NCIC CLINICAL TRIALS GROUP
2011 NEW INVESTIGATOR CLINICAL TRIAL COURSE

Correlative Studies in Phase III Trials:
Laboratory Aspects of Biomarker Studies

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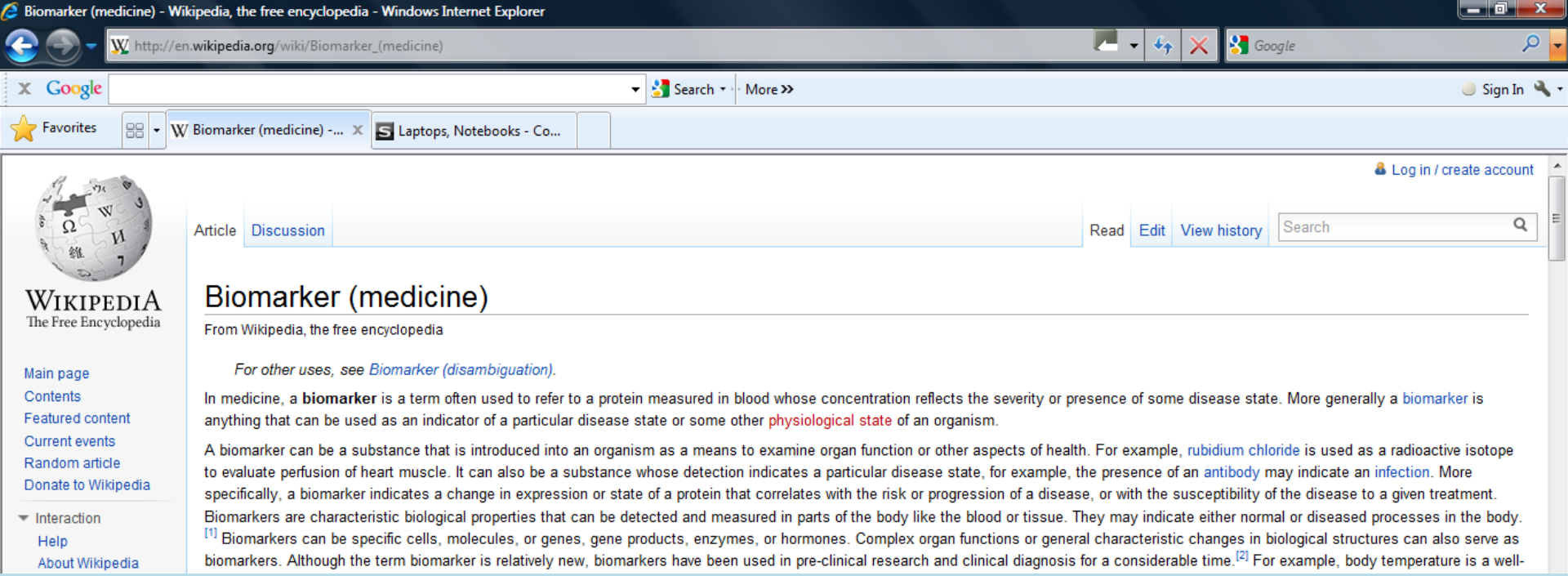
Conflict of Interest Declaration

- **Research grants from:**
  - Med Biogene (Vancouver, Canada)
  - Ventana Medical Systems (Tucson, Arizona)
  - Hoffmann-La Roche
  - Pfizer Canada
- **Honoraria from:**
  - AstraZeneca, Hoffmann-La Roche, Pfizer Canada, Lilly Canada, Boehringer-Ingelheim, Daiichi-Sankyo, Precision Therapeutics



Biomarker:

- **A parameter that can be used to measure the progress of disease or the effects of treatment**
- Can be specific cells, molecules, or genes, gene products, enzymes, or hormones.
- *In molecular terms* biomarker is "the subset of markers that might be discovered using genomics, proteomics technologies or imaging technologies.

Biomarker Studies in Phase 3 Trials

- **Prognostic markers:**
  - Identify patients who are at high risk of early death
  - High risk patients could potentially benefit from early aggressive treatment
- **Predictive markers:**
  - Identify patients most likely to benefit (or not benefit) from specific therapy
  - May tailor patients for more effective treatment and avoid potential harms

Relative Importance of Prognostic and Predictive Markers

	Early Stage	Advanced Stage
Prognostic Markers	+++	+
Predictive Markers	++	+++

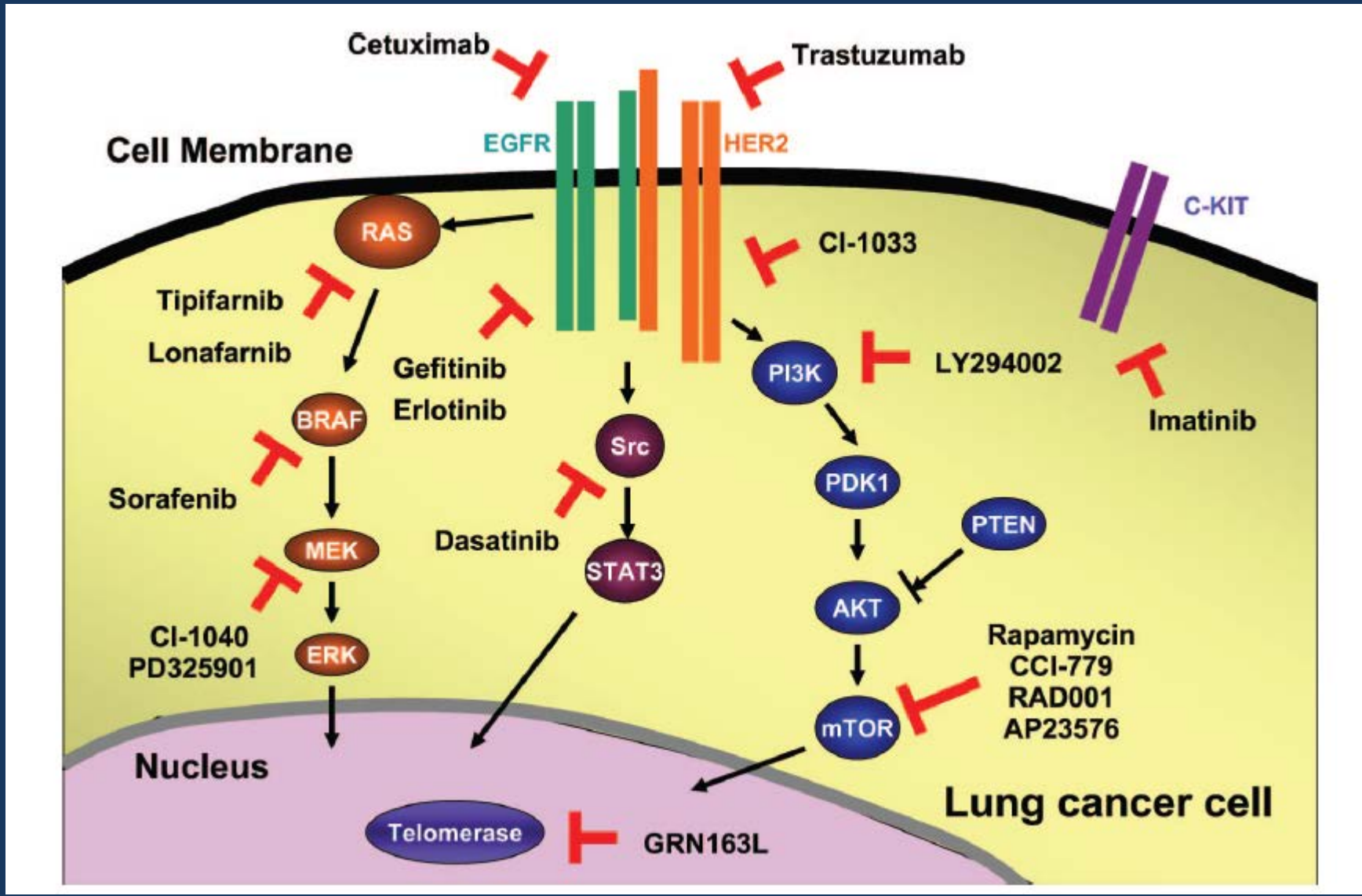
Role of Pathologist/Biomarker Scientist

- **Concept:**
  - Understanding potential biomarkers (drug targets)
  - Molecular aberrations linked to drug targets
- **Assay:**
  - Assays available to evaluate specific aberrations
  - Assay pros and cons
  - Reliability and cut-offs
  - Availability and adoptability
- **Sample:**
  - Sample types, availability and quality
  - Impact of tissue heterogeneity on assay

Essential Issues to Consider

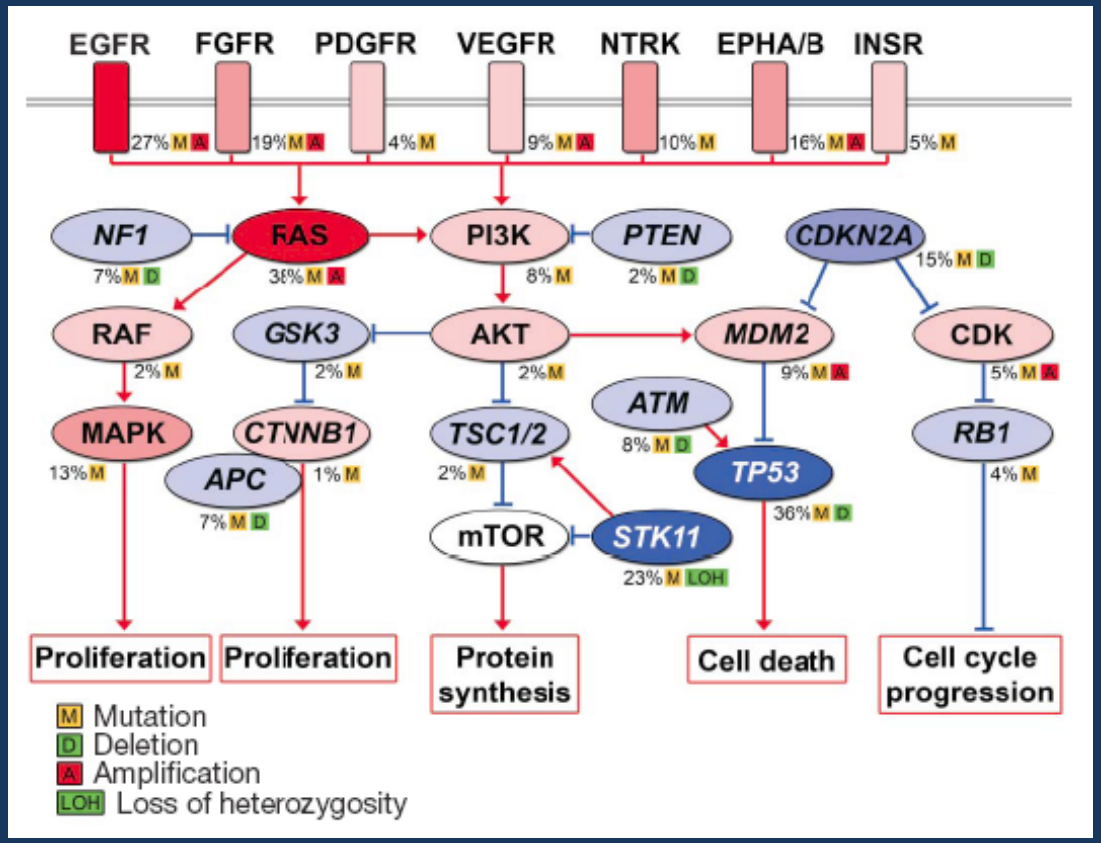
- Knowledge of drug targets (and related signaling pathways) improves:
  - Choices of markers to be studied
  - Development of diagnostic algorithm for clinical use of the biomarkers
- Biomarker frequency (prevalence) impacts on statistical power calculation
- Nature of an aberration determines the appropriate assays to use

