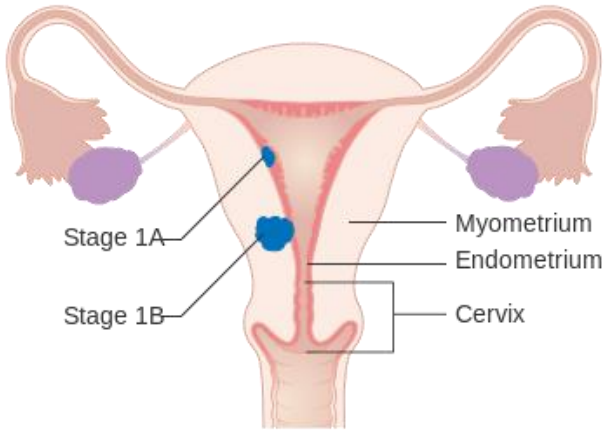
Product performance: screen precancerous lesions

Results of double-blind validation

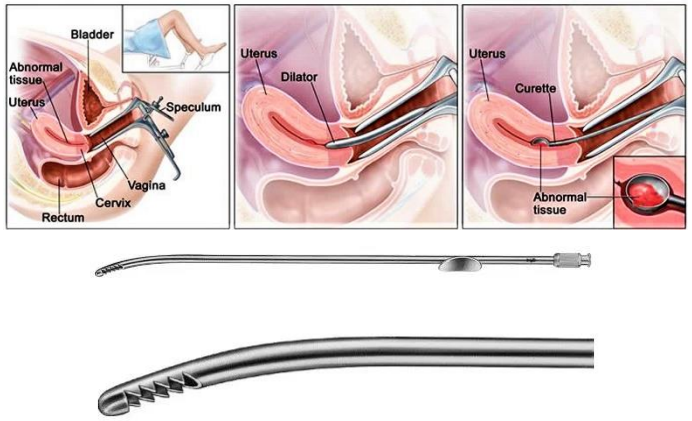
Further analysis of the detection performance of DNA methylation detection in different stages found that the detection rate in the cancer group was 100%, and the detection rate in the high-grade lesion group was 76.5%.



Clinical pain points of endometrial cancer screening



Early endometrial cancer is confined to the uterus



Endometrial biopsy may result in discomfort, bleeding, infection, and uterine perforation, with a high rate of missed tests

- Pain point: Lacking sensitive and accurate non-invasive screening method. Symptoms such as early irregular vaginal bleeding and vaginal drainage are easily overlooked, missing the opportunity for early diagnosis.

■Transvaginal ultrasonography:

Convenient and noninvasive, vaginal ultrasound is easy to miss diagnosis when the endometrium is <5 mm thick and hard to assess premenopausal endometrial lesions.

■Hysteroscopy:

Expensive, most patients require anesthesia, and has the risk of side effect(e.g. infection, water intoxication, air embolism, etc.), thus cannot be applied as as a routine screening method.

■Microscopic diagnostic curettage:

Invasive surgery, significant pain, has the risk of side effect(e.g. bleeding, infection, uterine perforation, uterine adhesions etc.), along with the possibility of missed scratch.

■Endometrial biopsy:

Gold standard, invasive surgery, and it is easy to get insufficient and inaccurate samples, especially for postmenopausal patients

Product performance: endometrial cancer

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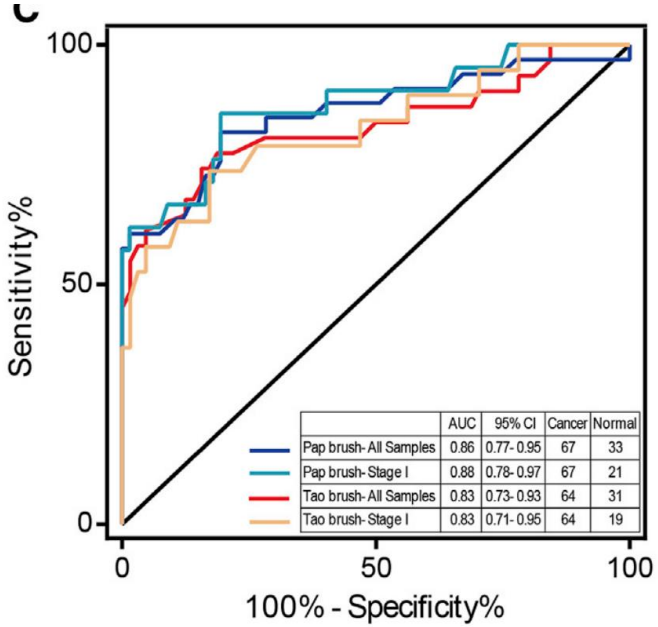
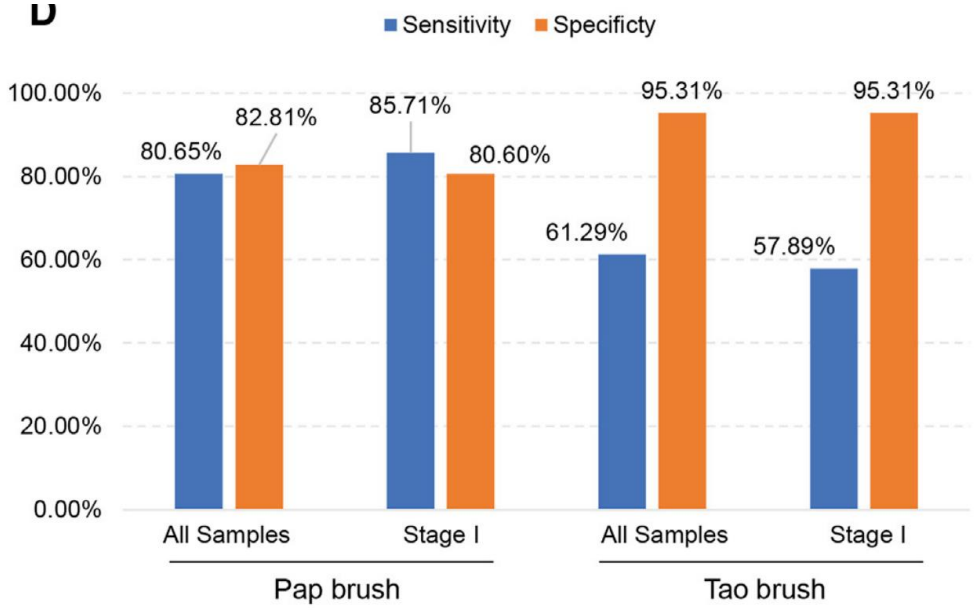
Double-blind samples:

① Pap sample（109）：NE 47，EH 20，AH 9，EC 33.
② Tao sample（103）：NE 44，EH 20，AH 8，EC 31.

