Molecular Diagnosis in Lung Cancer Treatment

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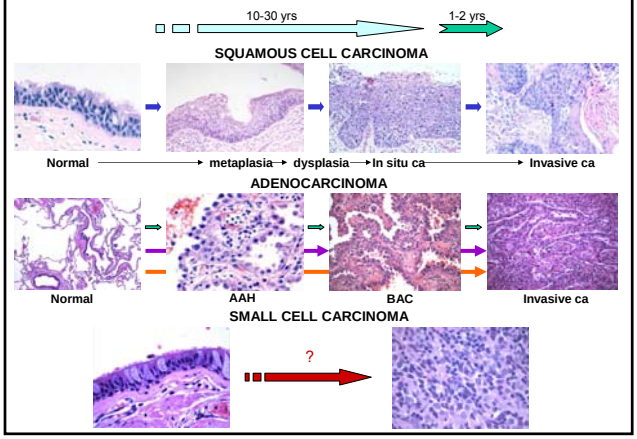
Lung cancer development is a multi-stage process



6 essential alterations for transformation of

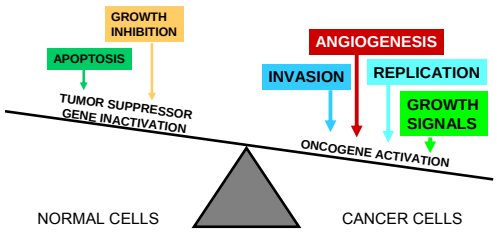
Normal cell

↓

Cancer cell

Hanahan D, Weinberg RA. Cell, 2000:100:57-71.

Imbalance in cellular homeostasis



Potential impacts of molecular aberrations

Clinical outcome:

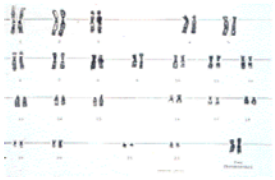
- Promote metastasis and poor prognosis
- Affect response to treatment

Opportunities:

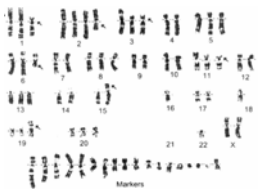
- Develop better disease/therapeutic markers
- Serve as therapeutic targets

Carcinoma cells invariably demonstrate complex chromosomal abnormalities

Normal Karyotype (2n=46)



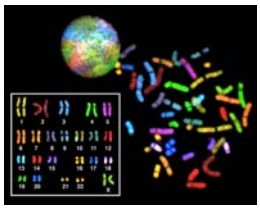
NSCLC cell line (2n=70)

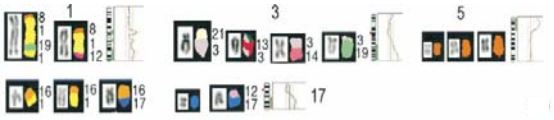


ABNORMALITIES OBSERVED:
- **aneuploidy or unbalanced gain or loss of chromosomes**
- **marker chromosomes that cannot be classified by usual G-banding technique**

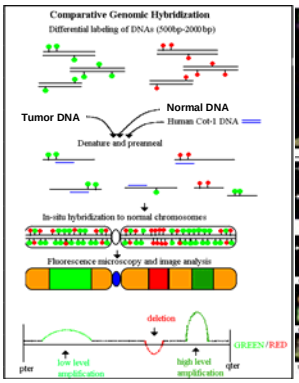
Abnormal (marker) chromosomes invariably represent complex translocations

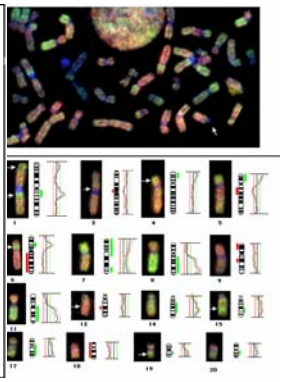
SKY: Spectral Karyotyping (Chromosomal painting)



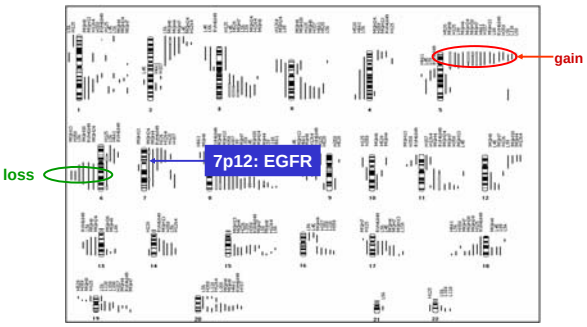


Comparative Genomic Hybridization (CGH)





