2nd International Congress on Oncological Sciences
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Liquid Biopsy for Precision Medicine in Mutation-Driven NSCLC

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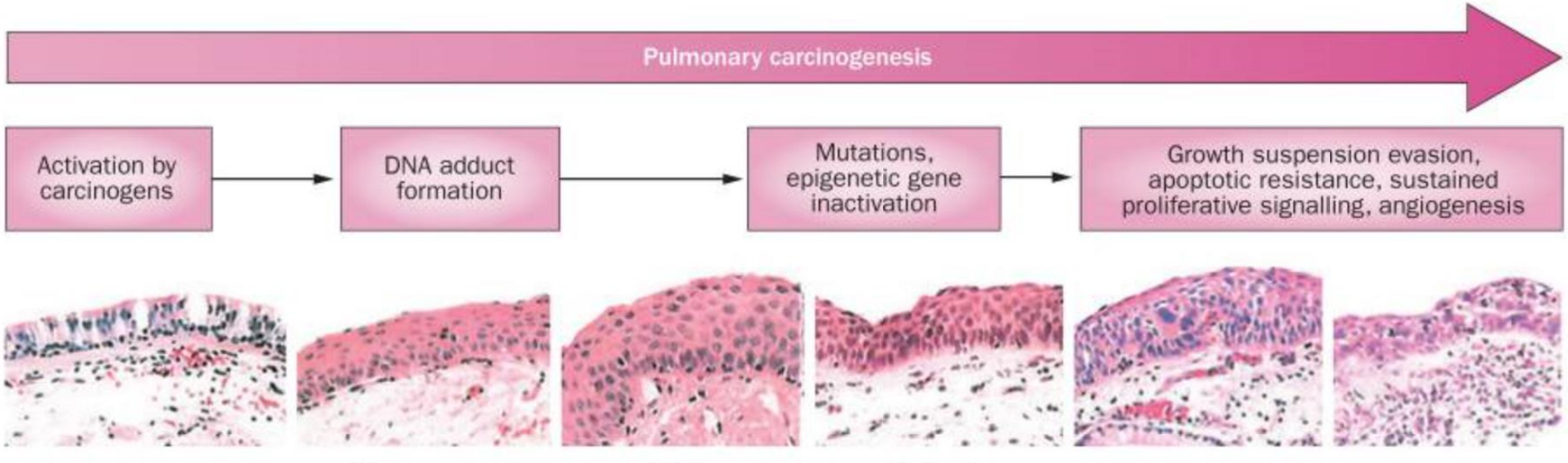
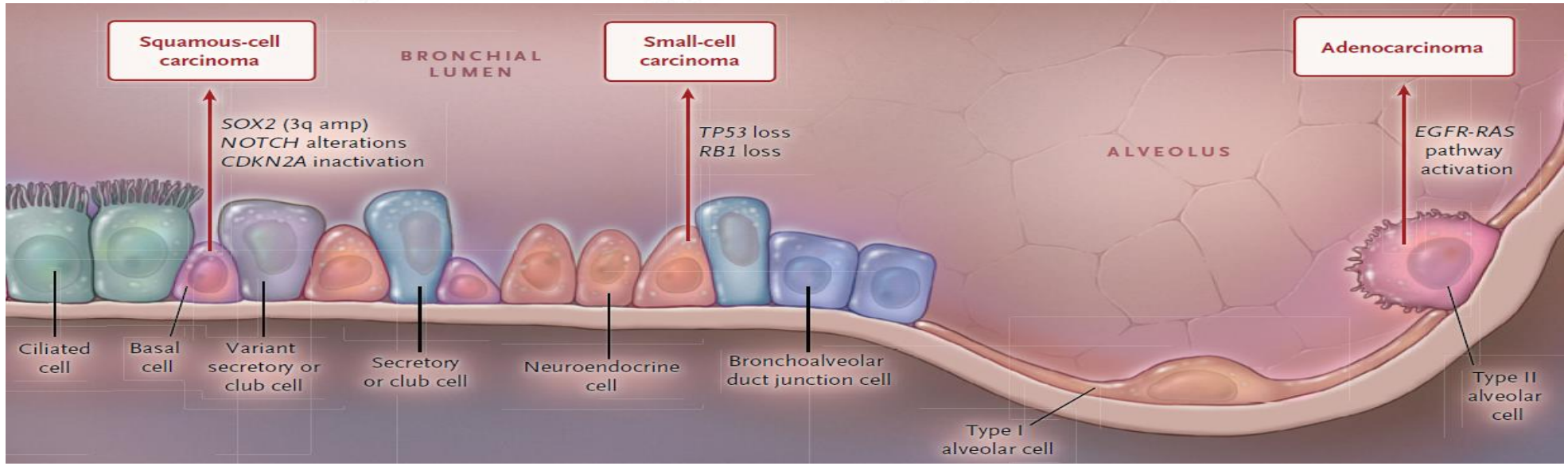
İstanbul, TÜRKİYE

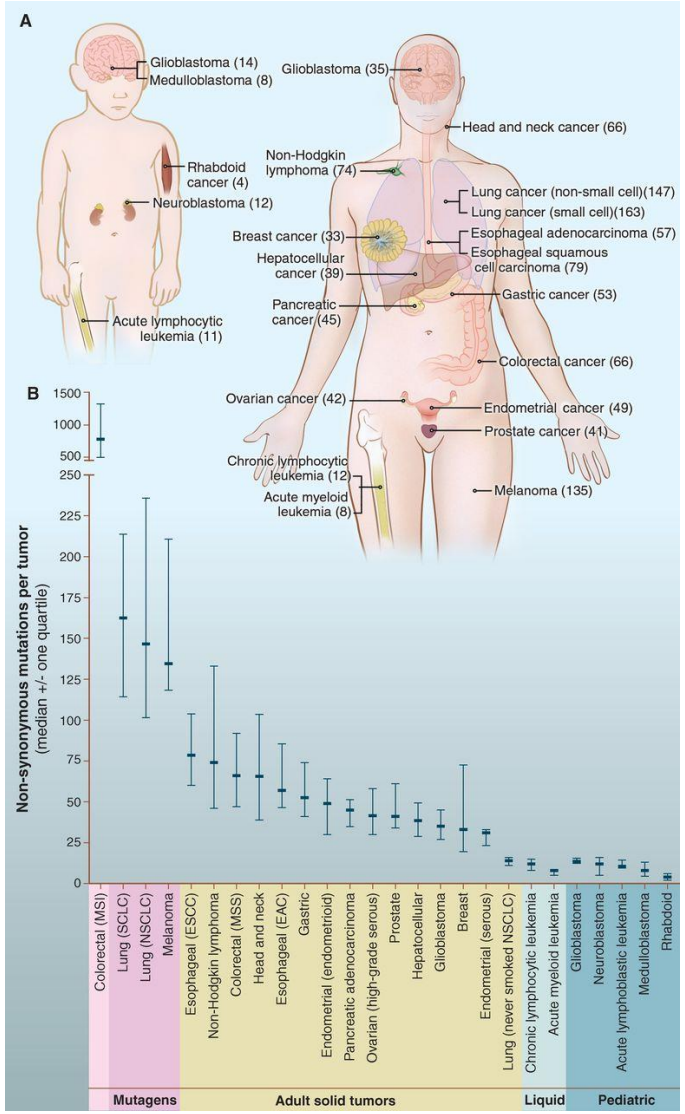
Lung Carcinogenesis





Keith & Miller. Nat Rev Clin Oncol, 2013
Swanton & Govindan. NEJM, 2016

Number of somatic mutations in representative human cancers, detected by genome-wide sequencing studies.



