Expert Review in Next Generation Sequencing:

Integration of Next Generation Sequencing Into Clinical Management of Solid Tumors

Reference Slide Deck

Moderator

Robert E. Coleman, MD, FRCP, FRCPE
Medical Director, prIME Oncology

Discussants

Fabrice André, MD, PhD
Institute Gustave Roussy, Villejuif, France

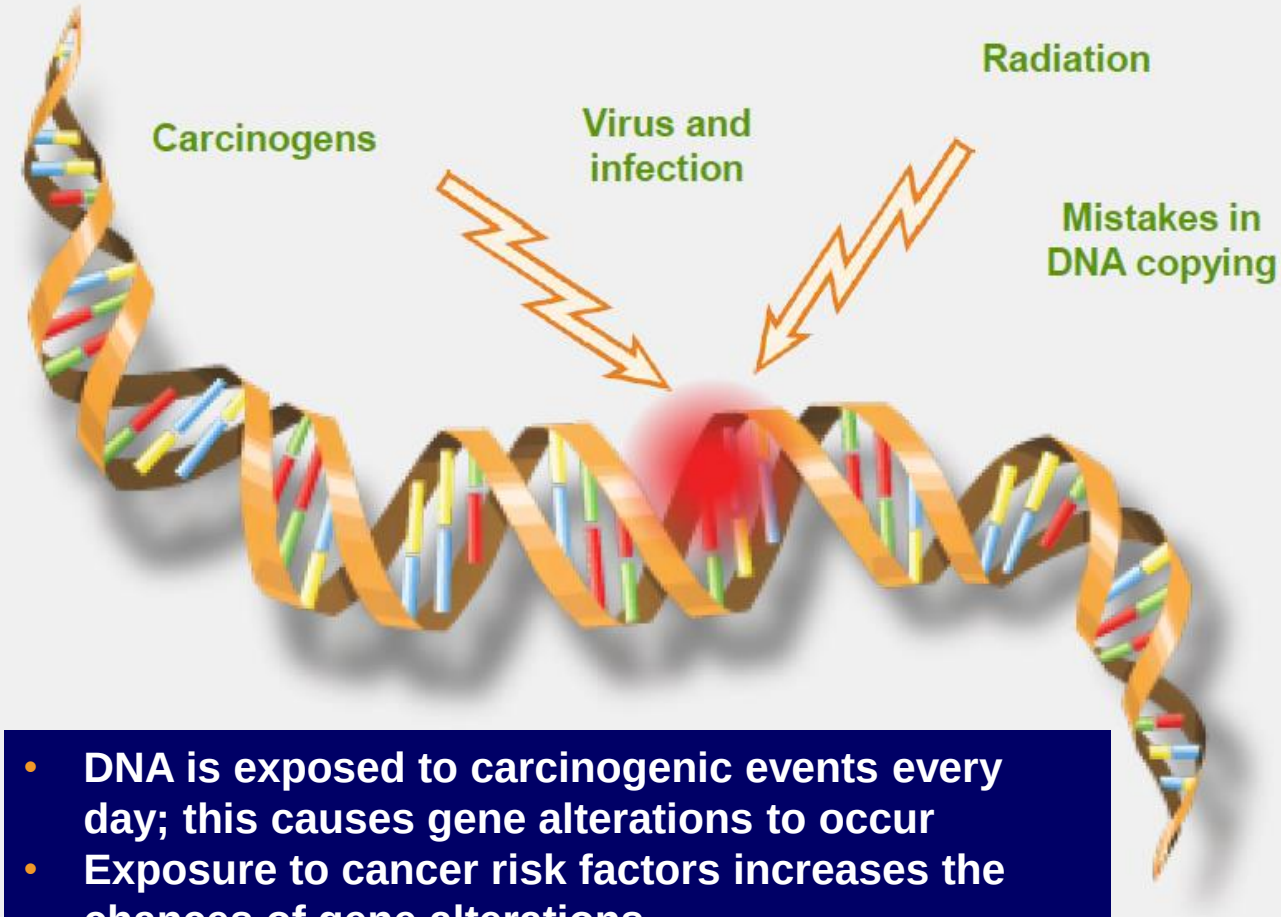
Dirk Arnold, MD, PhD
Tumor Biology Center Freiburg, Freiburg, Germany

Mary O'Brien, MD, FRCP
Royal Marsden Hospital, London, United Kingdom

prIME oncology

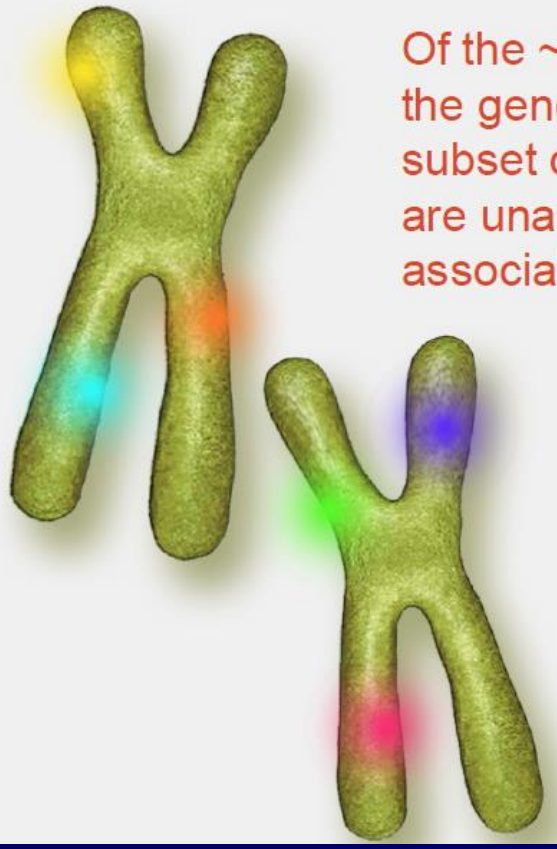
Introduction and Overview

Cancer Is a Disease of the Genome



- DNA is exposed to carcinogenic events every day; this causes gene alterations to occur
- Exposure to cancer risk factors increases the chances of gene alterations

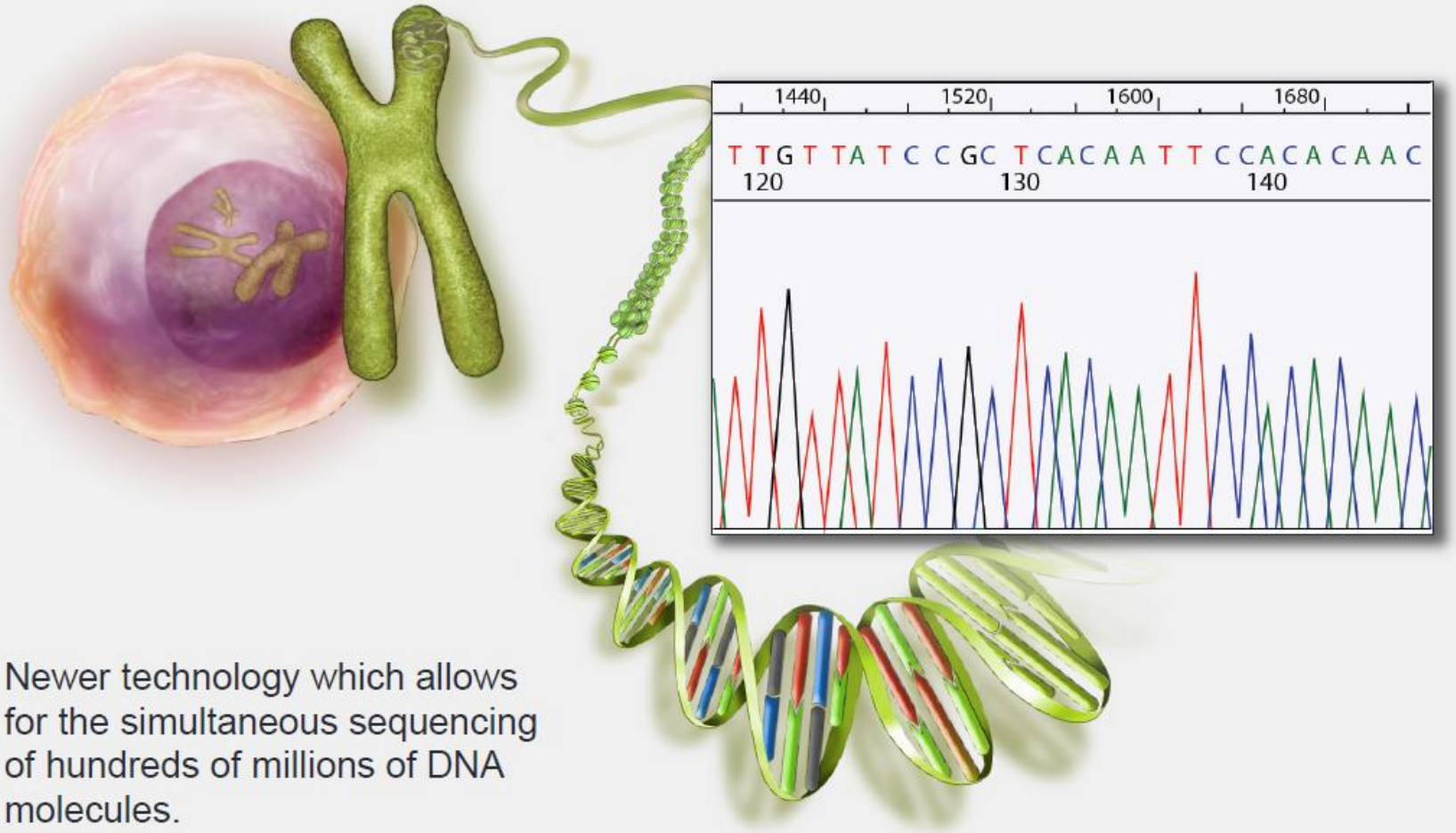
Cancer-Related Genes



Of the ~20,000 genes in the genome, only a subset of a few hundred are unambiguously associated with cancer

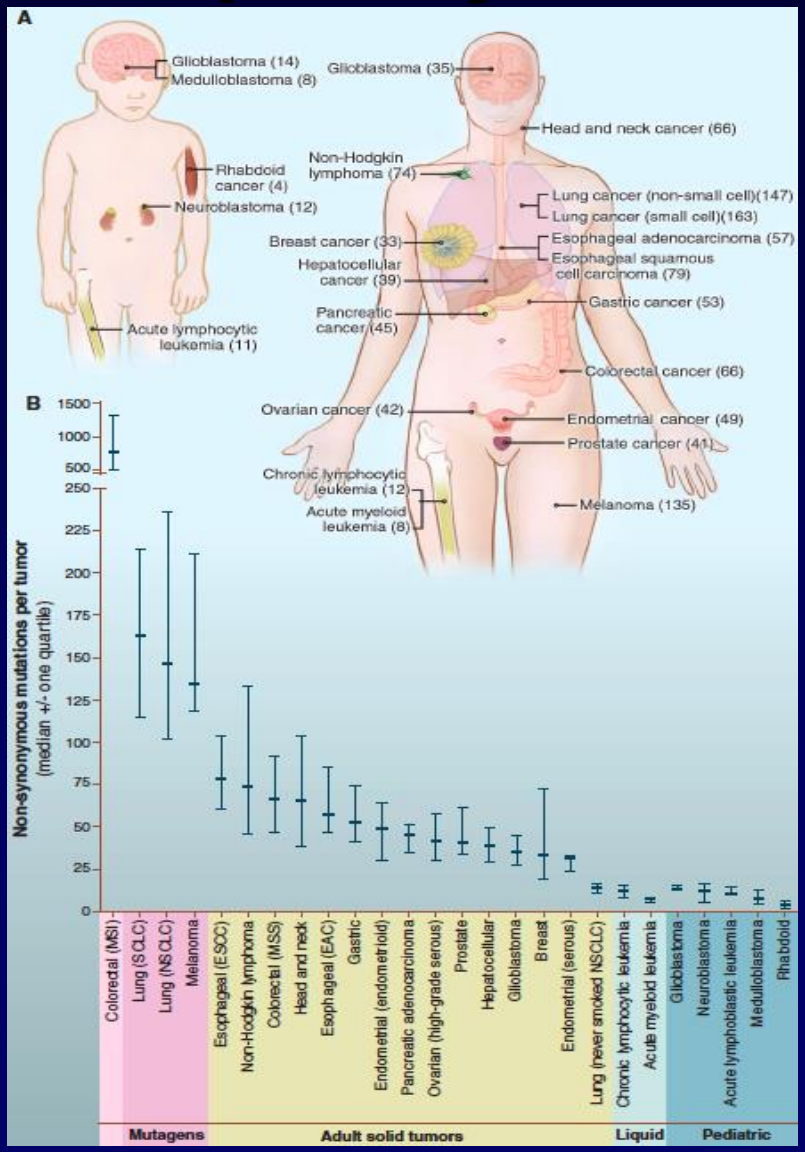
Gene names
BCR-ABL
ERBB2 (HER-2)
BRAF
KRAS
EGFR
KIT
BRCA1

What is Next Generation Sequencing (NGS)?



- Newer technology which allows for the simultaneous sequencing of hundreds of millions of DNA molecules.

Frequency of Mutations Across Cancer



Melanomas and lung tumors display many more mutations than average, with ~200 nonsynonymous mutations per tumor.

These larger numbers reflect the involvement of potent mutagens. Accordingly, lung cancers from smokers have 10 times as many somatic mutations as those from nonsmokers.

Vogelstein B, et al. *Science.* 2013;339(6127):1546-1558.

