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ACMG TECHNICAL STANDARD

Section E6.7-6.12 of the American College of Medical Genetics and Genomics (ACMG) Technical Laboratory Standards: Cytogenomic studies of acquired chromosomal abnormalities in solid tumors

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Clinical laboratory geneticists are encouraged to document the rationale for how a particular test was designed, its intended use and its performance specifications as well as whether or not it is in conformance with this technical standard. They also are advised to take notice of the date any particular technical standard was adopted, and to consider other relevant medical and scientific information that becomes available after that date. It also would be prudent to consider whether intellectual property interests may restrict the performance of certain tests and other procedures. Where individual authors are listed, the views expressed may not reflect those of authors' employers or affiliated institutions.

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ABSTRACT

Clinical cytogenomic studies of solid tumor samples are critical to the diagnosis, prognostication, and treatment selection for cancer patients. An overview of current cytogenomic techniques for solid tumor analysis is provided, including standards for sample preparation, clinical and technical considerations, and documentation of results. With the evolving technologies and their application in solid tumor analysis, these standards now include sequencing technology and optical genome mapping, in addition to the conventional cytogenomic methods, such as G-banded chromosome analysis, fluorescence in situ hybridization, and chromosomal microarray analysis. This updated Section E6.7-6.12 supersedes the previous Section E6.5-6.8 in Section E: Clinical Cytogenetics of the American College of Medical Genetics and Genomics Standards for Clinical Genetics Laboratories.
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6.7. Introduction

The genetic makeup of tumor cells includes vital information that not only describes the biology of the tumor but can also guide clinical patient care. Gross and submicroscopic chromosome alterations, including copy-number abnormalities (CNAs) and balanced rearrangements, are common oncogenic drivers in solid tumors and are also important clinical markers informing diagnosis and risk stratification and guiding treatment selection.[1-4] Cytogenomic studies comprise techniques that evaluate chromosome alterations, including established approaches such as G-banded chromosome analysis, fluorescence in situ hybridization (FISH), and chromosomal microarray analysis (CMA). They also include emerging technologies such as optical genome mapping (OGM), as well as the use of next-generation sequencing for detection of CNAs and balanced rearrangements/oncogenic gene fusions (Table 1).[5,6] The clinical laboratory practice of performing cytogenomic studies on patient samples in a clinically relevant time frame is critical to providing the best care to our patients. These standards provide structure and guidance to support laboratory geneticists in performing cytogenomic studies for solid tumors; however, comprehensive coverage of sequencing-based tumor testing is outside the scope of this document.

6.8. Methods

These technical laboratory standards were informed by a review of the literature and current guidelines. Resources consulted included PubMed, relevant American College of Medical Genetics and Genomics (ACMG) standards, National Comprehensive Cancer Network, and Children's Oncology Group guidelines, as well as current World Health Organization (WHO) guidelines. The workgroup members also used their expert opinion and empirical data to inform their recommendations. Conflicts of interest documentation for workgroup members is listed at the end of the paper. The ACMG Laboratory Quality Assurance Committee reviewed the document providing further input on the content, and a final draft was presented to the ACMG Board of Directors for review and approval to post on the ACMG website for member comment. Upon posting to the ACMG website, an email and link were sent to all ACMG members inviting participation in the 30-day open comment process. All members' comments and additional evidence received were assessed by the authors, and these recommendations were incorporated into the document as deemed appropriate. Member comments and author responses were reviewed by representatives of the ACMG Laboratory Quality Assurance Committee and the ACMG Board of Directors. The final document was approved for publication by the ACMG Board of Directors.

Table 1 Overview of cytogenomic assays

Comparison/Methodology	G-banded	FISH	CMA	Sequencing Panels		OGM
Analyte	Chromosome in dividing cells	DNA in Interphase nuclei and metaphase	DNA	DNA	RNA	DNA
Sample Type(s)	Fresh (Sterile)	Fresh, Frozen, Fixed	Fresh, Frozen, Fixed	Fresh, Frozen, Fixed	Fresh, Frozen, Fixed	Fresh, Frozen
Coverage	Whole genome	Targeted	Whole genome, Targeted (depending on assay design)	Whole genome, Targeted (depending on assay design)	Whole genome, Targeted (depending on assay design)	Whole genome
Distinction of individual cell clones	Yes	Yes	No	No	No	No
Throughput[a]	Low	Low	High	High	High	Low - Medium
Analytical sensitivity[a]	Low	Intermediate	Intermediate	High	High	Intermediate
Structural rearrangements	Yes	Yes	Unbalanced	Limited	Fusions	Yes
Copy-number abnormalities	Yes	Yes	Yes	Limited	No	Yes
Single-nucleotide variants	No	No	No	Yes	Limited	No

CMA, chromosomal microarray analysis; *FISH*, fluorescence in situ hybridization; *OGM*, optical genome mapping.

[a]These terms are relative and are not precisely defined.

6.9. General Considerations

6.9.1 Cytogenomic analysis of solid tumors is performed to detect and characterize chromosome alterations to support clinical care. This analysis may provide critical information for diagnosis, prognostication, and selection of therapy.[1-6] Cytogenomic studies of tumor tissues may be accomplished by G-banded chromosome analysis, FISH, CMA, OGM, sequencing, or a combination of these methodologies.

6.9.2 Study of tumor tissues will be influenced by several variables, including the amount of available tissue, whether the tissue is fresh, frozen, or fixed, the working differential diagnosis, and the available methods for testing.

6.9.3 The laboratory director and staff should be familiar with the recurrent cytogenetic and molecular aberrations associated with tumor types/subtypes and their clinical significance. Supplemental Tables 1 to 4 include clinically significant solid tumor chromosomal aberrations with known genes, clinical significance, and references.

6.9.4 Tumor processing, analytical variables, and turnaround time (TAT) should be determined by the laboratory based on the reason for referral, the sample(s) received, and the clinical application of the results (eg, selection of therapy). It is recommended that laboratories work with the oncologist and pathologist where possible to determine the method(s) to ascertain the clinically relevant genetic information, and the appropriate test utilization in the context of the patient's care.

6.9.5 The clinical significance of cytogenomic results must be interpreted within the context of the patient's pathologic and clinical findings. The presence of specific alterations or pertinent negative results should be communicated to the patient's care team as soon as feasible to contribute to timely clinical management.

6.9.6 A quality management plan spanning the pre-analytic, analytic, and post-analytic phases of testing is required. Specific metrics may include tracking of specimens and results, TAT, assay-specific metrics such as culture or hybridization failure rate, and correlation of results from different testing methods.

6.9.7 Cytogenomic studies may reveal germline and/or secondary findings. It is recommended that laboratories refer to their policies and procedures to address these situations.

6.10. Sample Collection and Processing

6.10.1. Sample collection

6.10.1.1 Sample collection and preparation are critical to the success of any assay. For example, G-banded chromosome analysis requires that fresh tissue be collected in a sterile manner. Other cytogenetic assays such as FISH or CMA may be successful with formalin-fixed paraffin-embedded (FFPE) tissue preparation which allows for optimal histopathologic evaluation. Frozen tissue may also be a viable option, particularly for bony specimens that are often decalcified in conventional pathology workflows and may have nucleic acid degradation. Evaluation of frozen tissue by a pathologist can be done by frozen section. For DNA or RNA based assays, nucleic acid isolation from fresh, frozen or FFPE tissue is preferred over cultured cells to avoid cultural artifacts.

6.10.1.2 Review of the tissue by a pathologist is recommended to identify and mark optimal areas of tumor for testing, specify the percentage of tumor in an area, and/or identify areas of necrosis or stromal tissue to avoid. In addition, evaluation of tumor cellularity in the selected sample is an important pre-analytic quality indicator that may subsequently influence analytic algorithms and clinical interpretation.