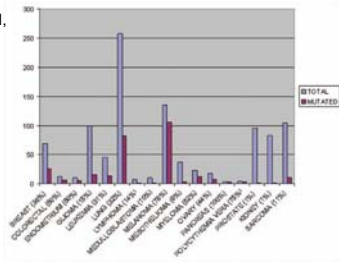
CGH studies have revealed chromosomal regions that are commonly involved in genetic gains and losses



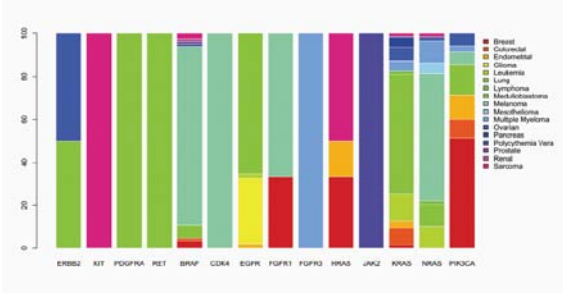
High-throughput oncogene mutation profiling in human cancer

(Thomas RK, et al. Nature Genetics 2007;39: 347-351)

- 1000 human cancers were studied, including 255 lung cancers
- Tumors were profiled for 238 known mutations in 17 oncogenes
- 30% of samples carry at least one mutation
- In lung cancer, 32% had at least one mutation

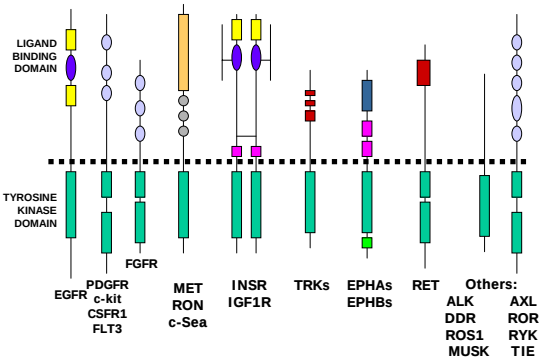


Tumor type distribution of oncogene mutations



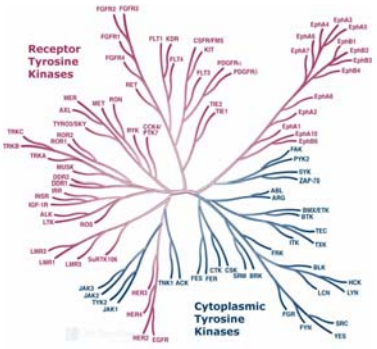
Thomas RK, *et al.* Nature Genetics 2007;39: 347-351

EGFR is a member of tyrosine kinase receptor family that transmit extracellular growth signals

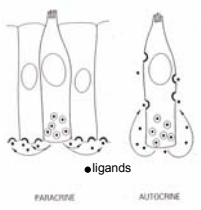


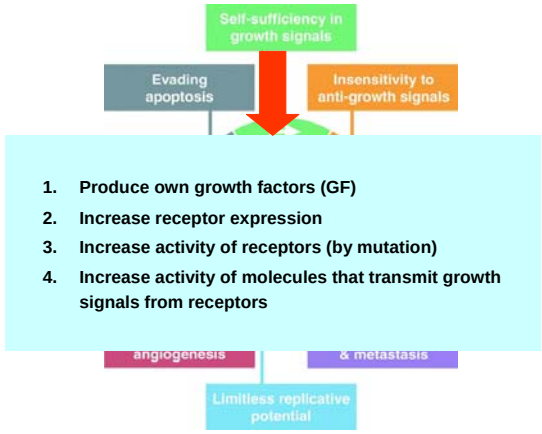
Tyrosine Kinase family tree

Manning G, *et al*. Science 2002;298:1912-1934

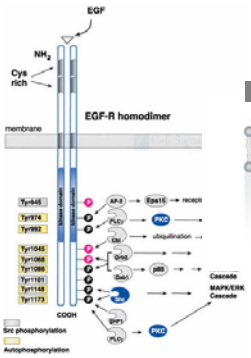


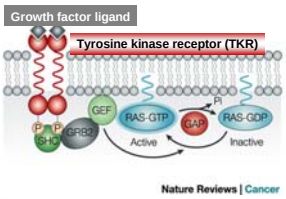
Tyrosine kinase receptor signaling loops





