Correlative Studies in Clinical Trials

Workshop #3

Ming Tsao and Lois Shepherd
August 2011

Correlative Studies in Clinical Trials can be:

- Integral – essential for randomization either as defining the allocation or population, or as a stratification factor
  - eg BR.10 – ras as a stratification factor
    MAC.12 – OncotypeDx
    MA.31 – HER2 +

- Integrated – defined in the protocol and mandatory – usually to better understand the treatment or toxicity profile eg. MA.32 – glucose and insulin

- Retrospective – studies which are usually done after the final analysis of a clinical trial which make use of the treatment regimens, outcomes, toxicity, quality of life

Translational Research Initiatives

- In 1997 a Correlative Science/ Tumour Bank Committee was convened to bring together pathologists, basic and clinical scientists, statisticians, epidemiologists to explore the concept of tumour banking for future research purposes

- A decision was taken to prospectively consider the inclusion of banking diagnostic FFPE tissue on all new Phase III trials and to attempt to collect retrospectively, material on older trials, to facilitate discovery and validation studies

- Over time, this has expanded to include collection as appropriate on Phase I and II studies as well as other biospecimens

-

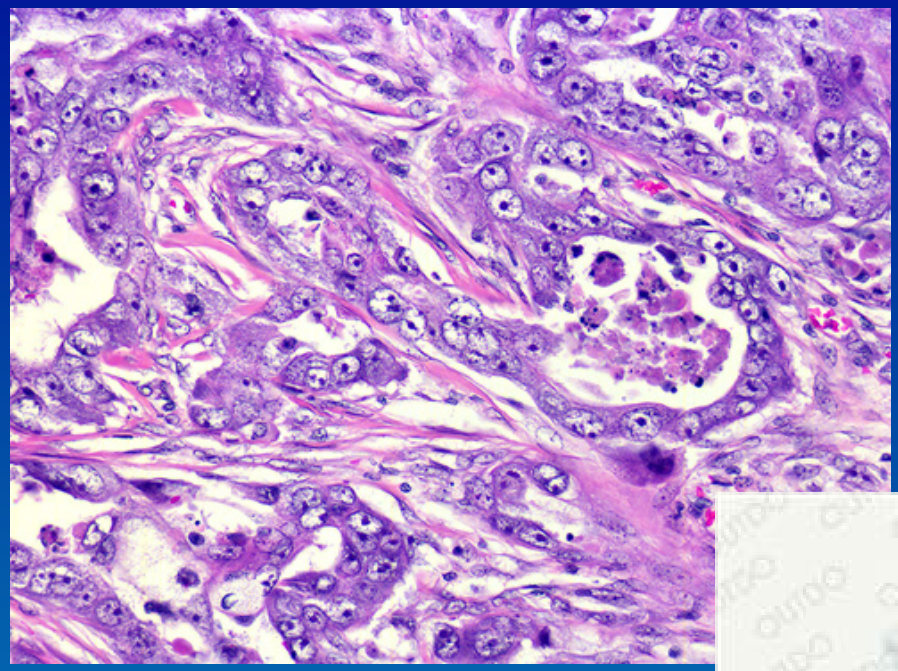
As a result the NCIC CTG has created…

- A national resource of clinical trial associated FFPE diagnostic material from many disease sites – breast, lung, colon, pancreas, ovary, prostate, endometrium, CNS

- A frozen tissue bank of NSCLC

- Virtual frozen breast bank

- Serum, plasma , urine, DNA, bone marrow on a growing number of studies



Growing number of Derivatives:

-TMAs

-DNA

-RNA

Protocols are essential:

- standardized protocols for collection, processing, shipping, storage

- laboratory manuals, labels, shipping specifications, safety issues all must be specified - “preanalytical”

- SOPs essential

Biobanks come in lots of shapes and forms ,,,

- REB approved
- "Accreditation" – CTRNet, CAP, OLA
- Adherence to ISBER Guidelines
- SOPs

Research /investigative studies for integral/integrated markers should be done in a GLP environment

- CLIA certified, CAP, OLA
- ? Use of diagnostic, validated assays
- retrospective studies in research laboratories may not meet this standard

Recent Publication or Guidelines

- REMARK
- R Simon. Using genomics in clinical trial design, Clinical Cancer Research 14:5984-93, 2008
- R Simon. Designs and adaptive analysis plans for pivotal clinical trials of therapeutics and companion diagnostics, Expert Opinion in Medical Diagnostics 2:721-29, 2008
- Use of Archived Specimens in Evaluation of Prognostic and Predictive Biomarkers. R. Simon, S. Paik, D. Hayes: JNCI,101,21 p1446

Some of our Successes ...

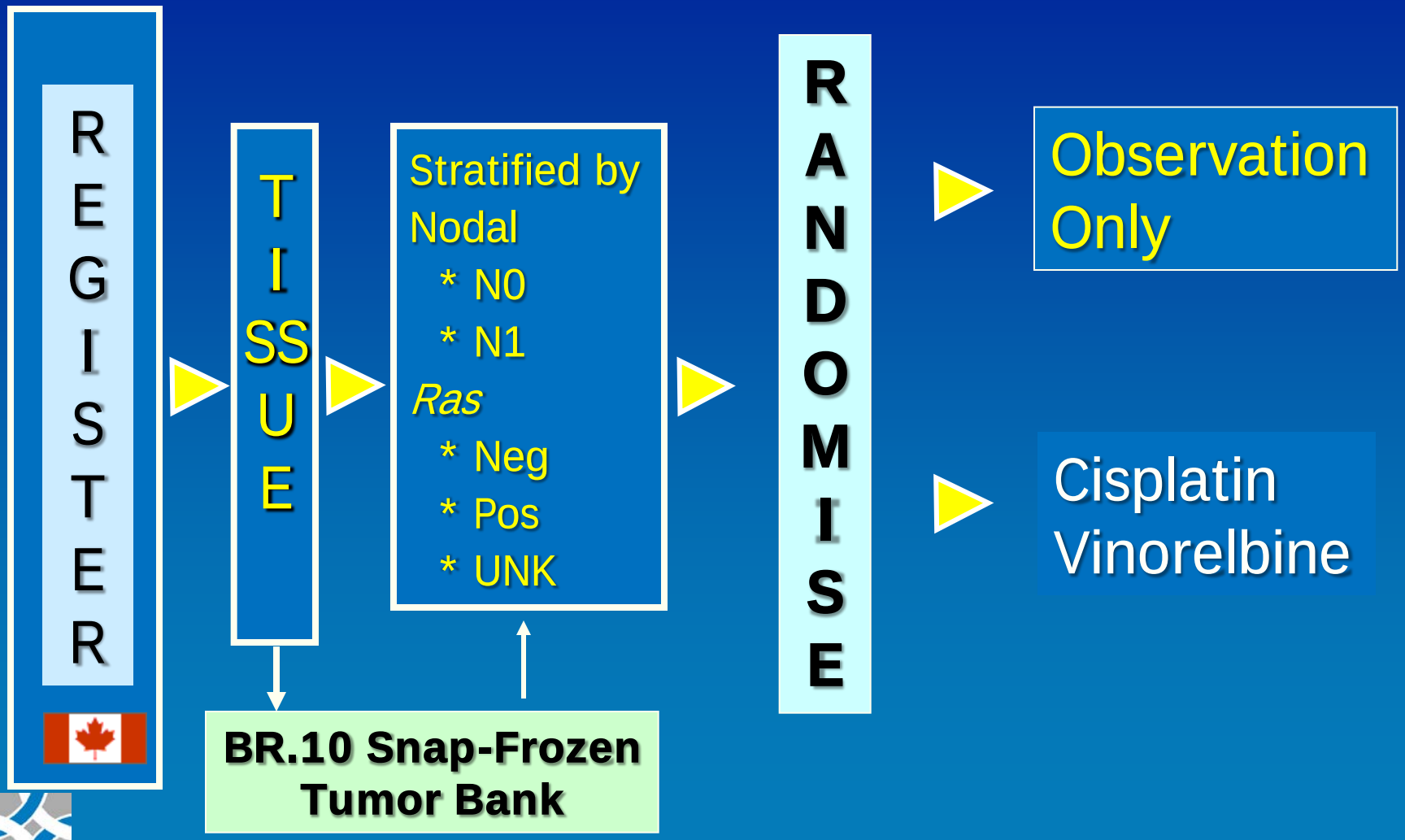
JBR.10 Adjuvant chemotherapy for resected non small cell lung cancer

Conducted with ECOG, CALGB and SWOG

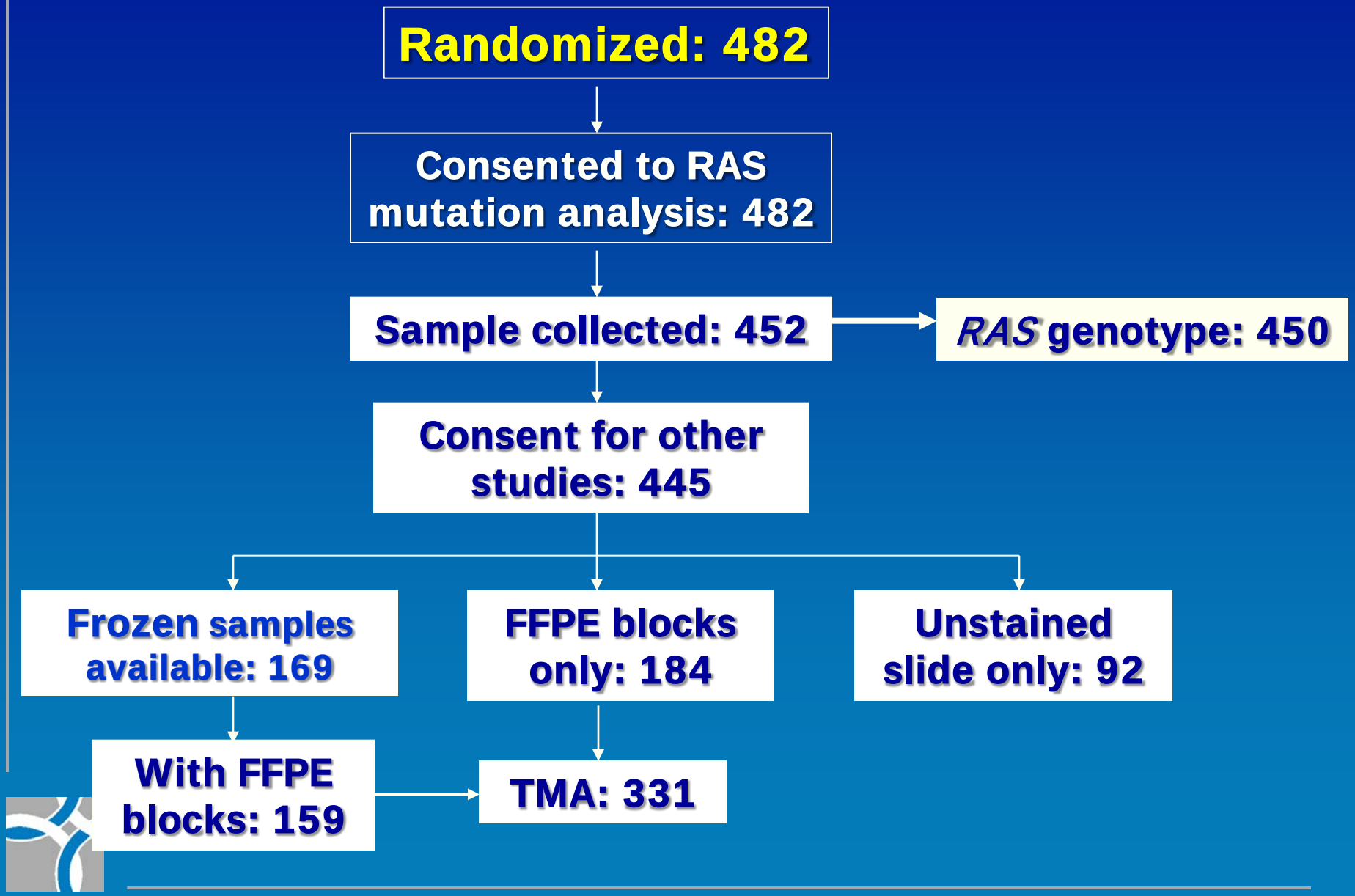
National Cancer Institute of Canada
Institut national du cancer du Canada