Diagnostic model	Endometrial cancer detection performance				
	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Pap Brush (Cutoff:<4.03)	80.65	82.81	86.49	62.69	84.21
Tao Brush (Cutoff:<1.25)	61.29	95.31	54.05	87.50	82.11
Either positive as positive (Cutoff: Pap<4.03, Tao<1.32)	90.32	73.44	62.22	94.00	78.95
Both positive as positive (Cutoff: Pap<2.5, Tao<4.55)	61.29	100.00	100.00	84.21	87.37





Double-blind test results show:

Pap brush: AUC =0.86, specificity=82.81%, sensitivity=80.65%;

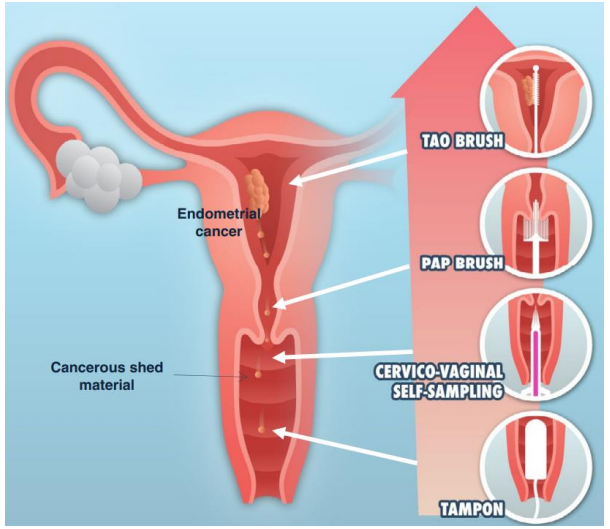
Tao brush: AUC=0.83, specificity=95.31%, sensitivity=61.29%;

NE: Normal endometrium

EH: Endometrial hyperplasia

AH: Atypical hyperplasia

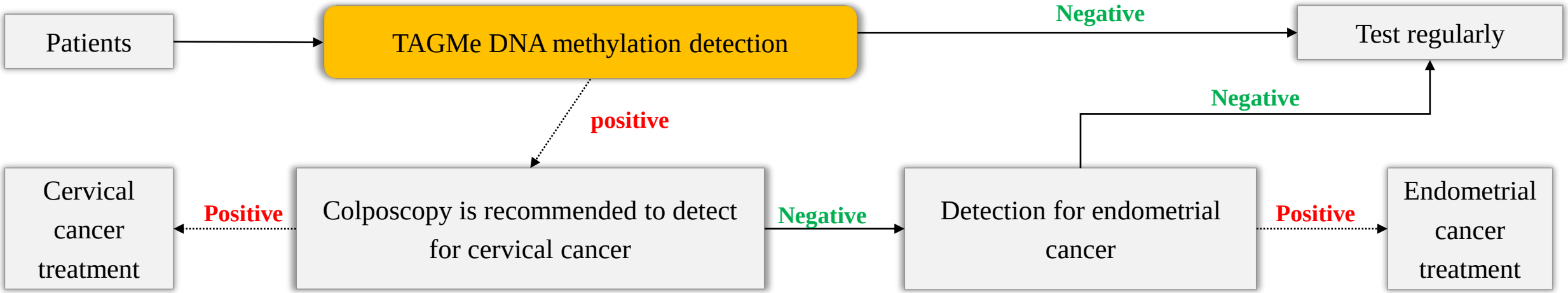
EC: Endometrial cancer



Combined with the existing clinical uterine cavity and cervical exfoliation cell sampling devices - Pap brush and Tao brush, the non-invasive screening of endometrial cancer is realized.

Summary

Project	Application scenarios	Sample types	Sample volume	Transportation requirement
TAGMe DNA methylation detection for female genital tract cancer -	• Early cancer screening • Auxiliary diagnosis • Risk monitoring • Recrudescence monitoring	Cervical scraping (TCT sampling method)	2～5 mL	Room temperature



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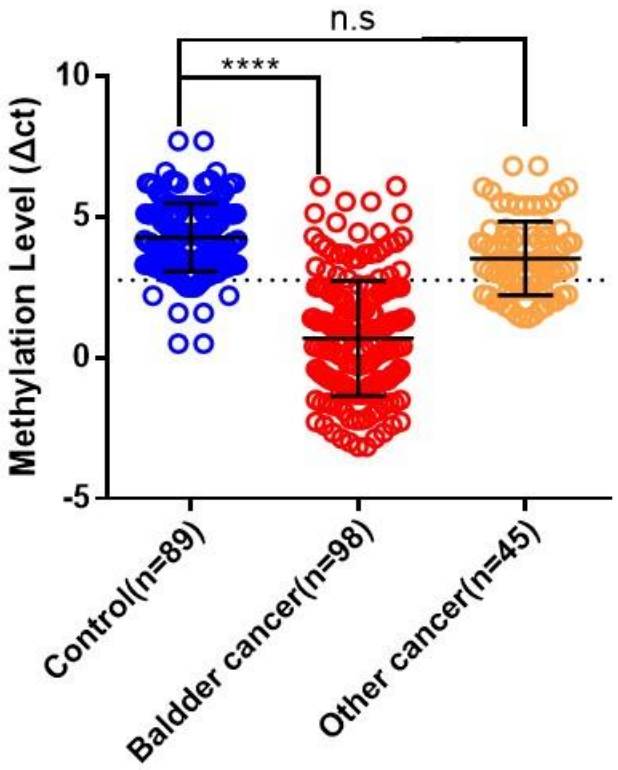
CE