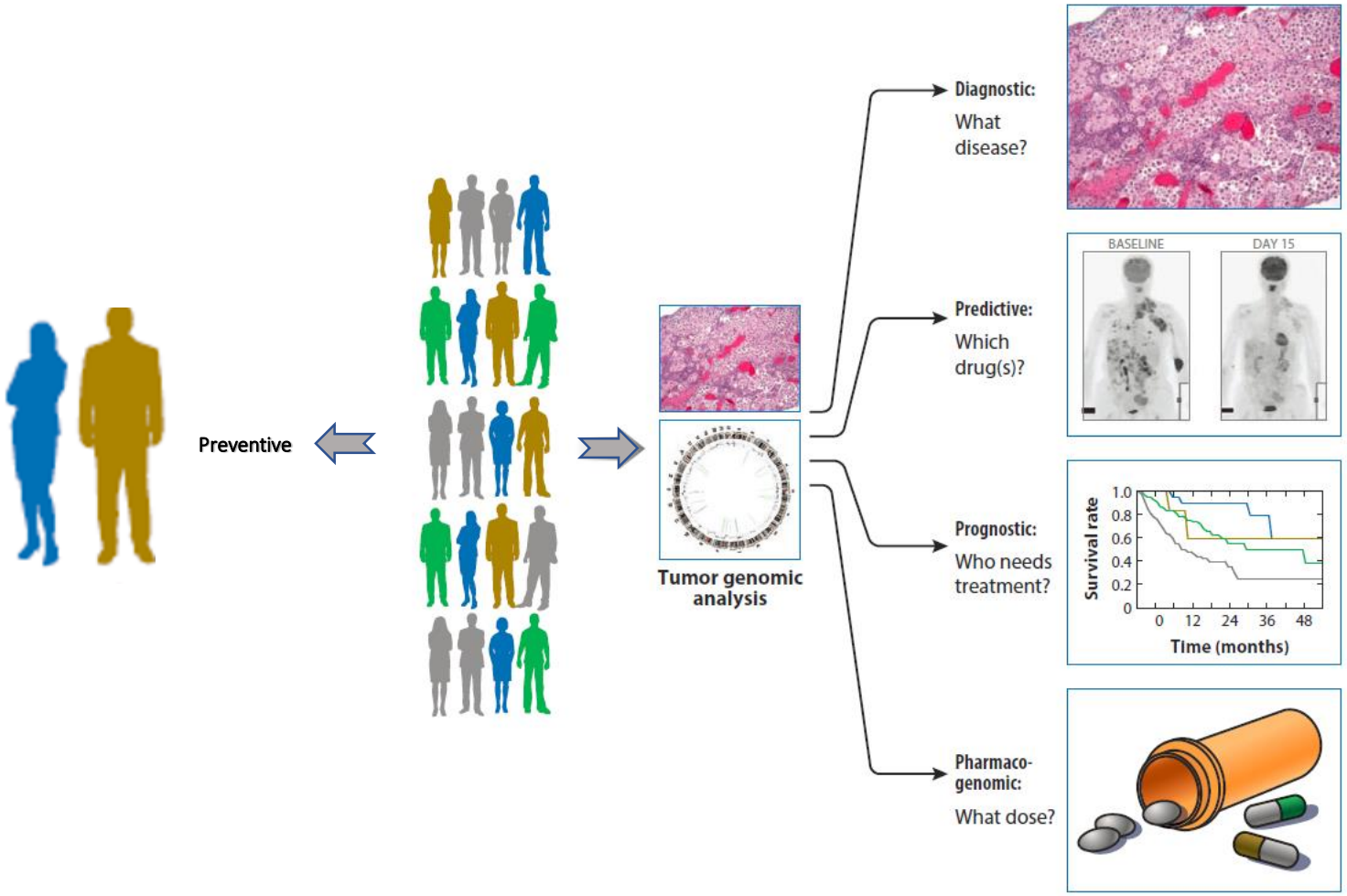
Vogelstein et al. Science; 2013

Recurrent Molecular Alterations in Lung Adenocarcinoma, Squamous-Cell Carcinoma, and Small-Cell Carcinoma

Type of Alteration	Adenocarcinoma	Squamous-Cell Carcinoma	Small-Cell Carcinoma
Cell-cycle mutations	*TP53* (46%), *CDKN2A*(4%)	*TP53* (91%), *CDKN2A* (17%), *RB1* (7%)	*TP53* (92%), *RB1* (75%)
	RTK/PI3K-MTOR signaling	*RTK/PI3K-MTOR* signaling	*RTK/PI3K-MTOR* signaling: *PTEN* (5%)
	KRAS (33%), *EGFR* (14%), *BRAF* (10%), *STK11* (17%), *MET* (8%), *NF1* (11%), *PIK3CA* (7%), *RIT1* (2%)	*PIK3CA* (16%), *PTEN* (8%), *HRAS* (3%)	
Other mutations	Oxidative stress response: *KEAP1* (17%), *MYC* pathway; *MGA* (8%)	Oxidative stress response: *CUL3* (6%), *KEAP1* (12%), *NFE2L2* (15%)	Epigenetic deregulation: *EP300* (11%), *CREBBP* (10%)
	Aberrant splicing: *U2AF1* (3%), *RBM10* (8%)	Squamous differentiation: *NOTCH1* (8%), *ASCL4* (3%), *NOTCH2* (5%)	Neuroendocrine differentiation: *NOTCH1* (15%), *NOTCH2* (5%), and *NOTCH3* (9%)
Rearrangements	*ALK* (3–8%), *ROS1* (2%), *RET* (1%), *NTRK1* (3%), *NRG1* (2%), *BRAF* (3% in those who never smoked), *ERBB4* (1%)	*FGFRs* (rare)	*RB1* (13%), *TP73* (7%), *CREBBP* (4%), *PTEN* (4%), *RBL1* (3%)
Amplifications	*TTF1* (14%), *TERT* (18%), *EGFR* (7%), *MET* (4%), *KRAS* (6%), *ERBB2* (3%), *MDM2* (8%)	Chr3q: *SOX2* (43%), *TP63* (29%), *PIK3CA* (38%), *HES1* (26%)†	*MYC* family members (16%): *MYC, MYCN, MYCL1, SOX2* (27%), *FGFR1* (8%), *IRS2* (2%)
Deletions	*CDKN2A* (20%)	*CDKN2A* (27%), *PTEN* (3%)	*TP53, RB1, CDKN2A,* Chr3p (e.g., *FHIT, ROBO1*)†
Commonly altered pathways	MAPK and PI3K signaling, oxidative stress response, cell-cycle progression, RNA splicing and processing, nucleosome remodeling	Squamous-cell differentiation, oxidative stress response, MAPK and PI3K signaling	Cell-cycle regulation, PI3K signaling, regulation of nucleosome transcriptional and remodeling, NOTCH signaling and neuroendocrine differentiation

Swanton & Govindan. NEJM, 2016

Genomics-Driven Biomarkers



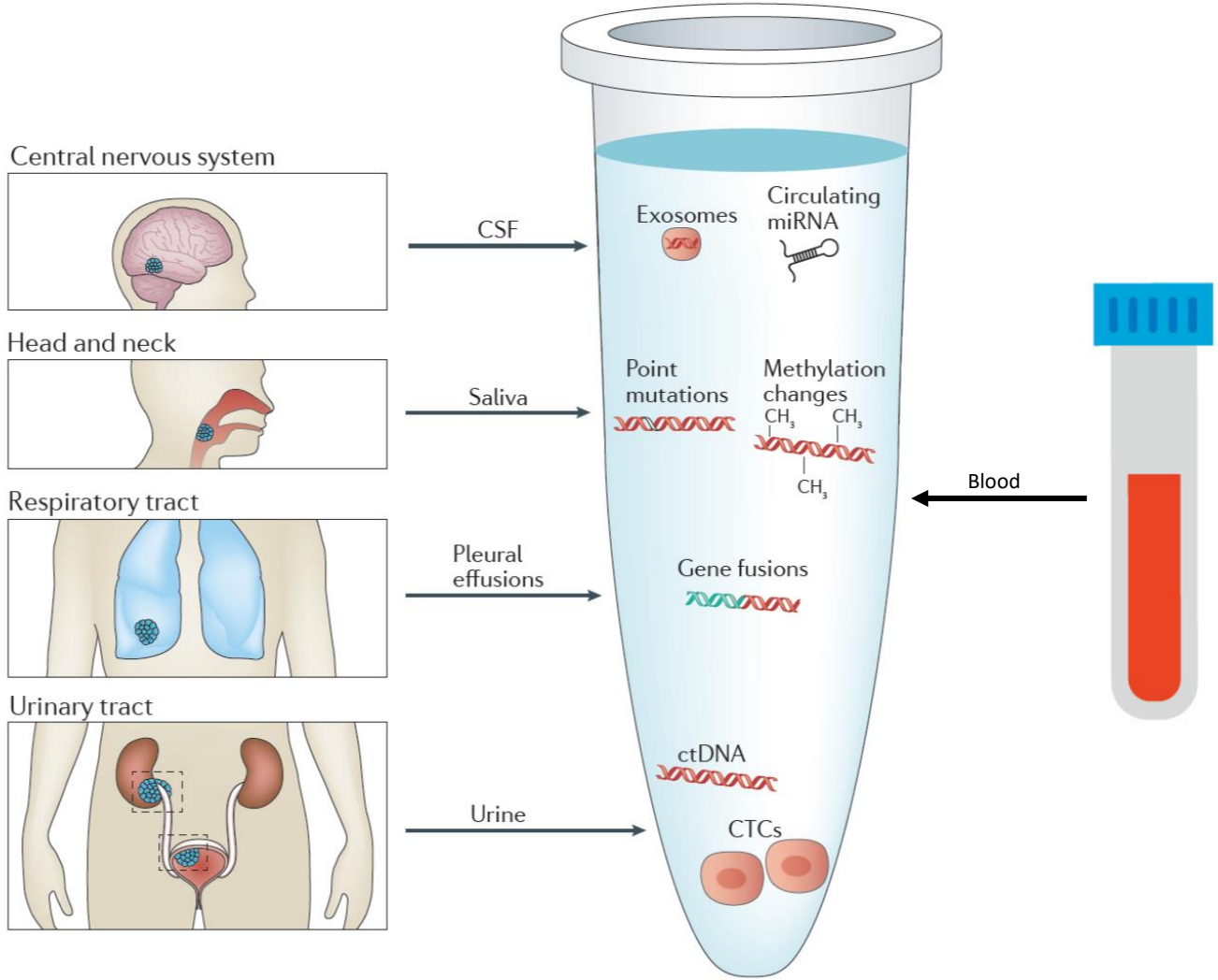
S. Roychowdhury & A.M. Chinnaiyan,
Annu. Rev. Genom. Human Genet. 2014

Precision Medicine

- The right drug
- The right patient
- The right time
- The right cost
- The right approach

What is a Liquid Biopsy?

