9b From Morphology to Molecular Pathology: A Practical Approach for Cytopathologists (Part 2: Molecular Testing)

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This presentation will illustrate how to apply molecular testing in cytology specimens. Lesions include lymphoproliferative disorders, soft tissue tumors, and other non-GYN lesions. We will present a discussion of molecular pathology, including basic molecular testing knowledge, how to select the appropriate tests, and how to interpret the molecular test results in conjunction with the cytology findings. The most common molecular tests will be covered.

- Integrate the various genetic test results with morphologic, clinical, and other relevant laboratory data to arrive at a single interpretation for diagnostic and prognostic purposes.
- Review how laboratories are using molecular diagnostics, particularly fluorescence in situ hybridization (FISH) technologies and outline key advantages and challenges of oncologic molecular testing relative to more traditional diagnostic methods.
- Recognize the advantages and limitations of the different testing modalities such that they are able to help physicians to prioritize test requests and eliminate redundant testing.

FACULTY:

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Practicing Pathologists
Cytopathology
Cytopathology (Non-Gynecologic)

1.0 CME/CMLE Credit

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From Morphology to Molecular Pathology: A Practical Approach for Cytopathologists
Part 2 – Molecular Pathology

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LSUHSC-S
I have no Conflict of Interest
Questions adapted from:

- Lefkowitch 2006: Anatomic Molecular Pathology
- Mais and Nordberg 2008: Quick Compendium of Pathology Board Review
- Lefkowitch 2006: Anatomic Molecular Pathology, ASCP Press

Acknowledgements
The corresponding figure shows a karyotype of a CD20, CD10, PAX5 and BCL6 positive lymphoid neoplasm. Which of the following statements about this type of tumor is TRUE?
(A) This tumor harbors a t(14;18)(q32;q21) translocation which causes overexpression of cyclin D1.

(B) Polymerase chain reaction (PCR) detects this translocation in a greater proportion of cases than do fluorescence in situ hybridization (FISH) or Southern blotting.

(C) Immunostaining for BCL2 in B cells is diagnostic of follicular lymphoma.

(D) Overexpression of BCL2 is associated with reduced apoptosis and resistance to chemotherapy.

(E) Detection of the BCL2 translocation by PCR is diagnostic of follicular lymphoma.
BCL2 protein expression – non-diagnostic
(although not present in non-neoplastic follicular center cells)

• Flow cytometry
• Immunohistochemistry
  – Normal mantle cells
  – B-cells in hyperplastic marginal zones
  – Normal plasma cells
  – T-cells
  – Follicular lymphomas
  – MALT lymphomas
  – Mantle cell lymphomas
  – Lymphocytic leukemias
  – ALL
Anti-apoptotic
BCL2 FISH
t(11;14)(q13;q32) BCL1/CCND1

- Diagnostic of mantle cell lymphoma
Oncogene
t(11;14)(q13;q32)
PCR for BCL2/IGH

- Wide variability of BCL2 breakpoints
- Standard primers for MBR and mbr
  - Detects only 35-65% of cases
- Can be seen at a low level in some reactive lymphoid proliferations
The corresponding figure is a fluorescence in situ hybridization (FISH) image of cells from a CSF sediment stained with probes for 15q22 and 17q21. Which of the following statements is TRUE?
Acute promyelocytic leukemia (APL) is characterized by t(15;17)(q22;q21), which results in a fusion transcript incorporating portions of the PML from chromosome 17 and the gene for retinoic acid receptor alpha (RARalpha) from chromosome 15.

APL cases with t(11;17)(q23;q21) (PLZF-RARalpha fusion) are resistant to treatment with retinoic acid derivatives.

The optimal method for demonstrating these translocations is DNA PCR.

Treatment with retinoic acid derivatives consistently results in complete loss of detectable transcript in the blood.

