Challenges of personalized medicine: from clinical trials to practice

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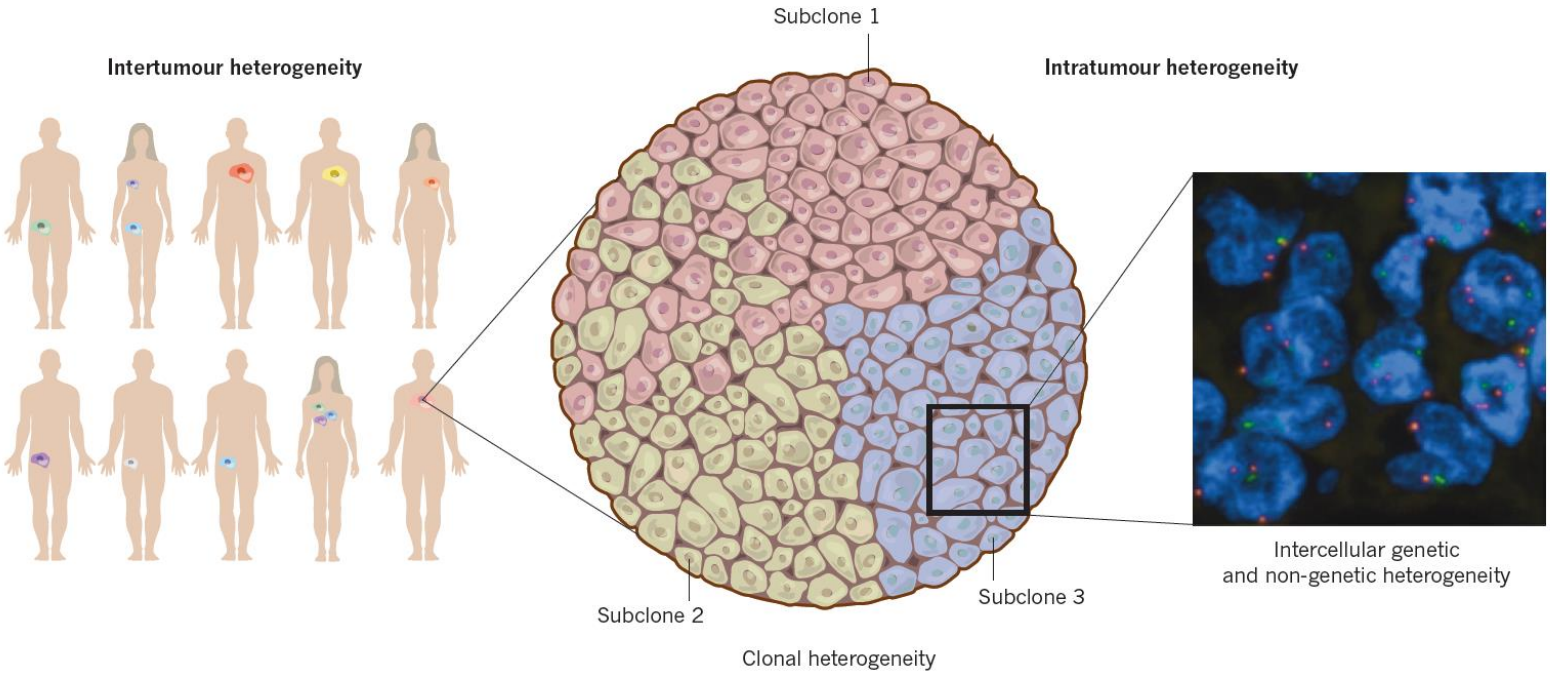
Issues for the development of molecular targeted therapies in cancer

- Identify a relevant molecular target for cancer development and/or progression.
- Develop anti-targeted agents which could be used as drugs.
- Identify patients whose cancers depend on the molecular target for growth and/or progression.
- Define one or more biomarkers for patient selection before treatment.
- Define optimal strategies for the use of the molecular targeted drug in combination and/or in sequence with conventional treatments (radiotherapy, surgery, chemotherapy).
- Manage novel side effects and toxicities.
- Identify and possibly overcome mechanisms of acquired resistance to molecular targeted therapies.

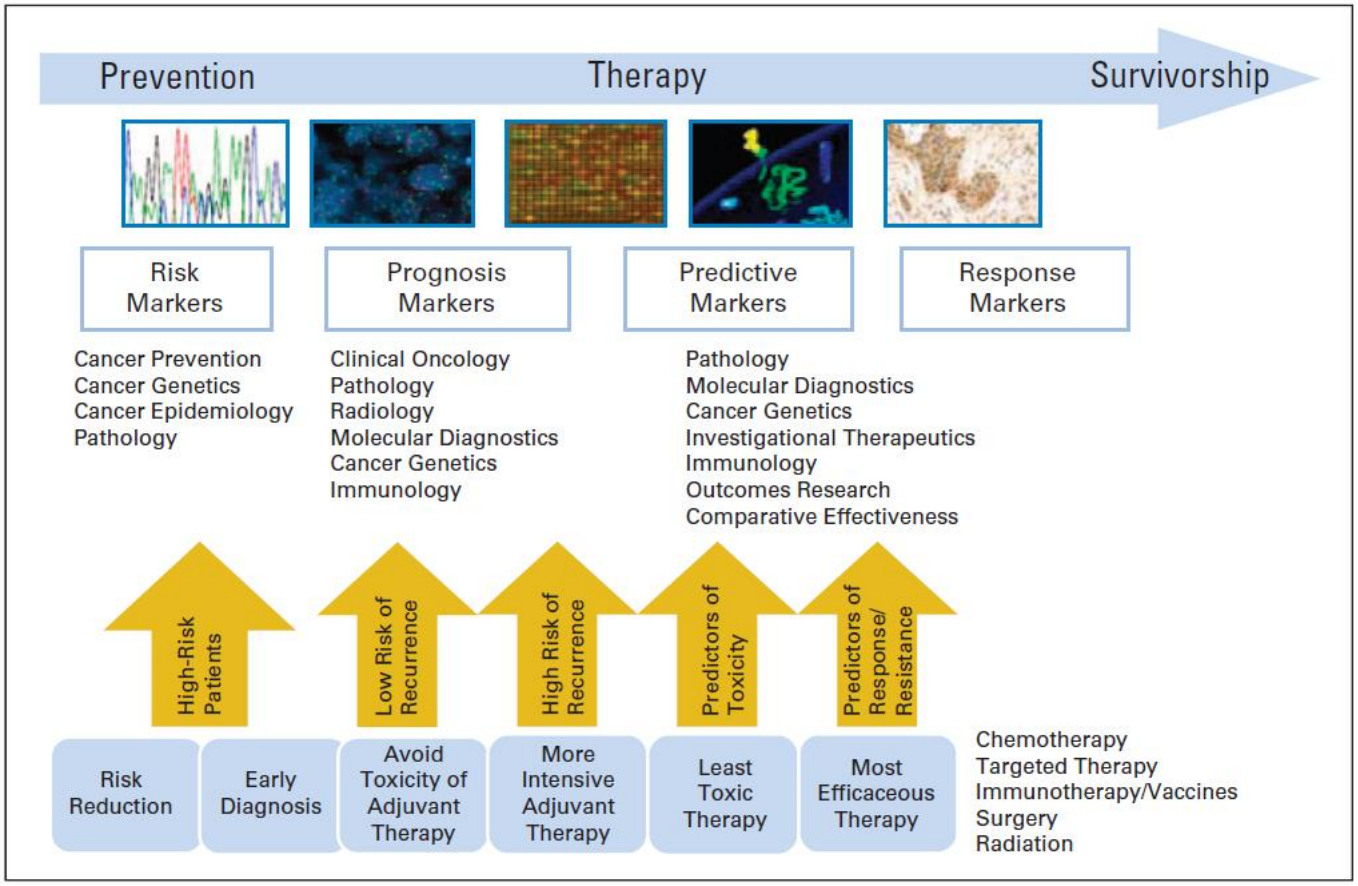
The efficacy of targeted therapy depends on