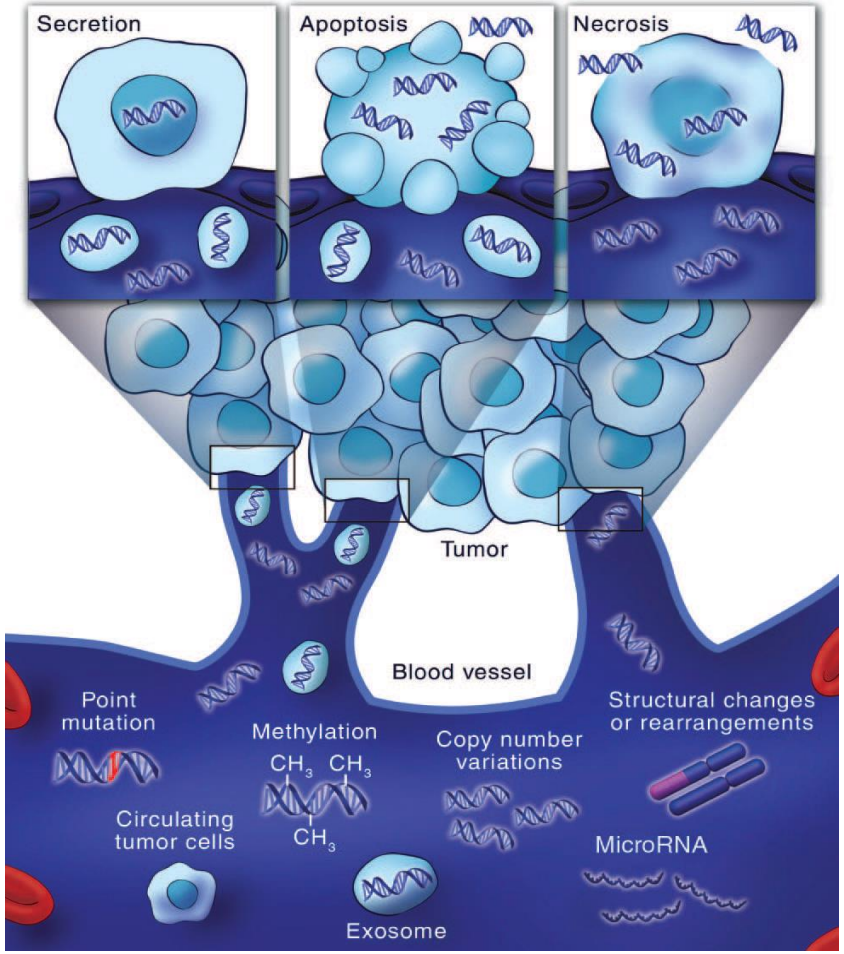
is the sampling and analysis of non-solid biological tissue, primarily blood.



Siravegna et al. Nat Rew Clincial Oncology, 2017

How Does Liquid Biopsy Work?

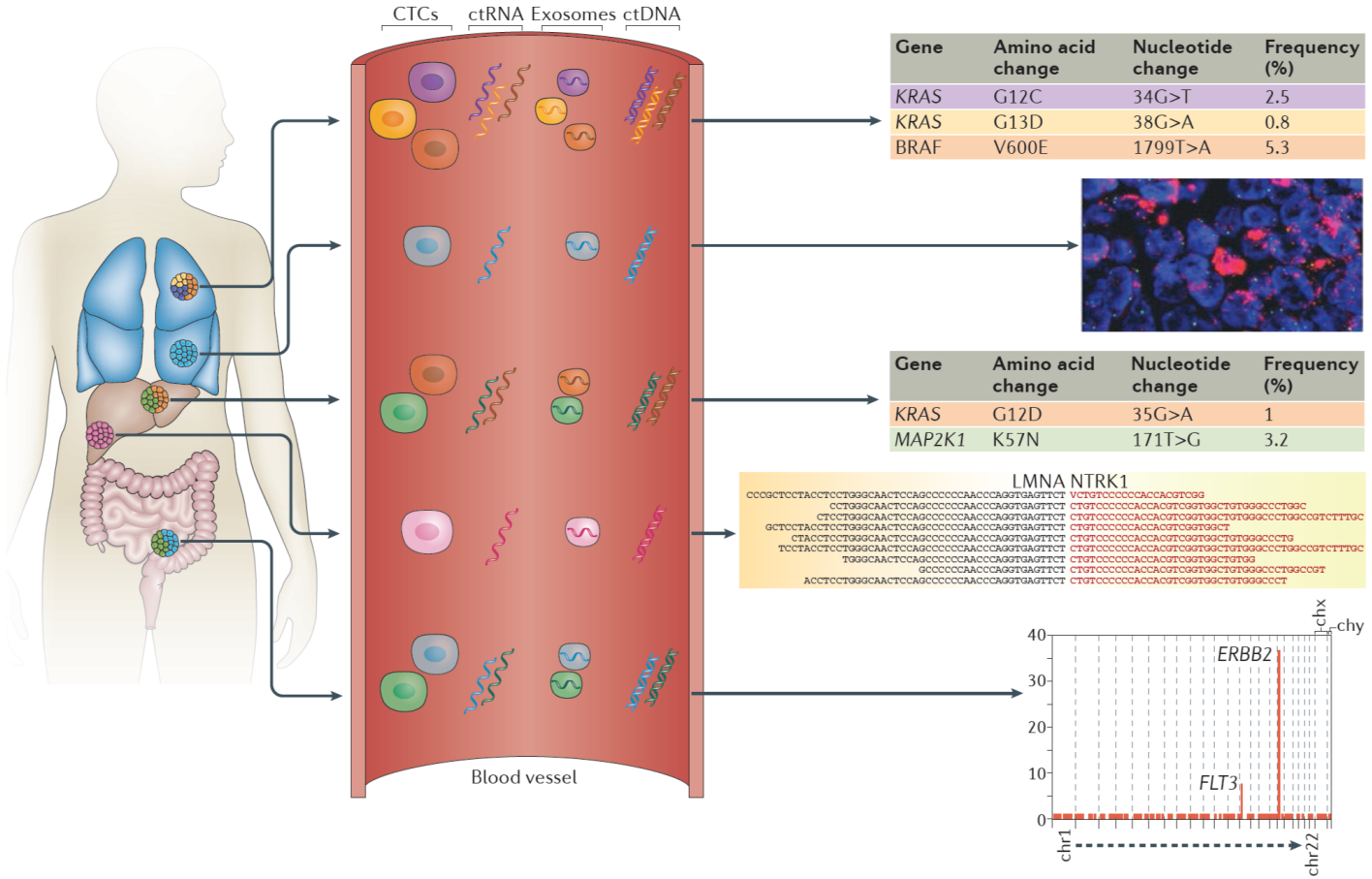
The localization of the primary tumour and of any metastatic lesions influences the presence of circulating tumour-derived nucleic acids, cells, and microvesicles in individual body fluids



Diaz and Bardelli, 2014 Journal of Clincial Oncology

Detection of Genetic Alterations and Genetic Heterogeneity

- ctCell
- ctDNA
- ctRNA
- Exosomlar
  - Point mutations
  - Amplyfications & Deletions
  - Translocations
  - Gene expression (mRNA/miRNA)
    - PCR (Conv., RT & Digital)
    - FISH
    - IHC
    - Sequencing (Sanger/NGS/Pyro)
    - Microarray



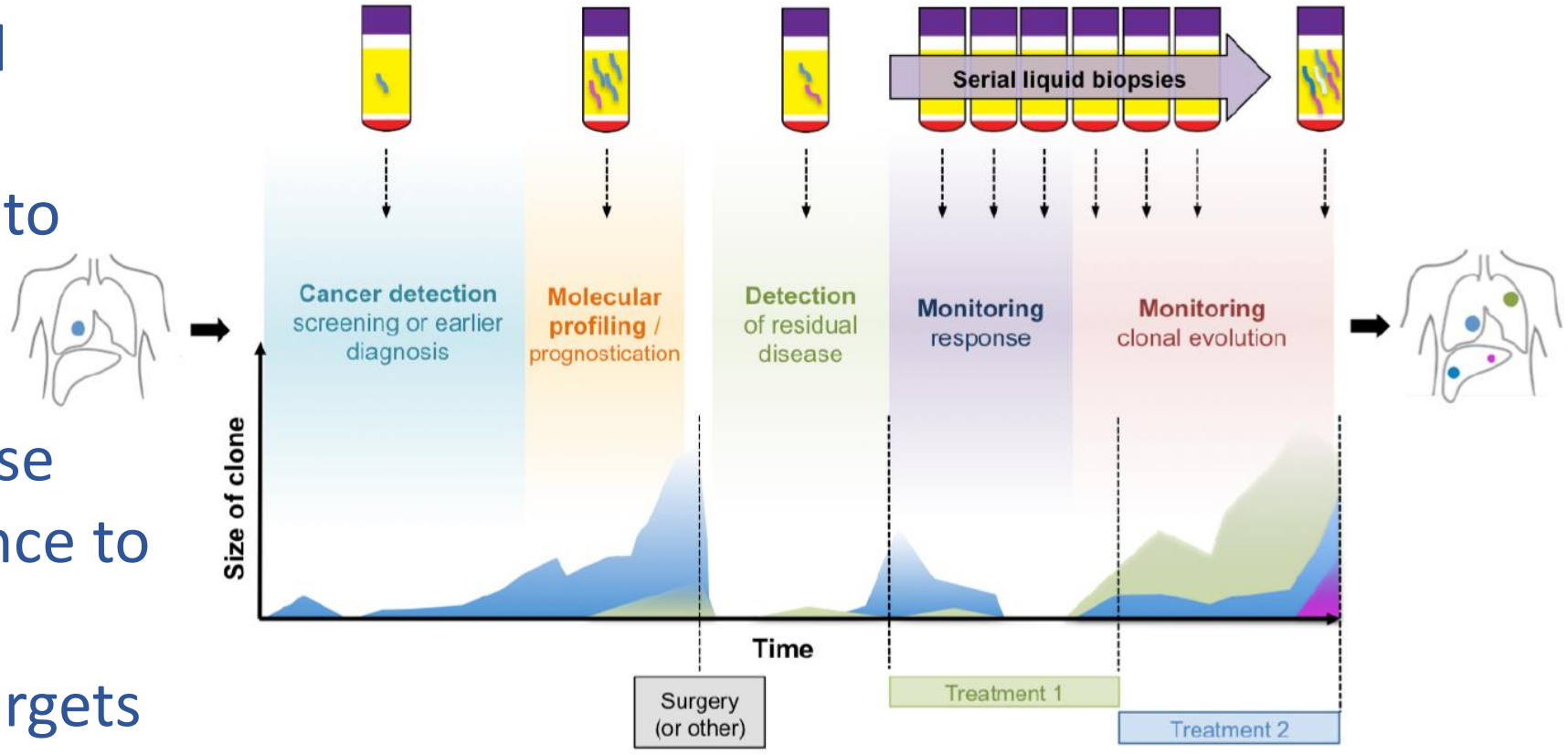
Gene	Amino acid change	Nucleotide change	Frequency (%)
KRAS	G12C	34G>T	2.5
KRAS	G13D	38G>A	0.8
BRAF	V600E	1799T>A	5.3