Real-time reverse transcription (RT)-PCR is not a reliable method for detecting fusion transcripts.
FISH for t(15;17)- Fusion probe
All-trans-retinoic acid (ATRA)

- t(15;17) inhibits PML and RARα function
- Binds to recruit HDAC to RA response genes
  - Chromatin condensation
  - Maturation arrest
- ATRA inhibits binding
  - Restores transcription of RA-responsive genes
  - Differentiation
t(11;17) PLZF-RARA

- Resistance to treatment with ATRA
- t(15;17) vs RARA variant
RT-PCR for t(15;17)

- Monitoring for treatment response
- Minimal residual disease
REQUIREMENT OF MOLECULAR CYTOGENETIC STUDIES

• I. Mandatory for diagnosis
  – CML: BCR-ABL; t(9;22)
  – Burkitt Lymphoma: C-MYC/IgH; t(8;14), t(14;22), t(2;14)
  – AML with AML1/ETO; t(8;21)
  – AML with CBFβ/MYH11; inv(16) or t(16;16)
• II. Integral part for treatment and prognosis
  – Anaplastic large cell lymphoma: ALK/NPM; t(2;5)
  – Acute promyelocytic leukemia: PML-RARα; t(15;17)
• III. Definitive diagnosis but not mandatory
  – Mantle cell lymphoma: BCL1/IgH; t(11;14)
  – Lymphoplasmacytic lymphoma: PAX/IgH; t(9;14)
  – Diffuse large cell lymphoma/high grade follicular: BCL6
• IV. Prognostic predictor
  – Plasma cell myeloma
• V. No specific cytogenetic abnormality
  – Natural killer cell lymphoma/leukemia
This polyacrylamide gel electrophoresis image shows a clonal control and a polyclonal sample product obtained after PCR with immunoglobulin heavy-chain joining region and framework region 3 primers. Which of the following statements about antigen receptor gene rearrangement analysis for detection of lymphoid clonality is TRUE?
(A) PCR detects a larger proportion of rearrangements than does Southern blotting.

(B) Unlike Southern blotting, which can be performed on paraffin-embedded tissue, PCR requires frozen tissue, because formalin inhibits DNA polymerases.

(C) PCR is especially sensitive for the detection of clonal rearrangements among follicular lymphomas.

(D) Use of PCR- either standard PCR or sequence-specific PCR- to detect residual disease in ALL is complicated by ongoing rearrangements.

(E) If a sample fails to show a clonal rearrangement by PCR, then Southern blotting will be of little value.
VDJ/VJ rearrangements of antigen receptor genes

- Normal response for immunologic competency
- Clonal rearrangements targeted for lymphoid proliferations
PCR for clonality (IGH/JH)

• Somatic hypermutations
• Deletions
• Failure of “consensus” primers to amplify different V families
  – Detects on 50-75% of all rearrangements
• Good for minimal residual disease
Southern blot for B-cell clonality

- Targets broader region of genome
- Laborious
- Requires more tissue
- Unreliable with paraffin
Question 4

- Direct epithelial growth factor receptor gene sequencing of paraffin-embedded lung tumor showed a 15-bp, in-frame deletion in exon 19 (shown here), which leads to a five amino acid deletion within the catalytic kinase domain of EGFR (delE746-A750). Which of the following statements about EGFR and tumors of this type is TRUE?
(A) Given the function of EGFR—a tyrosine kinase—this is likely to be an inactivating mutation and associated with sensitivity to gefitinib (Iressa), an EGFR inhibitor.

(B) This lung tumor is likely to be a small cell carcinoma.

(C) The patient from whom this sample was obtained is most likely a smoker.

(D) This carcinoma is likely to have an activating \textit{k-ras} mutation.

(E) This lung tumor is most likely an adenocarcinoma, and the patient more likely to be a nonsmoker and female.
EGFR Mutations by Race and Other Clinical Characteristics

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<th>Total</th>
<th>East Asian</th>
<th>Non-East Asian</th>
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<tr>
<td>Smokers</td>
<td>11%</td>
<td>17%</td>
<td>4%</td>
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<tr>
<td>Never-smokers</td>
<td>54%</td>
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<td>35%</td>
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<tr>
<td>Adenocarcinoma</td>
<td>42%</td>
<td>49%</td>
<td>16%</td>
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<tr>
<td>Non-AdenoCa</td>
<td>3%</td>
<td>4%</td>
<td>1%</td>
</tr>
<tr>
<td>Male</td>
<td>16%</td>
<td>22%</td>
<td>1%</td>
</tr>
<tr>
<td>Female</td>
<td>46%</td>
<td>58%</td>
<td>20%</td>
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Janne, ASCO Educational Session, 2007
Clinical management and EGFR status

The EGFR Axis

Inhibition Strategies:
- EGFR-Tyrosine Kinase inhibitors
- Anti-EGFR Antibody inhibitors
Molecularly targeted therapy

The RADIANT Trial: A Phase III Study of Erlotinib or Placebo with or without Adjuvant Chemotherapy for Patients with Resected, EGFR-Positive NSCLC

Protocol IDs: CSI-774-302, NCT00373425
Target accrual: 945 (Open)

Eligibility
- Resected Stage IB to IIIA
- EGFR-positive by FISH or IHC
- ≤4 cycles of platinum-based chemotherapy (optional)

Erbitux blocks the growth receptors on cancer cells.

