"Lung Cancer School 2017"
2017. 7. 22.

Current Status of Molecular Diagnostic Technique

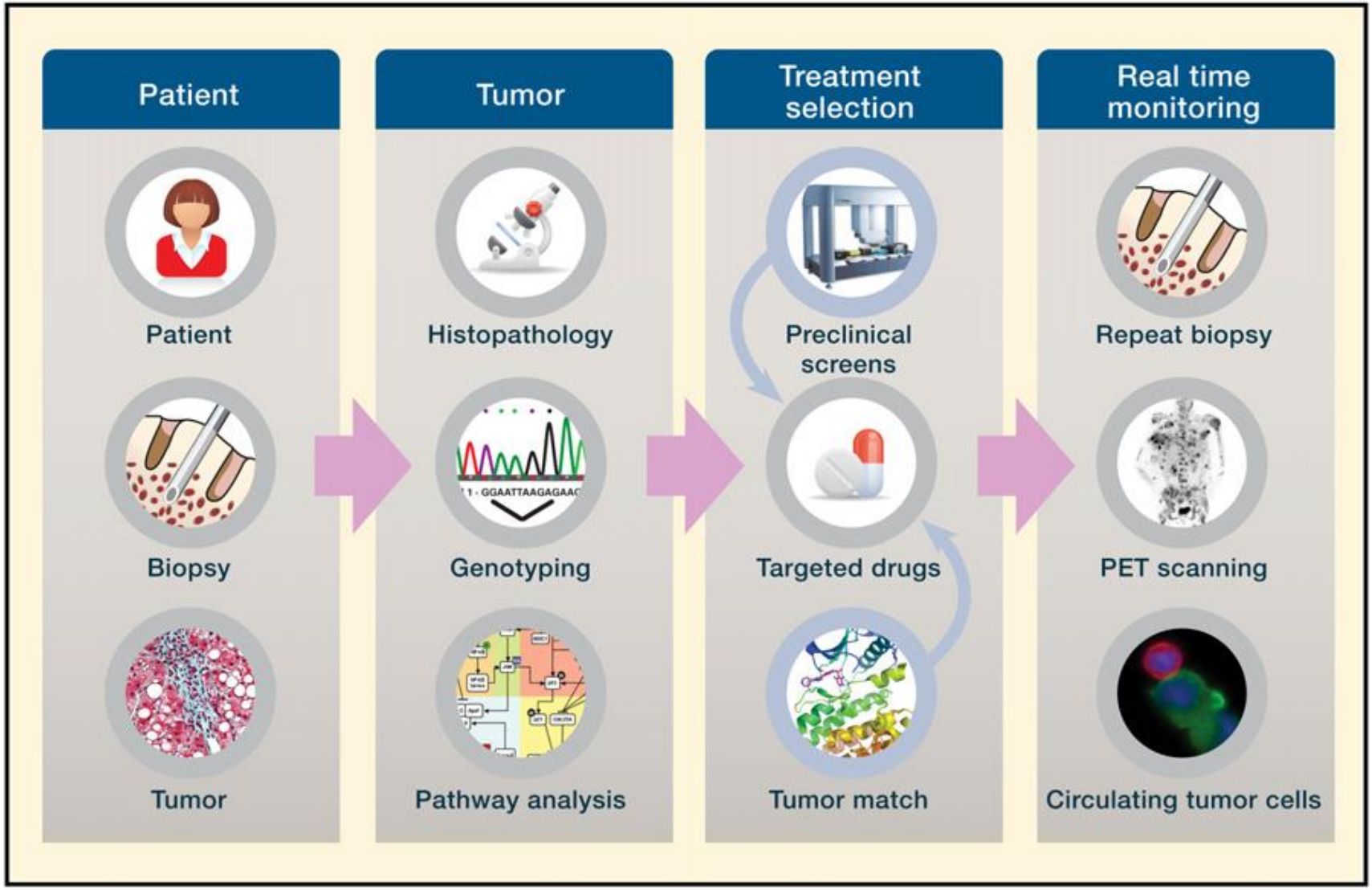
연세의대 병리학교실
세브란스병원 병리과
심효섭

Contents

- Current status of molecular testing in lung cancer
- Single gene testing
- Panel testing: Next generation sequencing
- Liquid biopsy

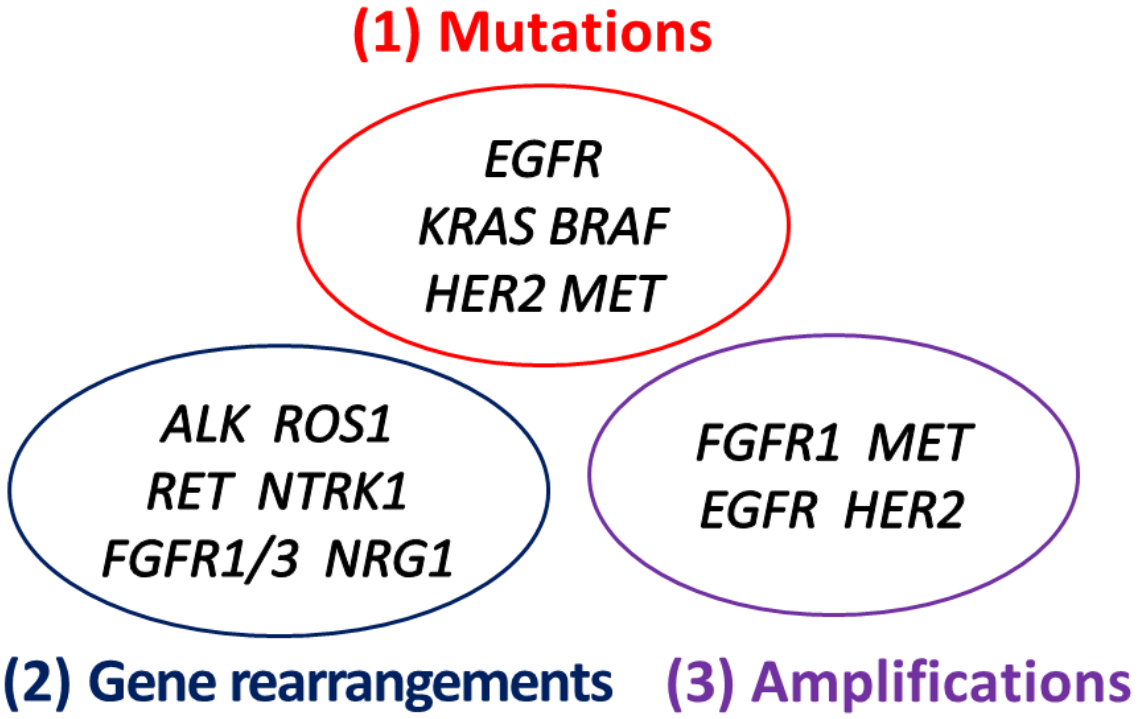
Overview

Matching Each Cancer with Individually Targeted Therapy



Essay: The Evolving War on Cancer *Cell* 2011;145:19-24

Three categories of targetable genetic alterations



J Pathol Transl Med. 2017; 51(3):242-254.

NCCN Guidelines Version 7.2017
Non-Small Cell Lung Cancer

NCCN Guidelines Index
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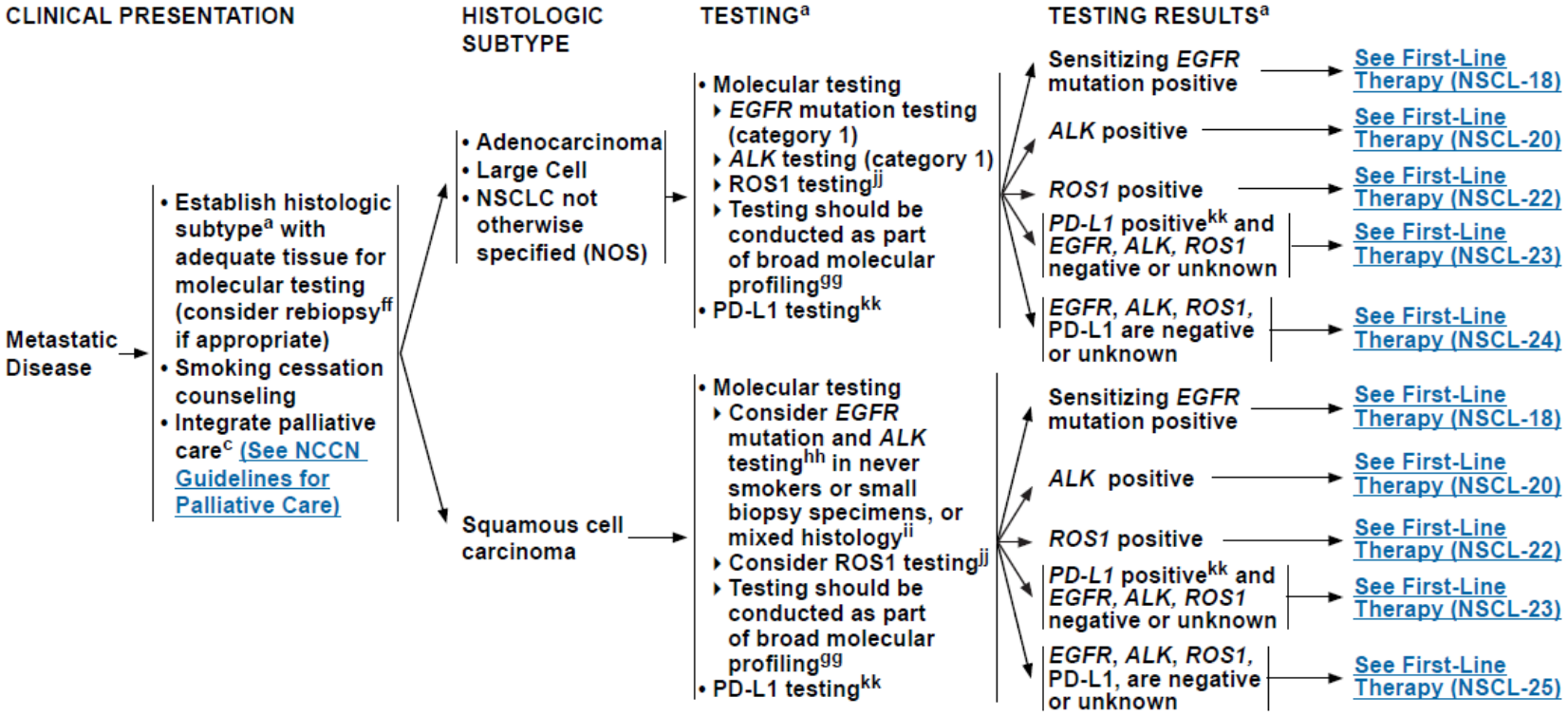
Discussion



[a] See Principles of Pathologic Review (NSCL-A).

[c] Temel JS, Greer JA, Muzikansky A, et al. Early palliative care for patients with metastatic non-small-cell lung cancer. N Engl J Med 2010;363:733-742.

[ff] If repeat biopsy is not feasible, plasma biopsy should be considered.

[gg] The NCCN NSCLC Guidelines Panel strongly advises broader molecular profiling with the goal of identifying rare driver mutations for which effective drugs may already be available, or to appropriately counsel patients regarding the availability of clinical trials. Broad molecular profiling is a key component of the improvement of care of patients with NSCLC. See Emerging Targeted Agents for Patients With Genetic Alterations (NSCL-H).