Gene	Amino acid change	Nucleotide change	Frequency (%)
KRAS	G12D	35G>A	1
MAP2K1	K57N	171T>G	3.2

Siravegna et al. Nat Rew Clincial Oncology, 2017

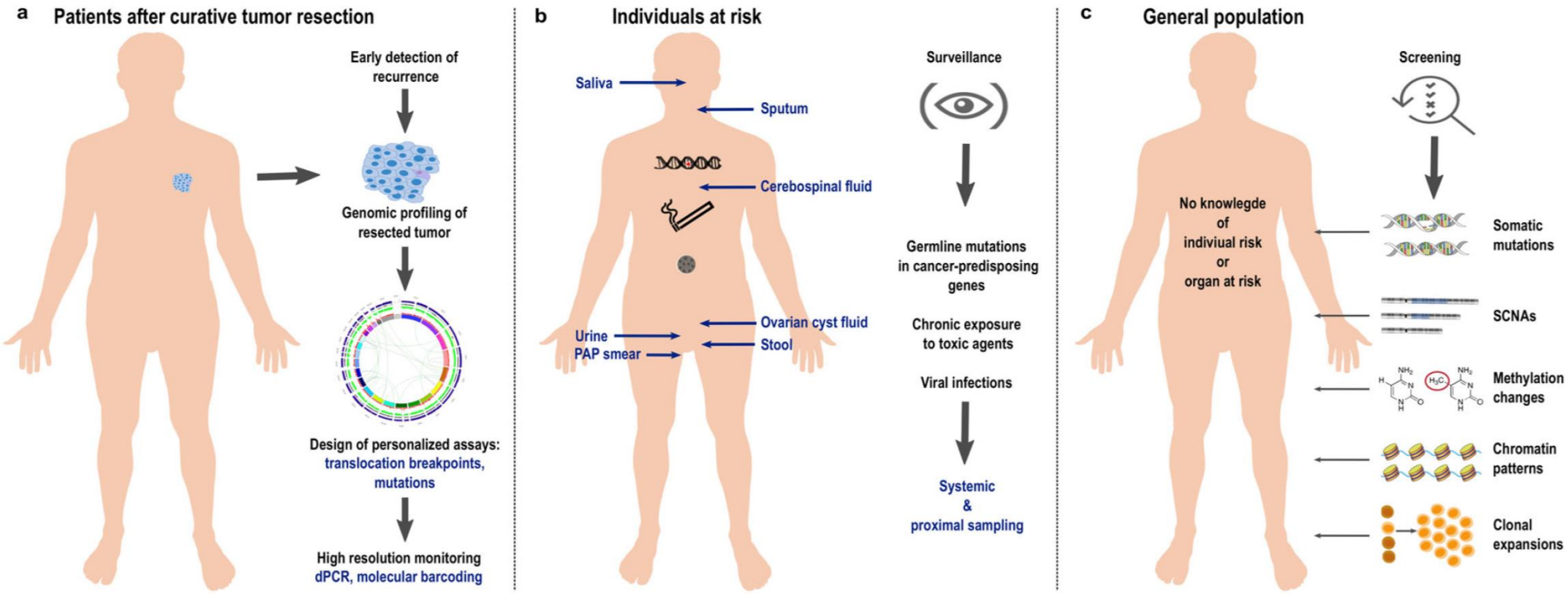
Clinical Applications of Liquid Biopsies

- Early Detection
- Profiling (prognostic)
- Detection of Residuel Disease/ Relaps
- Prediction (response to therapy)
- Monitoring
  - Evaluating response
  - Predicting resistance to therapy
  - Identifying new targets