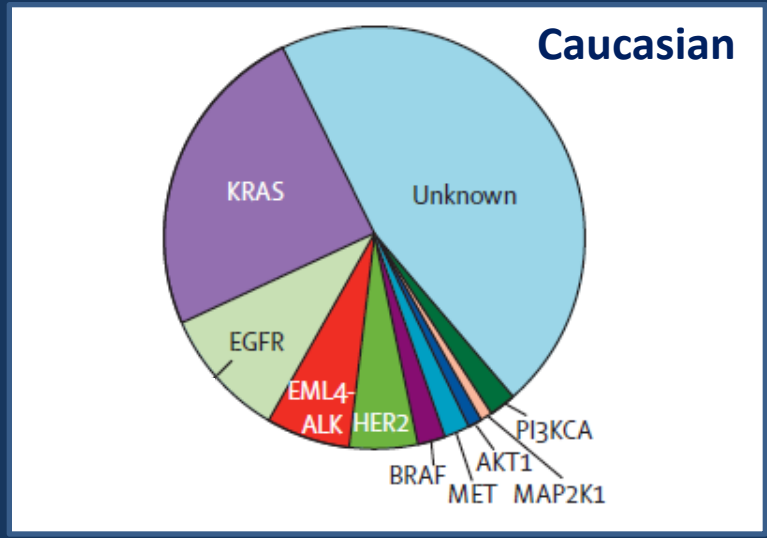
Drugs and Potential Targets

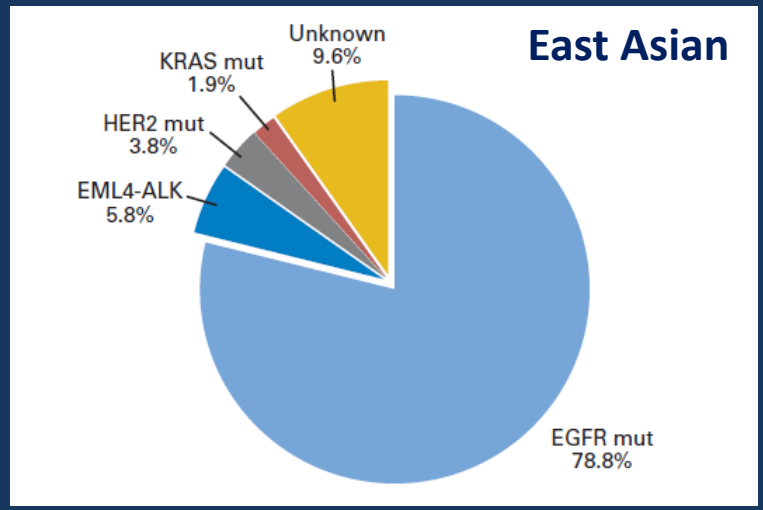


Sato M, et al. J Thorac Oncol 2007;2: 327–343

Genomic Aberrations as Potential Drug Targets in Lung Adenocarcinoma





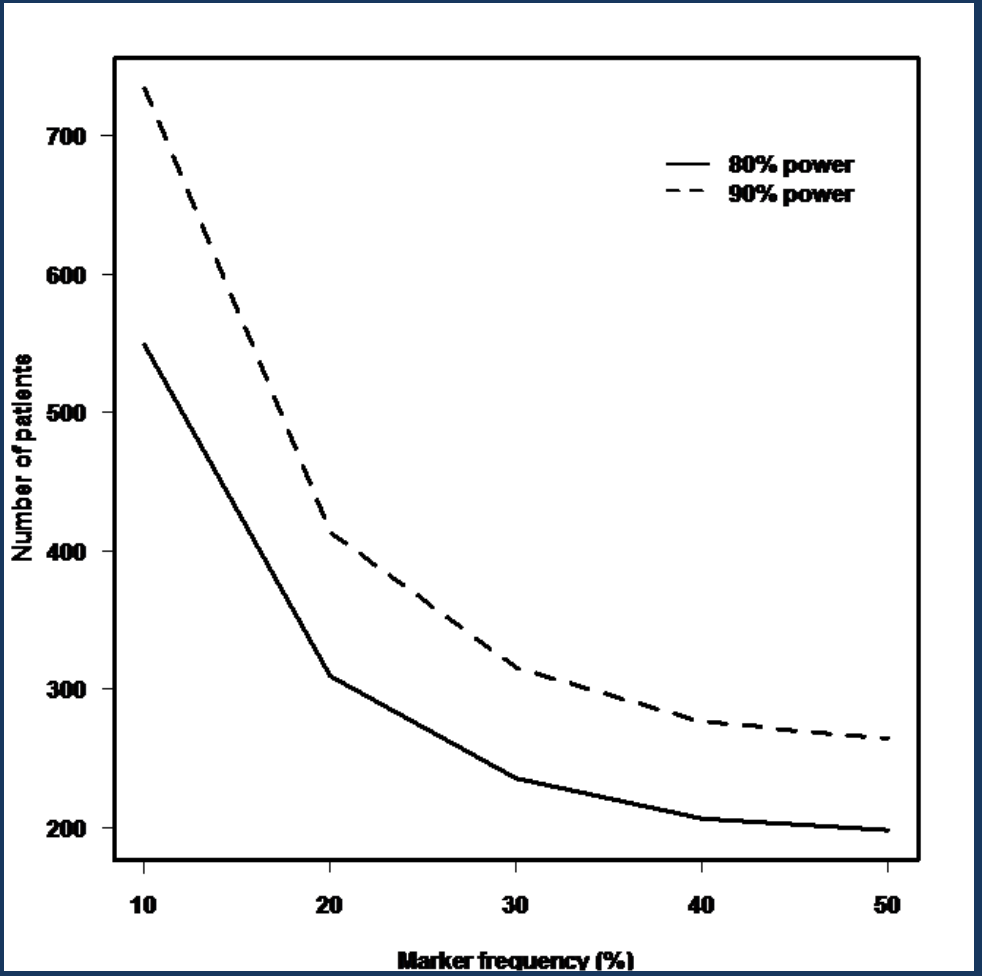


Sun Y, et al. J Clin Oncol 2010;28:4616-20
Pao W, Girard N. Lancet Oncol 2011;12:175-80

Marker Prevalence Impacts on Sample Size Requirement

A hypothetical prognostic marker analysis:

- **Hazard ratio: 2.0**
- **Survival at 5 yrs : 60%**
- **Accrual: 4 yrs**
- **Extra follow-up: 2 yrs**
- **Alpha: 0.05**
- **Power: 80% and 90%**



Courtesy of Melania Pintilie (biostatistician)

Few Classes of Drugs Have Found the Real Targets in Lung Cancer

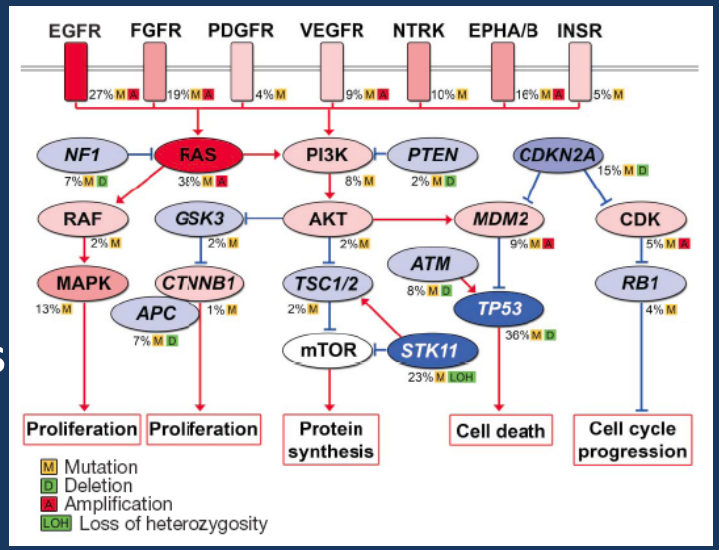
VEGF targeted agents
EGFR targeted agents
ALK inhibitor

mTOR inhibitors

Proteasome inhibitors

Cell cycle targeted agents
- PARP inhibitors
- CDK inhibitors
- Novel chemotherapy
- Proapoptotic agents

Vaccine Therapy



Other Kinase Inhibitors:
- PI3K
- AKT
- MAP kinase
- MEK (Ras, Raf)
- SRC
- Aurora kinase
- Polo-like kinases
- PKC

HSP 70, 90 targeted agents

HIF1-alpha antagonists

C-met inhibitors

Types of Biomarkers

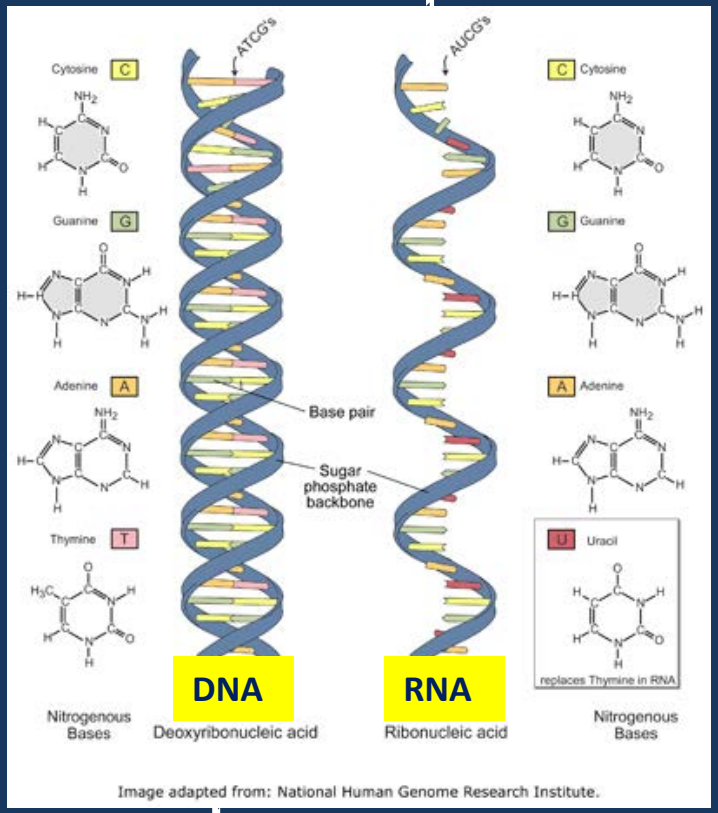


Image adapted from: National Human Genome Research Institute.