[hh] In patients with squamous cell carcinoma, the observed incidence of *EGFR* mutations is 2.7% with a confidence that the true incidence of mutations is less than 3.6%. This frequency of *EGFR* mutations does not justify routine testing of all tumor specimens. Forbes SA, Bharma G, Bamford S, et al. The catalogue of somatic mutations in cancer (COSMIS). Curr Protoc Hum Genet 2008;chapter 10:unit 10.11.

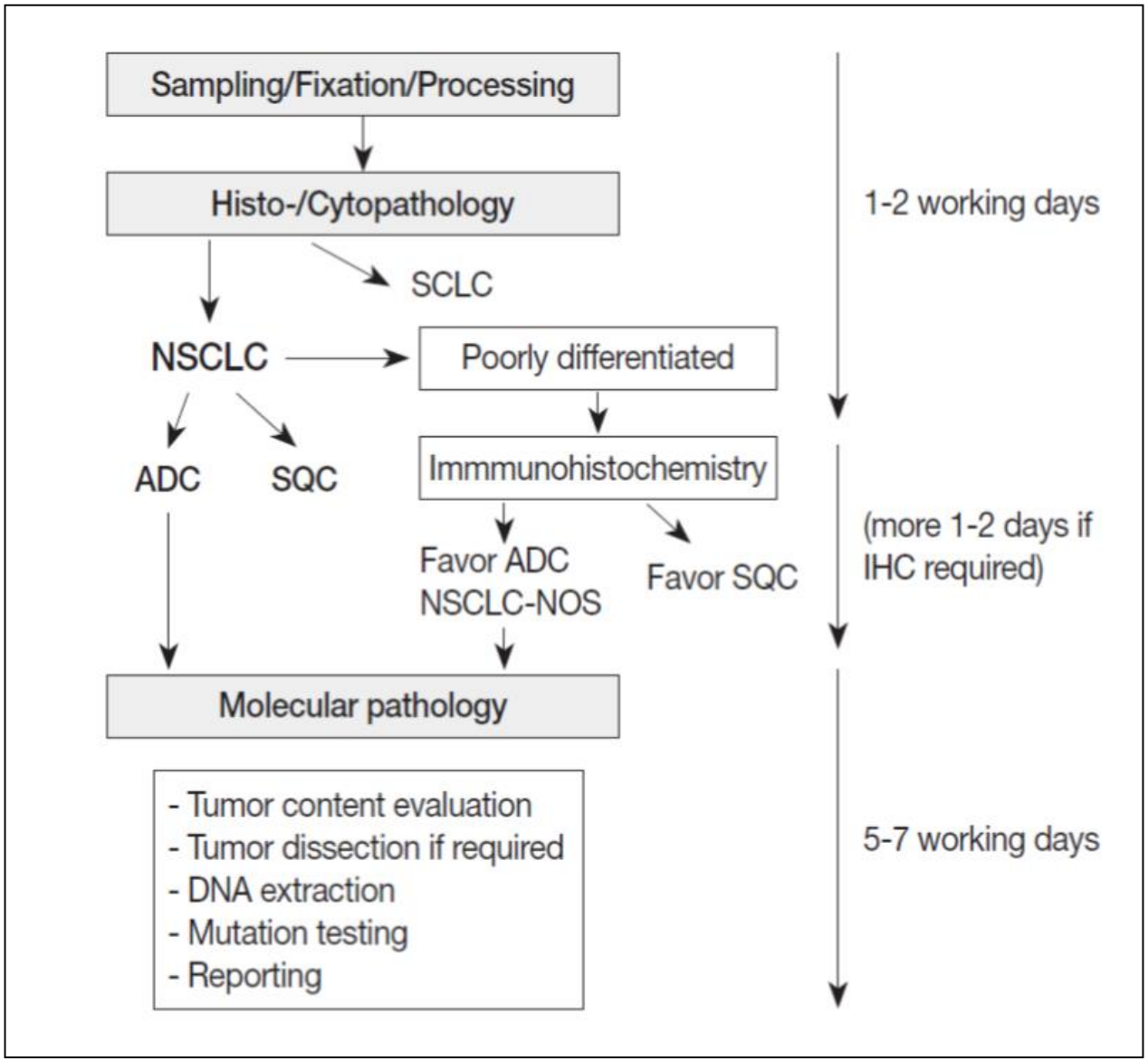
[ii] Paik PK, Varghese AM, Sima CS, et al. Response to erlotinib in patients with *EGFR* mutant advanced non-small cell lung cancers with a squamous or squamous-like component. Mol Cancer Ther 2012;11:2535-2540.

[jj] Shaw AT, Ou S-HI, Bang Y-J, et al. Crizotinib in ROS1-rearranged non-small cell lung cancer. N Engl J Med 2014;371:1963-1971.

[kk] PD-L1 expression levels of ≥50% are a positive test result for first-line pembrolizumab therapy.

Note: All recommendations are category 2A unless otherwise indicated.
Clinical Trials: NCCN believes that the best management of any patient with cancer is in a clinical trial. Participation in clinical trials is especially encouraged.

Integrative Pathologic Diagnosis



Korean journal of pathology 2013;47:100-6.

Sample types

- Tissue biopsy or resection: formalin-fixed paraffin-embedded (FFPE)
- Cytology: Aspirates, Washing, Fluid
- Liquid biopsy: Plasma

Biomarkers

- **DNA**
  - SNVs & Indels
  - Translocations/Fusions
  - Amplification/Copy number gains
- **RNA**
  - Fusion transcript
  - Splicing variants
  - Expression
- **Protein**
  - Overexpression
  - Aberrant expression

Methods

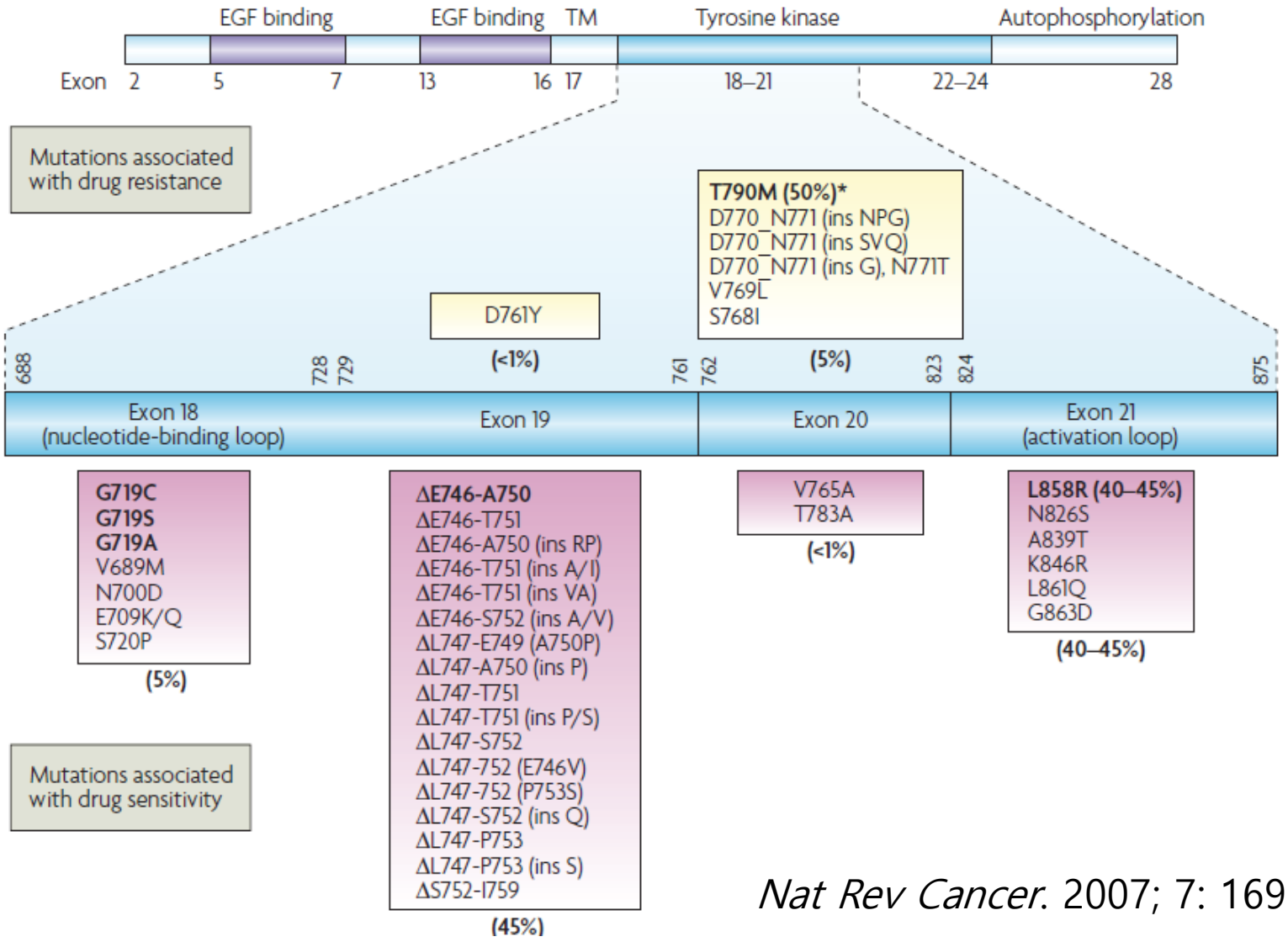
	Mutations	**Gene fusions**	**Amplifications**
DNA	Direct sequencing PCR-based method NGS	FISH NGS	FISH qPCR NGS
RNA		RT-PCR (fusion transcript) NGS	Real time PCR (mRNA overexpression)
Protein	IHC (Mutation-specific Ab)	IHC (protein expression)	IHC (protein overexpression)

Biomarkers + Methods

	Methods
***EGFR* mutations**	Direct sequencing Pyrosequencing PCR-based method (PNA clamping, cobas) PANA Mutyper
***ALK* or *ROS1* rearrangements**	Break apart FISH Immunohistochemistry RT-PCR Ventana ALK (D5F3) CDx Assay
Panel testing	Next generation sequencing (targeted DNA sequencing / RNA sequencing)
PD-L1 expression	PD-L1 IHC 22C3 pharmDx VENTANA PD-L1 (SP263) Assay

