

Molecular Diagnostics and the Emergence of Precision Medicine for Breast, Colorectal and Lung Cancer

Jeffrey S. Ross, M.D.

Cyrus Strong Merrill Professor and Chair.

Department of Pathology and Laboratory Medicine

Albany Medical College

Albany, NY

rossj@mail.amc.edu



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Jeffrey S. Ross, M.D. Conflict of Interest Disclosures

Major leadership position/advisory role for:

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Major stockholder in:

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SYFR, Inc.

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Various Oncology and Pathology textbooks

5 U.S. Patents in Molecular Diagnostics

Honoraria (lecture fee) from:

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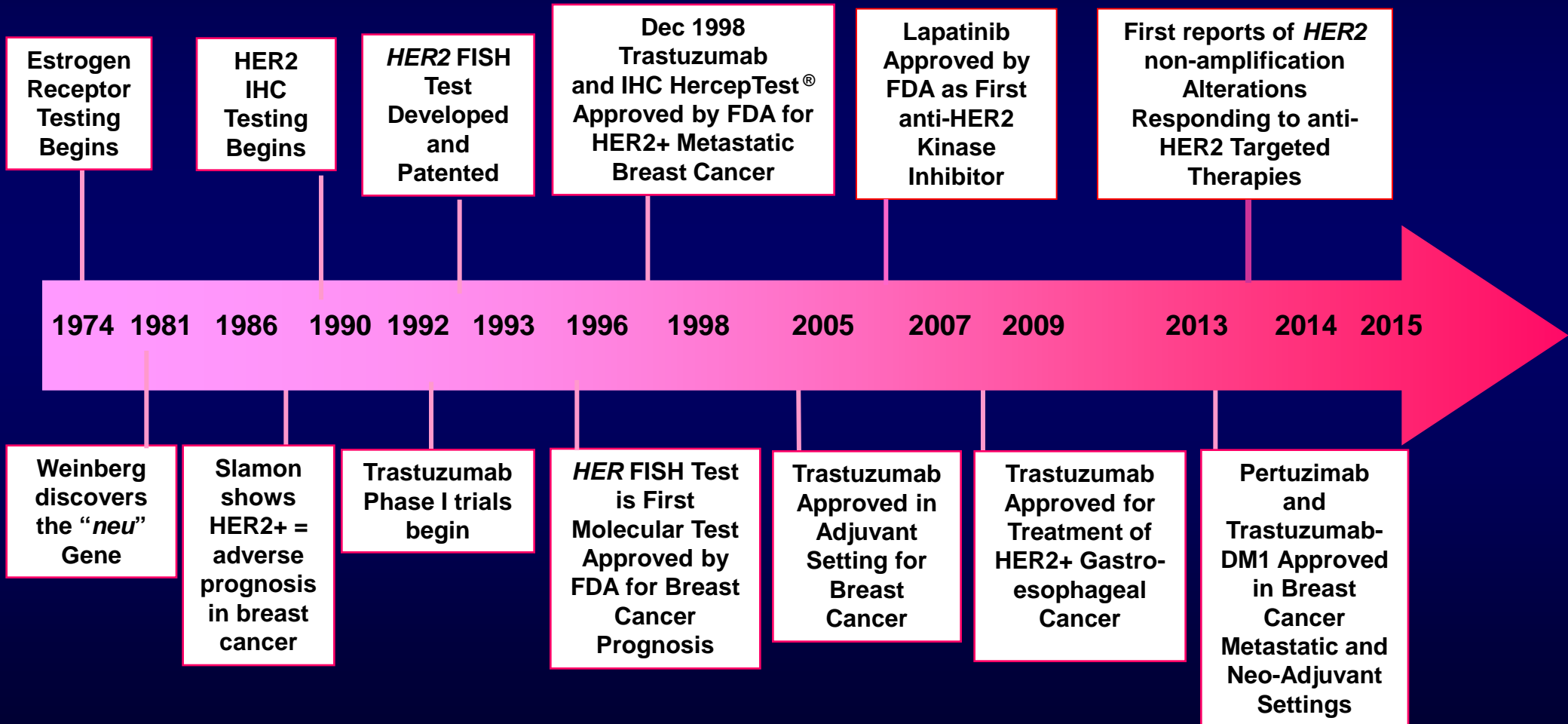
Affiliated Pathology Services at Albany
Medical Center

The Evolution of Molecular Pathology

- IHC widely used to confirm diagnosis and classify cancer
- Prognostic IHC markers emerge first in hematologic malignancies and then solid tumors
- OncotypeDx™ emerges as prognostic marker but used to decide therapy in early stage breast cancer
- mRNA based classification and therapy guidance achieves limited additional applications
- DNA sequencing era begins with Sanger method, advances to PCR methods, then evolves first into hot-spot NGS and then to CGP (comprehensive genomic profiling)

The *ERBB2* (*HER2*) Journey: a Paradigm for Precision Medicine





The First Companion Diagnostic



Traditional Molecular Testing Limitations

- Only a limited number of alterations screened at once
- Misses some types of mutations
- Exhaustive of tissue sample
- Results are specific for the test used; need to know ahead of time what questions to ask