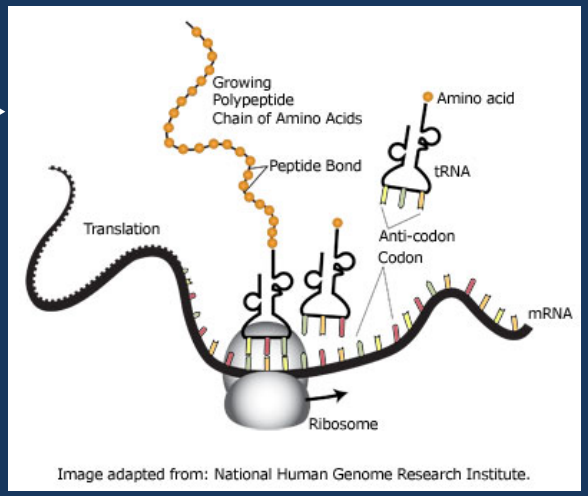
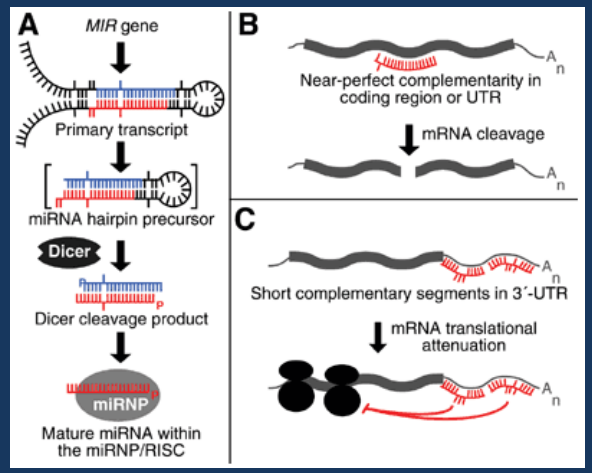


Image adapted from: National Human Genome Research Institute.

Protein



miRNA

Molecular Biomarker Assays

- **Protein:**
  - Immunohistochemistry (tissue)
  - Elisa (blood/fluid)
- **DNA/RNA/microRNA:**
  - Polymerase chain reaction (PCR) based
    - Mutations
    - Translocations
    - Single nucleotide polymorphisms
    - Transcripts (mRNA & microRNA)
  - Fluorescent in situ hybridization (FISH)
  - Microarrays
  - Other high throughput platforms

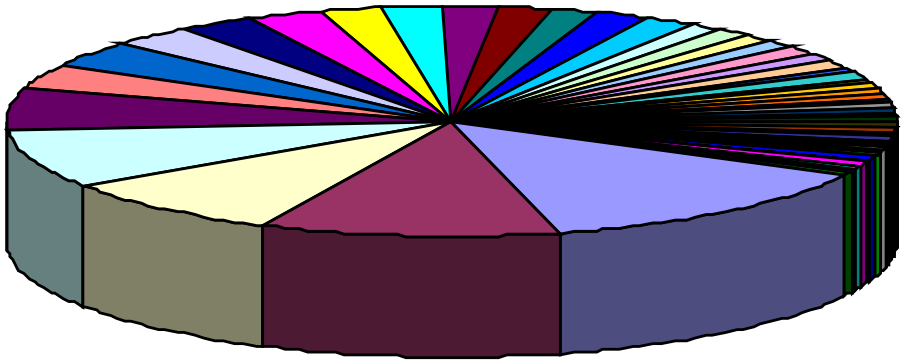
REVIEW

Journal of Clinical Pathology 2006;59:790

Immunohistochemical markers of prognosis in non-small cell lung cancer: a review and proposal for a multiphase approach to marker evaluation

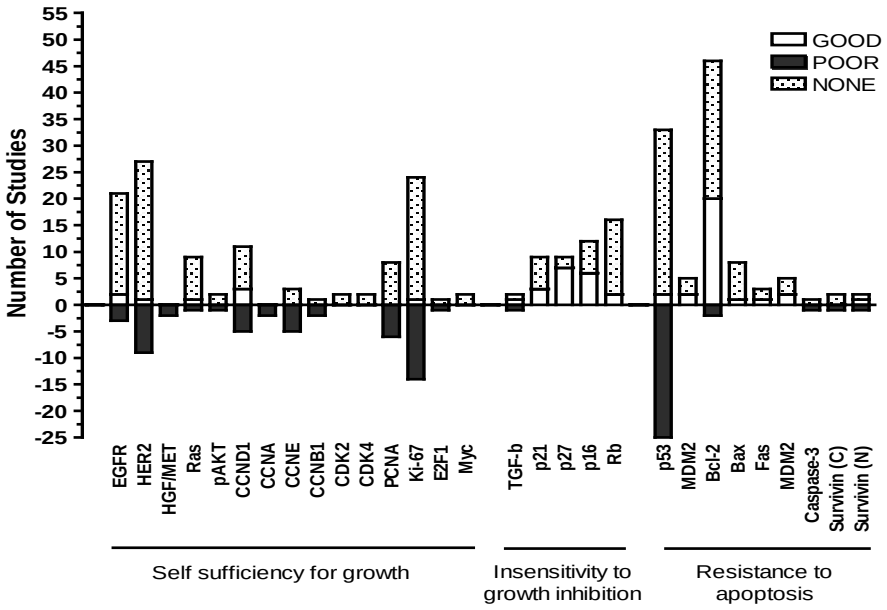
C-Q Zhu, W Shih, C-H Ling,* M-S Tsao

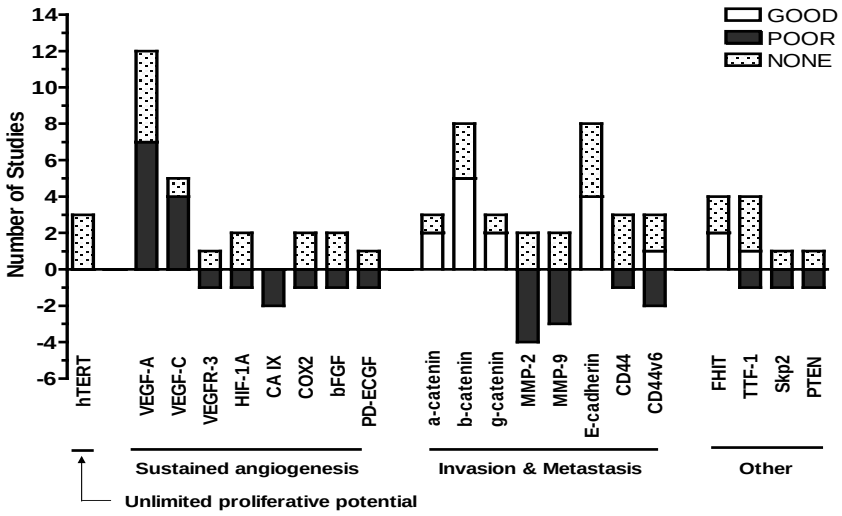
- **Pubmed search: May 1987 to October 2005**
- **462 reports and 12 reviews: ~50 markers studied by ≥ 2 groups**



p53 Bcl-2 Ki-67 HER2 EGFR CCND1 Rb
PCNA p16 VEGF-A Ras p21 p27 CCNE
Bax b-catenin E-cadherin MMP-2 MDM2 MDM2 VEGF-C
MMP-9 CD44v6 TTF-1 CD44 FHIT pAKT CCNB1
TGF-b Fas Survivin (C) Survivin (N) hTERT HIF-1A COX2
bFGF a-catenin g-catenin HGF/MET CCNA CDK2 CDK4
E2F1 Myc Caspase-3 VEGFR-3 CA IX PD-ECGF Skp2
PTEN

No Markers have been Validated Sufficiently for Clinical Application





Zhu CQ et al. J Clin Pathol 2006;59: 790-800.

Major Issues With IHC

- **Lack of uniform standards for:**
  - **Tissue processing (fixation time)**
  - **Antibodies**
    - **multiple sources**
    - **Variable sensitivity and/or specificity**
  - **Staining protocol**
  - **Scoring method**
  - **Statistical correlation with outcome**
- **Institutional biases**
- **Publication biases**