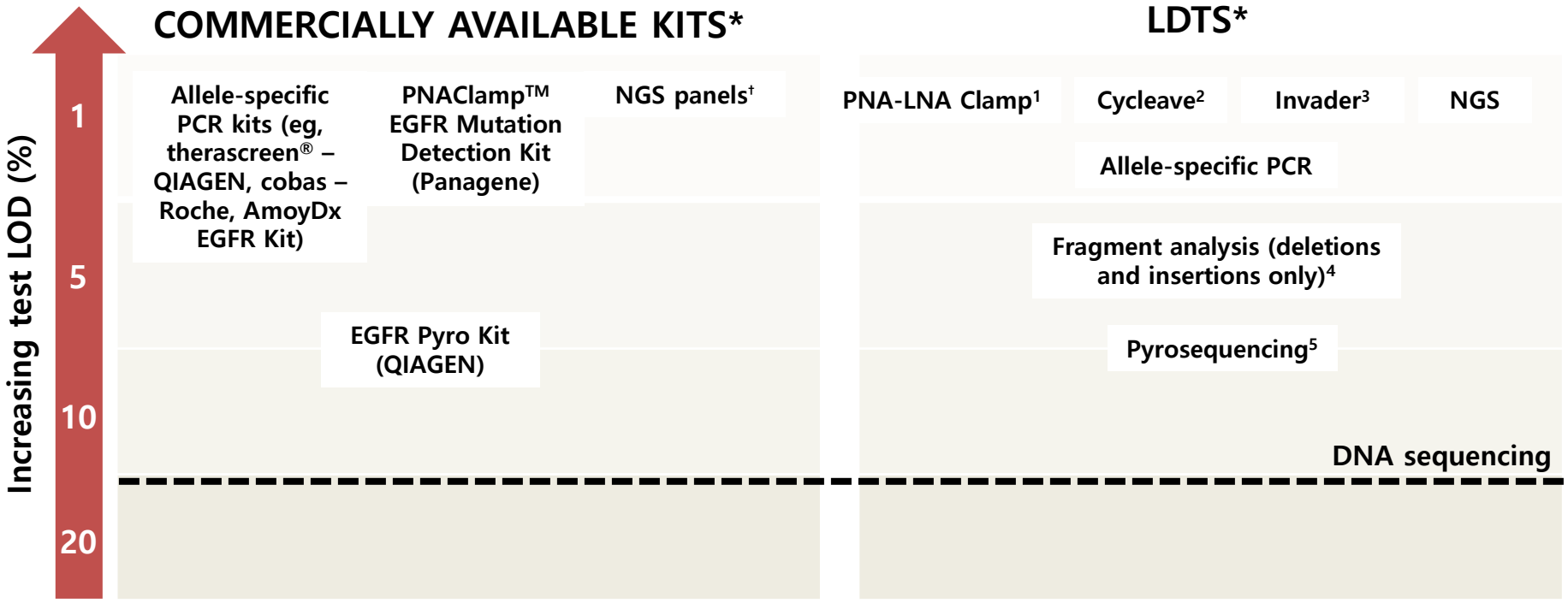
EGFR mutation testing

EGFR Mutations



Nat Rev Cancer. 2007; 7: 169-81.

EGFR Mutation Testing

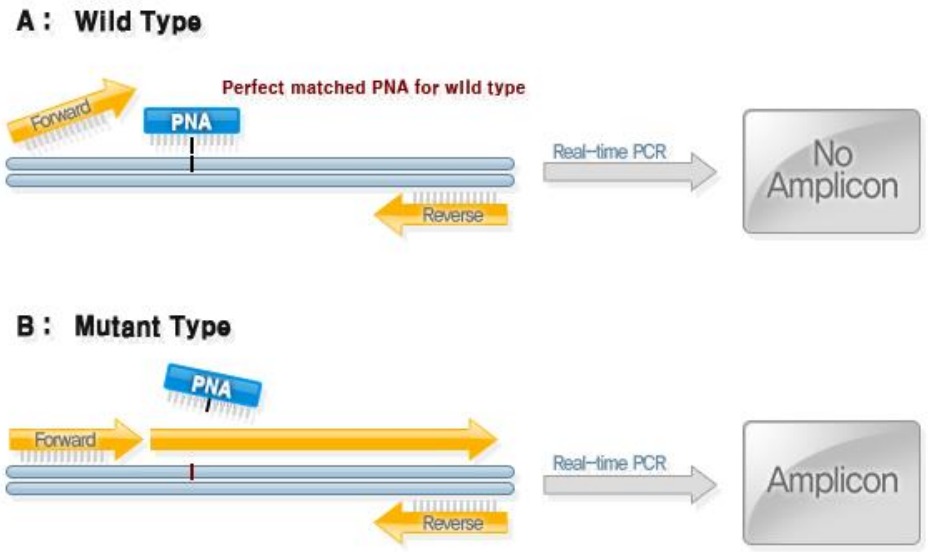


LOD, limit of detection.*This list is not comprehensive and only a selection of all available methods are listed.

†Eg, TruSight tumor sequencing panel, Illumina: GeneRead panels, Qiagen: Ion AmpliSeq cancer panels, Life Technologies.

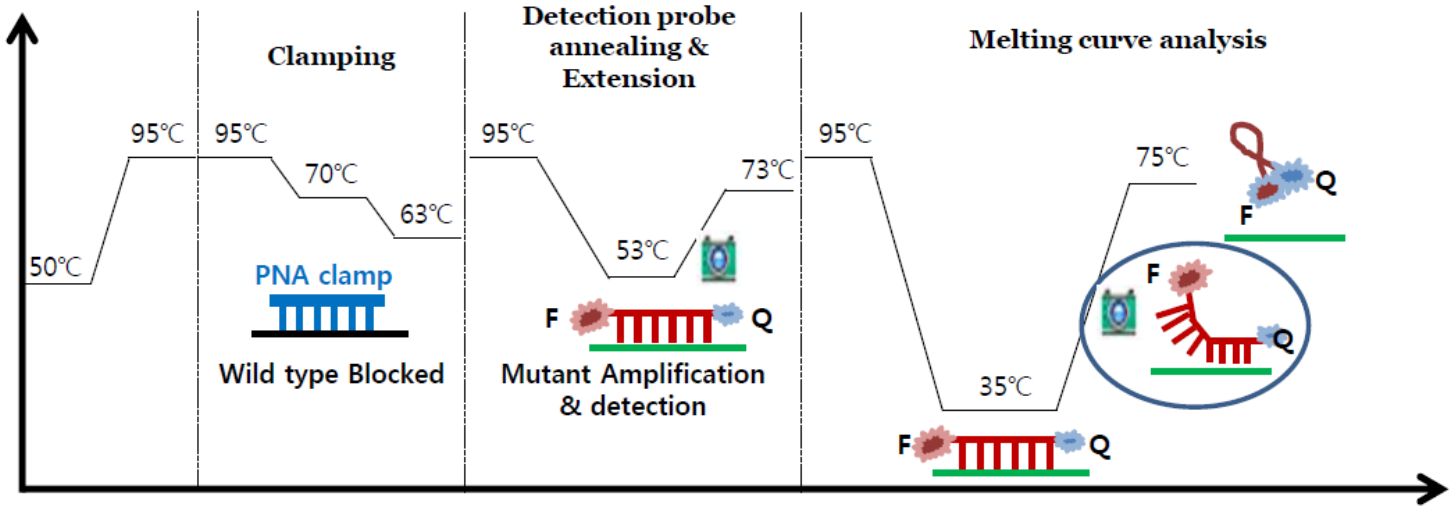
1. Nagai Y, et al. *Cancer Res.* 2005;65:7276–7282. 2. Yatabe Y, et al. *J Mol Diag.* 2006;8:335–341. 3. Naoki K, et al. *Int J Clin Oncol.* 2011;16:335–344. 4. Molina-Vila, M et al. *J Thorac Oncol.* 2008;3:1224–1235. 5. Dufort S, et al. *J Exp Clin Cancer Res.* 2011;30:57.

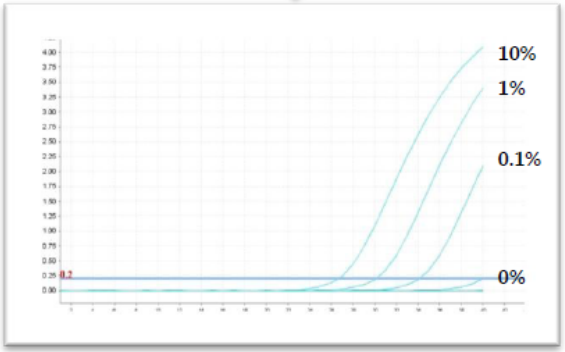
PNA-mediated Real-Time PCR Clamping (PNAClamp)

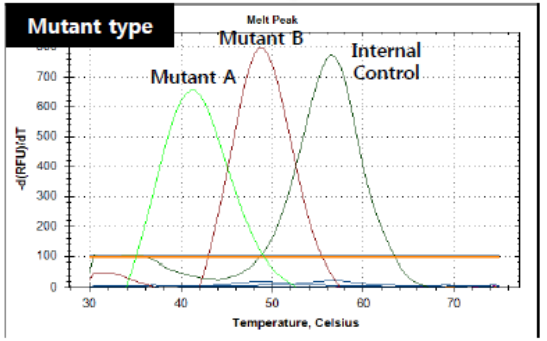


PANAMutyper™

- ✓ A novel technology that integrates PNAClamp™ and PANA S-Melting™
- ✓ Sensitivity : 0.1~ 0.01% & Genotyping

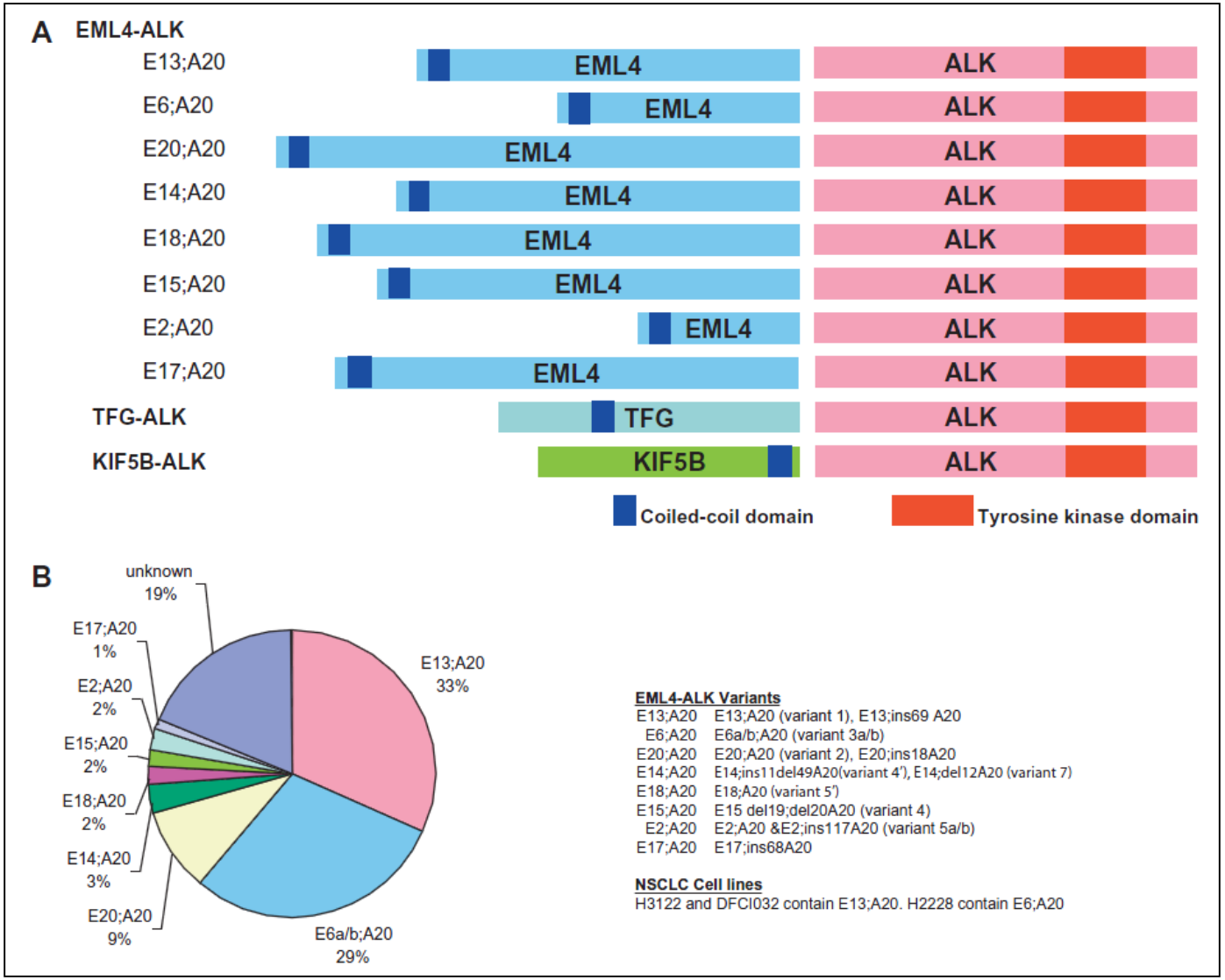






ALK or ROS1 rearrangement testing

Variants of *ALK* fusions



Eur J Cancer 2010;46:1773-1780

Vysis ALK Break Apart FISH Probe Kit

Product Description

Intended Use

The Vysis ALK Break Apart FISH Probe Kit is intended to detect rearrangements involving the ALK gene via fluorescence in situ hybridization (FISH) in formalin-fixed paraffin-embedded (FFPE) non-small cell lung cancer (NSCLC) tissue specimens.

