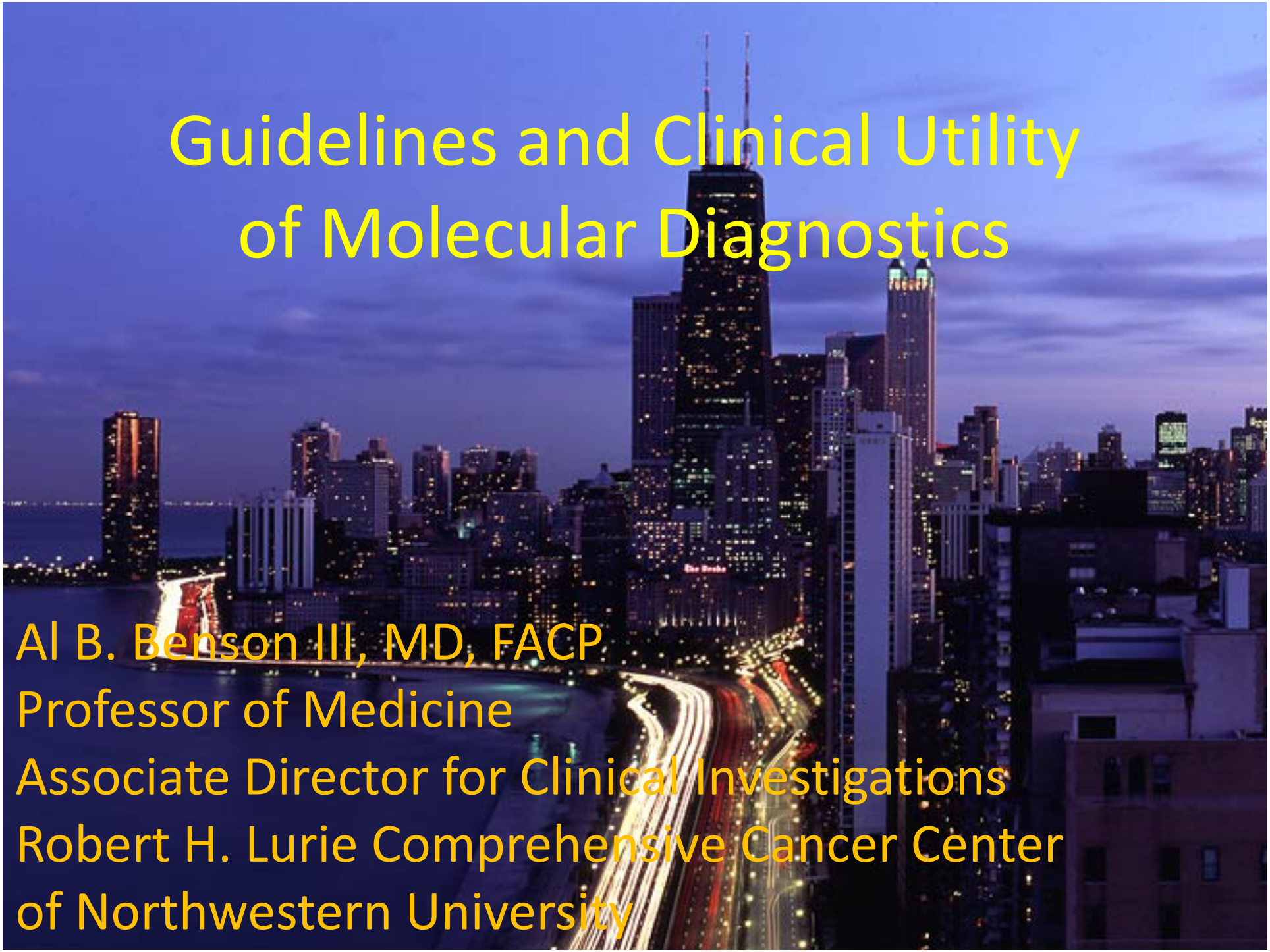

Guidelines and Clinical Utility of Molecular Diagnostics

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of Northwestern University

- Archie Chocrane identified three concepts related to the evaluation of a medical technology – efficacy, effectiveness, and efficiency:
  - **Efficacy** is the extent to which an intervention does more good than harm under ideal circumstances (i.e., in circumstances designed to maximize the effect of the intervention and eliminate confounding factors). (“Can it work?”)
  - **Effectiveness** is the extent to which an intervention does more good than harm when provided to real-world patients by physicians practicing in ordinary clinical settings. (“Does it work in practice?”)
  - **Efficiency** measures the effect of an intervention in relation to the resources it consumes. (“Is it worth it?”)