6.10.1.3 For G-banded chromosome studies, the laboratory should request a sample size of at least 0.5 to 1 cm^3 to be processed for cell cultures or other genomic assays. In cases with limited tissue (<0.5 cm^3), the laboratory may attempt to acquire as much as can be provided without compromising the histopathologic evaluation of the tumor. If the sample size precludes cell culture and G-banded chromosome evaluation, touch imprint preparations (TPs), cytospin preparations, or paraffin-embedded tissue sections may be used for FISH analysis and/or nucleic acid isolation for CMA or sequencing analysis. Because G-banded analysis is a technique that is established and available in clinical cytogenetic laboratories across the world, procuring viable tissue to culture tumor cells may be crucial for diagnosis. Depending on specimen size and availability of fresh tissue, short-term cultures may be initiated for G-banded chromosome analysis, which may lead, in some instances, to the detection of clinically relevant chromosomal abnormalities, therefore establishing the final diagnosis and avoiding additional testing. Examples include the detection of t(X;17)(p11.2;q35) in renal cell carcinoma with microphthalmia transcription factor translocation or t(12;15)(p13;q26.1) in congenital mesoblastic nephroma.[7,8]

6.10.1.4 Fresh tumor should be transported to the laboratory as soon as possible for immediate processing, including tissue culture for G-banded chromosome analysis, creating TPs for FISH analysis.

6.10.1.5 The fresh tumor sample is inspected, and details of the sample size, color, and other descriptive attributes are recorded.

6.10.1.6 Triage of the tumor sample as soon as possible is recommended to optimize a successful result from testing. Determining which cytogenomic methods will be used should be based upon the information contained in the requisition (including clinical information), laboratory policies, and the goals of testing.

6.10.2. Sample processing for fresh tissue culture

6.10.2.1 For tissue cultures, treatment with antibiotic- and/or antifungal-containing media may be warranted, particularly for tissues from a body region with high concentrations of bacteria (eg, tonsils and gut).

6.10.2.2 Disaggregation of solid tumor samples for tissue culture is required. Either mechanical and/or enzymatic methods may be used. For some tumor types, different growth characteristics can be seen with exposure vs no exposure to collagenase. If sufficient material is available, cultures should be initiated with and without enzyme exposure to address potential growth challenges.

6.10.2.3 Culture methods, culture medium, and culture conditions are chosen to best support cell growth in the type of tumor received. The diagnosis and histopathology of a tumor can be helpful in determining culture and harvest methods. Different cell types can be expected to respond differently to growth medium, harvest method, etc (Supplemental Table 5). If the diagnosis is unknown at the time of culture initiation, it can be helpful to know whether the pathologist would classify the tumor as a "small round cell tumor" (SRCT). SRCTs can be successfully grown in suspension, whereas non-SRCTs are best grown with monolayer culture methods (flask or coverslip), in situ coverslip cultures are recommended. Most SRCTs will also grow in monolayer culture. If adequate tissue is obtained, both culture types should be initiated for SRCTs, with duplicate cultures established whenever possible. Short culture durations are preferred to capture early dividing tumor cells and to avoid growth of normal tissues. It is recommended that monolayer cultures not extend longer than 3 to 7 days due to overgrowth of normal cells. Direct or overnight suspension cultures may also be used in conjunction with longer-term cultures to capture actively dividing tumor cells.

6.10.2.4 Experience with solid tumor culture will provide the laboratory with information regarding optimal growth conditions and harvest methods for different tumor types within each laboratory.

a) It is recommended that each laboratory maintain a database documenting how the different tumor types have grown, and which culture and harvest conditions yielded abnormal clones. This database can be used to optimize processing and harvesting methods.
b) Frequent (daily) observation of cells in culture is needed to determine cell growth rate and time to harvest. Time of harvest can be determined by laboratory policy and at the discretion of the technologists and laboratory directors based on the sample and differential diagnosis.
c) Conditions used for cell harvest will vary among tissue types, eg, type of mitotic inhibitor, concentration, and duration of exposure, and should be established by each laboratory.

6.11. Analytical methods

6.11.1. Conventional G-banded chromosome analysis

G-banded analysis of metaphase chromosomes from solid tumor specimens provides a comprehensive view of the entire genome at a single-cell level, albeit at a low resolution. Moreover, it allows the detection of chromosomal aberrations that may inform a specific diagnosis and potentially provide prognostic and therapeutic information, especially when the tumor type is unknown. Single-cell analysis also provides information about clonal heterogeneity and co-occurrence of genomic abnormalities in different clones.

6.11.1.1 Cell selection: Analysis of metaphase chromosomes ideally includes cells with both good and poor chromosome morphology when attempting to identify an abnormal clone. Once identified, clonal cells are karyotyped and imaged to provide the most accurate breakpoint assignments.

Cells that cannot be completely analyzed because of poor morphology should be scanned for obvious structurally abnormal chromosomes and abnormal chromosome counts. If feasible, metaphase FISH analysis from previous G-banded slides may further assist in resolving structural abnormalities.

Clonal abnormalities should be documented from 2 independent cultures, if possible, to ensure that in vitro culture artifacts are not mistakenly identified as a clinically significant abnormality. If only normal results are obtained from long-term in situ cultures, caution should be exercised, and the report should include a note that the negative results may be derived from the outgrowth of normal stromal cells.

6.11.1.2 Analytic standards

a) Analysis
   i) Analyze 20 metaphase cells and/or a sufficient number of cells to characterize all abnormal clones and subclones.
   ii) If all cells show a complex karyotype, analyze at least 10 cells.
   iii) For a recurrent or metastatic sample, additional cells may be scored for a specific abnormality that was identified in the primary diagnostic sample.
      (1) In addition to looking for the known clonal aberration(s) from the diagnostic study, analysis of a sample after therapy is performed with awareness of the possibility of new aberrations signifying clonal evolution and/or the appearance of a new clonal process.
      (2) FISH analysis may be considered in lieu of G-banded chromosomal analysis for diagnoses characterized by an abnormality for which FISH testing is available.
b) Documentation
   i) For abnormal cells:
      (1) If only 1 abnormal clone is present: 2 karyotypes.
      (2) If more than 1 related abnormal clone is present: 2 karyotypes of the stemline and 1 of each sideline.
      (3) If unrelated clones are present: 2 karyotypes for each stemline and 1 for each associated pertinent sideline.

ii) For normal cells:
(1) If only normal cells are present: 2 karyotypes.
(2) If normal and abnormal cells are present: one karyotype of a normal cell plus karyotypes for abnormal clone(s) as described above.

6.11.2. FISH analysis

6.11.2.1 FISH analysis may be used for primary, supplementary, or follow-up evaluation.

a) As a primary method for tumor evaluation, FISH is useful when (1) fresh tumor tissue is not available, (2) rapid diagnostic information is needed to narrow the differential diagnosis or planning of therapy, and (3) gene amplification or rearrangement for diagnostic or prognostic and/or therapeutic purposes is to be determined.
b) FISH may be used as an adjunct to the initial G-banded chromosome analysis, CMA, OGM, or sequencing, for example, to (1) confirm a specific molecular event, eg, gene rearrangement or fusion, (2) assess gene copy number, and (3) clarify level of clonality. It can also be added when no metaphase cells are obtained by culture of tumor material or G-banded chromosome analysis yields a normal result. Follow-up FISH studies may be indicated to assess recurrent disease or disease progression, and/or to differentiate recurrence of a tumor from a new disease process.

6.11.2.2 Characterization of FISH aberrations and signal patterns in diagnostic samples are useful for future monitoring of disease. Documentation of a unique FISH signal pattern at diagnosis can help establish a baseline for comparison in follow-up testing.