Reduce variability with ready-to-use components

- Premixed, optimized probes
- ALK positive control slides
- ALK negative control slides
- Ready-to-use slide preparation reagents

ALK FISH criteria

Table 1
Classification of Cells as Positive or Negative

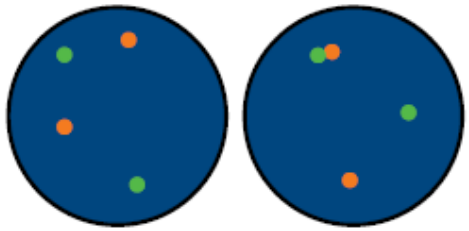
Signal Profile	No. of Adjacent or Fused Signals	No. of Single Orange Signals	No. of Single Green Signals	Cell Classification
1A, 1B	≥ 1	0	0	Negative
1C	≥ 1	0	≥ 1	Negative
2A, 2B, 2D	≥ 0	≥ 1	≥ 1	Positive
2C	≥ 1	≥ 1	0	Positive

51. Determine the number of cells classified as negative.
52. Determine the number of cells classified as positive.
53. A sample is considered negative if <5 cells out of 50 (5/50 or<10%) are positive.
54. A sample is considered positive if >25 cells out of 50 (25/50 or >50%) are positive.
55. A sample is considered equivocal if 5-25 cells (10-50%) are positive. If the sample is equivocal, a second reader should evaluate the slide.
    - The first and second cell count readings are added together and a percent is calculated out of 100 cells (average percent of positive cells).
    - If the average percent positive cells is <15% (15/100), the sample is considered negative.
    - If the average percent positive cells is 15% (15/100) or more, the sample is considered positive.

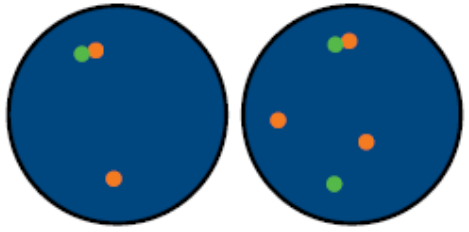
Signal Profile 2: Positive

Panel 2: Broken apart or deleted green

2A. 2B.



2C. 2D.



These nuc
rearranged
signals, 2 c
eters apart

A. A nucle
than one s
signals.

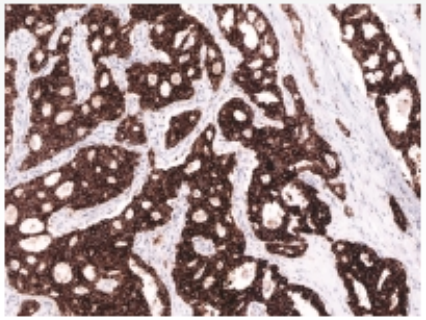
B. A nucle
signal(s) a
signal(s).

C. A nucle
single oran
green signa
fused and/
signals. N
with signal
should not

D. The san
have fused
apart signa

ALK IHC kit

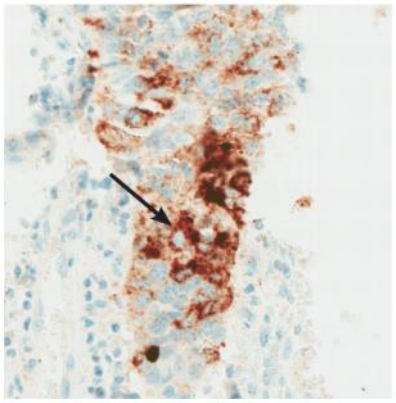
VENTANA ALK (D5F3) CDx Assay



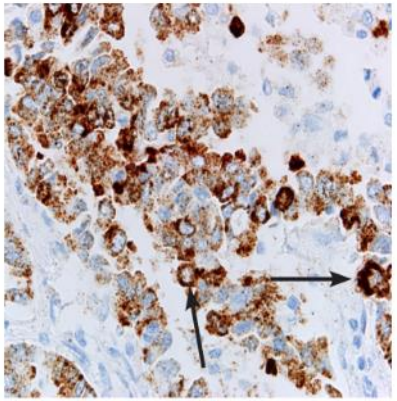
Catalog Number:	790-4796
Ordering Code:	06687199001
Quantity:	50 tests
Controls:	Appendix
Isotypes:	IgG
Clone Name:	D5F3
Species:	Rabbit Monoclonal
Localization:	Cytoplasmic
Regulatory Status:	IVD, FDA Approved (PMA)

VENTANA ALK (D5F3) CDx Assay is intended for the qualitative detection of the anaplastic lymphoma kinase (ALK) protein in formalin-fixed, paraffin-embedded (FFPE) non-small cell lung carcinoma (NSCLC) tissue stained with a BenchMark XT automated staining instrument. It is indicated as an aid in identifying patients eligible for treatment with XALKORI® (crizotinib).