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Two Types of Evidence-Based Guidelines

- Process map of integrated interventions over time
  - Addresses coordination of care
  - Illuminates continuum of care
  - Fewer references for each decision
- Exhaustive review of single issue
  - Scope is more restricted
  - May address single decision point
  - Comprehensive review and analysis of literature

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Examples of measures of effectiveness

- Level of evidence
- Probability of achieving a cure
- Impact on survival (e.g., overall, disease-free, progression-free)
- Impact on disease control
- Impact on improving performance status
- Impact on disease-related symptom control

From “Marker” to “Test”

- **Significant and independent value**
- **Validated by clinical testing**
- **Feasibility, reproducibility and widely available with quality control (robust)**
- **Performance should benefit the patient**

Ann Thor, ECOG, 2002

Clinical Utility

- Assay improves clinical decision-making and patient outcomes.
- Depends on the clinical situation, availability of effective therapies, magnitude of clinical benefit (or lack thereof) in one group versus another
- Relative value to patient, caregiver, and society place on the differences in benefits and risks
- Perceptions of these differences (patient, caregiver, society).

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Examples of Utility Questions

- Potential Clinical Utility
  - Marker + → treatment A
  - Marker - → treatment B
- No Clinical Utility
  - Marker + → treatment A
  - Marker - → treatment A
  - + analytic validity + clinical validity but strongly correlates with established clinical or histopathologic prediction
- Limited Clinical Utility
  - + prognostic marker but no predictive correlation with intervention

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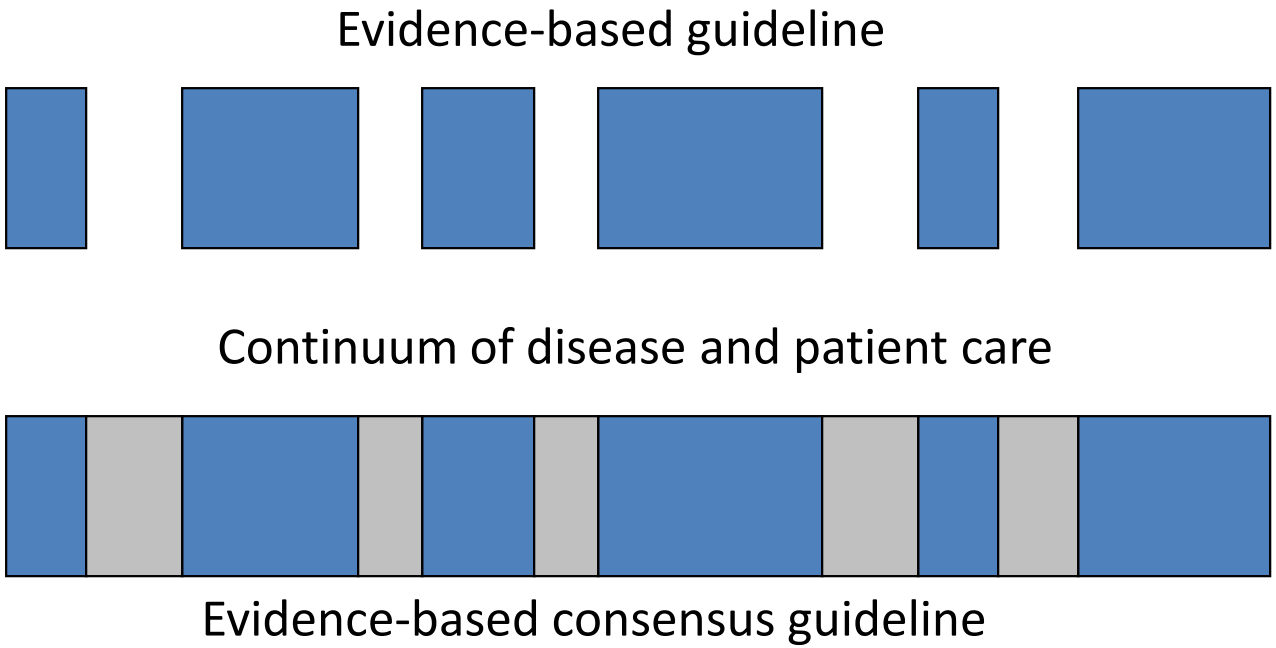
Challenges