Clinical Trials Group
Groupe des essais cliniques

JBR.10 - Study Design



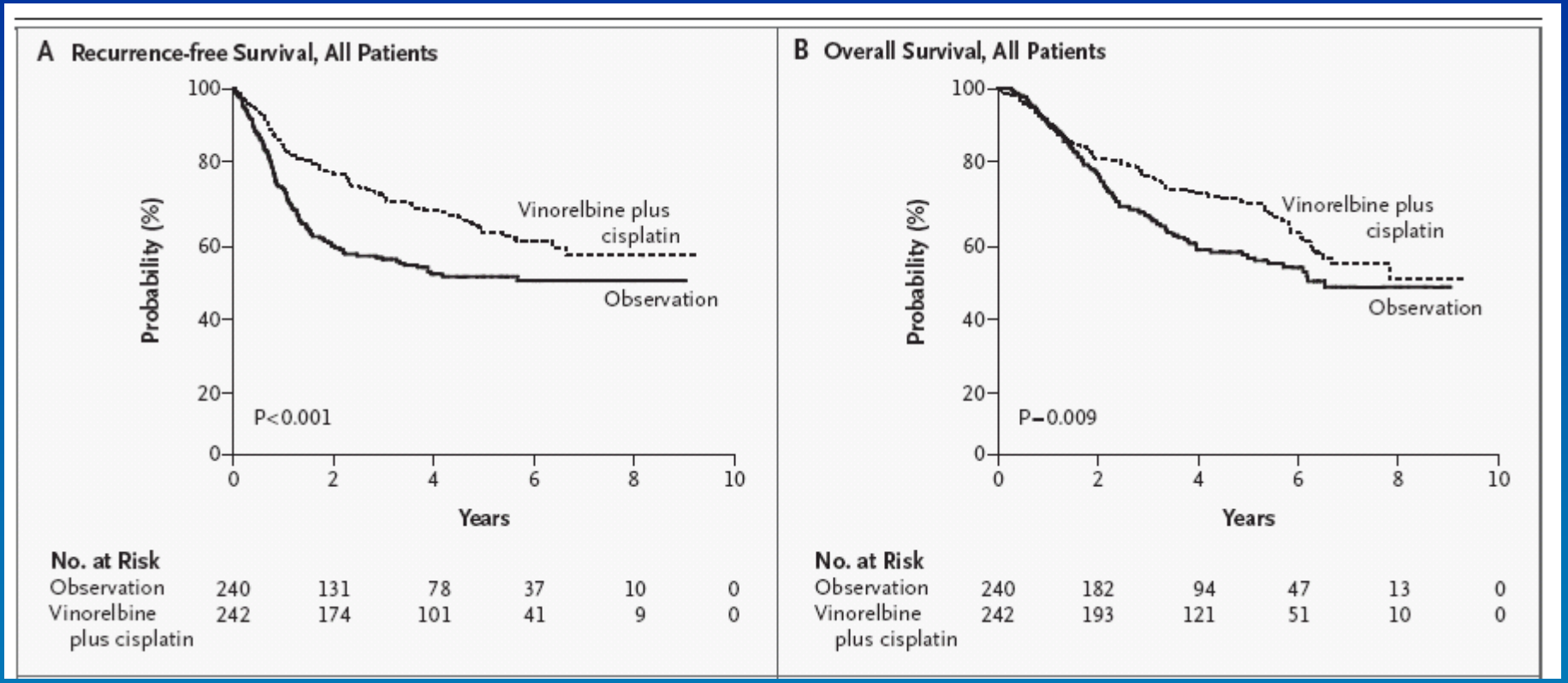
JBR.10 Tumor Samples and Completed Molecular Studies



What were the results?

- Chemotherapy improved the overall 5 year survival by **15% (69% vs 54%)**
- The risk of death was decreased by **31%**
- Toxicity was acceptable and changes in quality of life were relatively modest
- Elderly patients (>65) had a similar benefit as younger patients
- *ras* mutations were an adverse prognostic factor
- adjuvant chemotherapy suggested a survival advantage in wild-type *ras* patients but the test for interaction was not significant

BR.10



NEJM 352;25, 2005

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Vinorelbine plus Cisplatin vs. Observation in Resected Non–Small-Cell Lung Cancer

Timothy Winton, M.D., Robert Livingston, M.D., David Johnson, M.D., James Rigas, M.D., Michael Johnston, M.D., Charles Butts, M.D., Yvon Cormier, M.D., Glenwood Goss, M.D., Richard Inculet, M.D., Eric Vallieres, M.D., Willard Fry, M.D., Drew Bethune, M.D., Joseph Ayoub, M.D., Keyue Ding, Ph.D., Lesley Seymour, M.D., Ph.D., Barbara Graham, R.N., Ming-Sound Tsao, M.D., David Gandara, M.D., Kenneth Kesler, M.D., Todd Demmy, M.D., and Frances Shepherd, M.D., for the National Cancer Institute of Canada Clinical Trials Group and the National Cancer Institute of the United States Intergroup JBR.10 Trial Investigators

ABSTRACT

NEJM 352;25, 2005

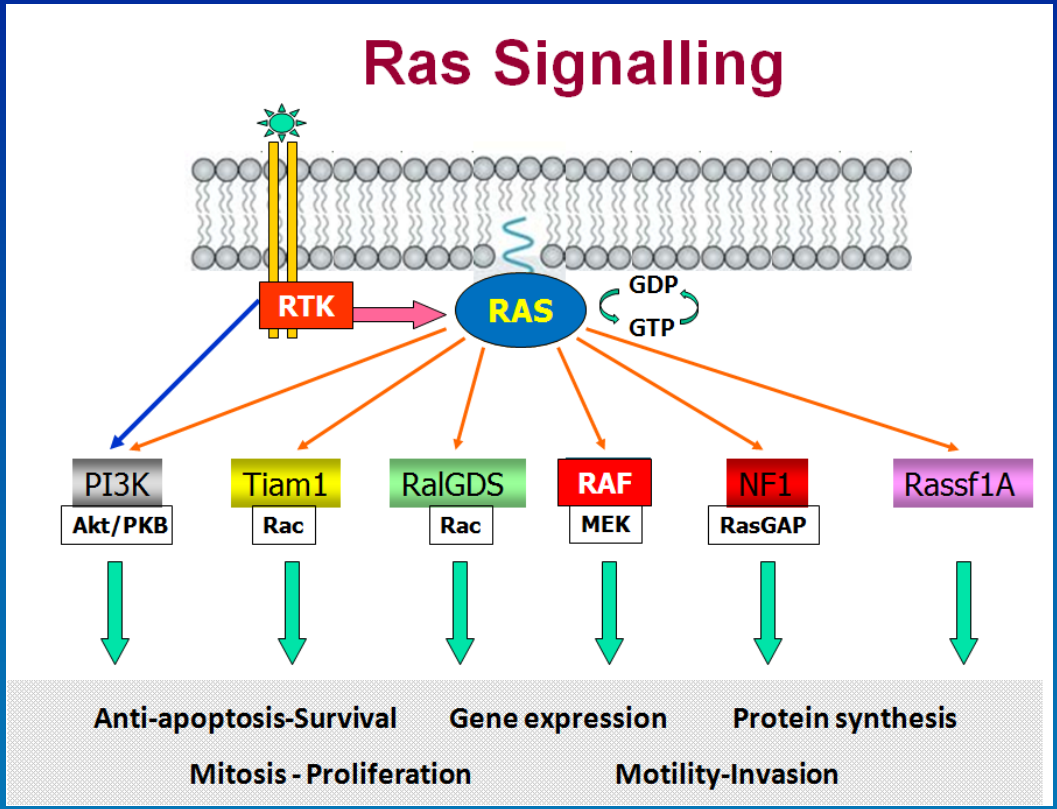
Adjuvant Chemotherapy for NSCLC
"The Smoke Clears"

On the basis of the data reported…., the controversy surrounding adjuvant chemotherapy for resectable NSCLC is over.

Additional research will enable us to select those patients most likely to benefit from adjuvant chemotherapy, to customize the therapy on the basis of the biology of the tumour….