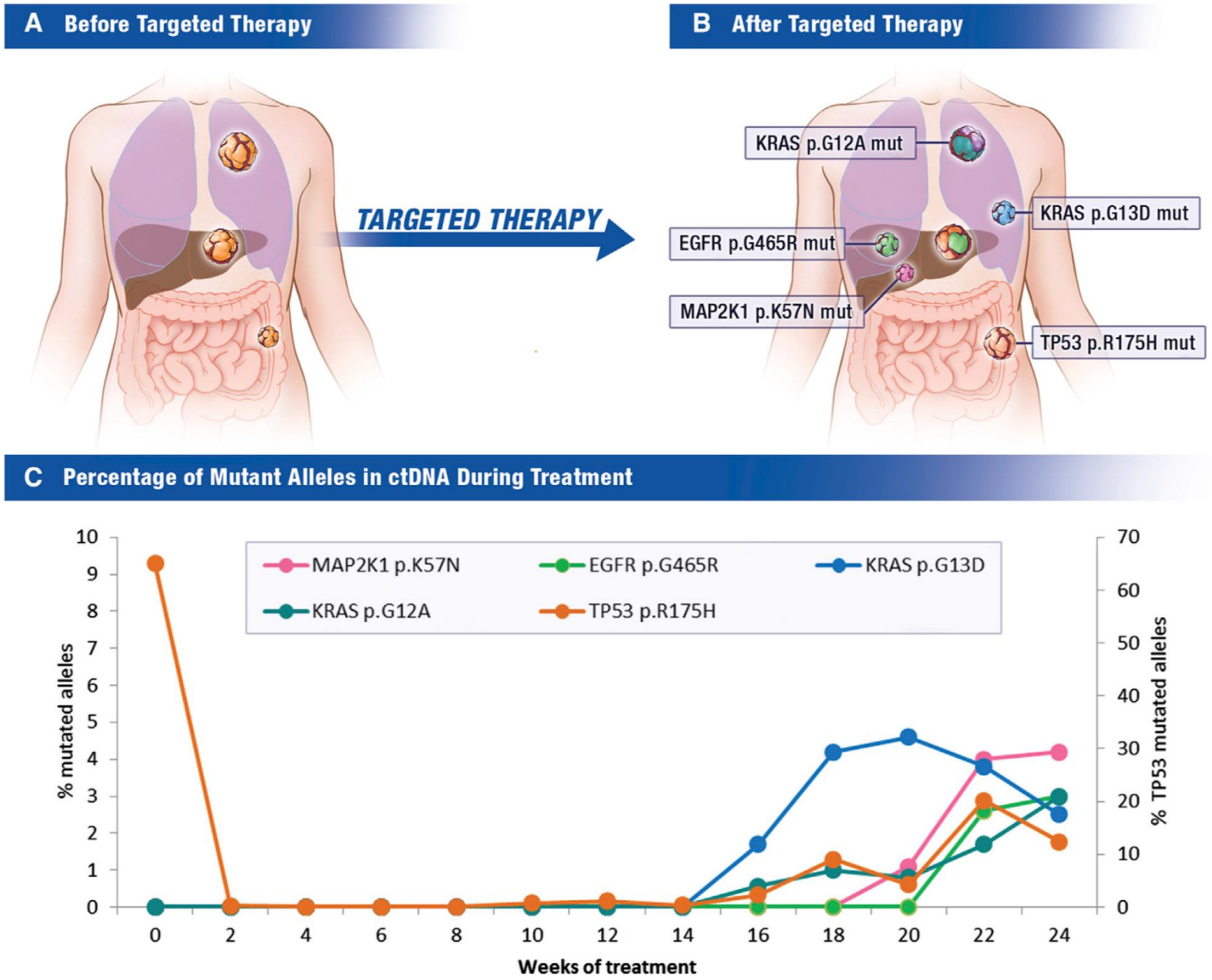
Wan et al. Nat Rew Cancer, 2017

Early Detection of Cancer



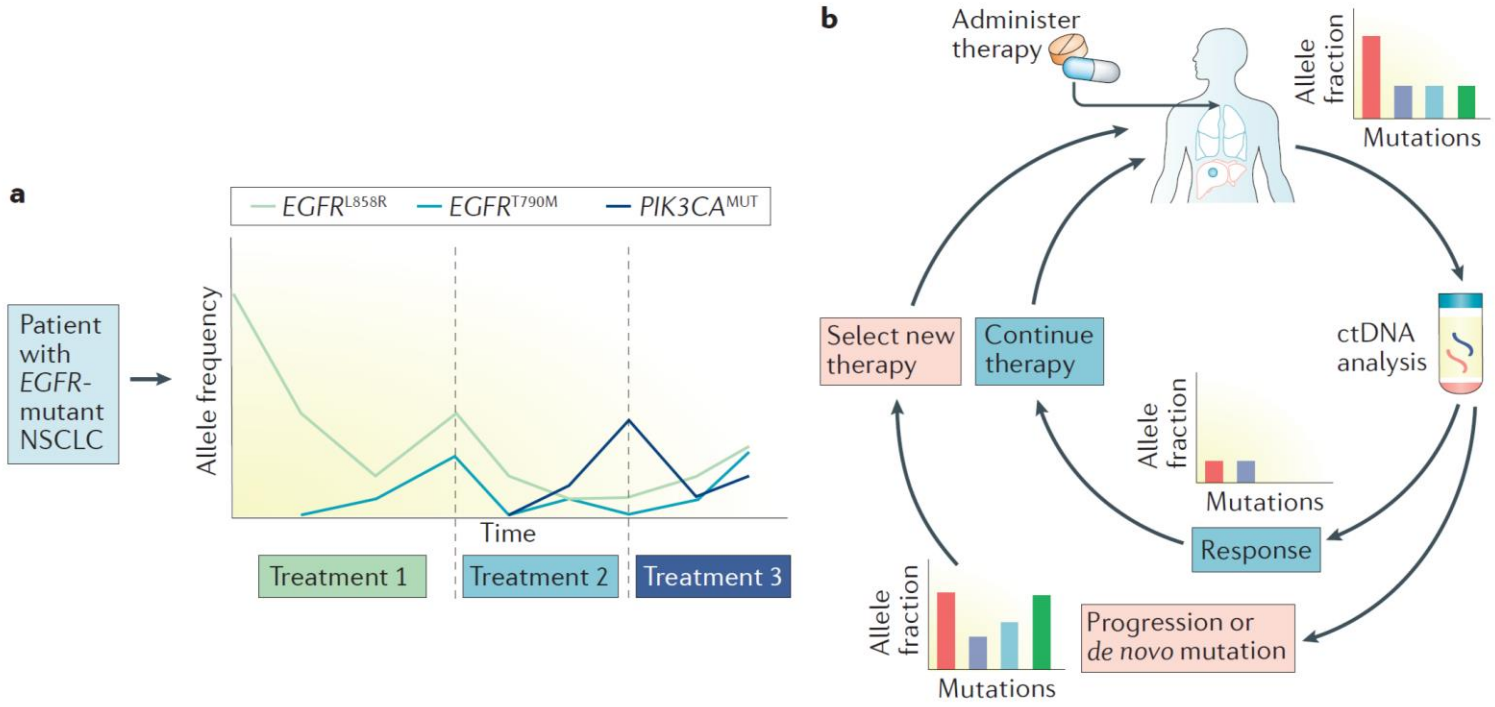
Heitzer et al. Precision Oncology, 2017

Liquid Biopsies to Monitor Cancer Evolution during Targeted Therapy



Bardelli & Klaus, Cancer Cell, 2017

Adaptive or reactive treatment paradigms using liquid biopsies



Wan et al. Nat. Rev. Cancer 2017

FDA Approved Liquid Biopsy Tests

Roche Molecular Diagnostics	Cobas EGFR Mutation test v2	PCR amplification of ctDNA to identify 42 EGFR mutations	FDA approved as companion diagnostic for Tarceva (erlotinib) (and Tagrisso (osimertinib) NSCLC in 2016
Veridex/Johnson & Johnson	CellSearch	Turnkey system for isolating and counting $EpCam^{+}$ cells from blood	FDA 501K clearance for cancers of breast since 2004, prostate since 2007, colon since 2008

Guideline from the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology

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ASCO SPECIAL ARTICLE

Molecular Testing Guideline for the Selection of Patients With Lung Cancer for Treatment With Targeted Tyrosine Kinase Inhibitors: American Society of Clinical Oncology Endorsement of the College of American Pathologists/ International Association for the Study of Lung Cancer/ Association for Molecular Pathology Clinical Practice Guideline Update

Gregory P. Kalemkerian, Navneet Narula, Erin B. Kennedy, William A. Biermann, Jessica Donington, Natasha B. Leighl, Madelyn Lew, James Pantelas, Suresh S. Ramalingam, Martin Reck, Anjali Saqi, Michael Simoff, Navneet Singh, and Baskaran Sundaram

Author affiliations and support information (if applicable) appear at the end of this article.

Published at jco.org on February 5, 2018.

G.P.K. and N.N. were Expert Panel co-chairs.

Clinical Practice Guideline Committee approval: October 2, 2017.

Editor's note: This American Society of Clinical Oncology clinical practice guideline provides recommendations, with comprehensive review and analyses of the relevant literature for each recommendation. Additional information, including a Methodology Supplement, slide sets, clinical tools and resources, and links to patient information at www.cancer.net, is available at www.asco.org/thoracic-cancer-guidelines and www.asco.org/guidelineswiki.

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0732-183X/18/3609w-911w/$20.00

ASSOCIATED CONTENT

Appendix

A B S T R A C T

Purpose

In response to advances in the field, the College of American Pathologists (CAP), the International Association for the Study of Lung Cancer (IASLC), and the Association for Molecular Pathology (AMP) recently updated their recommendations for molecular testing for the selection of patients with lung cancer for treatment with targeted tyrosine kinase inhibitors. ASCO has a policy and set of procedures for endorsing clinical practice guidelines that have been developed by other professional organizations.

Methods

The molecular testing guideline was reviewed for developmental rigor by methodologists. Then an ASCO Expert Panel reviewed the content and the recommendations.

Results

The ASCO Expert Panel determined that the recommendations from the CAP/IASLC/AMP molecular testing guideline are clear, thorough, and based upon the most relevant scientific evidence. ASCO endorsed the guideline with minor modifications.

Recommendations

This update clarifies that any sample with adequate cellularity and preservation may be tested and that analytical methods must be able to detect mutation in a sample with as little as 20% cancer cells. It strongly recommends against evaluating epidermal growth factor receptor (EGFR) expression by immunohistochemistry for selection of patients for EGFR-targeted therapy. New for 2018 are recommendations for stand-alone ROS1 testing with additional confirmation testing in all patients with advanced lung adenocarcinoma, and RET, ERBB2 (HER2), KRAS, and MET testing as part of larger panels. ASCO also recommends stand-alone BRAF testing in patients with advanced lung adenocarcinoma. Recommendations are also provided for testing methods for lung cancers that have a nonadenocarcinoma non–small-cell component, for patients with targetable mutations who have relapsed on targeted therapy, and for testing the presence of circulating cell-free DNA. Additional information is available at www.asco.org/thoracic-cancer-guidelines and www.asco.org/guidelineswiki.

J Clin Oncol 36:911-919. © 2018 by American Society of Clinical Oncology

New Recommendation Statements

Key Question 5: What is the role of testing for circulating, cell-free DNA for lung cancer patients?

No Recommendation: There is currently insufficient evidence to support the use of circulating cell-free plasma DNA (cfDNA) molecular methods for the diagnosis of primary lung adenocarcinoma.

Recommendation: In some clinical settings in which tissue is limited and/or insufficient for molecular testing, physicians may use a cell-free plasma DNA (cfDNA) assay to identify *EGFR* mutations.

New Recommendation Statements

Key Question 5: What is the role of testing for circulating cell-free DNA for lung cancer patients?

Expert Consensus Opinion: Physicians may use cell-free plasma DNA (cfDNA) methods to identify *EGFR* T790M mutations in lung adenocarcinoma patients with progression or secondary clinical resistance to EGFR-targeted tyrosine kinase inhibitors; testing of the tumor sample is recommended if the plasma result is negative.

No Recommendation: There is currently insufficient evidence to support the use of circulating tumor cell (CTC) molecular analysis for the diagnosis of primary lung adenocarcinoma, the identification of *EGFR* or other mutations, or the identification of *EGFR* T790M mutations at the time of *EGFR* TKI-resistance.

New Recommendation Statements

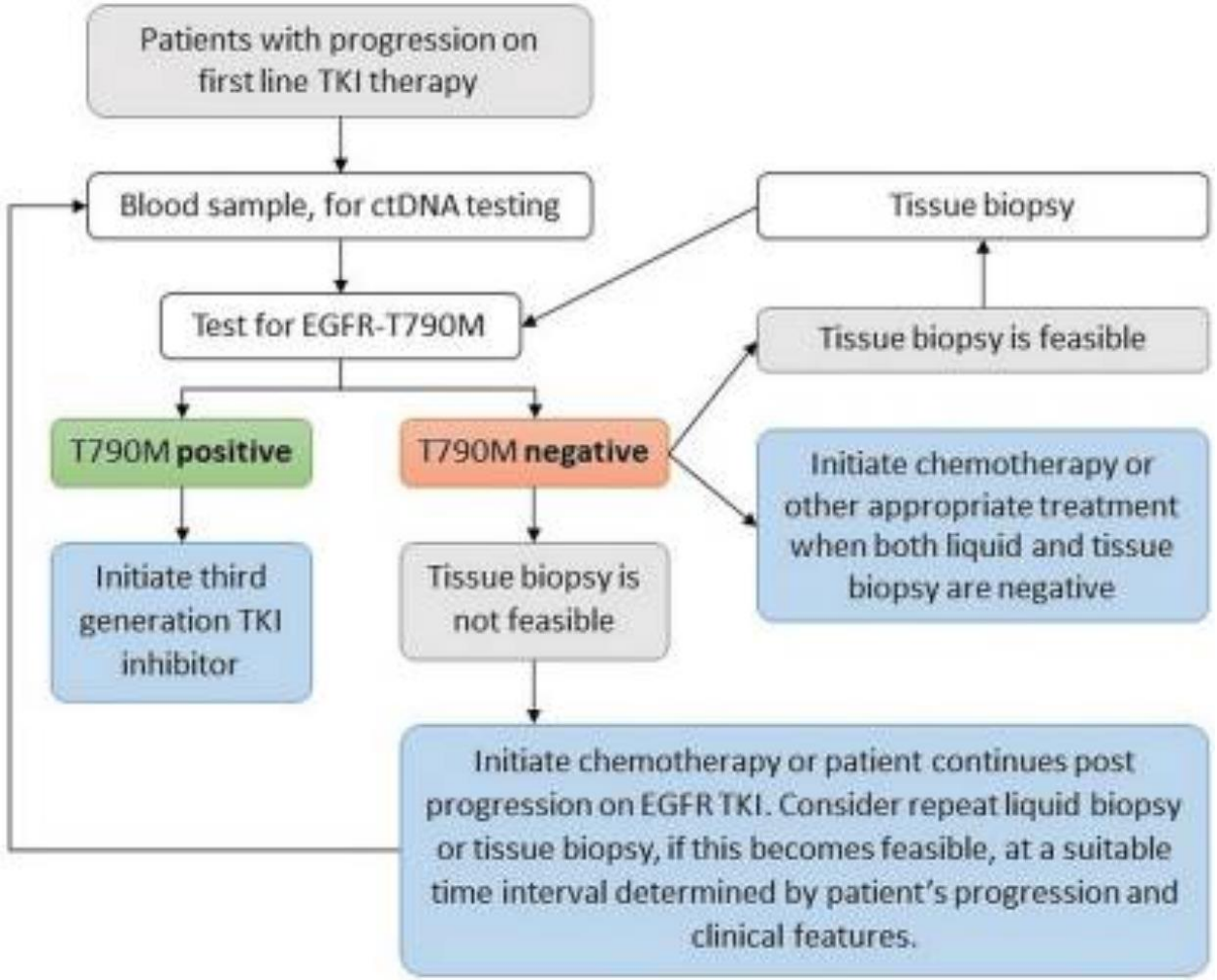
Key Question 3: Is molecular testing appropriate for lung cancers that do not have an adenocarcinoma component?