- Common diseases may become rare subsets
  - Trials with smaller numbers of patients probable
  - Evidence may be more limited
  - Reliance, in part, on databases including tumor banks for evidence
  - Screening eligible populations
  - Tissue: metastatic vs primary
  - Tumor heterogeneity and multiple markers

Evidence-based Consensus Allows Comprehensive Guideline



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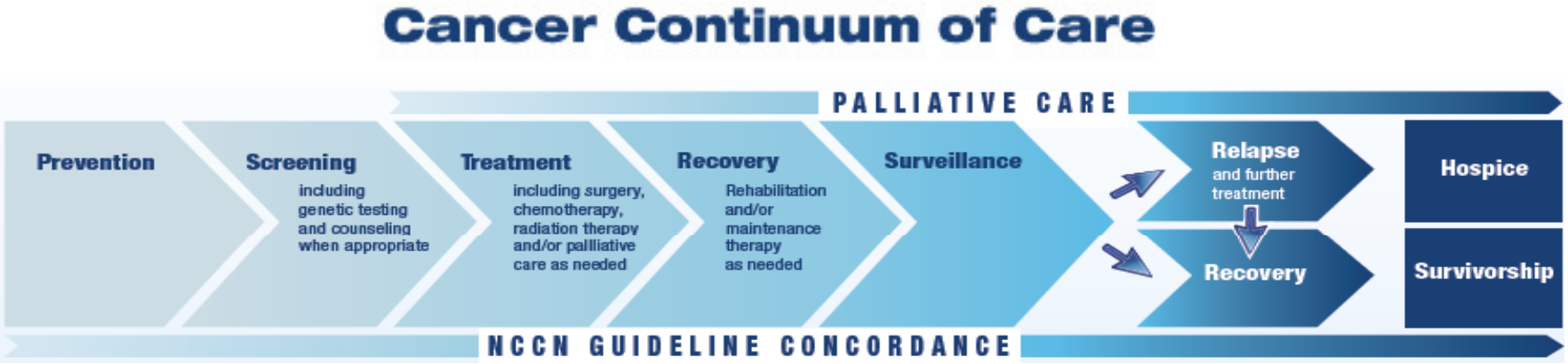
NCCN Guidelines Cover the Continuum of Care



- Provide a continuously updated fund of knowledge in the increasingly complex and evolving oncology space
- Process is highly structured and time intensive

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NCCN Clinical Practice Guidelines Multidisciplinary Panels

- Medical oncology
- Surgery/Surgical oncology
- Radiation oncology
- Hematology/Hematology oncology
- Bone Marrow Transplantation
- Urology
- Neurology/neuro-oncology
- Gynecologic oncology
- Otolaryngology
- Orthopedics/orthopedic oncology
- Pathology
- Dermatology
- Internal medicine
- Gastroenterology
- Endocrinology
- Diagnostic Radiology
- Interventional Radiology
- Nursing
- Cancer genetics
- Psychiatry, psychology
- Pulmonary medicine
- Pharmacology/Pharmacy
- Infectious diseases
- Allergy/immunology
- Anesthesiology
- Cardiology
- Geriatric medicine
- Epidemiology
- Patient advocacy
- Palliative, Pain management
- Pastoral care
- Oncology social work

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Evidence of Clinical Utility

NCCN Guidelines Panels require data supporting clinical utility for testing

- Data demonstrating that the biomarker affects treatment decisions
- Evidence that the biomarker can divide patients into specific clinically relevant subgroups
- Widespread availability of reliable testing