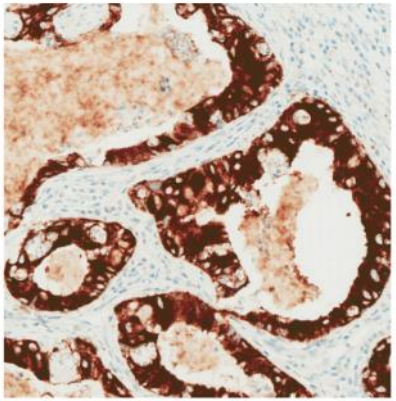
Clinical Diagnosis Positive



Few strong cytoplasmic staining tumor cells

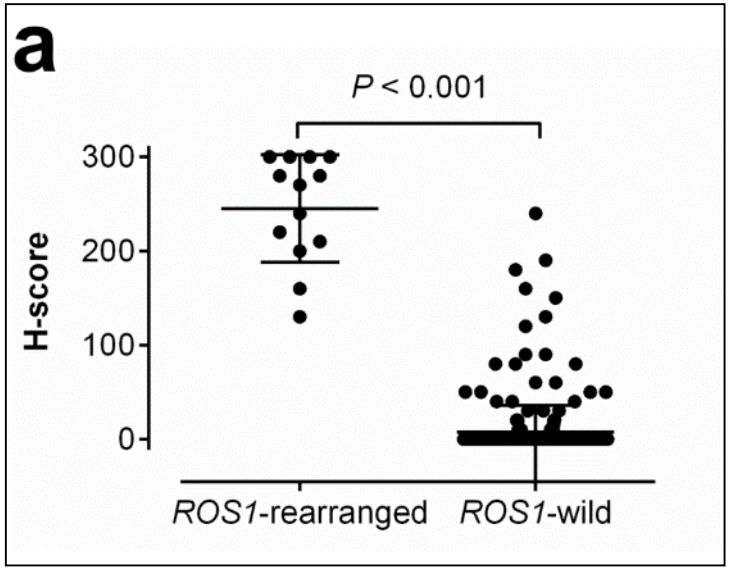


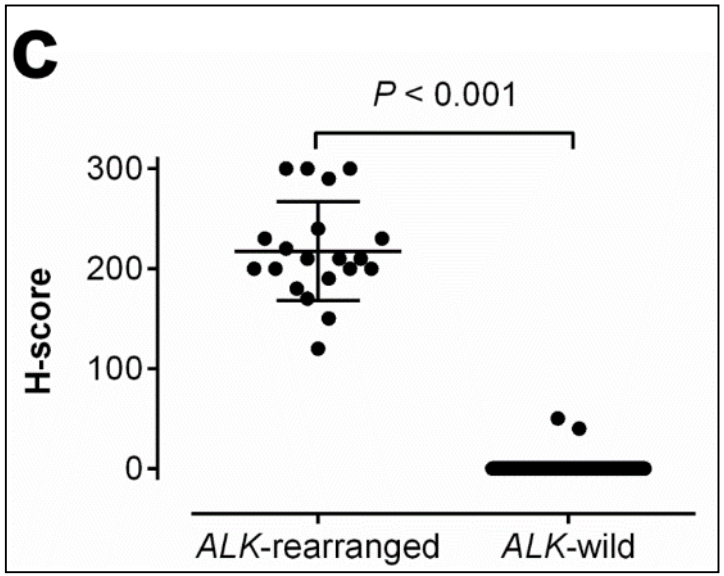
Strong cytoplasmic staining tumor cells

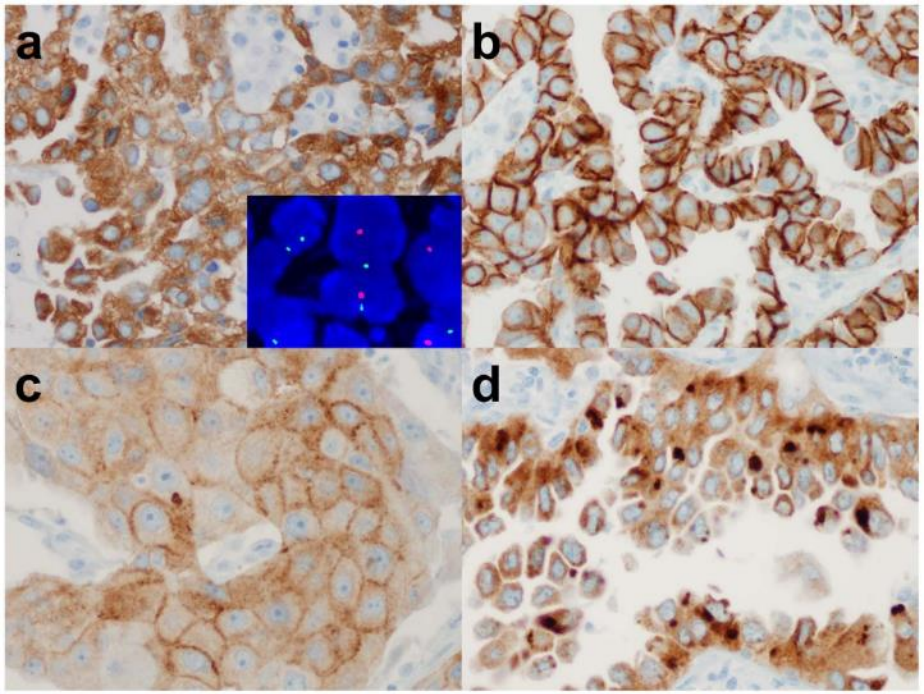


Homogeneously strong cytoplasmic staining within tumor cells

ROS1 IHC / FISH

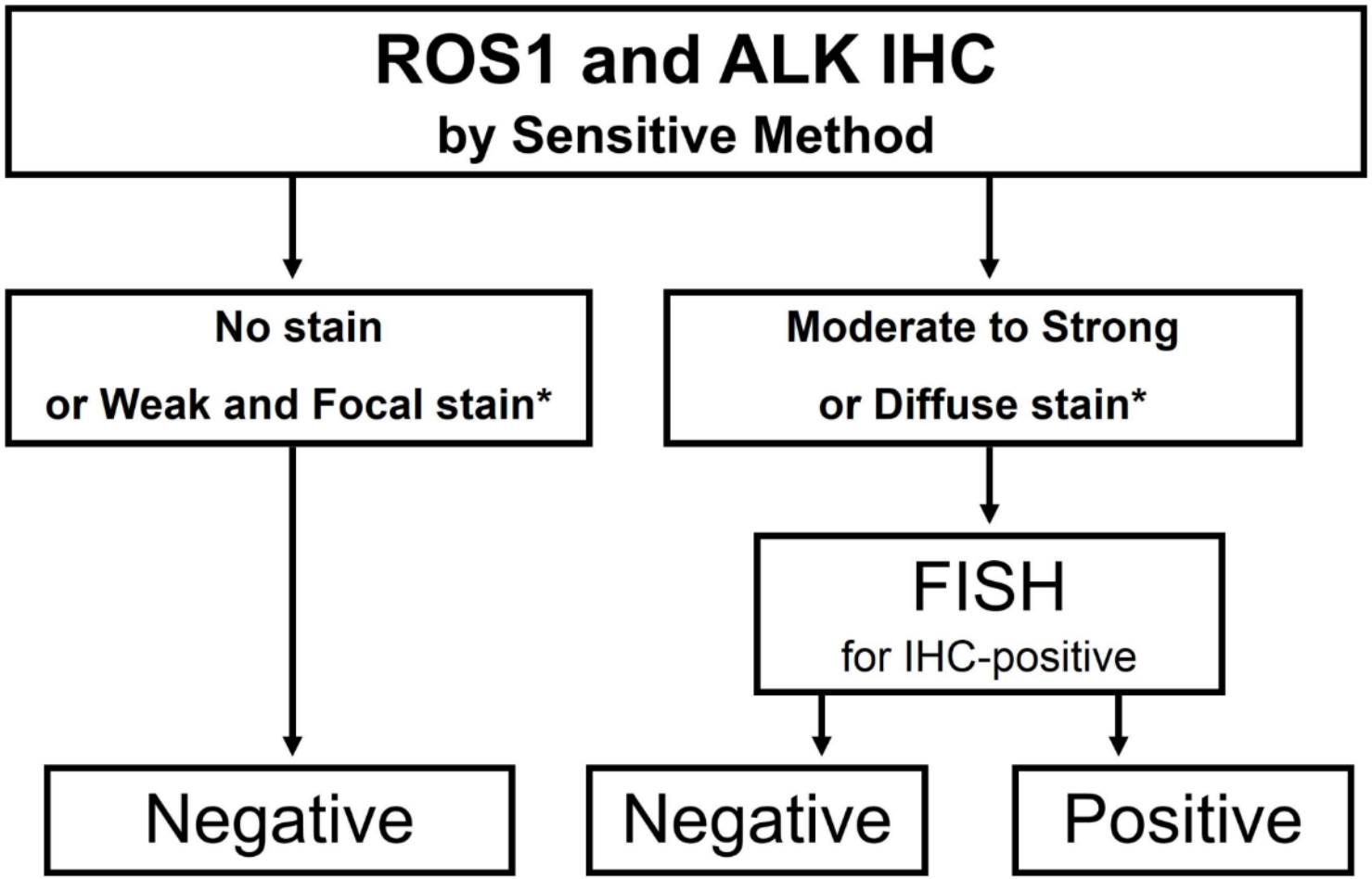






Cha YJ, *et al*. PLoS One 2014;9:e103333.

IHC screening



Cha YJ, *et al*. PLoS One 2014;9:e103333.

Korean Guidelines

The Korean Journal of Pathology 2013; 47: 100-106
http://dx.doi.org/10.4132/KoreanJPathol.2013.47.2.100

■ REVIEW & PERSPECTIVE ■

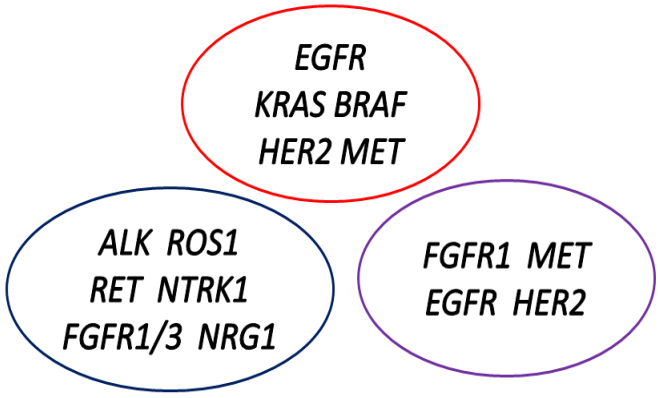
Guideline Recommendations for *EGFR* Mutation Testing in Lung Cancer: Proposal of the Korean Cardiopulmonary Pathology Study Group

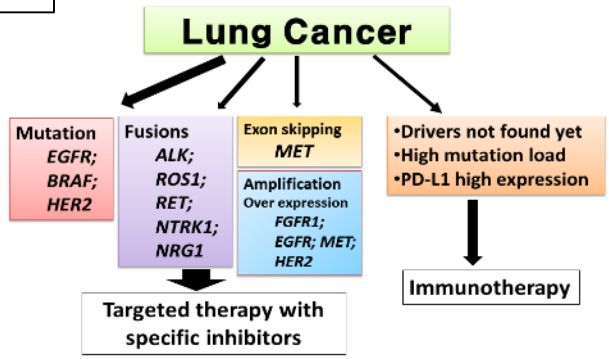
The Korean Journal of Pathology 2014; 48: 1-9
http://dx.doi.org/10.4132/KoreanJPathol.2014.48.1.1

■ REVIEW ■

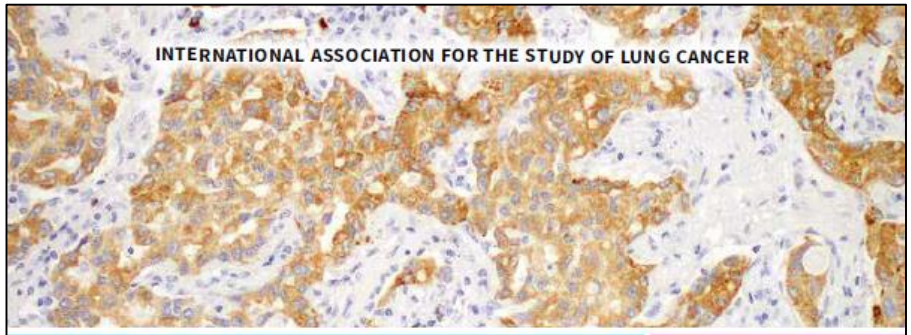
Guideline Recommendations for Testing of *ALK* Gene Rearrangement in Lung Cancer: A Proposal of the Korean Cardiopulmonary Pathology Study Group

Molecular testing of lung cancers





J Pathol Transl Med. 2017; 51(3):242-254.

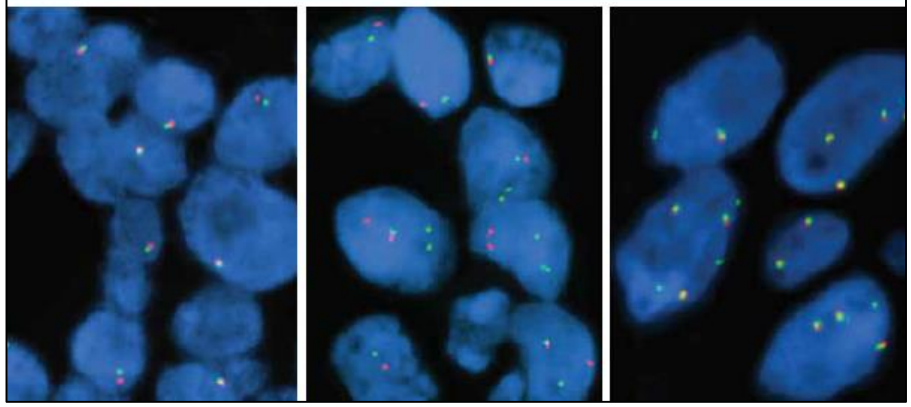


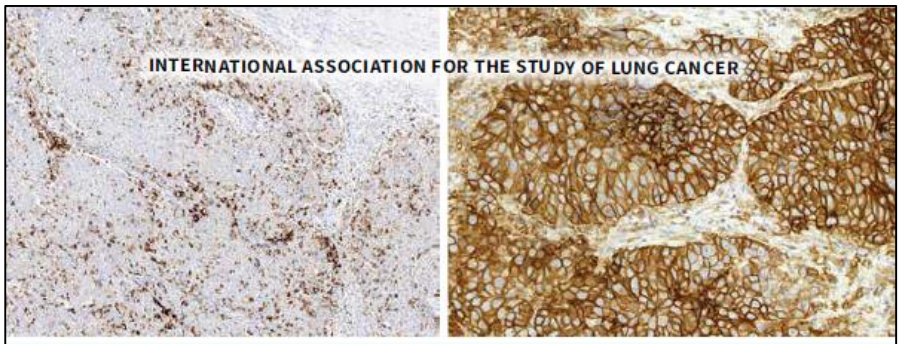
SECOND EDITION

IASLC ATLAS OF ALK AND ROS1 TESTING IN LUNG CANCER

IASLC
Conquering Thoracic Cancers Worldwide

EDITED BY
MING SOUND TSAO, MD, FRCPC
FRED R. HIRSCH, MD, PHD
YASUSHI YATABE, MD, PHD





IASLC ATLAS OF PD-L1 IMMUNOHISTOCHEMISTRY TESTING IN LUNG CANCER

IASLC
Conquering Thoracic Cancers Worldwide

EDITED BY
MING SOUND TSAO, MD, FRCPC
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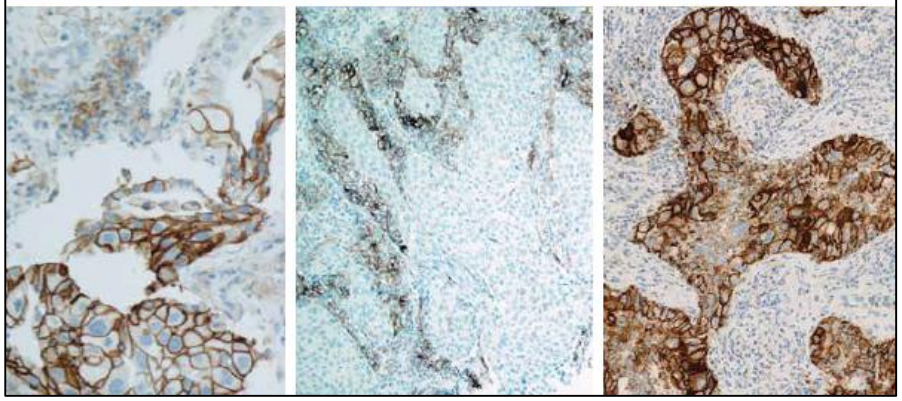
YASUSHI YATABE, MD, PHD
FRED R. HIRSCH, MD, PHD