Observation

Erlotinib 150 mg daily x 2 years

FISH analysis for EGFR copy number.
EGFR amplification
KRAS Mutations in NSCLC

- KRAS pathway links EGFR to cell proliferation and survival
- Activating mutations in KRAS could block EGFR signaling
  - Mediate resistance to EGFR inhibitors
- KRAS mutations occur in 10%-30% of NSCLC
  - Associated with smoking and poor prognosis
- Mutations rarely occur in both KRAS and EGFR

Question 5

• This image shows results ofMYCN FISH performed on a tumor sample from an 11-month-old child with neuroblastoma metastatic to the liver. An FNA of the lesion shows these cells. Which of the following statements is TRUE?
(A) This tumor is likely to have a hyperdiploid content and to be associated with a poor prognosis.

(B) This tumor is likely to show duplication of the short arm of chromosome 1 (1p), which is associated with a poor prognosis.

(C) This tumor is likely to express the TrkA neurotrophin receptor, which is associated with a poor prognosis.

(D) Amplification of the n-myc gene (MYCN) is not relevant in this case, because the patient has metastatic disease and is therefore a high-risk patient.

(E) MYCN amplification defines this patient as having high-risk disease.
Neuroblastoma

• Good prognosis
  – Hyperdiploid DNA content
  – No structural chromosomal abnormalities – gains of whole chromosomes
  – Expression of TrkA neutrophin receptor

• Poor prognosis
  – Diploid DNA content
  – Structural aberrations
    • 1p deletions
    • 11q
    • 17q gain
  – MYCN amplification
    • Survival drops from 93% to 10%
Question 6

• This image shows a spindle cell neoplasm in the leg of a 33-year-old woman. The tumor strongly expressed epithelial membrane antigen, BCL2 and CD99, and was focally positive for pancytokeratin. The following statements about this tumor are true EXCEPT:
(A) \( t(X;18)(p11;q11) \) is likely to be present in the above tumor.

(B) The above translocation results in a fusion encoding a protein incorporating the transactivation domain of SYT encoded on chromosome 18 and the C-terminal portion of SSX1, SSX3, or SSX4 encoded on the X chromosome.

(C) Immunohistochemistry for SYT protein, although not diagnostic, can help in the diagnosis of tumors of this type.

(D) Cases with the different molecular forms (SYT-SSX1, SYT-SSX2, SYT-SSX4) appear identical by classic karyotyping and by FISH.

(E) SYT-SSX2 is rarely seen in biphasic tumors of this type (tumors with distinct gland formation), and might be associated with a better prognosis.
FISH Technology

FISH Break-Apart Probes

Gene X
Question 7

- This lesion was seen in the cecum of a 40-year-old man who underwent screening colonoscopy because of endometrial carcinoma in a 43-year-old sister. His mother had a history of a “pelvic” cancer in her 40s, for which she had a total abdominal hysterectomy with bilateral salpingooophorectomy. Immunohistochemistry of the lesion showed complete loss of expression of MSH2 and “very weak” expression of MSH6. This lesion is LIKELY to:
(A) Exhibit microsatellite instability (MSI)

(B) Be associated with a germline mutation in MSH2

(C) Be associated with normal germline MSH6

(D) Be associated with impaired mismatch repair

(E) All of the above
FIGURE 2 - The adenoma-carcinoma sequence in sporadic CRC and in HNPCC - adapted from FEARON and VOGELSTEIN\(^{(17)}\)
>200 hereditary cancer syndromes
Hereditary Cancer

- Two or more relatives, same cancer
- Several generations
- Earlier ages
- Multiple primary cancers
- Other phenotypes
Unstable Areas
Mis-Match Repair

MLH 1

MSH 2

GT - GT - CA - CA - CA - CA - CA

GT - GT - CA - CA - CA - CA - CA

MSH 3/6
MSI Testing

- PCR analysis of microsatellites
- Number of repeats in normal
- Number of repeats in tumor
- Novel sized PCR amplicons in tumor
Question 8

- Karyotyping of a renal cortical tumor (A and B) from a 61-year-old man demonstrated a t(X;1) (p11.2;q23) translocation (C). The following statements about tumors of this type are true EXCEPT:
• Renal cortical tumors
  – Children and adolescents
• T(X;1) – TFE3 @ Xp11.2
• ASPL-TFE3 fusion product can be seen in alveolar soft part sarcoma
(A) They are more common in older individuals.