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Determination of Levels of Evidence Using Elements of Tumor Marker Studies

Level of Evidence	Category*	Validation Studies Availabile
I	A	None required
I	B	One or more with consistent results
II	B	None or inconsistent results
II	C	2 or more with consistent results
III	C	None or 1 with consistent results or inconsistent results
IV-V	D	N/A

A= prospective
B=prospective using archived samples
C=prospective/observational
D=retrospective/observational

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NCCN Categories of Evidence and Consensus
Category 1: Based upon high-level evidence, there is uniform NCCN consensus that the intervention is appropriate.
Category 2A: Based upon lower-level evidence, there is uniform NCCN consensus that the intervention is appropriate.
Category 2B: Based upon lower-level evidence, there is NCCN consensus that the intervention is appropriate.
Category 3: Based upon any level of evidence, there is major NCCN disagreement that the intervention is appropriate.

JNCCN 2011
JNCI 2009

Use of Archived Tissues to Determine Clinical Validity of Tumor Markers

Category Trial Design	A Prospective	B Prospective Using Archived Samples	C Prospective/Observational	D Retrospective/Observational
Clinical trial	PCT designed to address tumor marker	Prospective trial not designed to address tumor marker, but design accommodates tumor marker utility	Prospective observational registry, treatment and follow-up not dictated	No prospective aspect to study
Patients and patient data	Prospectively enrolled, treated and followed in PRCT	Prospectively enrolled, treated, and followed up in clinical trial and, especially if a predictive utility is considered, a PRCT addressing the treatment of interest	Prospectively enrolled in registry, but treatment and follow-up standard of care	No prospective stipulation of treatment or follow-up; patient data collected through retrospective chart review
Specimen collection, processing, and archival	Specimens collected, processed, and assayed for specific marker in real time	Specimens collected, processed, and archived prospectively using generic SOPs, assayed after trial completion	Specimens collected, processed, and archived prospectively using generic SOPs	Specimens collected, processed, and archived with no prospective SOPs
			Assayed after trial completion	
Statistical design and analysis	Study powered to address tumor marker question	Study powered to address therapeutic question and underpowered to address tumor marker question	Study not prospectively powered at all; retrospective study design confounded by selection of specimens for study	Study not prospectively powered at all; retrospective study design confounded by selection of specimens for study
		Focused analysis plan for maker question developed before performing assays	Focused analysis plan for maker question developed before performing assays	No focused analysis plan for marker question developed before performing assays
Validation	Result unlikely to be play of chance	Result more likely to be play of chance than A, but less likely than C	Result very likely to be play of chance	Result very likely to be play of chance
	Although preferred, validation not required	Requires one or more validation studies	Requires subsequent validation studies	Requires subsequent validation studies

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Current Molecular Biomarkers in Colon Cancer*

Biomarker	Molecular Compartment	Purpose	Analytic Validity Demonstrated	Levels of Evidence	NCCN Category of Evidence
Markers with accepted clinical utility					
KRAS mutations (except c.38G>A (p.G13D)]	Tumor DNA	Predictive (negative for anti-EGFR therapy), negatively prognostic in several first-line randomized studies (Lynch)	Multiple methods: PCR, multiplex assays, direct sequencing	Predictive: 1B Prognostic: IIB	2A
MSI and/or MMR protein loss	Tumor DNA for MSI testing with PCR; tumor IHC for MMR proteins	Screening (lynch syndrome) Prognostic (recurrence, overall survival) Predictive (lack of benefit, possibly worse outcome with adjuvant single-agent fluoropyrimidine therapy)	PCR, IHC	Screening: IB Prognostic: IB Predictive: IIB	2A
CEACAMS (CEA)	Patient serum	Surveillance	Immunoassay	IIC	2A
BRAF c. 1799 > A mutation (p.V600E)	Tumor DNA	Prognostic (strong negative prognostic marker) Predictive? (negative for anti-EGFR therapy)	Multiple methods: PCR, multiplex assays, direct sequencing	Prognostic: IB Predictive: IIIC	2A