Targeted approaches allow rapid screening of multiple genes for clinically relevant mutations

The Clinical Diagnostic Workflow

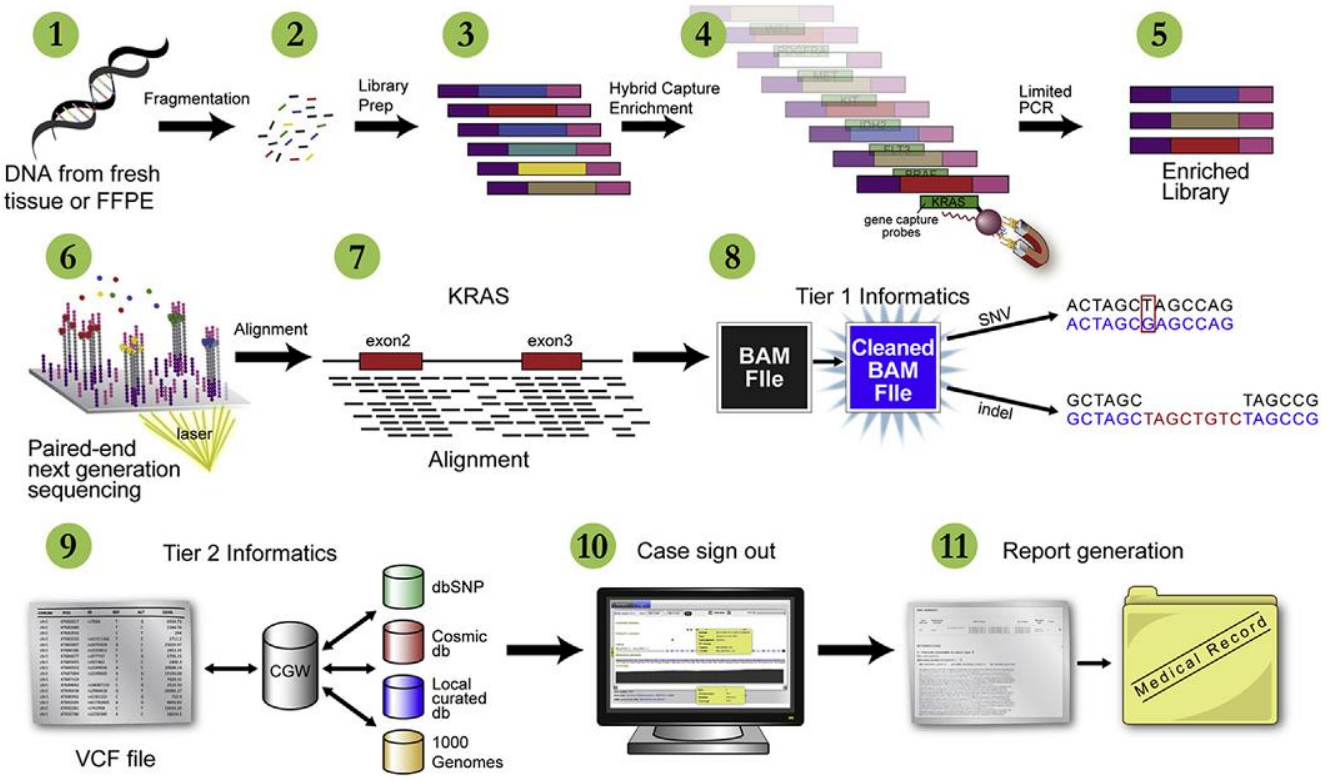


Figure 2 Schematic view of the WUCaMP assay workflow. DNA is extracted from tumor tissue (**1**) derived from fresh or FFPE specimens and fragmented by sonication (**2**). Libraries are prepared and amplified via limited-cycle PCR (**3**) and enriched for WUCaMP genes by fluid phase hybridization to custom cRNA capture reagents (**4**). The hybridized product is amplified (**5**) and sequenced on an Illumina HiSeq 2000 or Illumina MiSeq instrument (**6**). Paired-end reads are aligned to the genome (**7**), PCR duplicates are removed (**8**), and variant calls are made (**9**). Variants are annotated and classified by our internally developed CGW application, using publicly available and proprietary databases, and the case is reviewed and interpreted by a clinical genomicist for sign-out in CGW (**10**). A report is then issued to the medical record (**11**).

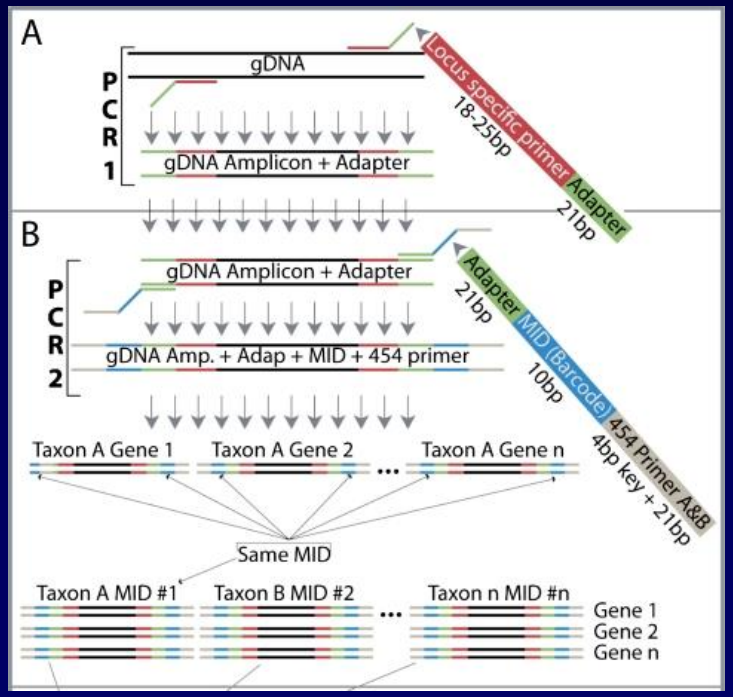
Cottrell CE, et al. *J Mol Diagn.* 2014;16(1):89-105.

NGS Modalities

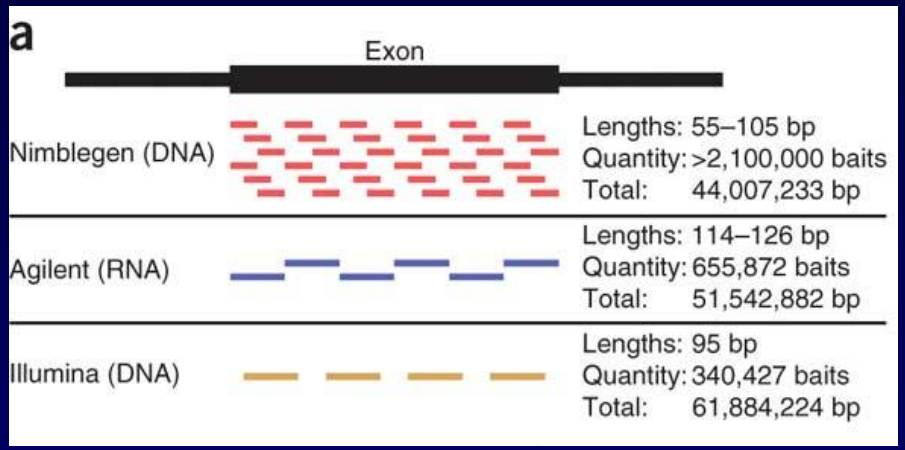
- **Whole-genome sequencing**
  - **Determines the complete DNA sequence of an organism's genome at a single time**
- **Whole-exome sequencing**
  - **Selectively sequences only the coding areas of the genome**
- **Targeted sequencing (hot spot)**
  - **Sequences only the hot spots of a subset of genes of interest**
- **Fully informative sequencing**
  - **Sequences a defined subset of genes of interest in their entirety**

Targeted Sequencing Approaches

Amplicon Sequencing



(Whole) Exome Sequencing



Clark MJ, et al. *Nat Biotechnol*. 2011;29(10):908-914.
Bybee SM, et al. *Genome Biol Evol.* 2011;3:1312-1323.

Mutation Detection in Next-Generation Sequencing

- **High throughput**
  - **Survey many cancer-related genes at once**
- **Mostly unbiased detection of all mutation types**
  - **Single-nucleotide variations**
  - **Copy-number alterations**
  - **Indels, inversions, translocations**
- **Readout of the mutation frequency**
  - **Quantification of mutations in heterogeneous samples**

NGS Data Quality and Quantity

- Origin
- Preparation
- Storage

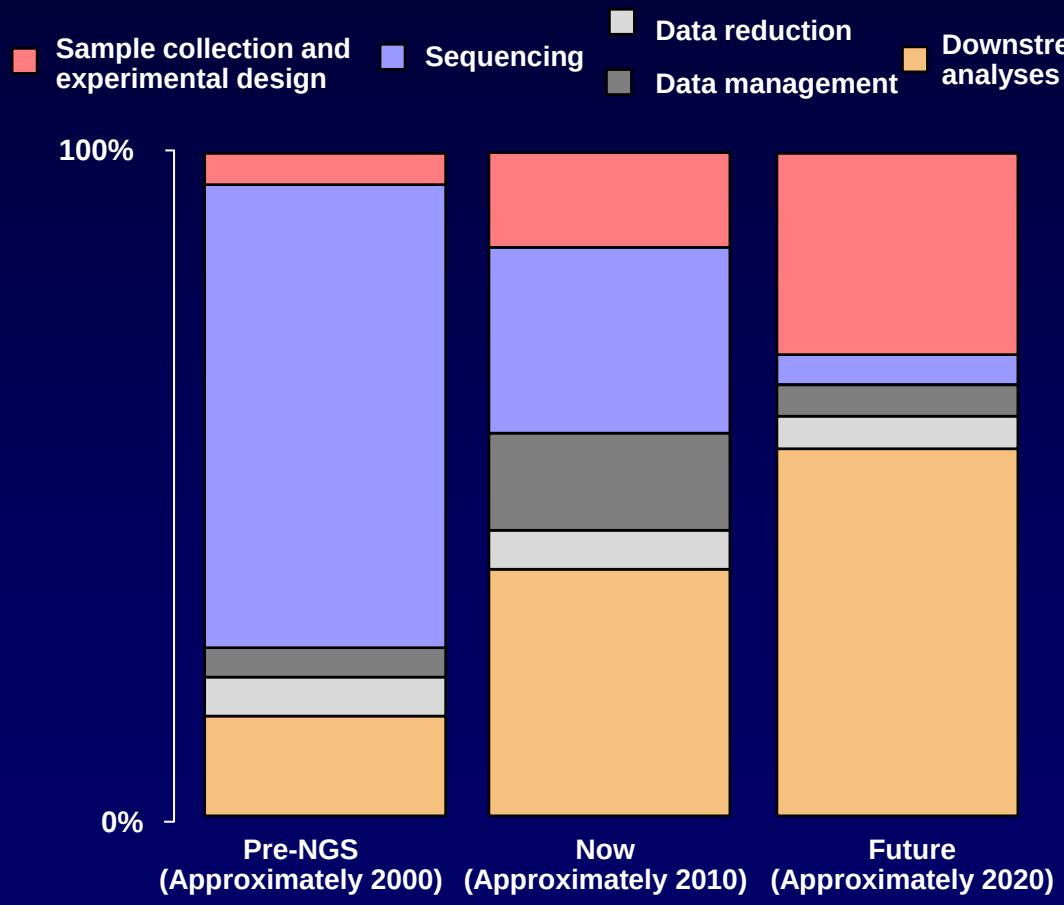
- Platform
- Application
  - Provider
  - Semi-customized
  - Fully-customized

- Provider
- Custom software packages
- Customized workflow (more advanced tools)

Development of the Most Suitable Bioinformatics Workflow

- **Fundamental element of an NGS pipeline is the mapping tool**
- **Determine the optimal mapper for your application (not trivial!) (choose from more than 80 mapping tools)**
  - **Compare most important features/characteristics of mapping tools**
  - **Select the mapping tools most probably addressing your needs**
  - **Evaluate the reduced subset of mappers in terms of their accuracy**
- **Additionally fundamental to a cancer NGS pipeline is the variant caller**

Sequencing vs Bioinformatic Costs



The higher the number of sequencing errors,

the higher the number of variations to be annotated,

the higher the bioinformatic costs.

Sboner A, et al. *Genome Biol*. 2011;12(8):125.

Factors to Consider When Interpreting Results

- Tumor purity
- Coding or non-coding mutation ?
- Rare germline single nucleotide polymorphism (SNP)?
- Known oncogene or tumor suppressor?
- Has variant been reported before?
- Is it clonally dominant?
- How many driver events?
- Is there functional evidence for mutation activity in the literature?
  - Hotspot mutation in an oncogene
  - Deleterious mutation in a tumor suppressor

Interpreting Results (cont)

- Does tumor type in question have a tendency for hypermutation?
  - Melanoma, NSCLC, MSI+ colorectal cancer
- Does mutation have the hallmarks of an FFPE artifact (v low frequency C>T or G>A mutations)?
- Read the small print - what exons are covered?
- Determine which codons were not effectively sequenced in the sequencing reaction:
  - Absence of a driver mutation does not necessarily mean the driver is not present
- Variant allele frequencies close to 50% or 100% - suspect a germline event
  - Vigilant for cancer-predisposing germline mutations

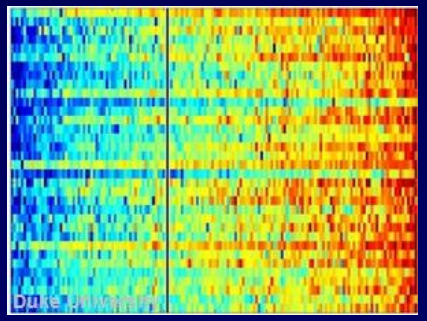
Possible Applications of NGS to Better Understand Tumor Biology

Future Application Of Genomics: Understand the Biology at the Individual Scale

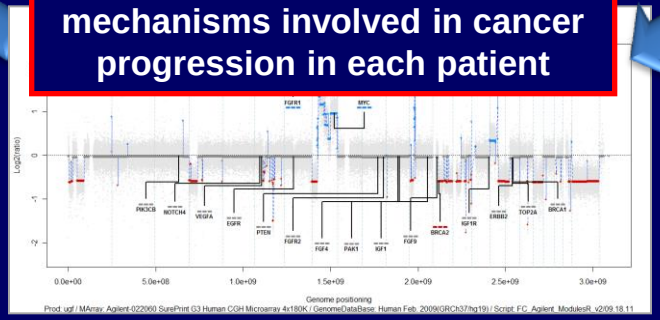
Patients with lethal cancer

Tumor specimen

Molecular profiling



The deliverable: A tool to decipher the molecular mechanisms involved in cancer progression in each patient



Targeted therapy according to the molecular profile

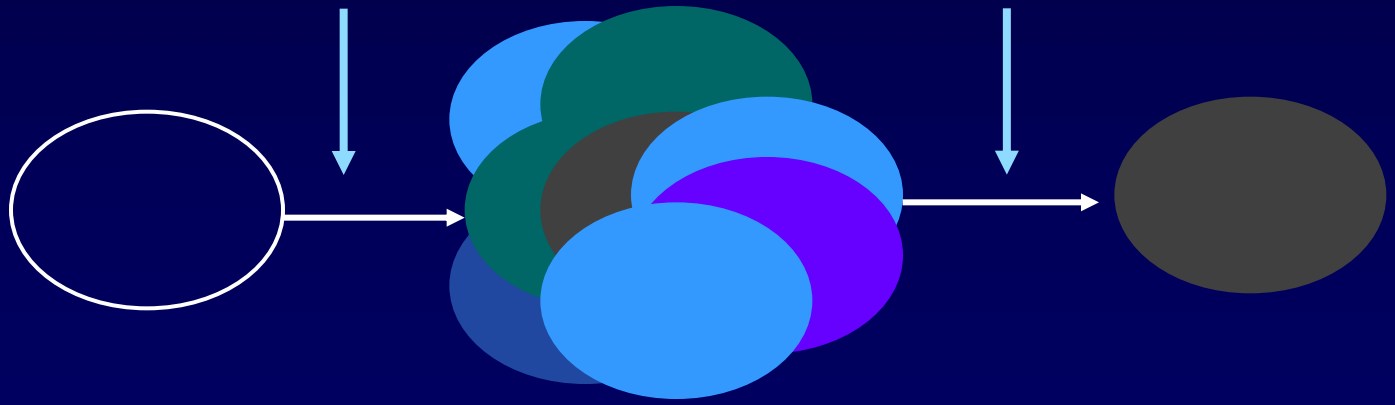
How Can Genomics Help the Oncologist Understand the Biology in Each Patient in Order to Provide Better Treatment?