(B) They may show positive immunohistochemical staining for TFE3 protein.

(C) They may contain papillary and solid areas with clear and eosinophilic cells.

(D) They stain variably with antibodies to cytokeratin.

(E) They share molecular similarities with alveolar soft part sarcoma.
This small round blue cell tumor (A) in the chest wall of a 14-year-old child showed strong immunohistochemical staining for CD99 and Fli-1, and was negative for desmin, smooth muscle actin, HHF-35, cytokeratin, CD45, CD3, CD20, MyoD1, myogenin, S-100, WT1 and epithelial membrane antigen. Classic cytogenetics (B) showed a t(11;22)(q24;q12), which was confirmed by FISH (C). Which of the following statements is FALSE?
(A) This tumor belongs to the Ewing’s sarcoma/primitive neuroectodermal tumor (ES/PNET) family.

(B) RT-PCR can be performed on paraffin-embedded tissue, and in this tumor would be likely to demonstrate an \( EWS-FLI1 \) fusion.

(C) When cytogenetics cannot be performed, a negative result for the \( EWS-FLI1 \) and \( EWS-ERG \), using a properly designed RT-PCR assay to include all \( EWS-FLI1 \) fusion variants, rules out a diagnosis of ES/PNET.

(D) Demonstration of “split” signals with a “break-apart” \( EWS \) probe would have strong evidence in favor of a diagnosis of ES/PNET in this case, had karyotyping been unsuccessful.

(E) In addition to ES/PNET, other tumors with fusion transcripts involving the EWS gene include clear cell sarcoma (“malignant melanoma”) of soft parts, desmoplastic small round cell tumors, extraskeletal myxoid chondrosarcoma and, rarely, myxoid liposarcoma.
ES/PNET Chromosomal Alterations

t(11;22)(q24;q12) detectable in 90% of cases

*EWSR* from 22q12 with *FLI1* on 11q24

Less commonly t(21;22)(q22;q12)

*EWSR* from 22q12 with *ERG* on 21q22
Fluorescent In-Situ Hybridization
Detecting \textit{ews} Translocations

\begin{itemize}
  \item \textit{No Translocation}
  \item \textit{Translocation}
\end{itemize}
FISH Reimbursement

CPT 88367
Medicare reimbursement per probe is $201.26 (402.52 per test)

CPT 88368
Medicare reimbursement per probe is $156.89 (313.78 per test)
This tumor from the leg of an 8-year-old girl was positive for HHF-35, MyoD1 and myogenin. The tumor was negative for cytokeratin, CD45, S-100 and epithelial membrane antigen. Classic cytogenetics showed a t(2;13) (q35;q14). The following are true EXCEPT:
Chromosomal Alterations
ARMS

t(2;13)(q35;q14) and t(1;13)(p36;q14)

Juxtaposition of
PAX3 (80-90%) at 2q35 or
PAX7 (10-20%) at 1p36

with

FOX01A (FKHR) at 13q14
The following statements about BCR-ABL translocations are true EXCEPT:
(A) They may be seen in chronic myelocytic leukemia (CML), during a blast crisis, and de novo (ALL).

(B) The 190-kilodalton fusion protein, resulting from a translocation within the minor cluster region of BCR, is most often associated with ALL.

(C) The oncogenic properties of the fusion protein are the result of constitutive activation of the serine-threonine kinase activity of $BCR$.

(D) Imatinib mesylate, which inhibits ABL kinase activity, is a specific treatment for chronic myelogenous leukemia.

(E) The $BCR-ABL$ transcript continues to be detectable by RT-PCR in most patients on imatinib treatment.
Question 12

- Amplification of HER 2/Neu in breast cancer:
(A) Correlates with increased estrogen and/or progesterone (ER/PR) receptor expression

(B) Is not an independent predictor of prognosis, because it is related to ER/PR expression

(C) Is best detected by immunohistochemistry using the Herceptest kit

(D) Results in increased HER2 protein expression, which helps tumors overcome the blocking effect of trastuzumab (Herceptin™), and causes resistance to this form of therapy.