6.11.2.3 Sample types that may be used for FISH include the following:

a) Paraffin-embedded tissue[9]
i) FFPE tissue is acceptable for FISH analysis. Tissues preserved in Bouin's or B5 fixative, or decalcified with strong acids are not suitable for FISH.
ii) Tumor sections cut to a validated thickness and mounted on positively charged organosilane-coated (silanized) slides work well. The cytogenetics laboratory may request several unstained sections, for potential repeat studies, and 1 hematoxylin and eosin (H&E)-stained sequentially cut section from the submitting laboratory.
iii) Before scoring a paraffin-embedded FISH slide it is crucial that a pathologist review the H&E-stained or immunohistochemistry (IHC)-positive slide to delineate the region of tumor cells to be scored because it can be difficult to differentiate normal cells from malignant cells using only 4',6-diamidino-2-phenylindole counterstain. The pathologist should make a clear boundary that can be overlaid onto an unstained slide. If needed, the technologist may seek clarification on where the malignant cells of interest are located on the slide before FISH scoring.

b) Cultured or direct-harvest tumor cells

Such preparations have multiple uses for both interphase and metaphase FISH evaluation including confirmation and clarification of suspected chromosome alterations or characterization of an apparently abnormal clone. Metaphase FISH evaluation may help clarify specific chromosome rearrangements.

c) TPs
i) A pathologist should be involved in selecting the tissue for TPs to ensure that the tumor is well represented.
ii) TPs are helpful when tissue architecture is not crucial.
iii) TPs can be made by lightly touching the piece of tumor to a glass slide without smearing, followed by air drying.

d) Cytospin preparations

Cytospin preparations are useful for concentration of samples with very low cellularity, eg, cerebrospinal fluid or urine.

e) Fresh-frozen tumor tissues
i) A frozen section performed for histologic evaluation of the sample by a pathologist will ensure that the tumor is well represented.
ii) Such tissues may also be useful in sequential analysis of tumors or in evaluation of archived samples.

6.11.2.4 Probe validation, analysis, quality assurance, and documentation of FISH results should be in accordance with Section E9 of these Technical Standards for Clinical Genetics Laboratories.[10,11]

6.11.3. CMA

6.11.3.1 CMA can provide valuable information to supplement that of G-banded chromosome and FISH analyses. In tumors where CNA, instead of gene rearrangements, play important roles in disease management, for example, neuroblastoma, Wilms tumor, and most central nervous system tumors, CMA may be the primary method for tumor evaluation. Isolated tumor DNA hybridized to whole-genome copy number and/or single-nucleotide polymorphism microarrays allows detection of loss, gain, and amplification of regions of DNA, which may not otherwise be detected by conventional cytogenetic methods. Single-nucleotide polymorphism probes allow for detection of regions with copy-neutral loss of heterozygosity, which may harbor critical tumor genes.[12]

6.11.3.2 Sample types that may be used for CMA analysis include (1) fresh tumor tissue, (2) FFPE tissue, (3) frozen tumor, and (4) cultured cells. If clinically indicated, metaphase preparations of cultured cells from fresh tumor tissue can assist in resolving unbalanced structural rearrangements detected by CMA. It is important to utilize appropriate CMA methodologies that can accommodate FFPE tumor tissue samples or fixed cultured cells.[4]

a) Fresh tumor tissue

A small piece of identified tumor is transferred to the laboratory as soon as possible for DNA isolation. During sampling, in cases of heterogenous tumors with areas of necrosis, normal tissue or prominent stroma, tumor dissection is essential to ensure that the extracted DNA is derived from the tumor tissue.

b) Paraffin-embedded tumor

A pathologist reviews the H&E-stained section of the tumor to identify an area of high tumor cellularity for DNA isolation.

c) Fresh-frozen tumor

Frozen stored tumor provides high-quality DNA for CMA. A pathologist's review of the corresponding H&E-stained slides from the frozen stored tumor can assure that the frozen sample contains adequate tumor.

d) Cultured tumor cells

Cultured tumor cells fixed in Carnoy's fixative may be used for DNA isolation for CMA. An early decision to use cells for CMA is best to minimize growth of normal stromal tissue.

6.11.3.3 Analysis and documentation of CMA studies should be in accordance with Section E10 of these Technical Standards for Clinical Genetics Laboratories.[12]

6.11.4. OGM

6.11.4.1 OGM is a cytogenomic tool that enables a genome-wide analysis of CNA, balanced rearrangements (translocations, inversions, and insertions), and complex rearrangements with higher resolution in a single assay compared with conventional methods. This method requires very-high-molecular-weight DNA.[13]

6.11.4.2 Sample types that may be used for OGM analysis include (1) fresh tumor tissue, (2) frozen tumor, and (3) cultured cells.

a) Fresh tumor tissue

For homogeneous tumors, fresh tissue is the ideal sample type and can be procured from surgical pathology for OGM analysis. The sample (~15 mg tissue) should be immediately stored at 4 °C to maintain intact cells for very-high-molecular-weight DNA isolation. For heterogeneous tumors, tissue can be cut from multiple sites to ensure representation from different portions of the tumor before DNA isolation.

b) Fresh-frozen tumor

The fresh-frozen tumor yields high-quality and high-molecular-weight DNA for OGM analysis. The fresh tumor tissue is transferred to cryovials (~30 mg viable tumor tissue) and either flash-frozen in liquid nitrogen or transferred to −80 °C for storage until DNA isolation.

c) Cultured tumor cells

Tumor cells that have been placed into culture may be used for isolating high-molecular-weight DNA using at least 1 million cells for OGM analysis. An early decision to use cells for OGM is best to minimize the growth of normal tissue components.

6.11.5. Sequencing

6.11.5.1 Sequencing technologies, including Sanger, NGS, and long-read methodologies, are powerful tools that can evaluate multiple types of genetic alterations, including single-nucleotide variants (SNVs), CNA, and rearrangements. RNA sequencing is an efficient method for evaluating gene fusions, whereas DNA sequencing can evaluate SNVs, CNA, and fusions depending on the assay design.[14] Long-read sequencing, which requires fresh or frozen tissue, may be used clinically for the identification of large and/or complex structural rearrangements and methylation status.

6.11.5.2 Fresh, frozen or FFPE tissues are acceptable sample types for sequencing assays. As discussed above, FFPE tissues are typically readily available in clinical pathology laboratories and provide a convenient source of both DNA and RNA. It is important that the assay of choice be specifically designed to accommodate the shorter fragments and relative damage associated with FFPE tissues, both in the library preparation steps and within the analytic pipelines. Tissues preserved in B5 fixative or decalcified are not suitable for most sequencing chemistries.

Cell-free DNA in body fluids can also serve as a source of tumor DNA, particularly for inoperative tumors or those with diffuse growth patterns.

6.11.5.3 Practical considerations: when deciding whether a sequencing assay is appropriate for evaluating the patient's diagnosis, prognosis, or therapeutic options, each of the following must be assessed:

a) The assay is suitable for the available material (eg, FFPE).
b) The assay evaluates the alteration type relevant to the patient's diagnosis (eg, SNVs, CNA, and/or gene fusion).
c) The assay sensitivity is sufficient to obtain a result given the estimated tumor cellularity. Note that a single assay may have a different sensitivity for different alteration types (for example, it may be highly sensitive for SNVs but have a lower sensitivity for CNA).
d) For targeted panels, take careful note of the assay design with respect to targeted regions. For example, some designs do not include all exons of a gene for evaluation of rearrangements or may exclude untranslated regions.
e) For RNA panels evaluating fusions, note whether the assay design allows for the detection of any fusion associated with a targeted gene (for example, *NTRK3* with any gene partner). Alternatively, some RNA panels may only assess known fusion pairings, for example, allowing for the detection of the canonical *ETV6::NTRK3* fusion, but would not be expected to identify *NTRK3* fused to a novel fusion partner. RNA panels also cannot identify fusions that are not transcribed.

6.11.5.4 Analysis and documentation should be in accordance with the Technical Standards for the interpretation and reporting of CNA,[12] SNV,[2,15,16] and gene fusions[17] in cancer.

6.12. TAT and Reporting

6.12.1. TAT

6.12.1.1 TAT should be appropriate for the intended purpose of the test. The laboratory should have a written policy for TAT and when to prioritize based on the clinical application (with respect to each other and with respect to other sample types) such that the genetic information provided can be used for appropriate and timely clinical management. The laboratory will also monitor the TAT for continuous quality improvement.

6.12.1.2 Because of the multiplicity of tumor types and the different tumor growth characteristics in culture, TATs will vary. Ideally, the final report for each tumor is available as soon as possible given such factors.

