Madrid, 9 de mayo de 2012

USCAP & AACR HIGHLIGHTS

Avances en Patología Pulmonar

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Roche

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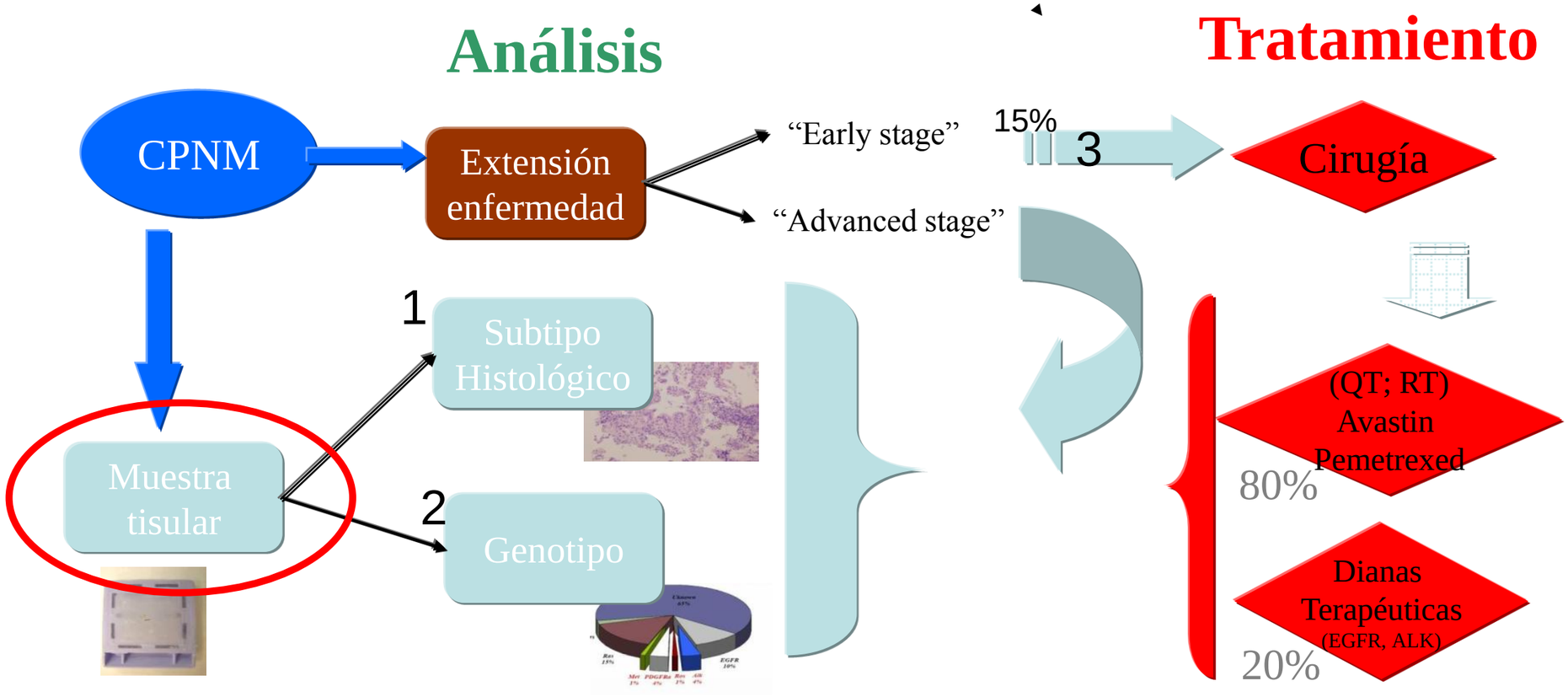
Manejo del cáncer de pulmón no microcítico



- **Un cambio de paradigma: Tratamiento personalizado:**
  1) La morfología como primer biomarcador
  2) Estudio de alteraciones moleculares predictoras de respuesta al tratamiento: IHQ, HIS o PCR.
  3) Estadificación: Imagen, PET, AP…

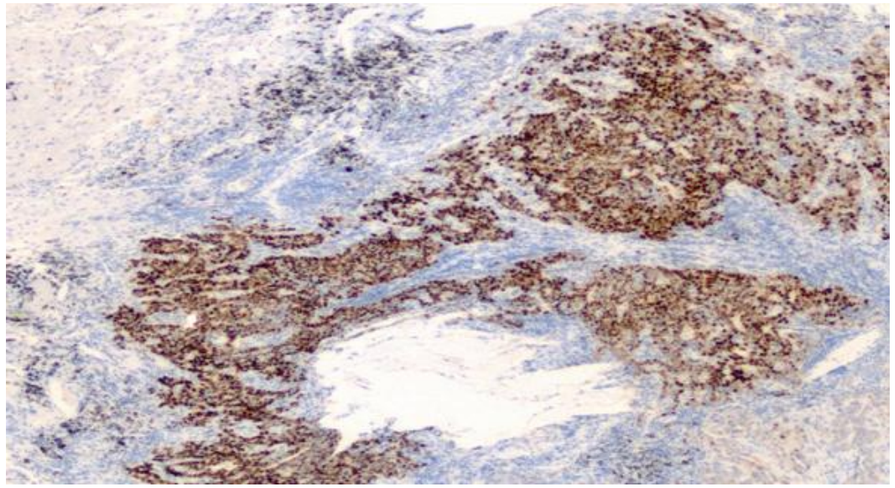
"El patólogo es la pieza más importante del tratamiento personalizado. Sin biomarcador no hay tratamiento personalizado" ELCC. 2012.

1.- Morfología

1967 : p40 is superior to p63 for the diagnosis of pulmonay SCC.

Bishop et al, JH, MSKCC, NCI, Milano..