- **Identify the driver:**
  - Targeted sequencing + capacity to quantify CNA
  - ctDNA to scale-up
- **Track & kill the lethal clone**
  - Intratumor heterogeneity and resistance to therapy
  - Ultradeep sequencing and ctDNA to find the lethal clone
- **Long-term perspectives:**
  - Define the mutational process: whole-exome sequencing
  - Understand the dialogue between cancer cell & immune system: whole-exome sequencing

Genomic Tests to Identify Oncogenic Drivers at the Individual Scale

Oncogenic drivers:
Malignant transformation
Cancer progression

Targeting oncogenic drivers:
oncogene de-addiction
= tumor shrinkage

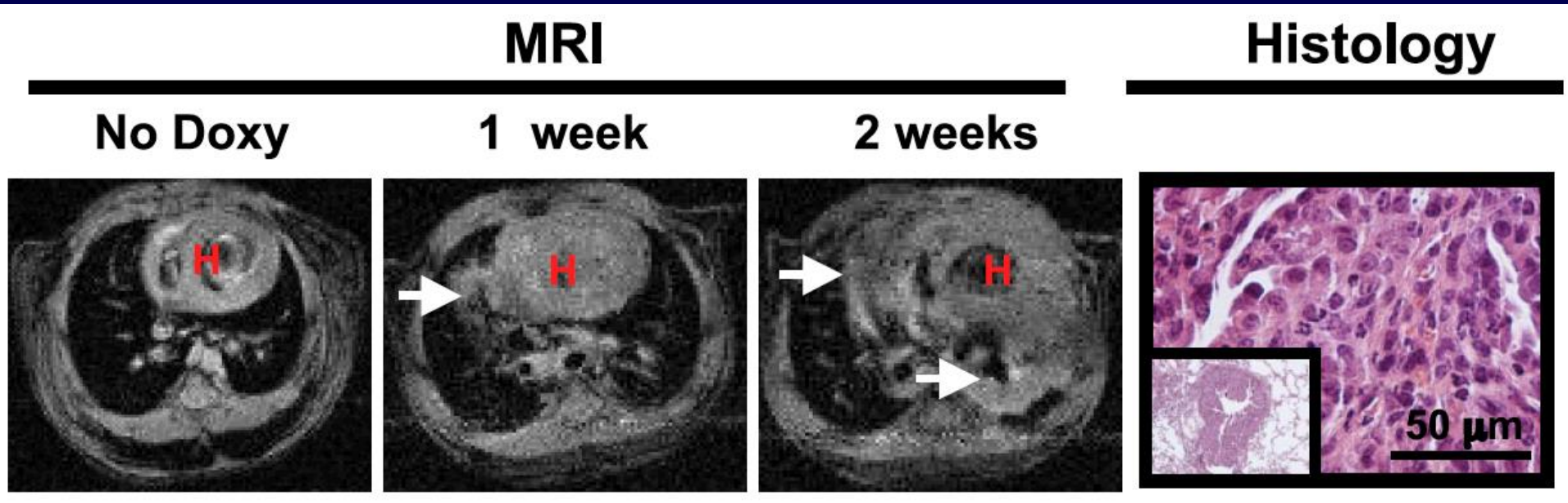


Normal cell | Cancer progression | Residual disease

Can we identify oncogenic drivers in individuals, in order to shrink the tumor?

A Preclinical Example of an Oncogenic Driver

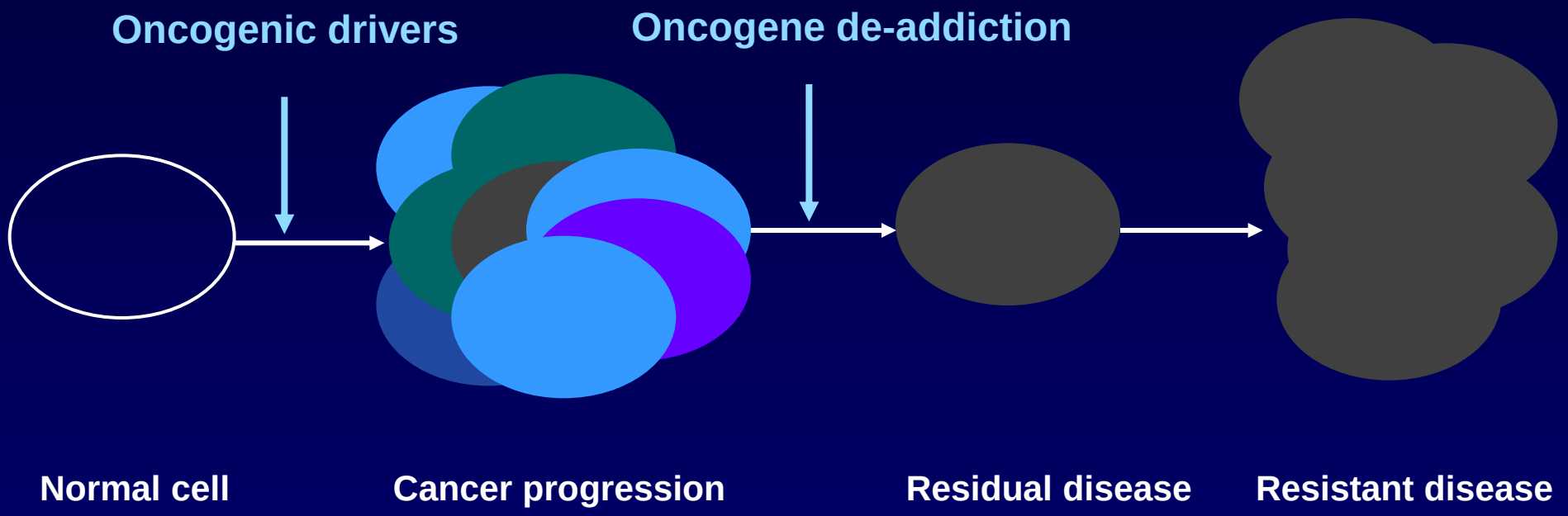
Inducible expression of mutated HER2 (HER2YVMA): Rapid development/maintenance of adenosquamous lung tumors in mice



Doxy, doxycycline

Perera SA, et al. *Proc Natl Acad Sci U S A.* 2009;106(2):474-479.

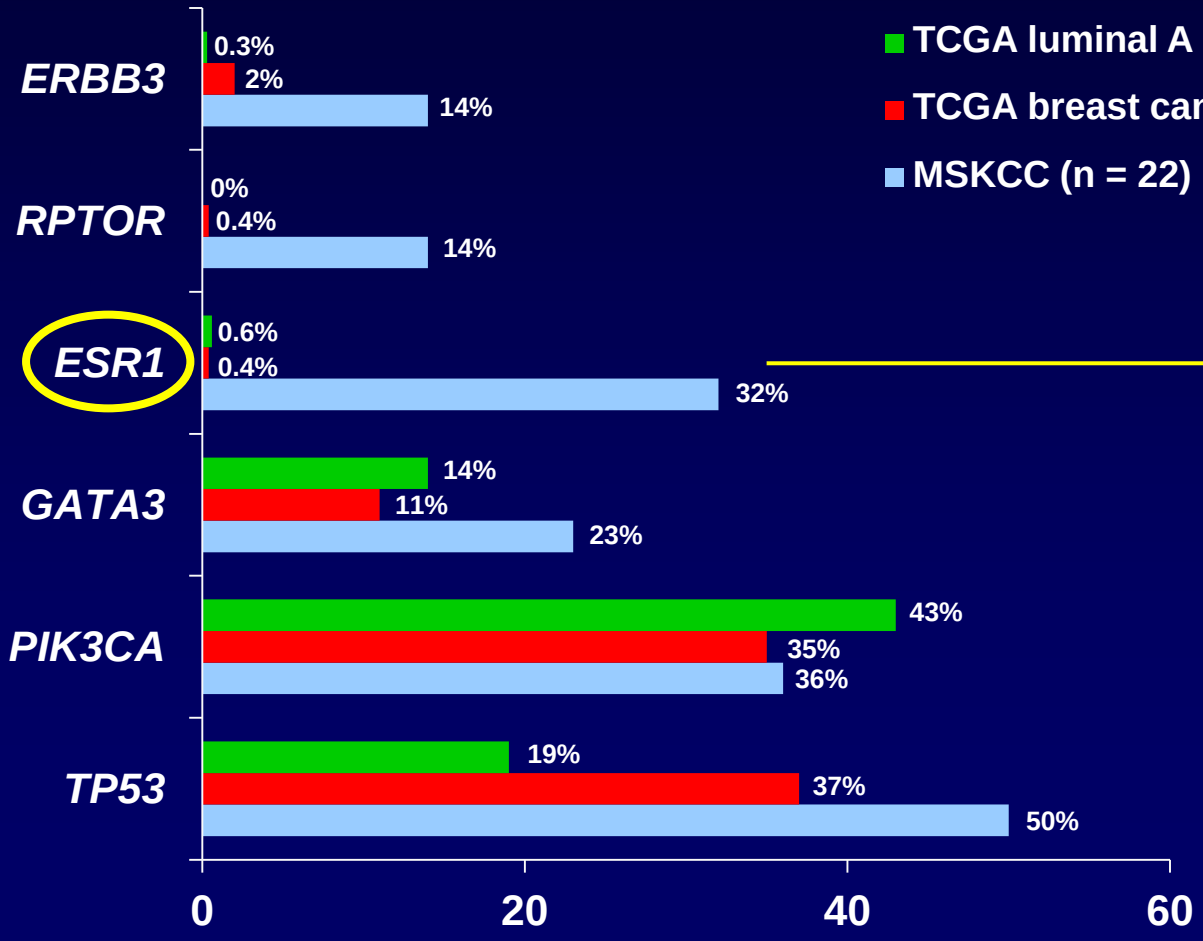
Beyond the Drivers: How the Use of Genomic Tests Could Help Avoid Resistance?



How Could the Use of Genomic Tests Help Avoid Resistance?

- Does intratumor heterogeneity predict resistance to therapy?
- Is it possible to detect the lethal subclone in primary tumor using deep sequencing…and kill it?
- Is it possible to detect the appearance of lethal subclone by using circulating DNA… and kill it?

Which Gene Is a Good Candidate to Work on This Concept in BC ?



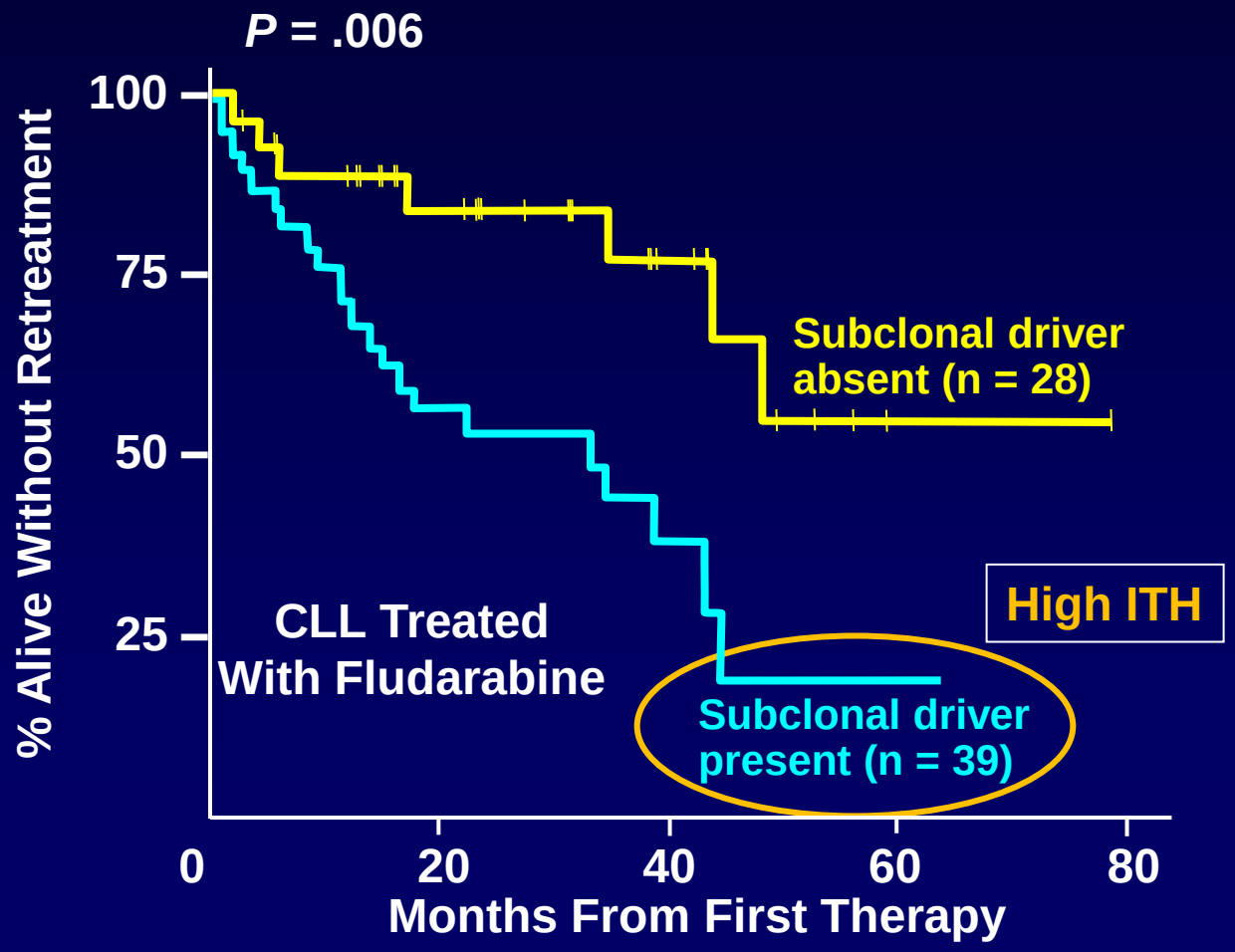
Mutated in metastases

Not in primary (using low coverage)

Mediates resistance to therapy

Toy W, et al. *Nat Genet.* 2013;45(12):1439-1445.