Expert Consensus Opinion: Physicians may use molecular biomarker testing in tumors with histologies other than adenocarcinoma when clinical features indicate a higher probability of an oncogenic driver.

Clinical indications for liquid biopsy for EGFR mutation detection in lung cancer

- No tissue sample available
- Low percentage of cancer cells in FFPE sample
- DNA of low quality and/or no DNA amplification
- Inappropriate fixation
- Long turnaround time to get the results

Clinical indications for liquid biopsy for EGFR mutation detection in lung cancer at progression under EGFR TKI

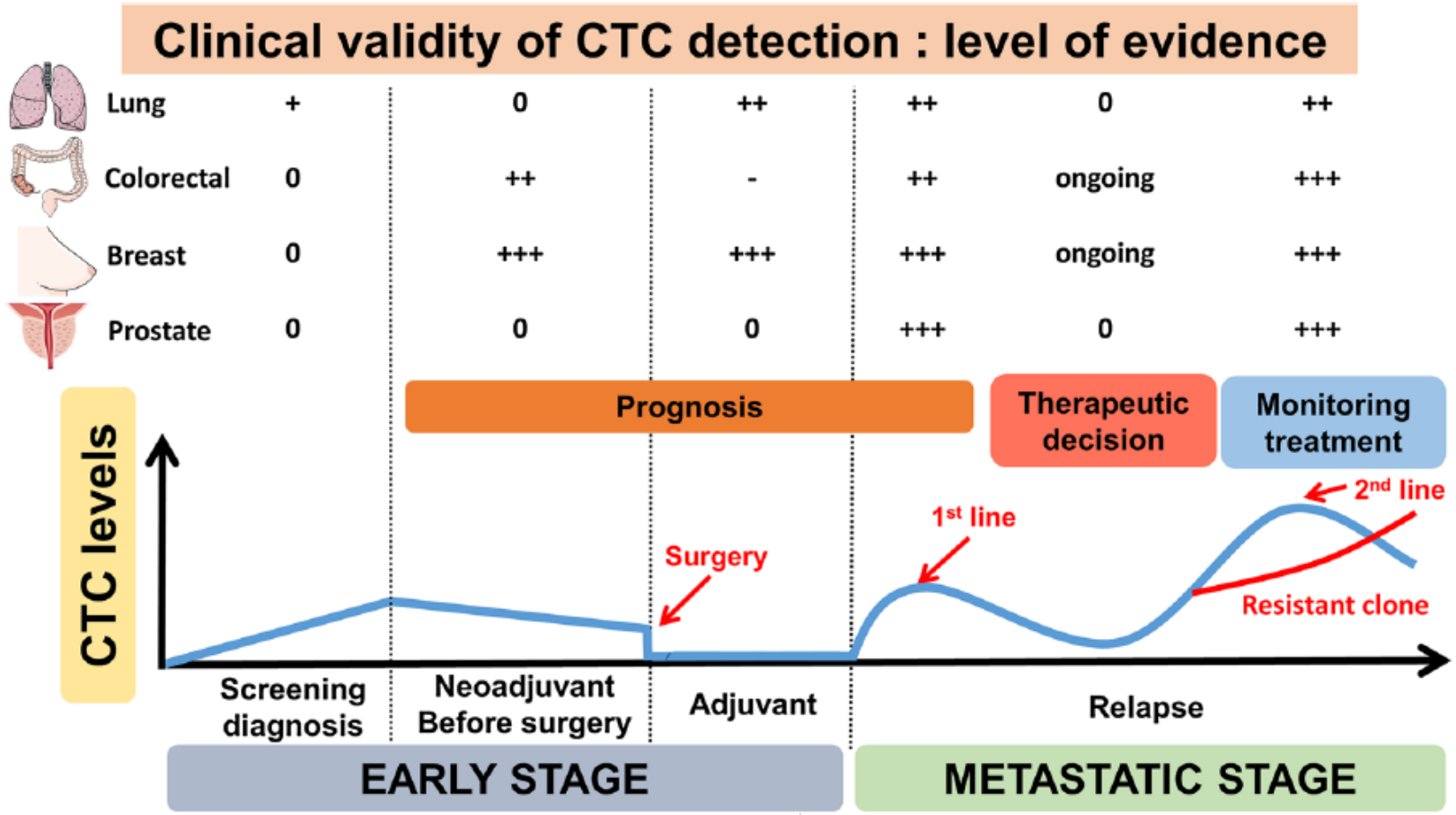


Stockley et al. Curr Oncol. 2018

Liquid Biopsy for Precision Medicine in Mutation-Driven NSCLC

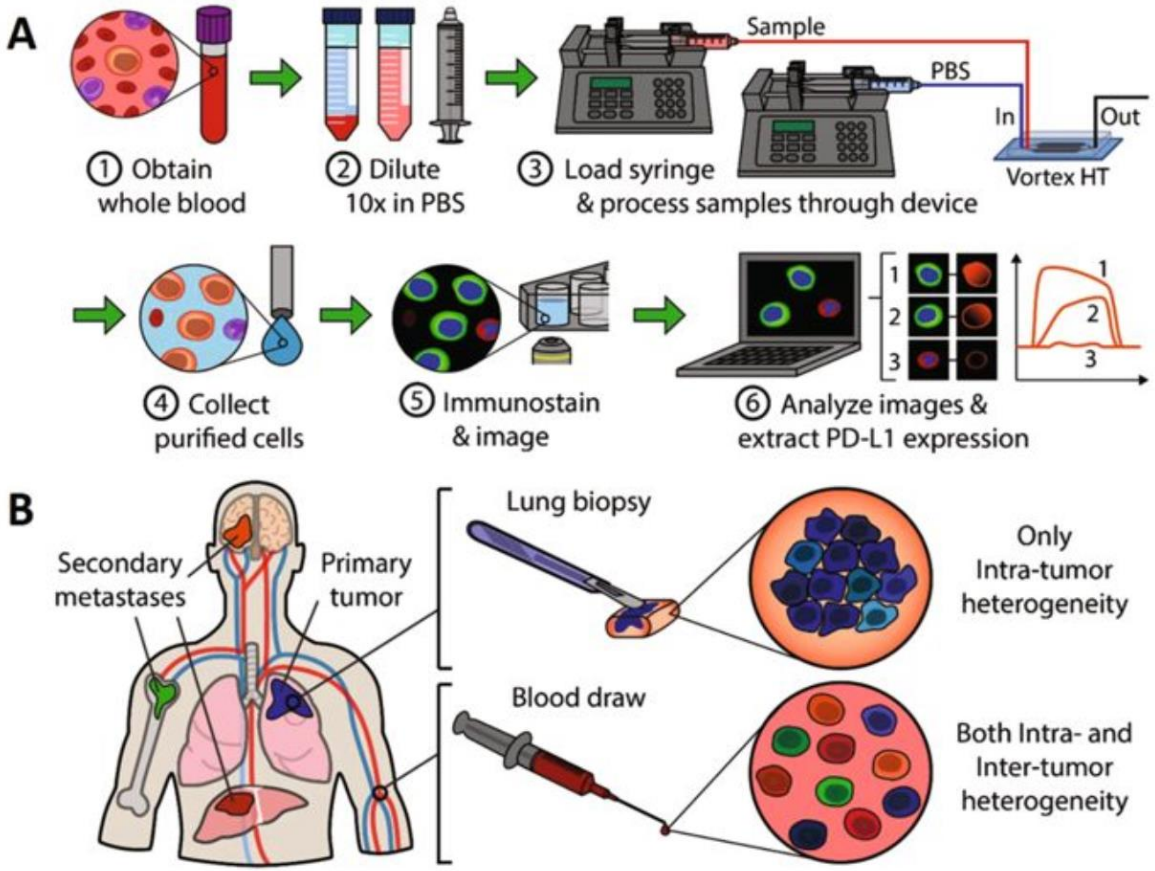
- Liquide biopsy for lung cancer in 2018
  - In routine now, assessment of EGFR status in cf-DNA
- Coming soon
  - assessment of various genomic alteration by NGS
- Perspectives:
  - Early diagnosis of lung cancer (RNA, microRNA, CTCs, DNA methylation)
  - Predictive biomarker(s) for immunotherapy response
  - Screening of lung cancer onset in a high risk population