NGS cancer panel testing

보건복지부 고시 제2017-15호, 25호

*시설, 인력, 장비, 유전자패널에 대한 요건을 충족하여 사전에 승인

시설	1)「생명윤리 및 안전에 관한 법률」제49조에 따른 유전자검사 기관으로 신고된 **요양기관**이면서, 2) 한국유전자검사평가원의 "**유전자검사 정확도 평가"를 3회 이상** 받은 이력이 있고, 승인신청 직전 평가 결과 '**A'등급**(우수)인 기관
인력	1) **전문의 자격 취득 후 5년 이상**의 경험이 있는 **병리과** 또는 진단검사의학과 **전문의** 1인 이상 상근하고, 2) 검사 실시인력(임상병리사) 1인 이상이 상근해야 함.
장비	1) 식품의약품안전처장('이하 식약처') **허가 또는 신고**를 받은 '차세대염기서열분석장비'를 사용하거나, 2)「식약처 NGS 임상검사실 인증」요양기관의 경우에는 식약처 허가 또는 신고를 받지 않은 '유전자서열검사장비' 사용을 인정

보건복지부 고시내용: 고형암

***NGS기반 유전자 패널 검사는 현재 '조건부 선별급여'**

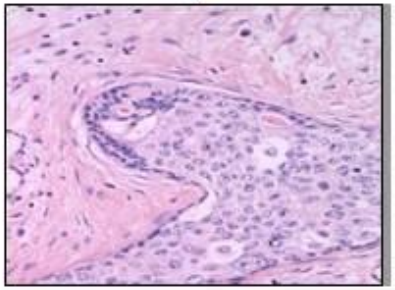
고형암 급여 대상 질환	**위암, 폐암, 대장암, 유방암, 난소암, 흑색종, 위장관 기질종양, 뇌척수의 악성종양, 소아신경모세포종, 원발불명암**
필수 유전자	HER2, EGFR, ALK, KRAS, NRAS, BRAF, BRCA1, BRCA2, KIT, PDGFRA, IDH1, IDH2, MYC(C-myc), N-myc(MYCN)
수가 산정 방법	Level Ⅰ : 유전자수 5~50개 이거나 유전자 길이 150kb 이하인 경우 LevelⅡ : 유전자수 51개이상 이거나 유전자 길이 150kb 초과한 경우
인정횟수	진단시 1회 인정을 원칙으로 함. 다만, 재발 및 치료불응 시에 한하여 추가 1회를 인정함
동의서	보건복지부 고시 제 2017-25호에 의하면 NGS 검사 처방시 "인체유래물 기증동의서"를 받도록 권고하고 있습니다. 작성된 동의서는 EMR에 등록하여 주시고 사본 1부를 환자에게 제공하시기 바랍니다.

병리과 'NGS 검사' 흐름도

암 환자

임상 병력 및 정보

조직 및 세포 검사

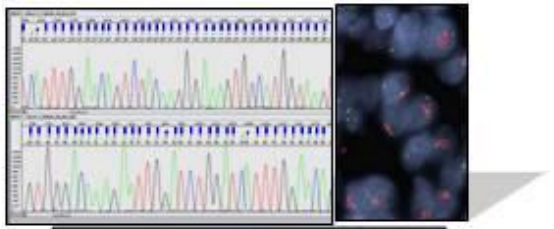


병리 진단

대상자 슬라이드 검색

NGS 검사

분석 결과 검증



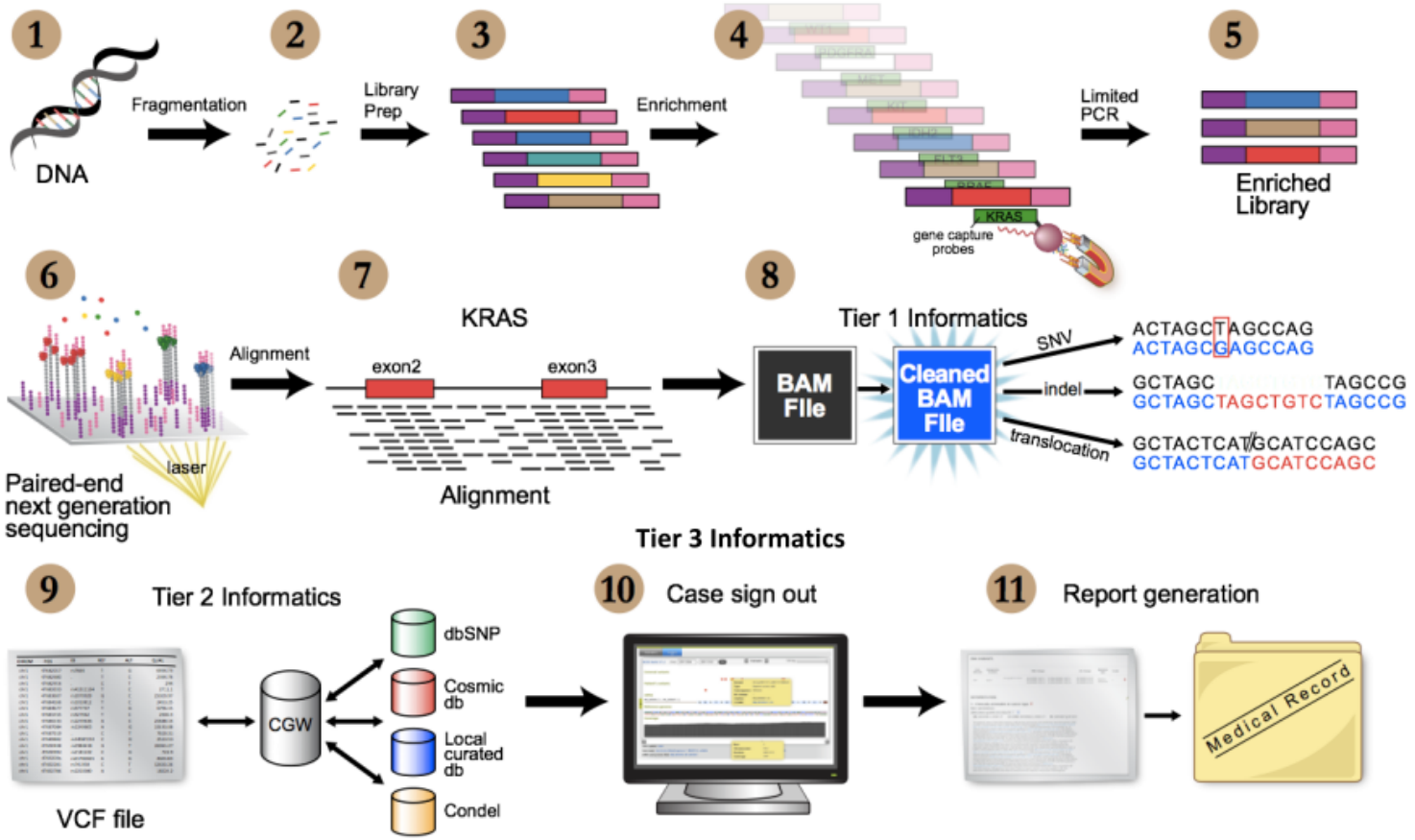
Direct Seq, FISH, IHC

최종 결과 보고

약물 선택 및 치료 결정

Clinical NGS

- Clinical NGS: Wet lab + Dry lab



Reference: Cottrell CE, et al. *J Mol Diagn* 2014;16:89-105

Things to consider in NGS testing

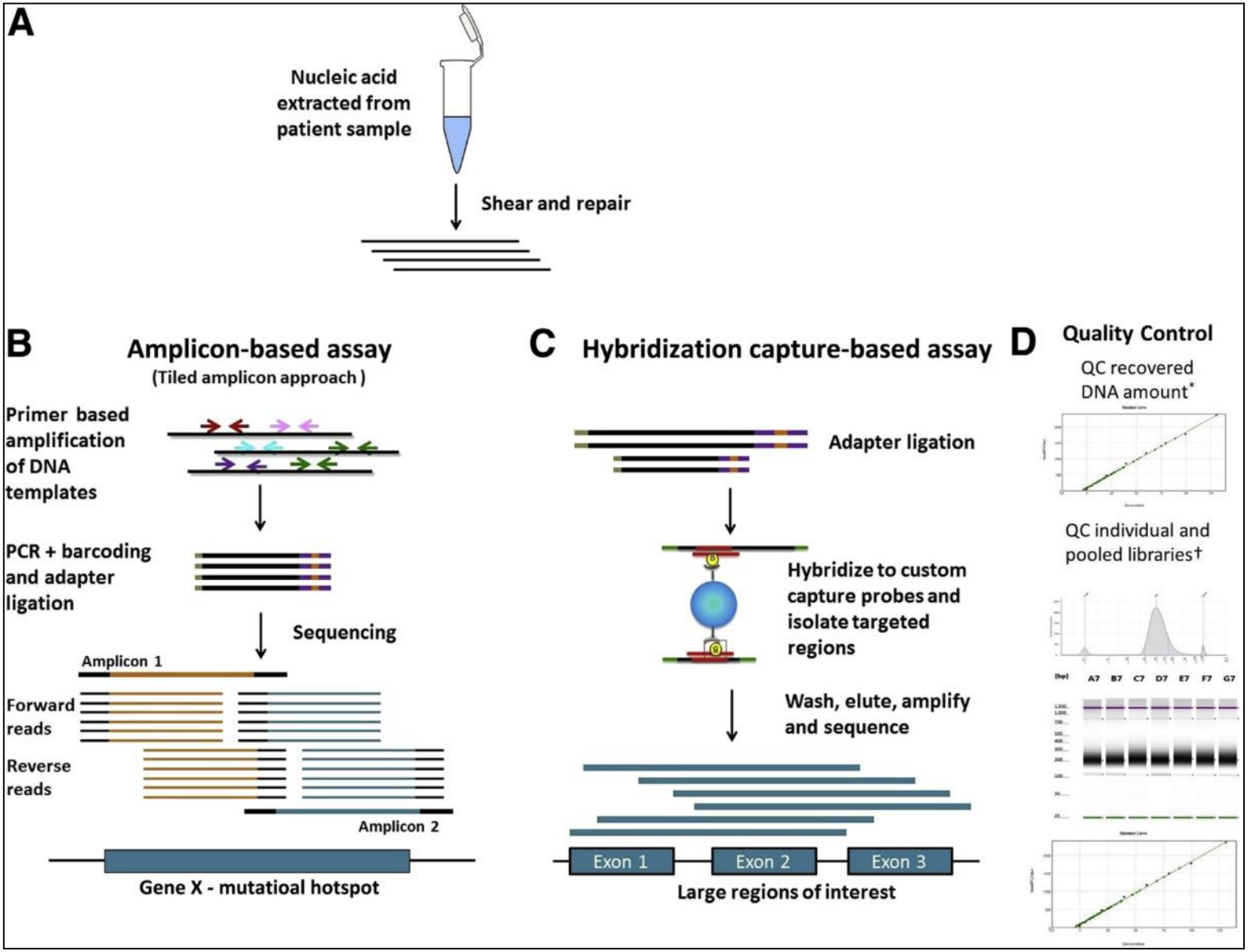
- Platform
- Panel type
  - Custom panel
  - Commercially available panel
- Panel scope
  - Genes / Exons / Hot spots
  - Types of genetic alterations
    - SNVs, Indels
    - Fusions
    - Amplifications (copy number gains)

Target Enrichment

- **Clinical targeted sequencing is focused on a particular gene or set of genes**

- **Targeted sequence enrichment methods**
  - **Hybridization-capture**
  - **PCR-based (Amplicon)**