TUMOR HETEROGENEITY



Burrell Nature 2013

Target-based agents + predictive biomarkers: PERSONALIZED MEDICINE



Meric-Bernstam JCO 2013

Predictive biomarkers and personalized medicine

- **Biomarkers that are associated with response to drugs (positive selection)**
  - **EGFR mutations and ALK rearrangements in NSCLC**
  - **BRAF mutations in melanoma**
  - **ERBB2 gene amplification in breast/gastric cancer**
- **Biomarkers that are associated with resistance to drugs (negative selection)**
  - **RAS mutations and resistance to EGFR monoclonal antibosies in CRC**

Challenges in biomarker testing in NSCLC

- The number of potential biomarkers is increasing
- Oncogenic pathways are activated through different, peculiar genomic alterations in NSCLC
- The molecular landscape may affect tumor response to targeted agents even in tumors with driver mutations
- The molecular profile of NSCLC changes following treatment with target based agents
- Need of methods for molecular profiling of NSCLC
- Assessment of somatic mutations in blood samples

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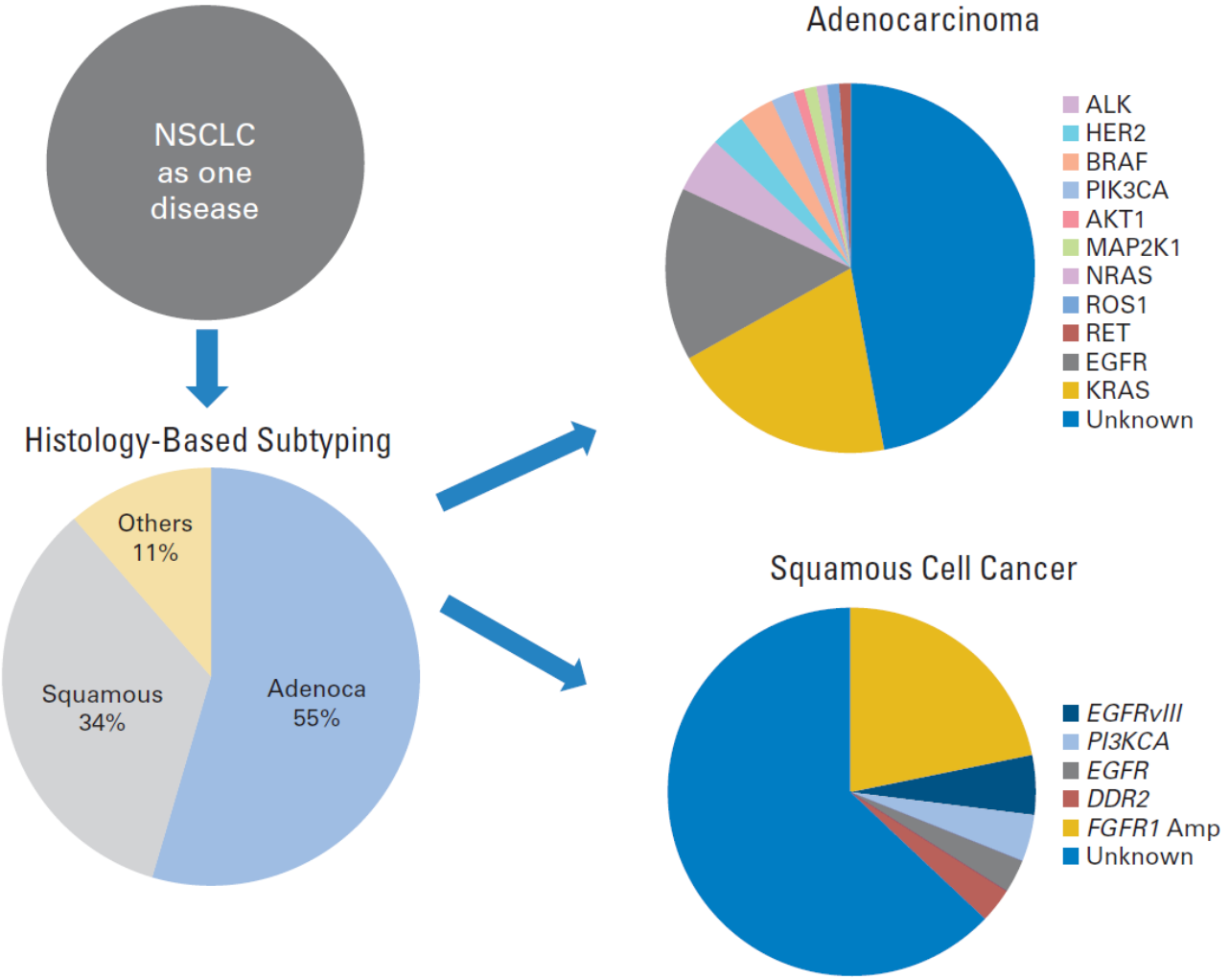
Molecular subsets of lung carcinoma



Li JCO 2013

Biomarkers in NSCLC

	Biomarker	Drug
Approved	EGFR mutations	Gefitinib, erlotinib
Approved	ALK rearrangements	Crizotinib
	ROS-1 rearrangements	Crizotinib
	RET rearrangements	Cabozantinib
	MET amplification	Crizotinib
	DDR2 mutations	Dasatinib
	NRAS mutations	Selumetinib/Trametinib (preclinical)
	ErbB-2 mutations	Afatinib/Lapatinib/Trastuzumab
	KRAS mutations	Selumetinib
	FGFR1 amplification	Dovitinib
	BRAF V600E	Vemurafenib
	BRAF Y472C	Dasatinib