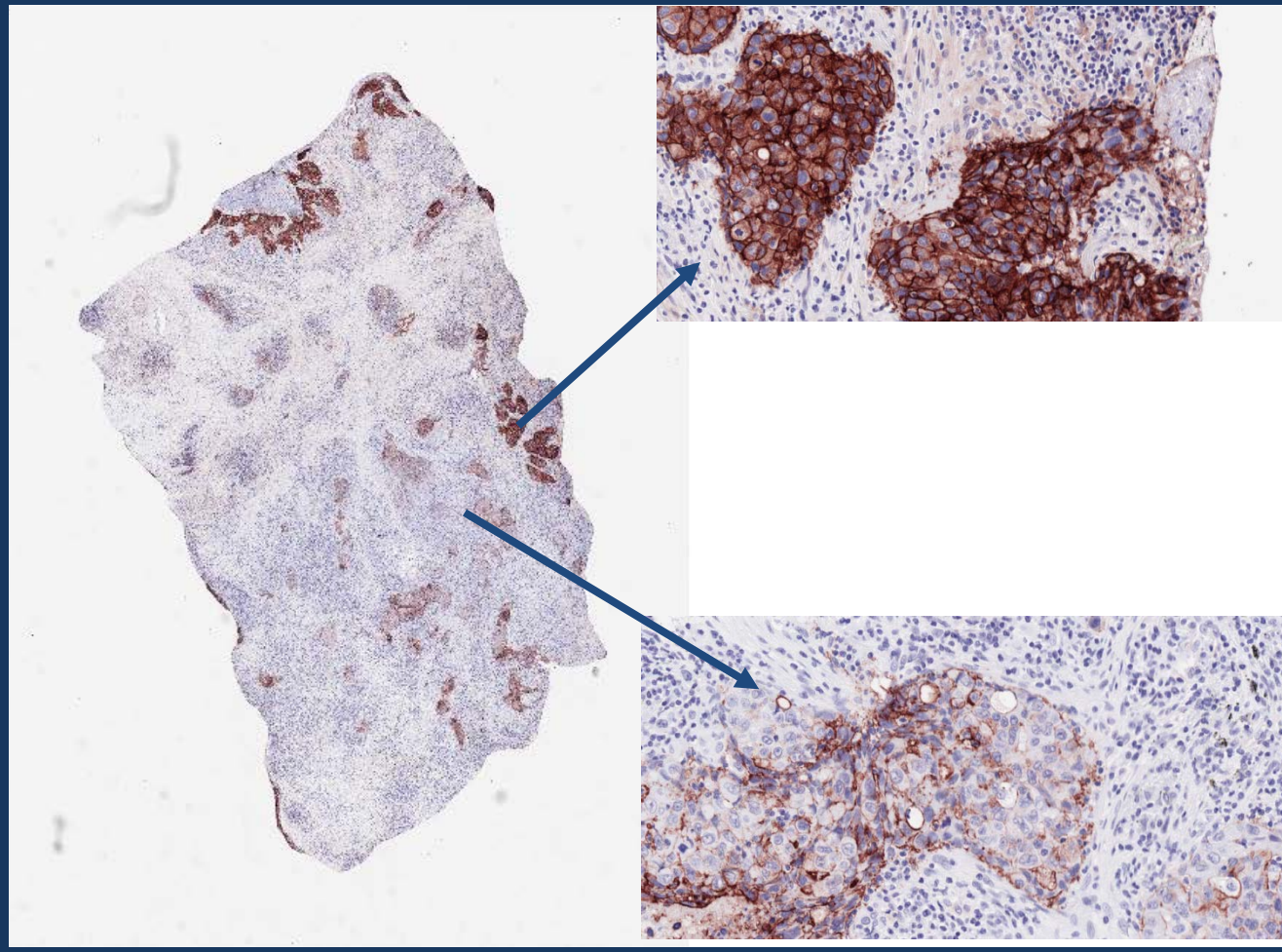
Example: Cyclin D1 Studies

Marker	Author	Year	No. of	Antibody Source	MC (clone)/PC	Ab dilution	Univariate	multivariate	Criteria/cut-offs
Cyclin D1	Kwa HB	1996	96	Non-commercial	PC	1:80	no	no	>10% nuclei stained
Cyclin D1	Caputi M	1999	135	Non-commercial	PC	1:100	poor	NA	0; 1-30%; 30-60%; >60%
Cyclin D1	Keum JS	1999	69	Novocastra	MC(P2D11F11)	1:200	poor	no	>5% cells stained
Cyclin D1	Brambilla E	1999	168	Dako	NA	NA	no	no	>5% cells
Cyclin D1	Anton RC	2000	467	PharMingen	MC(G124-326)	1:500	Good for SQ	N/A	>10% cells
Cyclin D1	Volm M	2000	145	Santa Cruz	MC(Ab-3)	1:10	no	no	moderate-strong staining
Cyclin D1	Nguyen VN	2000	89	Dako	MC(DCS-6)	NA	no	NA	cytoplasmic staining
Cyclin D1	Gugger M	2001	92	Novocastra	MC(P2D11F11)	1.6 ug/ml	Good	yes	any nuclear staining (?)
Cyclin D1	Jin M	2001	106	PharMingen	MC(G124-326)	1:50	poor	yes	> nuclear background or cytoplasm staining
Cyclin D1	Dosaka-Akita H	2001	217	Oncogene Science	MC(DCS-6)	1:40	no	N/A	any nuclear staining
Cyclin D1	Ikehara M	2003	72	Novocastra	PC	1:200	poor	NA	>20% cells
Cyclin D1	Au NHC	2004	284	Dako	MC(DCS-6)	1:300	Good for AD	no	4 tiers system; cut-off for positive not stated
Cyclin D1	Burke L	2005	106	Oncogene Science	MC(DCS-6)	1:40	no	no	Intensity (0-3) + % cells (0-3); positive: 4 or >
Cyclin D1	Esposito V	2005	105	NA	NA	NA	Poor	yes	>5% cells
Cyclin D1	Dworakoska D	2005	111	Dako	MC(DCS-6)	1:100	no	no	any cell stain

Ideal Scoring System

- Simple & reproducible independently
- Minimize dependency on technical variability
- Minimize observers‘ subjectivity
- Capture heterogeneity

With Most IHC Markers, Staining Pattern is Heterogeneous



Scoring Systems for IHC

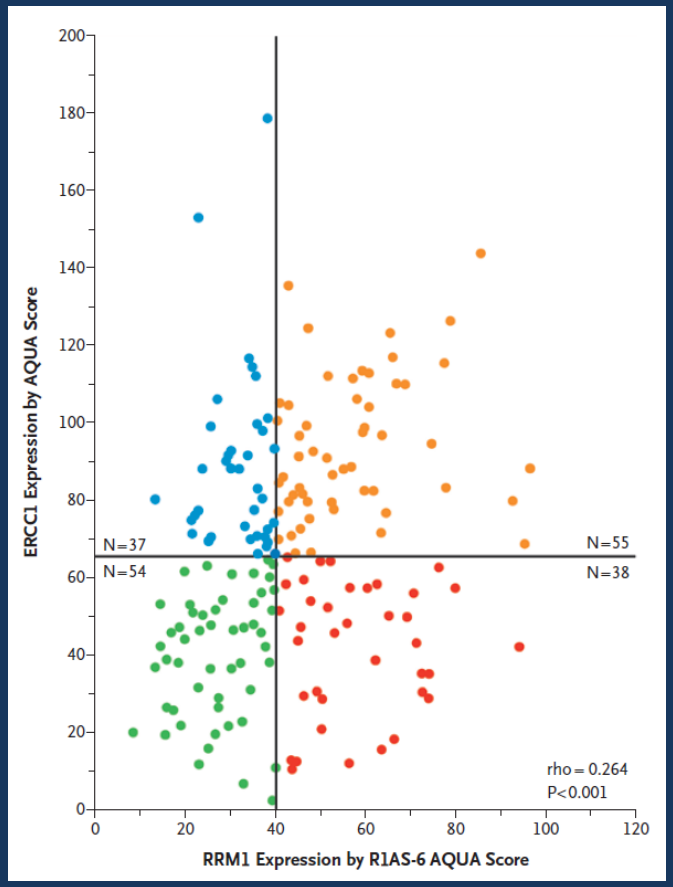
Direct Score

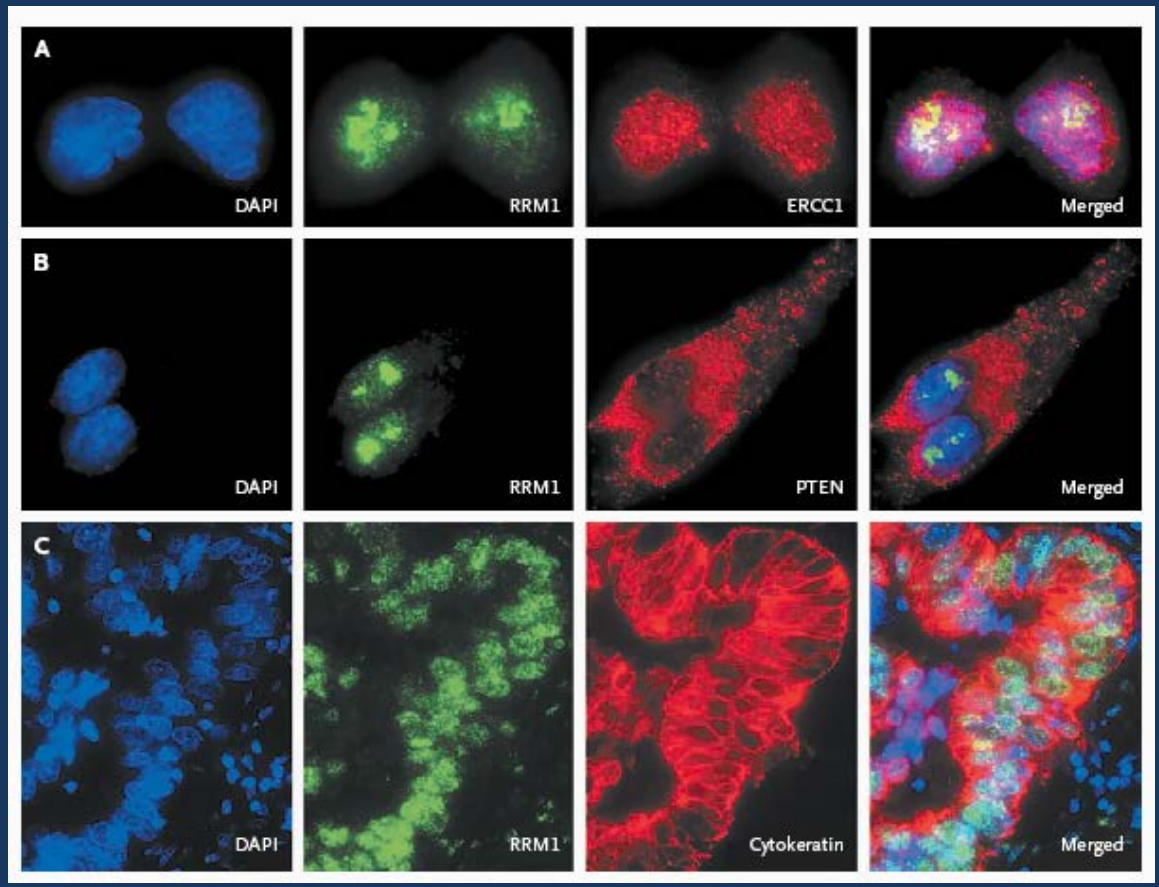
- **Staining intensity:**
  - **Absent: 0**
  - **Weak: 1**
  - **Moderate: 2**
  - **Strong: 3**
- **Percent tumor cells stained:**
  - **0 to 100%**

H-Score

- **Attempt to represent overall staining features:**
  - **Intensity (I) x percent (%)**
- **Capture heterogeneity:**
  - **$0 \times \%(I_0) + 1 \times \%(I_1) + 2 \times \%(I_2) + 3 \times \%(I_3)$**

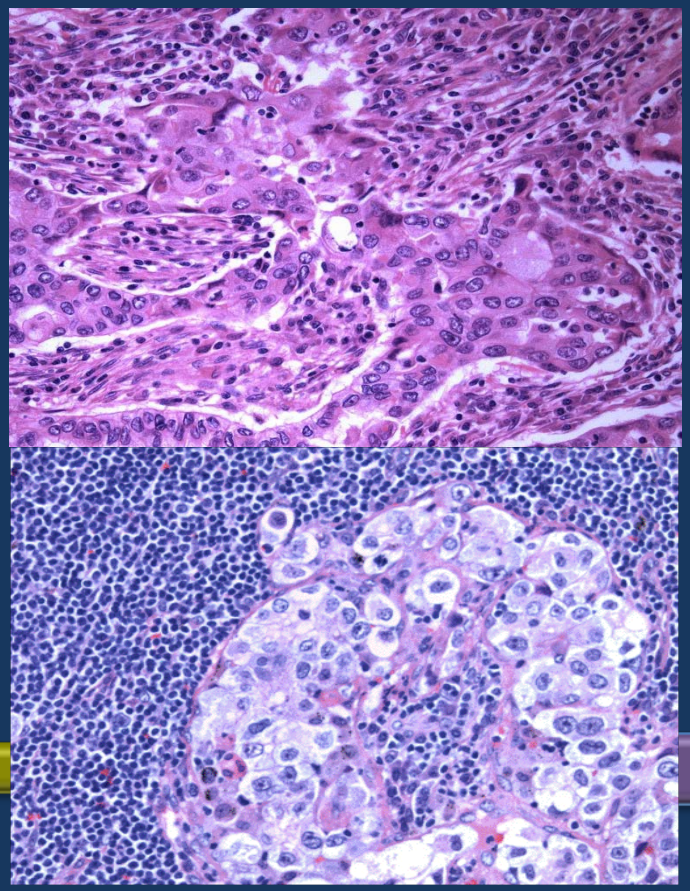
Quantitative Image Analysis





Zheng Z, et al. N Engl J Med 2007;356:800-8.