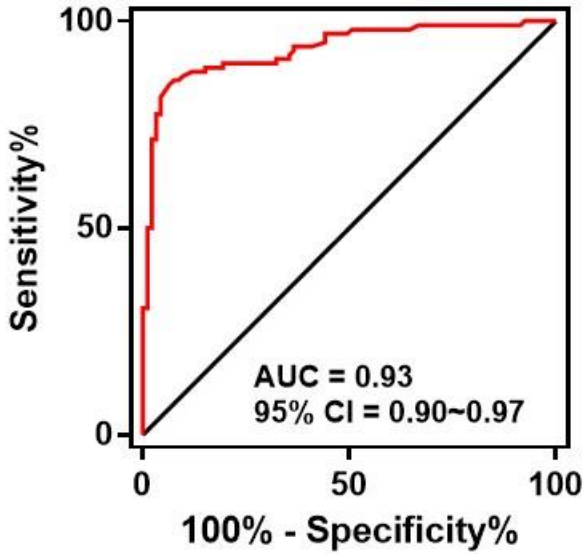
Full-process solution for urinary tract cancer

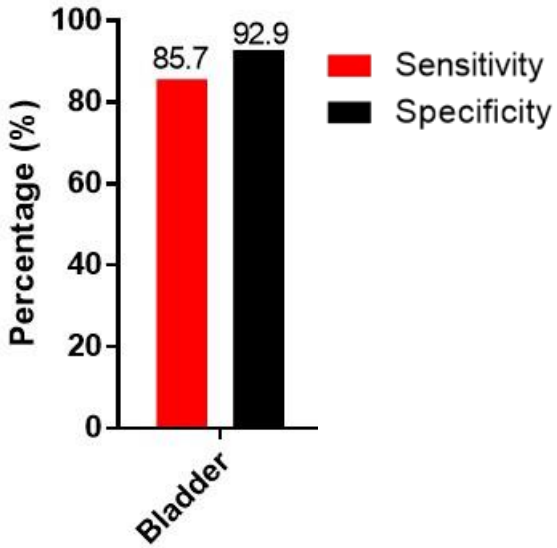
TAGMe-UrCan

Product performance

Urine detection for bladder cancer





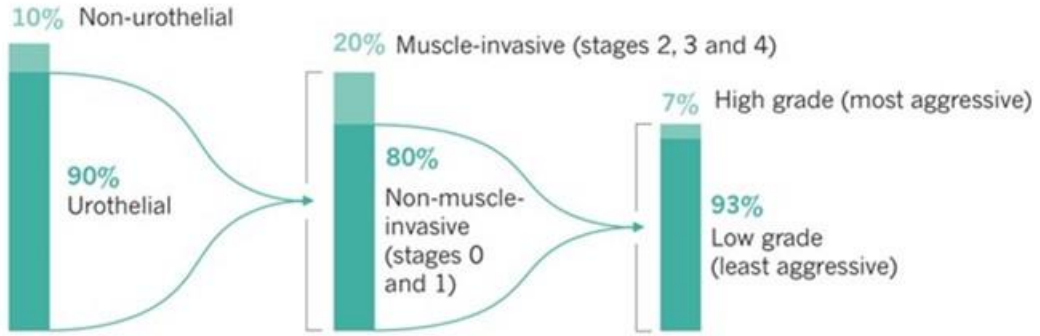


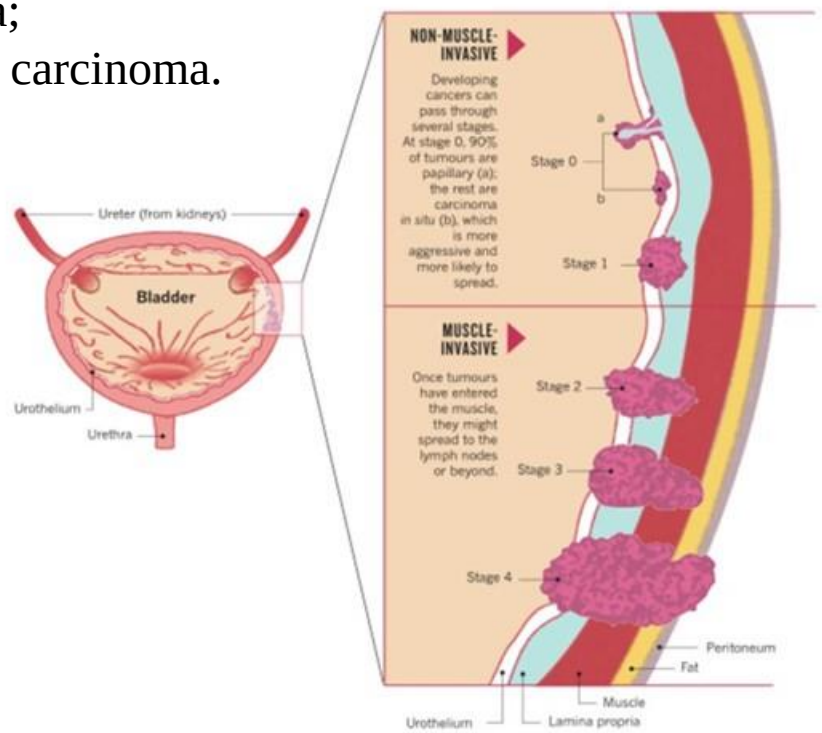
Double-blind validation of urine samples for bladder cancer,

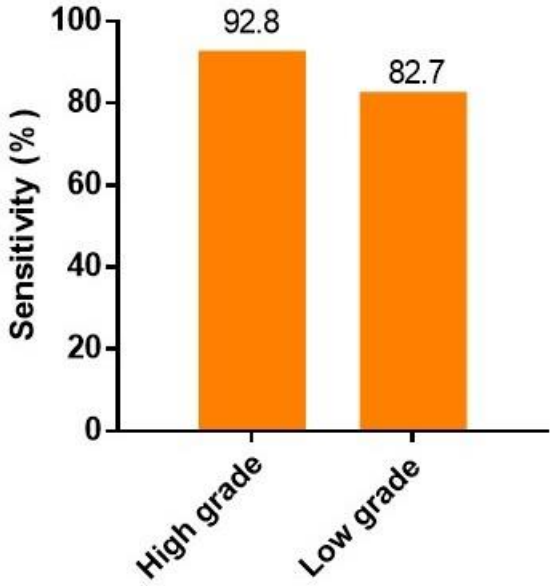
AUC=0.93, specificity = 92.9%, sensitivity = 85.7%;

