

DNA METHYLATION BIOMARKERS IN CANCER DIAGNOSTICS

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Abstract

Cancer development and progression are characterized by intricate genetic and epigenetic alternations, with DNA methylation playing a crucial role in this transformative process. Recent advancements in DNA methylation mapping technologies have significantly contributed to the identification of novel cancer biomarkers. We provide a short overview of Conformité Européenne-marked in vitro diagnostic tests designed to detect DNA methylation changes in oncology practice.

Keywords: DNA methylation, cancer, biomarker

INTRODUCTION

Cancer development is intricately linked to the gradual genetic changes that involve mutations in oncogenes, tumor suppressor genes (caretaker and gatekeeper genes), and chromosomal abnormalities. Beyond this genetic landscape, it has been confirmed that epigenetic modifications also play a substantial role in initiating and advancing cancer. These modifications entail heritable alterations to DNA without any change in its nucleotide sequence.

The most extensively studied epigenetic modification within the human genome is DNA methylation. This process involves the covalent addition of a methyl group to the 5-carbon of the cytosine ring in CpG sequences, ultimately yielding 5-methylcytosine. The methyl group transfer occurs through a reaction catalyzed by DNA methyltransferases, utilizing S-adenosylmethionine as the methyl group donor. In the context of tumor cells, DNA methylation undergoes a distinctive redistribution, manifesting as a combination of global genomic hypomethylation and localized CpG island hypermethylation. Subsequently, the hypermethylation of promoter regions associated with tumor suppressor genes or genes governing cell cycle control, apoptosis, and drug sensitivity leads to transcriptional silencing (1). This epigenetic modulation contributes to the inactivation of critical pathways, promoting uncontrolled cell proliferation and resistance to therapeutic interventions that are often characteristic of cancer development and progression.

The early occurrence of epigenetic modifications in cancer pathogenesis suggests their potential as promising biomarkers for screening and early detection. The further potential lies also in their reversibility, offering a unique possibility for the development of innovative treatment options. Moreover, the recent advancements in DNA methylation mapping

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technologies have significantly increased opportunities for epigenetic research. This progress has facilitated the identification of novel epigenetic biomarkers with potential practical application in clinical care (2). However, before the tests based on these biomarkers can be integrated into clinical practice in the European Union (EU), they must undergo clinical validation to ensure their results align with the In Vitro Diagnostic (IVD) Device Regulation.

In the following section, we provide an overview of IVD oncology tests designed to detect DNA methylation changes that obtained Conformité Européenne (CE) marking.

DNA METHYLATION CANCER BIOMARKERS APPROVED FOR CLINICAL USE IN THE EUROPEAN UNION

Globally, over thirty DNA methylation-based assays designed to assist in clinical decision-making in various types of cancer have been introduced to the market (3,4). Not all of them have fulfilled the requirements of EU IVD regulation. The tests approved for use in the EU are summarized in **Table 1**.

Currently, there are three IVD tests available to help with the diagnosis of lung cancer. Epi proLung BL Reflex Assay, manufactured by Epigenomics AG, Berlin, Germany, detects the methylation of the *SHOX2* gene in bronchial aspirates from patients at an increased risk of the disease (5). The manufacturer has adapted the test for use in plasma samples, resulting in Epi proLung, which enables the discrimination between lung cancer and non-cancerous diseases based on a combined methylation analysis of *SHOX2* and *PTGER4* genes (6). A novel blood-based test, named PulmoSeek from AnchorDx Medical Company, Guangzhou, China, detects methylation in a panel of 100 pre-selected lung cancer-specific methylation regions and can differentiate malignant from benign pulmonary nodules (7).

Another DNA methylation-based test developed by Epigenomics AG is intended for use in screening for colorectal cancer. The Epi proColon test uses peripheral blood samples to analyze the methylation status of the *SEPT9* gene (8,9). The test has also been adapted for the early detection of hepatocellular carcinoma under the name HCCBloodTest (10). Completely non-invasive is the EarlyTect Colon Cancer test, manufactured by Genomictree, Daejeon, South Korea, that targets *SDC2* gene methylation in DNA extracted from stool (11).