KRAS Mutation and NSCLC

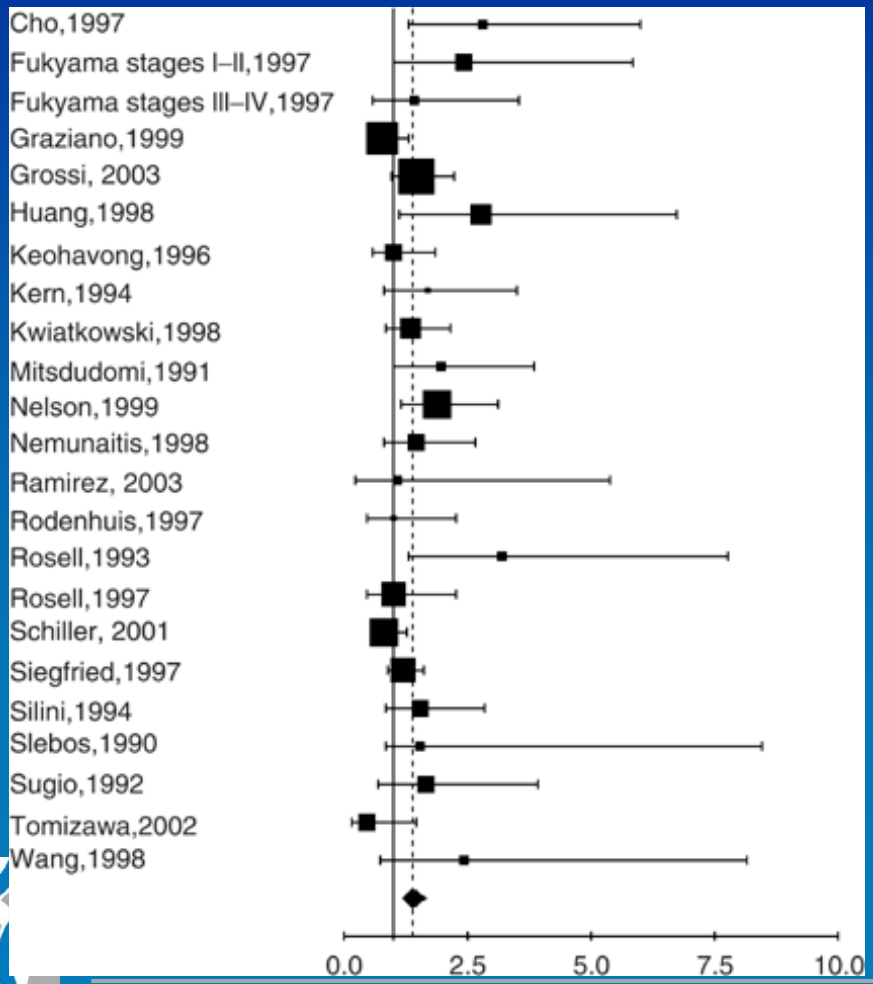
- *RAS* mutations were the first transforming genes (oncogenes) identified in human cancer cells
- *RAS* mutations occur in 15-20% NSCLC, with >90% involving *KRAS*
- In 1990, KRAS mutation was first reported as a prognostic marker in lung adenocarcinoma
  (Slebos RJC, et al. NEJM 1990;323:561-5)



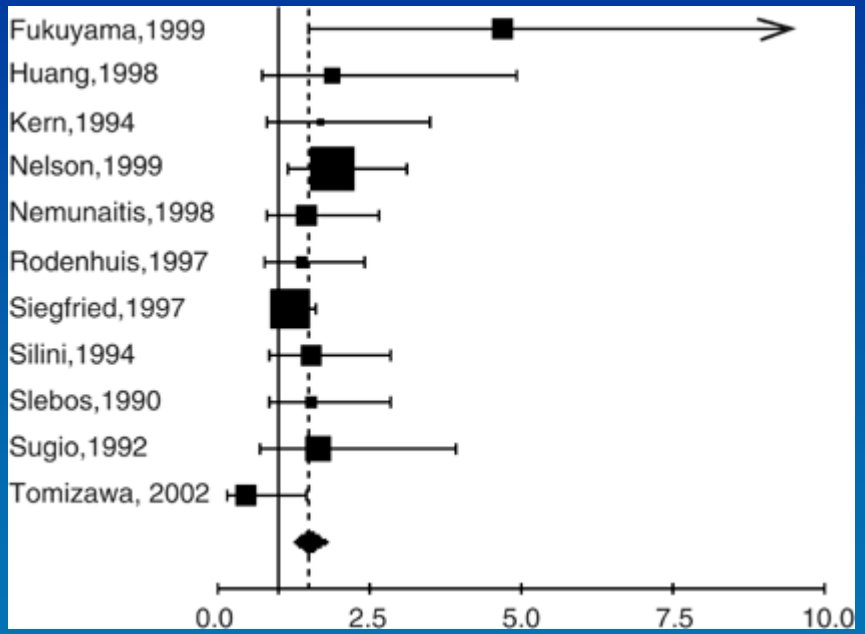
The role of RAS oncogene in survival of patients with lung cancer: a systematic review of the literature with meta-analysis

C. Mascaux, N. Iannino, B. Martin B, et al. Br J Cancer 2005;92:131-9

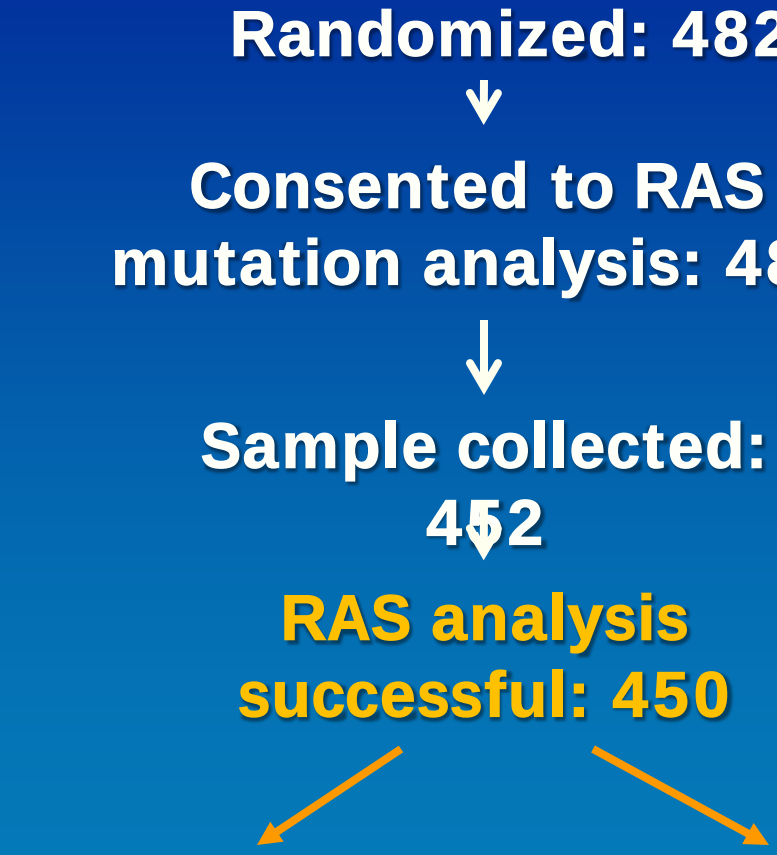
NSCLC (n=2631)
HR:1.40; CI 95% 1.18–1.65



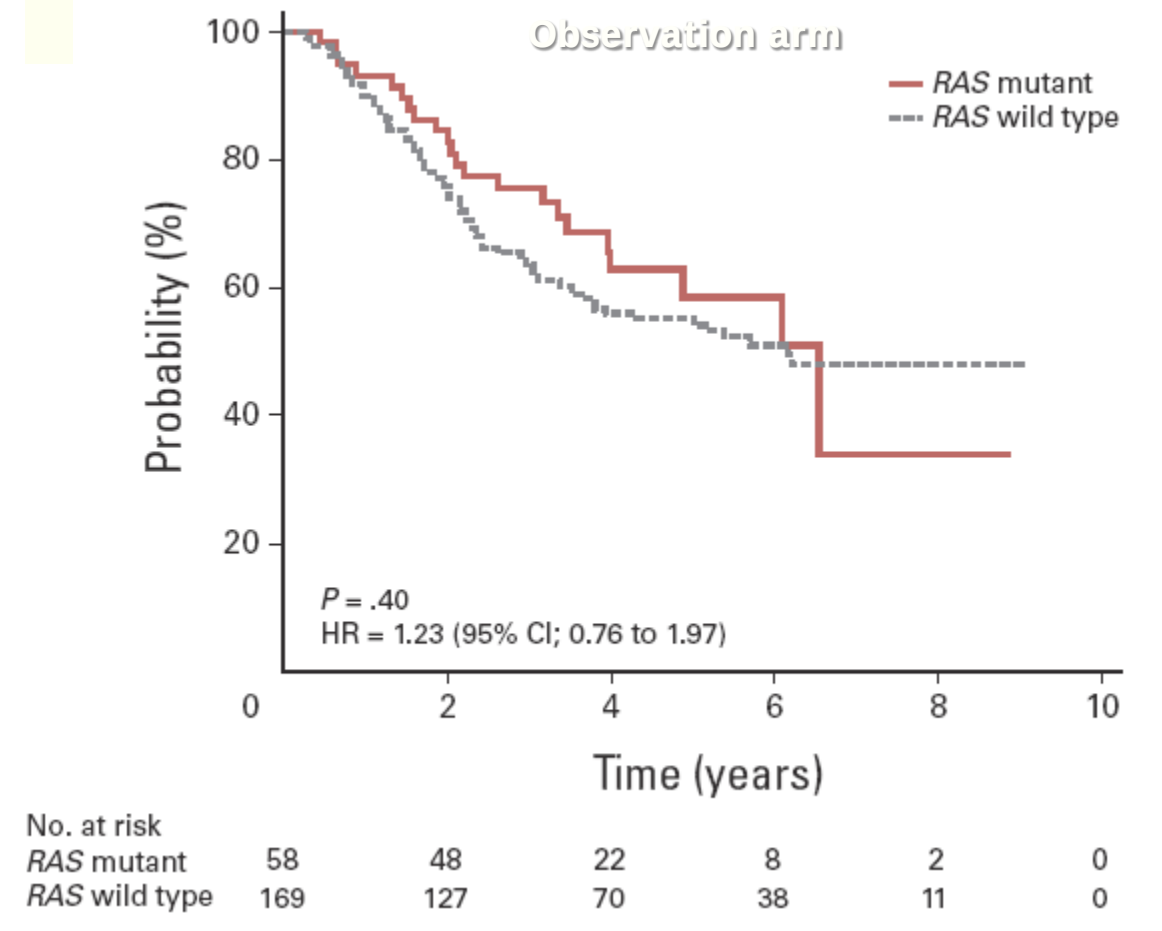
Adenocarcinoma (n=1170)
HR=1.50; CI 95% 1.26–1.80