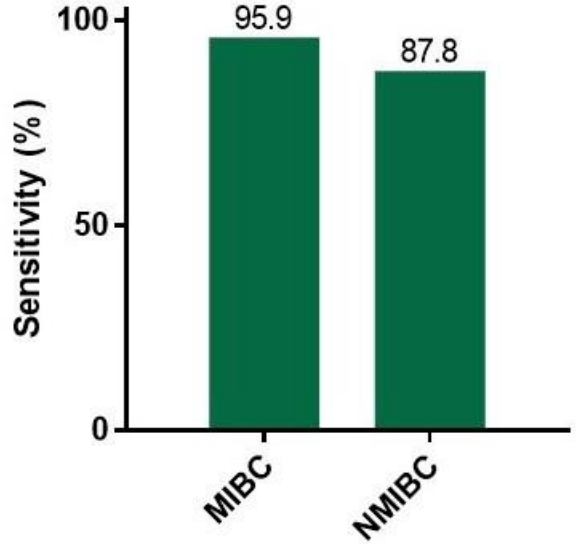
Product performance

- Bladder cancer: 90% of which is transitional epithelial cell carcinoma; 80% of which is non-invasive carcinoma; 93% of which is low-grade carcinoma.









- The detection rate of high-grade bladder cancer is **92.8%** and that of low-grade bladder cancer is **82.7%**;
- The detection rate of invasive carcinoma (MIBC) is **95.9%**, The detection rate for non-invasive carcinoma (NMIBC) is **87.8%**.

Product performance

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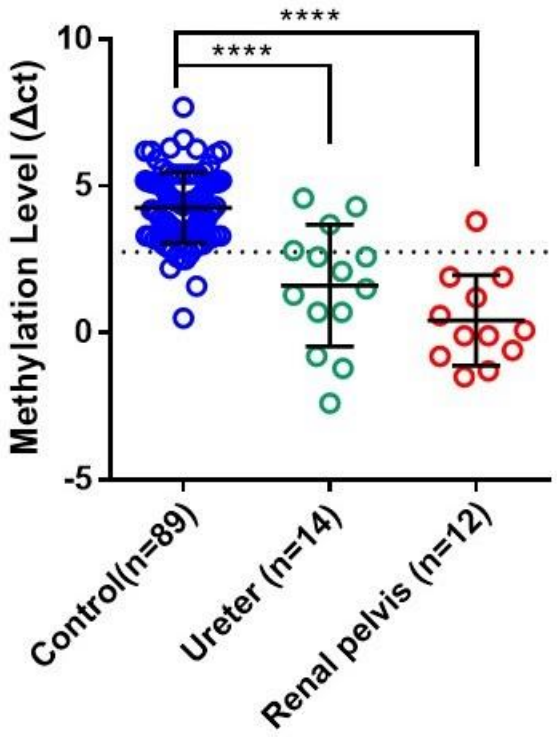
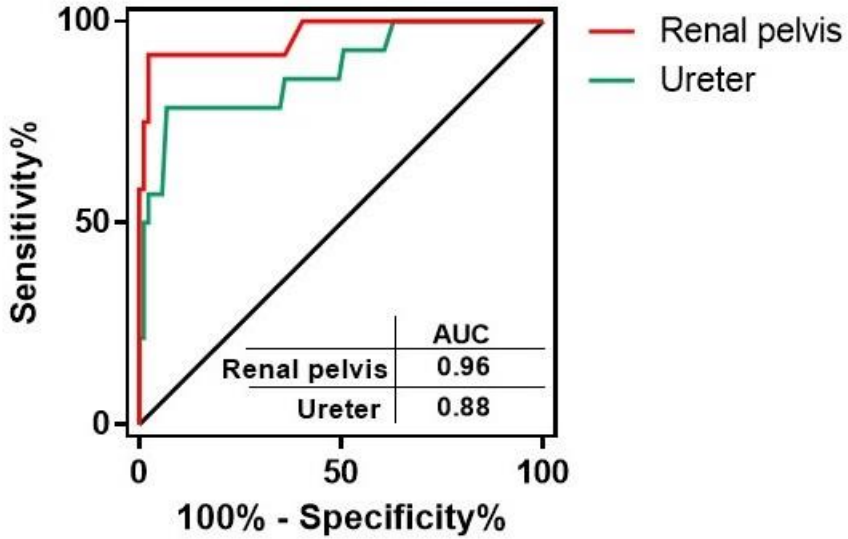
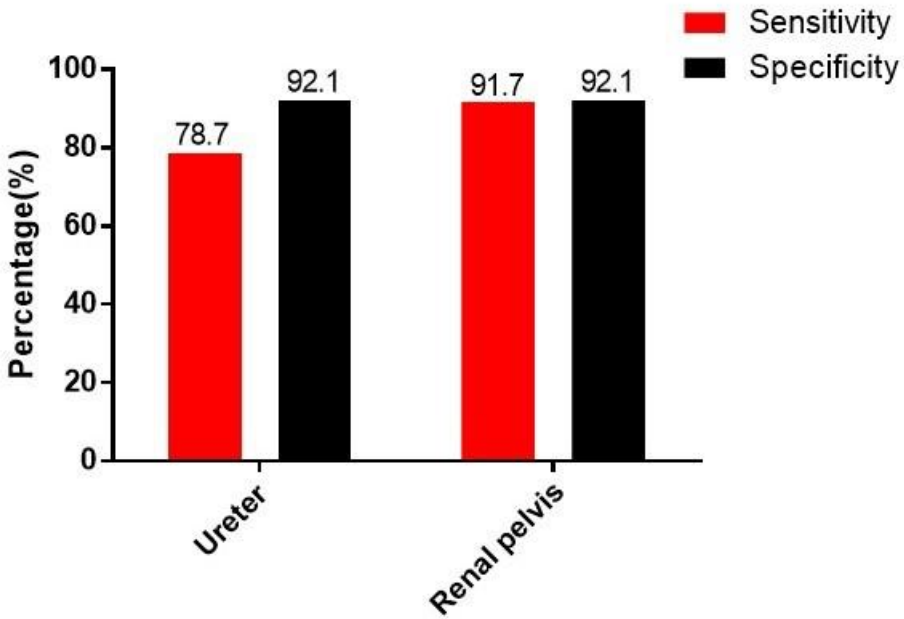
Urine detection for renal pelvis cancer and ureteral cancer