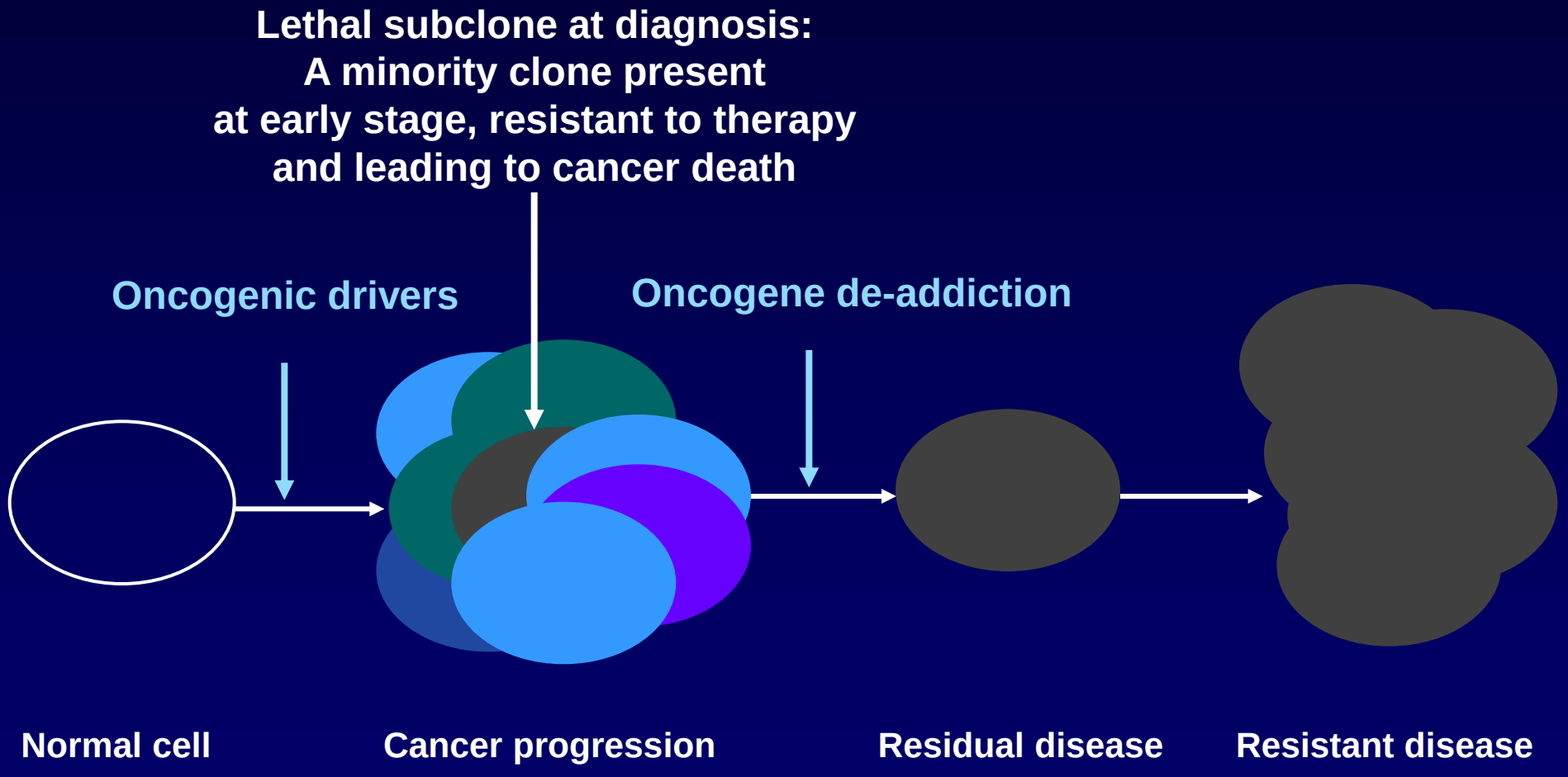
Does Intratumor Heterogeneity Predict Resistance to Therapy?



Intratumor heterogeneity could define a disease resistant to therapy

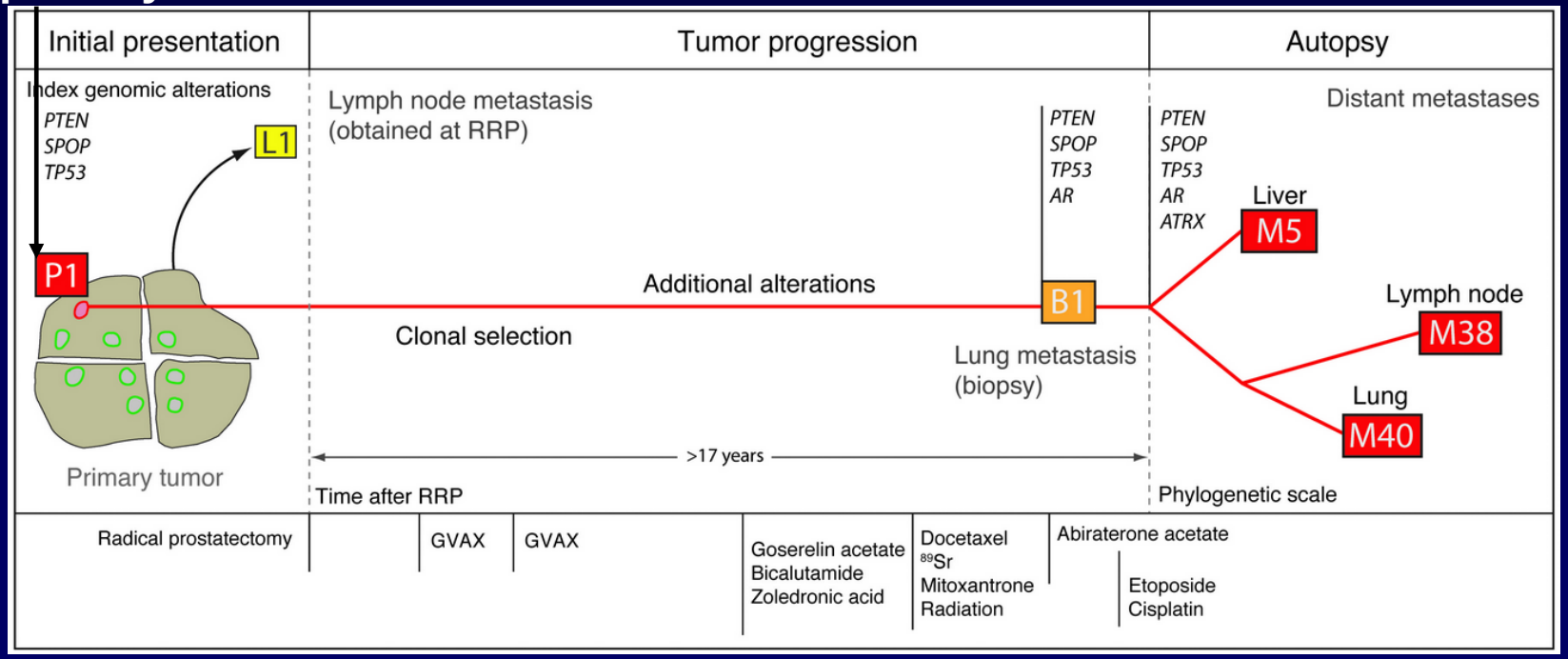
Landau DA, et al. *Cell.* 2013;152(4):714-726.

Is It Possible to Detect the Lethal Subclone in Primary Tumor ?



Is It Possible to Detect the Lethal Subclone in Primary Tumor?

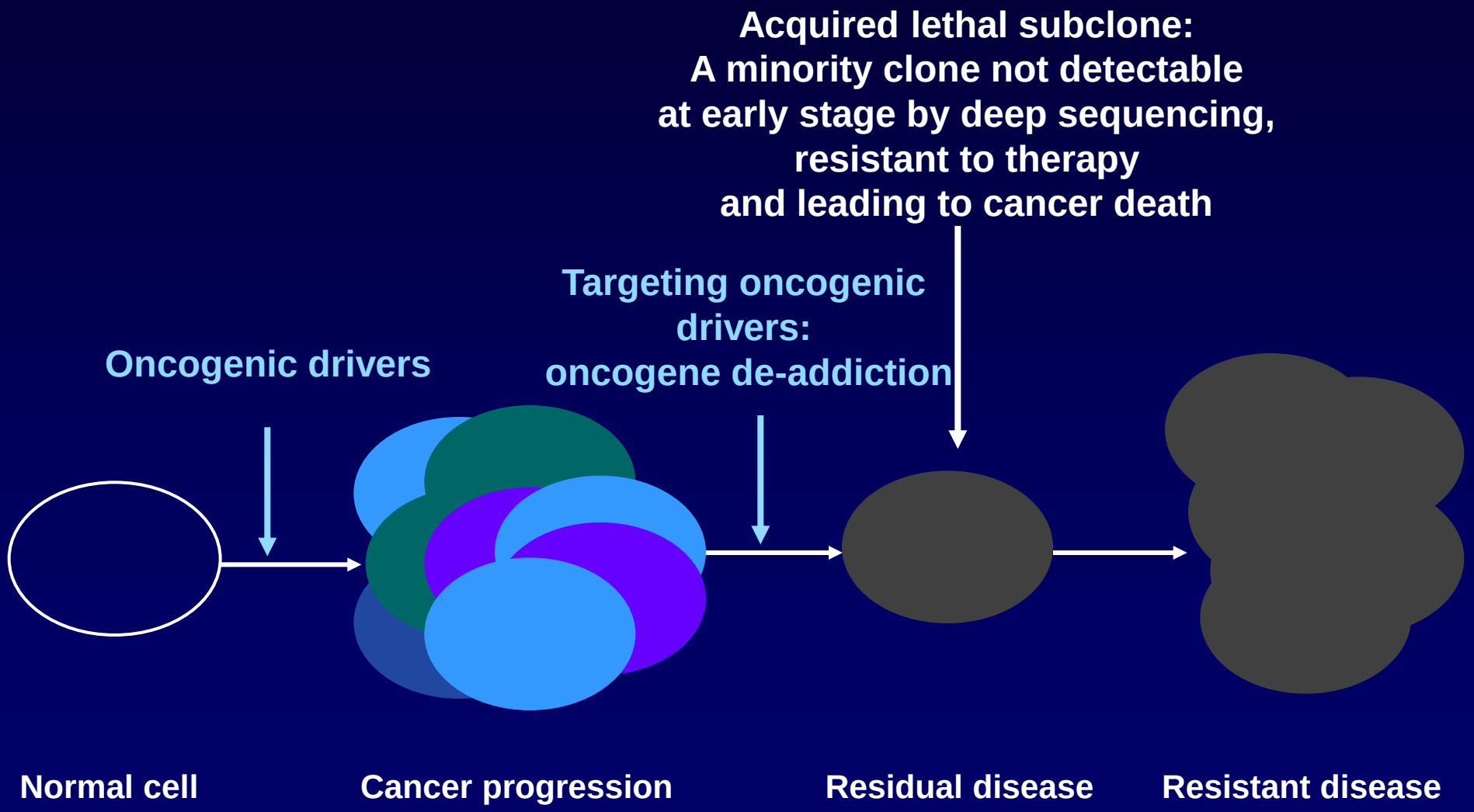
Lethal clone present in a minority of cells in the primary tumors



Ultradeep sequencing (x40,000) could detect the lethal minority subclone in primary tumor

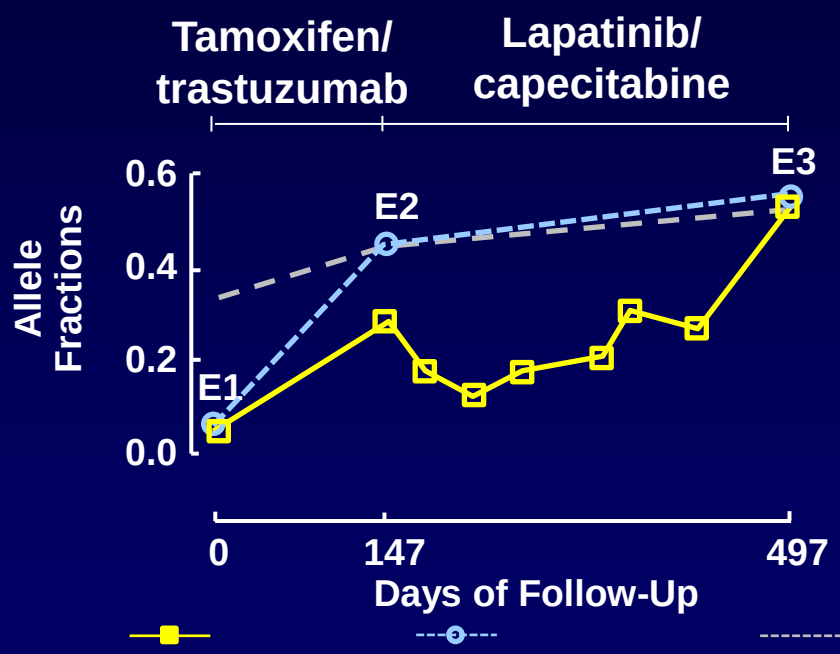
Hafner MC, et al. *J Clin Invest.* 2013;123(11):4918-4922.

Is It Possible to Detect the Appearance of Lethal Subclone ?



Is It Possible To Detect The Appearance Of Lethal Subclone?

Breast cancer, study ID: DETECT-52

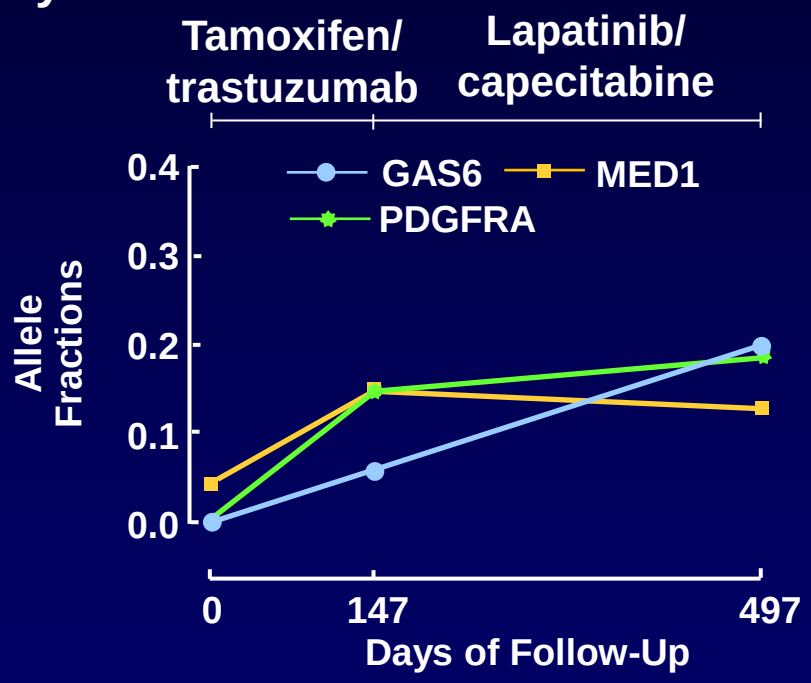


‘Anchor’ mutations (TAm-Seq/digital PCR)

‘Anchor’ mutations (exome sequencing)

‘Tumor burden in plasma (exome-wide sequencing)

‘Anchor’ mutations were measured using digital PCR or Tam-Seq for all available plasma samples, and using exome sequencing at selected timepoints indicated by E1, E2, and E3.

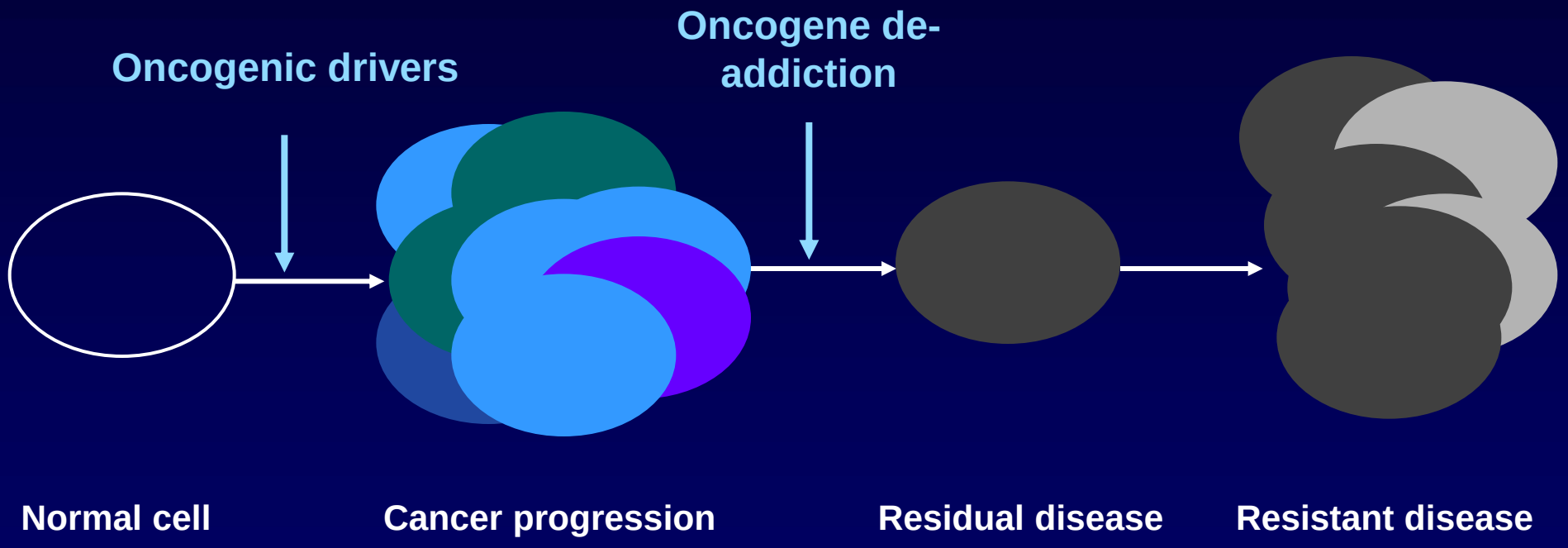


Examples of mutations showing significant changes in allele fractions

Circulating DNA to monitor appearance of lethal clones

Murtaza M, et al. *Nature*. 2013;497(7447):108-112.