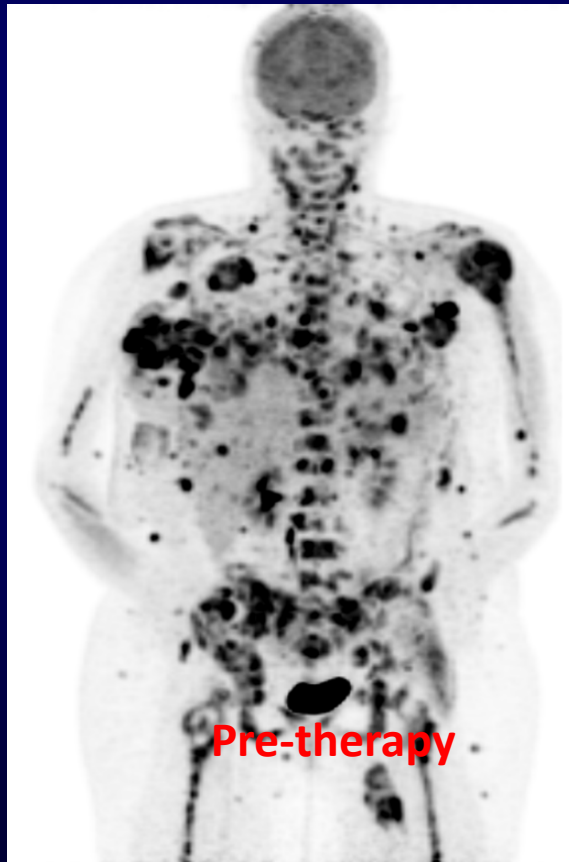
DNA Mutations Detected or Missed by Traditional Testing

| Test | Detects | Can Miss |
|-----------------|--|---|
| IHC | Protein expression | Any alteration not known of ahead of time |
| FISH | Copy number alterations, rearrangements, substitutions  | Any alteration not known of ahead of time Indels  |
| Hot Spot Panels | Substitutions  | Any alteration not known of ahead of time Indels, copy number alterations, rearrangements  |

"Why the Cancer Genome Matters"

Comprehensive Genomic Profiling → Precision Oncology

Patient with **BRAF V600E** mutated malignant melanoma treated
with selective inhibitor of BRAF (vemurafenib)



Pre-therapy



15 days post-therapy

Slide courtesy of Dr. Grant McArthur

Inflammatory Breast Cancer with ERBB2 Base Substitution (IHC-/FISH-) Responds to Anti-HER2 Targeted Therapy