(E) Is associated with response to treatment with trastuzumab, but this response is blunted or lost in tumors that lose PTEN expression.
Question 13

- The following statements about KIT and PDGFRA mutations in gastrointestinal stromal tumors (GISTs) are true EXCEPT:
(A) GISTs characteristically contain mutations of exons 9, 11, 13 or 17 of c-KIT, or exon 12 or 18 of PDGFRA.

(B) These mutations cause constitutive activation of the KIT or platelet-derived growth factor receptor alpha (PDGFRA) tyrosine kinases, produced by the above genes.

(C) Tumors with PDGFRA mutations tend to be negative for KIT expression by immunohistochemistry.

(D) Studies have shown a correlation between specific c-KIT and PDGFRA mutations and response to therapy with the tyrosine kinase inhibitor imatinib (Gleevec™).

(E) KIT mutations identify GIST as “high risk” or “malignant.”
Mutant KIT Signaling

Effective for metastatic GIST
Also has significant benefit in adjuvant setting
Detection of Kinase Gene Mutations

- DNA from paraffin-embedded tumor
- Screen for mutations by:
  - denaturing HPLC
  - real-time PCR
- Mutations confirmed by direct sequencing
# Molecular Classification of GISTs: Genotype Predicts Benefit of Imatinib

<table>
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<tr>
<th><strong>KIT</strong></th>
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<tr>
<td>Exon 11 (62%)</td>
<td>Best outcome on imatinib</td>
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<tr>
<td>Exon 9 (10%)</td>
<td>More likely to progress than exon 11</td>
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<tr>
<td></td>
<td>May benefit from higher dose (800 mg/d)</td>
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<tr>
<td>Exons 13 &amp; 17 (2%)</td>
<td>Responsive to imatinib</td>
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<th><strong>PDGFRA</strong></th>
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<td>Exons 12 &amp; 14 (2.5%)</td>
<td>Responsive to imatinib</td>
</tr>
<tr>
<td>Exon 18 D842V (5%)</td>
<td>Predicts imatinib failure</td>
</tr>
<tr>
<td>Exon 18 other (1%)</td>
<td>Responsive to imatinib</td>
</tr>
<tr>
<td>‘Wild-type’ (18.5%)</td>
<td>More likely to progress than exon 11</td>
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<tr>
<td></td>
<td>May be more responsive to sunitinib</td>
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Allele-Specific Real-Time PCR For KIT Exon 9 Mutation

Exon 9: FAM-probe-Q

Exon 17: TR-probe-Q

[Mutation = 6 bp insertion]

FAM Signal: Mutant Tumors, Wildtype Tumor, and Negative Control

TR Signal: Mutant Tumors, Wildtype Tumor, Negative Control
Question 14

- The following statements regarding hereditary nonpolyposis colon cancer (HNPCC) and/or MSI are true EXCEPT:
(A) HNPCC has been associated with mutations in MLH1, MSH2, MSH6, PMS1 and MLH3 genes.

(B) According to the modified Bethesda criteria, MSI testing should be performed in carcinomas occurring before the age of 50.

(C) HNPCC is associated with a high frequency of somatic mutations of the *BRAF* gene

(D) Other tumors in the spectrum of HNPCC include endometrial carcinoma, urothelial carcinoma, and carcinoma of the small intestine, stomach and hepatobiliary ducts.

(E) High MSI in colon cancers is associated with a reduced likelihood of metastatic disease, and better overall survival.
Question 15

• Which of the following statements about FISH, using the Urovysion™ assay to detect bladder cancer, is TRUE?
(A) FISH is more specific but less sensitive than cytologic examination.

(B) Loss of at least one copy of any two of chromosomes 3, 7 or 17 identified by centromeric probes, and amplification of chromosome 9p21 with a sequence-specific probe, are the most important markers of urothelial carcinoma.

(C) The probe set is chosen to evaluate for polysomy of chromosome 3, 7 and 17, and/or homozygous loss of 9p21.

(D) Evaluation of 100 successive cells is more sensitive than evaluating 25 morphologically abnormal cells.