*Note: references also provided for each marker

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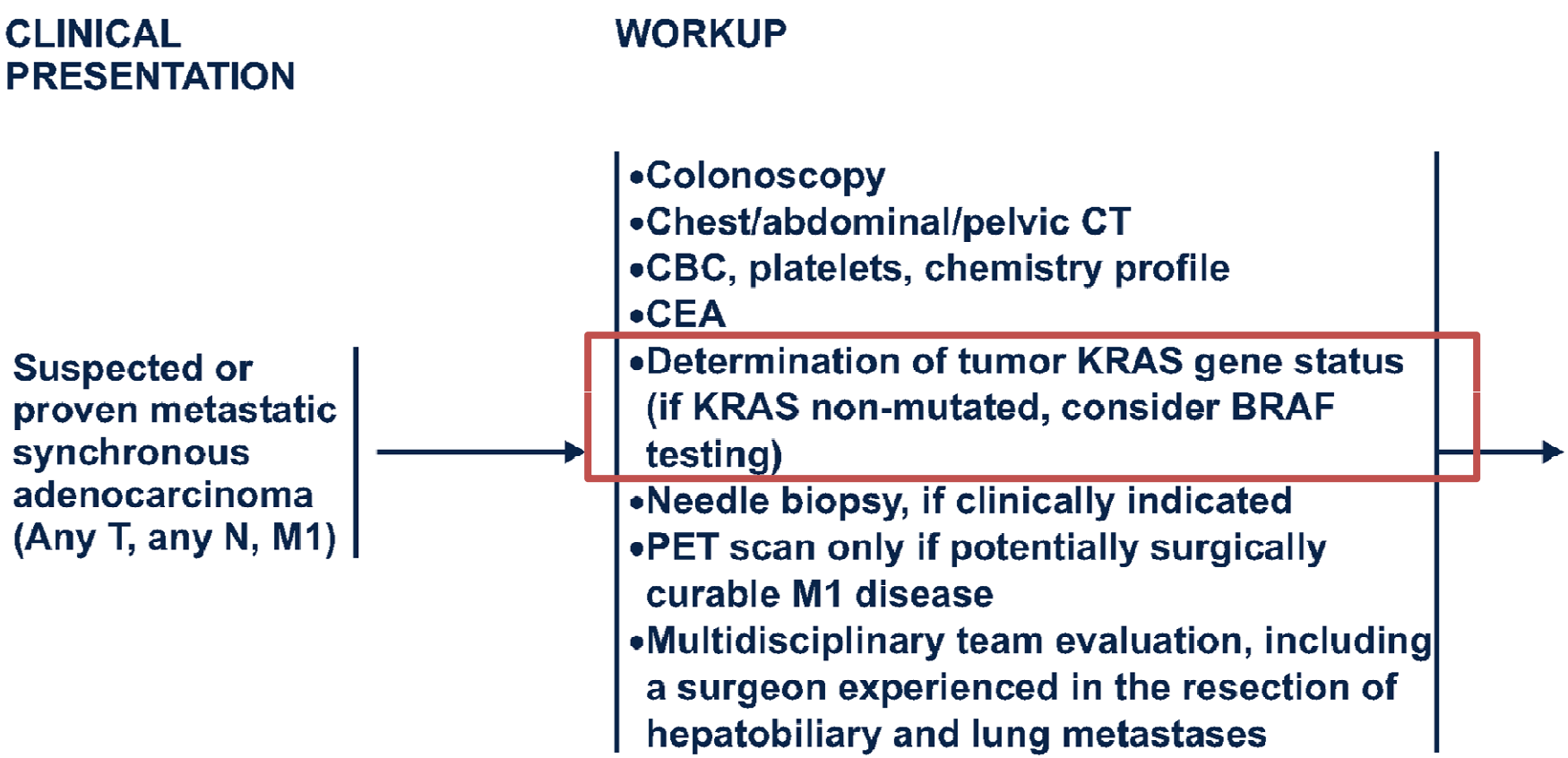
Colon Cancer



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PRINCIPLES OF PATHOLOGIC REVIEW

KRAS Mutation Testing

- Mutations in codons 12 and 13 in exon 2 of the coding region of the KRAS gene predict lack of response to therapy with antibodies targeted to the epidermal growth factor receptor.
- Testing for mutations in codons 12 and 13 should be performed only in laboratories that are certified under the clinical laboratory improvement amendments of 1988 (CLIA- 88) as qualified to perform high complex clinical laboratory (molecular pathology) testing. No specific methodology is recommended (sequencing, hybridization, etc.).
- The testing can be performed on formalin fixed paraffin embedded tissue. The testing can be performed on the primary colorectal cancers and/or the metastasis as literature has shown that the KRAS mutations are similar in both specimen types.