Hybridization-capture vs. PCR-based



J Mol Diagn. 2017; 19(3):341-365

Hybridization-capture vs. PCR-based capture

	Hybridization	PCR-based
Pros	• High scalability (can target full exomes to small gene panels) • Allows for translocation and copy number variant detection • More uniform coverage • Can track duplicate reads	Rapid, high on-target efficiency
Cons	• Lower on-target efficiency (needs more input DNA) • Hybridization can be slow (1-2 days)	Can't track duplicate reads, PCR conditions largely influence effectiveness

NGS Data files

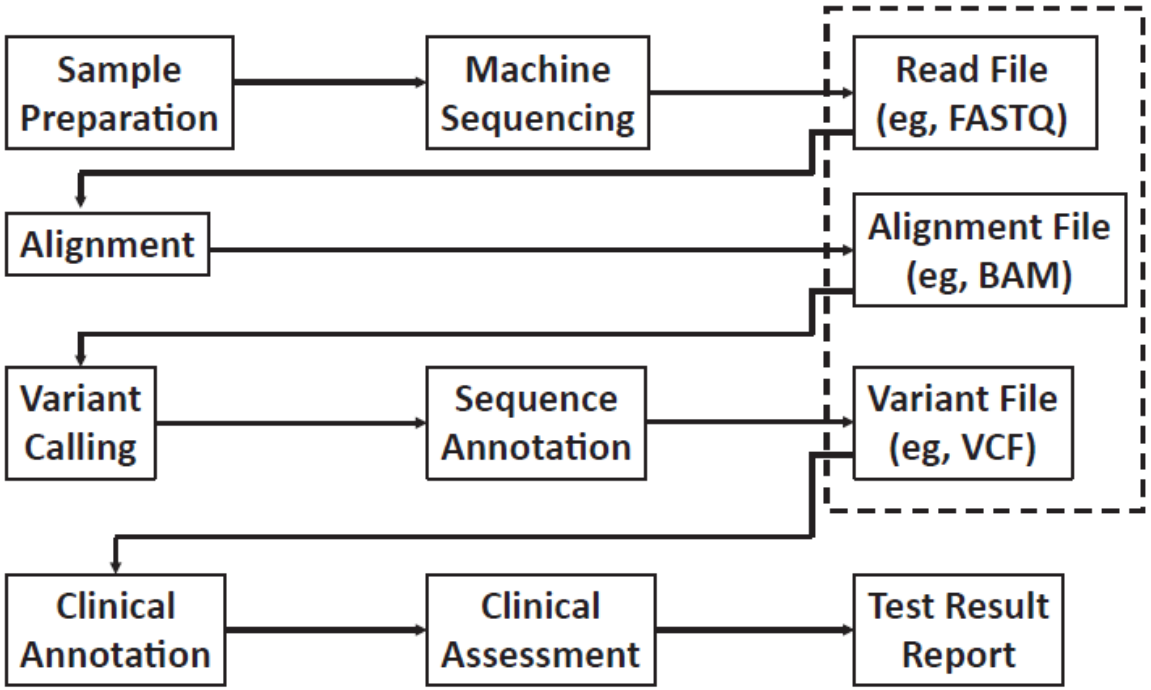


Figure 1 Next-generation sequencing workflow and associated data files (designated with a **dashed-line box**). Machine sequencing of the patient sample produces a large number of short reads deposited in a file with associated quality scores (eg, FASTQ). These reads are aligned to a reference assembly or sequence and the results are deposited in an alignment file (eg, BAM). Variants are called and their properties relevant to the sequence (eg, type of variant) are annotated and deposited in the variant file [eg, variant call format (VCF)]. The data in the variant file are further analyzed to determine what findings are clinically relevant and reportable to the physician to inform medical decision making.

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SPECIAL ARTICLE

Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer

A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists

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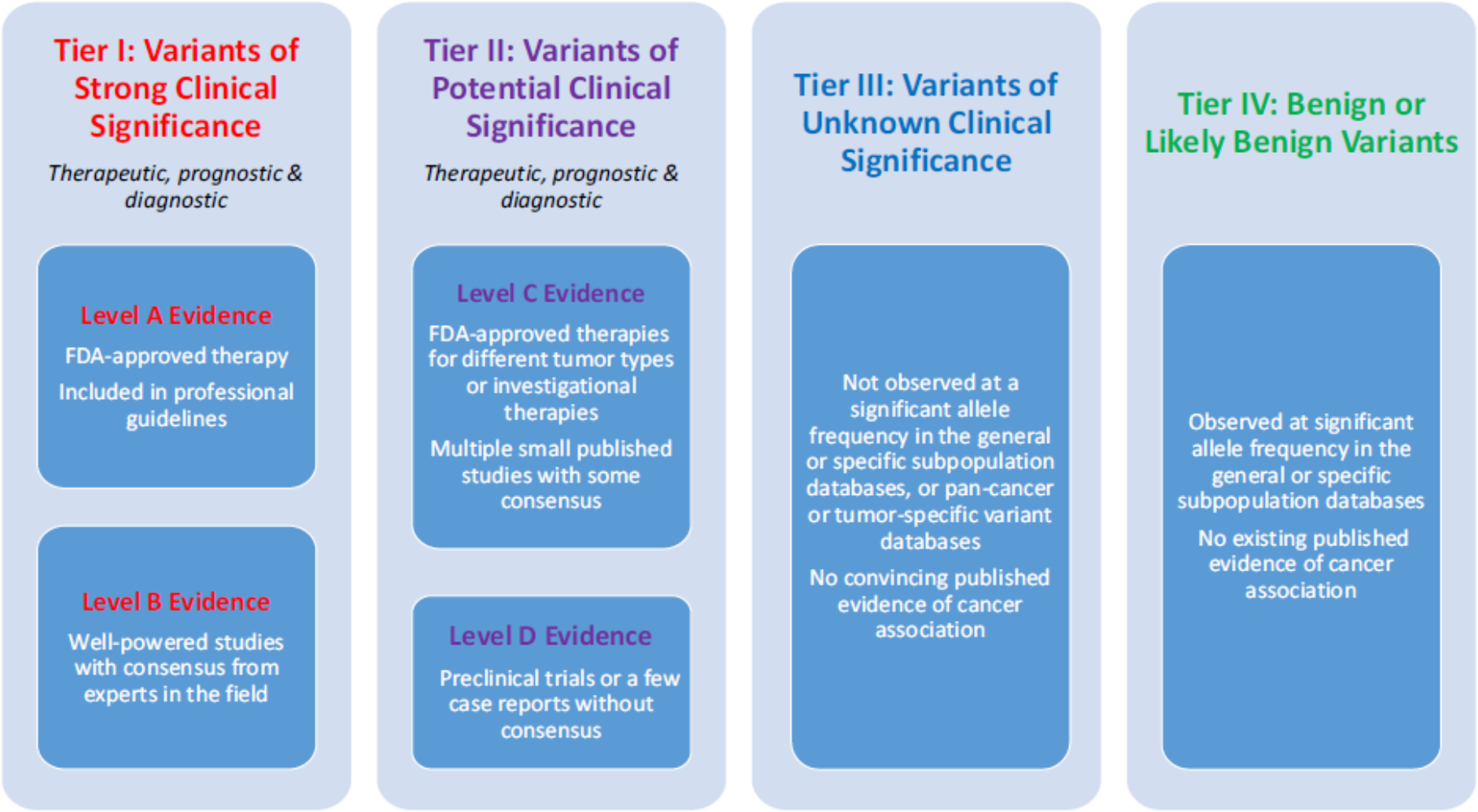
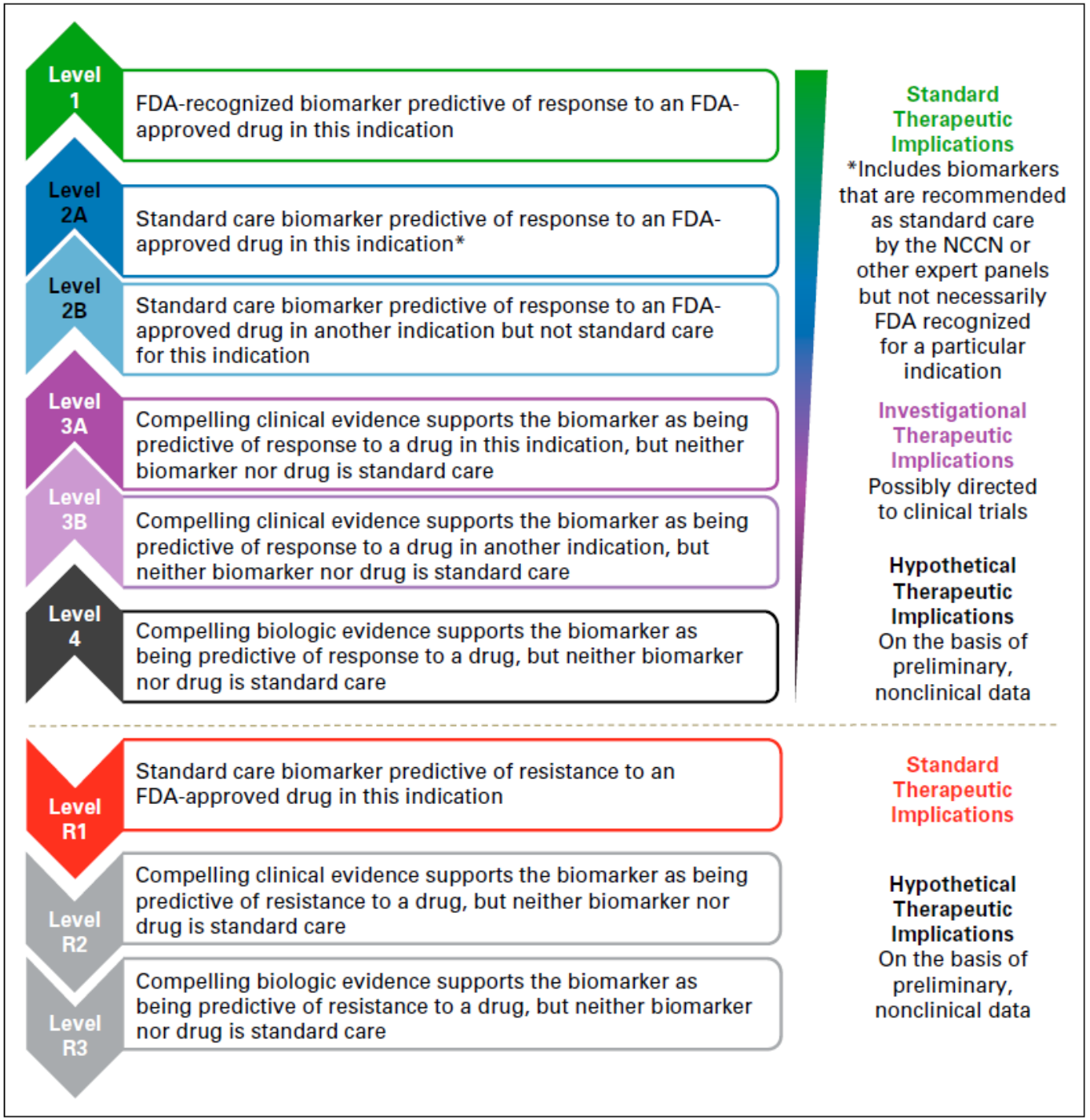
Widespread clinical laboratory implementation of next-generation sequencing–based cancer testing has highlighted the importance and potential benefits of standardizing the interpretation and reporting of molecular results among laboratories. A multidisciplinary working group tasked to assess the current

Evidence-based variant categorization



J Mol Diagn. 2017;19: 4-23.