(E) Papillary urothelial carcinoma is more likely to be FISH positive than is carcinoma in situ.
UroVysion™

(p16 gene)
UroVysion™ - Positive Results
Multiple Chromosomal Gains - CEP 3, 7, 17
Question 16

- This tumor from the leg of an 8-year-old girl was positive for HHF-35, MyoD1, and myogenin. This tumor was negative for cytokeratin, CD45, S-100 and epithelial membrane antigen. Classic cytogenetics showed a t(2;13)(q35;q14). The following are true EXCEPT:
(A) This tumor is an alveolar rhabdomyosarcoma (ARMS).

(B) RT-PCR is likely to show a fusion transcript involving the 5' portion of *PAX3* and the 3' portion of *FOXOA1* (FKHR).

(C) A more common translocation in alveolar rhabdomyosarcoma is t(1;13) (p36;q14), which results in a *PAX7-FOXOA1* fusion.

(D) This transcript is associated with a higher rate of bone marrow involvement than the *PAX7-FOXOA1* transcript.

(E) The *PAX7-FOXOA1* translocation is more likely to be amplified as *double minutes* (*dmin*).
Question 17

- Which of the following statements about gene expression analysis of lymphomas using microarrays is/are TRUE?
(A) Gene expression analysis of diffuse large B-cell lymphomas using cDNA microarrays differentiates a germinal center B cell-like group with a good prognosis, an activated B cell-like group with a poor prognosis and a third, heterogeneous, group with a poor prognosis.

(B) Immunohistochemistry with CD10, BCL6 and MUM1 can approximate this separation into germinal center-like and nongerminal center-like groups.

(C) The prognosis of follicular lymphomas is related to gene expression patterns of non-neoplastic cells in the microenvironment.

(D) It has demonstrated a unique gene expression pattern in primary mediastinal B-cell lymphoma, distinct from that of other diffuse large B-cell lymphomas.

(E) All of the above.
Question 18

- Which of the following statements about genetic alterations in thyroid neoplasms is/are TRUE?
(A) RET/PTC translocations are common among papillary thyroid carcinomas seen in younger individuals, and in those following radiation exposure.

(B) BRAF mutations occur more commonly in papillary thyroid carcinomas in older individuals, and are associated with an increased likelihood of late-stage disease and anaplastic carcinoma.

(C) BRAF mutations, RAS mutations and RET/PTC rearrangements rarely occur together in papillary thyroid carcinoma.

(D) PAX8-PPARgamma translocations are frequent in follicular thyroid carcinomas and less frequent in follicular adenomas.

(E) All of the above.
Question 19

• When performing tests on RNA from paraffin-embedded tissue, which one of the following factors should be considered?
(A) The amount of RNA retrieved depends on the type of fixative used, time prior to fixation and duration of fixation.

(B) The larger the amplicon, the greater the likelihood of successful amplification.

(C) Formalin is a better fixative for RNA preservation than is ethanol.

(D) RNA integrity in routinely processed, paraffin-embedded tissue is best assessed by gel electrophoresis to determine the ratio of 18S and 28S rRNA.

(E) RNA extraction from paraffin-embedded tissue is fiction.
Question 20

- Which of the following statements about gliomas is TRUE?
(A) Oligodendrogliomas are characterized by the loss of the short arm of chromosome 1 (1p) and the long arm of chromosome 19 (19q) in the majority of cases.

(B) Oligodendrogliomas are characterized by the loss of the long arm of chromosome 1 (1q) and the short arm of chromosome 19 (19p) in the majority of the cases.

(C) LOH at chromosome 1 is associated with resistance to chemotherapy.

(D) Activating mutations in the ATP binding region of EGFR are common in gliomas and are associated with increased sensitivity to gefitinib.

(E) Loss of expression of methylguanine methyltransferase (MGMT) is associated with resistance to chemotherapy.
Question 21

• Which of the following statements about controls for PCR or RT-PCR is FALSE?
(A) When setting up a PCR, one should always add template to the no DNA control first, to avoid accidentally contaminating it.

(B) When setting up a PCR, one should add template to test samples first, followed by the positive control and the negative/no DNA controls.

(C) When setting up a RT-PCR, in addition to a no template (no RNA control) one should set up a no RT control for each RNA sample being tested.

(D) When setting up a PCR following a RT reaction, one should set up a separate no template control, in addition to the product of the no RNA RT reaction.