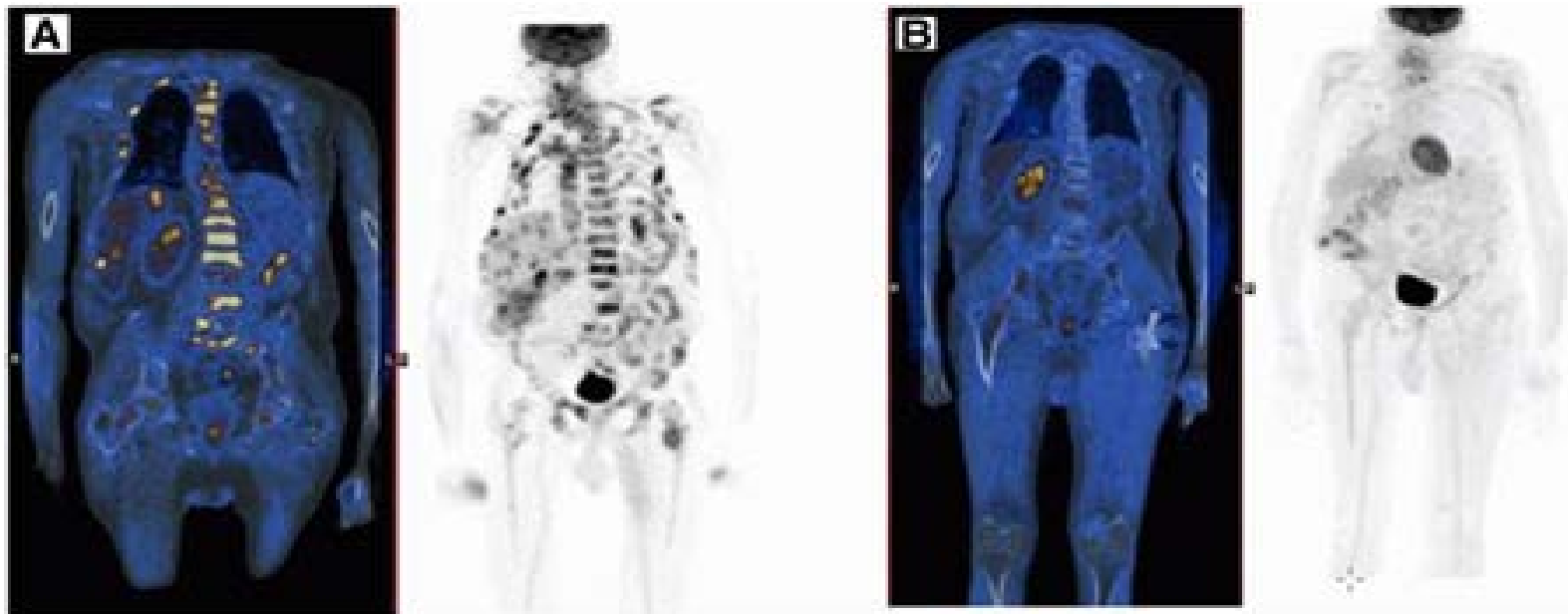


**Pre-therapy: extensive
active disease**



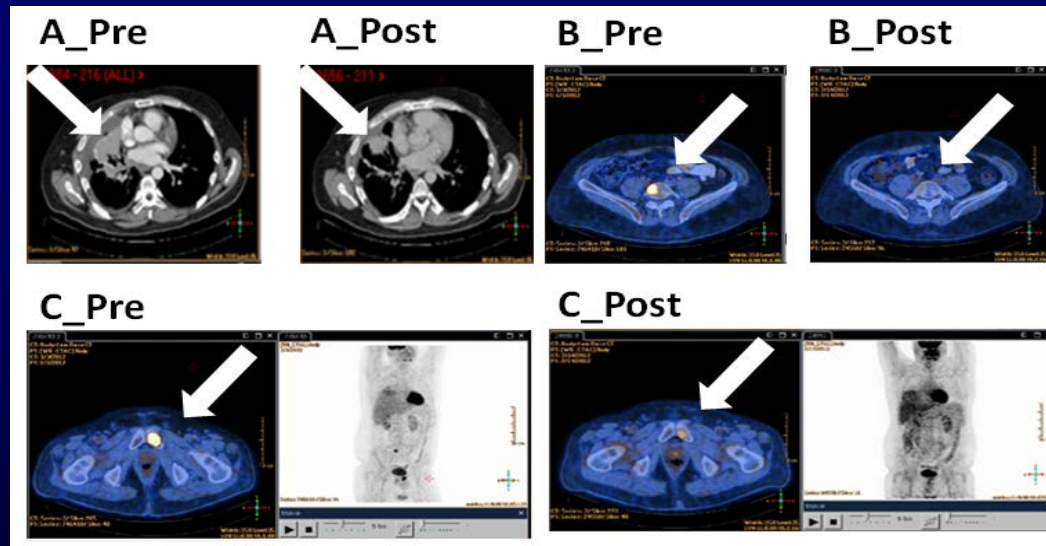
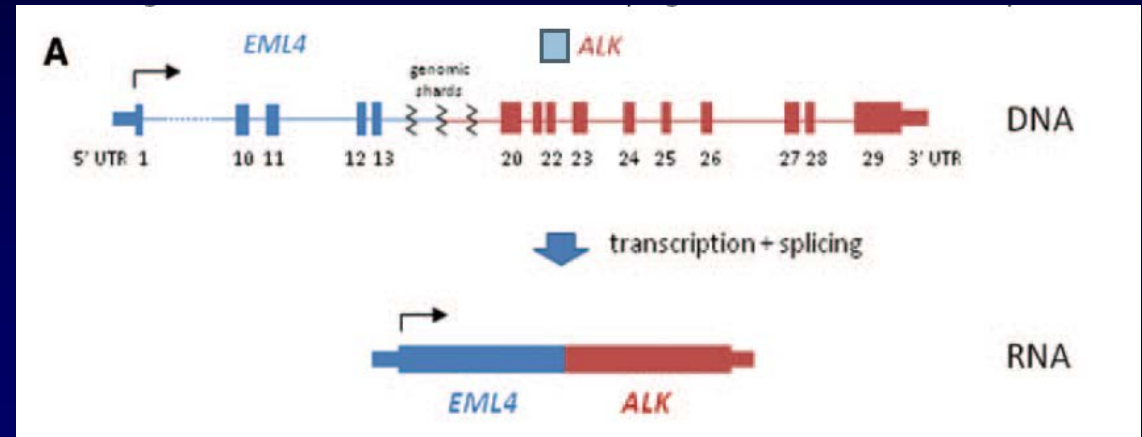
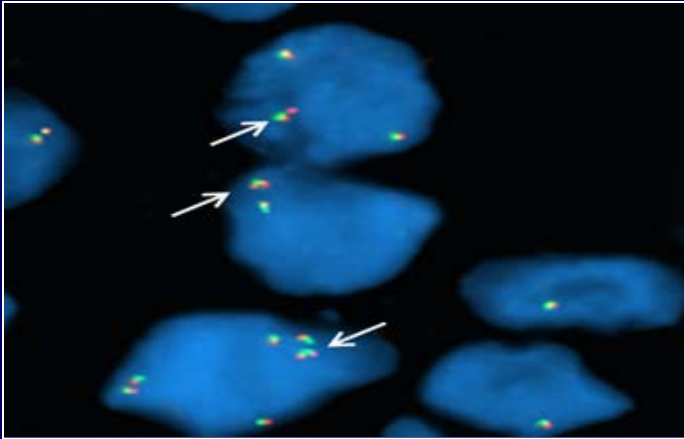
**Post-therapy: good response
with lower/less activity**

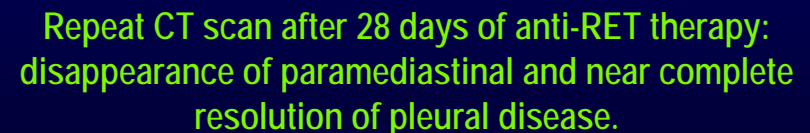
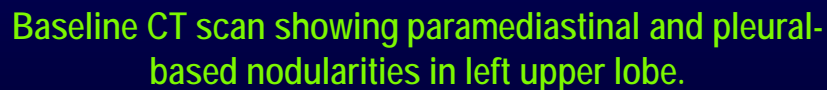
Response of a HER2 FISH/IHC Negative Cutaneous Adnexal Carcinoma with an *ERBB2* S310F Mutation to anti-HER2 Targeted Therapy



Vornikova O et al. The Oncologist 2014;19:1006-1007

EML4-ALK FISH Negative NSCLC: Comprehensive Genomic Profiling Positive for Novel ALK Fusion



[illegible]

- 8/386 (2.1%) RET fusions identified in clinical NSCLC cases to date

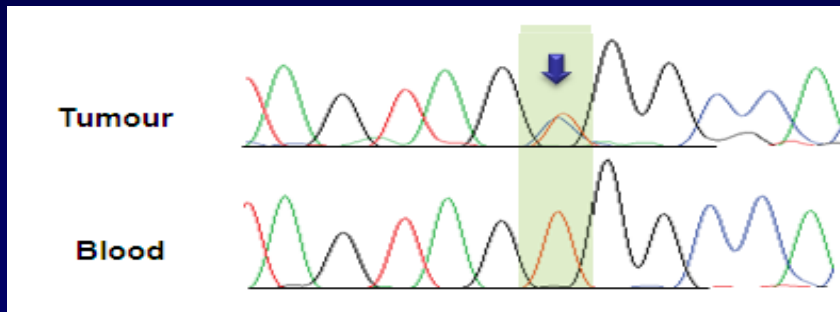
Analytic Validation

- Test accurately/reliably measures analyte or genotype of interest
 - False negatives more frequent than the false positives
 - No internal controls in normal human tissues
 - So analytic validation requires controls be created for the test system and run in parallel with the patient samples