BRAF Mutation Testing

- Patients with a V600E BRAF mutation appear to have a poorer prognosis. Retrospective subset analyses suggest potential benefit from anti-EGFR monoclonal antibodies in the first-line setting with active chemotherapy regardless of V600E mutation status. Limited available data suggest lack of antitumor activity from anti-EGFR monoclonal antibodies in the presence of a V600E mutation when used after patient has progressed on first-line therapy.
- Testing for the BRAF V600E mutation can be performed on formalin fixed paraffin embedded tissues. This is usually performed by PCR amplification and direct DNA sequence analysis. Allele-specific PCR is another acceptable method for detecting BRAF V600E mutation. This testing should be performed only in laboratories that are certified under the clinical laboratory improvement amendments of 1988 (CLIA-88) and qualified to perform highly complex clinical laboratory (molecular pathology) testing.

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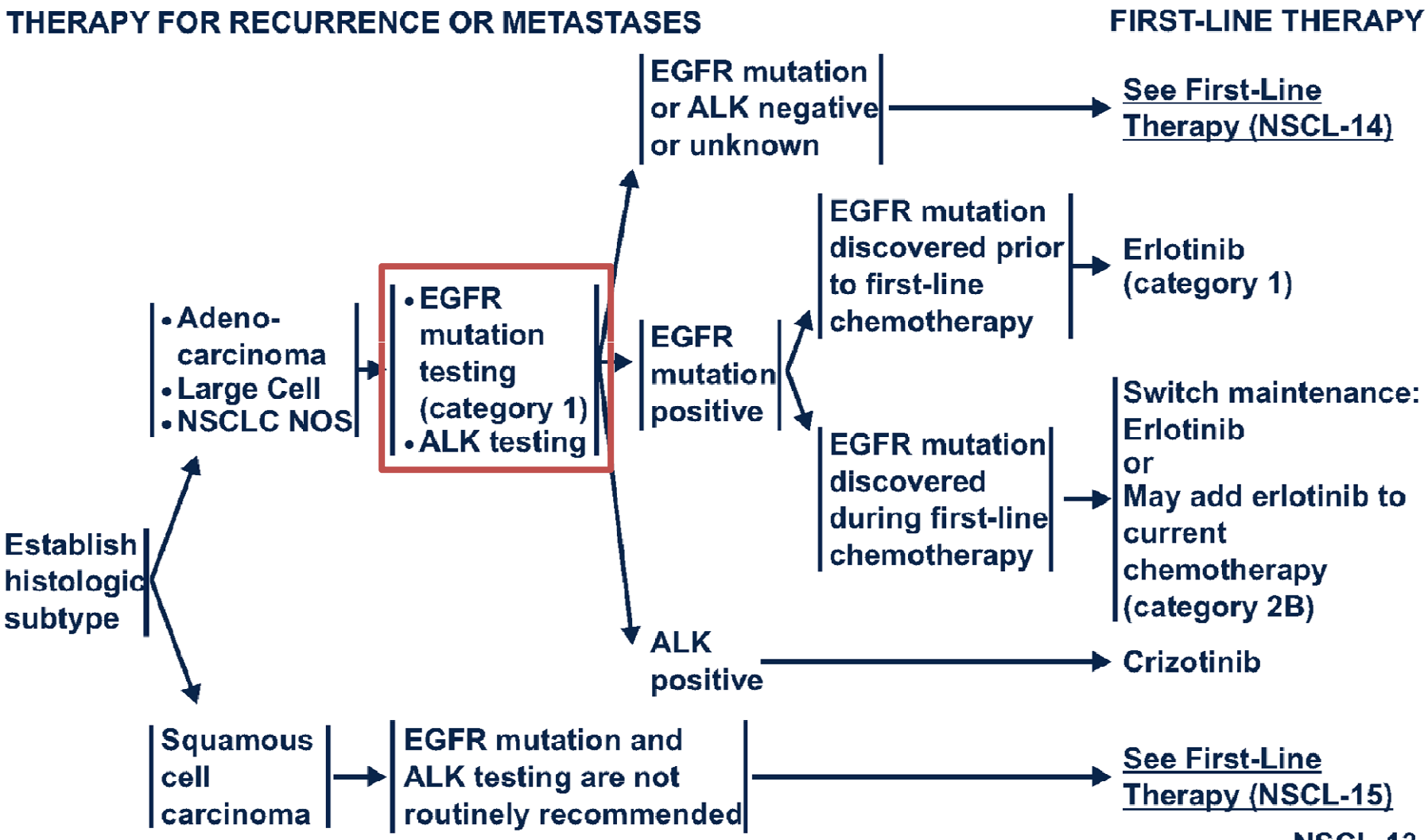
Non-Small Cell Lung Cancer