6.12.2. Reporting

6.12.2.1 The most recent edition of the International System for Human Cytogenetic Nomenclature (ISCN) and Human Genome Variation Society (HGVS) should be used to report the chromosomal, FISH, CMA, and sequencing results.[18,19]

6.12.2.2 Preliminary verbal reports may be appropriate for some cases and should be documented appropriately.

6.12.2.3 If an aberration is suspected to be germline, analysis of uninvolved blood, buccal, or skin/tissue sample is recommended to clarify the germline vs somatic nature of the aberration so that genetic counseling may be recommended as appropriate.

6.12.2.4 The final report(s) for tumor samples should contain the following information:

1) Patient identification using 2 unique identifiers
2) Referring physician name
3) Sample information (type, dates of collection and receipt, date of report)
4) Reason for referral or suspected diagnosis
5) ISCN or HGVS nomenclature as appropriate
6) Cells analyzed (both normal and abnormal) when applicable
7) Narrative description of the aberrations observed. The report should correlate the results of all assays performed on the same tissue. The interpretation will correlate the genetic testing results with the histopathology report and patient-specific clinical information. Discussion can include the clinical significance of the results for the diagnosis, prognosis, and/or therapeutic management of the patient with reference to current literature.
8) References may be included to support the interpretation and to provide helpful information for the health care professional.

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Conflict of Interest

All members of this workgroup are directors of clinical laboratories that offer cytogenomic testing or molecular diagnostic services to patients with solid tumors.

Additional Information

The online version of this article (https://doi.org/10.1016/j.gim.2024.101070) contains supplemental material, which is available to authorized users.

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Supplementary Information

Section E6.7-6.12 of the ACMG Technical Laboratory Standards: Cytogenomic studies of acquired chromosomal abnormalities in solid tumors

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Supplemental Table 1. Central nervous system tumors with diagnostic or clinically significant chromosome aberrations				
Tumor	**Chromosome aberrations/location**	**Genes involved**	**Clinical significance***	**References (PMID)**
Adult-type diffuse gliomas				
Astrocytoma, *IDH*-mutant	Lack of 1p/19q co-deletion		D	WHO 5th
	TP53 deletion, 17p13.1		D	WHO 5th
	ATRX deletion, Xq21.1		D	WHO 5th
	CDKN2A and/or *CDKN2B* homozygous deletion, 9p21.3	*CDKN2A/B*	P	WHO 5th
	RB1 homozygous deletion, 13q14.2	*RB1*	P	31996992
	CDK4 amplification, 12q14.1	*CDK4*	P	31996992
	MYCN amplification, 2p24.3	*MYCN*	P	31996992
	PDGFRA amplification, 4q12	*PDGFRA*	P	31996992
Oligodendroglioma, *IDH*-mutant and 1p/19q co-deleted	1p/19q whole-arm co-deletion		D	WHO 5th
	Homozygous deletion of *CDKN2A*, 9p21.3		D, P	WHO 5th, 31832685
	Polysome 1q and 19p		P	19808867, 22710961, 31140557
Glioblastoma, *IDH*-wildtype	+7/-10		D	WHO 5th
	EGFR amplification, 7p11.2	*EGFR*	D, P	WHO 5th
	PDGFRA (4q12), MET (7q31.2), FGFR3 (4p16.3), MYC (8q24.2), CDK4 (12q14.1), CDK6 (7q21.2), GLI1 (12q13.3), MDM2 (12q15) amplification	*PDGFRA, MET, FGFR3, MYC, CDK4/6, GLI1, MDM2*		3335011, 24120142
	Deletion of *CDKN2A/B (9p21.3), RB1 (13q14.2), PTEN (10q23.3), TP53 (17p13.1)*	*CDKN2A/B, RB1, PTEN, TP53*		24120142
	Gene fusion of *EGFR (7p11.2), MET (7q31.2), FGFR3 (4p16.3), NTRK1/2/3 (*1q23.1/9q21.3/15q25.3)	*EGFR, MET, FGFR3, NTRK1/2/3*		WHO 5th
Pediatric type diffuse low-grade glioma				
Diffuse astrocytoma, *MYB*- or *MYBL1*-altered	*MYB* (6q23.3) or *MYBL1* (8q13.1) altered	*MYB::PCDHGA1, MYB::MMP16, MYB::MAML2, most common*	D	23583981, 26810070, 31563982, 31595312
Angiocentric glioma	*MYB* rearrangement or amplification, 6q23.3	*MYB::QKI* (majority), *MYB::PCDHGA1; MYB::ESR1*	D	WHO 5th, 21046410
Pediatric type diffuse high-grade glioma				
Diffuse midline glioma, H3 K27-altered	*TP53* deletion, 17p13.1	*TP53*	T	31481512
	PDGFRA amplification, 4q12	*PDGFRA*		22389665
Diffuse pediatric-type high-grade glioma, H3-wildtype and IDH-wildtype	*MYCN* amplification, 2p24.3	*MYCN*	D, P	WHO 5th, 28401334
	PDGFRA (4q12), EGFR (7p11.2) amplification	*PDGFRA, EGFR*	D	28401334
	TP53 (17p13.1), NF1 (17q11.2) deletion	*TP53, NF1*	D	28966033

Infant-type hemispheric glioma	Fusion of *NTRK1/2/3 (1q23.1/9q21.3/15q25.3), ROS1 (6q22.1), ALK (2p23.2),* or *MET (7q31.2)*	*NTRK1/2/3, ROS1, ALK,* or *MET*	D, T	WHO 5th
Circumscribed astrocytic gliomas				
Pilocytic astrocytoma	7q34 duplication	*KIAA1549::BRAF*	D	WHO 5th, 18974108, 19016743
	BRAF fusions with partners other than *KIAA1549*, 7q34	*BRAF*	D	29141672
	FGFR1 fusions (especially *FGFR1::TACC1*), 8p11.2	*FGFR1::TACC1*		23817572, 31729570
	NTRK family fusions	*NTRK* family		23817572, 32082673
	NF1 deletion*, 17q11.2*	*NF1* (often germline)	D	10931370, 23222849
High-grade astrocytoma with piloid features	*NF1* deletion*, 17q11.2*	*NF1*	D	WHO 5th
	KIAA1549::BRAF fusion*, 7q34*	*KIAA1549::BRAF*	D	WHO 5th
	FGFR1::TACC1 fusion*, 8p11.2*	*FGFR1::TACC1*	D	WHO 5th
	CDKN2A/B homozygous deletion, 9p21.3	*CDKN2A/B*	D	29564591
	CDK4 amplification, 12q14.1	*CDK4*	D	29564591
	ATRX deletion, Xq21.1	*ATRX*	D	29564591
Pleomorphic xanthoastrocytoma	Homozygous *CDKN2A* and/or *CDKN2B* deletion, 9p21.3	*CDKN2A/B*	D	WHO 5th
Subependymal giant cell astrocytoma	Biallelic inactivation of the *TSC1* (9q34.1) or *TSC2* (16p13.3) gene (Tuberous sclerosis), with the second hit frequently observed as deletion or loss of heterozygosity	*TSC1, TSC2*	D	9403714, 9007104, 29221145
Astroblastoma, *MN1*-altered	*MN1* fusion, 22q12.1	*MN1::BEND2, MN1::CXXC5*	D	WHO 5th, 28960623, 31111274
	CDKN2A homozygous deletion, 9p21.3	*CDKN2A*		28960623, 30876455
Ependymoma				
Supratentorial ependymoma, *ZFTA* fusion-positive	*ZFTA* fusion, 11q13.1	*ZFTA::RELA*; most common	D	24553141
	Secondary *CDKN2A* homozygous deletion, 9p21.3	*CDKN2A*	P	32514758
Supratentorial ependymoma, *YAP1* fusion-positive	*YAP1* fusion; 11q22.1	*YAP1::MAMLD1*, most common	D	WHO 5th
Spinal ependymoma, *MYCN*-amplified	*MYCN* amplification, 2p24.3	*MYCN*	D,P	WHO 5th, 31414211
	Secondary loss of chromosome 10, deletion of chromosome 11q			
Spinal ependymoma (*MYCN* amplification absent)	Loss of chromosome 22q, 22q12.2	*NF2*		WHO 5th, 35307892