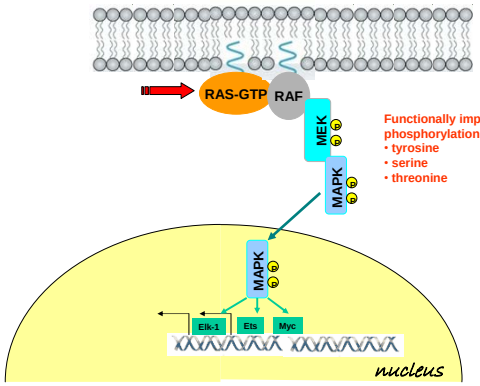
RTK activation pathways





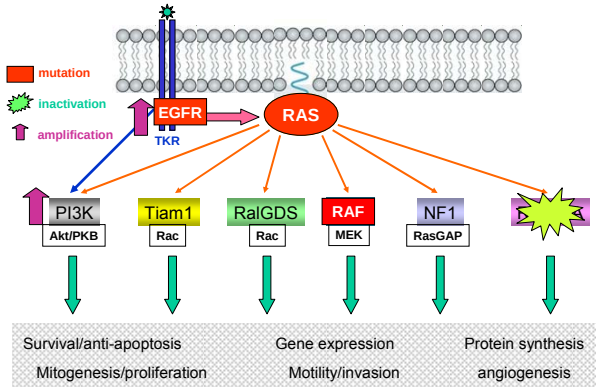
Nature Reviews | Cancer

Ras-Raf-MAPK signaling



Functionally important protein phosphorylation sites include:
- tyrosine
- serine
- threonine

Ras downstream pathways



Aberrant RTK signaling pathways in NSCLC

- **Amplification of RTK:**
  - EGFR (~10%), high polysomy (~30%)
  - PI3K-alpha (60% of SQCC)
- **Activating mutations:**
  - EGFR (10-60%)
  - RAS (20%; mainly ADC)
  - Met/HGF receptor (occasional)
  - Collagen (discoid domain) receptors (DDR 1&2)
- **Overexpression of RTK:**
  - EGFR (60%: 90% SQCC, 50% ADC)
  - Met/HGFR (70%, mainly ADC)

Frequency of high EGFR protein expression in NSCLC

	SQCC	ADC	LCC
Rusch (1997)	94%	57%	63%
Fontanini (1998)	57%	35%	23%
Hsieh (2000)	92%	53%	60%
Hirsch (2003)	82%	46%	33%

SQCC: squamous cell ca, ADC: adenoca; LCC: large cell ca

EGFR tyrosine kinase domain mutations

EGFR Mutations in Lung Cancer: Correlation with Clinical Response to Gefitinib Therapy.

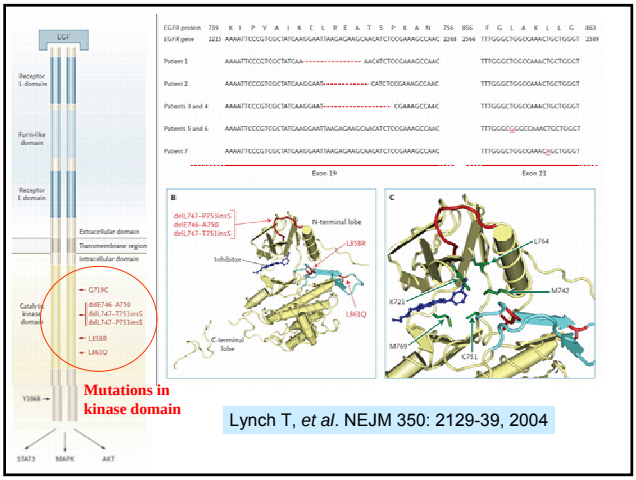
Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, Herman P, Kaye FJ, Lindeman N, Boggon TJ, Naoki K, Sasaki H, Fujii Y, Eck MJ, Sellers WR, Johnson BE, Meyerson M.