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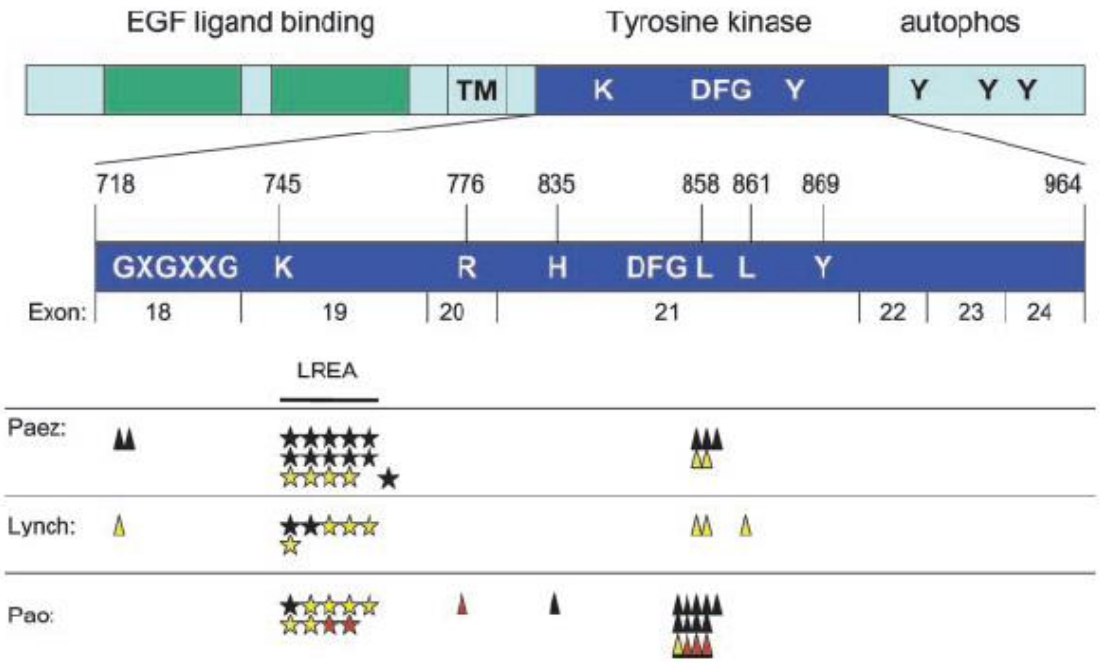
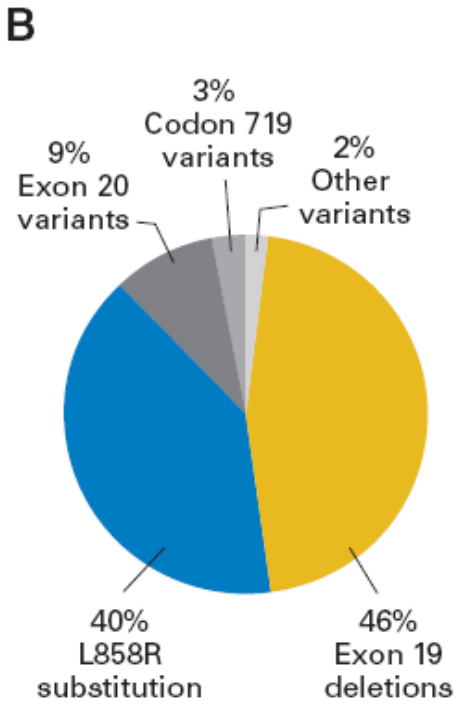
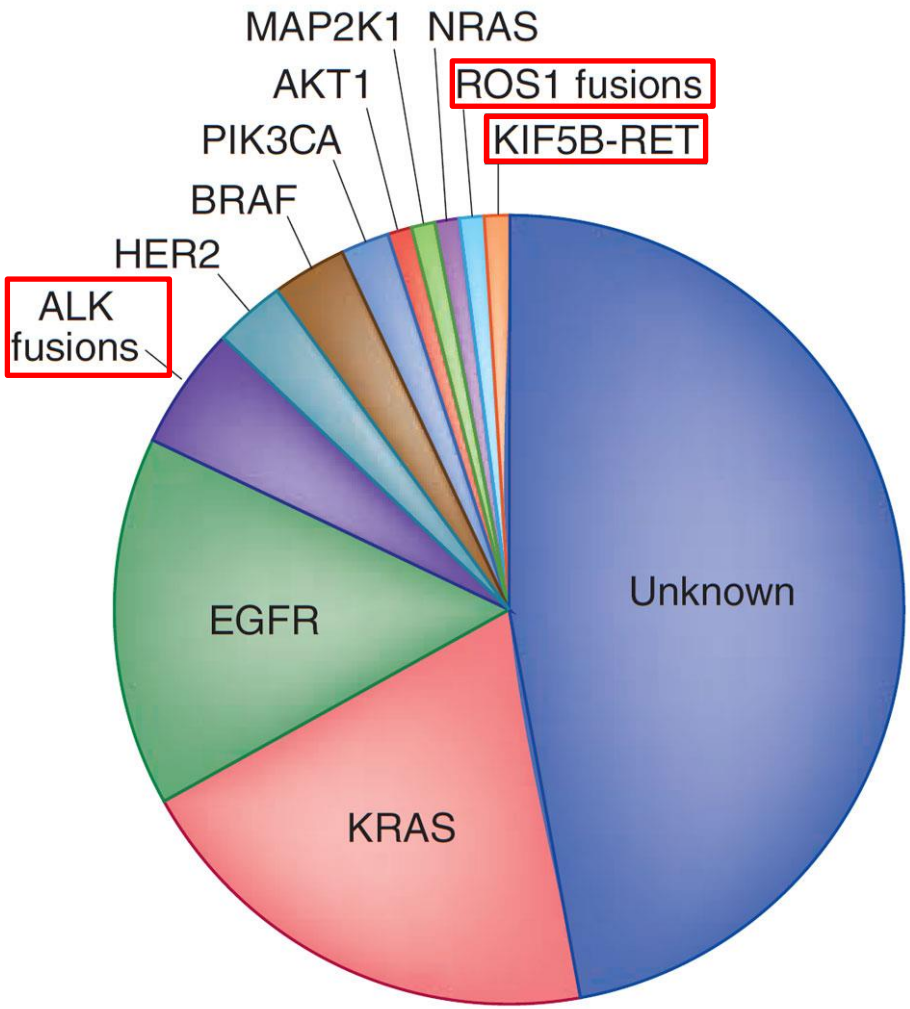
EGFR mutations and NSCLC





Lynch NEJM 2004; Paez Science 2004; Pao PNAS 2004; Sequist JCO 2007

Genetic alterations in Lung Adenocarcinomas



Pao & Hutchinson, Nat Medicine 2012

Therapeutic Implications

EGFR mutant: tyrosine kinase inhibitors

ALK fusion: ALK inhibitors

ROS1 fusion: crizotinib

RET fusion: cabozantinib, vandetanib

NTRK1: AZ64, PLX7486 (in clinical development)

Fusion transcripts in lung adenocarcinomas

3’ Gene	5’ Partners
ALK	EML4, HIP1, KIF5B, KLC1, TPR
ROS1	CD74, EXR, GOPC, LRIG3, SDC4, SLC34A2, TPM3
RET	CCDC6, CUX1, KIF5B
NTRK1	CEL, NFASC, IRF2BP2, TFG, QSTM1, SSBP2, DYNC2H1, CD74, MPRIP