RAS Mutation Analysis on JBR.10 tumor samples

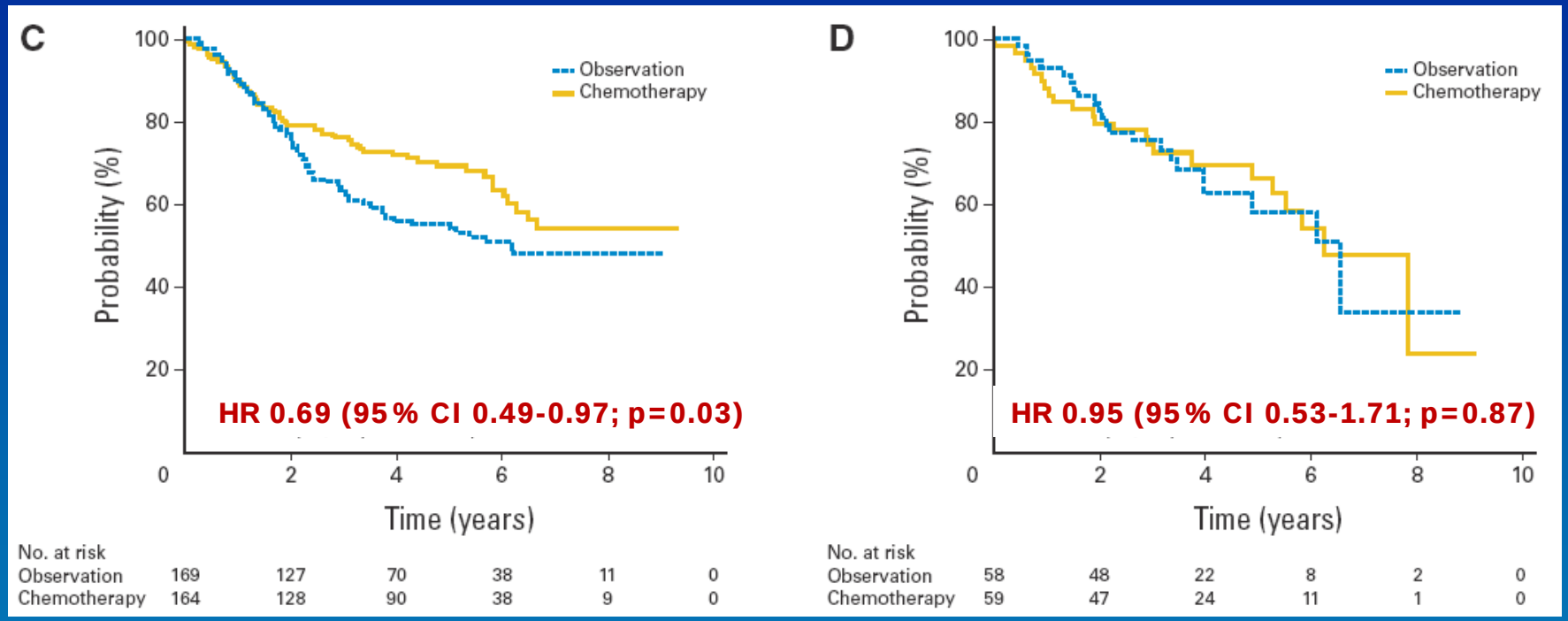


RAS Mutant Patients had Slightly but not Statistically Significant Poorer Survival



Winton T, *et al.* NEJM 2005;352:2589.
Tsao MS, *et al.* J Clin Oncol 2007;25:5240-47.

JBR.10: Mutant *Ras* Patients Had Little Benefit from Adjuvant Chemotherapy



Interaction P value = 0.29

(Insufficient evidence to say that the differences seen between mutant and wild type patients are statistically significant)

Winton T, *et al.* NEJM 2005;352:2589.
Tsao MS, *et al.* J Clin Oncol 2007;25:5240-47.

LACE-BIO Project

- **L**ung **A**djuvant **C**hemotherapy **E**valuation - **Bio**marker Consortium
- JBR.10, IALT, ANITA, CALGB 9633
- Meta- or pooled analysis of promising prognostic/predictive biomarkers
- Total number of patients with samples available for marker studies: 1400-1700

Validation of Prognostic and/or Predictive Markers

	Prognostic	Predictive
b-tubulin	Yes	No
p27, p16, cyclin E	No	No
Mucin	No	No
p53 mutation	No	Yes (Sqcc)
p53 IHC	No	NO
KRAS	No	No
Bax	No	Yes
Intense Lymphocytic infiltrate	Yes	No

ESMO congress
Milan 2010

LACE-Bio Pooled Analysis of the Prognostic and Predictive Value of KRAS Mutation in Completely Resected Non-Small Cell Lung Cancer (NSCLC)

M.S. Tsao, P. Hainaut, A. Bourredjem, P.A.Janne, X. Ma, J.-P. Pignon, J.-Y. Douillard, J.-C. Soria, L. Seymour, F.A. Shepherd
on behalf of the LACE-Bio Collaborative Group

ESMO congress Milan 2010

Pooled Analysis of *KRAS* Mutation in LACE-Bio

Trial	Patients in Trial	Patients with sample for *KRAS* analysis	ADC	SCC	Others	*KRAS* Mutation Rate (%)
ANITA	840	143 (17%)	33 (30%)	56 (51%)	21 (19%)	22/110 (20%)
IALT	1867	783 (42%)	223 (31%)	408 (57%)	87 (12%)	98/718 (14%)
JBR10	482	452 (94%)	210 (47%)	156 (34%)	84 (19%)	113/450 (25%)
CALGB 9633	344	343 (>99%)	136 (53%)	85 (33%)	37 (14%)	70/258 (27%)
LACE-Bio	**3533**	**1721 (48.7%)**	**602 (39%)**	**705 (46%)**	**229 (15%)**	**303/1536 (19.7%)**

ADC: adenocarcinoma; SCC: squamous cell carcinoma

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Pooled Prognostic Value of *KRAS* Mutation

	Deaths/ All patients	Hazard ratio	95% CI	P value
Overall Survival				
Wild type	602/1233	1		0.09
Mutant	149/303	1.18	0.97-1.44	
Disease-Free Survival				
Wild type	679/1233	1		
Mutant	167/303	1.15	0.96-1.39	0.13

Inter-trial heterogeneity: p=0.60 for OS and p=0.30 for DFS

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Predictive Value of Benefit from Adjuvant Chemotherapy (Disease-Free Survival)

	Chemotherapy (Deaths/ Patients in Group)	**Control** (Deaths/ Patients in Group)	**Hazard ratio CT vs. Surgery [95% CI]**
***KRAS* wild-type (n=1233)**	329/621	350/612	0.86 [0.74-0.99] p=0.045
***KRAS* mutated (n=303)**	85/155	82/148	0.92 [0.67 - 1.24] p=0.57

Test for interaction *KRAS* *Treatment p=0.70

Interaction HR (95% CI): 1.07 [0.76 ; 1.51]

Between trial heterogeneity: p=0.41

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CONCLUSION

- ***KRAS*** **mutation cannot be used to select or exclude patients from cisplatin-based adjuvant chemotherapy**
- ***KRAS*** **mutation is only weakly prognostic, and**

VOLUME 28 · NUMBER 29 · OCTOBER 10 2010

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Prognostic and Predictive Gene Signature for Adjuvant Chemotherapy in Resected Non–Small-Cell Lung Cancer

Chang-Qi Zhu, Keyue Ding, Dan Strumpf, Barbara A. Weir, Matthew Meyerson, Nathan Pennell, Roman K. Thomas, Katsuhiko Naoki, Christine Ladd-Acosta, Ni Liu, Melania Pintilie, Sandy Der, Lesley Seymour, Igor Jurisica, Frances A. Shepherd, and Ming-Sound Tsao

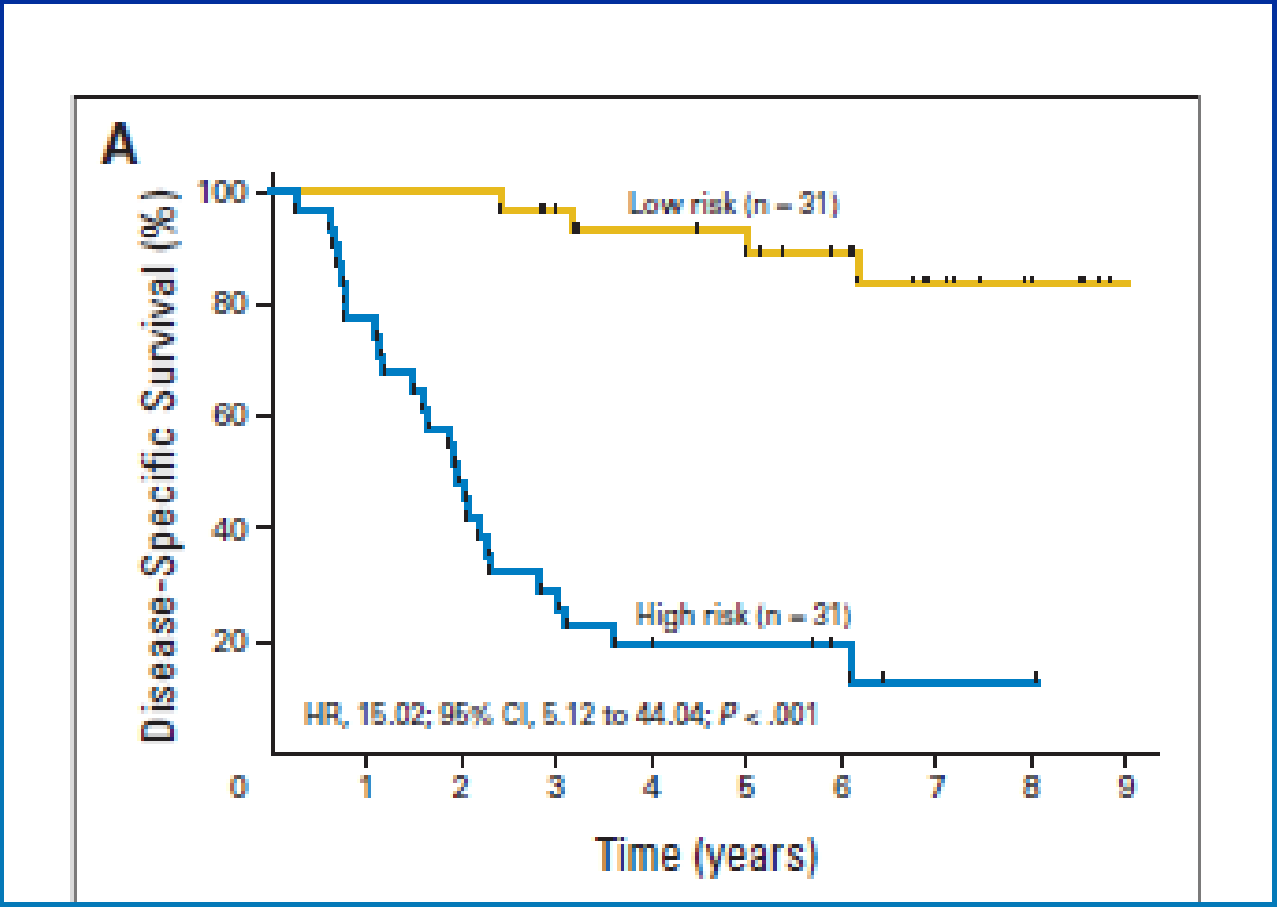
Gene expression profiling

mRNA extracted from 133/166 frozen tissue >20% tumour cellularity
62 OBS / 71 ACT

A 15 gene signature selecting greatest separation of good and poor prognosis subgroups in the OBS patients identified