Clinical Validity of Circulating Tumor Cells



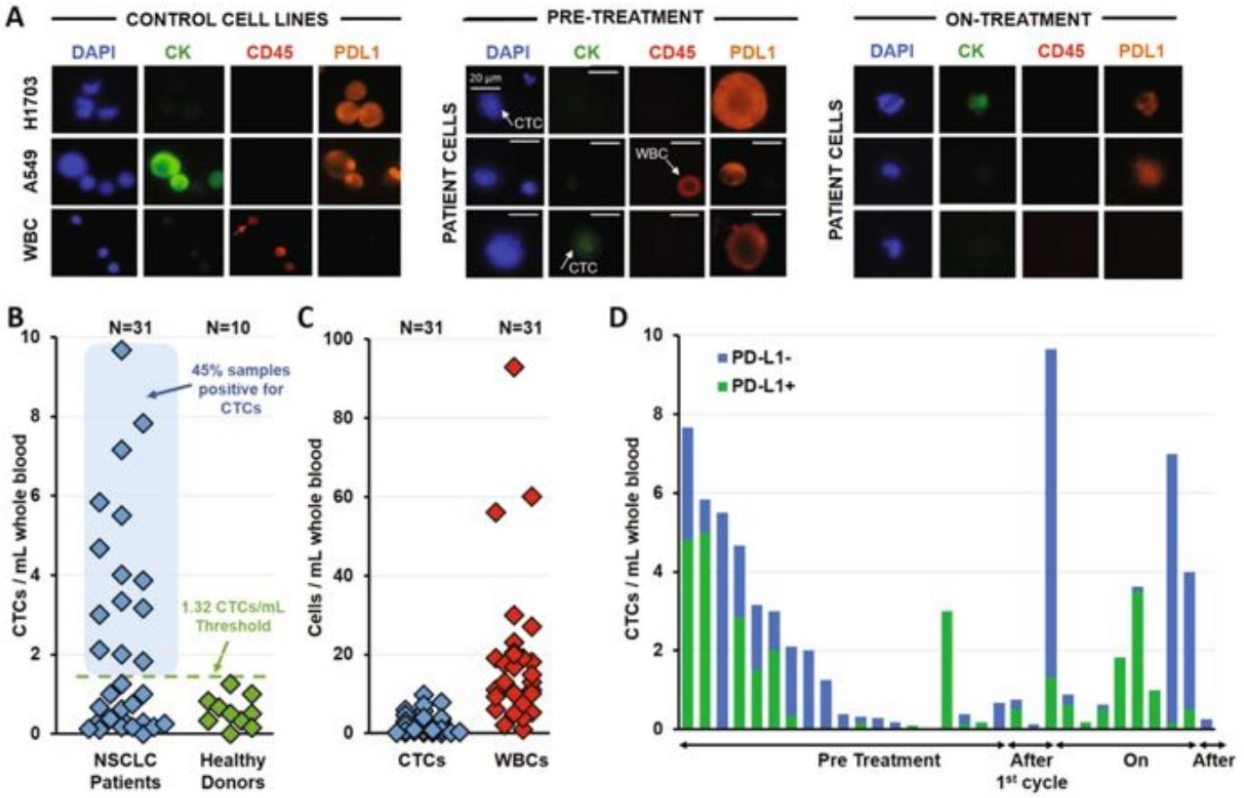
Cabel et al. Int J Clin Oncol. 2017

PD-L1 expression in NSCLC CTCs



Dhar et al. Sci. Reports, 2018

Comparison of PD-L1 expression in CTCs and matched primary tumor



Dhar et al. Sci. Reports, 2018

Precision Medicine and Imaging

Clinical Cancer Research

Validation of a Plasma-Based Comprehensive Cancer Genotyping Assay Utilizing Orthogonal Tissue- and Plasma-Based Methodologies

Justin I. Odegaard[1], John J. Vincent[1], Stefanie Mortimer[1], James V. Vowles[1], Bryan C. Ulrich[2], Kimberly C. Banks[1], Stephen R. Fairclough[1], Oliver A. Zill[1,3], Marcin Sikora[1], Reza Mokhtari[1], Diana Abdueva[1], Rebecca J. Nagy[1], Christine E. Lee[1], Lesli A. Kiedrowski[1], Cloud P. Paweletz[2], Helmy Eltoukhy[1], Richard B. Lanman[1], Darya I. Chudova[1], and AmirAli Talasaz[1]

Abstract

Purpose: To analytically and clinically validate a circulating cell-free tumor DNA sequencing test for comprehensive tumor genotyping and demonstrate its clinical feasibility.

Experimental Design: Analytic validation was conducted according to established principles and guidelines. Blood-to-blood clinical validation comprised blinded external comparison with clinical droplet digital PCR across 222 consecutive biomarker-positive clinical samples. Blood-to-tissue clinical validation comprised comparison of digital sequencing calls to those documented in the medical record of 543 consecutive lung cancer patients. Clinical experience was reported from 10,593 consecutive clinical samples.

Results: Digital sequencing technology enabled variant detection down to 0.02% to 0.04% allelic fraction/2.12 copies with ≤0.3%/2.24–2.76 copies 95% limits of detection while maintaining high specificity [prevalence-adjusted positive predictive values (PPV) >98%]. Clinical validation using orthogonal plasma- and tissue-based clinical genotyping across >750 patients demonstrated high accuracy and specificity [positive percent agreement (PPAs) and negative percent agreement (NPAs) >99% and PPVs 92%–100%]. Clinical use in 10,593 advanced adult solid tumor patients demonstrated high feasibility (>99.6% technical success rate) and clinical sensitivity (85.9%), with high potential actionability (16.7% with FDA-approved on-label treatment options; 72.0% with treatment or trial recommendations), particularly in non–small cell lung cancer, where 34.5% of patient samples comprised a directly targetable standard-of-care biomarker.

Conclusions: High concordance with orthogonal clinical plasma- and tissue-based genotyping methods supports the clinical accuracy of digital sequencing across all four types of targetable genomic alterations. Digital sequencing's clinical applicability is further supported by high rates of technical success and biomarker target discovery.
Clin Cancer Res; 24(15); 3539–49.

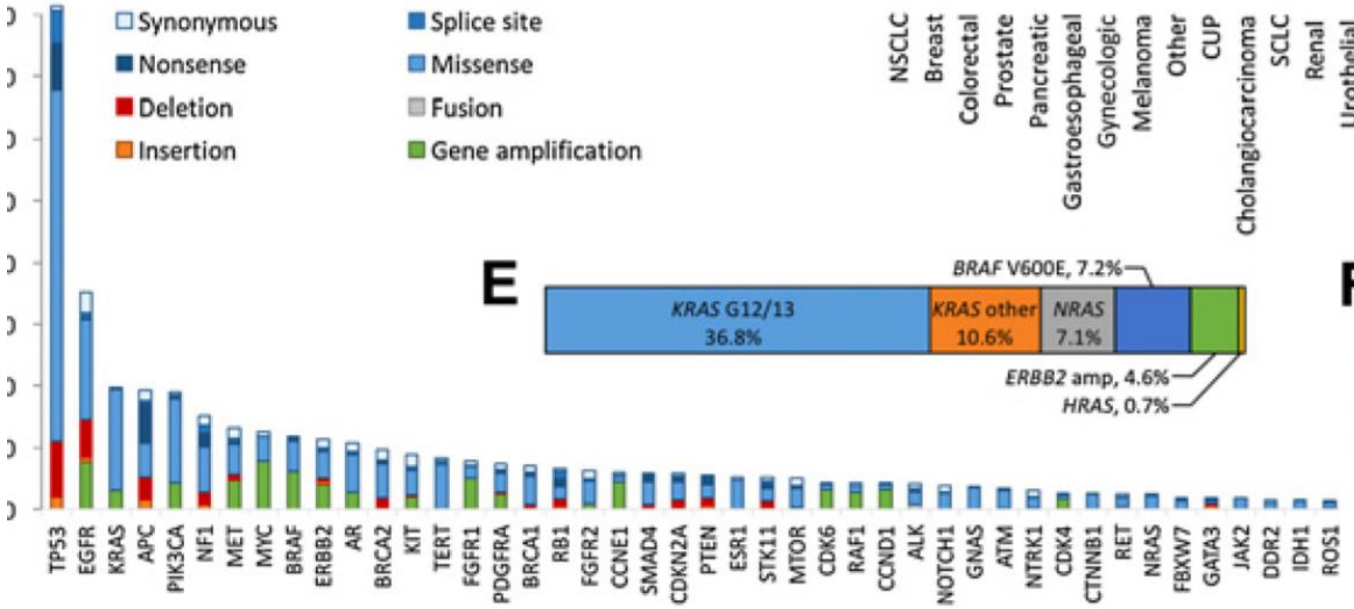
Point Mutations (SNVs) *(73 Genes)*							Indels *(23 Genes)*		Amplifications (CNVs) *(18 Genes)*		Fusions *(6 Genes)*
AKT1	*ALK*	*APC*	*AR*	*ARAF*	*ARID1A*	*ATM*	*ATM*	*APC*	*AR*	*BRAF*	*ALK*
BRAF	*BRCA1*	*BRCA2*	*CCND1*	*CCND2*	*CCNE1*	*CDH1*	*ARID1A*	*BRCA1*	*CCND1*	*CCND2*	*FGFR2*
CDK4	*CDK6*	*CDKN2A*	*CTNNB1*	*DDR2*	*EGFR*	*ERBB2*	*BRCA2*	*CDH1*	*CCNE1*	*CDK4*	*FGFR3*
ESR1	*EZH2*	*FBXW7*	*FGFR1*	*FGFR2*	*FGFR3*	*GATA3*	*CDKN2A*	*EGFR*	*CDK6*	*EGFR*	*NTRK1*
GNA11	*GNAQ*	*GNAS*	*HNF1A*	*HRAS*	*IDH1*	*IDH2*	*ERBB2*	*GATA3*	*ERBB2*	*FGFR1*	*RET*
JAK2	*JAK3*	*KIT*	*KRAS*	*MAP2K1*	*MAP2K2*	*MAPK1*	*KIT*	*MET*	*FGFR2*	*KIT*	*ROS1*
MAPK3	*MET*	*MLH1*	*MPL*	*MTOR*	*MYC*	*NF1*	*MLH1*	*MTOR*	*KRAS*	*MET*	
NFE2L2	*NOTCH1*	*NPM1*	*NRAS*	*NTRK1*	*NTRK3*	*PDGFRA*	*NF1*	*PDGFRA*	*MYC*	*PDGFRA*	
PIK3CA	*PTEN*	*PTPN11*	*RAF1*	*RB1*	*RET*	*RHEB*	*PTEN*	*RB1*	*PIK3CA*	*RAF1*	
RHOA	*RIT1*	*ROS1*	*SMAD4*	*SMO*	*STK11*	*TERT***	*SMAD4*	*STK11*			
TP53	*TSC1*	*VHL*					*TP53*	*TSC1*			
							VHL				