- n=470
- P63 (4A4) vs deltaNp63
- 3% adenoca +, con < 5% núcleos



- 2028
- 2022:
  - n=150 Adca, 35 SCC.
  - P63 + in all Adca subtypes , except mucinous

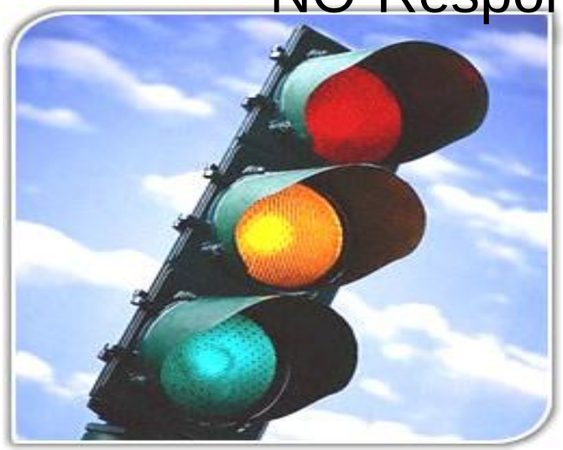
	p63	p40
Escamoso (n=81)	100%	100%
Adenocarc (n=237)	17-31%	0- 3%*
Linfoma Cél grande (n=152)	54%	0%

La morfología es el primer biomarcador:

Biomarcadores:

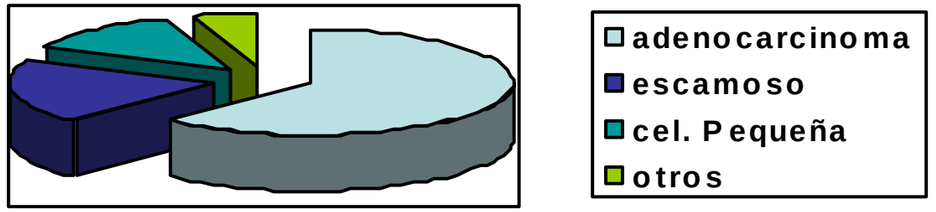
"Cambios medibles,
ya sean estos moleculares,
bioquímicos, fisiológicos o morfológicos,
que se asocian con respuesta
al tratamiento":

NO-Respondedor



Respondedor

- **El 70% de los casos son diagnosticados en biopsias pequeñas o citologías**
- **Entre un 10-30% de los casos son diagnosticados como carcinomas no microcíticos**



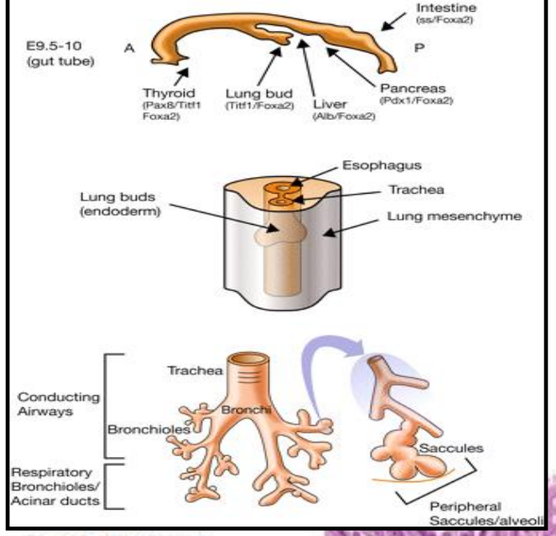
1) Microcítico (NE):
Etopside + cisplatino