NSCL-13

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PRINCIPLES OF PATHOLOGIC REVIEW

Molecular Diagnostic Studies in Lung Cancer

- EGFR and KRAS
  - EGFR is normally found on the surface of epithelial cells and is often overexpressed in a variety of human malignancies. Presence of EGFR-activating mutations represents a critical biological determinant for proper therapy selection in patients with lung cancer.
  - There is a significant association between EGFR mutations — especially exon 19 deletion, exon 21 mutation (L858R), and exon 18 (G719X) — and response to TKIs.
  - EGFR and KRAS mutations are mutually exclusive in patients with lung cancer.
  - KRAS mutations are associated with intrinsic TKI resistance, and KRAS gene sequencing could be useful for the selection of patients as candidates for TKI therapy.
  - The prevalence of EGFR mutations in adenocarcinomas is 10% of Western and up to 50% of Asian patients, with higher EGFR mutation frequency in non-smokers, women, and non-mucinous cancers. KRAS mutations are most common in non-Asians, smokers, and in mucinous adenocarcinoma. The most common EGFR mutations result in an arginine for leucine substitution at amino acid 858 in exon 21 (L858R) and in frame deletions at exon 19. Mutations are more common in non-mucinous lung adenocarcinoma with lepidic pattern (former BAC pattern) and in lung adenocarcinoma with papillary (and or micropapillary) pattern.
  - Resistance to TKI therapy is associated with KRAS mutation and with secondary asquired EGFR mutations, such as T790M.

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PRINCIPLES OF PATHOLOGIC REVIEW

Molecular Diagnostic Studies in Lung Cancer

- **EML4-ALK**
  - Anaplastic lymphoma kinases (ALK) gene rearrangements, in a subset of anaplastic large cell lymphomas (ALCL), have been recognized for over 15 years. The fusion between echinoderm microtubule--associated protein-like 4 (EML4) and ALK has recently been identified in a subset of patients with NSCLC. EML4-ALK NSCLC represents a unique subset of NSCLC patients for whom ALK inhibitors may represent a very effective therapeutic strategy. Crizotinib is an oral ALK inhibitor that was recently approved by the FDA for patients with locally advanced or metastatic NSCLC who have the ALK gene rearrangement. (ie, ALK positive).
  - EML4-ALK NSCLC occurs most commonly in a unique subgroup of NSCLC patients who share many of the clinical features of NSCLC patients likely to harbor EGFR mutations. However, for the most part, EML4-ALK translocations and EGFR mutations are mutually exclusive. EML4-ALK translocations tend to occur in younger patients and in those with more advanced NSCLC while this relationship has not been reported for EGFR mutant NSCLC.
  - The current standard method for detecting EML4-ALK NSCLC is fluorescence in situ hybridization (FISH), although other methods are currently being evaluated, including polymerase chain reaction (PCR) and IHC. A big advantage of FISH is that a commercially available probe set, developed for the diagnosis of ALK-rearranged ALCLs, is applicable for the diagnosis of ALK-rearranged lung adenocarcinomas. The IHC tests used to diagnose ALK-rearranged ALCLs in clinical laboratories worldwide are inadequate for the detection of the majority of ALK-rearranged lung adenocarcinomas. This is due to the lower level of ALK expression in ALK-rearranged NSCLCs compared with ALK-rearranged ALCLs. A molecular diagnostic test that uses FISH was recently approved by the FDA to determine which patients with lung adenocarcinoma are ALK positive.