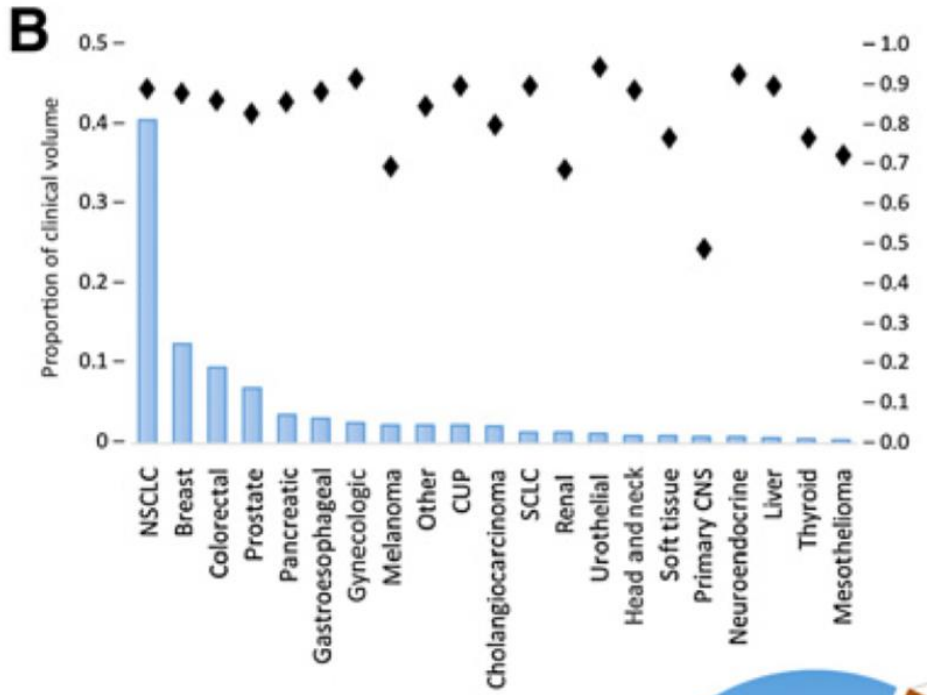
*** includes TERT promoter region*

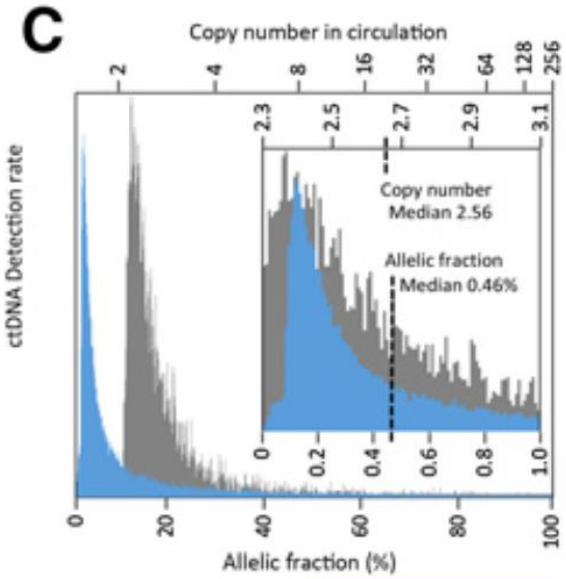
Validation of a Plasma-Based Comprehensive Cancer Genotyping Assay

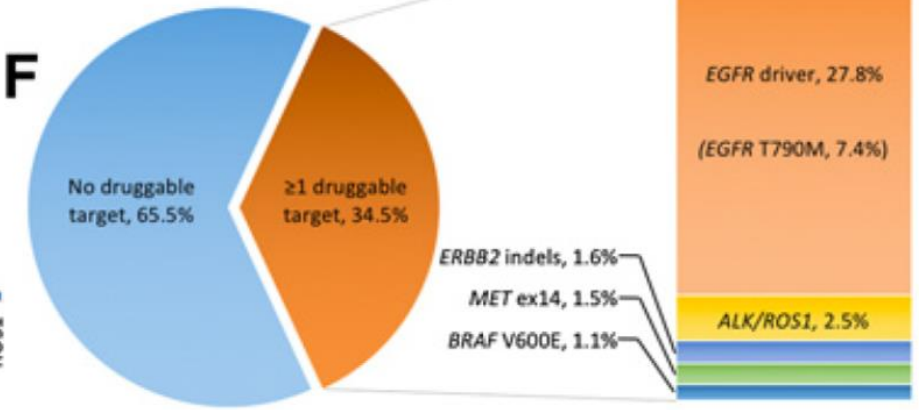
- Using orthogonal plasma- and tissue-based clinical genotyping across >750 patients
  - high accuracy and specificity [positive percent agreement (PPAs) and negative percent agreement (NPAs) >99% and positive predictive values (PPVs) 92%–100%].
- Clinical use in 10,593 advanced adult solid tumor patients
  - high feasibility (>99.6% technical success rate)
  - clinical sensitivity (85.9%), with
  - high potential actionability (16.7% with FDA-approved on-label treatment options; 72.0% with treatment or trial recommendations),
  - particularly in non–small cell lung cancer, where 34.5% of patient samples comprised a directly targetable standard-of-care biomarker

mples	10,593
successfully reported	99.6% (10,547)
with detectable ctDNA	85.3% (9,000)
m VAF, median across samples (range)	1.6% (0.01–97.7%)
VAF, per variant (range)	0.46% (0.01–97.7%)
SNV/indel/CNA #, per sample (range)	3.6/0.5/0.8 (0–102)
roved on-label therapies	16.7% (1,766)









NSCLC Cancer Panel

Hizmet Kodu	Test Adı	Test içeriği	Endikasyon
70027250	Likit biyopsi EGFR	EGFR geni 18,19,20,21. Ekzon Mutasyonları (T790M dahil)	EGFR inhibitörü tedavisi altında direnç takibi
70027226	Akciğer Panel 1 (Klasik Panel)	EGFR + ALK + ROS Minimal panel	Tirozin kinaz inhibitörü seçimi amaçlı minimal panel
70027227	Akciğer Panel 2 (TKİ paneli)	EGFR + KRAS + BRAF + ALK + ROS	Tirozin kinaz inhibitörü seçimi ve direnç analizi
70027228	Akciğer Panel 3 (Genişletilmiş TKI panel)	EGFR + ALK + ROS + MET + HER2 + FGFR1 + RET	Tirozin kinaz inhibitörü ve direnç analizi seçimi, genişletilmiş panel
70027230	Akciğer Panel 4 (PAN NSCLC-NGS)	Yeni nesil dizi analizi (Next generation sequencing) ile 19 gen analizi (Mutasyon, translokasyon, amplifikasyon gibi tüm genetik değişiklikler)	Hedefe yönelik tedavilere yanıtın öngörülmesi ve tedaviye direnç amaçlı 19 genin eşzamanlı değerlendirilmesi
70027251	Likit Biyopsi Akciğer Panel 4 (PAN NSCLC-NGS)	Yeni nesil dizi analizi (Next generation sequencing) ile 19 gen analizi (Mutasyon, translokasyon, amplifikasyon gibi tüm genetik değişiklikler)	Hedefe yönelik tedavilere yanıtın öngörülmesi ve tedaviye direnç amaçlı 19 genin eşzamanlı değerlendirilmesi

- **Likit Biyopsi (70027251): Akciğer Panel 4 (PAN NSCLC-NGS) testi aynı panel olup kandan hücre dışı serbest DNA'dan çalışılmaktadır.**
- **Tüm testler için parafin bloktaki tümör dokusu, likit biyopsi için patoloji laboratuvarından temin edilecek özel tübe alınacak 5 ml periferik kan yeterlidir.**
- **PAN NSCLC-NGS haricindeki tüm testler istek yapılarak ayrı ayrı da çalışılmaktadır.**

Liquid biopsies become a reality in cancer care

Thank you