Posterior Fossa-A ependymoma	Chromosome 1q gain or 6q loss			35307892
Posterior Fossa-B ependymoma	Loss of chromosome 6, 22q and gain of chromosome 18 (over 50%) and loss of chromosome 10, 17 and gain of chromosomes 1q, 5 and 8 (under 50%)			35307892
	Loss of chromosome 13q		P	
Myxopapillary ependymoma	Loss of chromosomes 1, 10 and 22, gain of chromosome 7, concurrent gain or 9 and 18			35307892, 22516549
Subependymoma	Loss of chromosome 6		P	35307892
	Loss of chromosome 19			30053291
Meningioma				
Meningioma	Homozygous *CDKN2A* and/or *CDKN2B* deletion, 9p21.3	*CDKN2A/B*	P	WHO 5th
	Deletion 22q12.2	*NF2*		WHO 5th
	Losses on chromosome 1p, 6p/q, 10q, 14q, and 18p/q, and (less frequently) losses on 2p/q, 3p, 4p/q, 7p, and 8p/q,		P	WHO 5th; 20682713
Chordoid meningioma	Deletion of chromosome 2p			WHO 5th, 30382370
Angiomatous, microcystic, and metaplastic meningiomas	Gain of chromosome 5			WHO 5th
Medulloblastoma				
SHH-activated SHH-1	*GLI1,* 12q13.3 or *GLI2* amplification, 2q14.2	*GLI1* or *GLI2*		WHO 5th, 28545823
SHH-activated SHH-2	Loss of chromosome 9q, 9q22.32 and 10q, 10q24.32	*PTCH1* and *SUFU*		WHO 5th, 28545823
SHH-activated SHH3 and *TP53*-mutant	*MYCN* amplification, *2p24.3; GLI2* amplification, 2q14.2; 17p LOH or deletion, 17p13.1, chromothripsis	*MYCN*, *GLI2*, *TP53*	P,D	WHO 5th, 28545823
SHH-activatedSHH3 and *TP53*-wildtype	Chromosome 9p gain, 9q loss; *PPM1D* amplification, 17q23.2			WHO 5th, 28545823
SHH-activated SHH-4	Gains of chromosome 3q, loss of chromosomes 9q, 10q and 14q			WHO 5th, 28545823
WNT	Loss of chromosome 6		D	WHO 5th, 28545823
Non-WNT/non-SHH				
group 3	*MYCN* amplification*, 2p24.3,* isodicentric 17q [(idic(17)(p11.2)]	*MYCN*	P	WHO 5th
group 4	chromosome 7 gain, 8 loss, 11 loss, and 17 gain		P	WHO 5th
Choroid Plexus				
Choroid Plexus Papilloma	Gains of chromosome 7, 9, 12 and 20 (over 50%); less frequently chromosomes 8, 11, 15q, 18 and 19. Infrequent losses		D	25575132

Atypical Choroid Plexus Papilloma	Gains of chromosomes 7, 8, 9, 12 and 20 (50% or over) less frequently gains of chromosome 11. No recurrent chromosome losses		D	25575132
Choroid Plexus Carcinoma	Deletions of chromosomes 3, 11p, 6, 11q, 16p, 22q, 16q, 17p, 5q, 8p, 13q, 15q, 5p, 9, 10p, 18 (over 50%) and 8q, 10q, 17q, and 19p (under 50%).		D	25575132

*D: diagnostic; P: prognostic; T: therapeutic

Supplemental Table 2. Genitourinary tumors with diagnostic or clinically significant chromosome aberrations				
Tumor	**Chromosomal Aberrations**	**Genes Involved**	**Clinical Significance***	**References (PMID)**
RENAL				
Renal cell tumors (RCT)				
Clear cell renal cell carcinoma (ccRCC)	3p deletion	*VHL, BAP1*	D	WHO 5th edition, 2022; PMID: 32434132
	9p loss		P	
Multilocular cystic renal neoplasm of low malignant potential	loss of 3 or 3p	*VHL*	D	WHO 5th edition, 2022
Clear cell papillary renal cell carcinoma	lack of 3p loss	NOT *TSC1, TSC2, MTOR* or *ELOC*		WHO 5th edition, 2022
Papillary renal cell carcinoma	gain 7 and 17, loss Y	*MET*	D	WHO 5th edition, 2022; PMID: 32434132
Chromophobe RCC	hypodiploid due to loss of 1, 2, 6, 10, 13, 17, 21		D	WHO 5th edition, 2022; PMID: 32434132
Oncocytoma	loss 1q, loss Y		D	PMID: 32434132
TFE3-rearranged renal cell carcinoma (RCC)	rea Xp11.23; t(X;1)(p11.2;q21), t(X;17)(p11.2;q25), t(X;1)(p11.2;p34)	*TFE3*::var (most common *PRCC, ASPSCR1* (*ASPL*), *SFPQ*; also *CLTC, PARP14, RBM10, NONO, MED15, DVL2, KAT6A, NEAT1, MATR3, FUBP1, EWSR1*	D, P	WHO 5th edition, 2022, PMID: 35980471, 34704642
TFEB-altered RCC	rea 6p21.1; t(6;11)(p21;q12)	*TFEB*::var (most common *MALAT1*; also *COL21A1, CADM2, EWSR1, PPP1R10, KHDRBS2, ACTB, CLTC, NEAT1*); *TFEB* amp	D, P	WHO 5th edition, 2022, PMID: 26536169, 33208882
	6p21 amplification including TFEB			
ELOC (formerly TCEB1)-mutated RCC	8q21	hotspot mutations in *ELOC*	D	WHO 5th edition, 2022
Fumarate hydratase-deficient RCC	LOH or loss 1p/1q, 13q, 18; gain 2, 16, 17	germline mutation *FH*; somatic mutations *FH*. *NF2, FAT1, PTPRT, EP300*	D, P	WHO 5th edition, 2022, PMID: 35288096

Succinate dehydrogenase-deficient RCC	LOH or loss 1p (SDHB); gain 1q	germline mutation *SDHB*; less commonly *SDHC*, *SDHA*, *SDHD*; somatic mutations uncommon		WHO 5th edition, 2022, PMID: 35288096
ALK-rearranged RCC	rea 2p23	*ALK*::var (commonly *VCL* in sickle cell trait carriers or *TPM3*)	D, P	WHO 5th edition, 2022, PMID: 36370168, 34704642
SMARCB1-deficient renal medullary carcinoma	rea 22q11.23 or loss 22q11.2	SMARCB1::var (MALAT1, CAPN2, RORA, MAML2)	D, P	WHO 5th edition, 2022, PMID: 26433572
Metanephric tumors (adenoma/adenofibroma/ stromal)	t(9;15)(p24;q24	somatic *BRAF*; *KANK1*::*NTRK3*	D	WHO 5th edition, 2022
PROSTATE				
Glandular neoplasms of the prostate				
Intraductal carcinoma of the prostate (IDC-P)	Loss 10q23.31 *PTEN*, 16q22.1 *CDH1*, and 16q23.1 *BCAR1*	*PTEN*, *CDH1* and *BCAR1*	P, D	WHO 5th edition, 2022; PMID: 29295717
	Gain 8q24.21 *MYC*	*MYC*	P, D	WHO 5th edition, 2022; PMID: 29295717
	rea 21q22.2 *ERG*	*ERG*::var (majority of cases of IDC-P)	D	WHO 5th edition, 2022; PMID: 20220513
	Loss 13q13.1 *BRCA2* (biallele)	*BRCA2*	D	WHO 5th edition, 2022; PMID: 33626496
Prostatic acinar adenocarcinoma	Loss 10q23.31 *PTEN* Loss 17p13.1 *TP53* (inactivation)	*PTEN* *TP53*	P	WHO 5th edition 2022; PMID: 22705054, 29029453, 31359337, 31502941, 32129857, 31411988, 22684219
	rea 21q22.3 *TMPRSS2* (ETS transcription factors rearrangements)	*TMPRSS2*::var (e.g., *TMPRSS2::ERG*)	P, D	WHO 5th edition, 2022; PMID: 30082453
	Gain 8q24 *MYC*	*MYC*	P	WHO 5th edition, 2022
Prostatic ductal adenocarcinoma	rea 21q22.2 *ERG*		P, D	WHO 5th edition, 2022; PMID: 19151660