The Four Types of Clinically Relevant Genomic Alteration Each Pose Different Diagnostic Challenges

Base Substitution

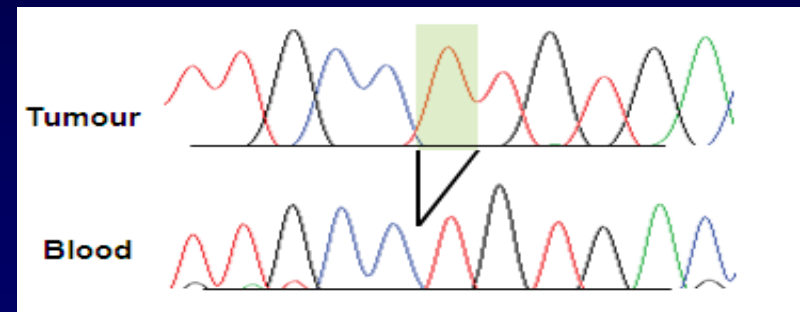
e.g. *BRAF*, *EGFR*



Capillary sequencing, Mass Spectrometry

Short Insertions/Deletion

e.g. *EGFR*, *ERBB2*

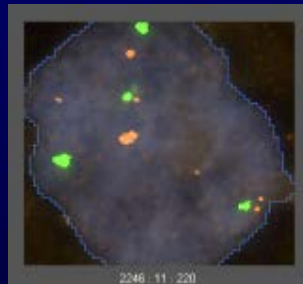
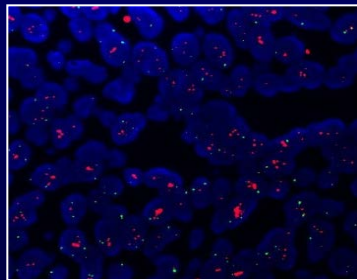


Capillary sequencing, gel size shift assays

Focal Amplification & Homozygous Deletion

e.g. *HER2*, *MET*

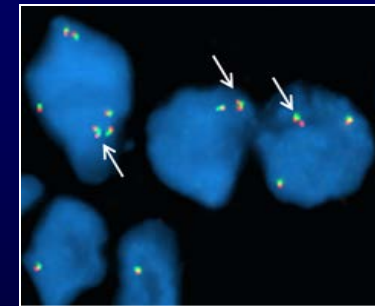
e.g. *PTEN*, *TSC1/2*



Fluorescence *In Situ* Hybridization (FISH)

Gene Fusion

e.g. *ALK*, *ROS1*, *RET*



RT-PCR FISH

Multiple different diagnostic tests may exhaust precious biopsy material

Analytic Validation

Demonstration of high accuracy and reproducibility required for clinical use

Base Substitutions

(MAF 5-100%)

Sensitivity: >99.9% PPV: >99.9%

Insertions/Deletions

(1-40bp, MAF 10-100%)

Sensitivity: 98% PPV: >99%

Copy Number Alterations

(>20% tumor content, zero or ≥ 8 copies)

Sensitivity: >95% PPV: >99%

Gene Fusions

(>20% tumor content, select introns)

Sensitivity: >99% PPV: >99%

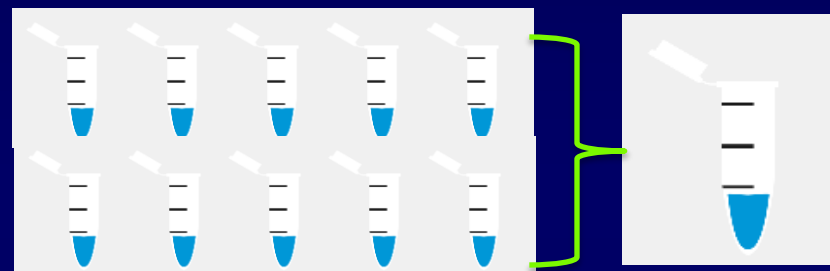
Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing

Frampton et al, *Nature Biotechnology* 2013

Controlled validation studies:

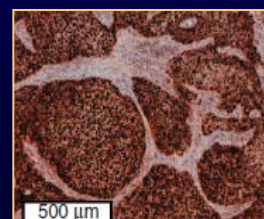
Cell-line pools with known alterations:

- 2056 subs 227 indels
- 210 CNAs 32 fusions



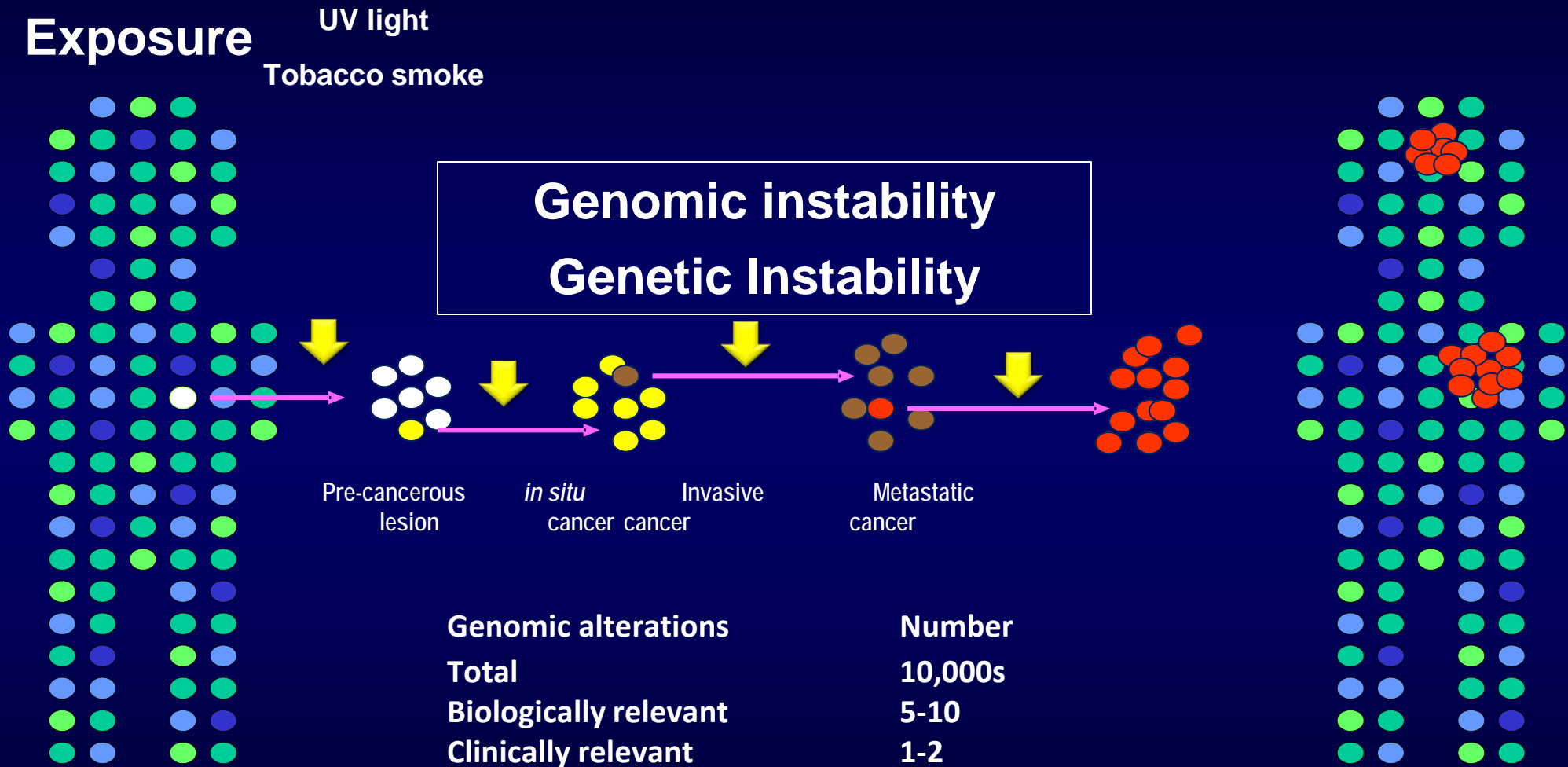
Concordance studies with existing platforms on clinical samples:

- 118 subs/indels: Sequenom, PCR
- 185 CNAs: FISH, IHC
- 43 fusions: break-apart FISH



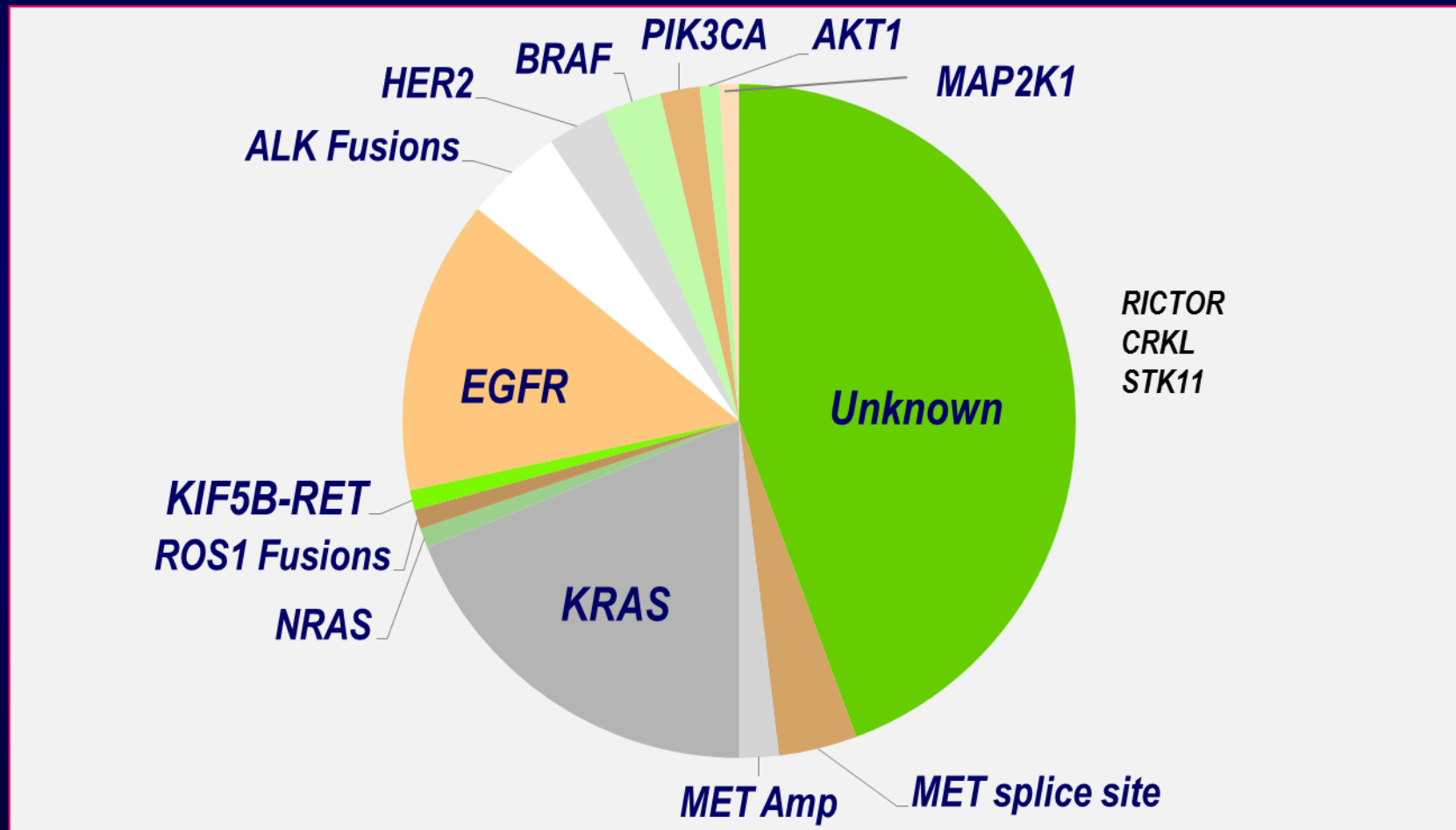
Solid Tumor Genomes are Complex:

Driver Alterations Must be Separated from Passenger Alterations

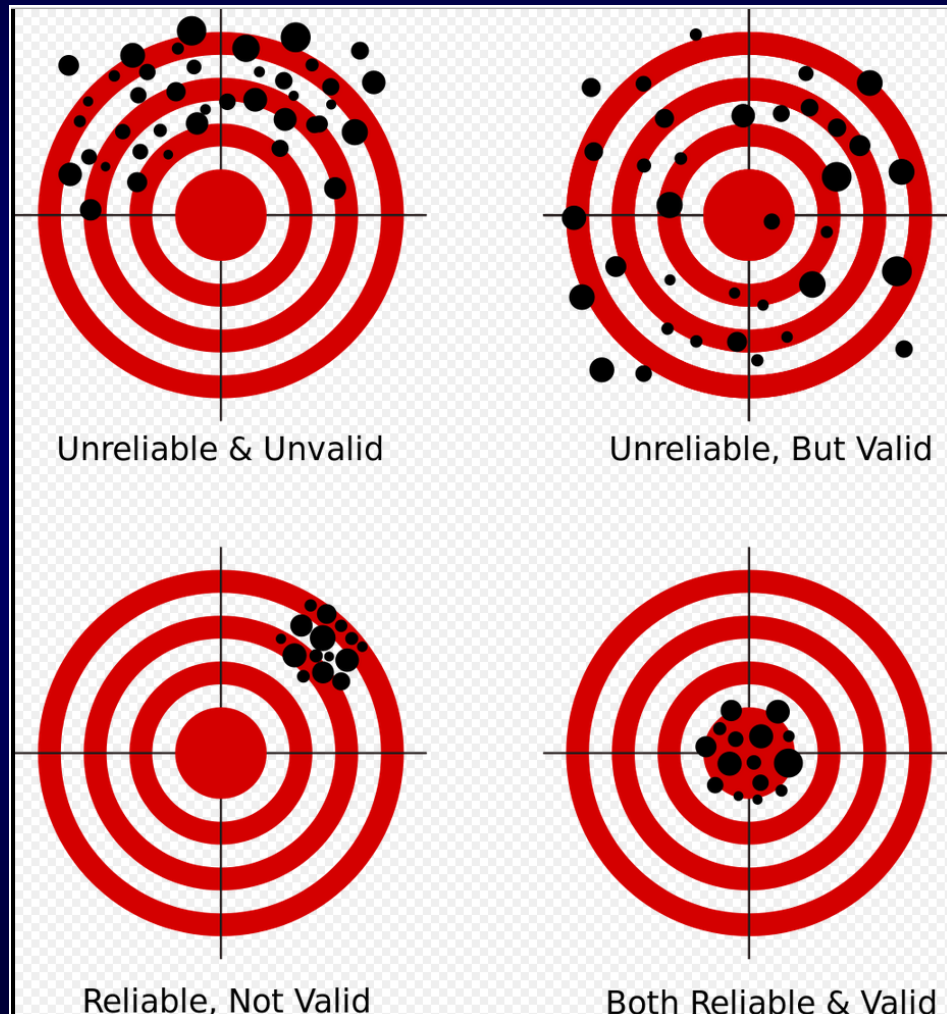


*The number of “clinically relevant” alterations in a single patient is LOW
buried amongst 1,000s of passenger genomic alterations*

The Modern Landscape of NSCLC



Clinical Validation of a Diagnostic Test (Does Test Result Predict Treatment Response?)