Probe Set	Gene Symbol	Gene Name
201243_s_at	ATP1B1	ATPase, Na+/K+ transporting, beta 1 polypeptide
203147_s_at	TRIM14	Tripartite motif-containing 14
221591_s_at	FAM64A	Family with sequence similarity 64, member A
218881_s_at	FOSL2	FOS-like antigen 2
202814_s_at	HEXIM1	Hexamethylene bis-acetamide inducible 1
204179_at	MB	Myoglobin
204584_at	L1CAM	L1 cell adhesion molecule
202707_at	UMPS	Uridine monophosphate synthetase
208399_s_at	EDN3	Endothelin 3
203001_s_at	STMN2	Stathmin-like 2
210016_at	MYT1L	Myelin transcription factor 1-like
202490_at	IKBKAP	Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein
206426_at	MLANA	Melan-A
205386_s_at	MDM2	Mdm2, transformed 3T3 cell double minute 2
219171_s_at	ZNF236	Zinc finger protein 236

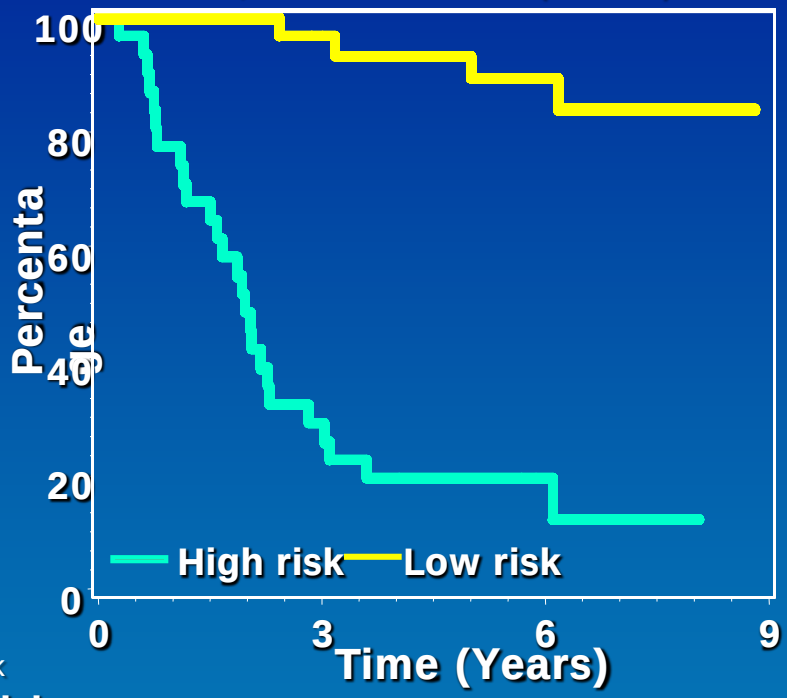
The 15-Gene Signature is Prognostic in Observation Patients (Stages IB and II)



HR,15.02 (95%CI 5.12-44.04) p<0.0001

Signature is Prognostic in Observation but not in Chemotherapy Treated Patients

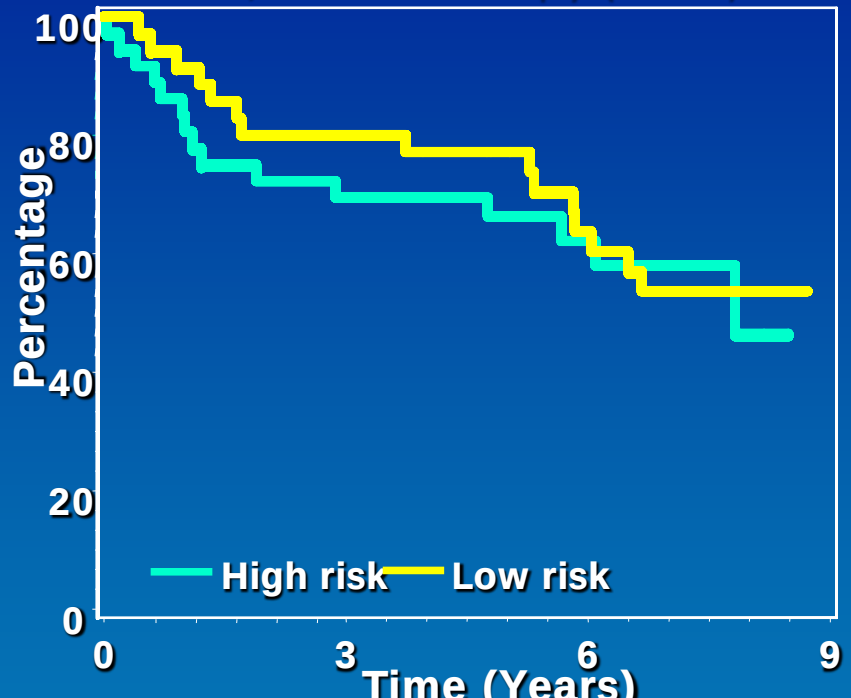
JBR.10, observation (n=62)



# at Risk				
Low Risk	31	28	20	1
High Risk	31	9	3	0

HR 15.02 (95% CI 5.12-44.04) p<0.0001

JBR.10, chemotherapy (n=71)

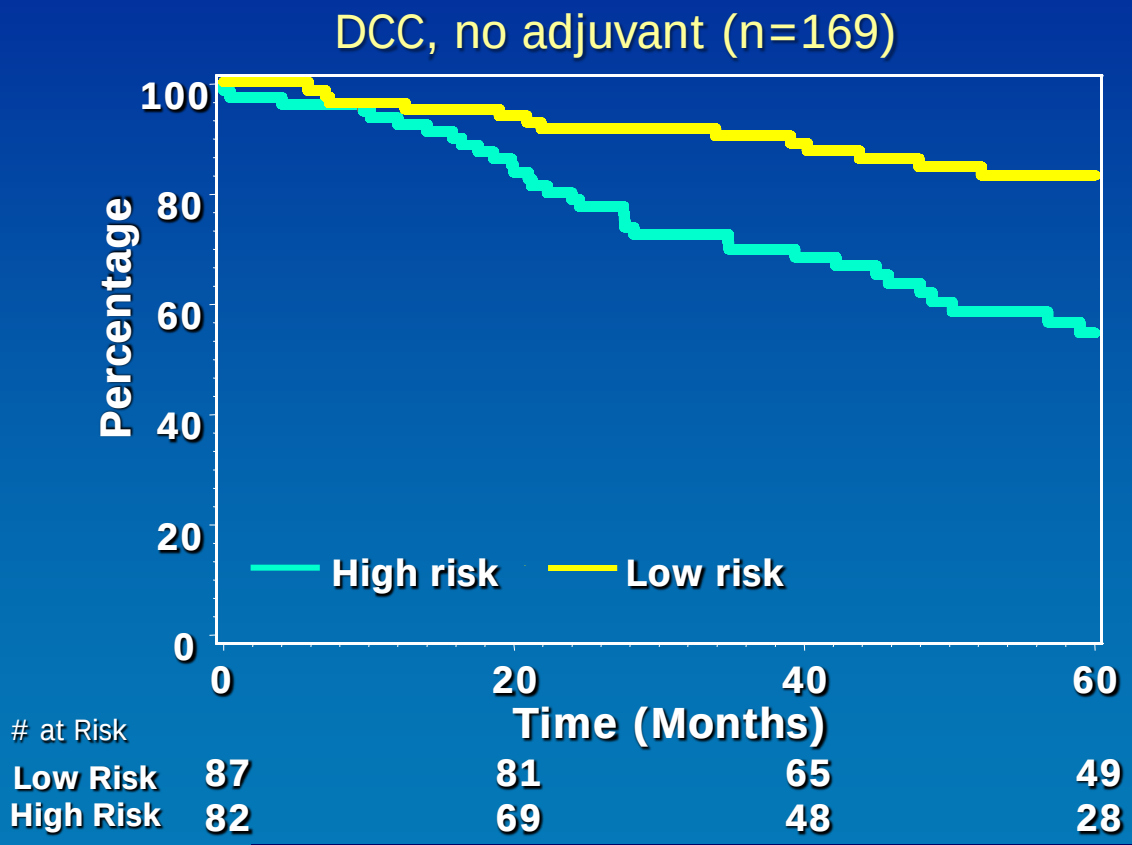


	35	28	19	3
	36	25	15	1

HR 1.15 (95% CI 0.56-2.37) p=0.6942

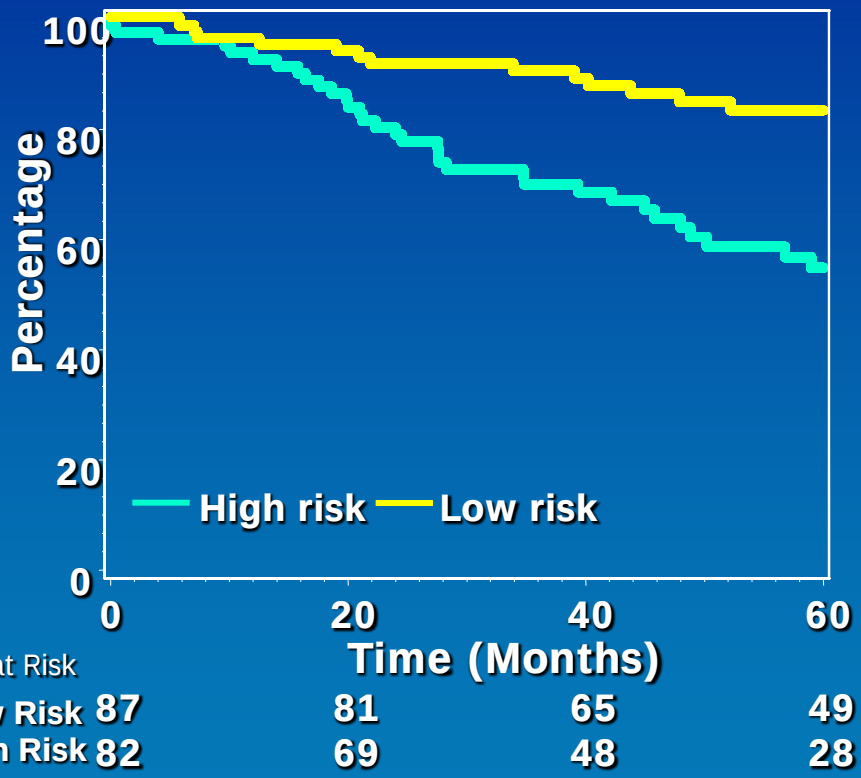
Validation of 15-gene Signature in the NCI Director's Challenge