2) Adenocarcinoma y Célula grande:
Pemetrexed,
Bevacizumab

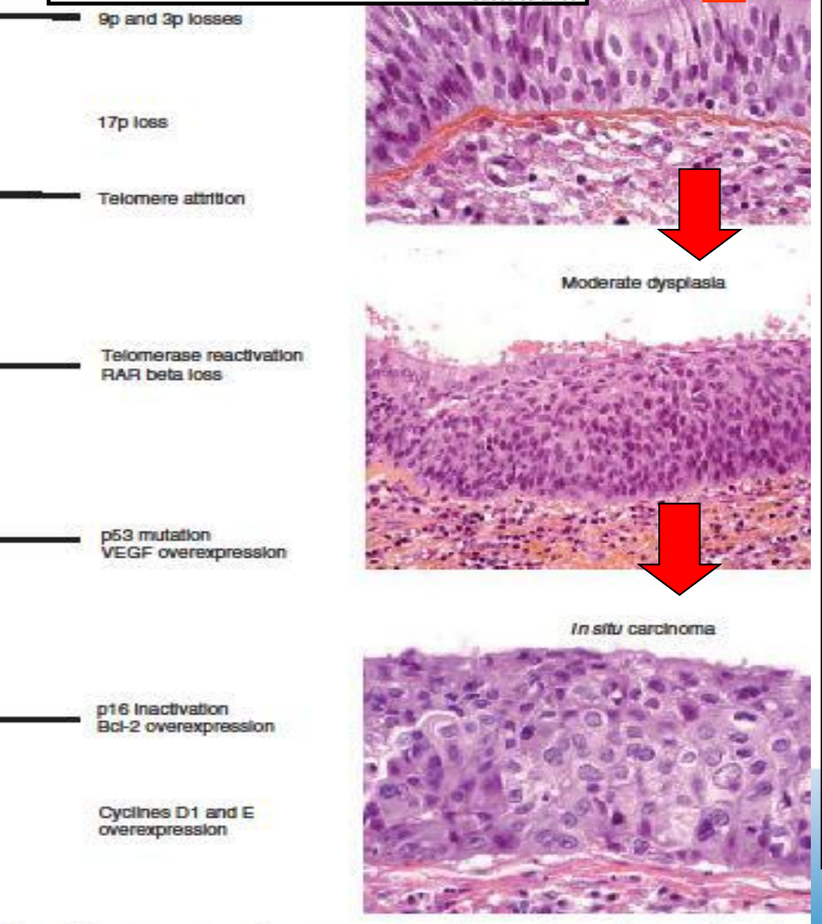
3) Escamoso:
Gemcitabine

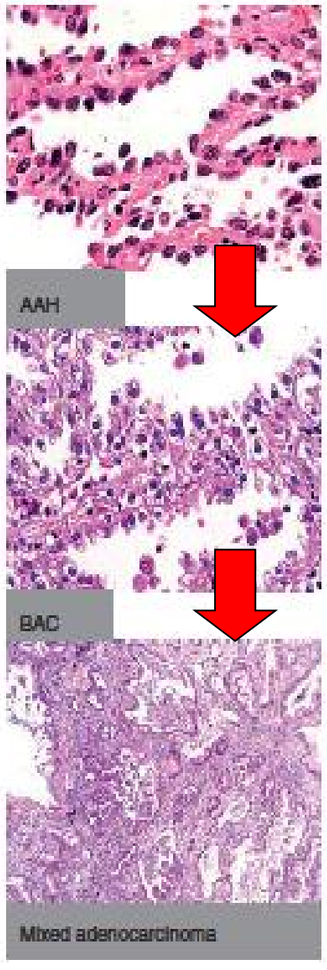
¿Cáncer de pulmón?

Al menos 3 entidades con morfogénesis, lesiones precursoras, fenotipo y genotipo distintos



1





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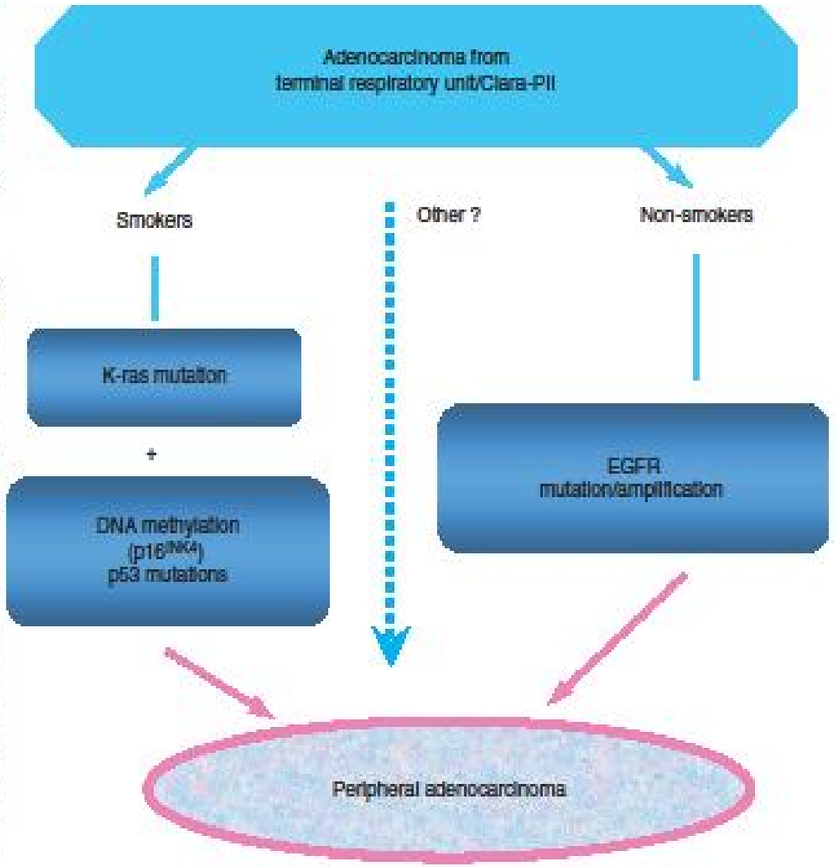
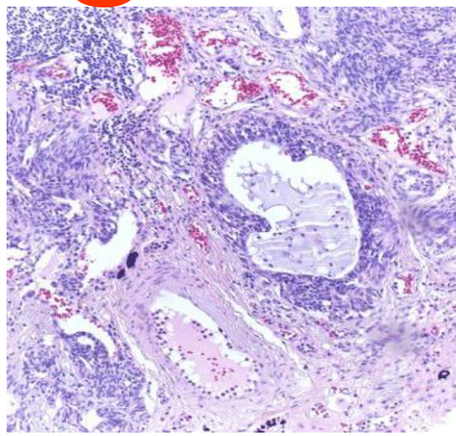