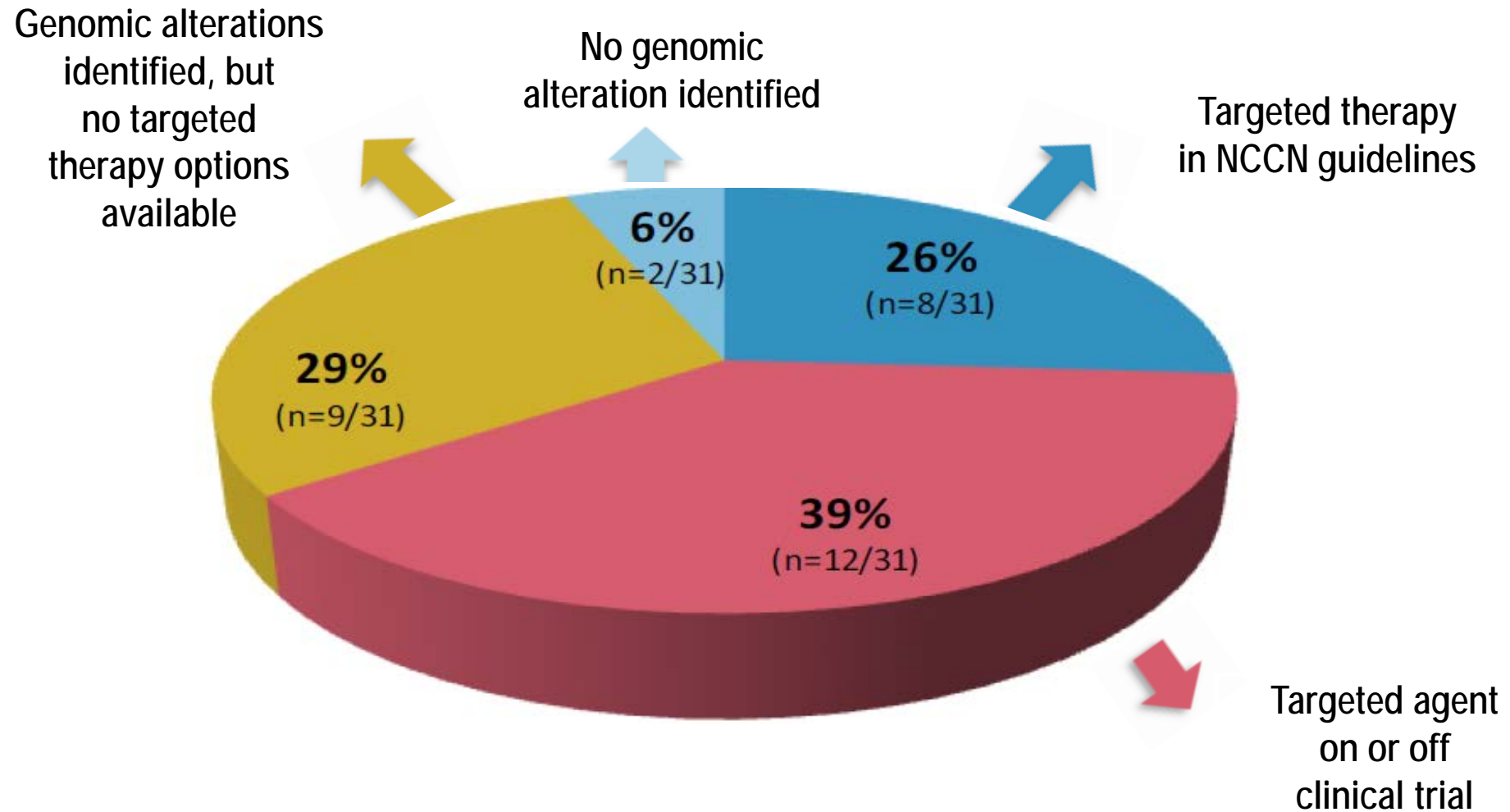
For Cancer predictive tests and Companion Diagnostics:

- PPV never 100% but directionally impactful. Patient is eligible for treatment, + response likely, not guaranteed
- NPV never 100% either, but directionally impactful. Responses much less likely.