Stage IB-II Patients <u>without</u> Adjuvant Chemotherapy

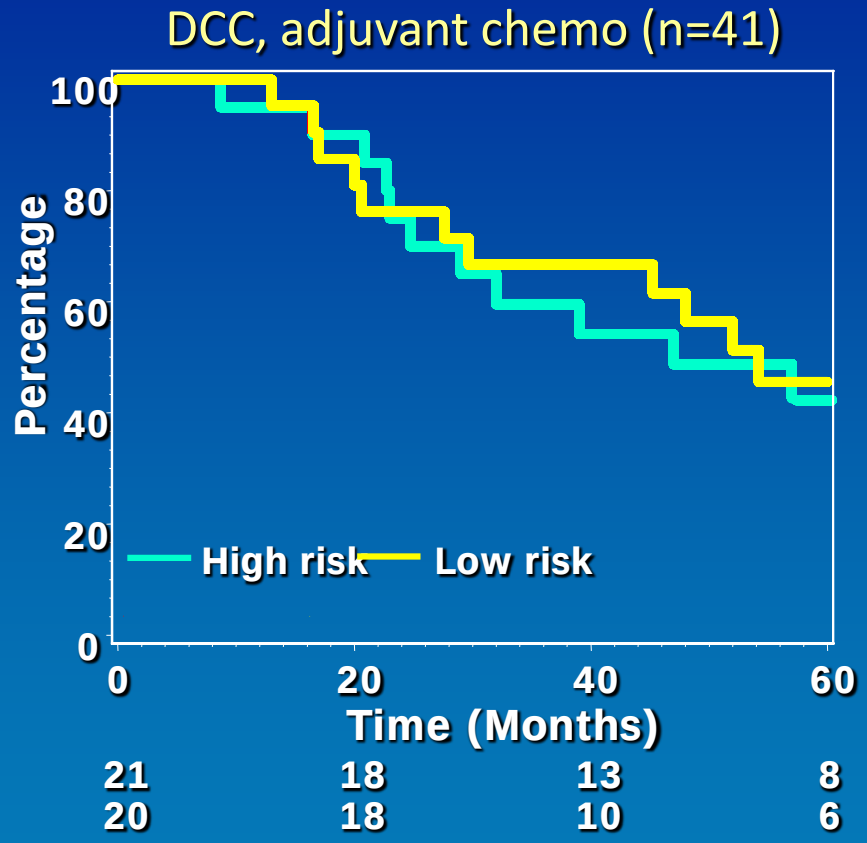


HR 3.21 (95% CI 1.69-6.11)
p=0.0002

Not Prognostic in the DCC's Stage I-II Patients *with* Adjuvant Chemotherapy



HR 3.21 (95% CI 1.69-6.11)
p=0.0002

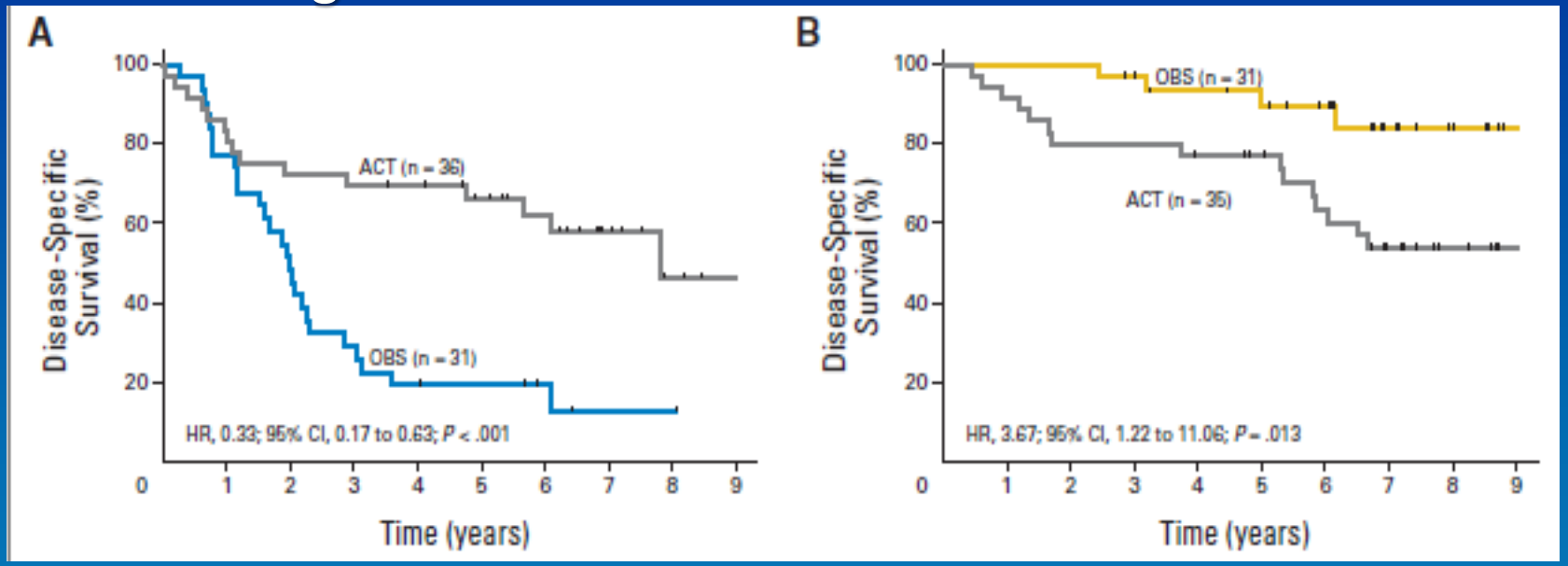


HR 1.10 (95% CI 0.47-2.53)
p=0.8294

Chemotherapy Benefits JBR.10 High Risk but Not Low Risk Patients

High risk

Low risk



HR 0.33 (95% CI 0.17-0.63) p=0.0005

HR 3.67 (95% CI 1.22-11.06) p=0.0133

Interaction p = 0.0001

BR.10 Gene Signature Discovery

- A novel 15-gene signature may identify early stage non-small cell lung cancer patients who are most likely to benefit from chemotherapy after complete surgical resection

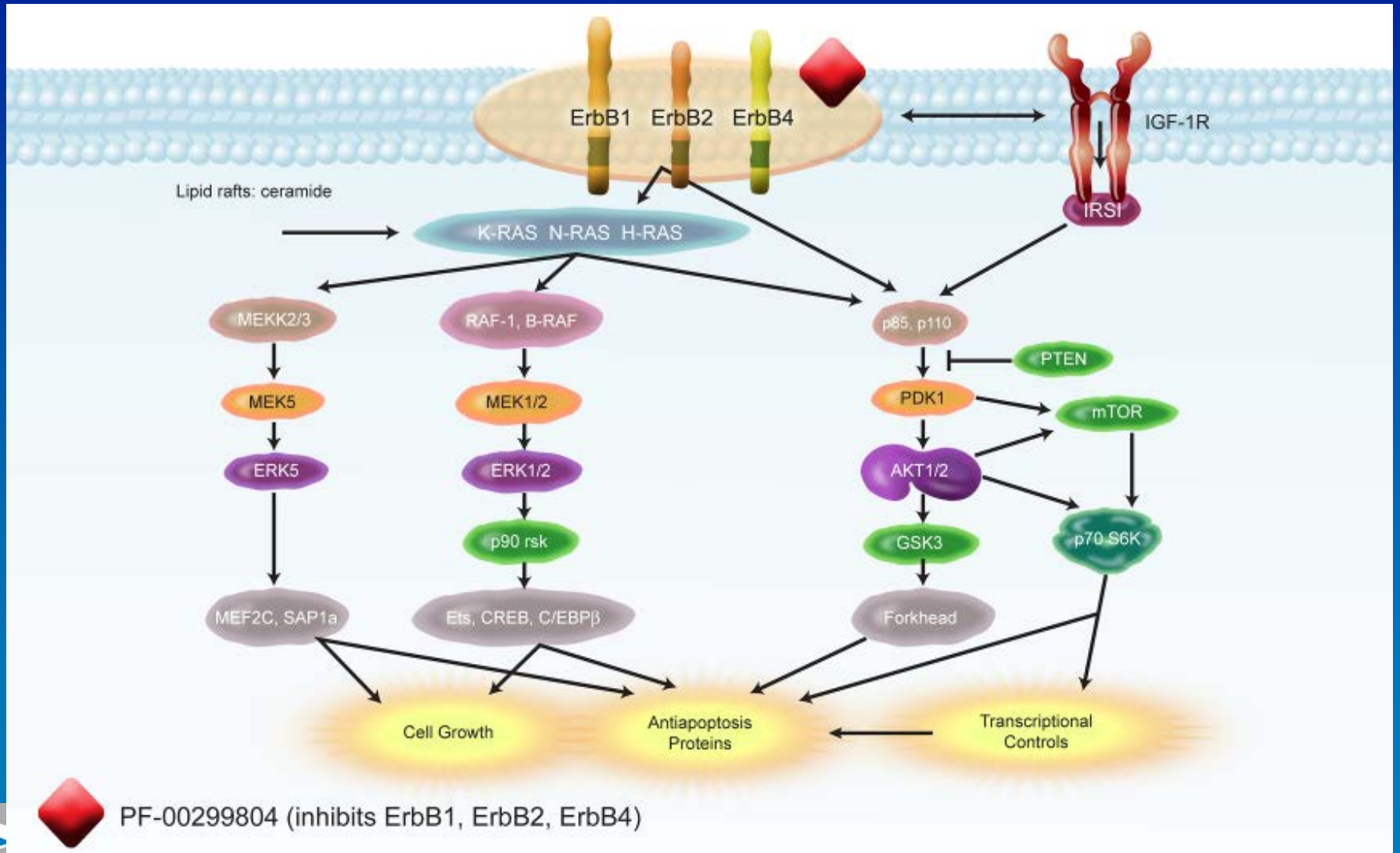
- If validated by further testing, the signature may improve the current method for deciding which patients should receive adjuvant chemotherapy

NCIC CTG BR.26: A double blind placebo controlled trial of PF-804 in patients with incurable stage IIIB/IV NSCLC after failure of standard therapy for advanced of metastatic disease

PF-00299804 (PF804)

