CLINICAL AND LABORATORY STANDARDS INSTITUTE®

2nd Edition

CLSI MM23™

Molecular Diagnostic Methods for Solid Tumors (Nonhematologic Neoplasms)

CLSI MM23 covers the current state of molecular diagnostic methods intended for characterization of solid tumors and covers a range of clinical applications, including diagnosis, prognosis, therapeutic response prediction for available drugs and drugs still in clinical trials, monitoring, and presymptomatic and predisposition testing.

A guideline for global application developed through the Clinical and Laboratory Standards Institute consensus process.

CLSI MM23-Ed2
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Replaces CLSI MM23-Ed1

Molecular Diagnostic Methods for Solid Tumors (Nonhematologic Neoplasms)

Lawrence J. Jennings, MD, PhD, D(ABHI), FCAP
Jason Merker, MD, PhD
Haydar Celik, PhD
Fei Dong, MD
Jianli Dong, MD, PhD, FACMG
Julie Gastier-Foster, PhD, FACMG
George A. Green, IV, PhD
Susan J. Hsiao, MD, PhD
Cynthia L. Jackson, PhD
Robert F. Klees, PhD
Annette Leon, PhD, MS, FACMG, CGMBS, CCS
Ronald M. Przygodzki, MD
Honey V. Reddi, PhD, FACMG
Alexander Sartori
Elizabeth Sheppard, HT(ASCP), MBA
Nirupama Singh
Shalini Verma, MD
Richard Y. Wang, DO

Abstract

Clinical and Laboratory Standards Institute MM23—*Molecular Diagnostic Methods for Solid Tumors (Nonhematologic Neoplasms)* describes development and implementation of nucleic acid biomarker assays for accurate detection of somatic alterations, with applications to clinical decision-making for cancer patients with solid tumors. CLSI MM23 is intended for molecular diagnostic laboratory directors, industry laboratory professionals, health care professionals (including anatomic and clinical pathologists), manufacturers and developers, and regulatory and accreditation organizations. The methods and recommendations discussed in CLSI MM23 focus primarily on detection of tumor-specific (ie, somatic) genetic abnormalities that are acquired during tumorigenesis and that are distinct from normal variations in nonmalignant cells of the same tissue.

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Foreword

With the completion of the Human Genome Project and subsequent large-scale international cancer genomics projects, researchers have identified germline and somatic changes involved in many diverse aspects of tumor biology. Identification of genetic changes that drive neoplastic transformation of normal tissue, as well as progression to more advanced disease states, provides insight into tumor biology and associated therapies. Massively parallel sequencing technologies, also referred to as "next-generation sequencing" (NGS), have been rapidly adopted for detection of somatic variants in the medical laboratory to guide therapy selection, as well as to assist with prognostication, cancer diagnosis, and classification. The field has witnessed the development of validated predictive cancer biomarkers that are independent of the tumor of origin. Additionally, identification of somatic variants in molecular oncology is increasingly used as an inclusion or stratifying criteria in cancer clinical trials. It is essential that these new tests be useful for medical decision-making purposes and that their clinical validity and utility be evaluated as quickly and efficiently as possible.

Given ongoing advancements in molecular testing of tumor specimens, guidelines are needed to address the performance and reporting practices of such tests. CLSI MM23 covers the current state of molecular diagnostic techniques intended for characterization of solid tumors, as well as a range of clinical applications, including diagnosis, prognosis, monitoring of tumor burden, presymptomatic and predisposition testing, and therapeutic response prediction for both available drugs and drugs still in clinical trials. CLSI MM23 includes a brief discussion of heritable cancer syndromes and pharmacogenetics, which are covered in more depth in CLSI MM01[1] and CLSI MM19.[2] The rapid development of new molecular diagnostic techniques might render CLSI MM23 incomplete after its publication.

The methods and QC approaches described in CLSI MM23 are not absolute or immutable. They represent expert consensus recommendations presented by the document development committee and are intended for use by diagnostic laboratories. Such use is intended to facilitate interlaboratory comparisons of results and diagnostic interpretations and to ensure accuracy in diagnosis and tumor characterization.