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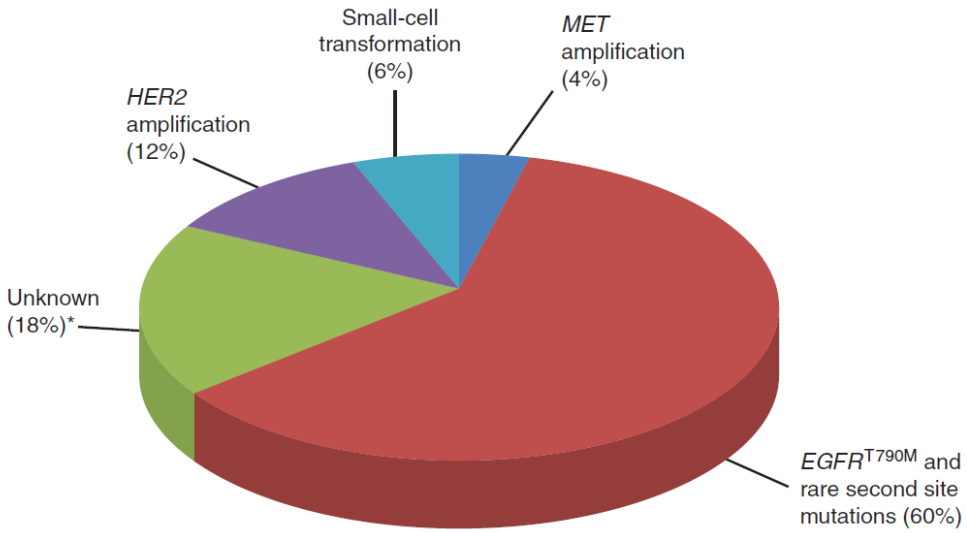
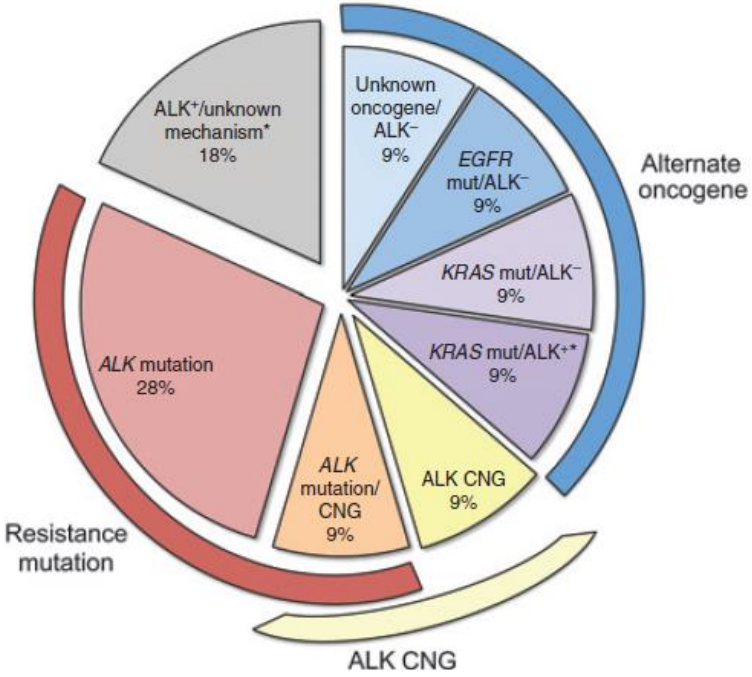
Frequency of resistance mechanisms to TKIs in NSCLC