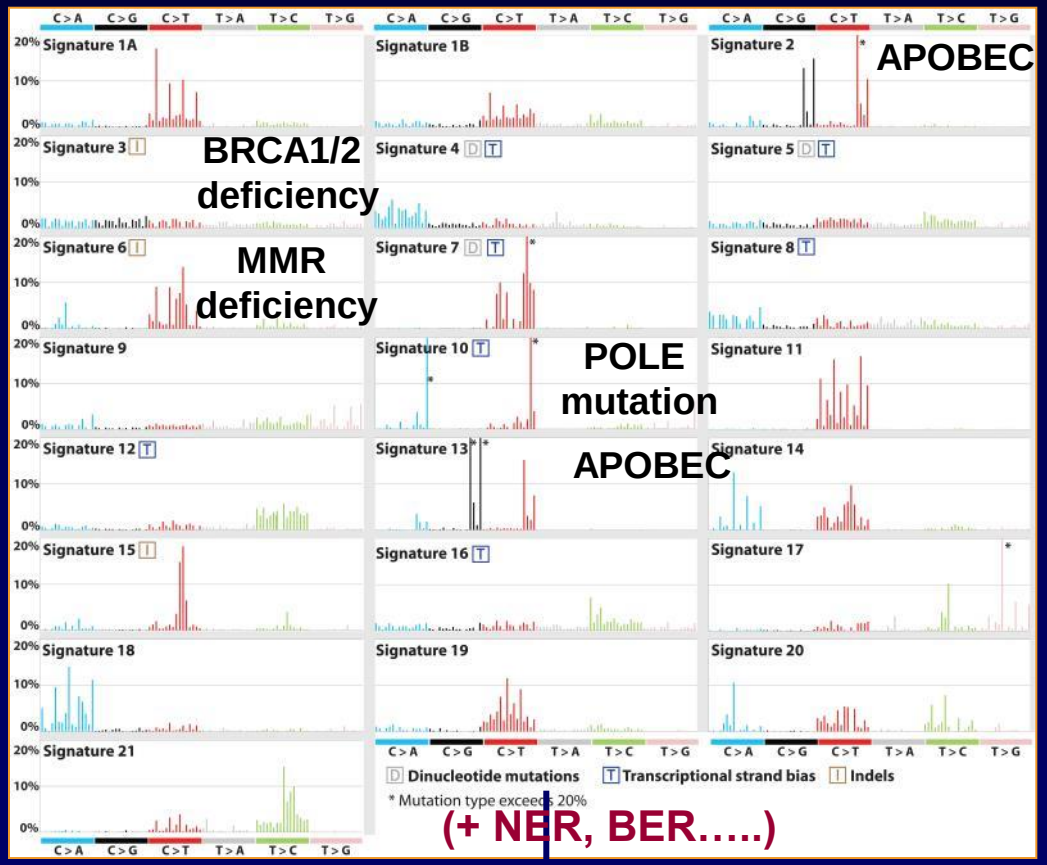
Identify Mutational Processes and DNA Repair Defects in Each Single Patient



Mutational processes & DNA repair defects:
Generate accumulation of DNA alterations

Deciphering the Mutational Processes and DNA Repair Defects in Each Patient

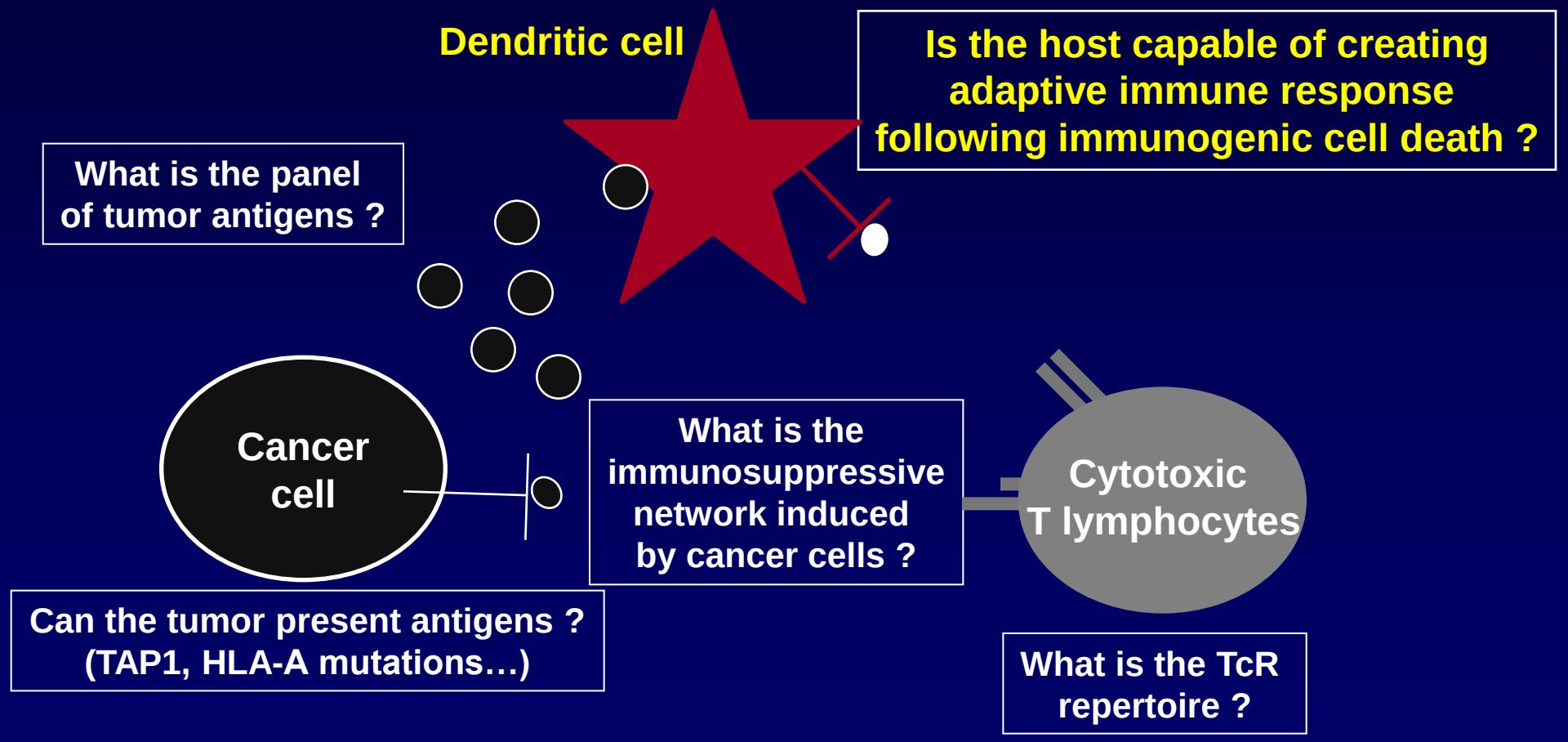
Pattern of mutations by sequencing (without focus on specific genes)



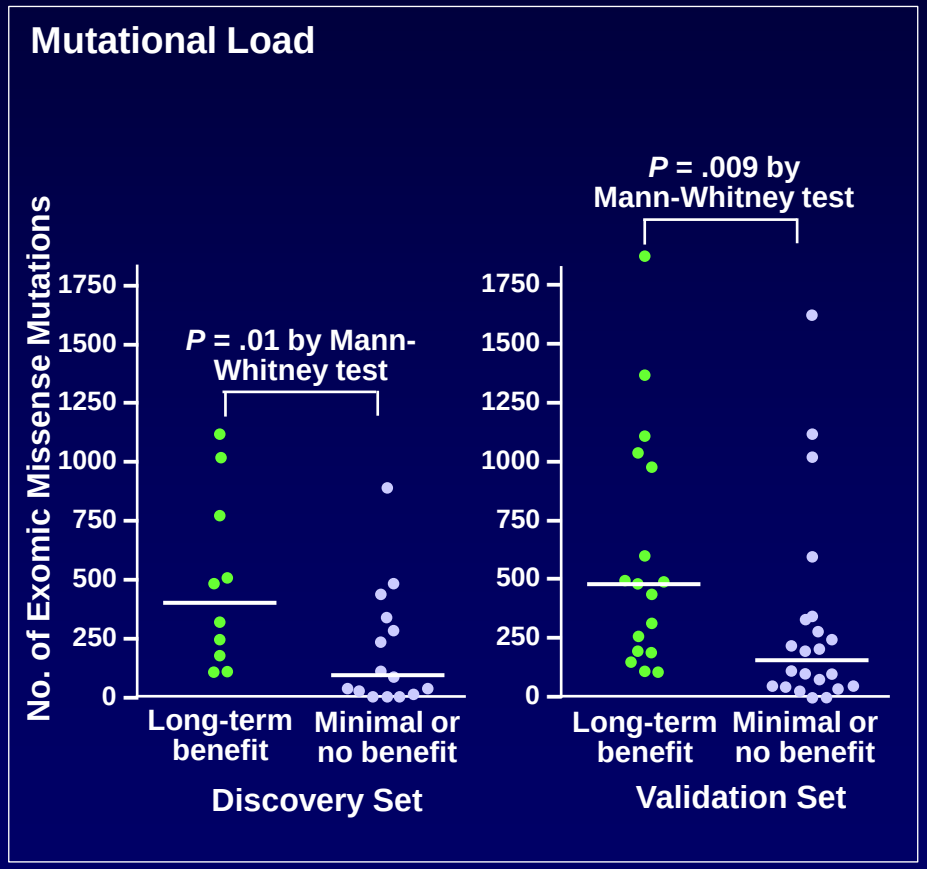
Synthetic lethality with DNA repair defect (PARPinh and *BRCA* defects)
Targeting mutational process?

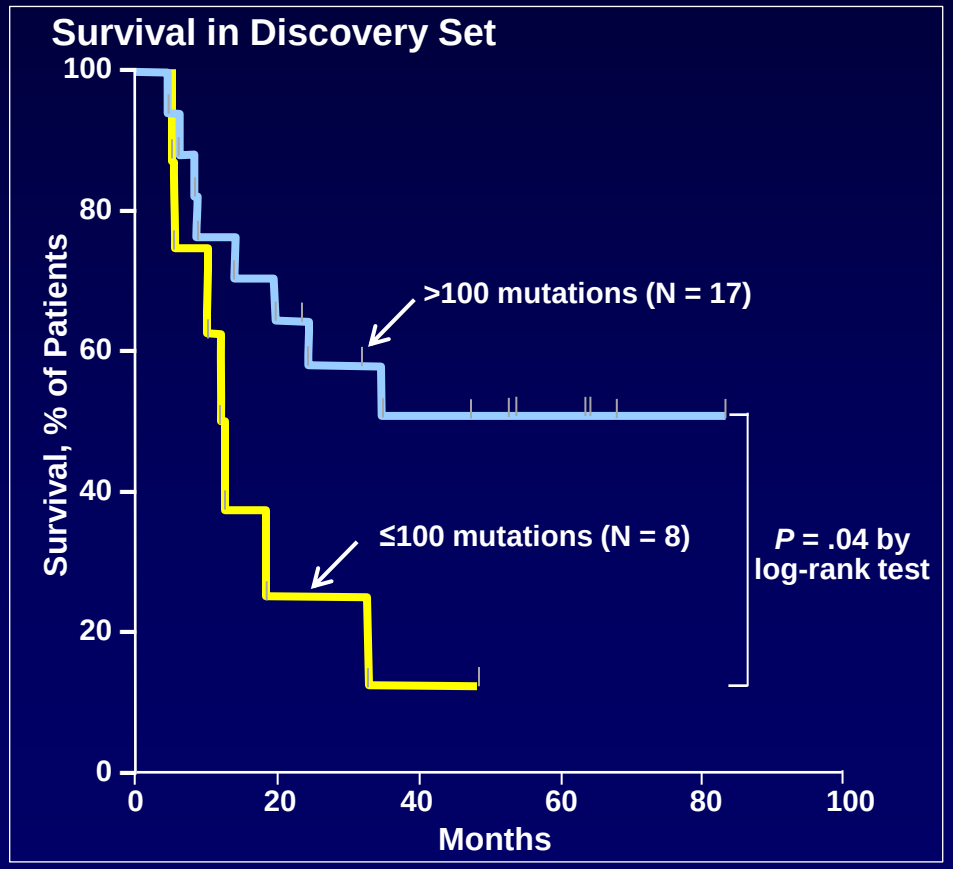
Alexandrov LB, et al. *Nature.* 2013;500(7463):415-421.

Potential Applications of Genomics to Identify Immune Defects in Individuals With mBC



Mutational Load and Efficacy of Anti-CTLA4 Ab





Snyder A, et al. *N Engl J Med.* 2014;371(23):2189-2199.