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Melanoma

SYSTEMIC THERAPY OPTIONS FOR ADVANCED OR METASTATIC MELANOMA[1]

- **Clinical trial (preferred)**
- **Ipilimumab (category 1)[2,3]**
- **Vemurafenib (category 1)[4,5]**
- **Dacarbazine**
- **Temozolomide**
- **High-dose Interleukin-2[6,7]**
- **Dacarbazine-or temozolomide-based combination chemotherapy/biochemotherapy (including cisplatin and vinblastine with or without IL-2, interferon alfa) (category 2B)[7]**
- **Paclitaxel (category 2B)**
- **Paclitaxel/cisplatin (category 2B)**
- **Paclitaxel/carboplatin (category 2B)**

[1]Patients who progress after initial therapy may be offered subsequent therapy if they maintain a performance status of ECOG 0-2 or Karnofsky score ≥ 60.

[2]Ipilimumab has the potential for significant immune-mediated complications. Participation in the risk evaluation and mitigation strategy (REMS) program and/or experience in use of the drug as well as resources to follow the patient closely are essential. Ipilimumab should be used with extreme caution, if at all, in patients with serious underlying autoimmune disorders.

[3]Re-induction with ipilimumab may be considered for select patients who experienced no significant systemic toxicity during prior ipilimumab therapy and who relapse after initial clinical response or progress after stable disease > 3 months.

[4]Vemurafenib is recommended for patients with V600 mutation of the BRAF gene documented by an FDA-approved or Clinical Laboratory Improvement Amendments (CLIA)-approved facility.

[5]Vemurafenib has the potential for significant dermatologic complications including cutaneous squamous cell carcinoma and extreme photosensitivity. Regular dermatologic evaluation with referral to a dermatologist as clinically indicated. Patients should also be carefully monitored for the development of other adverse reactions such as joint pain and swelling.

[6]High-dose interleukin-2 should not be used for patients with inadequate organ reserve, poor performance status, or untreated or active brain metastases. For patients with small brain metastases and without significant peritumoral edema, IL-2 therapy may be considered (category 2B).

[7]Administration of multiagent regimens and high-dose interleukin-2 is complex and associated with significant toxicities. Therapy should be restricted to an institution with medical staff experienced in the administration and management of regimens.

ME-E

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In Development

- To ensure access to appropriate testing as recommended by NCCN Guidelines
- Identify the utility of a biomarker to screen, diagnose, monitor, or provide predictive or prognostic information
- Discriminate between clinically useful biomarkers and those that are not yet clinically indicated

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NCCN Guidelines Panels Existing Recommendations for Testing

Currently more than 800 biomarker recommendations in NCCN Guidelines:

- Determine risk of disease (BRCA-1/BRCA-2)
- Screening (PSA for prostate)
- Diagnostic (BCR/ABL in CML)
- Prognostic (CA 19-9 in pancreas)
- Predictive (ER/PR status in breast)
- Risk of toxicity (UGT1A1*28 allele for irinotecan)
- Response/disease monitoring (AFP; HCG in testicular)

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NCCN Biomarkers Compendium™

Specific Indication	Molecular Abnormality	Test Purpose	Methodology	NCCN Level of Evidence	Specimen Types	NCCN Recommendation
Breast Cancer: DCIS Newly DX Stage I-IV	ER expression	Prognostic Predictive	IHC	2A	FFPE tumor tissue	Positive result predicts responsiveness to hormone therapy in invasive disease and possible prevention in DCIS
CML: Chronic Phase Adult CML	BCR-ABL t(9;22) translocation	Diagnosis	FISH	2A	Bone marrow, peripheral blood	Philadelphia chromosome (BCR-ABL, t(9;22) translocation is diagnostic for CML. If bone marrow is not feasible….

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