Mutation Analysis by PCR-Sequencing



Assay sensitivity is limited by amount of contaminating normal DNA of non-cancer cells

Macro-dissection to Enrich for Tumor Cells

Sensitivity of Mutation Assays

Method	Sensitivity	Mutations identified
Direct Sequencing	25%	Known and new
PCR-SSCP	10%	Known and new
TaqMan PCR	10%	Known only
Loop-hybrid mobility shift assay	7.5%	Known only
Cycleave PCR	5%	Known only
PCR-RLFP (fragment length analysis)	5%	Known only
MassARRAY genotyping	5%	Known only
LNA -PCR clamp	1%	Known only
Scorpion ARMS (DxS)	1%	Known only
dHPLC	1%	Known only
COLD-TaqMan PCR	0.05%	Known only

Adapted from: *Pao and Ladanyi. Clin Cancer Res 2007;13:4954-55*

BR.21 erlotinib study:

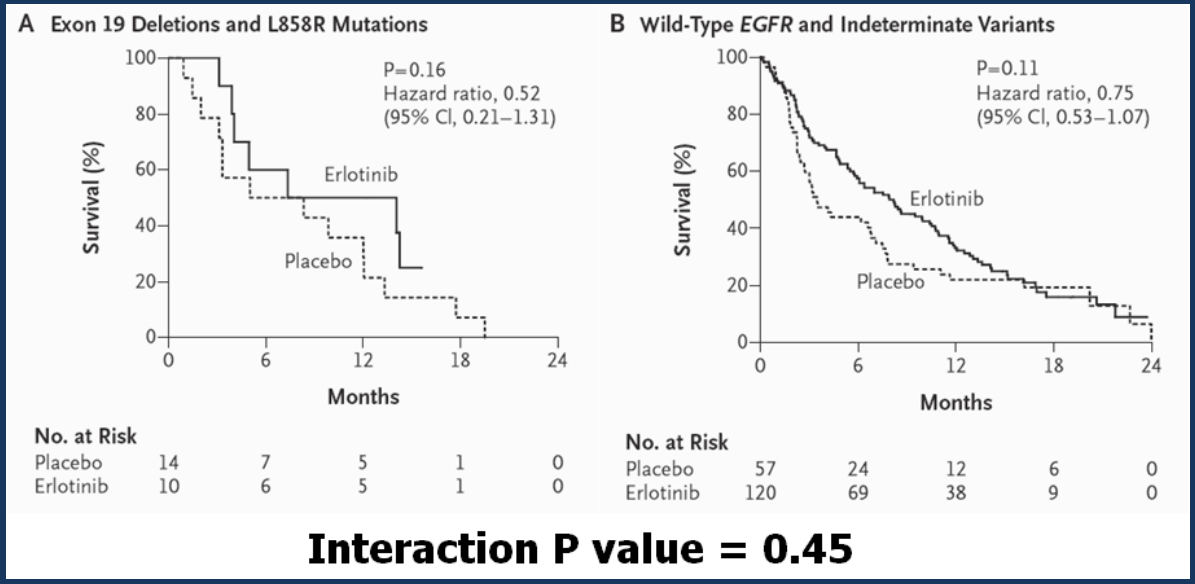
Higher Sensitivity Method Identified 40% More Mutations

Number of Patients	Direct Sequencing	Sequencing + ARMS/RLFP
In the trial	731	
Successful EGFR mutation analysis	201	204
Ex-19 del + L858R	24 (12%)	34 (17%)

Tsao et al, NEJM 2005; 353: 133-44
Zhu CQ, et al. J Clin Oncol 2008;26:4268-75

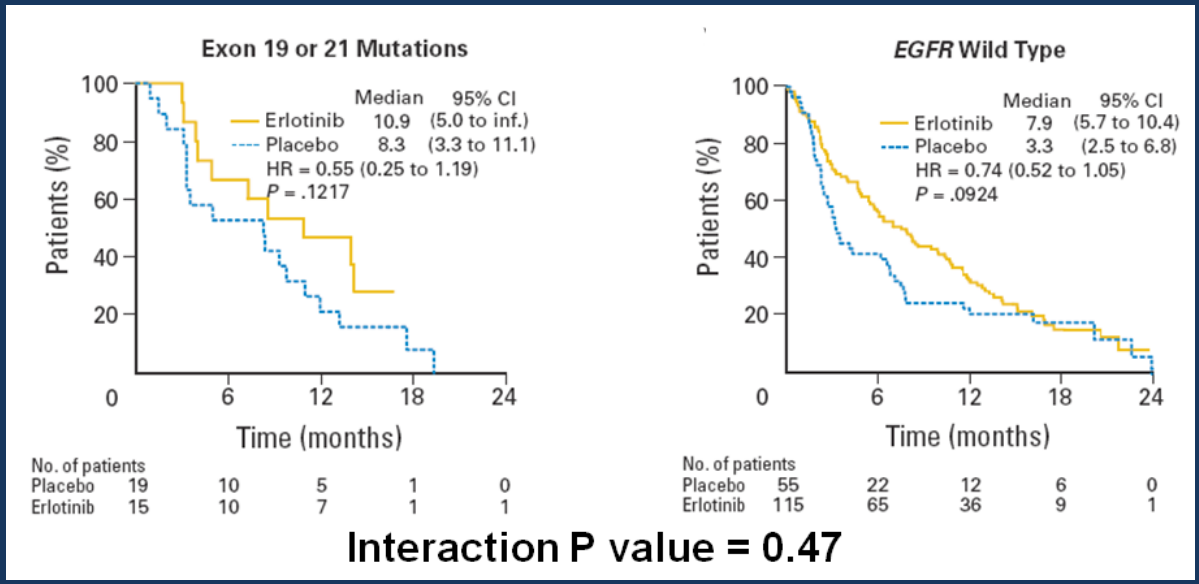
Impact on Clinical Outcome

NEJM 2005



Interaction P value = 0.45

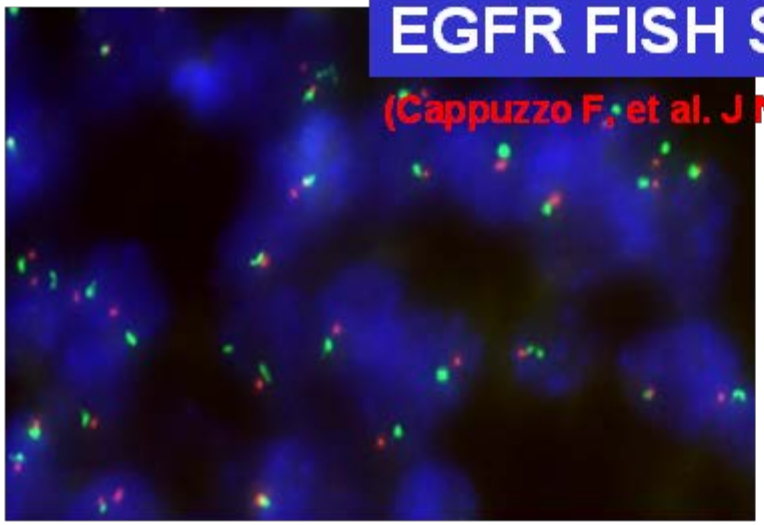
JCO 2008



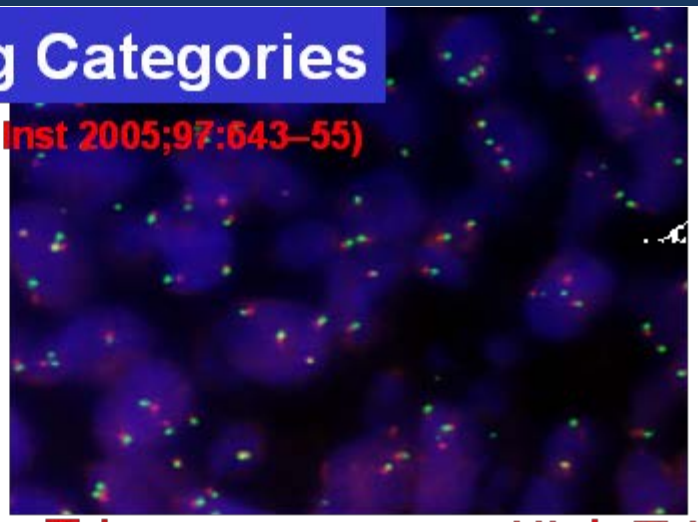
Interaction P value = 0.47

EGFR FISH Scoring Categories

(Cappuzzo F, et al. J Natl Cancer Inst 2005;97:643–55)

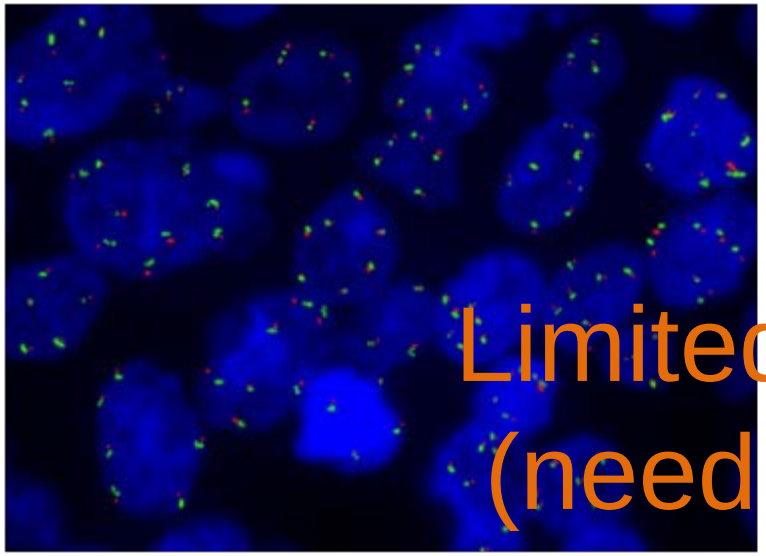


Disomy
≤2 gene copies in >90% cells

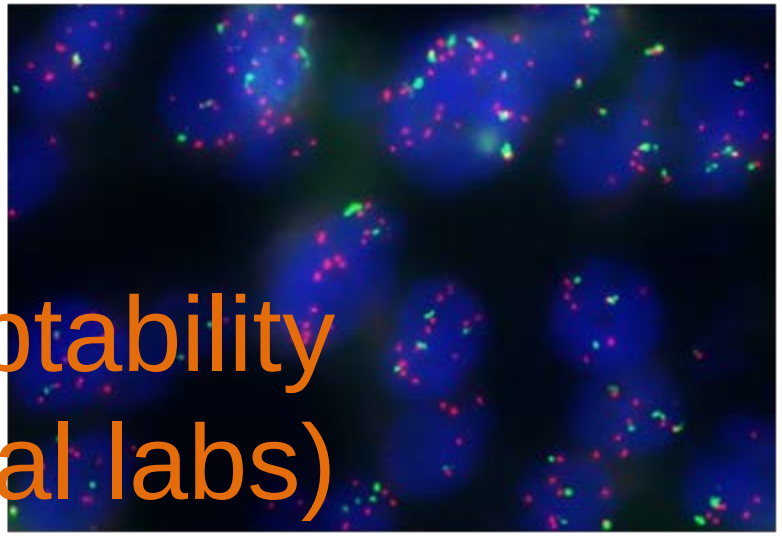


Low Trisomy
3 gene copies in
>10% <40% cells

High Trisomy
3 gene copies in
≥40% cells



Limited adoptability
(need central labs)



Low Polysomy
≥4 gene copies in
>10% but <40% cells

High Polysomy
≥4 gene copies
in ≥40% cells

Gene Amplification
Gene/chromosome ratio >2 or
≥15 gene copies in ≥10% cells

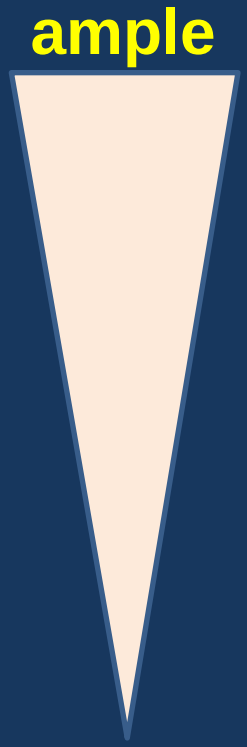
Common Tissue Fixatives and DNA/RNA Quality