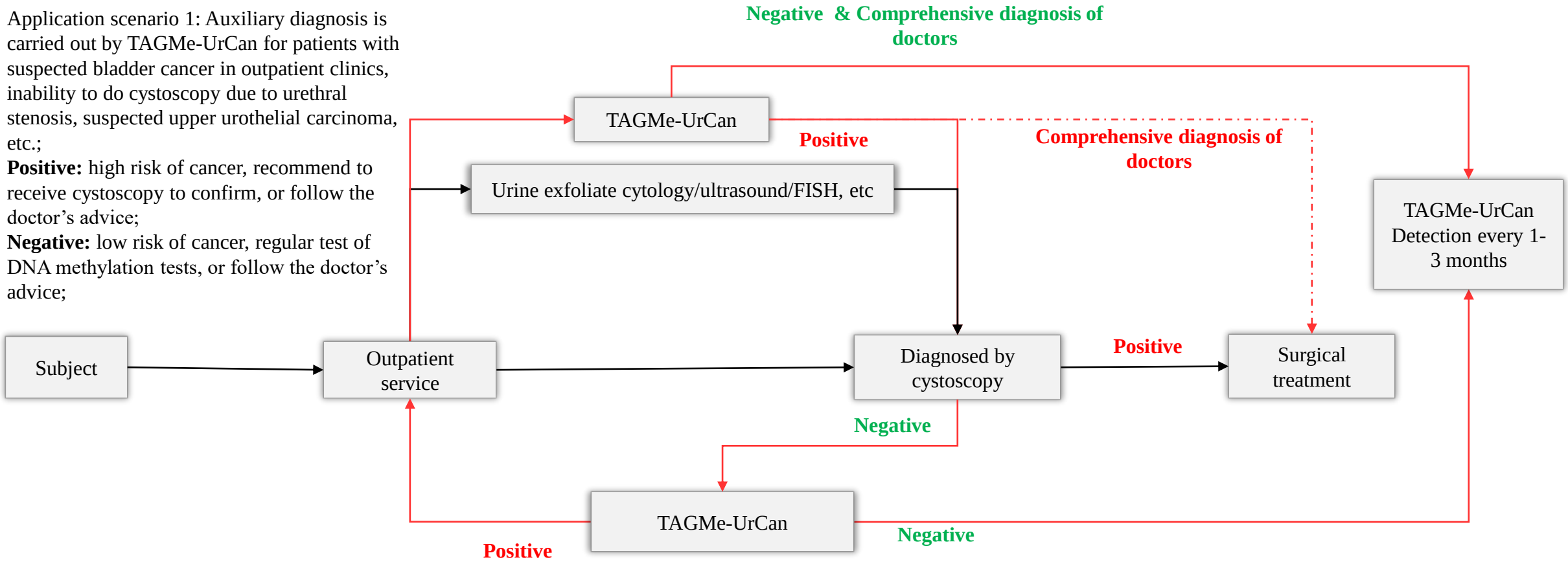


- **Renal pelvis cancer: sensitivity=91.7%, specificity=92.1%;**
- **Ureteral cancer: sensitivity=78.7%, specificity=92.1%;**

Application scenario: Auxiliary diagnosis

Negative

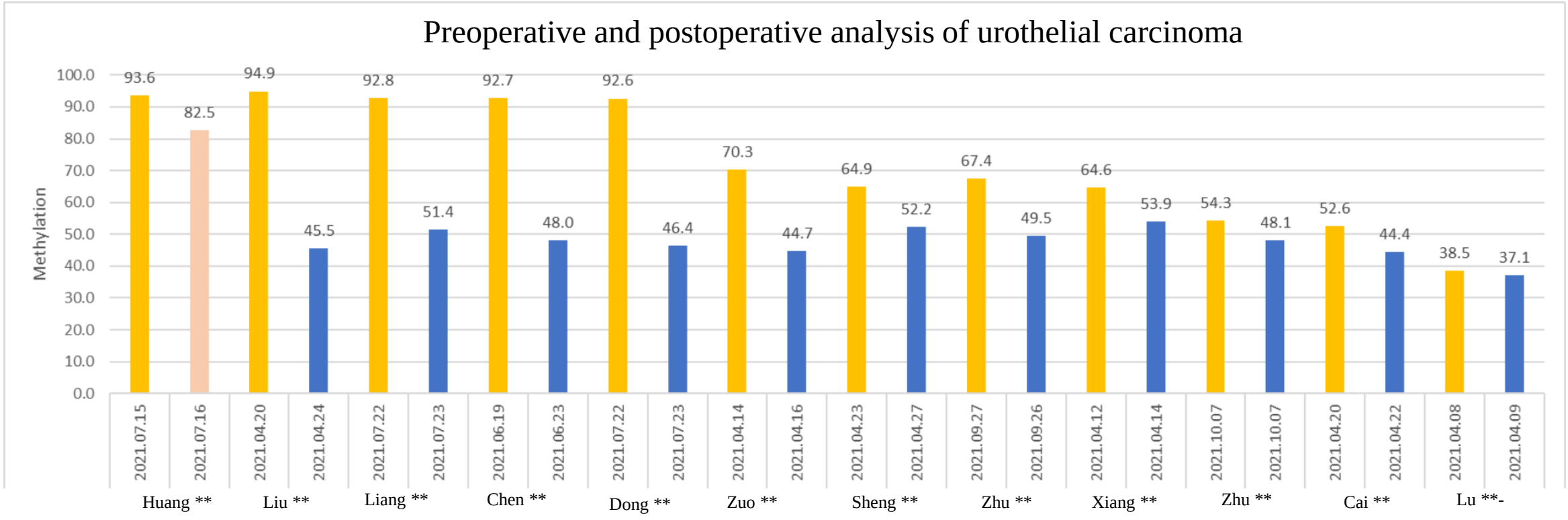
Application scenario 1: Auxiliary diagnosis is carried out by TAGMe-UrCan for patients with suspected bladder cancer in outpatient clinics, inability to do cystoscopy due to urethral stenosis, suspected upper urothelial carcinoma, etc.;
Positive: high risk of cancer, recommend to receive cystoscopy to confirm, or follow the doctor's advice;
Negative: low risk of cancer, regular test of DNA methylation tests, or follow the doctor's advice;



Application scenario 2: For patients with clinical symptoms such as space-occupying lesions, hematuria, etc., but the cystoscopy comes back negative;
Positive: high risk of cancer, recommend to re-examine for cystoscopy, close monitoring, and follow the doctor's advice;
Negative: low risk of cancer, regular check-up, cancer risk monitoring;

Case study of surgical efficacy determination

All patients (12/12) had lower postoperative DNA methylation level than before, but postoperative DNA methylation level of yellow SS was still a strong positive, suggesting a high suspicion of postoperative tumor residue. It was strongly recommended to review and monitor.