(E) RT-PCR can be performed in a single tube.
Question 22

• A thyroid tumor from a 9-year-old girl shows an insular growth pattern, and is immunoreactive for thyroid transcription factor 1 (TTF-1), carcinoembryonic antigen and chromogranin. There is no history of thyroid cancer in the family. Which of the following statements is TRUE?
(A) This patient is likely to harbor an inactivating germline mutation of the \textit{RET} tumor suppressor gene.

(B) The majority of patients with this type of tumor, even in the absence of a family history, have a germline activating mutation of the \textit{RET} proto-oncogene.

(C) A significant proportion of tumors of this type harbor a translocation involving the \textit{RET} proto-oncogene (PTC1, PTC2 or PTC3).

(D) Activating somatic mutations of the RET proto-oncogene may be seen in at least 25-35\% of sporadic cases of this tumor type, and may be associated with an increased likelihood of recurrence.

(E) Germline testing for RET mutations is not indicated in this patient, in the absence of a family history.
Question 23

• Which of the following statements about loss of imprinting (LOI) of IGF2 in Wilms’ tumor is TRUE?
(A) IGF2 LOI is more common in older children with Wilms’ tumors.

(B) IGF2 LOI is seen predominantly in "perilobar nephrogenic rest-like" tumors.

(C) IGF2 LOI is present in Wilms’ tumors that develop in Beckwith-Weidemann syndrome (BWS).

(D) IGF2 LOI is absent in tumors with LOH of 11p15 or WT1 mutations.

(E) All of the above.
Question 24

- Pheochromocytoma is associated with germline mutations in:
(A) RET proto-oncogene

(B) Neurofibromin

(C) VHL

(D) Succinate dehydrogenase subunit D

(E) All of the above.
Question 25

• Which of the following statements about alterations of tumor suppressor genes and oncogenes in cancers is TRUE?
(A) Oncogenic mutations of tumor suppressor genes invariably occur at “hot spots,” allowing one to test only for specific mutations.

(B) Because cancer-associated mutations of tumor suppressor genes involve loss of function, they can occur throughout the gene, and never involve “hot spots.”

(C) Activating mutations of receptor tyrosine kinases occur at a limited number of sites.

(D) Direct sequencing of the entire coding region of a tumor suppressor gene should detect all significant alterations-barring technical error.

(E) Tests for hypermethylation of tumor suppressor gene promoters require pure tumor, and can be performed only on microdissected samples.
Question 26

- Which of the following statements about fibroblast growth factor receptor 3 (FGFR3) and p53 (TP53) mutations in bladder cancer is TRUE?
(A) FGFR3 mutations act synergistically with p53 mutations in aggressive bladder cancer.

(B) FGFR3 and p53 mutations are independent markers of poor prognosis in bladder cancer.

(C) Both FGFR3 and p53 mutations are associated with a poor prognosis, but are not independent predictors of prognosis.

(D) FGFR3 and p53 mutations are almost mutually exclusive, and are both associated with high-grade bladder cancer.

(E) FGFR3 mutations are more common in low grade and low-stage urothelial carcinomas with a low rate of grade and stage progression.
Question 27

- Which of the following statements about gene expression profiling of node-negative, tamoxifen-treated breast cancer are TRUE?
(A) A “recurrence score” based on the normalized quantitative expression of 16 genes predicts long-term distant recurrence-free survival.

(B) The test requires frozen tissue, as quantitative analysis of RNA from paraffin-embedded tissue is unreliable.

(C) Elevated expression of genes involved in the ER signaling pathway is associated with an increased likelihood of recurrence.

(D) HER2 expression is not used in this profile, as it requires evaluation of gene amplification using DNA.

(E) Elevated expression of genes involved in proliferation is associated with a reduced likelihood of recurrence.
Question 28

• Which of the following associations of tumor/genetic alteration is TRUE?
(A) t(17;22), COL1A1-PDGFB with dermatofibrosarcoma protuberans, giant cell fibroblastoma and Bednar tumor

(B) t(12;15)(p13;q25), ETV6-NTRK3 with infantile fibrosarcoma and cellular congenital mesoblastic nephroma

(C) t(X;17)(q11.2;q25), ASPL-TFE3 with alveolar soft part sarcoma

(D) t(7;17)(p15;q21), JAZF1-JJAF1 with endometrial stromal sarcoma

(E) All of the above.