Base Fixative	DNA	RNA
Buffered formaldehyde	Fair	Fair
Glutaraldehyde	Good	Unknown
Methanol-chloroform	Good	Good
Ethanol-chloroform	Good	Good
Picric acid (Bouin)	Poor	Poor
Mercuric Cl (B5, Zenker)	Poor	Poor
Decalcifying acids	Poor	Poor

Hunt JL, Arch Pathol Lab Med 2008;132:248-60

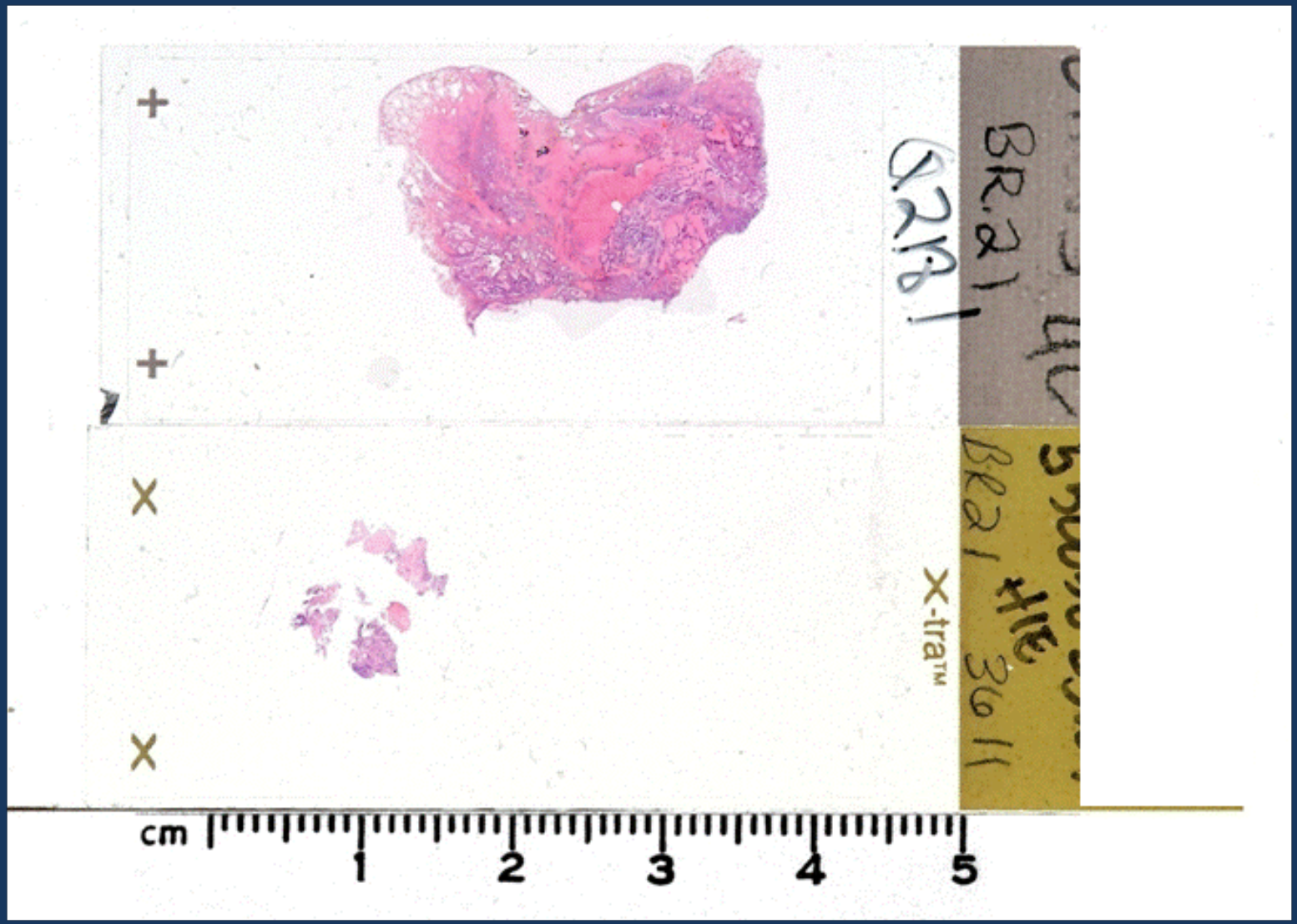
Biological Samples

- Resection
- Open biopsy
- Needle core biopsy
- Needle aspiration biopsy
- Effusion
- Sputum

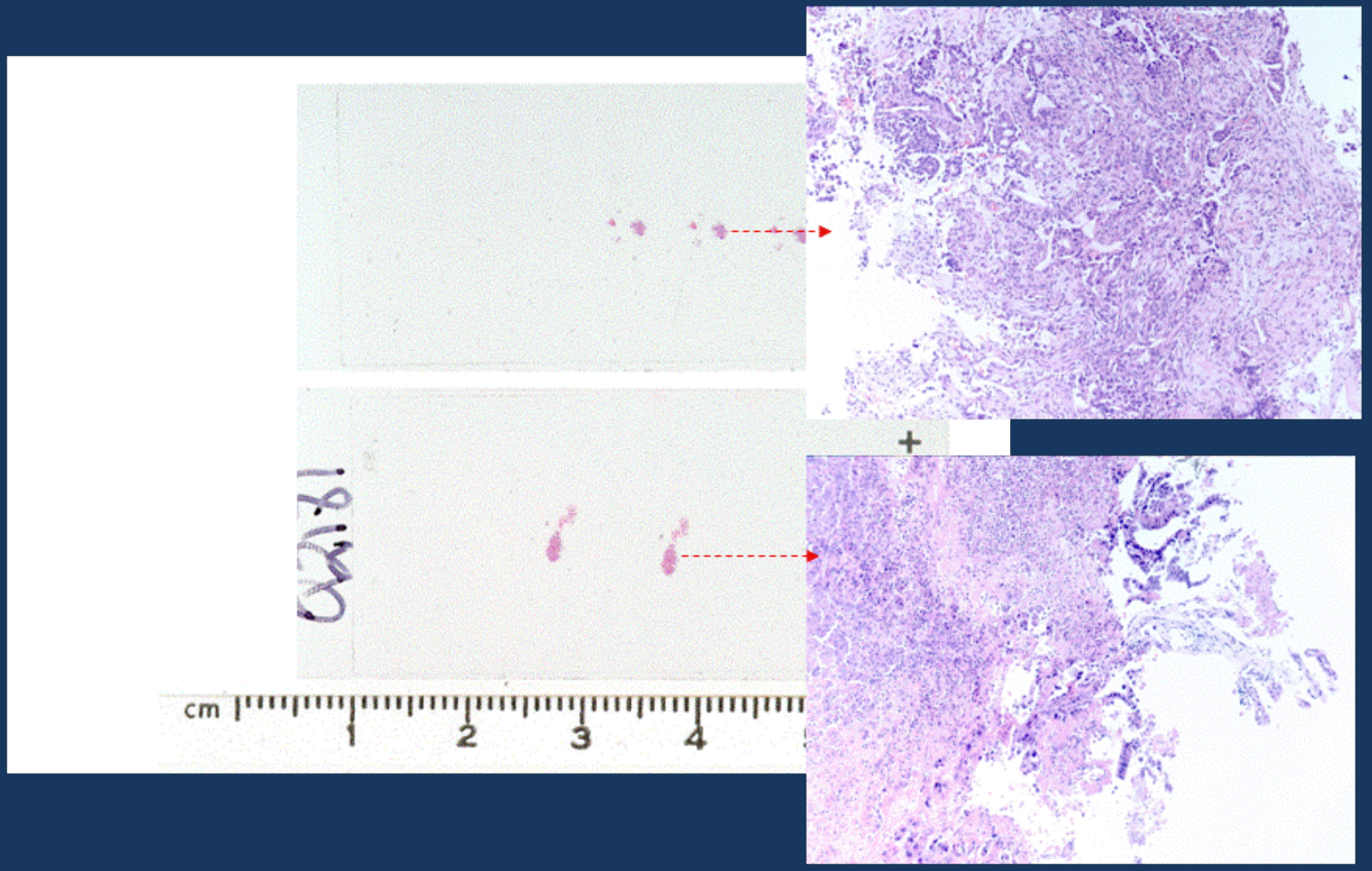


- Blood: Protein +++; DNA/RNA +

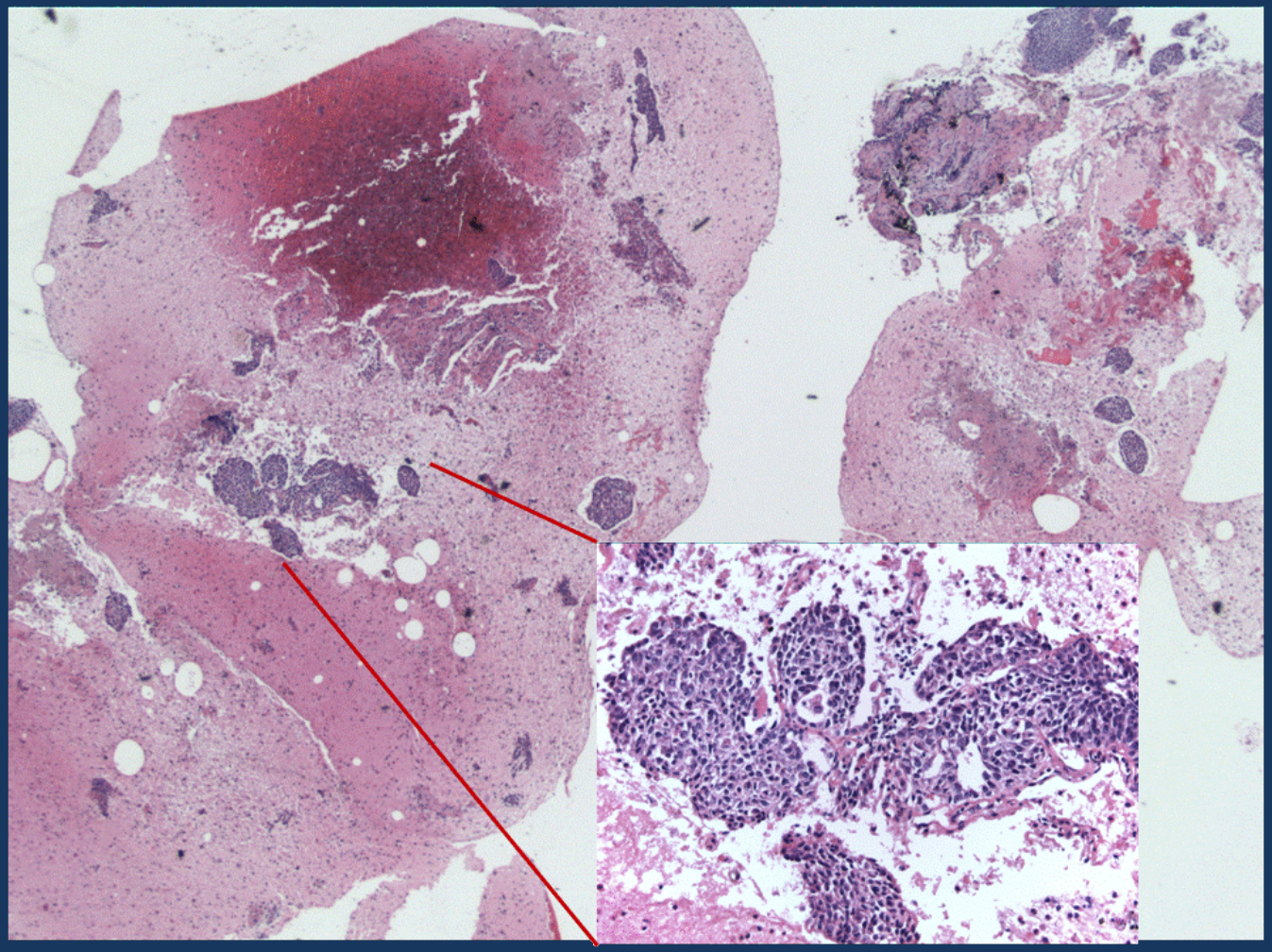
Ideal specimens (resection/biopsy)