Application scenario: Treatment efficacy assessment

Negative

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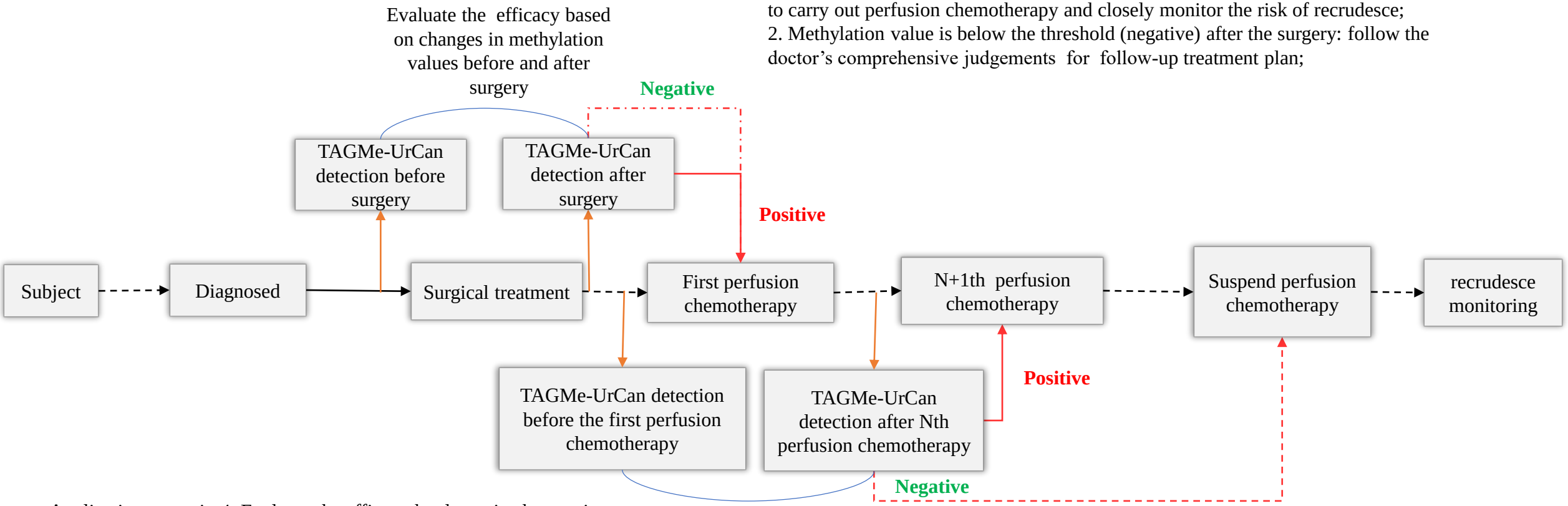
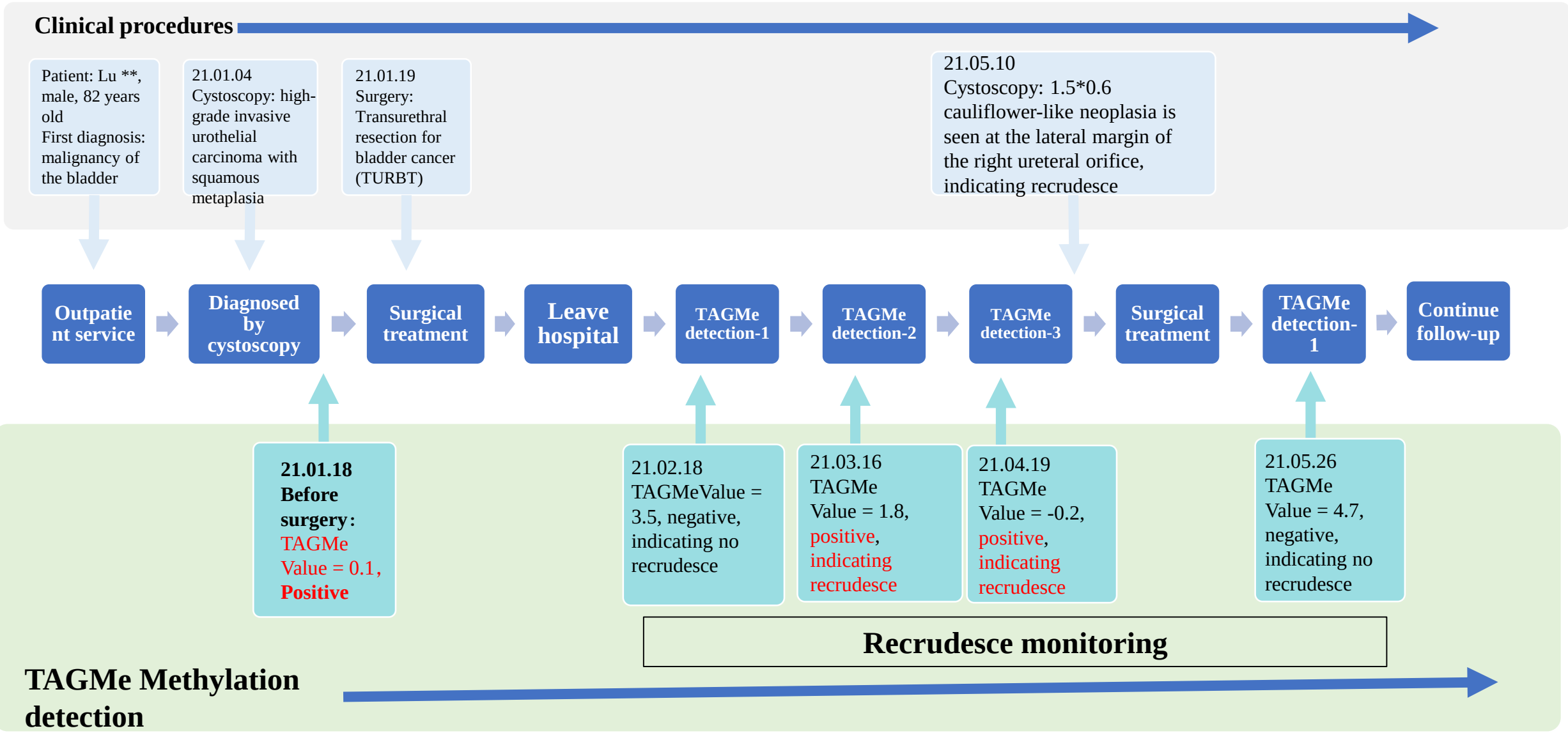
Application Scenario 3: Evaluation of Surgical Efficacy:
1. Methylation value is still above the threshold (positive) after the surgery: recommend to carry out perfusion chemotherapy and closely monitor the risk of recrudesce;
2. Methylation value is below the threshold (negative) after the surgery: follow the doctor's comprehensive judgements for follow-up treatment plan;