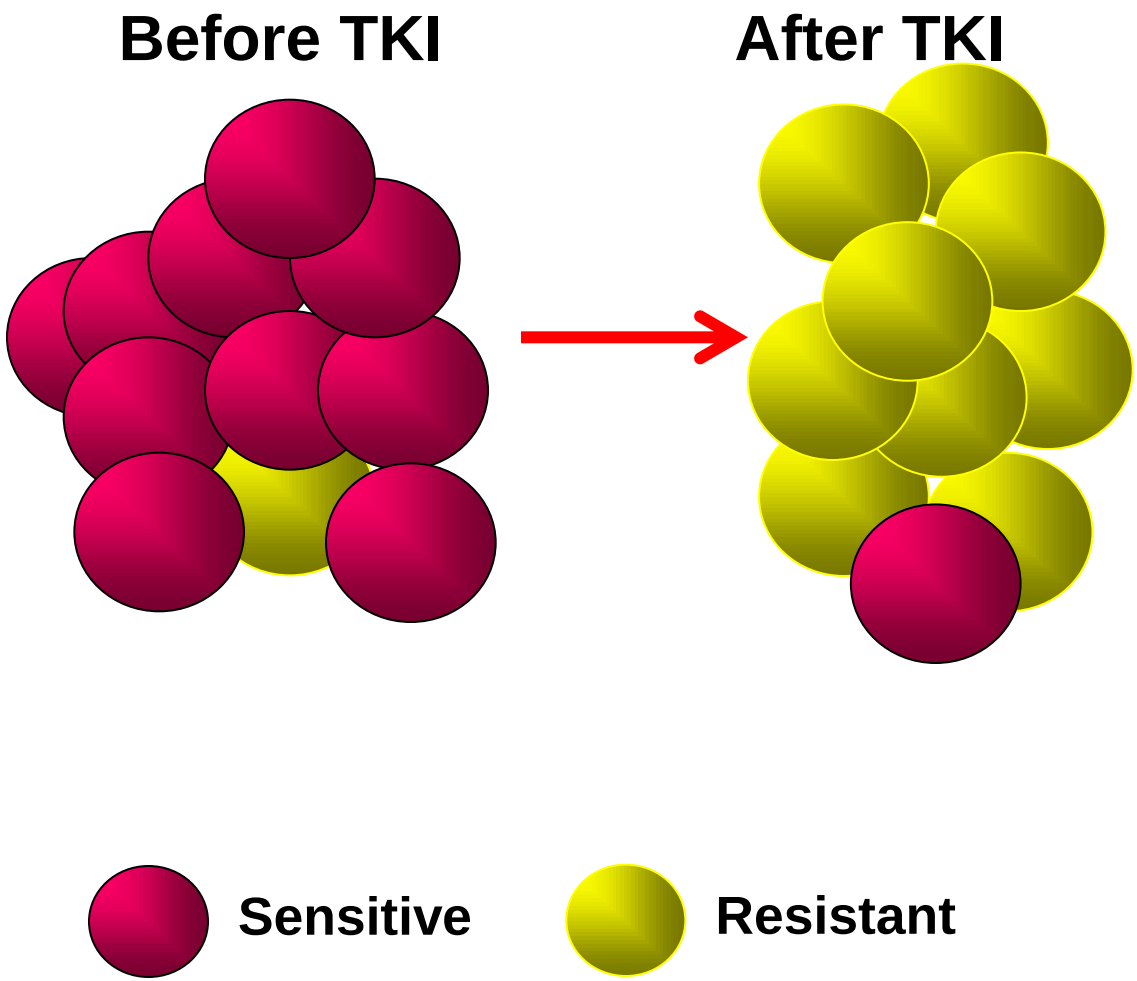


- MAPK1 amplification
- AXL kinase activation
- BRAF mutations

Takezawa Cancer Discov 2012

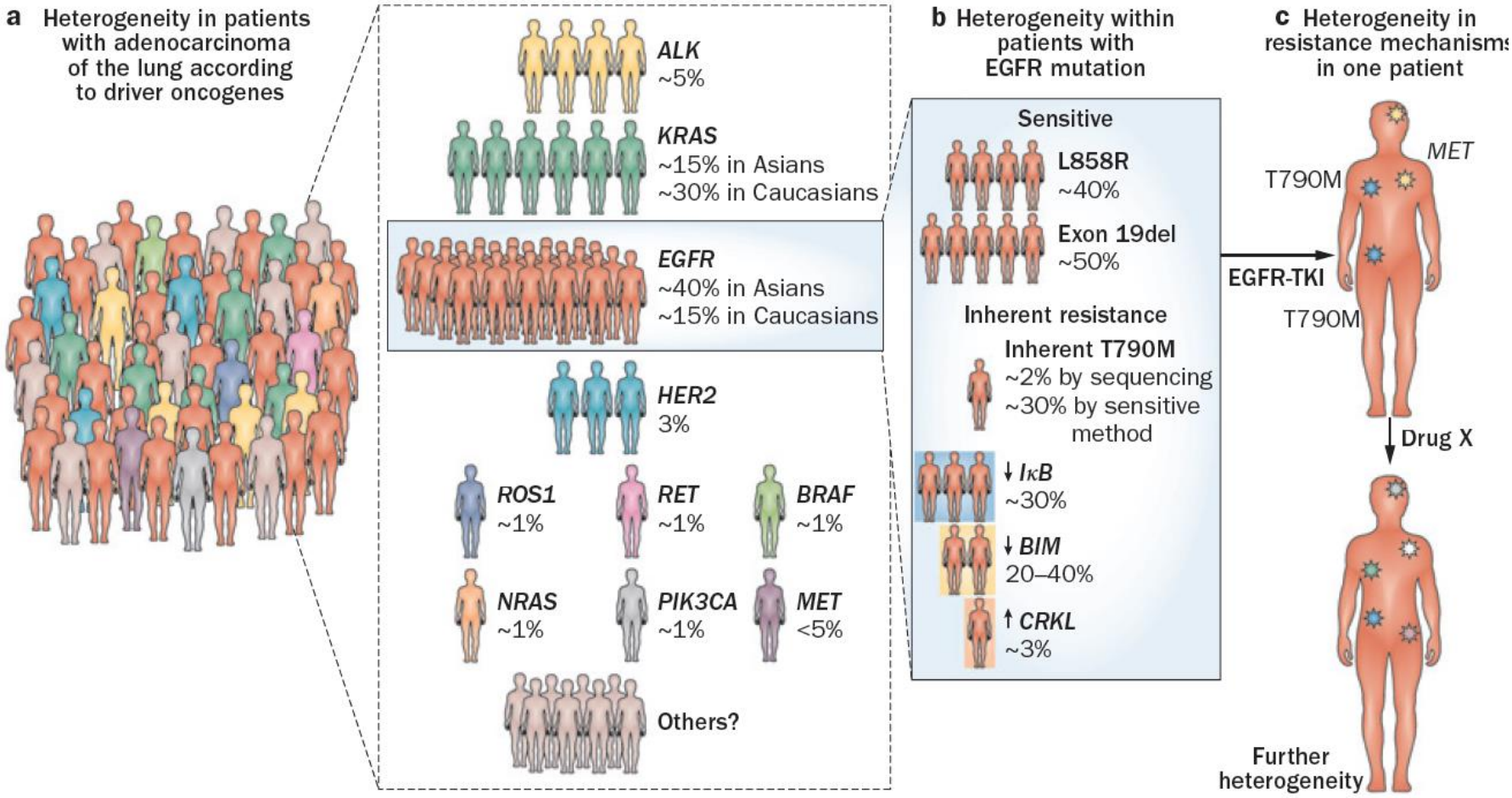
Doebele Clin Cancer Res 2012

Resistant cells are selected by Tyrosine Kinase Inhibitor (TKI) treatment



The efficacy of targeted therapy is affected by

TUMOR HETEROGENEITY



Mitsudomi Nat Rev Clin Oncol 2013

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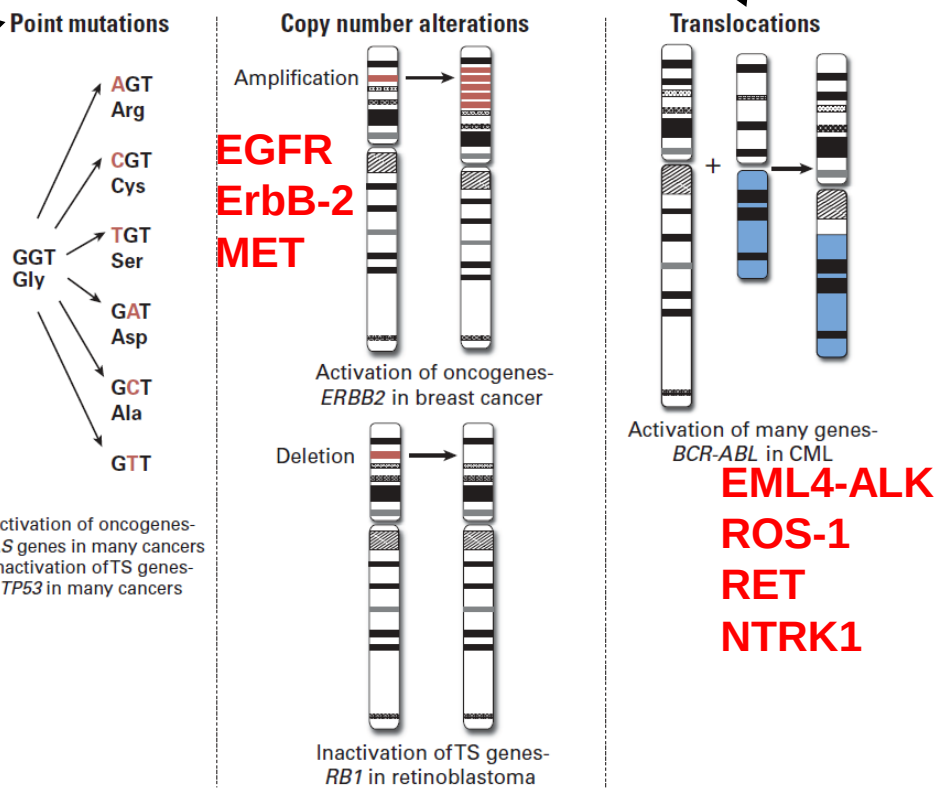
The major classes of genomic alterations that give rise to cancer

FISH, Immunohistochemistry

Sequencing, Real Time PCR etc.



EGFR
ErbB-2
BRAF
KRAS
NRAS
PIK3CA
AKT1
MAP2K1

EGFR
ErbB-2
MET

EML4-ALK
ROS-1
RET
NTRK1

Modified from McConaill JCO 2010

Clinical cancer genomics

High-throughput genotyping platforms (multiplexed screens): detection of selected somatic mutations. Allows identification of rare mutations (such as *BRAF*^{V600E} in lung cancer) but are limited to the known variants that have been chosen for analysis. Favor oncogenes over tumor suppressor genes, for which only frequently mutated sites are evaluated. Not suited for the analysis of gene copy number variations and does not allow the identification of gene rearrangements. Difficulties in detecting small insertions or deletions. Decreased sensitivity in tumor samples with high stromal admixture.

Targeted massively parallel sequencing: detection of somatic mutations with increased sensitivity. Permits increased sequencing coverage of predefined regions of interest, such as coding exons of known oncogenes and tumor suppressor genes, in addition to pharmacogenomic polymorphisms. Also suited for detection of gene copy number variations and predefined gene rearrangements. Identification of small insertions/deletions remains difficult with current algorithms.

Whole-exome sequencing: detection of point mutations, small insertions/deletions, pharmacogenomic polymorphisms, and gene copy number variations with increased sensitivity. Not suited for discovery of gene rearrangements.