Squamous neoplasms of the prostate				
Adenosquamous carcinoma of the prostate	t(21:21)(q22.2;q22.3) t(3;7)(q27.1;q34)	*TMPRSS2::ERG* fusion *FAM131A::BRAF* fusion	P, D	WHO 5th edition, 2022; PMID: 32639612, 31882336
Squamous cell carcinoma of the prostate	t(21:21)(q22.2;q22.3)	*TMPRSS2::ERG* fusion	D	WHO 5th edition, 2022; PMID: 29629426, 31882336, 32628337
Adenoid cystic (basal cell) carcinoma of the prostate	t(6;9)(q23.3;p23-p22.3)	*MYB::NFIB* fusion	D	PMID: 31189999, 26089205
BLADDER				
Urothelial cell carcinoma (transitional cell carcinoma)				
Urothelial carcinoma in situ	Copy number changes: chromosomes 3, 7, 17, and 9p21		D	11447756, 31467041
Non-invasive papillary urothelial carcinoma, high-grade	Loss 17p	*TP53*	D, P	2208176, 21106220
	rea 4p16.3 FGFR3	*FGFR3*::var fusion and amplification	D	21106220
	Loss 9p21.3 *CDKN2A*	*CDKN2A*	D	2208176, 8895761, 9516934, 8208555
Invasive urothelial carcinoma	Focal loss: 9p21.3, 13q14.2, 17p12-p11.2, and 10q23.31	*CDKN2A, RB1, NCOR1,* and *PTEN*	D	24476821, 28988769
	Focal gain: 6p22.3, 3p25.2, 11q13.3, 19q12, 8q24.21, 12q15, 8q22.3, 1q23.3, 20q11.21, and 8p11.23.	*E2F3, PPARG, CCND1, CCNE1, MYC, MDM2, YWHAZ, NECTIN4 (PVRL4), BCL2L1,* and *ZNF703*, respectively	D	24476821, 28988769
Squamous cell carcinoma of bladder	Trisomy 7 Monosomy 9 Rearrangements of chromosomes 3, 8, 10, 13, and 17		D	9546064
	Deletion 9p	*CDKN2A*	D, P	7658499
	Loss: 17p and 18p (schistosomiasis-associated squamous carcinoma)		D	10964104
REPRODUCTIVE				
Endometrial stromal cell sarcomas				
Endometrial stromal nodule	t(7;17)(p21;q15)	*JAZF1::SUZ12* fusion	D	11371647, 17667554, 15043312,

				21836477, 21420714
Low-grade endometrial stromal sarcoma	Polycomb family gene fusions: t(7;17)(p15.2-15.1;q11.2) t(6;7)(p21.32;p15.2-15.1) t(6;10)(p21.32;p11.22) t(6;10;10)(p21.32;q22;p11.22) t(10;17)(q22.3;p13.3)	*JAZF1::SUZ12* (most common) *JAZF1::PHF1* *EPC1::PHF1* *MEAF6::PHF1*	D	21836477, 11371647, 12850374, 15043312, 16049311, 16397222, 17197920, 17667554, 18580489, 22918161, 23211293, 24592973, 25288234, 27154512, 27219024, 22761769, 24530230, 2434229
	t(X;17)(p11.22;q21.33) t(5;6)(q31.2;p21.32) t(2;6)(q23.1;p21.32) t(10;17)(p11.22;q11.2)	*MBTD1::EZHIP* (*CXorf67*), *BRD8::PHF1* (implicated in high grade tumor) *EPC2::PHF1* *EPC1::SUZ12* (< 3 reported cases each)	D, P	23959973, 28758277, 29721194, 30144186, 30789359
High-grade endometrial stromal sarcoma	t(10;17)(q22.3;p13.3)	*YWHAE::NUTM2A/B* fusions	D, T (Anthracycline-based therapy)	22223660, 22456610, 23599159, 24592973, 27219024, 28390819
	t(X;22)(p11.4;q13.2)	*ZC3H7B::BCOR* fusions (common)	D	23580382, 29192652, 27631520, 30789359
	t(X;10)(p11.4;p11.22) t(X;17)(q26.1;p15.2-p15.1) t(5;6)(q31.2;p21.32)	*EPC1::BCOR* fusion *JAZF1::BCORL1* fusion *BRD8::PHF1* fusion	D	30144186, 28331900, 30789359
Mixed epithelial and mesenchymal tumors				
Adenosarcoma of the uterine corpus	Amplification 8q13.1 *MYBL1* (sarcomatous overgrowth)	*MYBL1*	D	26974998, 25231023
	rea 8q13.3 *NCOA2* rea 20q13.12 *NCOA3*	*NCOA2*::var fusion *NCOA3*::var fusion	D	26592504

Endometrial carcinomas				
Endometrioid carcinoma of the uterine corpus	POLE-ultramutated endometrioid carcinoma, mismatch repair–deficient endometrioid carcinoma, p53-mutant endometrioid carcinoma, no specific molecular profile (NSMP) endometrioid carcinoma		D	WHO 5th edition, 2022
Serous carcinoma of the uterine corpus	Copy-number–high subgroup i(1q) Gain: 1q, 2, 7, 10		D	23636398, 7736425, 9115961, 8174089
	Amplification 17q12 *ERBB2* (*HER2*) (>30% of endometrial serous carcinomas)	*ERBB2 (HER2)* amp	D, T (benefit from trastuzumab to a carboplatin and paclitaxel regimen)	24123408, 31550396, 23765245, 29584549
Undifferentiated and dedifferentiated carcinomas of the uterine corpus	Copy-number–low		D	20305618; 23018216; 27491810; 26743474; 28863077
Carcinosarcoma of the uterine corpus	Copy-number-high hyperdiploidy (60–78%) Copy-number–low hypodiploidy (22–38%)		D	27499902, 28292439
Mesenchymal tumors of the uterus				
Intravenous leiomyomatosis	t(12;V)(12q14.3;V)	*HMGA2*::var fusion	D	11904348, 12508249, 26892441
	Recurrent loss: 22q and 1p regional Recurrent gain: 12q		D	11904348, 12508249, 26892441
Uterine leiomyomas	t(6;V)(p21.31;V) Other rea(6p21.31) and t(12;14)(q14.3;q23-24)	*HMGA1*::var fusion *HMGA2*::var fusion	D	16504804, 25106763
	Loss: 7q22, 22q, and 1p	*CUX1, DEPDC5* and *SMARCB1,* and *NPHP4*	D	22965931, 26787895, 23738515, 26787895, 24525513, 24412114, 19602464