Three CE-IVD tests (therascreen *MGMT* Pyro Kit, Qiagen, Hilden, Germany; Human *MGMT* Gene Methylation Detection Kit, Xiamen Spacegen, Xiamen, China; and *MGMT* Methylation Detection Kit, EntroGen, Los Angeles, CA, USA) were developed to predict the effectiveness of temozolomide therapy in the most common malignant brain tumor in adults, glioblastoma (12). All the tests detect the methylation of distinct CpG sites of the *MGMT* gene in the DNA extracted from formalin-fixed paraffin-embedded (FFPE) tissue samples.

Several methylation-based tests are also available for early diagnosis of cervical cancer or advanced cervical intraepithelial neoplasia, a premalignant lesion that precedes cervical cancer. For this purpose, Qiagen offers a test named QIASure that analyzes the methylation status of the *FAM19A4* gene and microRNA hsa-mir124-2 in cervical or vaginal specimens (13). GynTect (oncgnostic, Jena, Germany) targets the methylation of the six-gene panel (*ASTN1*, *DLX1*, *ITGA4*, *RXFP3*, *SOX17*, and *ZNF671*) in the DNA extracted from cervical smears of HPV-positive women (14). The iStat Biomedical (New Taipei City, Taiwan) developed two tests for the detection of either cervical or oral cancer in the DNA from cells collected by the scraping: *PAX1* DNA Detection Kit and *ZNF582* DNA Detection Kit (15).

Another CE-IVD test from Qiagen, the therascreen *PITX2* RQq PCR Kit, is intended for predicting the outcomes after anthracycline based chemotherapy in patients with high risk breast cancer. The test detects the methylation of three CpG sites within the *PITX2* gene in the DNA extracted from FFPE tissue (16).

To help with bladder cancer diagnostics and surveillance, two non-invasive tests are available based on urine DNA methylation detection. The UriFind Bladder Cancer Detection Kit from AnchorDx detects *ONECUT2* and *VIM* methylation (17). The Bladder EpiCheck (Nucleix, Rehovot, Israel) is a post-treatment monitoring test that targets methylation changes in 15 proprietary biomarkers and is intended for recurrence detection (18).

The EsoGuard assay developed by Lucid Diagnostics (Gilbert, AZ, USA) can assist with detecting the precursor condition of esophageal cancer called Barrett's Esophagus. The minimally invasive test targets the hypermethylation of the *CCNA1* and *VIM* genes in esophageal brush cells (19).

The microarray DNA methylation signatures detected in fresh frozen or FFPE tissue are the base for the EPICUP test developed by Grupo Ferrer Internacional SA (Barcelona, Spain). This primary tumor type classifier presents an invaluable tool for improving the diagnosis of cancers of an unknown primary and the following choice of treatment (20).

OverC Multi-cancer Detection Blood Test (Burning Rock Biotech, Guangzhou, China) was designed to aid in the early detection of multiple cancers, including esophageal, liver, lung, ovarian, and pancreatic cancers (21). The test is intended for liquid biopsies and targets the circulating tumor DNA methylation.

The IVD tests listed above have received approval for use in the EU. However, there is a potential for the additional kits approved in the USA by the Food and Drug Administration to extend their reach to the European market.

CONCLUSION

The absence of definitive biomarkers in oncology poses a significant challenge in cancer research. The dynamic and responsive nature of epigenetic modifications, such as DNA methylation, makes them an attractive target and underlines their potential diagnostic and prognostic values. As indicated by the number of previously mentioned tests, the integration of novel methylation biomarkers into clinical practice has clearly begun and holds the promise to improve cancer diagnostic and treatment strategies in the foreseeable future.