3

DIPNECH



Tumores neuro-endocrinos

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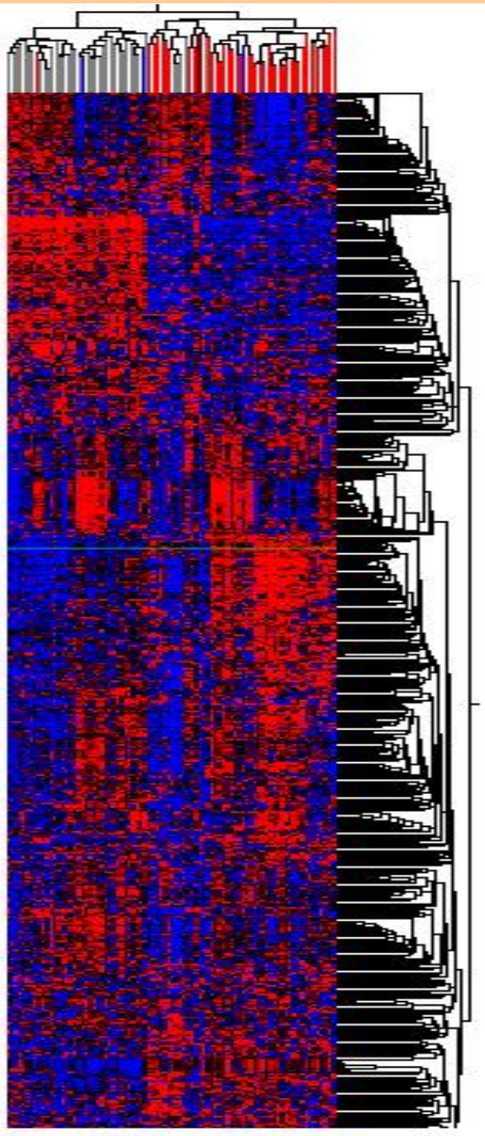
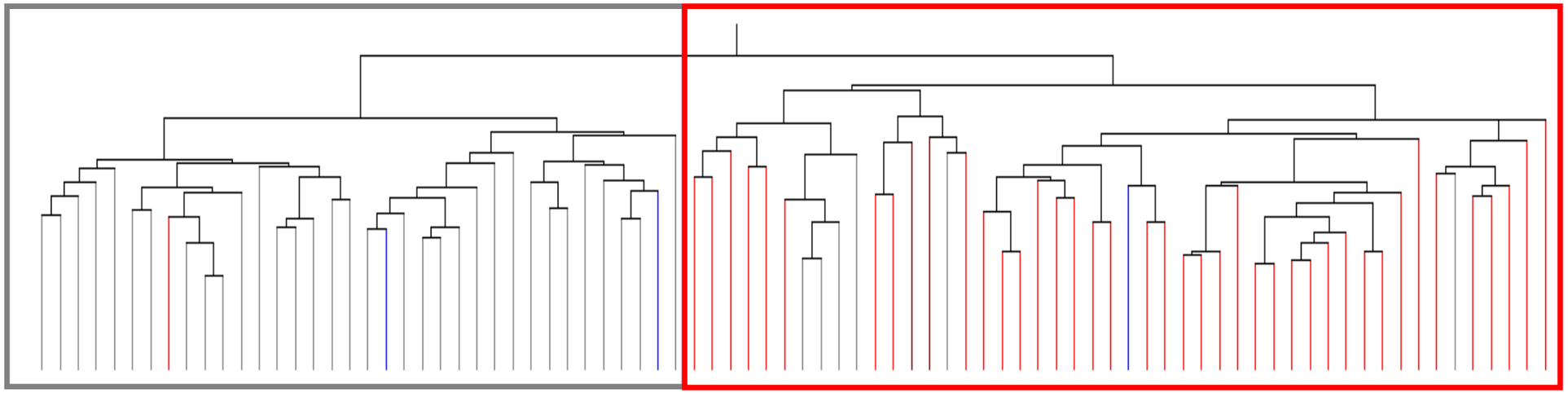
DESCUBRIMIENTO DE GRUPOS MOLECULARES

Clustering jerarquico. Centrado Pearson y Average linkage



Perfil de expresión 1

Perfil de expresión 2



Epidermoides (40)

Adenocarcinomas (39)

Adenoescamosos (3)

Célula Grande (2)

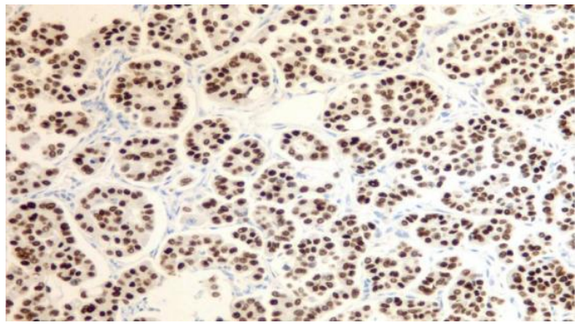
Objetivo: uso correcto de un panel inmunohistoquímica

- **Diagnóstico:** elegir uno para cada subtipo.
  - Adenocarcinoma: TTF1 (mejor), napsina; PE-10
  - Escamoso: p63 (mejor) ¿p40?, CK5/6, HMWCK

- **Dianas terapéuticas:** EGFR, ALK, c-met, HER2, PTEN..

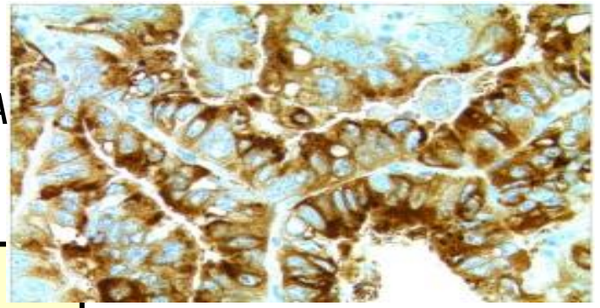
1996: TTF1 expression correlates with predominant histologic subtypes and recurrence in stage I adenocarcinoma.

Kadota et al, MSKCC



Subtipo	TTF1
AIS, AMI	100%
lepidico	96%
papilar	88%
acinar	87%
micropapilar	75%
Sólido	77%
Mucinoso inv	44%
Coloide	33%

- 2060: Comparison of Napsin A in tumours with polyclonal and monoclonal antibodies. *Zhu et al, PA*