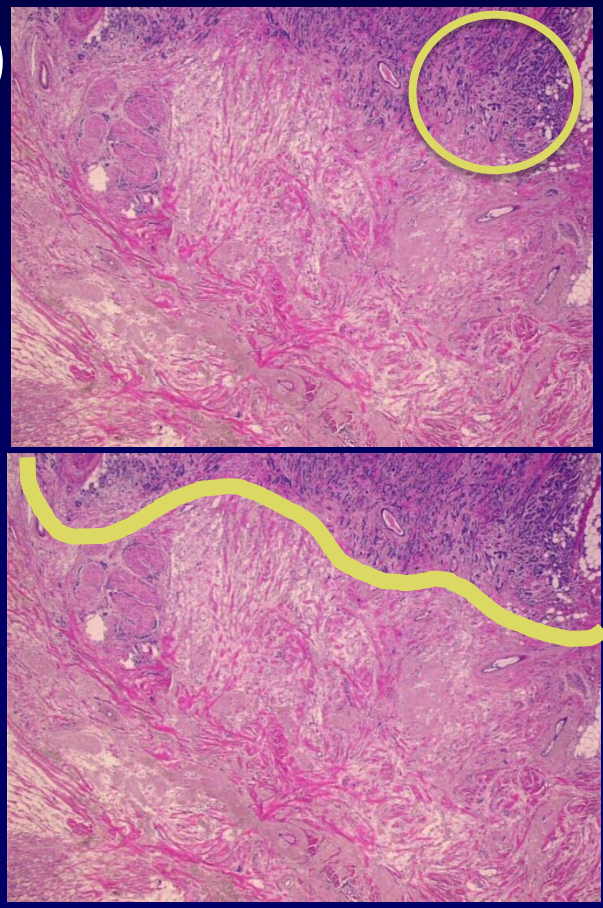
Clinical Utility of NGS

Information Needed for Adequate Sample Provision

- DNA or RNA?
  - RNase-free environment
- Quantity
  - cellularity
- Quality and integrity
  - Pre-analytic variables
- Platform to be used

Sample Preparation

- Choice of the most representative block
  - Invasive vs noninvasive vs precursors
  - Tumor cellularity (area vs % nuclei)
- Whole sections vs cores
- Age of the tissue sections
- Macrodissection
- Microdissection
  - Cytology smears



Potential Indications for Requesting NGS in Patients With Solid Tumors

- Stage IV non-small cell lung cancer (NSCLC) – mainly adenocarcinomas
- Carcinoma of unknown primary (CUP)
- Rare solid tumors
- Newly diagnosed patients with selected stage IV poor prognosis solid tumors

Whom to Molecularly Assess For Target Identification in Advanced Cancer

- **NSCLC adeno**
  EGFRm, ALKr, RETr, ROSr, HER2m, METa
- **Melanoma**
  BRAFm, (NRASm, KITm (a)?)
- **Colorectal**
  KRASm, NRASm, BRAFm
- **GIST**
  KITm, PDGFRαm
- **Glioblastoma:**
  MGMTmethyl
- **Breast:**
  HER2ae, ERe, PRe
- **Dermatofibrosarcoma:**
  PDGFRβr
- **Stomach:**
  HER2ae

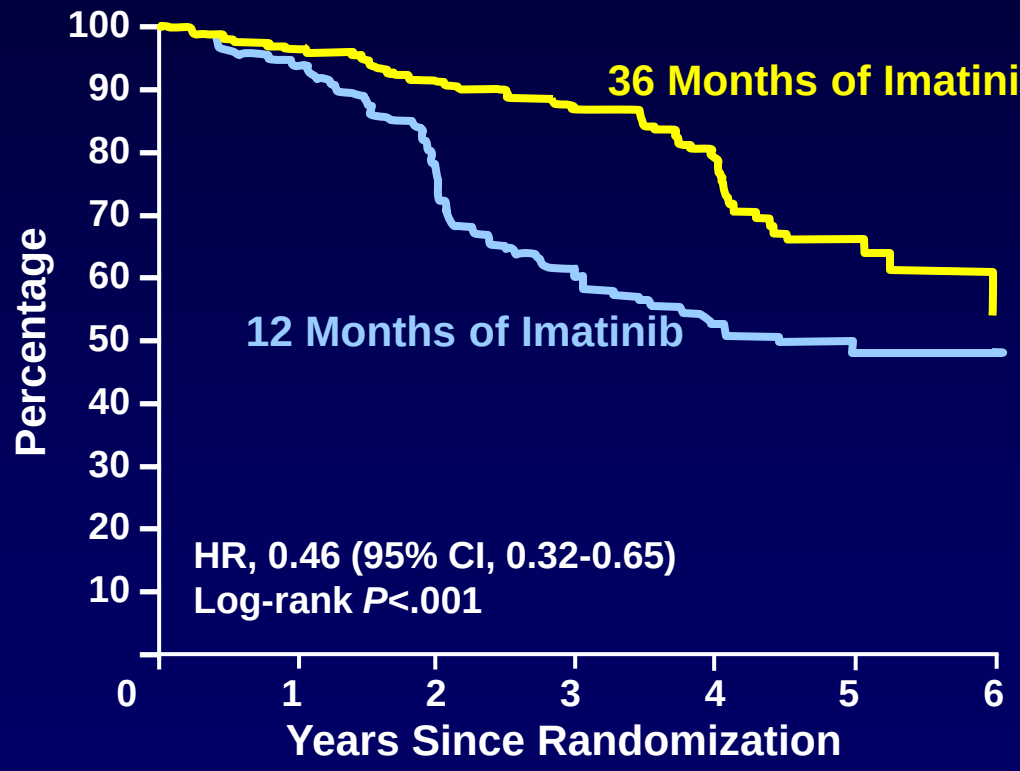
m, mutated
r, rearranged
a, amplified
methyl, methylated
e, (over)expression

Whom to Molecularly Assess For Target Identification in Early Cancer

- **GIST**
  - Imatinib increases OS in adjuvant setting
- **Glioblastoma**
  - Temozolomide increases OS
- **Breast**
  - Trastuzumab, tamoxifen, and aromatase inhibitors in adjuvant setting improve OS
- **Colorectal**
  - Cetuximab failed in adjuvant setting
- **Lung**
  - EGFR TKI being tested
- **Melanoma**
  - BRAF and BRAF/MEK TKI in adjuvant setting being tested

Whom to Molecularly Assess For Target Identification in Early Cancer

GIST – Adjuvant Treatment



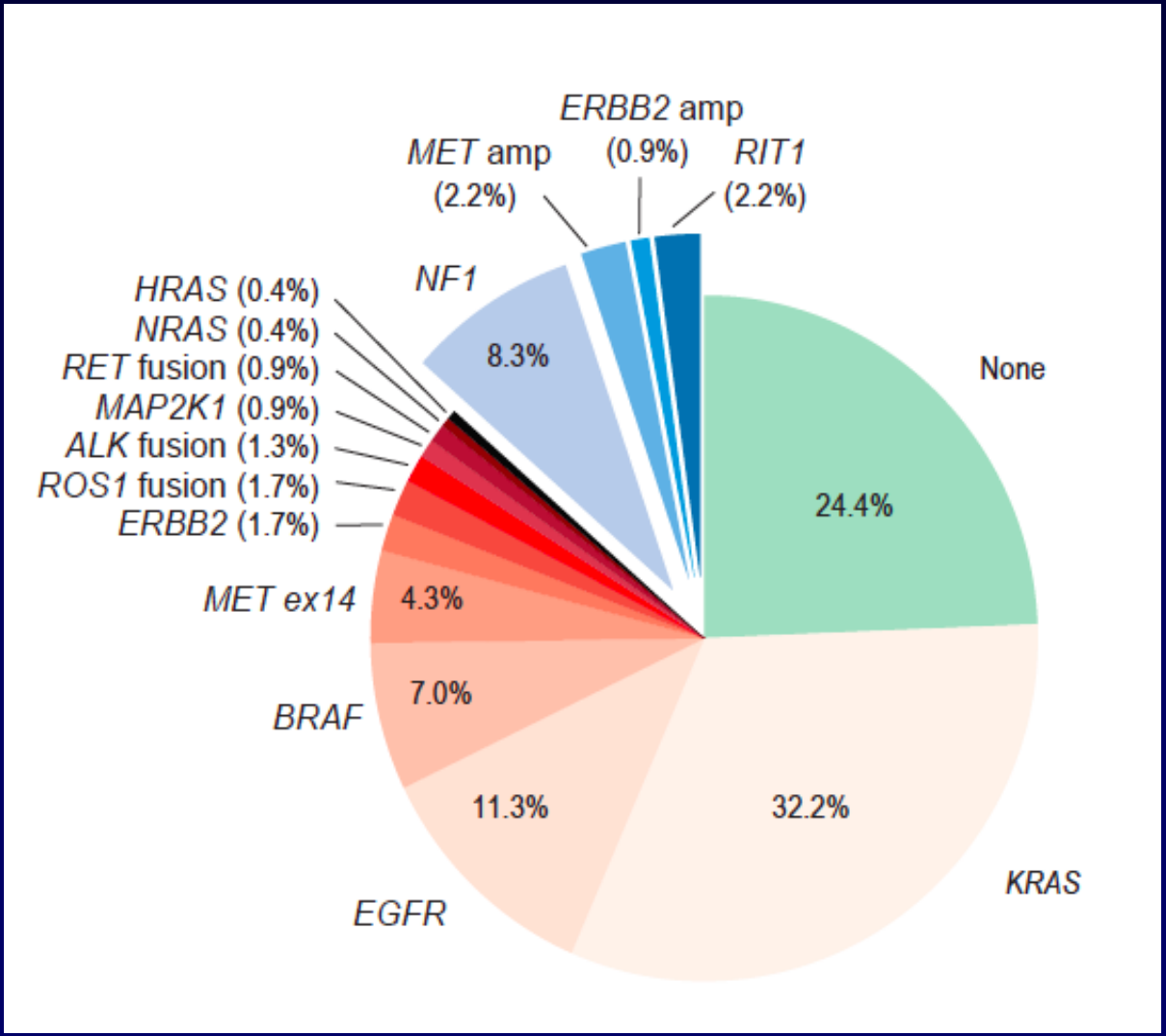
No. of patients							
36 Months of imatinib	198	184	173	133	82	39	8
12 Months or imatinib	199	177	137	88	49	27	10

5-year survival rate 92% vs 81.7%
(HR 0.45 95% CI 0.22-0.89, *P* = .02)

Joensuu H, et al. *JAMA.* 2012;307(12):1265-1272.

Potential Indications: NSCLC

Frequency of Driver Alterations in Lung Adenocarcinoma



The Cancer Genome Atlas. *Nature.* 2014;511(7511):543-550.

Driver Alterations: What Should We Test For?

- EGFR
- ALK
- HER-2
- BRAF
- RET
- ROS
- MET

Lindeman NI, et al. *J Thorac Oncol.* 2013;8(7):823-859.

Driver Alterations: What Should We Test For?

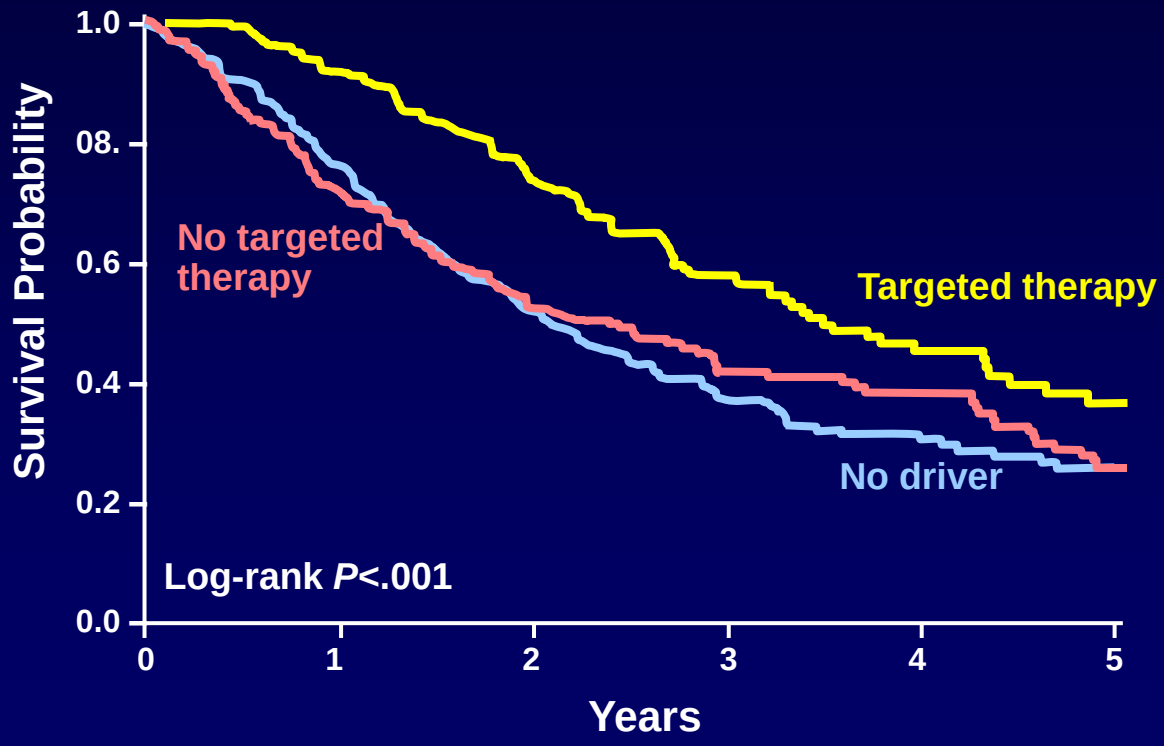
- EGFR
- ALK
- HER-2
- BRAF
- RET
- ROS
- MET

Tissue should be prioritized for EGFR and ALK testing. EGFR and ALK results should be available within 2 weeks (10 working days).

Lindeman NI, et al. *J Thorac Oncol.* 2013;8(7):823-859.

Survival of Patients With Drivers: Targeted Therapy vs No Targeted Therapy

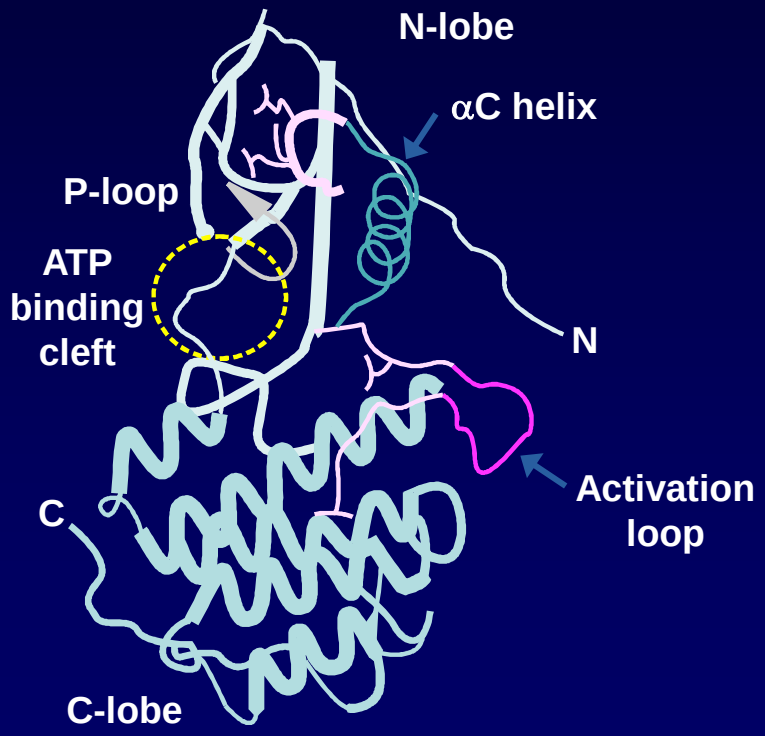
Patients with an oncogenic driver mutation who did and did not receive targeted therapy, and patients without an oncogenic driver