SCIENCE April 29, 2004

Activating Mutations in the Epidermal Growth Factor Receptor Underlying Responsiveness of Non –Small-Cell Lung Cancer to Gefitinib

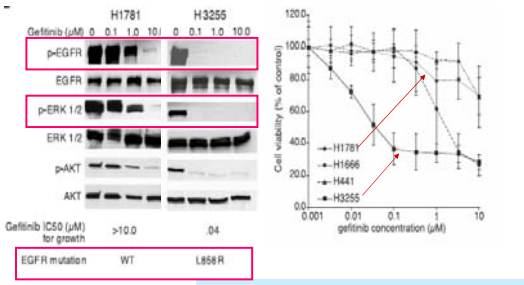
Thomas J.Lynch,M.D.,Daphne W.Bell,Ph.D.,Raffaella Sordella,Ph.D.,Sarada Gurubhagavatula,M.D., Ross A.Okimoto,B.S.,Brian W.Brannigan,B.A.,Patricia L.Harris,M.S.,Sara M.Haserlat, B.A., Jeffrey G.Supko,Ph.D.,Frank G.Haluska,M.D.,Ph.D.,David N.Louis,M.D.,David C.Christiani,M.D., Jeff Settleman,Ph.D.,and Daniel A.Haber,M.D.,Ph.D.

NEW ENGLAND JOURNAL OF MEDICINE, MAY 20, 2004



Lynch T, *et al*. NEJM 350: 2129-39, 2004

Mutant EGFR are more sensitive to inhibition of kinase activity by EGFR inhibitors



Paez *et al*: Science 2005;304:1497-1500

"Oncogene addiction" theory

(Weinstein IB. Science 2002;297:63-4)

- Dependency of tumor cells on single and predominant oncogenic activity to sustain their proliferation and/or survival
- Oncogene addiction can be the achilles heal of cancer.

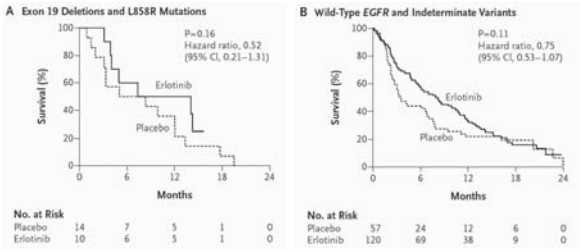
EGFR tyrosine kinase domain mutations

- **More common in:**
  - Adenocarcinoma
  - Women
  - East Asian NSCLC patients
  - Never smokers
- **Tumors with mutations** demonstrate significantly greater response rate to EGFR inhibitor drugs gefitinib (Iressa) or erlotinib (Tarceva)

EGFR mutation predicts higher response rate to EGFR TKI treatment

Study	No. Pts	Pts selection	ethnicity	Agent	RR of WT pts	RR of Mut pts	Resp Criteria
Tokumo	21	recurrent disease	japanese	gefitinib	2/12 (17%)	8/9 (89%)	ECOG
Takano	66	recurrent disease	japanese	gefitinib	3/27 (11%)	32/39 (82%)	SWOG imaging
Kim	27	sample availability	korean	gefitinib	2/21 (10%)	6/6 (100%)	RECIST
Han	90	consecutive	korean	gefitinib	10/73 (14%)	11/17 (65%)	WHO
Chou	54	sample availability	chinese	gefitinib	4/21 (19%)	17/33 (52%)	ECOG
Mean					21/154 (14%)	74/104 (71%)	
Cortes-Funes	83	sample availability	spanish	gefitinib	6/73 (9%)	6/10 (60%)	RECIST
Cappuzzo	89	sample availability	italian	gefitinib	4/74 (5%)	8/17 (53%)	ECOG
Ebehard	274	TRIBUTE	US	erlotinib+chemo	18/99 (18%)	8/15 (53%)	RECIST
Mean					28/246 (11%)	22/42 (52%)	

How about survival benefit from erlotinib/gefitinib?



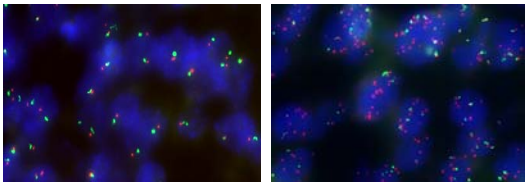
BR.21 study: EGFR TK domain mutation is not a good predictor of differential survival benefit from erlotinib compared to placebo

Tsao MS *et al.* NEJM 2006;354:527-28

High increases in EGFR gene copy number also predicts response to EGFR TKI drugs

(Cappuzzo *et al.* J Natl Cancer Inst 2005;97: 643-55)

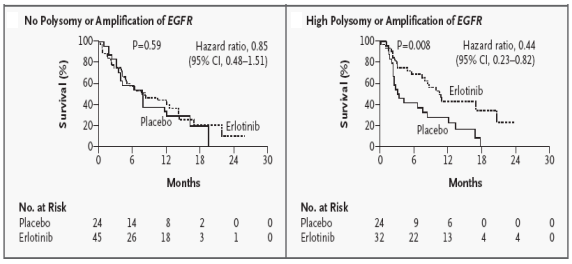
Gene copy number assessed by Fluorescent In Situ Hybridization (FISH)