Whole-genome sequencing: discovery of novel gene rearrangements, complex insertions/deletions, and microbial infections as well as identification of copy number alterations. Accurate point mutation detection remains a limitation, requiring significantly more coverage.

Dienstmann JCO 2013

Next Generation Sequencing-based Cancer Panels

Panel	Source	N. genes	DNA (ng)
TruSight Tumor Sequencing Panel	Illumina	26	30-300
TruSeq Amplicon Cancer Panel	Illumina	48	250
Ion AmpliSeq™ Cancer Hotspot Panel v2	Life Technologies	50	10
Ion AmpliSeq™ Colon and Lung Cancer Research Panel v2	Life Technologies	22	10
GeneRead DNAseq Tumor Actionable Mutations Panel	Qiagen	8	10
GeneRead DNAseq Clinically Relevant Tumor Panel	Qiagen	24	10

Slide courtesy of Nicola Normanno

Ion AmpliSeq™ Colon & Lung Cancer Panel

Developed and verified 8 labs experienced in colon & lung cancer screening

Prof. Ian Cree
Warwick Medical School
United Kingdom

Dr. Marjolijn Ligtenberg & Dr. Bastiaan Tops
Radboud University
Nijmegen Medical Centre
The Netherlands

Prof. Orla Sheils
Trinity College Dublin,
Ireland

Dr. Cristoph Noppen &
Dr. Henriette Kurth
VIOLLIER AG Basle,
Switzerland

Dr. Ludovic Lacroix
Institut Gustave Roussy
Paris, France

Prof. Aldo Scarpa
ARC-NET University of
Verona Italy

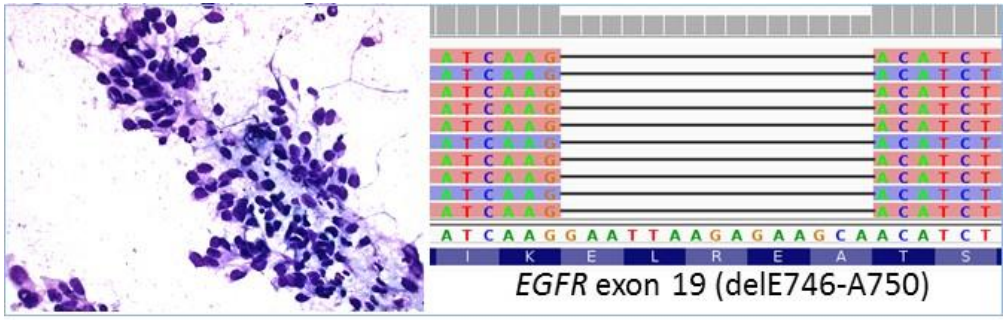
Prof. Pierre Laurent Puig
Université Paris Descartes,
France

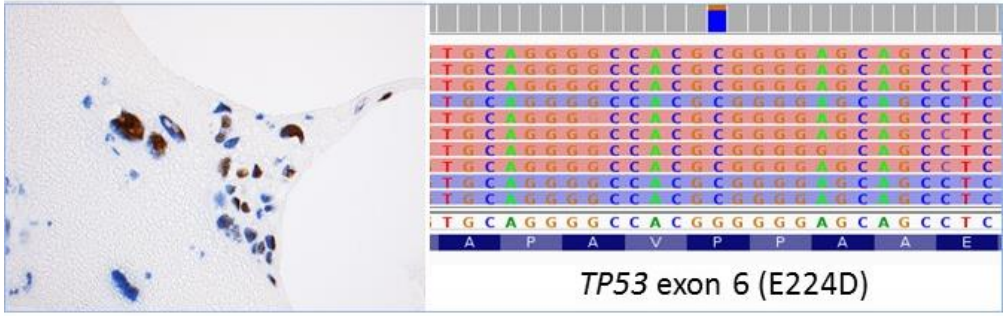
Dr. Nicola Normanno
Centro Ricerche
Oncologiche Mercogliano,
Italy

Ion AmpliSeq™ Colon and Lung Cancer Panel
Panel design and relevance

- **Genes included**: KRAS, EGFR, BRAF, PIK3CA, AKT1, ERBB2, PTEN, NRAS, STK11, MEK1, ALK, DDR2, CTNNB1, MET, TP53, SMAD4, FBXW7, FGFR3, NOTCH1, ERBB4 , FGFR1, FGFR2
  - **New genes DDR2 and MEK1**
  - KRAS exon4 to include codons 117 to 146
  - EGFR exon12 to include codon 492
  - BRAF exon11 to include codons 466 and 469
- Comparison with Ion AmpliSeq™ Cancer Hotspot Panel v2 (CHP2)
  - 11 of 90 amplicons are new
    - DDR2, ALK, EGFR, MEK1

Molecular typing of lung adenocarcinoma on cytological samples using a multigene next generation sequencing panel





36/38 (95%) adequate libraries

EGFR	6/36 (16%)
KRAS	10/36 (28%)
TP53	7/36 (18%)
PIK3CA	3/36 (8%)
BRAF	2/36 (5%)
STK11	1/36 (3%)