Gene abbreviations

<i>ASTN1</i>	<i>Astrotactin 1</i>
<i>CCNA1</i>	<i>Cyclin A1</i>
<i>DLX1</i>	<i>Distal-Less Homeobox 1</i>
<i>FAM19A4</i>	<i>TAFA Chemokine Like Family Member 4</i>
<i>ITGA4</i>	<i>Integrin Subunit Alpha 4</i>
<i>MGMT</i>	<i>O-6-Methylguanine-DNA Methyltransferase</i>
<i>ONECUT2</i>	<i>One Cut Homeobox 2</i>
<i>PAX1</i>	<i>Paired Box 1</i>
<i>PITX2</i>	<i>Paired Like Homeodomain 2</i>
<i>PTGER4</i>	<i>Prostaglandin E Receptor 4</i>
<i>RXFP3</i>	<i>Relaxin Family Peptide Receptor 3</i>
<i>SDC2</i>	<i>Syndecan 2</i>
<i>SEPT9</i>	<i>Septin 9</i>
<i>SHOX2</i>	<i>Short Stature Homeobox 2</i>
<i>SOX17</i>	<i>Sex Determining Region Y-Box Transcription Factor 17</i>
<i>VIM</i>	<i>Vimentin</i>
<i>ZNF582</i>	<i>Zinc Finger Protein 582</i>
<i>ZNF671</i>	<i>Zinc Finger Protein 671</i>

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Table 1 Commercially available DNA methylation-based tests in oncology with CE-IVD certification

Test name	Methylation biomarker	Biological sample	Company	EU certification
Lung cancer				
Epi proLung BL Reflex Assay	SHOX2	Bronchial aspirate	Epigenomics AG, Germany	2010
Epi proLung	SHOX2, PTGER4	Plasma	Epigenomics AG, Germany	2017
PulmoSeek	100 biomarkers	Plasma	AnchorDx, Hong Kong	2022
Colorectal cancer				
Epi proColon	SEPT9	Plasma	Epigenomics AG, Germany	2011
EarlyTect Colon Cancer	SDC2	Stool	Genomictree, South Korea	2017
Glioblastoma				
therascreen MGMT Pyro Kit	MGMT	Tumor tissue	Qiagen, Germany	2012
Human MGMT Gene Methylation Detection Kit	MGMT	Tumor tissue	Xiamen Spacegen, China	2016
MGMT Methylation Detection Kit	MGMT	Tumor tissue	EntroGen, USA	2018
Cancers of Unknown Primary				
EPICUP	multiply CpG sites	Tumor tissue	Ferrer Internacional, Spain	2015
Cervical cancer				
QIAsure	FAM19A4/miR124-2	Cervical/vaginal specimen	Qiagen, Germany	2016
PAX1 DNA Detection Kit	PAX1	Cervical/oral scrapes	iStat Biomedical, Taiwan	2016
GynTect	ASTN1, DLX1, ITGA4, RXFP3, SOX17, ZNF671	Cervical smear	oncgnostic, Germany	2019
Oral cancer				
ZNF582 DNA Detection Kit	ZNF582	Cervical/oral scrapes	iStat Biomedical, Taiwan	2016
Bladder cancer				
Bladder EpiCheck	15 proprietary markers	Urine	Nucleix, Israel	2017
UriFind Bladder Cancer Detection Kit	ONECUT2, VIM	Urine	AnchorDx, Hong Kong	2020
Hepatocellular carcinoma				
HCCBloodTest	SEPT9	Plasma	Epigenomics AG, Germany	2018
Esophageal precancer				
EsoGuard	CCNA1, VIM	Brush cells	Lucid Diagnostics, USA	2021
Breast cancer				
therascreen PITX2 RGQ PCR Kit	PITX2	Tumor tissue	Qiagen, Germany	2018
Multiple cancer types				
OverC Multi-cancer Detection Blood Test	ctDNA markers	Plasma	Burning Rock Biotech, China	2022