Low copy number:
≤4 gene copies in <40% cells

High copy number:
≥4 gene copies in ≥40% cells *or*
Gene/chromosome ratio >2

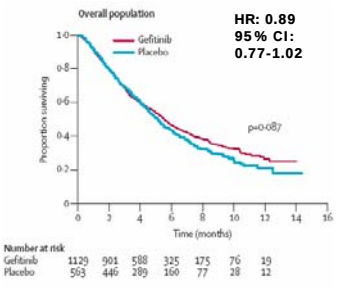
BR.21 study: high EGFR gene copy number is a better predictor of survival benefit from erlotinib than mutation



P value for interaction = 0.10

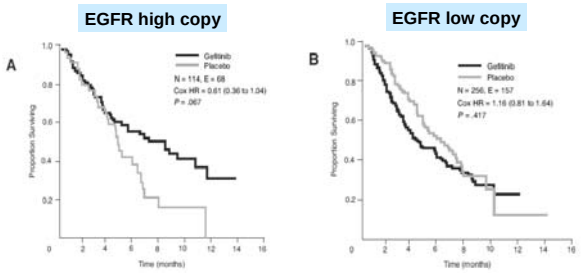
Tsao MS *et al.* NEJM 2005;353:133-44

Iressa Survival Evaluation in Lung Cancer (ISEL): Phase 3 placebo controlled trail of gefitinib (Iressa)



Thatcher N, *et al*. Lancet 2005;366:1527-37.

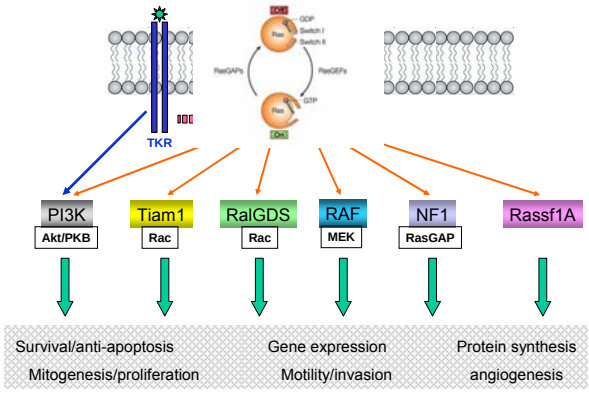
Survival benefit of Iressa appears mainly in EGFR high copy (by FISH) patients



Hirsch F, *et al*. J Clin Oncol 2006;24:5034-41.

Patients with high EGFR gene copy have poorest prognosis, yet they are most likely to benefit from drug treatment

Ras downstream pathways



K-ras mutations could be a negative selection marker for erlotinib therapy

TRIBUTE TRIAL

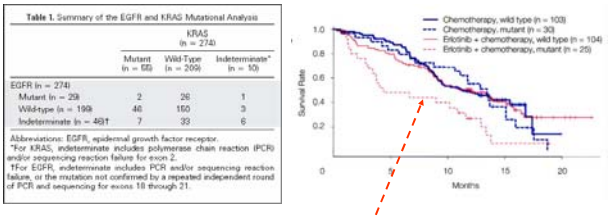


Table 1. Summary of the EGFR and KRAS Mutational Analysis

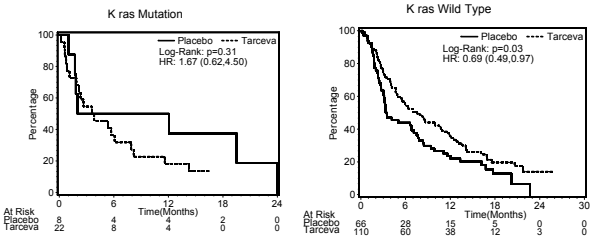
	KRAS (n = 274)		
	Mutant (n = 55)	Wild-Type (n = 209)	Indeterminate* (n = 10)
EGFR (n = 274)			
Mutant (n = 29)	2	26	1
Wild-type (n = 199)	46	150	3
Indeterminate (n = 46)†	7	33	6

Abbreviations: EGFR, epidermal growth factor receptor.
*For KRAS, indeterminate includes polymerase chain reaction (PCR) and/or sequencing reaction failure for exon 2.
†For EGFR, indeterminate includes PCR and/or sequencing reaction failure, or the mutation not confirmed by a repeated independent round of PCR and sequencing for exons 18 through 21.