24/36 (67%) at least one
9/36 (25%) multiple

Colon and Lung Panel - a single workflow solution

DNA analysis

Colon and Lung AmpliSeq ™ panelv2

DNA mutations

Tumor Sample

RNA lung fusion research panel

ALK, ROS,RET, NTKR1 fusions & expression

Ion PGM ™ System

semiconductor sequencing

Ion Reporter ™ Software

automated analysis & reporting

RNA Analysis

Sample to report in less than 36 hours

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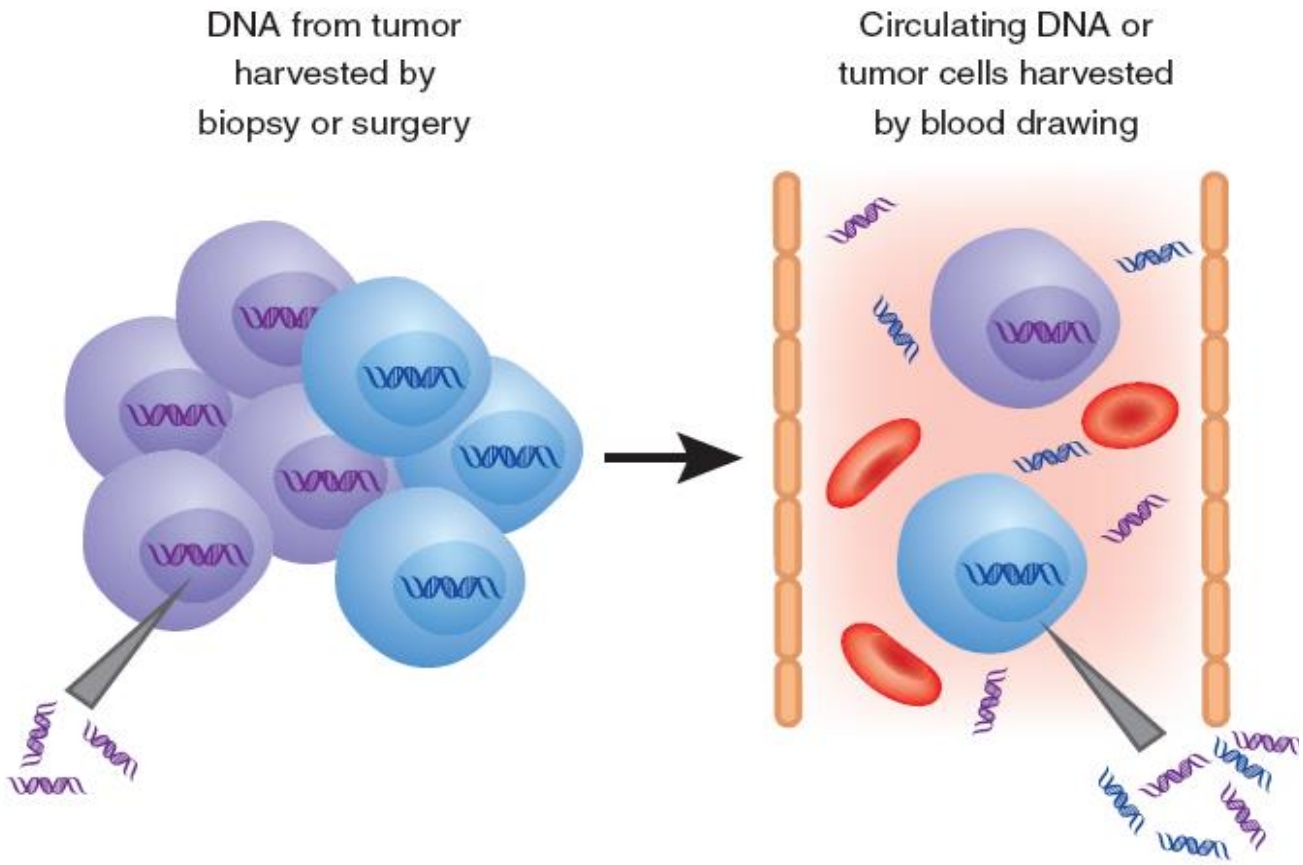
Different sources of tumor DNA



Fleischacker & Schmidt Nat Med 2008

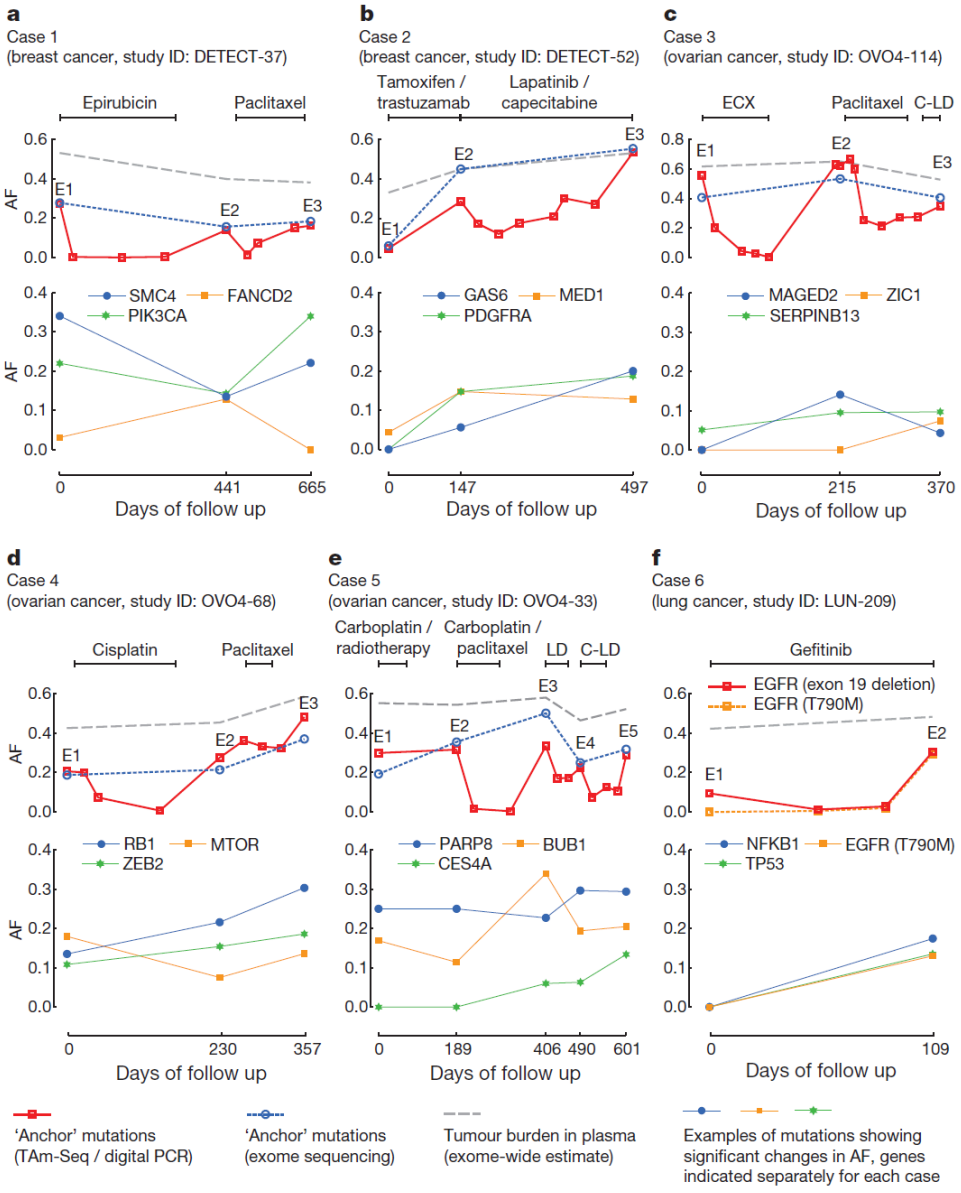
Detection of EGFR mutations in plasma

Different methods have been used to detect EGFR mutations in the plasma/serum of NSCLC patients

Method	Sensitivity*	Reference
Therascreen	43.1%	Goto et al. J Thorac Oncol 2011
PNA clamp	59.1%	Rosell et al. N Engl J Med 2009
Digital PCR	92%	Yung et al. Clin Cancer Res 2009