Napsina A

Zhu et al, Mod Pathol 2012

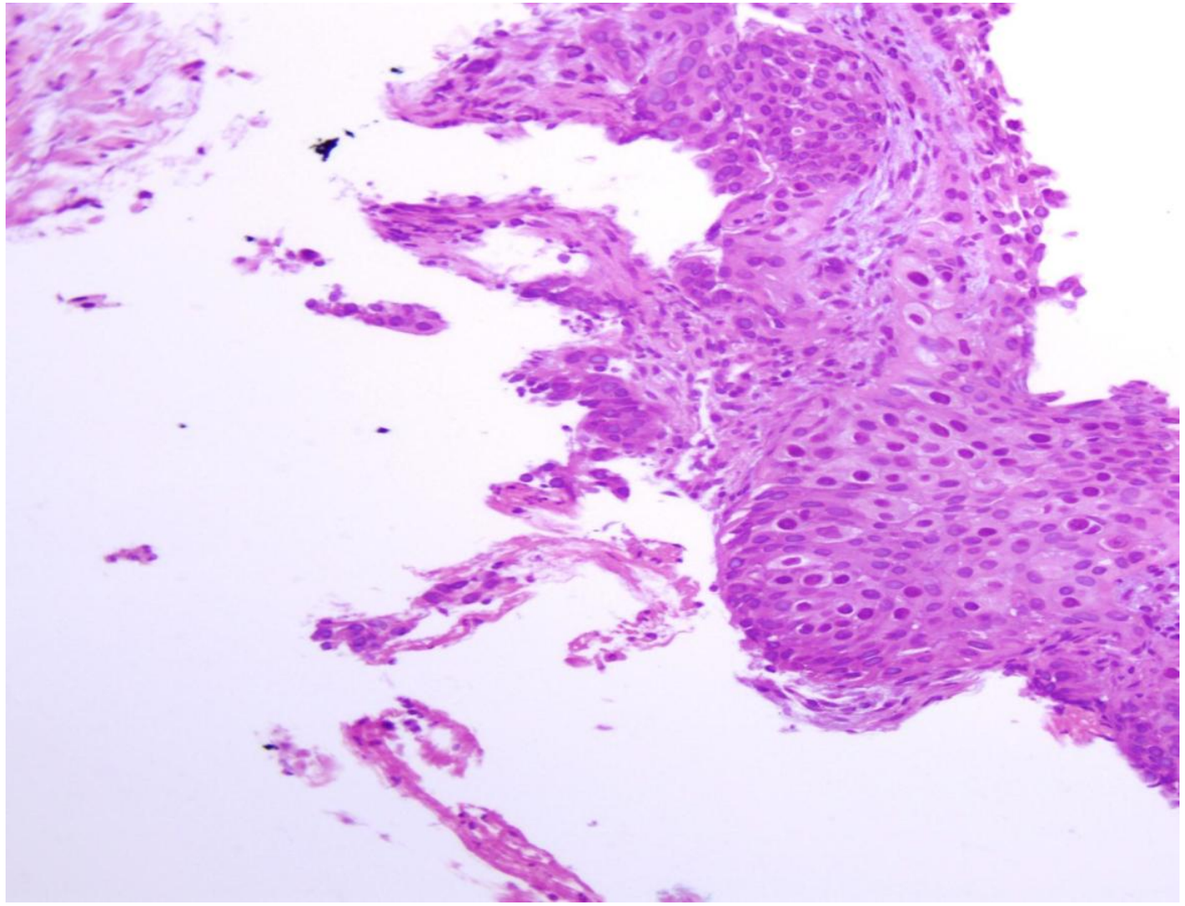
Tumor	monoclonal	policlonal
Adenoca Pulmón	**72%**	**83%**
Riñón papilar	50%	75%
Tiroides papilar	15%	12%
Riñón cels claras	2.5%	12%
Adenoca esófago	0%	11%
ovario	1.4%	7%
endocervical	7%	7%
PANCREAS	0%	6%
NE pulmón	7%	5%
Escamoso pulmón	2%	2%
Mama lobulillar	0%	1.2%

Unknown Origin

SSC vs Adeno

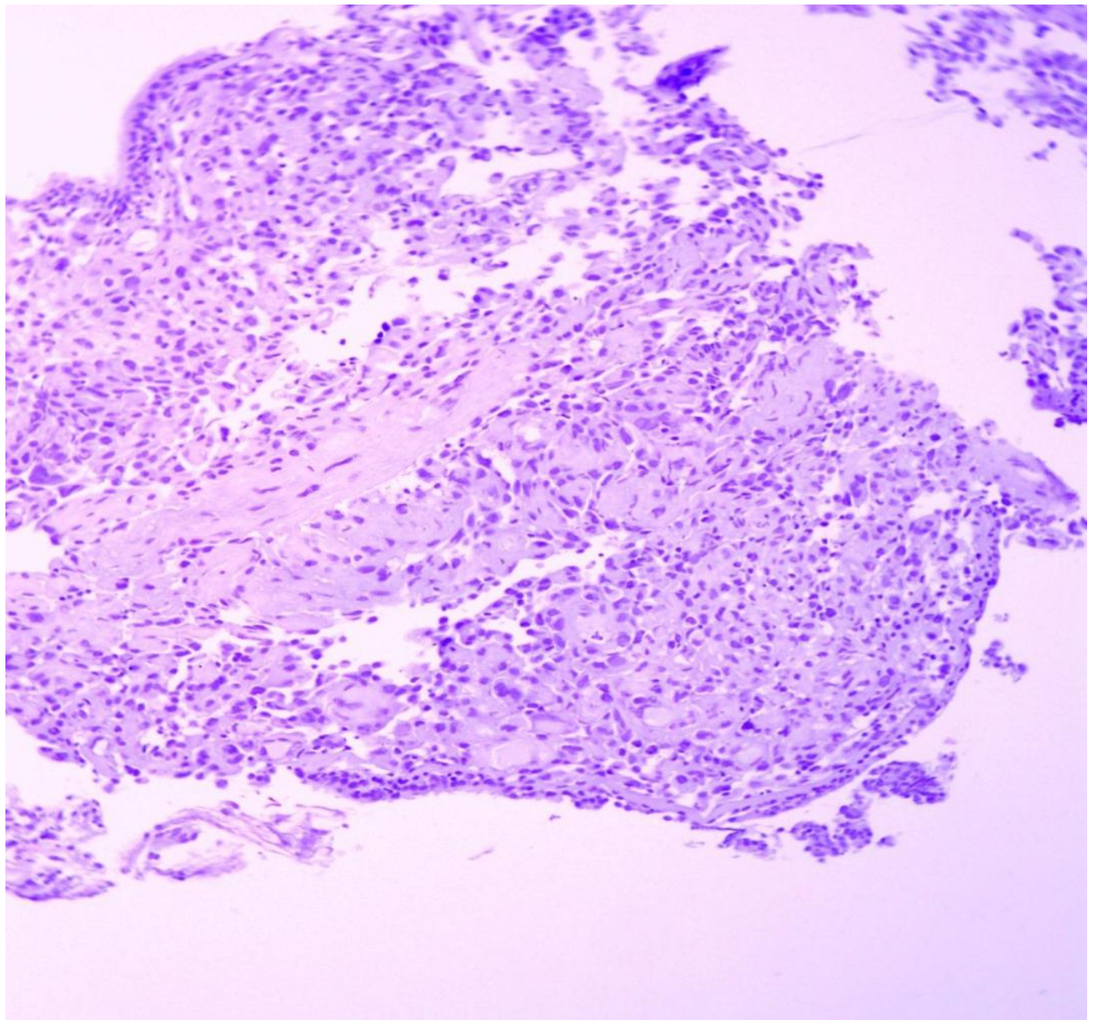
	p63	p40
Escamoso (n=81)	100%	100%
Adenocarc (n=237)	17-31%	0-3%*
Linfoma Cél grande (n=152)	54%	0%