K-ras mutations: 55/ 264 (21%)

D. Eberhard *et al*, J Clin Oncol 2005;23:1-14

BR.21: KRAS mutant patients show no benefit from erlotinib, but the number of patients is small

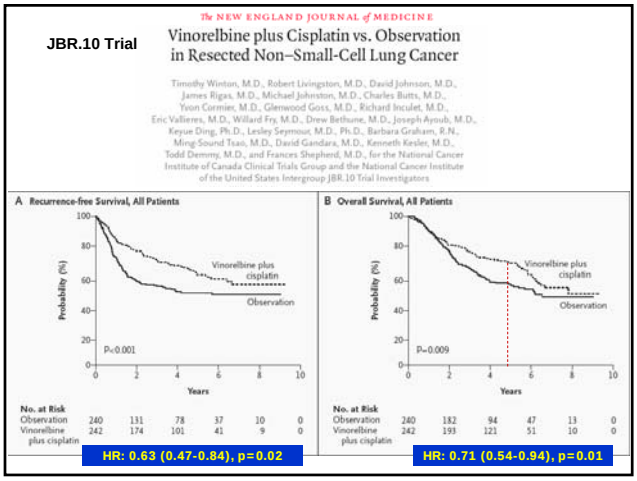


Tsao *et al*, ASCO 2006: Abstract #7005

Early stage NSCLC

Is there a role for molecular markers?

JBR.10 Trial

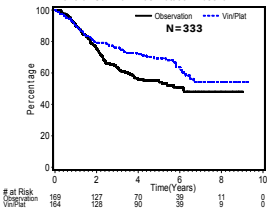


HR: 0.63 (0.47-0.84), p=0.02

HR: 0.71 (0.54-0.94), p=0.01

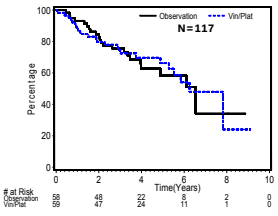
JBR.10: Patients with *ras* mutation are not likely to benefit from adjuvant chemotherapy

***Ras* Wild Type**



HR=0.69 (0.49-0.97), p=0.03

***Ras* Mutant**



HR=0.95 (0.53-1.71), p=0.87

Interaction P value = 0.25

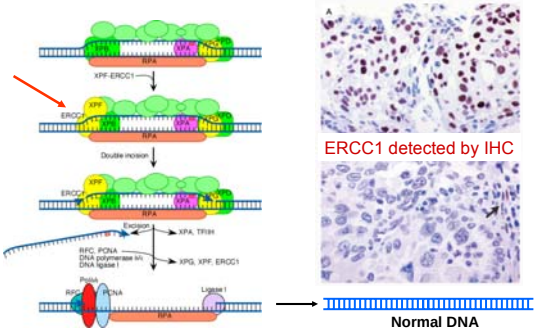
DNA Repair by ERCC1 in Non–Small-Cell Lung Cancer and Cisplatin-Based Adjuvant Chemotherapy

Ken A. Olaussen, Ph.D., Ariane Dunant, M.S., Pierre Fouret, M.D., Ph.D., Elisabeth Brambilla, M.D., Ph.D., Fabrice André, M.D., Ph.D., Vincent Haddad, M.S., Estelle Taranchon, M.S., Martin Filipits, Ph.D., Robert Pirker, M.D., Helmut H. Popper, M.D., Rolf Stahel, M.D., Ph.D., Laure Sabatier, Ph.D., Jean-Pierre Pignon, M.D., Ph.D., Thomas Tursz, M.D., Ph.D., Thierry Le Chevalier, M.D., and Jean-Charles Soria, M.D., Ph.D., for the IALT Bio Investigators*

International Adjuvant Lung Trial (IALT)

- 1867 patients with completely resected NSCLC I-II-IIIA
- HR= 0.86 [0.76-0.98], p<0.03
- Absolute benefit : 4.1% mprovement of 5 year OS
- Tumor from 761 pts were available for marker analyses

Cisplatin-DNA Adduct and Nucleotide Excision Repair (NER)



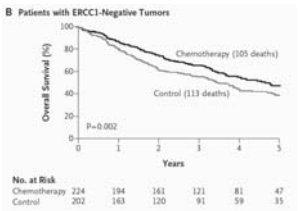
ERCC1 detected by IHC

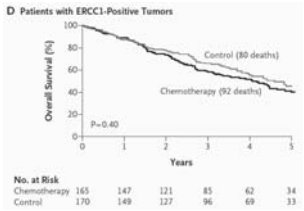
Normal DNA

Hypothesis: ERCC- patients are not able to repair damages from cisplatin

IALT study:

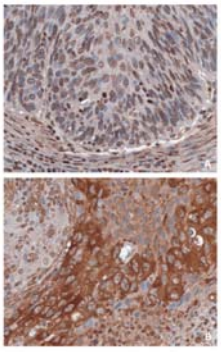
1. **Lack of ERCC1 expression is a poor prognostic marker**
2. **Adjuvant chemotherapy mainly benefits ERCC1 negative patients**

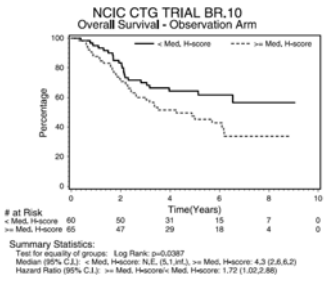




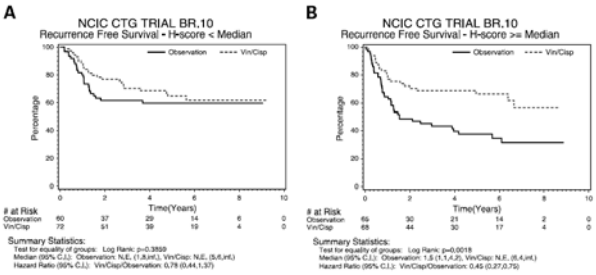
Olaussen KA, *et al*. NEJM 2006;355:983-91

JBR.10: Patients with high class III β-tubulin expression have poorer survival (prognosis)





JBR.10: Survival benefit from adjuvant cis/vin appears greater in patients with high class III β-tubulin



HR: 0.78; p=0.39 **HR: 0.45; p=0.002**

Question: Are high tubulin expressing tumors addicted to their tubulin level?

Biomarkers for adjuvant therapy in NSCLC patients

- Poor prognostic markers: to select patients with high risk for death from recurrence and possibly benefit from adjuvant therapy.
- Predictive markers: to select patients who are most likely to benefit from a specific adjuvant therapy

What about microarrays?

- Good evidence that gene expression profiles by microarray can distinguish:
  - tumors of different histological types
  - patients with different prognosis
- But signatures for histological typing do not overlap with those for prognosis
- Prognosis signatures are more reflective of molecular pathways important for the biology of lung cancers

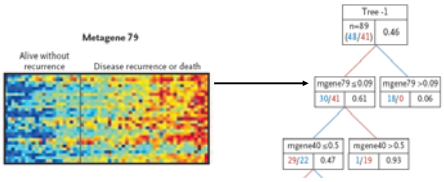
The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

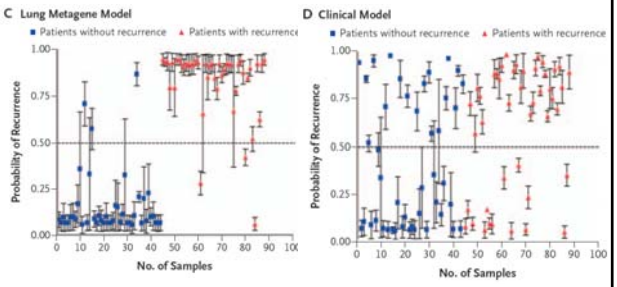
N Engl J Med 2006;355:570-80.

A Genomic Strategy to Refine Prognosis in Early-Stage Non–Small-Cell Lung Cancer

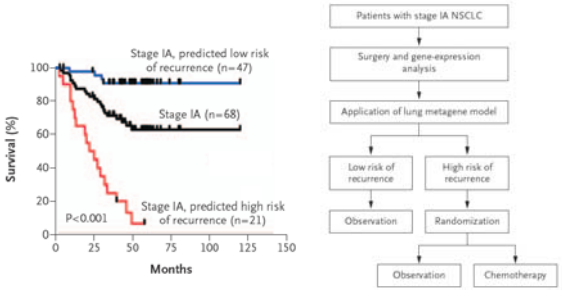
Anil Potti, M.D., Sayan Mukherjee, Ph.D., Rebecca Petersen, M.D., Holly K. Dressman, Ph.D., Andrea Bild, Ph.D., Jason Koontz, M.D., Robert Kratzke, M.D., Mark A. Watson, M.D., Ph.D., Michael Kelley, M.D., Geoffrey S. Ginsburg, M.D., Ph.D., Mike West, Ph.D., David H. Harpole, Jr., M.D., and Joseph R. Nevins, Ph.D.