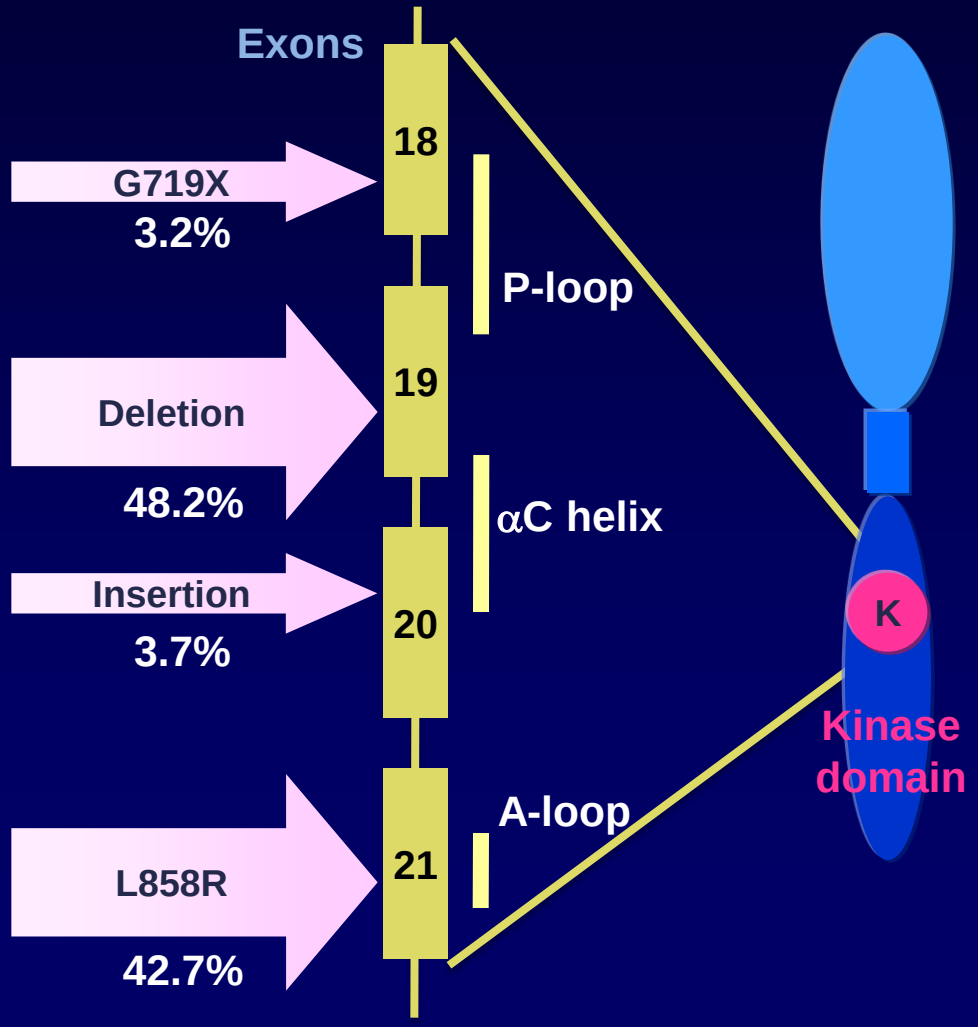


Driver, no targeted therapy (A)	313	2.4 years (1.8 to 2.9)
No driver (B)	361	2.1 years (1.8 to 2.5)
Driver, targeted therapy (C)	264	3.5 years (3.2 to 4.6)

Kris MG, et al. *JAMA.* 2014;311(19):1998-2006.

EGFR Mutations Are Located Predominantly on Exons 19 and 21





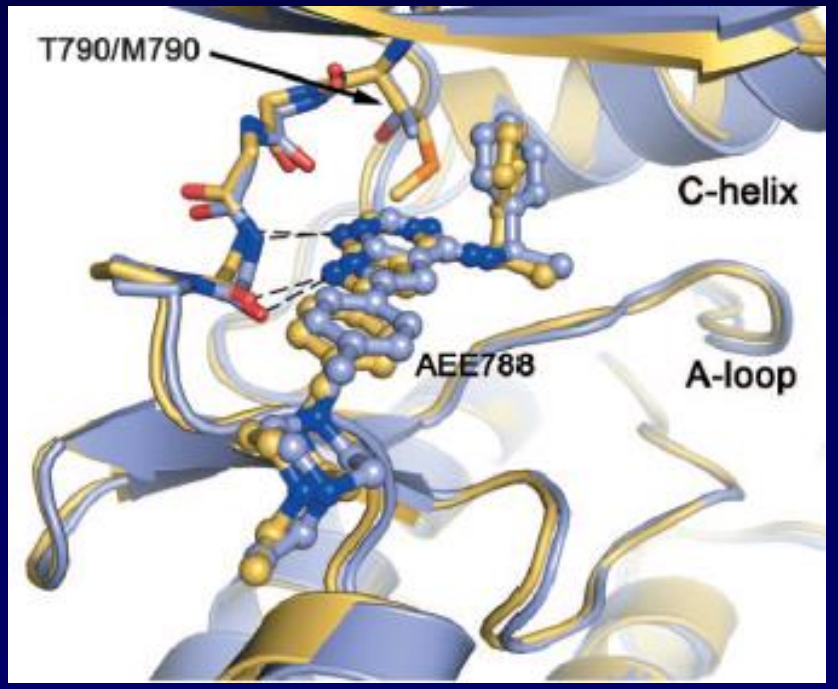
Approximately 10-15% of all NSCLC Patients have EGFR-mutated disease

Mitsudomi T, et al. *Int J Clin Oncol.* 2006;11(3):190-198. Rosell R, et al. *N Engl J Med.* 2009;361(10):958-967. Riely GJ, et al. *Clin Cancer Res.* 2006;12(24):7232-7241.

T790M Mechanism at Resistance

The T790M mutation in EGFR kinase causes drug resistance by increasing the affinity for ATP

Kinase	$K_{d,}$nM Gefitinib
WT	35.3 ± 0.4
T790M	4.6 ± 0.1
L858R	2.4 ± 0.1
L858R/T790M	10.9 ± 0.6

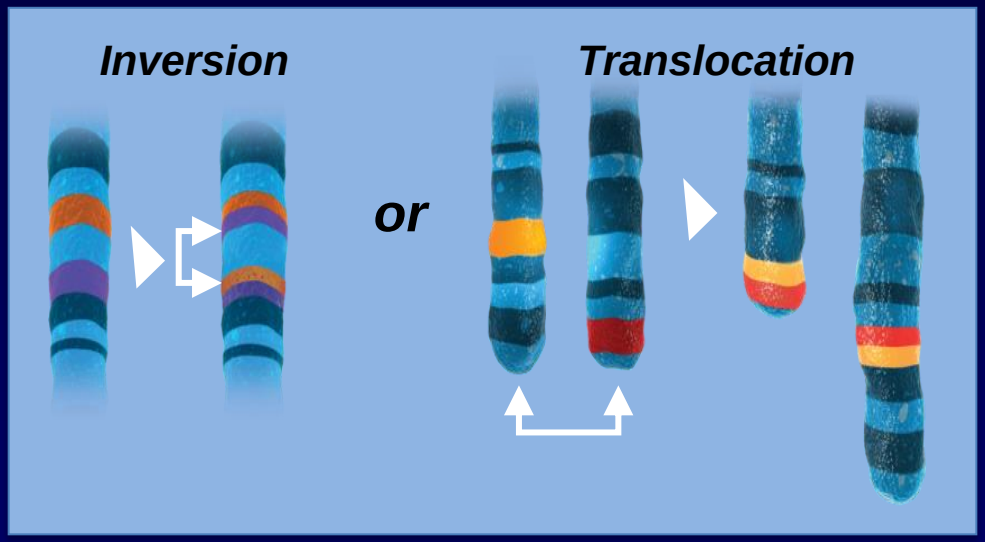


Enyme Kinetic Parameters of WT and Mutant EGFR

Kinase	$K_{m[ATP],\ \mu M}$	$K_{cat,\ s}$-1	$k_{cat}/K_{m[ATP],\ \mu M}$-1*S-1
WT	5.2 ± 0.2	0.026	5.00E-3
T790M	5.9 ± 0.1	0.137	2.32E-2
L858R	148 ± 4	1.484	1.00E-2
L858R/T790M	8.4 ± 0.3	0.456	5.43E-2

Yun CH, et al. *Proc Natl Acad Sci.* 2008;105(6):2070-2075.

EML4-ALK Fusion Oncogene Key Driver in 2% to 7% NSCLC



ALK rearrangements induce ALK protein expression.

Constitutive ALK activation → oncogene addiction

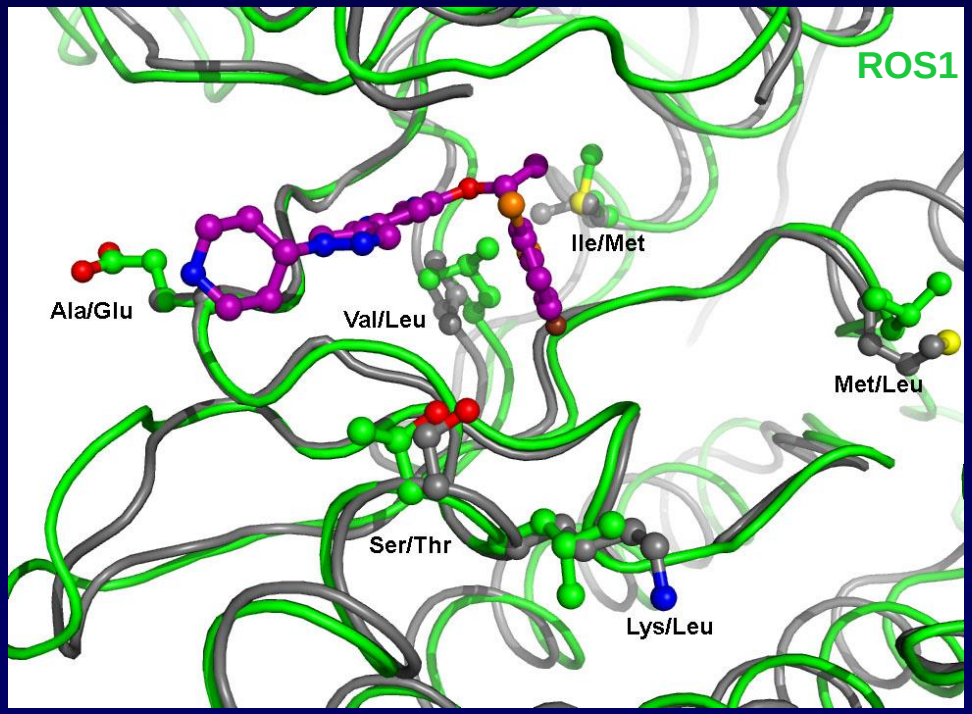
ALK, anaplastic lymphoma kinase; EML4, echinoderm microtubule-associated protein like 4

Soda M, et al. *Nature.* 2007;448(7153):561-566. Kwak EL, et al. *N Engl J Med.* 2010;363(18):1693-1703.

ROS1 and ALK TK Domains Are Similar

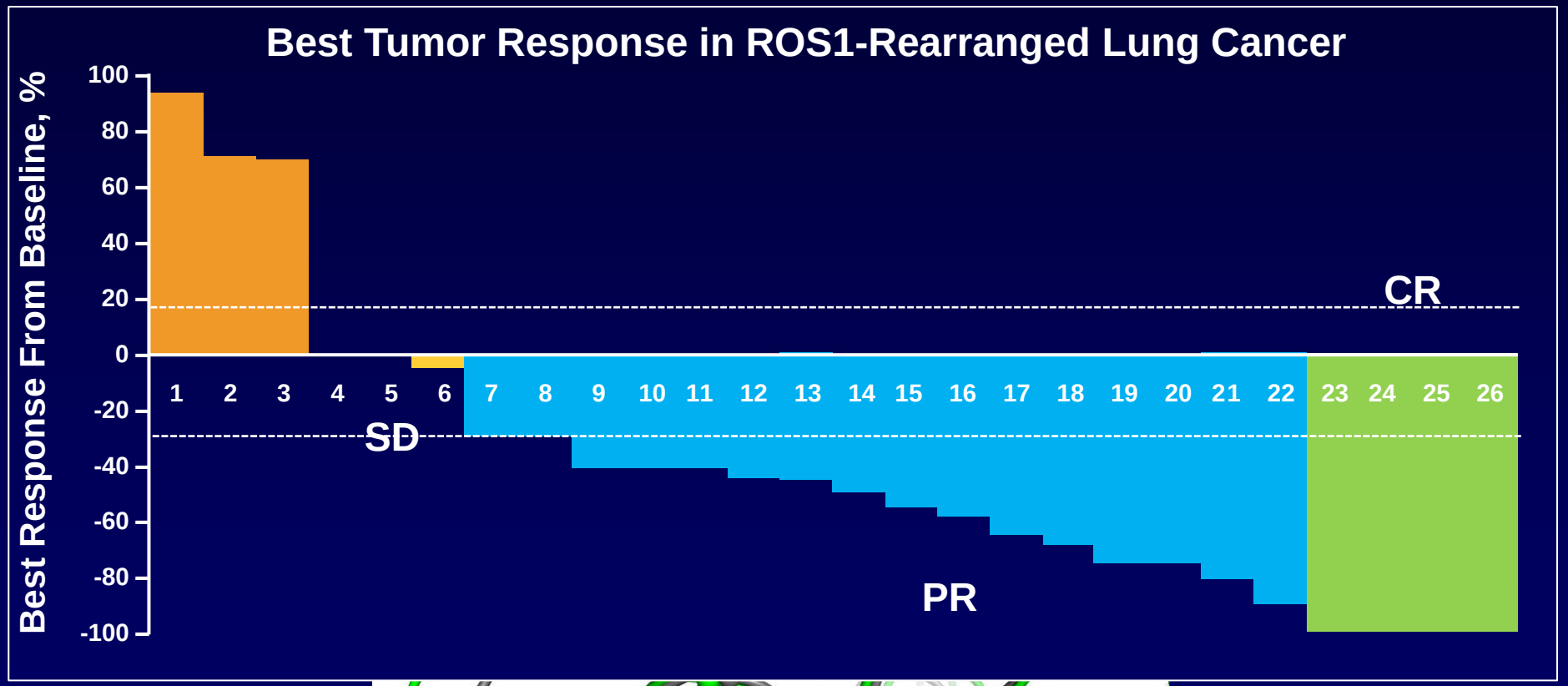
77% Identity in ATP-Binding Site

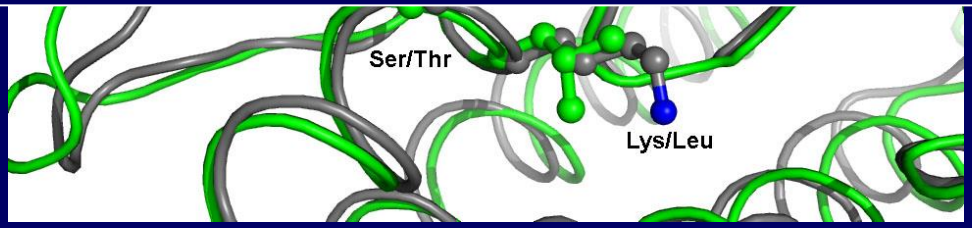
	G-Loop		N-Terminus					GK										Extended Hinge Region								C-Terminus							
Kinase	**L1**	**L9**	**N1**	**N2**	**N3**	**N4**	**N5**	**H1**	**H3**	**H4**	**H5**	**H6**	**H7**	**H8**	**H9**	**H10**	**H11**	**H12**	**H13**	**C1**	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**
ALK	L	V	A	K	E	I	V	I	L	E	L	M	A	G	G	D	L	K	S	R	N	L	G	D	F	G	M
ROS1	L	V	A	K	E	M	L	I	L	E	L	M	E	G	G	D	L	L	T	R	N	L	G	D	F	G	L



ROS1 and ALK TK Domains Are Similar

77% Identity in ATP-Binding Site





A More Complex Picture
Missing Driver Proof of Concept

Molecular Pathways: ROS1 Fusion Proteins in Cancer

Kurtis D. Davies and Robert C. Doebele

1%-2% NSCLC, responsive to ROS TKIs

- **8.7% (2/23) in cholangiocarcinomas**
- **0.5% (1/200) in ovarian cancers**
- **0.6% (3/495) in gastric adenocarcinomas**
- **0.8% (2/ 236) in colorectal cancers**
- **7.7% (2/26) in inflammatory myofibroblastic tumors**
- **2.9% (1/34) in angiosarcomas**
- **5% (1/20) in epithelioid hemangioendotheliomas**

Will all these tumor sites respond to ROS-targeted therapy?