Challenging Specimens: Needle Core Biopsies



Cytology Cell Blocks: Can Be Excellent Materials for Molecular Analyses



Need the pathologist to evaluate section

EGFR Mutation Analyses on Aspiration/Fluid Materials

	Sample	Fixative	NSCLC	Analysis Method	Ex 19 del	Ex 21 L858R	Others	Yield
Nomoto	FNA	Ethanol	37 (35A)	HRMA	13	9	2	59%
Smith	FNA	Air-dried	11 (6A)	HRMA	3	0	0	27%
Lim	FNA/Bx	RNAlater	88 (42A)	Dseq/WGA	7	10	4	24%
Wu	Effusion	-80C	136 (93A)	DSeq	32	50	11	68%
Kimura	Effusion	-80C	43 (30A)	DSeq	9	2	0	26%
Kimura	Effusion	-80C	24 (23A)	DxS	6	2	0	33%
Horiike	TBNA	-80C	94 (58A)	DxS/DSeq	17	14	0	33%
Fassina	FNA	FineFix	77 (61A)	HRMA	0	2	1	4%

FNA: fine needle aspiration
HRMA: high resolution melting analysis
DxS: Scorpion ARMS
TBNA: transbronchial needle aspiration
Dseq: direct sequencing

Laboratory Requirement

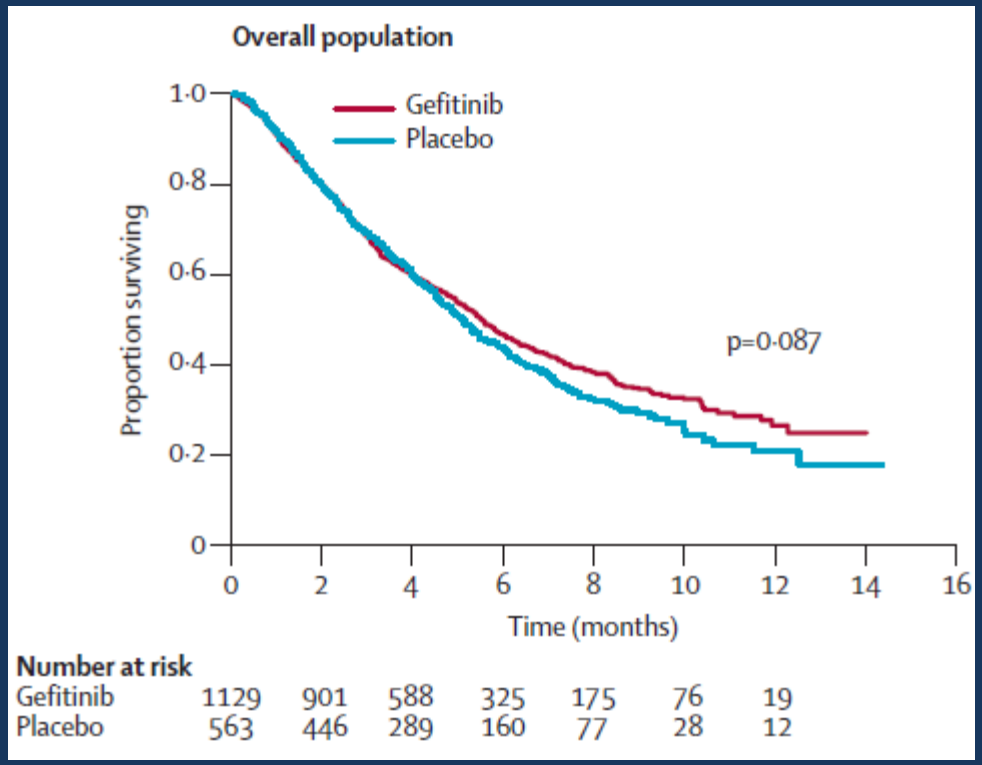
Stage of Study	Lab Requirement	Assay requirement
Basic research (target identification)	Research laboratory	Research Lab. assays
Preclinical and exploratory studies	Research laboratory	Reliable assays
Clinical development	Accredited clinical laboratory	Validated assays

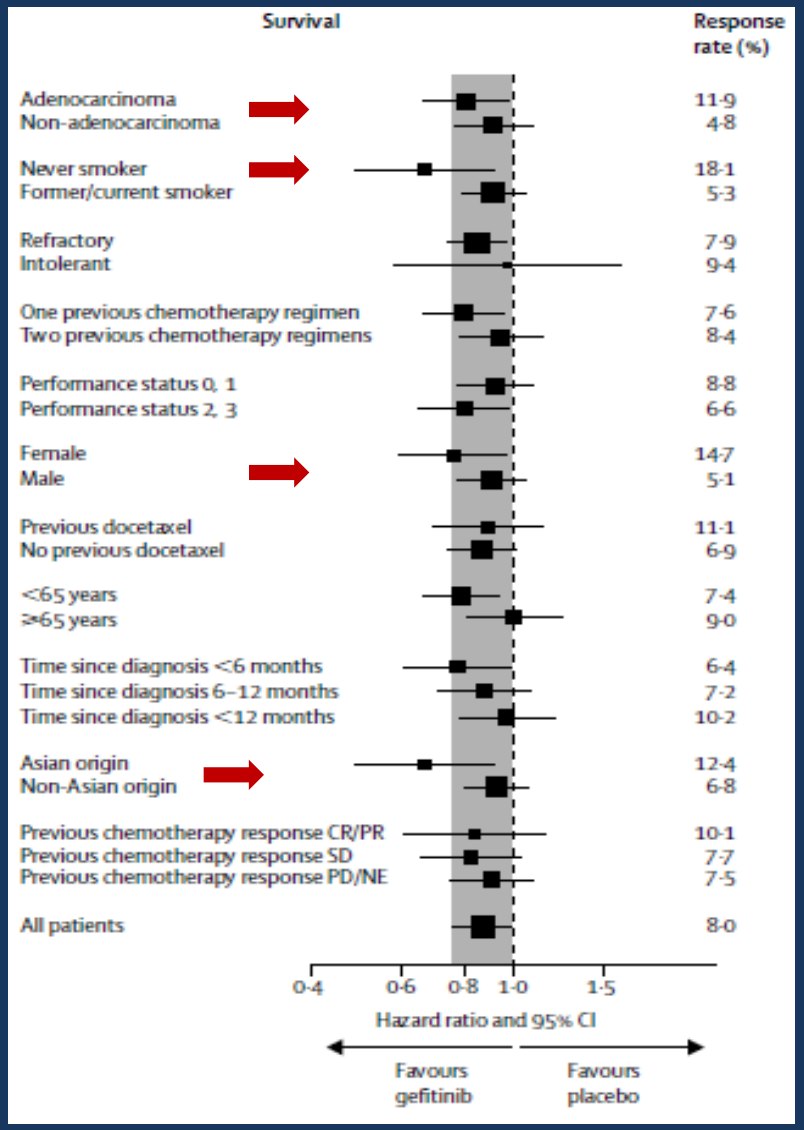
Sample Availability in Pivotal Phase 3 Lung Cancer Trials

Trial	Sample Collection	Mandatory (yes/no)	Patients in Trial	Patients with samples collected
TRIBUTE	Retro	No	1079	274 (25%)
ISEL	Retro	No	1692	379 (22%)
INTEREST	Retro	No	1433	380 (27%)
Br.21	Pro	No	731	325 (45%)
IPASS	Pro	No	1217	437 (36%)
IALT	Retro	No	1867	761 (41%)
JBR10	Pro	Yes (KRAS)	482	450 (90%)
SATURN	Pro	Yes (ihc)	889	742 (83%)

ISEL – Unselected Patients

Overall Survival





ISEL: Gefitinib vs. Placebo (1692 patients)

Markers	Patient with Result
EGFR IHC	379 (22%)
EGFR FISH (gene copy)	370 (22%)
EGFR mutation	215 (13%)
KRAS mutation	152 (9%)
BRAF mutation	118 (7%)

Hirsch FR, *et al.* J Clin Oncol 2006;24:5034-42.

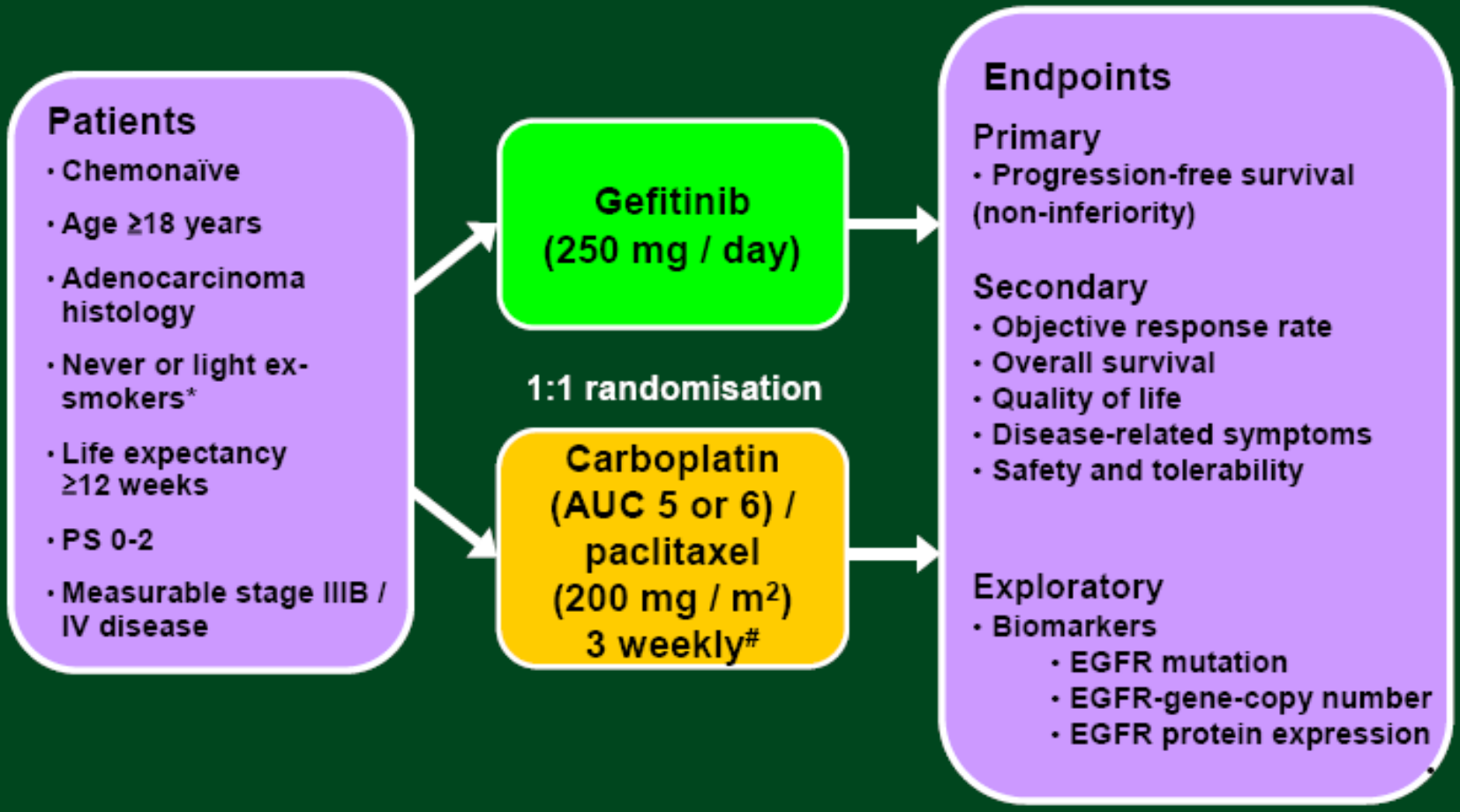
ISEL: Response Rate to Gefitinib

Mutation rate	Response Rate	
	Mutant	Wild type
EGFR (11%)	37.5% (6/16)	2.6% (3/116)
KRAS (8%)	0% (0/6)	8% (7/87)
BRAF (0%)	NA	NA