Metagenes classify patients for their risk of recurrence better than clinical model



Planned clinical CALGB Trial



Outstanding questions:

- **Is metagene method sufficiently validated?**
- **Is microarray approach most cost-effective?**

The NEW ENGLAND JOURNAL of MEDICINE

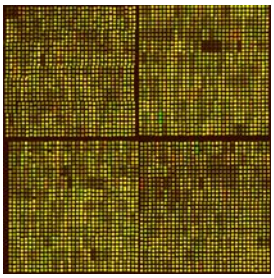
ESTABLISHED IN 1812 JANUARY 4, 2007 VOL. 356 NO. 1

A Five-Gene Signature and Clinical Outcome in Non–Small-Cell Lung Cancer

Hsuan-Yu Chen, M.Sc., Sung-Liang Yu, Ph.D., Chun-Houh Chen, Ph.D., Gee-Chen Chang, M.D., Ph.D., Chih-Yi Chen, M.D., Ang Yuan, M.D., Ph.D., Chiou-Ling Cheng, M.Sc., Chien-Hsun Wang, M.Sc., Harn-Jing Terng, Ph.D., Shu-Fang Kao, M.Sc., Wing-Kai Chan, M.D., Han-Ni Li, M.Sc., Chun-Chi Liu, M.Sc., Sher Singh, Ph.D., Wei J. Chen, M.D., Sc.D., Jeremy J.W. Chen, Ph.D., and Pan-Chyr Yang, M.D., Ph.D.

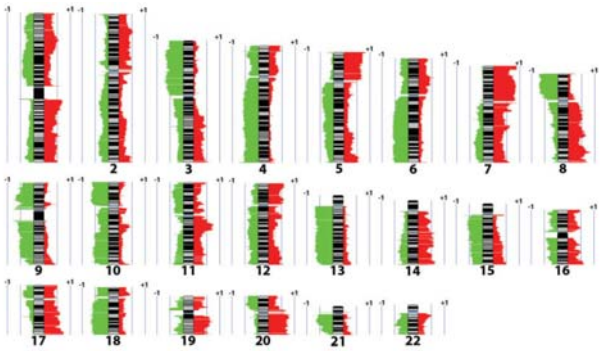
Fine mapping of chromosomal abnormalities by Array-CGH

- **Chromosomes are cut into fragments that are ~100 kilobases long**
- **Fragments are arrayed on glass slide**
- **Differentially labeled DNA from tumor and normal are co-hybridized to the microarrays and signals for each type of sample are detected and compared**

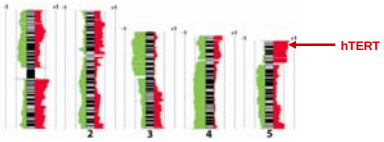


Chromosomal abnormalities in NSCLC cell lines detected by array-CGH

(Garnis C *et al*. Int J Cancer 2006;118: 1156-64)



Chromosome 5p: telomerase (hTERT)

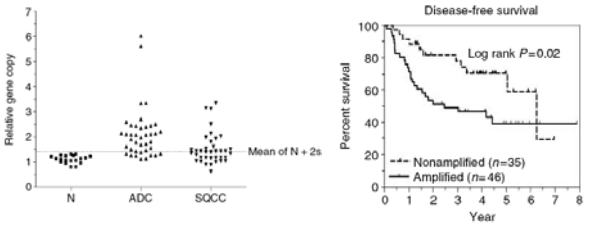


- Role of telomerase complex is to maintain the length of telomeres
- Progressive shortening with continuous replication can trigger apoptosis or growth arrest
- Activation of telomerase is an obligate step during carcinogenesis

Amplification of telomerase (*hTERT*) gene is a poor prognostic marker in non-small-cell lung cancer

C-Q Zhu[1], J-C Cutz[2], N Liu[1], D Lau[1], FA Shepherd[3,4], JA Squire[1,2,5,6] and M-S Tsao[0,1,2,5,6]

British Journal of Cancer (2006) 94, 1452–1459



CONCLUSIONS

- Currently there are several very promising predictive markers for selection of NSLC patients to receive targeted or adjuvant chemotherapy
- These markers still require additional validation before they can be implemented as routine clinical tests
- Best way to validate these markers are in patients involved in large phase 3 clinical trials
- The use of molecular markers for stratifying cancer patients to therapeutic options will likely come to reality during the next decade