Overview of Changes

CLSI MM23-Ed2 replaces CLSI MM23-Ed1, published in 2015. Several changes were made in this edition, including:

- Removing most of the discussion on heritable disease testing, except for a brief discussion of heritable cancer syndromes and pharmacogenetics for solid tumor therapies
- Adding cancer biomarkers and updating nomenclature introduced into clinical practice or late phase clinical trials since the previous edition was published
- Expanding the discussion of test development, with a focus on somatic, solid tumor testing
- Updating methods and technologies commonly used in the molecular pathology and genetics laboratory for solid tumor testing, with an emphasis on NGS methods
- Adding discussion of bioinformatics pipelines, computer and storage infrastructure, and related topics, with a focus on tumor NGS
- Revising considerations for preexamination, examination, and postexamination phases of testing, with an emphasis on molecular oncology testing
- Incorporating discussion of cell-free circulating tumor DNA assays and other liquid biopsy assays throughout the relevant subchapters
- Expanding discussion of quality systems, with a focus on molecular oncology testing

NOTE: The content of CLSI MM23 is supported by the CLSI consensus process and does not necessarily reflect the views of any single individual or organization.

KEY WORDS

biomarkers
cancer
cell-free DNA (cfDNA)
circulating tumor DNA (ctDNA)
companion diagnostic devices
genetics
genomics
laboratory-developed test (LDT)
liquid biopsy
methylation
next-generation sequencing (NGS)
oncology
solid tumor
somatic variants
test development

Chapter 1

Introduction

Molecular Diagnostic Methods for Solid Tumors (Nonhematologic Neoplasms)

1 Introduction

1.1 Scope

CLSI MM23 focuses on descriptions of various technologies and method selection for specific oncology applications. It describes the development and implementation of nucleic acid biomarker assays for accurate detection of somatic alterations, with applications for clinical decision-making in oncology. The methods and recommendations discussed focus primarily on detection of tumor-specific genetic abnormalities that are acquired during tumorigenesis and that are distinct from normal variations in nonmalignant cells of the same tissue. CLSI MM23 also includes new biomarkers described for a wide variety of tumors and newer approaches with multigene panels and complex data interpretations developed since the previous edition was published.

CLSI MM23 focuses on the underlying nucleic acid tumor markers and variants but does not examine cell-surface antigens, immunohistochemistry (IHC), or protein markers. CLSI MM23 focuses on neoplasms that are not hematopoietic or lymphoid. Other CLSI documents provide more detailed guidance on molecular testing for heritable genetics and specimen identification (CLSI MM01[1]), molecular hematopathology (CLSI MM05[3]), FISH (CLSI MM07[4]), microarrays (CLSI MM12[5]), and multiplex nucleic acid assays (CLSI MM17[6]). Although also covered in CLSI MM23, additional details on next-generation sequencing (NGS), including multigene DNA panels, RNA sequencing, and liquid biopsy, are covered in CLSI MM09.[7]

CLSI MM23 is intended for molecular diagnostic laboratory directors, industry laboratory professionals, health care professionals (including anatomic and clinical pathologists), manufacturers and developers, and regulatory and accreditation organizations.

1.2 Background

1.2.1 Tumorigenesis

Tumorigenesis is a multistep process involving multiple factors. The hallmarks of cancer, first proposed in 2000,[8] was expanded in 2011 to include eight hallmark capabilities and two enabling characteristics (see Figure 1).[9] Recently, additional emerging hallmarks and enabling characteristics have been proposed, including unlocking phenotypic plasticity, nonmutational epigenetic reprogramming, polymorphic microbiomes, and senescent cells.[10] It is anticipated that the understanding of tumorigenesis will continue to evolve with increasingly sophisticated experimental investigations.