No Survival Analysis due to inadequate sample size

IPASS

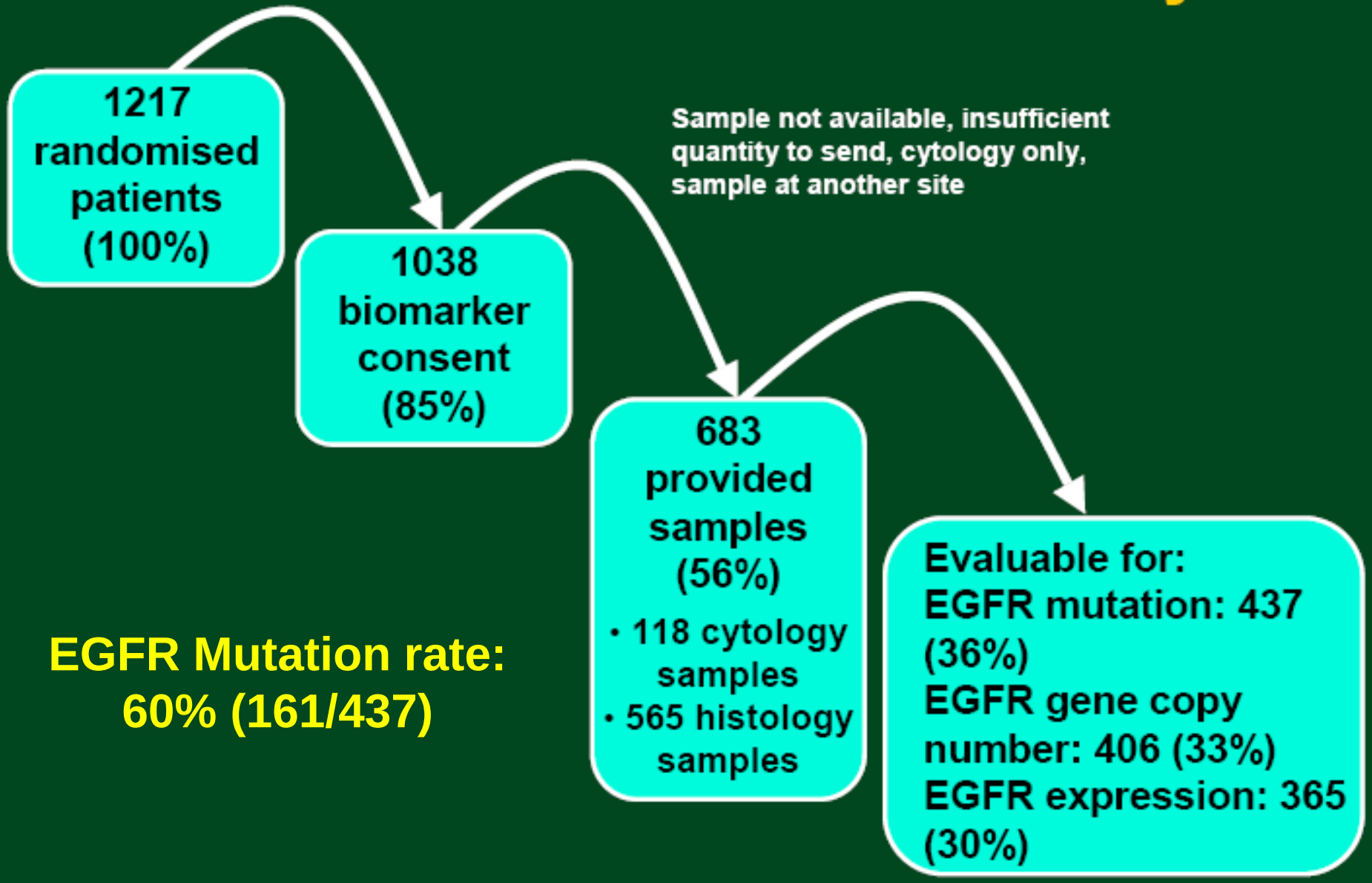
Study design



*Never smokers, <100 cigarettes in lifetime; light ex-smokers, stopped ≥15 years ago and smoked ≤10 pack years; #limited to a maximum of 6 cycles
Carboplatin / paclitaxel was offered to gefitinib patients upon progression
PS, performance status; EGFR, epidermal growth factor receptor

Mok et al, Chicago 2008

Attrition rates in biomarker analysis



EGFR Mutation rate: 60% (161/437)

Mok et al, Chicago 2008

Gefitinib or Carboplatin–Paclitaxel in Pulmonary Adenocarcinoma

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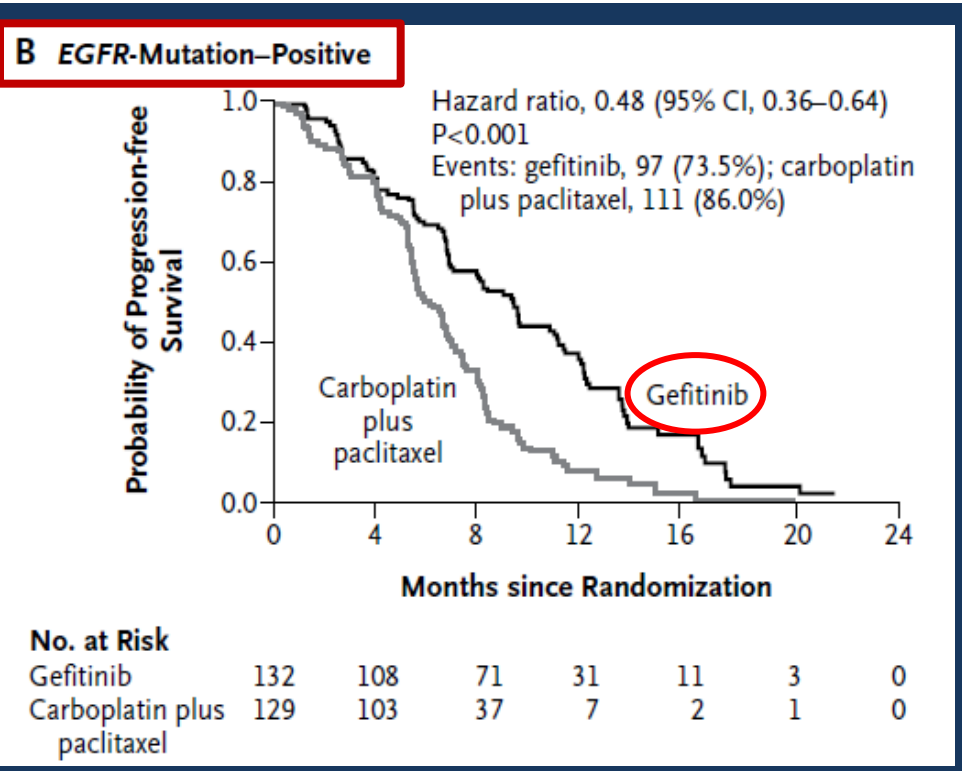

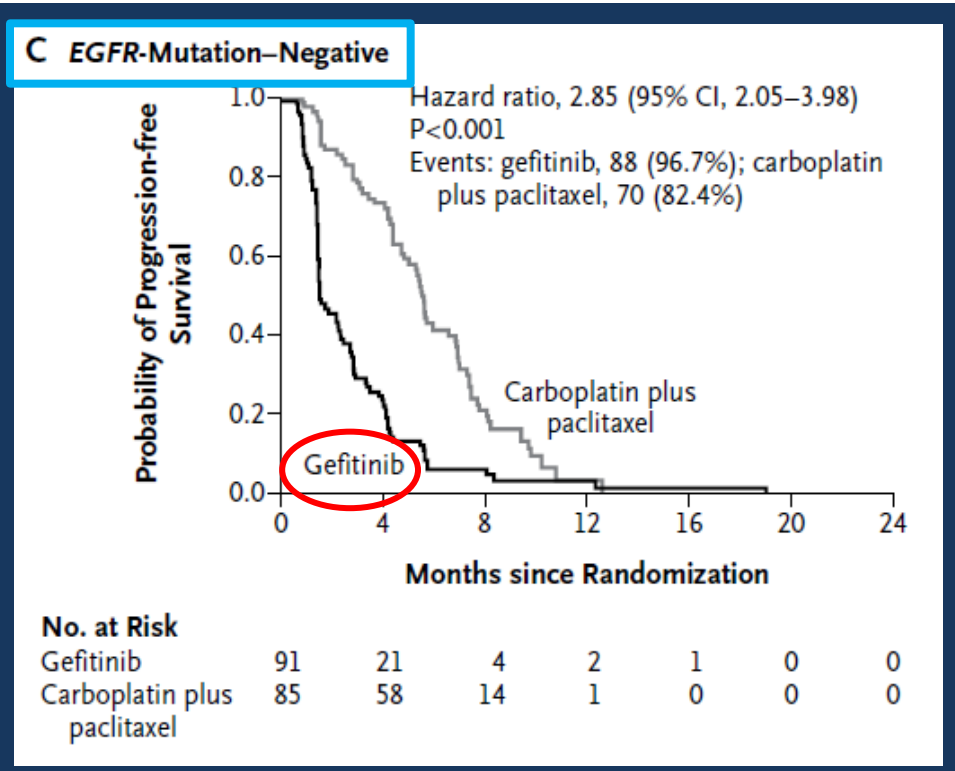

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Conclusions

- **Clinical trials of targeted drugs are "risky" without inclusion of biomarker correlative studies**
- **Role of pathologists/biomarker scientists in Clinical Trial Protocol Design:**
  - **Proper selection of best candidate markers**
  - **Protocol for appropriate sample acquisition**
  - **Proper selection of "best" assays**
  - **Assist Statistician in the interpretation of data in the right biological context**