- **OncoKB:**
  Levels of evidence



Korean Guidelines

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■ REVIEW ■

Good Laboratory Standards for Clinical Next-Generation Sequencing Cancer Panel Tests

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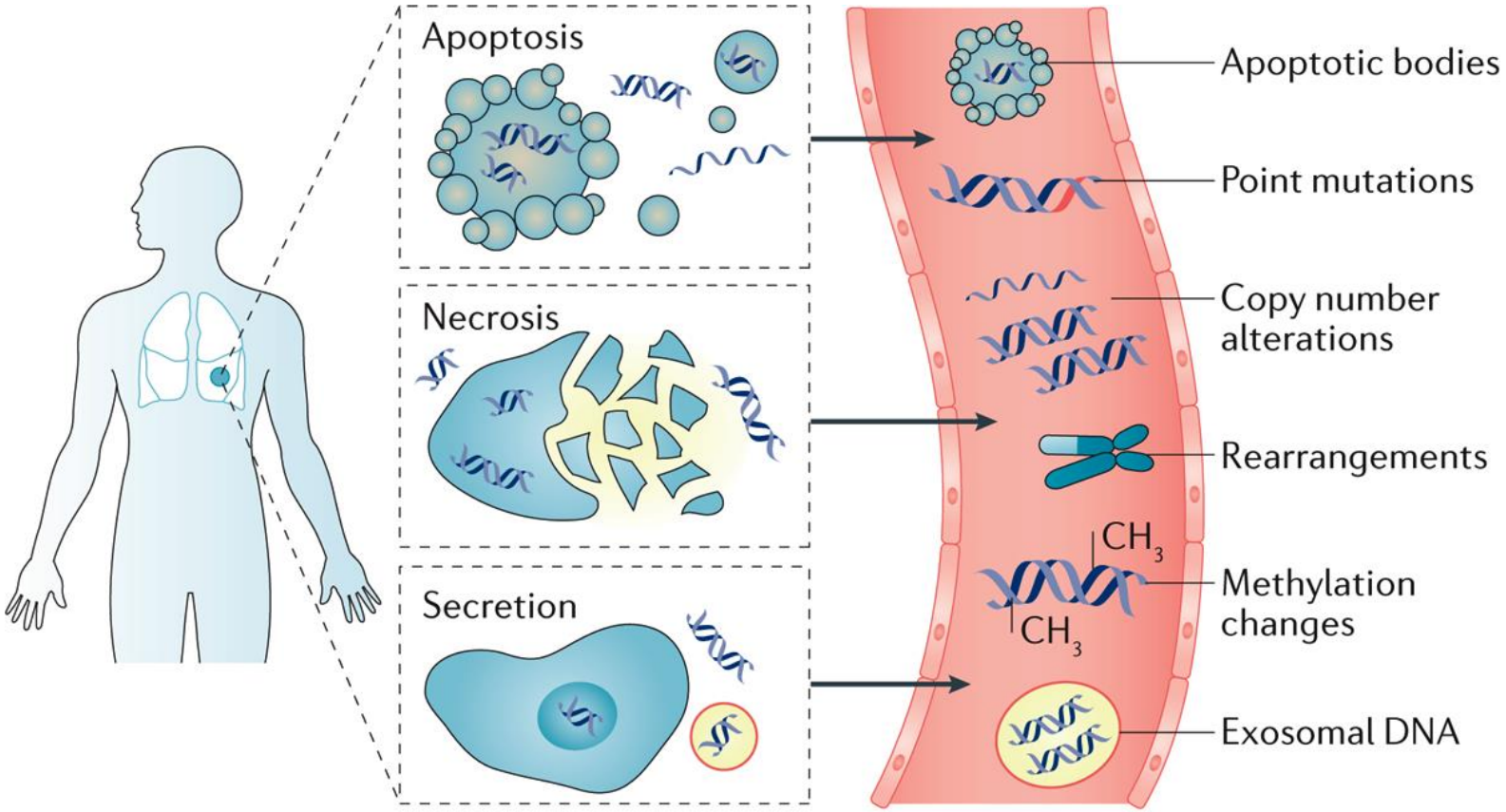
Next-generation sequencing (NGS) has recently emerged as an essential component of personalized cancer medicine due to its high throughput and low per-base cost. However, no sufficient guidelines for implementing NGS as a clinical molecular pathology test are established in Korea. To ensure clinical grade quality without inhibiting adoption of NGS, a taskforce team assembled by the Korean Society of Pathologists developed laboratory guidelines for NGS cancer panel testing procedures and requirements for clinical implementation of NGS. This consensus standard proposal consists of two parts: laboratory guidelines and requirements for clinical NGS laboratories. The laboratory guidelines part addressed several important issues across multistep NGS cancer panel tests including choice of gene panel and platform, sample handling, nucleic acid management, sample identity tracking, library preparation, sequencing, analysis and reporting. Requirements for clinical NGS tests were summarized in terms of documentation, validation, quality management, and other required written policies. Together with appropriate pathologist training and international laboratory standards, these laboratory standards would help molecular pathology laboratories to successfully implement NGS cancer panel tests in clinic. In this way, the oncology community would be able to help patients to benefit more from personalized cancer medicine.

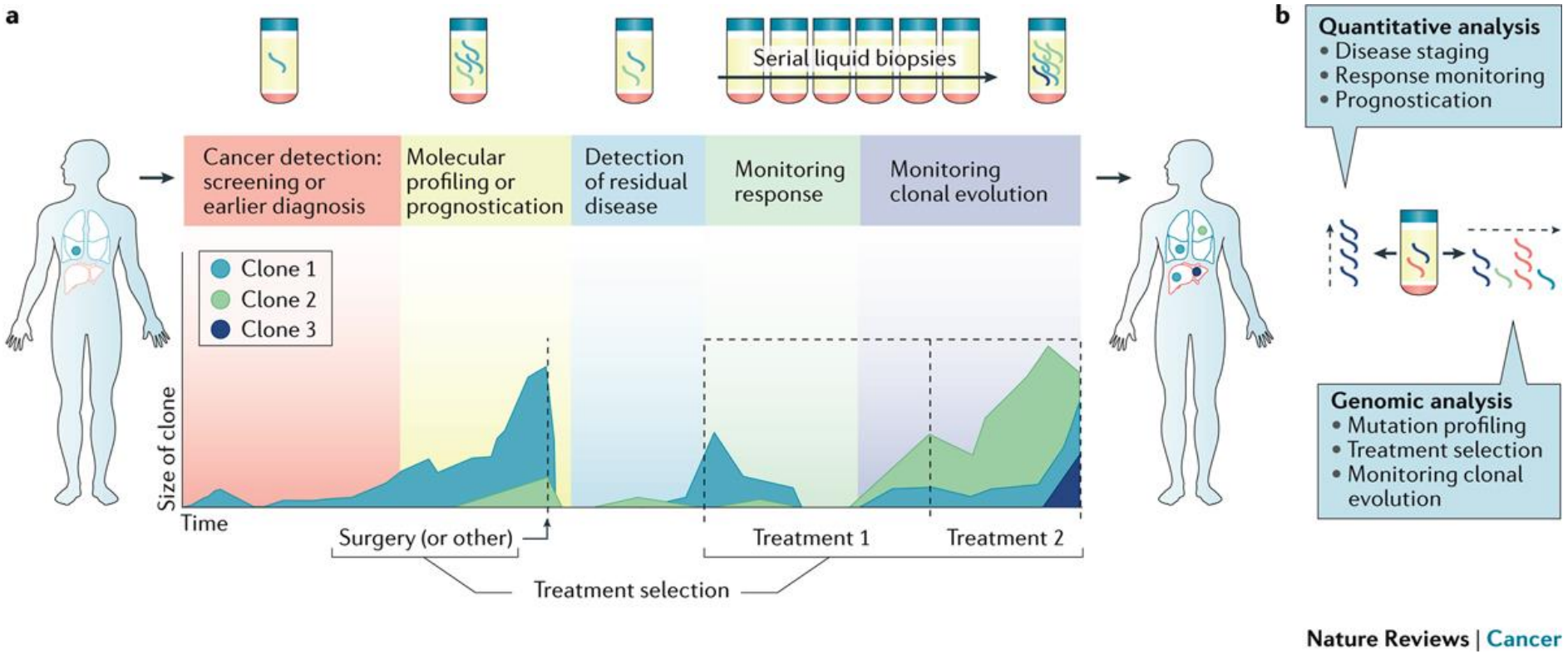
Liquid biopsy

Origins and range of alterations in cell-free DNA



Nature Reviews | Cancer

Applications of ctDNA analysis



*ctDNA: circulating tumor DNA

Comparison and utility of technology platforms for ctDNA analysis

Scale of analysis	Example technologies	Loci interrogated	Indicative limit of detection (mutant allele fraction or concentration)	Clinical utility
Single-locus or multiplexed assays	Microfluidic or allele-specific PCR: • Digital PCR[28,101,103,194] • BEAMing[29,30] • Intplex[3,122]	Microfluidic or allele-specific PCR: • 1–10 loci • Both ctDNA and cfDNA (Intplex)	Varies by method, optimal implementations can reach sensitivity of 0.001%–0.01% or individual mutant copies per millilitre[30,122,243,244]	• Detecting and quantifying recurrent hot-spot mutations • Monitoring for recurrent resistance mutations • Rapid turnaround time
	Enrichment for mutant alleles: • COLD-PCR[108] • SCODA[105,106] • NaME-PrO[107]	Enrichment for mutant alleles: 10–100 loci		
	Allele-specific or ARMS-PCR kits for companion diagnostics: • Cobas EGFR[99] • Therascreen EGFR[98]	• Cobas EGFR: 7 mutation assays covering multiple variants • Therascreen EGFR: 3 mutation assays covering multiple variants	Stated limit of detection (≥95% sensitivity): • Cobas EGFR: 25–100 copies per millilitre[99] • Therascreen EGFR: median 1.42% (range 0.05%–12.47% for different variants)[98]	Approved for *in vitro* diagnostic use: • Cobas EGFR: FDA approved • Therascreen EGFR: CE marked
Targeted sequencing approaches	Amplicon-based: • TAm-Seq[34] • Enhanced TAm-Seq[117] • Safe-SeqS[115]	10 kb to 50 Mb	• <0.01%–0.50% for purpose-built panels[34,111,114,115,117] • 1% for off-the-shelf multiplexed panels[45,112] • 5% for exome sequencing[39]	• Profiling gene panels • Monitoring for *de novo* resistance mutations • Monitoring clonal evolution in response to therapy • Sensitivity for disease burden can be increased by testing multiple loci in parallel (FIG. 4)
	Hybrid capture: • Exome sequencing[39] • CAPP-Seq[110,114] • Digital sequencing[111,118,185]			
Genome-wide	WGS: • Plasma-Seq[38] • PARE[197]	• 3.2 Gb (whole genome) • 21.6 kb unique to LINE-1 (REF. 184)	5%–10%[38]	• Identifying structural variants • Stratifying patient samples on the basis of disease burden • Detecting the presence of chromosomal aberrations
	Amplicon-based: • FAST-SeqS[184] • mFAST-SeqS[119]			

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Summary

- Molecular diagnostics for precision therapeutics have become the standard of care in the management of lung cancer patients
- As a result, molecular diagnostic technology is rapidly evolving to provide integrated and sensitive detection.