Dtco: CNM con IHQ epidermoide



- Mujer 49 años exfumadora
- Cefaleas (mts cerebrales)
- Citologías:
  - Aspirado: negativo
  - PAAF ganglio: No-microcítico probable epid.
- IHQ:
  - TTF1 –
  - P63 –
  - CK5/6 + ALK +, EGFR-

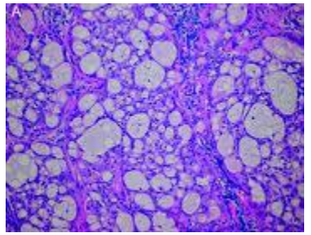
Dtco: CNM con IHQ epidermoide



- Mujer 70 años
- Citologías
  - Aspirado: negativo
  - Aspirado: No-microcítico.
- IHQ:
  - TTF1 –
  - P63 –
  - CK5/6 +

ALK -, EGFR+ (L858R)

1995 Cribiform pattern identifies a poor prognostic subset of acinar predominant tumours in stage I adenocarcinoma, Kadota et al, MSKCC



2004 WHO Classification	SMALL BIOPSY/CYTOLOGY: IASLC/ATS/ERS
ADENOCARCINOMA Mixed subtype Acinar Papillary Solid	*Morphologic adenocarcinoma patterns clearly present:* Adenocarcinoma, describe identifiable patterns present (including micropapillary pattern not included in 2004 WHO classification) Comment: If pure lepidic growth – mention an invasive component cannot be excluded in this small specimen
Bronchioloalveolar carcinoma (nonmucinous)	Adenocarcinoma with lepidic pattern (if pure, add note: an invasive component cannot be excluded)
Bronchioloalveolar carcinoma (mucinous)	Mucinous adenocarcinoma (describe patterns present)
Fetal	Adenocarcinoma with fetal pattern
Mucinous (colloid)	Adenocarcinoma with colloid pattern
Signet ring	Adenocarcinoma with (describe patterns present) and signet ring features
Clear cell	Adenocarcinoma with (describe patterns present) and clear cell features
No 2004 WHO counterpart – most will be solid adenocarcinomas	*Morphologic adenocarcinoma patterns not present (supported by special stains):* Non-small cell carcinoma, favor adenocarcinoma
SQUAMOUS CELL CARCINOMA Papillary Clear cell Small cell Basaloid	*Morphologic squamous cell patterns clearly present:* Squamous cell carcinoma
No 2004 WHO counterpart	*Morphologic squamous cell patterns not present (supported by stains):* Non-small cell carcinoma, favor squamous cell carcinoma

TABLE 1 2011 International Association for the Study o Lung Cancer/American Thoracic Society/ European Respiratory Society classification of lung adenocarcinoma in resection specimens

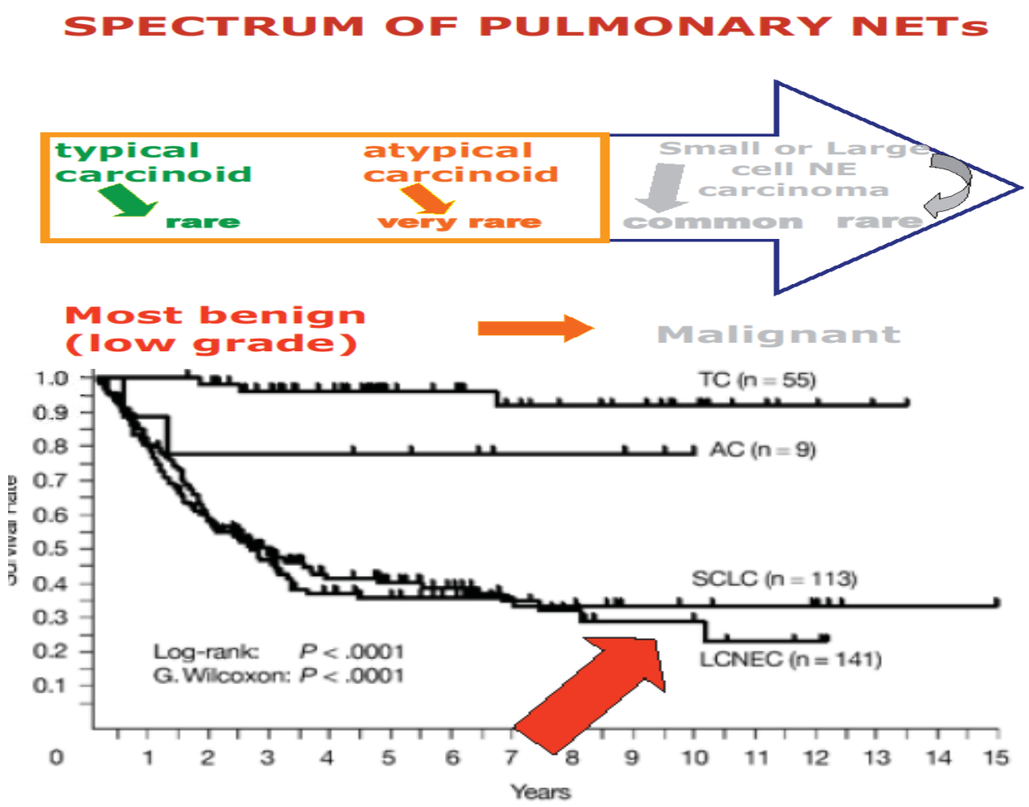
Pre-invasive lesions
- Atypical adenomatous hyperplasia
- Adenocarcinoma *in situ* (⩽3 cm formerly solitary BAC)
  - Nonmucinous
  - Mucinous
  - Mixed mucinous/nonmucinous