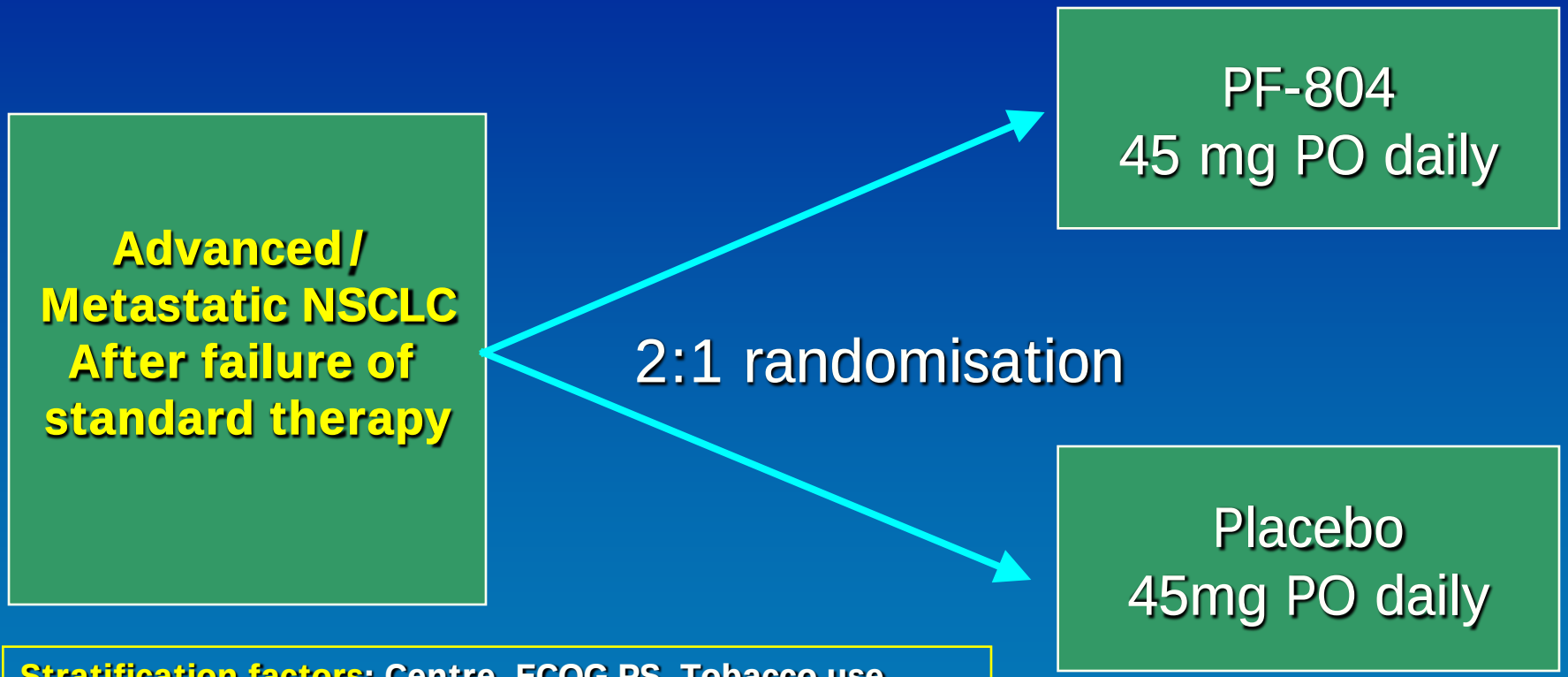
- **PF-00299804 (PF804)**
  - Selective, irreversible inhibitor of HER family of tyrosine kinases
  - HER receptor inhibition via Irreversible covalent modification of ATP-binding site
  - Overcomes resistance to gefitinib/ erlotinib in T790M +ve tumours
  - Oral, RP2D 45mg continuous oral dosing daily

HER Biology


ErbB1
ErbB2
ErbB4
IGF-1R
Lipid rafts: ceramide
IRS1
K-RAS N-RAS H-RAS
MEKK2/3
RAF-1, B-RAF
p85, p110
PTEN
MEK5
MEK1/2
PDK1
mTOR
ERK5
ERK1/2
AKT1/2
p90 rsk
GSK3
p70 S6K
MEF2C, SAP1a
Ets, CREB, C/EBPβ
Forkhead
Cell Growth
Antiapoptosis Proteins
Transcriptional Controls
PF-00299804 (inhibits ErbB1, ErbB2, ErbB4)

Study Overview: NCIC CTG BR.26 Design

Randomised double blind placebo controlled trial



Stratification factors: Centre, ECOG PS, Tobacco use, Best response to prior EGFR TKI, Weight Loss, Ethnicity

NCIC CTG BR.26

- **Primary Endpoint**
  - Overall survival
- **Secondary endpoints**
  - OS in *K-Ras* WT patients
  - OS in *EGFR* mutation +ve patients
  - Progression Free Survival
  - Objective RR
  - Time to Response and response Duration
  - Toxicity
  - Quality of life
  - Economic evaluations
  - Correlation of tumour and blood markers with outcomes

Study Overview: Statistical Design

- Randomised double blind placebo controlled trial
- Sample Size n= 720 patients
  - placebo arm estimated median OS of 4 months
  - 90% power to detect 33% improvements with PF-804
  - 1-sided 2.5% significance test
- Interim analysis
  - For futility
  - Performed at approximately 200 deaths
- Accrual Aims
  - 30 patients per month
  - 720 patients accrued over 2 years

BR.26 correlative analyses

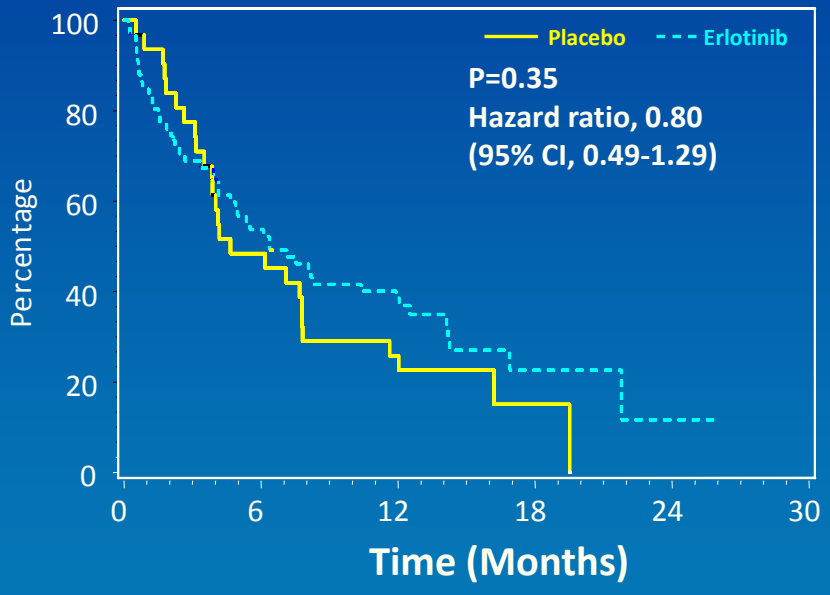
Background

- **Biomarkers have the potential to inform which patients most likely to benefit from a therapy**
  - **Save toxicity for those unlikely to benefit**
  - **Reduce societal costs if ineffective in a subset**
  - **Allow identification of subset for whom other therapies can and should be developed**

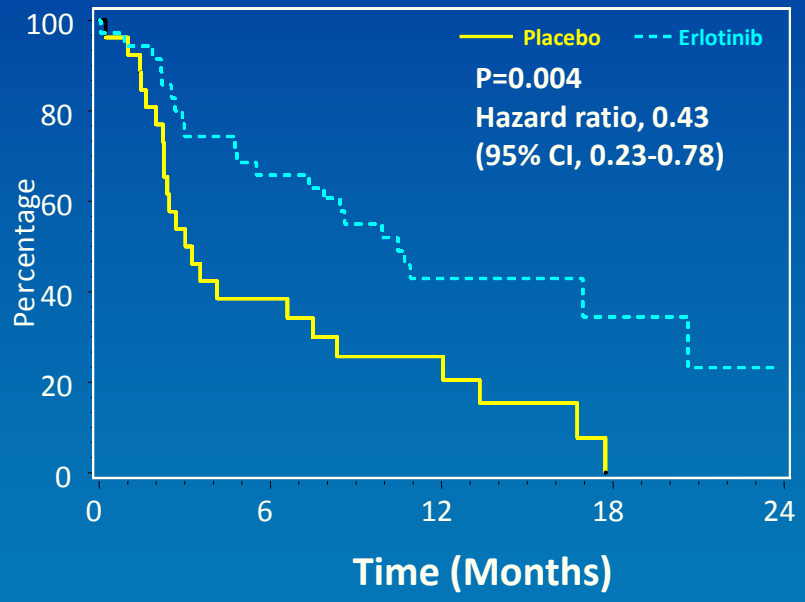
- **EGFR pathway has well characterised biomarkers of interest**
  - ***EGFR gene mutation / copy number***
  - ***KRAS* mutations**
  - **Acquired *EGFR* mutations predictive of resistance**