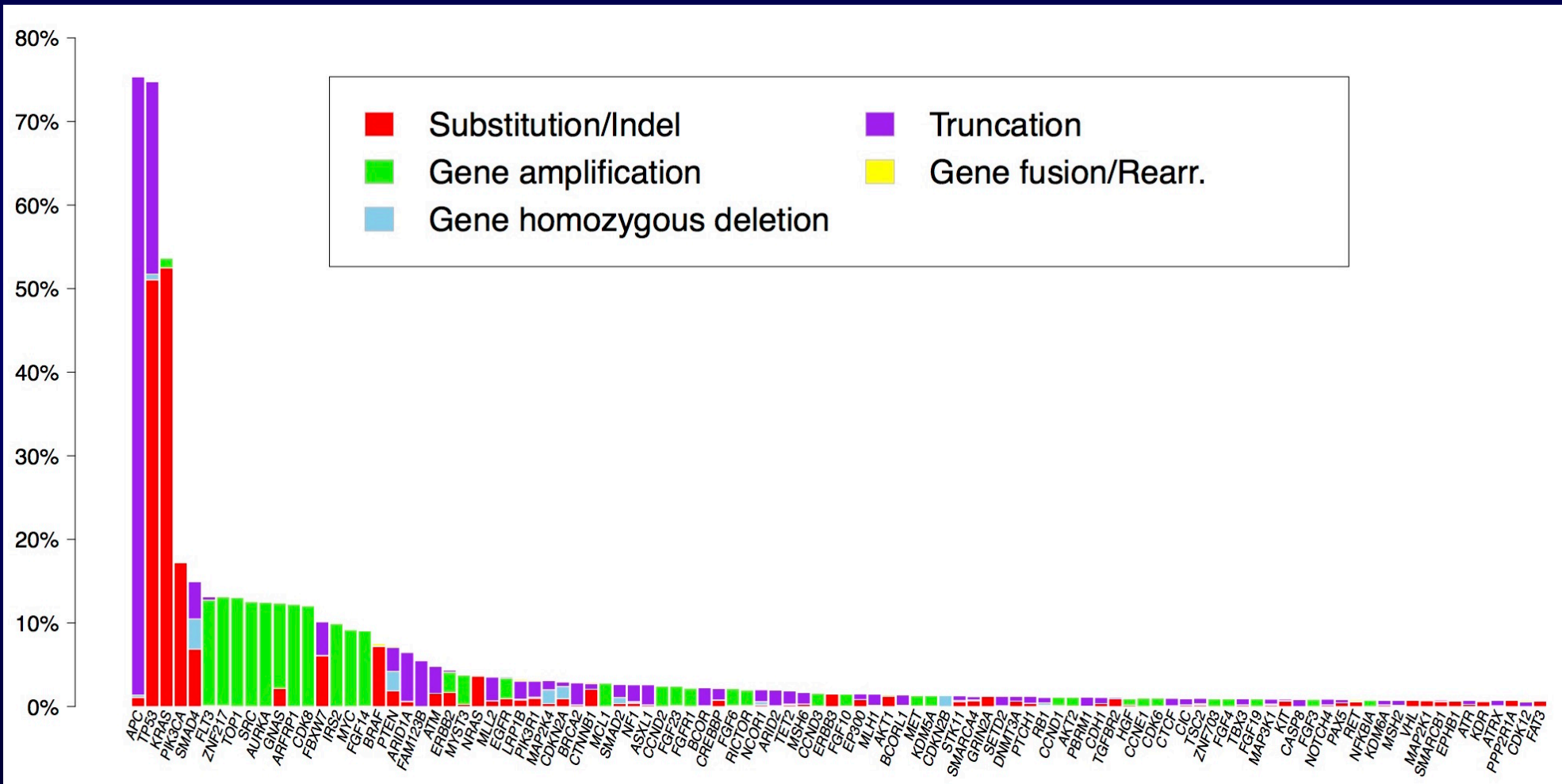
Clinical Utility of a Diagnostic Test

| Elements of clinical utility | Explanation |
|------------------------------|--|
| Health outcomes | Health outcomes are outcomes that matter to patients and society: to prevent premature death, to restore or maintain functional health. |
| Strategy | Outcomes are generated not only by testing only but also by a management strategy that starts with testing but includes all downstream consequences of subsequent clinical management. |
| Probabilistic | Not all outcomes will be observed in everyone tested; evaluations will be made at the group level and expressed in terms of a distribution of outcomes. |
| Comparative | Utility is defined relative to a comparator strategy: current best standard practice. |

A Hybrid Capture Comprehensive Genomic Profiling Test Detects a Wide Range Alterations Missed by Hotspot Testing



Finding New Targets for Metastatic CRC



Now to the Questions:

The answers are: "it all depends."

Question 1

- ...*analytical validity* of the molecular pathology test to estimate prognosis for Medicare beneficiaries?
- IT DEPENDS:
 - Some tests address prognosis, but do not guide therapy
 - Some tests have strong analytic validation, others DON'T
 - Some tests have built-in internal controls (eg HER2 FISH), but most do not
 - Some tests have created "surrogate controls like running cell lines in parallel with patient samples, but most do not"

Question 2

- ...is there evidence to conclude that using the molecular pathology test to estimate prognosis affects health outcomes?
- IT DEPENDS:
 - Predictive tests identifying genomic alterations have to lead to effective therapies or beneficial clinical trials to improve outcomes
 - Greatest outcome benefit comes from detecting all types of genomic alterations that drive therapeutic response:
 - base substitutions, insertions/deletions, copy number changes and rearrangements

Q2 continued: What's this business about clinical trials improving outcomes?

- **Entry into Clinical Trials for Cancer in the targeted therapy era is very different than for the non-targeted anti cancer drug trials conducted years ago.**
- **High response rates are expected, so benefit expected**
 - “Conventional therapy might give a response rate of 10 or 20 percent,” Dr. Pazdur said. “The newer drug has a response rate of 50 or 60 percent. Does it make sense to do a randomized trial?” And even if a trial were planned, he said: “Who would go on that trial? Would you go on that trial?” “When you are having a big effect, it is kind of jaw dropping,” Dr. Pazdur added. “These are response rates we haven’t seen before in diseases.”
- **Single arm studies starting to lead to approval**
- **Targeted therapies soon may be ready for approval on Phase I data**
- **This is a cautious paradigm shift, but it seems to be supported by experience**

Question 3

- ...is there evidence to conclude that using the molecular pathology test to estimate prognosis has clinical utility?
- IT DEPENDS:
 - Tests in breast and lung cancers clearly improving outcomes across multiple alterations
 - Colon cancer not there yet, *KRAS/NRAS testing* used only as negative selectors of specific therapy
 - Many other examples of success in targeting genomic alterations in a wide variety of tumor types:
 - Leukemias and lymphomas
 - Gastrointestinal stromal tumors
 - Melanoma

I.M.H.O. Scoresheet

| Cancer Type | Test to Estimate Prognosis | Q1a | Q1b | Q2 | Q3 |
|---|---------------------------------|-----|-----|----|----|
| Adenocarcinoma of the colon and rectum | BRAF | 4 | 4 | 4 | 4 |
| | KRAS | 5 | 5 | 5 | 5 |
| | Microsatellite instability | 2 | 2 | 2 | 2 |
| | MLH1 promoter methylation | 1 | 1 | 1 | 1 |
| | Oncotype DX [®] Colon | 1 | 1 | 1 | 1 |
| Breast cancer (invasive duct and lobular cancers) | MammaPrint [®] | 4 | 4 | 4 | 4 |
| | Oncotype DX [®] Breast | 4 | 4 | 4 | 4 |
| Non-small cell lung cancers | ALK | 5 | 5 | 5 | 5 |
| | EGFR | 5 | 5 | 5 | 5 |
| | KRAS | 2 | 2 | 2 | 2 |

The next round of questions for the MEDCAC?

| Cancer Type | Test to Estimate Prognosis | Q1a | Q1b | Q2 | Q3 |
|----------------------------|----------------------------|-----|-----|----|----|
| Non-small cell Lung Cancer | ROS1 | ? | ? | ? | ? |
| | RET | ? | ? | ? | ? |
| | ERBB2 | ? | ? | ? | ? |
| | NTRK1 | ? | ? | ? | ? |
| | MET splice site | ? | ? | ? | ? |
| Ovarian Serous Carcinoma | “BRCAness” | ? | ? | ? | ? |
| | ERBB2 | ? | ? | ? | ? |
| Sarcomas | CDK4/MDM2 | ? | ? | ? | ? |
| | MET amplification | ? | ? | ? | ? |
| | Multiple gene fusions | ? | ? | ? | ? |

Thank You