	Deletion Xq22.3 *COL4A5* and *COL4A6*	*COL4A5* and *COL4A6*	D	25106763
	Abnormal karyotypes (40% of uterine leiomyomata)		D	16504804
	t(10;17)(q22;q21)	*KAT6B::KANSL1* fusion	D	31027501
	t(12;14)(q15;q24)	*HMGA2* overexpression	D	30292626, 23738515
Metastasizing leiomyoma	Loss: 19q and 22q terminal deletion		D	17460458
Uterine tumor resembling ovarian sex cord tumor	rea 6q25.1-q25.2 ESR1 rea 2p25.1 GREB1	*ESR1::var* *GREB1::var* (Partners including *NCOA1*, *NCOA2*, *NCOA3*, *CTNNB1*, *NR4A3*, and *SS18*)	D	30350331, 30273195, 31094921, 31464709
Perivascular epithelioid cell tumor (PEComa)	rea Xp11.23 TFE3 rea 14q24.1 RAD51B	*TFE3::var* *RAD51B::var*	D	20871214, 25517951, 25651471, 30001237
	t(5;8)(q32;8q24.22)	*HTR4::ST3GAL1* fusion	D	18085521
Inflammatory myofibroblastic tumor	rea 2p23.2-p23.1 (ALK rearrangements)	*ALK::var* (common partners include *IGFBP5*, *THBS1*, and *TIMP3*)	D	27874193, 22646268, 28490045, 28664932, 25321329
		RANBP2::ALK and *RRBP1::ALK* fusions	P (aggressive IMT with epithelioid morphology)	21164297, 27874193
	Complex genetic rearrangements (RNA sequencing)	*ALK* negative	D	28490045, 29794871, 28664932, 30741845, 28731868
		ETV6::NTRK3 (uterine IMTs) *RET::*var fusion	D	29900760, 31917155
GERM CELL TUMORS (GCTs)				
Post-pubertal GCTs	i(12p), amp(12p)		D	9461002, 34680371, 15738984, 15167939, 17020968
	inv(10)(q11q11)	*RET::NCOA4 (PTC3)*	D, T	8290261, 35957881

	Gain: 1q, 7, 8, 12p, 21, 22, and X Loss: 1p, 4, 5, 11q, 13q, 18		D (distinguishes GCTs; Mediastinal GCT associated with Klinefelter syndrome)	15738984, 34068019, 9461002, 25609015
Pre-pubertal GCTs	i(12p)		D, Less frequent in types I and II; 12p gain rare in prepubertal GCT distinguishes from adult GCT; Prepubertal GCT karyotypes generally less complex compared to adult GCTs	10908150, 10779021, 11921289, 32144540
	Gains in 1q, 3, 11q, 20q, and 22 Loss: 1p, 4q, 6q			24577549, 34068019, 17285132, 29515628

*D: diagnostic; P: prognostic; T: therapeutic

Supplemental Table 3. Gastrointestinal, dermal, and neural crest tumors with diagnostic or clinically significant chromosome aberrations

Tumor	Chromosomal Aberrations	Genes Involved	Clinical Significance*	References (PMID)
HEAD AND NECK				
Eye				
Uveal melanoma	monosomy 3		P	21658465; 28810145; 26556006 (WHO)
	gain of 6p and 8q			
Salivary gland				
Pleomorphic adenoma	8q12 or 12q14.3 rearrangement	*PLAG1* or *HMGA2* rearrangements	D	23821214 (WHO)
		concurrent or isolated *HMGA2* amplification	D	18828159; 34324456 (WHO)
Warthin tumor		**NO** *MAML2* rearrangement	D	24121173; 32222825 (WHO)
Mucoepidermoid carcinoma	mostly t(11;19)(q21;p13)	*CRTC1*::*MAML2*	D, P	20588178; 23018873; 32860299 (WHO); 24856188
	rarely t(11;15)(q21;q26)	*CRTC3*::*MAML2*	D	19749740 (WHO)
	very rarely t(6;22)(p21;q12)	*EWSR1*::*POU5F1*	D	18338330 (WHO); 24856188
Adenoid cystic carcinoma	t(6;9)(q22–23;p23–24) or t(8;9)(q13;p22)	*MYB*::*NFIB* or *MYBL1*::*NFIB*	D	28594149 (WHO)
	loss: 1p, 6q, 15q			22505352; 29619555 (WHO)
	loss: 14q			22505352 (WHO)
Acinic cell carcinoma	t(4;9)(q13;q31)	*NR4A3* upregulation	D	30664630; 31094928; 32341238 (WHO)
Secretory carcinoma	mostly t(12;15)(p13;q25)	*ETV6*::*NTRK3*		20410810 (WHO)
Hyalinizing clear cell carcinoma	mostly t(12;22)(q13;q12)	*EWSR1*::*ATF1*	D	21484932 (WHO)
Intraductal carcinoma	usually inv(10)(q11q11)	*NCOA4*::*RET*	D	29443014 (WHO)
Myoepithelial carcinoma		*PLAG1* fusions are identified in over 50%		29084941; 33027073 (WHO)
Carcinoma ex pleomorphic adenoma		*PLAG1* or *HMGA2* rearrangements and/or amplification		11839563; 15920557; 18828159; 24468654; 27379604 (WHO)
		amplification of *MYC* and/or *EGFR*		

GASTROINTESTINAL				
Esophagus				
Esophageal squamous cell carcinoma	amplification in 7p11.2	*EGFR*	P	26376349; 28757263 (WHO)
	amplification in 11q13	*CCND1*; *CTTN*		
	amplification in 8p11.23	*FGFR1*		
	amplification in 8q24.21	*MYC*		
	amplification in 12p12.1	*KRAS*		
	amplification in 12q15	*MDM2*		
	amplification in 3q26	*TP63* and *PRKCI*		
	amplification in 3q26.32–q26.33	*SOX2* and *PIK3CA*		
	amplification in 14q13.3	*NKX2-1*		
	homozygous deletion 9p21.3	*CDKN2A* and *CDKN2B*		
	homozygous deletion 2q22.1–q22.2	*LRP1B*		
	homozygous deletion 9p24.1	*PTPRD*		
	homozygous deletion 3p14.2	*FHIT*		
Stomach				
Gastroblastoma	t(11;12)(q13;q13)	*MALAT1::GLI1*	D	28731043 (WHO)
Gastrointestinal stromal tumors (GIST)	loss of 14q, 22q, 1p and 15q		P	28632504 (WHO); 17226762
		KIT or *PDGFRA* mutation leading to constitutive activation	T	9438854; 9588894; 12522257; 25605837 (WHO)
Liver				
Fibrolamellar carcinoma (synonym: fibrolamellar HCC)	400Kb deletion of 19p13.12	*DNAJB1*::*PRKACA*	D	28110996; 24578576 (WHO)
Hepatoblastoma	gain of 1q, 8q and 2p (trisomy of 2, 8, 20)		P	19061838 (WHO); 15981236; 20461752
Hepatic mesenchymal hamartoma	t(11;19)(q13;q13)	*MALAT1*		15325096
Pancreas				
Pancreatoblastoma	loss (or LOH) of 11p			11696422 (WHO)
SKIN				
Low-CSD melanoma (superficial spreading melanoma)	loss at chromosomes 9, 10, 6q, and 20	*CDKN2A, PTEN*		14578177 (WHO)
	gain of chromosomes 1q, 6p, 7, 8q, 17q, and 20q	*BRAF*		
Spitzoid melanocytic neoplasms (spitzoid melanoma, Spitz naevus and atypical Spitz tumor)		fusions of *ROS1, ALK, BRAF,*		24445538 (WHO)

		NTRK1, NTRK3, MET, or *RET*		
Hidradenoma	(11;19)(q21;p13)	*CRTC1*::*MAML2*	D	17334997 (WHO)
	t(6;22)(p21;q12)	*EWSR1*::*POU5F1*	D	18338330
NEURAL CREST				
Neuroblastoma	deletion of 1p		P; often concurrent with *MYCN* amplification	8608986; 16306521; 20145112; 20558371; 10379019; 18923191; 19536264 (WHO); 32903140
	deletion of 11q		P; inversely associated with *MYCN* amplification	
	deletion of 3p		P	
	deletion of 4p		P	
	gain of 1q		P	
	gain of 17q		P	
	gain of 2p, including 2p24.3 and 2p23 amplification (dmin, hsr)	*MYCN* and *ALK*	P	20558371; 6719137; 4047115 (WHO); 20719933; 18923525; 25517749
Neuroendocrine				
Follicular thyroid carcinoma	t(2;3)(q13;p25)	*PAX8*::*PPARG*	D	23738683 (WHO)
Papillary thyroid carcinoma	rearrangements of 10q11.2	*RET* (*CCDC6*::*RET* and *NCOA4*::*RET*)	D	10882153; 26868437; 29281951 (WHO)
		NTRK1 and *NTRK3* fusions	T	26784937; 29281951; 33923728 (WHO)
Medullary thyroid carcinoma		*RET* mutation leading to constitutive activation		26868437 (WHO)
	deletion of 9p21	*CDKN2A*	P	27610696 (WHO)
LUNG				
NSCLC [adenocarcinoma (AdC), squamous cell carcinoma (SCC), large cell lung cancer (LCLC), etc.]	gain/amplification of chromosomes 3q	*SOX2, TP63, PIK3CA*	mostly SCC; T	19801978; 15983384; 24461890; 24174329 (WHO); 23026827; 22363766;
	amplification of 7p12	*EGFR*	AdC and SCC	
	amplification of 8p11	*FGFR1*	mostly SCC; T	
	amplification of 7q31	*MET*	mostly AdC; T	
	deletion of 9p21	*CDKN2A*	mostly SCC	
	7p12 amplification	*EGFR*		
	2p23 rearrangement (mostly inv(2)(p21p23))	*ALK* (mostly *EML4*::*ALK*)	mostly AdC, LCLC; T	
	6q22 rearrangement	*ROS1*	mostly AdC; T	