NCIC CTG BR.21: Survival According to Updated *EGFR* Copy Number

EGFR FISH Low Copy



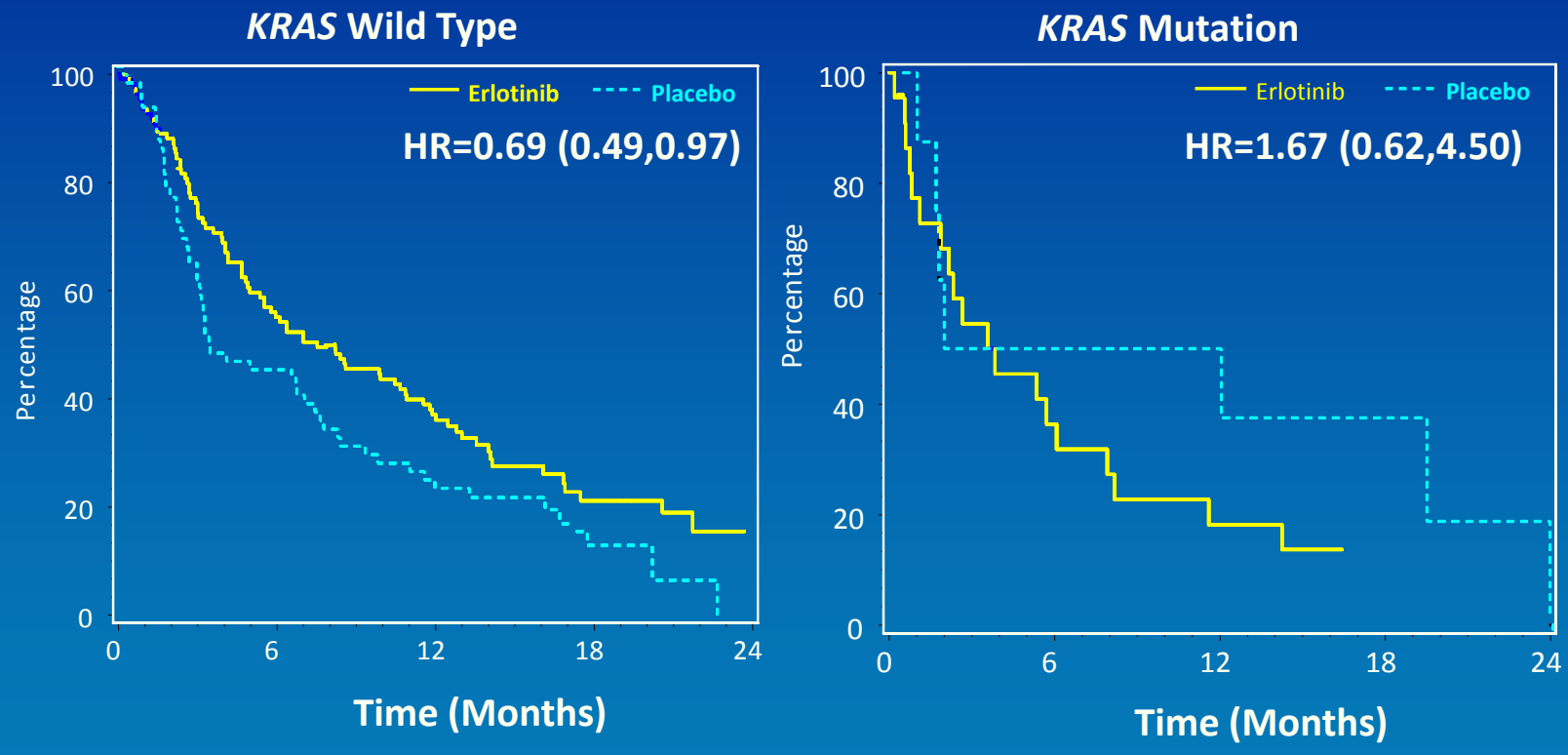
EGFR FISH High Copy



Interaction P value = 0.12

Zhu et al. J Clin Oncol, 2008

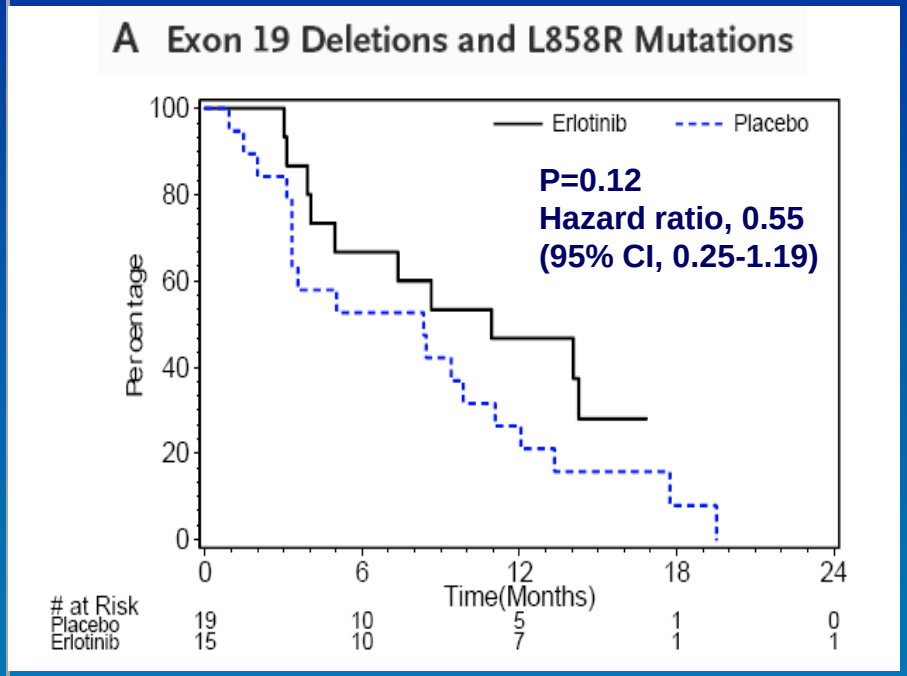
NCIC CTG BR.21: Survival According to Updated *KRAS* Mutation Status

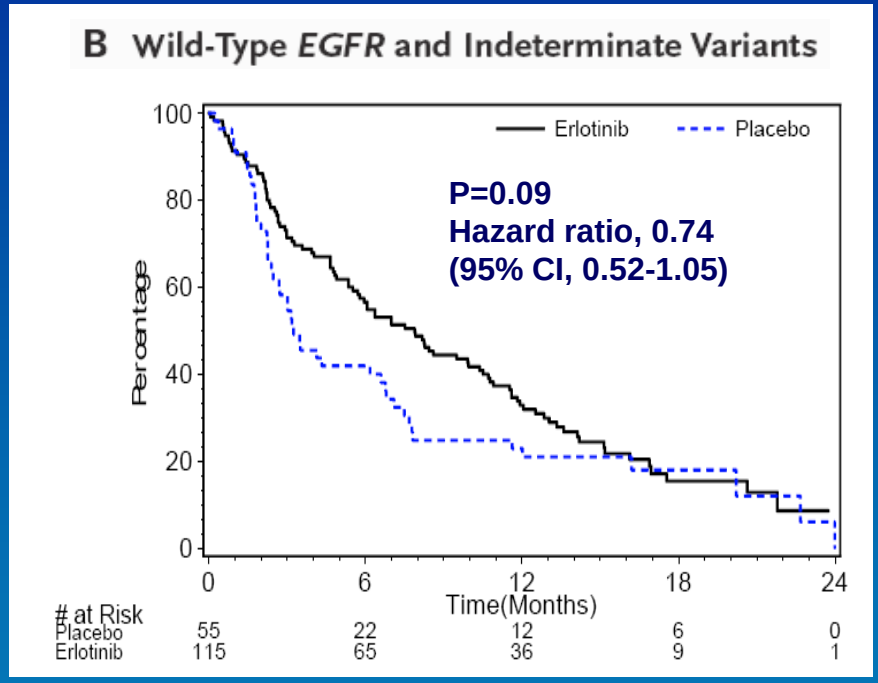


Interaction P value = 0.09

Zhu et al. J Clin Oncol, 2008

BR.21Survival According to Updated *EGFR* Mutation Status

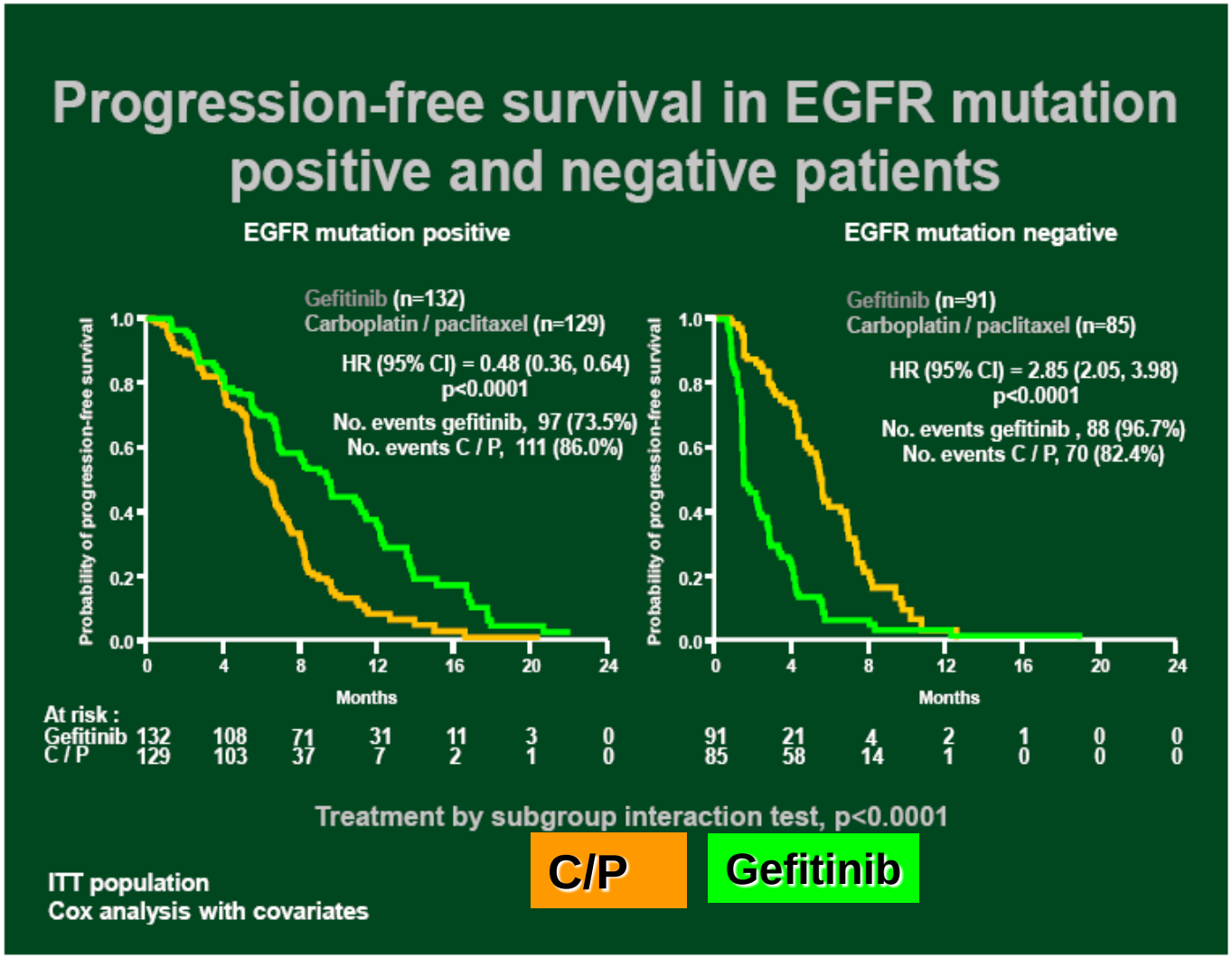




Interaction P value = 0.47

Zhu et al. J Clin Oncol, 2008

First-line gefitinib vs. carboplatin / paclitaxel patients with adenocarcinoma (IPASS)



T Mok et al. NEJM 361 2009

BR.26 Correlative Sciences

- **Strong rationale to evaluate these biomarkers prospectively in BR.26**

- **To evaluate a biomarker requires adequate proportion of samples from the clinical trial participants**
  - **BR.21 clinical trial included an optional tissue consent**
  - **Only a subset of tissues were available**
  - **Some available tissue not sufficient to yield data**
  - **Small proportion of samples severely compromises the biomarker analysis and impacts on the clinical utility of the biomarker**

- **Therefore, tissue collection mandatory in BR.26**
  - **But, recognising difficulty of collecting tissue in practice, inclusion of evaluation of biomarkers from blood samples**

BR.26 Correlative Studies

- **Prospectively planned analyses to evaluate prognostic and predictive biomarkers**
  - **Secondary endpoints:**
    - **OS in patients with baseline *EGFR* gene mutation positive tumours**
    - **OS in patients with baseline *KRAS* WT tumours**
- **Additional prognostic / predictive assays planned**
  - **serum EGFR extracellular domain (ECD), serum HER2 ECD, SNPs, E-cadherin ELISAs, TGF-α and HGF**
- **Only prognostic / predictive biomarkers will be evaluated**
  - **NO testing for inherited diseases**
- **Collection of plasma to validate a blood based biomarker assay**

BR.26 Samples

	Collection Time Point		
Sample	**Baseline**	**Every second cycle**	**Off protocol therapy**
Tissue (Archival or Fresh sample)	√		√ (if possible)
Plasma	√	√	√
Serum	√	√	√
Blood for DNA/RNA	√	√	√