Davies KD, et al. *Clin Cancer Res*. 2013;19(15):4040-4045.

Potential Indications: Cancer of Unknown Primary, Rare Tumors, and Patients Who Have Exhausted Standard Treatments

- **Cancer of Unknown Primary (CUP)**
  - **More molecular information may help identify the potential site of origination and any mutations that may potentially be targeted with therapy**
- **Rare tumors with aggressive biology**
  - **May not have time for trial and error with different treatments; NGS may identify targetable mutations**
- **Patients who have exhausted standard treatments**
  - **NGS may identify new targetable mutations**

A Specific Driver Mutation in Anaplastic Thyroid Cancer

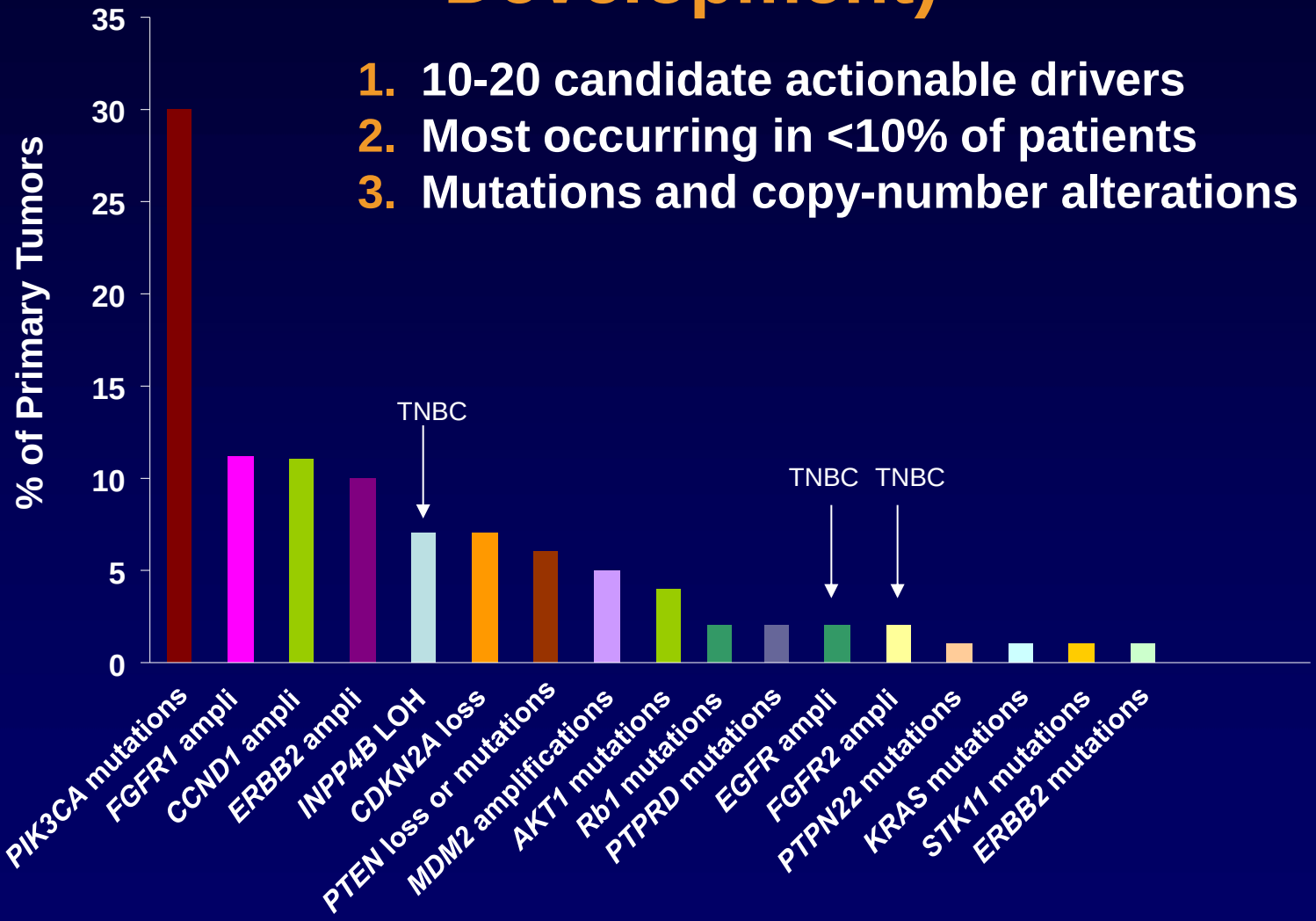
BRAF V600E Inhibition in Anaplastic Thyroid Cancer

Rosove MH, et al. *N Engl J Med.* 2013;368(7):684-685.

Potential Indications: Breast Cancer

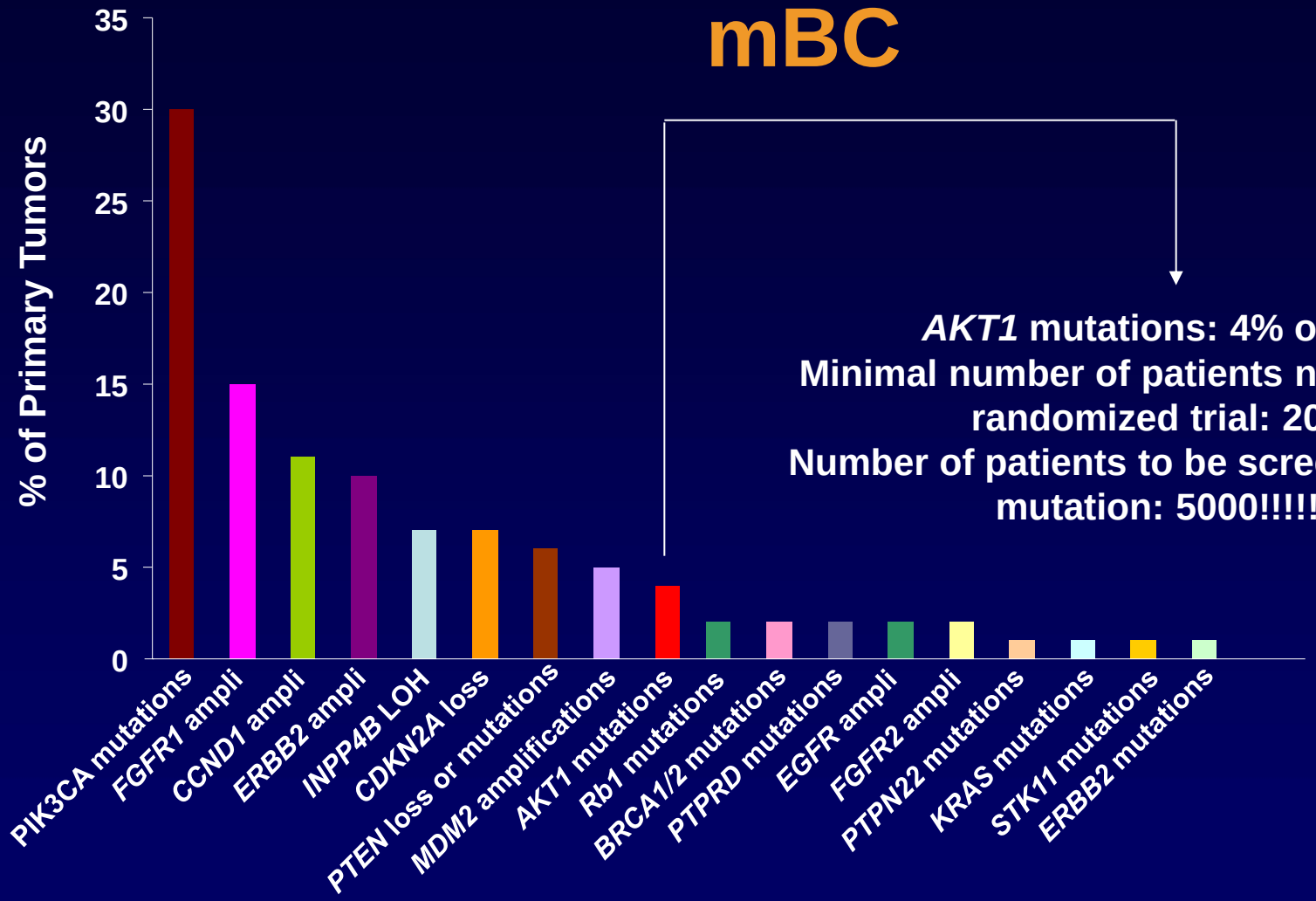
Candidate Actionable Genomic Alterations in Breast Cancer (With Drugs Under Development)

1. 10-20 candidate actionable drivers
2. Most occurring in <10% of patients
3. Mutations and copy-number alterations



Stephens PJ, et al. *Nature.* 2012;486(7403):400-404. The Cancer Genome Atlas Network. *Nature.* 2012;490(7418):61-70. Gewinner C, et al. *Cancer Cell*. 2009;16(2):115-125. André F, et al. *Clin Cancer Res.* 2009;15(2):417-419. Turner N, et al. *Oncogene.* 2010;29(14):20136-2023.

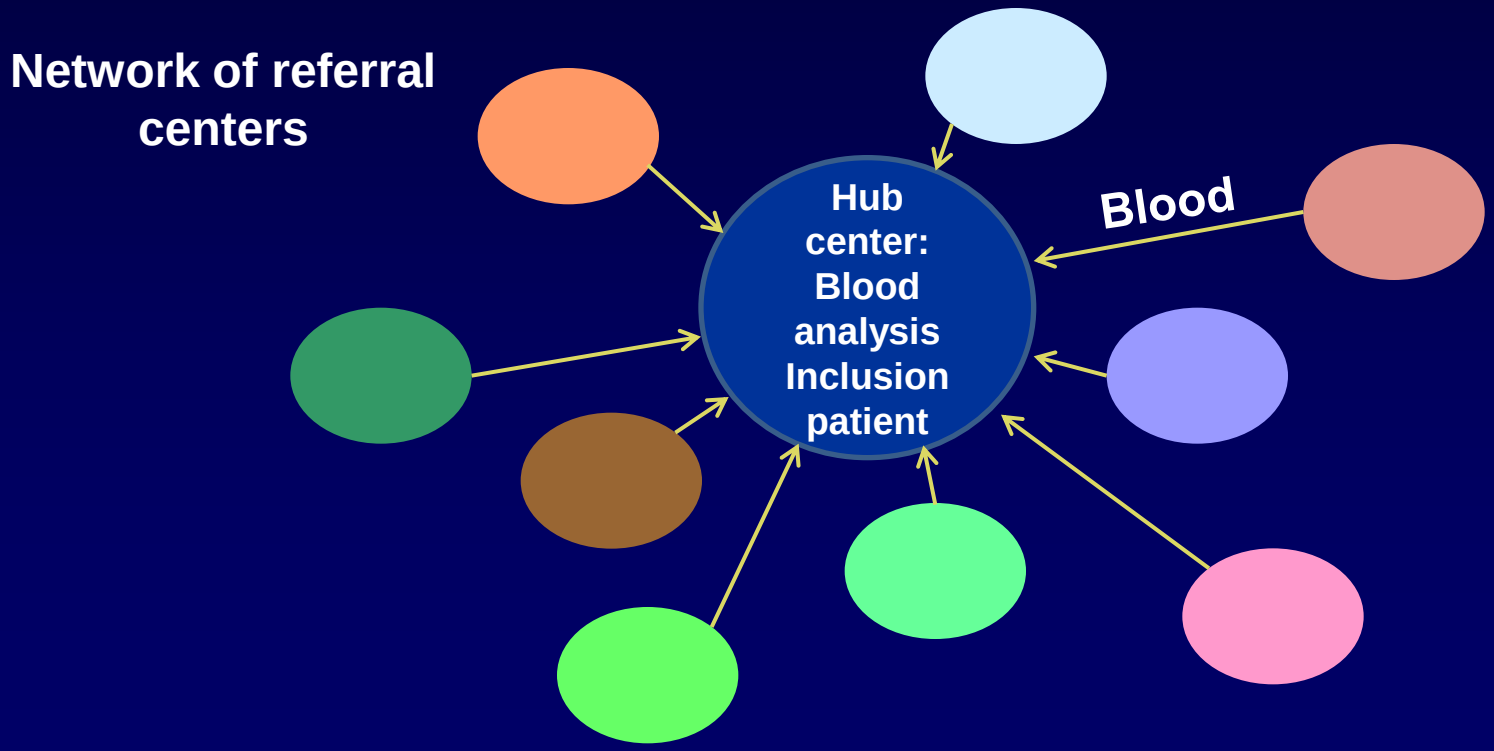
Challenges in Stratified Medicine for mBC



Accrual is the challenge of stratified medicine in mBC:
How to screen genomic alterations in 5000-10,000 metastatic breast cancer patients?

Stephens PJ, et al. *Nature.* 2012;486(7403):400-404. The Cancer Genome Atlas Network. *Nature.* 2012;490(7418):61-70. Gewinner C, et al. *Cancer Cell*. 2009;16(2):115-125. André F, et al. *Clin Cancer Res.* 2009;15(2):417-419. Turner N, et al. *Oncogene.* 2010;29(14):20136-2023.

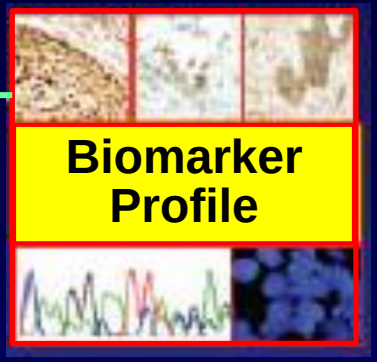
Possible Solution: Use of Circulating DNA to Screen Genomic Alterations in Centers That Do Not Routinely Perform Biopsies



Example of a Basket Study: The Battle Trial

Umbrella Protocol

Core Biopsy



EGFR KRAS/BRAF
VEGF RXR/CyclinD1

Randomization:
Equal → *Adaptive*

- Erlotinib
- Vandetanib
- Erlotinib + Bexarotene
- Sorafenib

Primary endpoint: 8-week disease control (DC)

Conclusions and prIME Points™

prIME Points™

- Molecular targeted treatments are providing new therapeutic options across multiple malignancies
- Predictive biomarkers needed to utilize targeted treatments
- NGS provides detailed information on actionable mutations
- Technical developments improving accuracy and reducing costs
- Interpretation requires specialist knowledge

prIME Points™

- Provides information on tumor biology and patient selection for clinical trials
- May soon replace multiple individual biomarker testing in some cancers, eg, lung adenocarcinoma
- Of potential clinical value in rare solid tumors and for patients of good performance status with advanced disease who have exhausted standard treatments