	10q11.2 rearrangement	*RET*	mostly AdC; T	
Malignant pleural mesothelioma	homozygous deletion of 9p21	*CDKN2A*	P	16540645
BREAST				
Secretory breast carcinoma	t(12;15)(p13.2;q25.3)	*ETV6*::*NTRK3*	D; T	16888913; 12450792
Invasive breast carcinoma	dmin, hsr	*ERBB2* (*HER2*) amp	T	19548375; 22417857; 23539740
	gains of 1q and 16p, loss of 16q (as der(16)t(1;16) or der(1;16)); lack of *ERBB2* amplification		mostly ER-positive	20500230
	loss of 1p, 8p, and 17p; gain of 1q and 8q; amplification of 17q12 (*ERBB2*)		mostly ER-negative	
	8p12 rearrangement	*NRG1* fusions	T	29858224
		Homologous recombination deficiency (HRD) score	T	26957554

*D: diagnostic; P: prognostic; T: therapeutic

Supplemental Table 4. Bone and soft tissue tumors with diagnostic or clinically significant chromosome aberrations				
Tumor	**Chromosome aberrations**	**Genes involved**	**Clinical significance***	**References (PMID)**
Adipocytic tumors				
Lipoblastomatosis	8q12.1	*PLAG1 rearrangement*	D	WHO 5th
Atypical lipomatous tumor	12q15	*MDM2* amplification	D,P	WHO 5th
Myxoid liposarcoma	t(12;16)(q13;p11.2)	*FUS::DDIT3*	D	WHO 5th
Fibroblastic and myofibroblastic tumors				
Nodular fasciitis	17p13.2	*USP6*	D	WHO 5th
Solitary fibrous tumor	inv(12)(q14q24.1)	*NAB2::STAT6*	D	WHO 5th
Dermatofibrosarcoma protuberans	t(17;22)(q21.22;q13.1)	*COL1A1::PDGFB*	D	WHO 5th
Infantile fibrosarcoma	t(12;15)(p13.2;q25.3)	*ETV6::NTRK3,* other kinase fusions	D,T	WHO 5th
Inflammatory myofibroblastic tumor	2p23.2	*ALK,* other kinase fusions	D,T	
Low-grade fibromyxoid sarcoma	t(7;16)(q33;p11.2)	*FUS::CREB3L2*	D	WHO 5th
Sclerosing epithelioid fibrosarcoma	t(11;22)(p11.2;q12.2)	*EWSR1::CREB3L1*	D	WHO 5th
Vascular tumors				
Epithelioid hemangioendothelioma	t(1;3)(p36.31;q25.1), t(X;11)(p11.23;q22.1)	*WWTR1::CAMTA1, YAP1::TFE3*	D	WHO 5th
Skeletal muscle tumors				
Alveolar rhabdomyosarcoma	t(2;13)(q36.1;q14.11), t(1;13)(p36.13;q14.11)	*PAX3::FOXO1, PAX7::FOXO1*	D,P	WHO 5th
Spindle cell rhabdomyosarcoma	6q22.1, 6q24.1, 8q13.3, 2p14, 22q12.2, 12q13.12	*VGLL2, CITED2, NCOA2, MEIS1, EWSR1, TFCP2* rearrangements	D	WHO 5th
Tumors of uncertain differentiation				
Angiomatoid fibrous histiocytoma	t(2;22)(q33.3;q12.2)	*EWSR1::CREB1*	D	WHO 5th
Ossifying fibromyxoid tumor	6p21.32, Xp11.23	*PHF1, TFE3* rearrangements	D	WHO 5th
Synovial sarcoma	18q11.2	*SS18* fusions	D	WHO 5th
Epithelioid sarcoma	22q11.23	*SMARCB1*	D	WHO 5th
Alveolar soft part sarcoma	der(17)t(X;17)(p11.23;q25.3)	*ASPSCR1::TFE3*	D	WHO 5th
Clear cell sarcoma of soft tissue	t(12;22)(q13.12;q12.2)	*EWSR1::ATF1*	D	WHO 5th
Desmoplastic small round cell tumor	t(11;22)(p13;q12.2)	*EWSR1::WT1*	D	WHO 5th
Rhabdoid tumor	22q11.23	*SMARCB1*	D	WHO 5th
Undifferentiated small round cell sarcomas of bone and soft tissue				
Ewing sarcoma	22q12.2	*EWSR1*	D	WHO 5th

CIC-rearranged sarcoma	19q13.2	*CIC*	D	WHO 5th
Sarcoma with BCOR genetic alterations	Xp11.4	*BCOR*	D	WHO 5th
Chondrogenic tumors				
Mesenchymal chondrosarcoma	Deletion of the region between 8q13.3 and 8q21.1	*HEY1::NCOA2*	D	WHO 5th

*D: diagnostic; P: prognostic; T: therapeutic

Supplemental Table 5. Tumor nomenclature for solid tumor culture method selection

Tumor types may histologically be divided into small round cell tumors (SRCTs) and non-small round cell tumors (NSRCTs) based on cellular features. SRCTs may grow in suspension or attach to the culture dish and grow as a monolayer. NSRCTs will not grow in suspension. When the sample is received in the laboratory, if the histopathologic diagnosis is not yet known, it can be helpful if the pathologist can tell you if the tumor is a 'SRCT' for the purposes of initiating cultures. Some tumors may grow with either method. If sufficient sample is provided for a SRCT, initiate cultures using both methods. If a very small amount of tumor is received, a coverslip culture is best. Observation of growth will allow one to determine if cells attach or float. If cells float and form balls, a suspension microharvest can be done. Suspension direct or overnight harvest may provide material for FISH if culture growth fails.

Suspension and monolayer - Small round cell tumors (SRCTs)

- Ewing sarcoma or peripheral primitive neuroectodermal (pPNET)
- Medulloblastoma or central primitive neuroectodermal tumor (PNET)
- Neuroblastoma
- Osteosarcoma
- Retinoblastoma
- Rhabdomyosarcoma

Monolayer Culture - Non-small round cell tumors (NSRCTs)

Brain tumors

- Astrocytoma
- Choroid plexus tumor
- Ependymoma
- Glial tumors, glioblastoma, ganglioglioma
- Meningioma
- Oligodendroglioma

Mesenchymal tumors or sarcomas or “spindle cell” tumors

- Clear cell sarcoma
- Desmoplastic small round cell tumor
- Fibrosarcoma
- Hemangiosarcoma
- Hepatoblastoma, hepatocellular carcinoma
- Leiomyosarcoma, leiomyoma
- Liposarcoma, lipoma
- Malignant fibrous histiocytoma (MFH)
- Mesothelioma
- Synovial sarcoma
- Wilms tumor

Germ cell tumors

- Embryonal carcinoma, yolk sac tumors
- Seminoma
- Teratoma

Epithelial tumors (carcinomas)

- Breast

Gastrointestinal
Lung
Prostate
Renal cell