Minimally invasive adenocarcinoma (⩽3 cm lepidic predominant tumour with ⩽5 mm invasion)
- Nonmucinous
- Mucinous
- Mixed mucinous/nonmucinous

Invasive adenocarcinoma
- Lepidic predominant (formerly nonmucinous BAC pattern with >5 mm invasion) Bajo riesgo
- Acinar predominant Riesgo intermedio (Alto riesgo si cribiforme >30%!!)
- Papillary predominant Riesgo intermedio
- Micropapillary predominant Alto riesgo
- Solid predominant Alto riesgo

Variants of invasive adenocarcinoma
- Invasive mucinous adenocarcinoma (including formerly mucinous BAC) Alto riesgo
- Colloid Alto riesgo
- Fetal (low and high grade)
- Enteric

Objetivo: ~~Microcítico o no Microcítico~~ (Neuroendocrino y cual o no-NE)



XS
S
M
L
XL

- **Dd: Carcinoide o Cel. pequeña:**
  - Hipercelularidad, patrón
  - Pleomorfismo
  - >5-10 mitosis/10CGA, Ki67
  - Necrosis
- **Dd Cel Pequeña vs Escamoso basalioide:**

Travis et al, Mod Pathol 25; 2012

	Cel pequeña	basaliodeSSC
p63	-	+
HMWCK	-	+
TTF1	+ (70%)	-
Cromogr/sinap	+ (60-80%)	-
CD56	+ (90%)	-
ki67	70-80%	70-80%

2052 Gene expression profilling NE tumours*; Wang , MSKCC, Cornell:* SCLC-LCNEC/ Carcinoid central / periferico

2000 Histone 1.5 *Ko, Mount Sinai*

2016 Comparative IHC analysis to distinguish malignant mesothelioma from reactive mesothelial cells, *Minato et al, Japan*

	GLUT1	CD146	IMP3	EMA	Desmin
MM	26/31	19/31	29/31	23/31	1/31
RMC	0/40	0/40	5/40	5/40	19/40

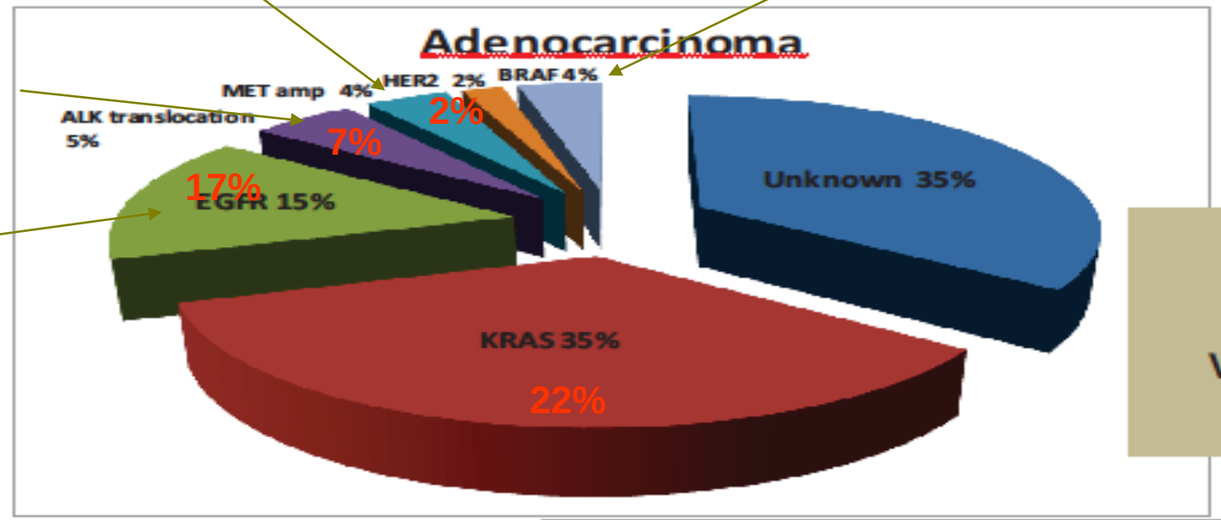
2.- Genes

IHQ,FISH/ crizotinib

PCR/ dasatinib

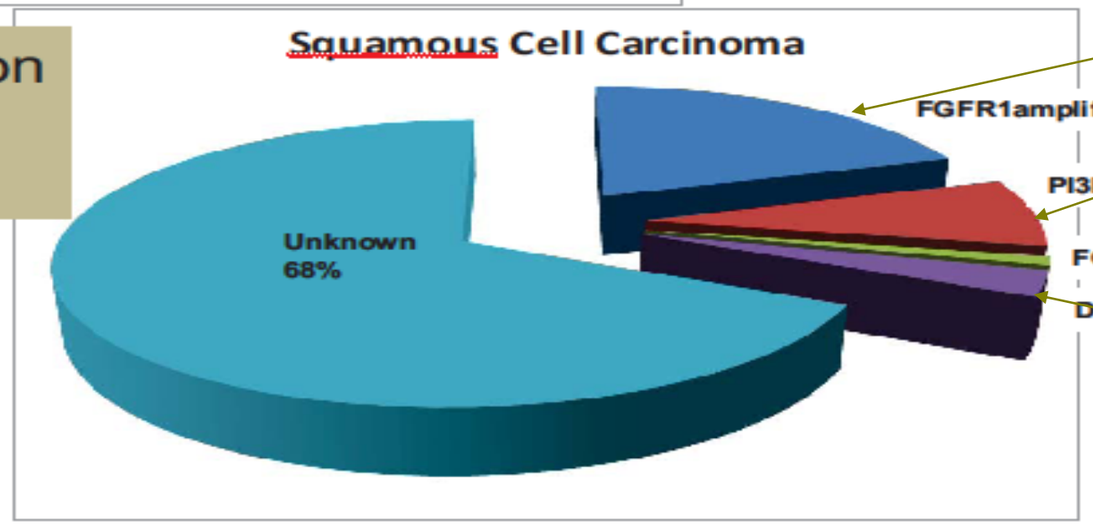
★ FISH/crizotinib

★ PCR/ TKIs



'Drugable' mutation profiles will vary according to Tumour histology

Biomarker selection may be driven by histology?