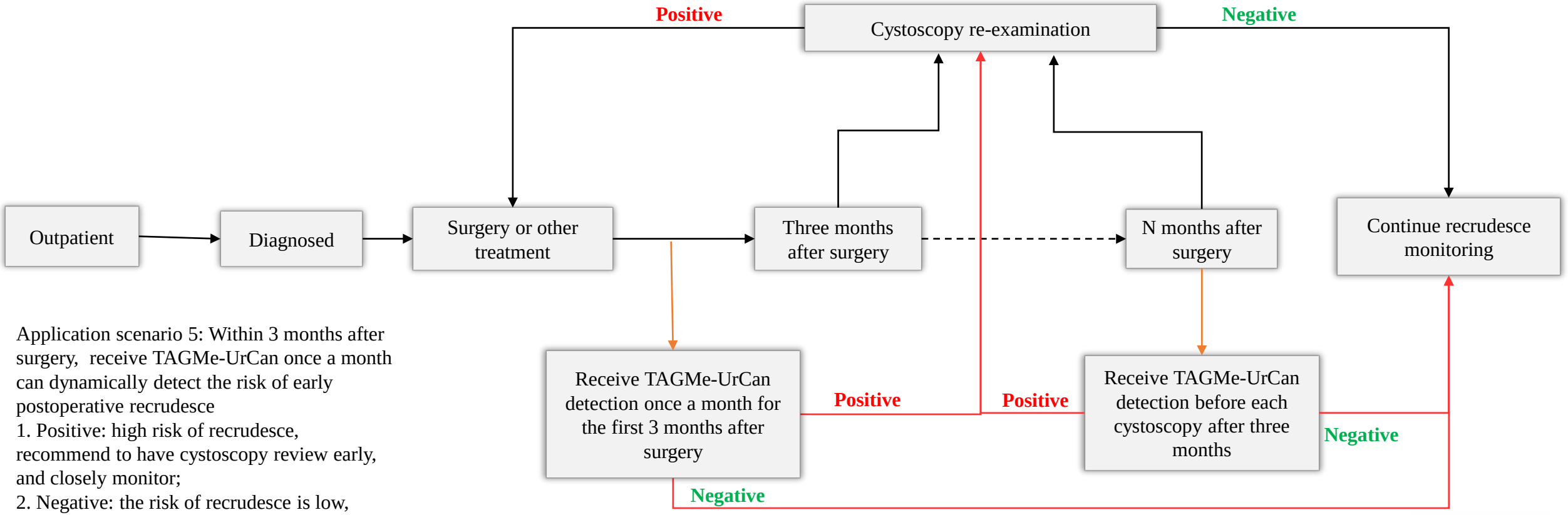
Application scenario 4: Evaluate the efficacy by dynamic changes in methylation values after each perfusion chemotherapy
1. The postoperative methylation value is still above the threshold (positive): recommend to continue (or change the regimen) for perfusion chemotherapy and closely monitor the risk of recrudesce;
2. The postoperative methylation value is below the threshold (negative): follow the doctor comprehensive judgements on whether to stop perfusion chemotherapy;

Recrudesce of urinary urothelial carcinoma

Case studies of recrudesce monitoring



Application scenario: Recrudesce monitoring



Application scenario 5: Within 3 months after surgery, receive TAGMe-UrCan once a month can dynamically detect the risk of early postoperative recrudesce

1. Positive: high risk of recrudesce, recommend to have cystoscopy review early, and closely monitor;
2. Negative: the risk of recrudesce is low, follow the doctor's comprehensive judgment and advice.

Application scenario 6: After 3 months of surgery, before each cystoscopy, have TAGMe-UrCan to detect the risk of recrudesce after surgery for long-term dynamic detection

1. Positive: high risk of recrudesce, recommend to have cystoscopy review early, and closely monitor:
2. Negative: the risk of recrudesce is low, follow the doctor's comprehensive judgment and advice.

Changhai Hospital - Full Process Case Study

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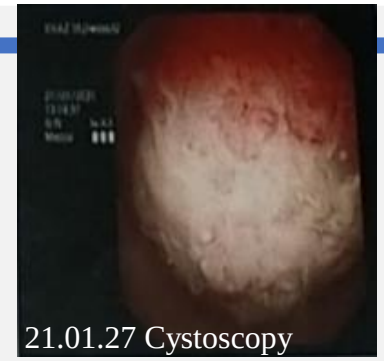
Clinical procedures

Patient: Liu **, female, 82 years old Initial diagnosis: high-grade varus papillary urinary tract carcinoma of the bladder

20.03.31 Cystoscopy: Cauliflower-like neoplasia is seen at the ureteral opening on the left side of the bladder, and cauliflower new organism is seen at the top of the bladder

20.05.22 Surgery: Transurethral resection for bladder cancer (TURBT)

20.06~20.12：
Seven months after the operation, due to the inconvenience of her location, cystoscopy pain and other reasons, the patient's compliance was poor, and she did not return to the hospital for re-examination


21.01.27 Cystoscopy

21.01.27 Cystoscopy: Cauliflower-like neoplasia on the anterior wall of the bladder; 21.02.01 Pathology: High-grade papillary urothelial carcinoma

Outpatient service → **Diagnosed by cystoscopy** → **Surgical treatment** → **Leave hospital** → **TAGMe recrudesce detection-1** → **TAGMe recrudesce detection-2** → **Cystoscopic review**

20.03.24
TAGMe Value = 92, positive, indicating a high risk of urinary urothelial cancer

20.05.22
Before surgery: TAGMe Value = 94, positive

20.05.22
After surgery: TAGMe Value = 42, negative, indicating successful surgery

20.12.26
TAGMeValue = 85, positive, indicating recrudesce

21.01.26
TAGMeValue = 95, positive, indicating recrudesce and progression; doctors strongly recommend returning to the hospital for re-examination!