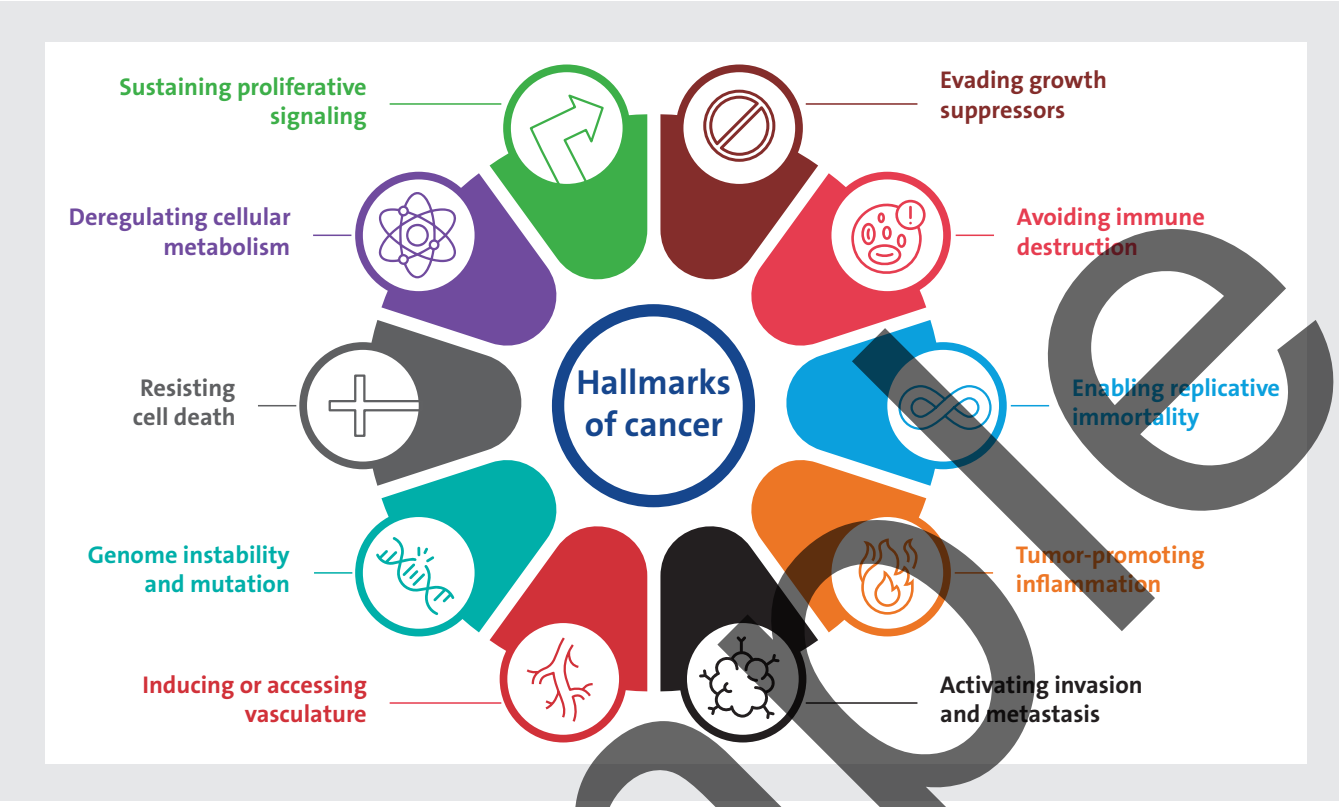

Figure 1. Hallmarks of Cancer.[9] (Reprinted from *Cell*, Vol. 144 / No. 5, Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation, pp. 646-674, Copyright ©2011, with permission from Elsevier.)

1.2.2 Cancer Genetics

Cancer is caused by variants or other genetic alterations in certain genes. Variants are inherited (germline) in a minority of cases, which can result in a heritable cancer predisposition syndrome. In > 90% of cancer cases, the variants or other genetic alterations are acquired (somatic) during the life of the individual. Variants are most commonly introduced by errors in DNA replication and repair. DNA damage also might be caused by environmental exposures, such as sunlight, tobacco, viruses, and other carcinogens. Development of solid tumors needs multiple genetic events. Although there are common hallmark features of cancer, many different genes and pathways might be perturbed to generate causal alterations for the same type of cancer, thus contributing to the significant genetic heterogeneity observed among the same tumor type (eg, lung adenocarcinoma).

Genes associated with cancer can be grouped into two categories[11]:

- **Oncogenes:** Proto-oncogenes encode proteins involved in promoting cell growth, division, and related processes. When these genes acquire an activating variant, they directly promote cell growth and survival and are consequently classified as oncogenes. Variation of a single allele is sufficient to contribute to cancer development; therefore, oncogenes have also been referred to as "dominant genes."[12] *BRAF, EGFR*, and *KRAS* are common, clinically relevant oncogenes.
- **Tumor suppressor genes:** These genes encode proteins involved in regulating cell growth and division. When these genes are inactivated, loss of normal regulation contributes to cancer development. Loss-of-function (LOF) variants in both alleles are needed to contribute to cancer development; thus, tumor suppressor genes are also called "recessive genes." *APC, BRCA1,* and *TP53* are common tumor suppressor genes examined in clinical practice of some advanced cancer types.[13-15]

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