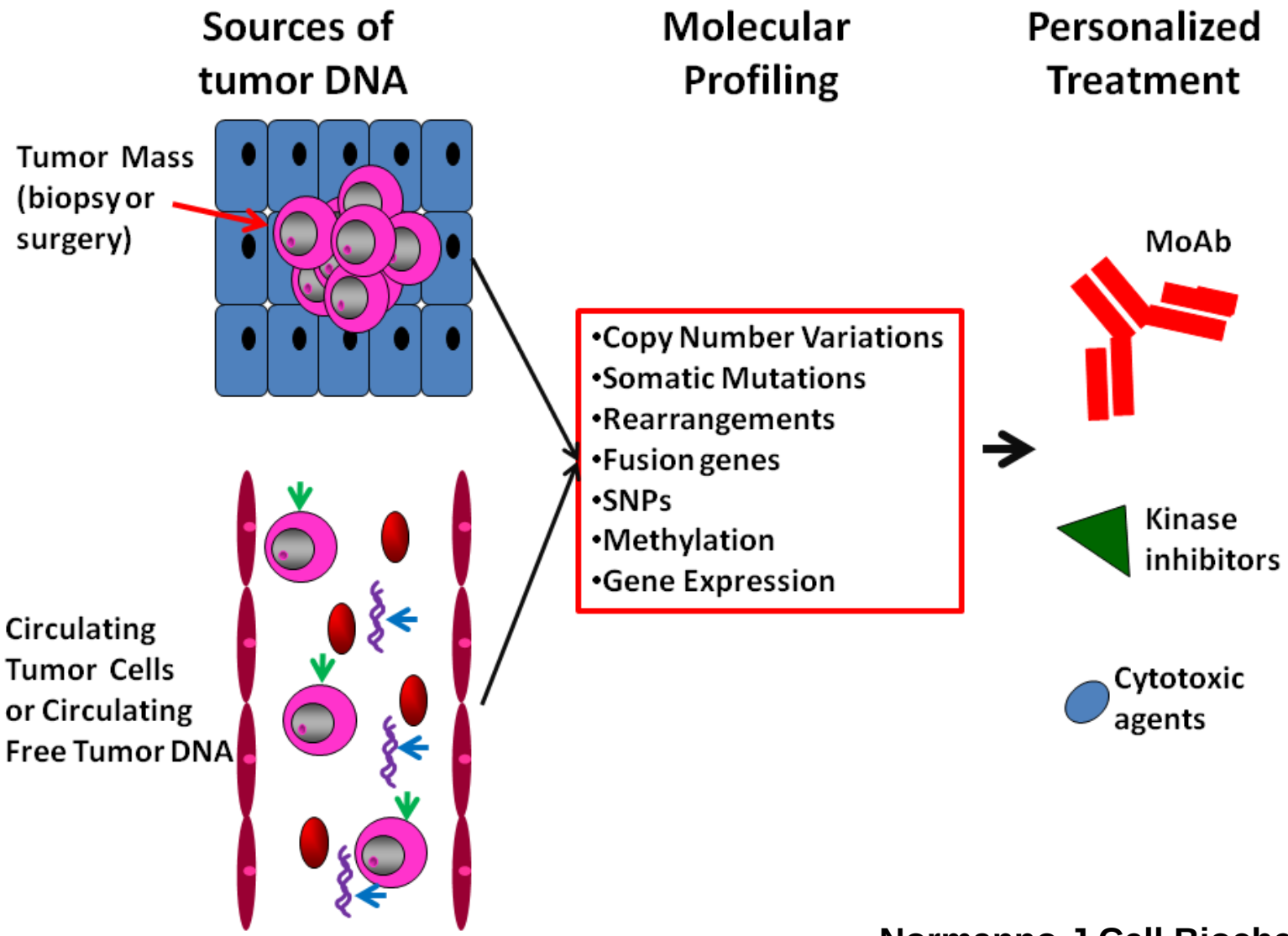
*ratio between positive cases on plasma and positive cases on tissue

Whole exome sequencing of plasma DNA



Murtaza Nature 2013

The future of biomarker testing in NSCLC

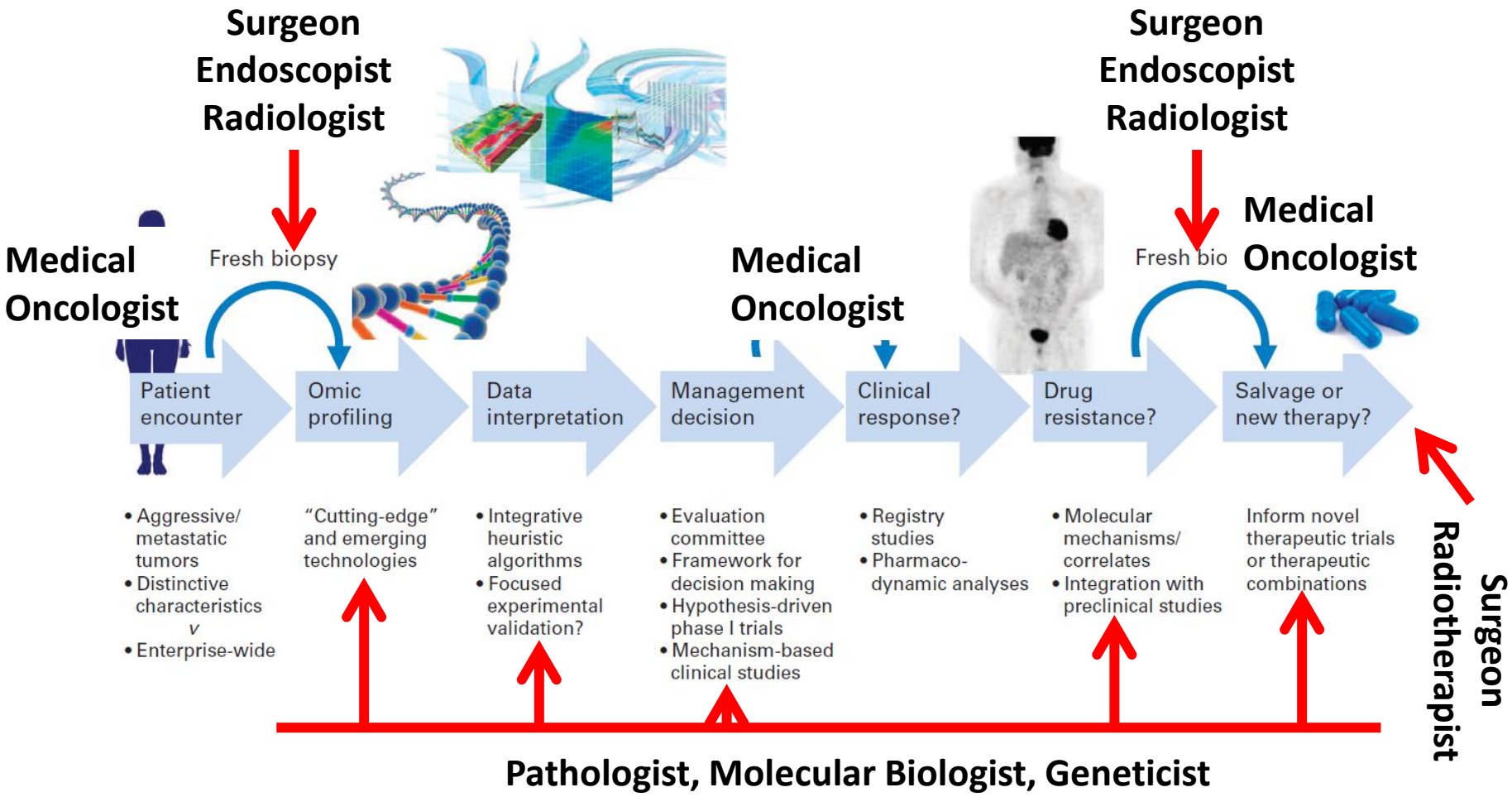


Normanno J Cell Biochem 2013

Challenges in genomics-driven oncology

- **Alterations of “actionable” oncogenic pathways are not always predictive of response to targeted agents.**
- **Different molecular alterations of the same oncogene may not be equivalent.**
- **Intra-tumor heterogeneity may affect response to targeted agents.**
- **The molecular profile of solid tumors may significantly change following treatment with target based agents.**
- **Genomic testing programs should be strongly linked to matched clinical trials.**

Genomics-Driven Oncology



Garraway JCO 2013