Auxiliary diagnosis | **Efficacy assessment** | **recrudesce monitoring**

TAGMe-UrCan

- **Sampling tubes are mailed directly to patients. Urine can be taken at home, which is convenient!**
- **TAGMe can be applied to whole process from early screening, auxiliary diagnosis, efficacy evaluation to recrudesce monitoring of urinary urothelial cancer.**

Summary of methylation detection for urothelial carcinoma

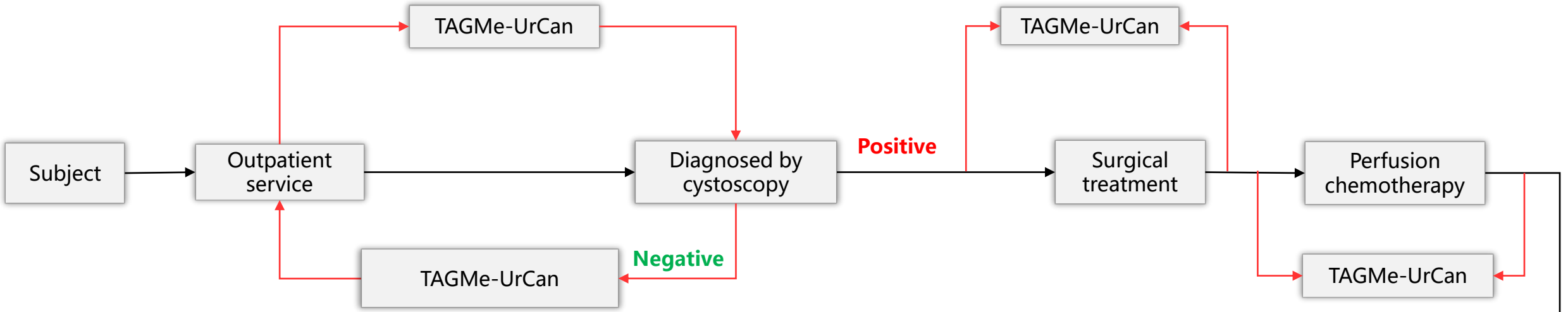
Sample requirements

Noninvasive: Only 30 ml of urine is required
Convenient: Samples can be taken at home
Simple: Storage and shipping at room temperature for 15 days
Fast: Electronic reports takes only 3 to 5 work days

Scenarios

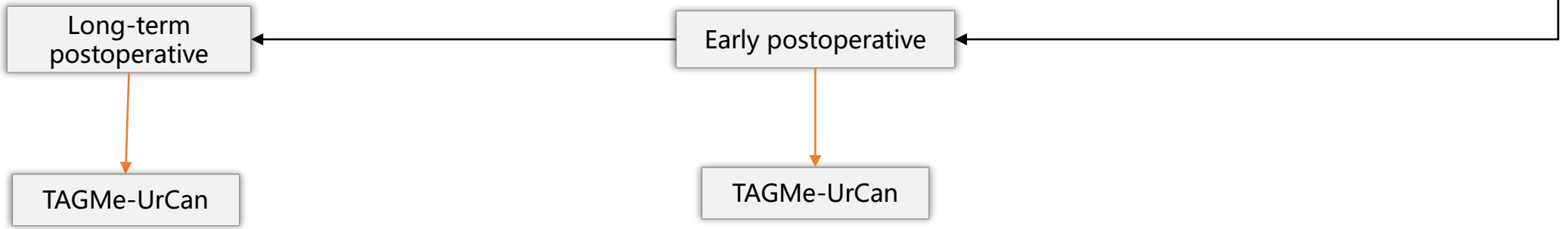
High-risk populations, early screening
Clinical-suspicious people, assist cystoscopy for diagnosis
Perfusion chemotherapy, efficacy assesment
Initial prognosis, risk assessment of recrudesce
Prognostic recrudesce, long-term recrudesce monitoring

Epiprobe TAGMe DNA methylation molecular detection provides a full-process solution for urinary tract carcinoma



Before diagnosis:-Adjunctive diagnosis and recrudesce monitoring

During treatment. : Evaluation of surgical efficacy and perfusion therapy efficacy



After surgery and long-term recrudesce monitoring

Appendix : Academic Publications

Academic Publications

Genome Research, 2019.01 (**IF=11.1**)
Cancer Research, 2019.10 (**IF=12.7**)
Clinical and Translational Medicine, 2021.06 (**IF=11.5**)
Frontiers in Molecular Biosciences (**IF=5.2**)
Signal Transduction and Targeted Therapy（**IF=38.104**）

About Epiprobe

As a high-tech enterprise founded in 2018 by top epigenetic experts, Epiprobe focuses on the molecular diagnosis of cancer DNA methylation and precision theranostics industry. With a profound technology basis, EPIPROBE aims to lead the era of new products to nip cancer in the bud!

Based on Epiprobe core team's long-term research, development and transformation in the field of DNA methylation with the cutting-edge innovations, combined with the unique DNA methylation targets of tumors, Epiprobe uses a unique multivariate algorithm combining big data and artificial intelligence technology to independently develop an exclusive patent-protected liquid biopsy technology. By analyzing the methylation level of specific sites of free DNA fragments in the sample, the shortcomings of traditional examination methods and the limitations of surgery and puncture sampling are avoided, which not only achieves accurate detection of early tumors, but also enables real-time monitoring of tumor occurrence and development dynamics.

Epiprobe's tumor molecular detection technology can be used for early tumor screening, auxiliary diagnosis, preoperative and postoperative evaluation, recrudescence monitoring, which runs through the whole process of tumor diagnosis and treatment, providing better solutions for doctors and patients.

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