FISH/ AZD4547

FISH,PCR/ BKM120

PCR/ Dasatinib

(%) Lung cancer mutation consortium

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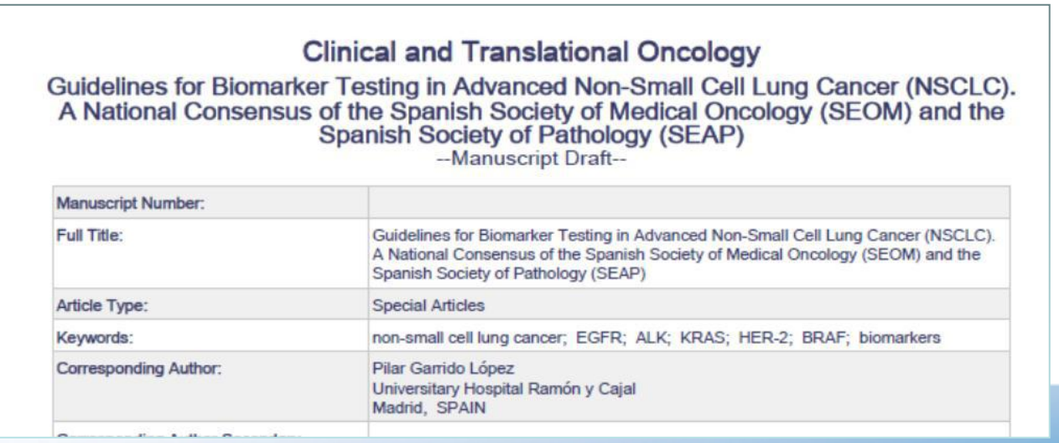
2032 **Resolving the controversy on EGFR/KRAS mutations in SCC**.

Recktman et al , MSKCC, DanaFarber

- N=95 SCC p63+, TTF-: no mutaciones
- N=16 SCC con mutaciones EGFR/Kras reclasificados como adenoescamoso (63%), o adenocarcinoma poco diferenciado “Escamoide” (31%)



Clinical and Translational Oncology

Guidelines for Biomarker Testing in Advanced Non-Small Cell Lung Cancer (NSCLC). A National Consensus of the Spanish Society of Medical Oncology (SEOM) and the Spanish Society of Pathology (SEAP)

--Manuscript Draft--

Manuscript Number:	
Full Title:	Guidelines for Biomarker Testing in Advanced Non-Small Cell Lung Cancer (NSCLC). A National Consensus of the Spanish Society of Medical Oncology (SEOM) and the Spanish Society of Pathology (SEAP)
Article Type:	Special Articles
Keywords:	non-small cell lung cancer; EGFR; ALK; KRAS; HER-2; BRAF; biomarkers
Corresponding Author:	Pilar Garrido López Universitary Hospital Ramón y Cajal Madrid, SPAIN

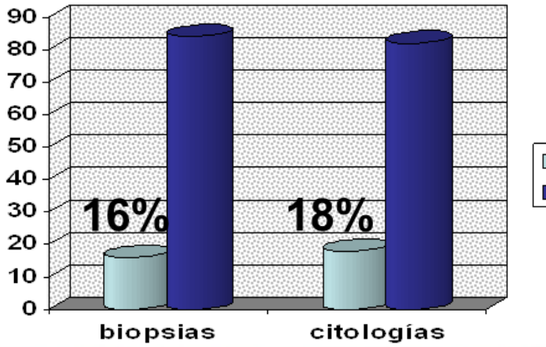
2049 **Molecular histologic correlations in TCGA study of lung SCC;**

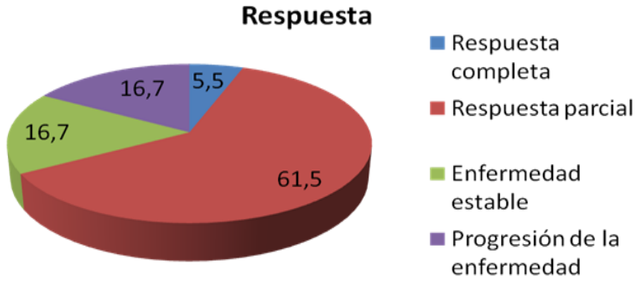
Travis et al:

213 submitted SCC reevaluated:

83% SCC (16% queratinizantes, 69% no-queratinizantes y 15% basaliodes)

17% reclassified





Inmunohistoquímica específica para mutaciones de EGFR

- 2003 The performance of E746-A750del mutation specific Antibody;
  - 62% sensibility for exon 19 EGFR deletions,
  - 100% specificity
- 2004 The performance of L858R antibody; *Kyshtoobayeva, CA*
  - 100% sensibilidad
  - 100% especificidad

1961 Are we there yet? *Arora et al, Japon, NCI, Bethesda:* NO. Low sensitivity, for both, exon 19 false positive if 1:25 dilution

1992 **ALK rearrangement by FISH and IHC methods. Prevalences and clinical outcomes**. *Hernandez-Losa et al, Val d Hebron*

- N=99 selected (80% Adca) caucasian.
- ALK rearrangement = 8.5%, better prognosis, younger, never smokers, adenocarcinomas..
- 5/7 positive with IHC (D5F3 mAb)
  - 1971 Ab clone 5 A4 100%
  - 1980 clone 5 A4 Not ready

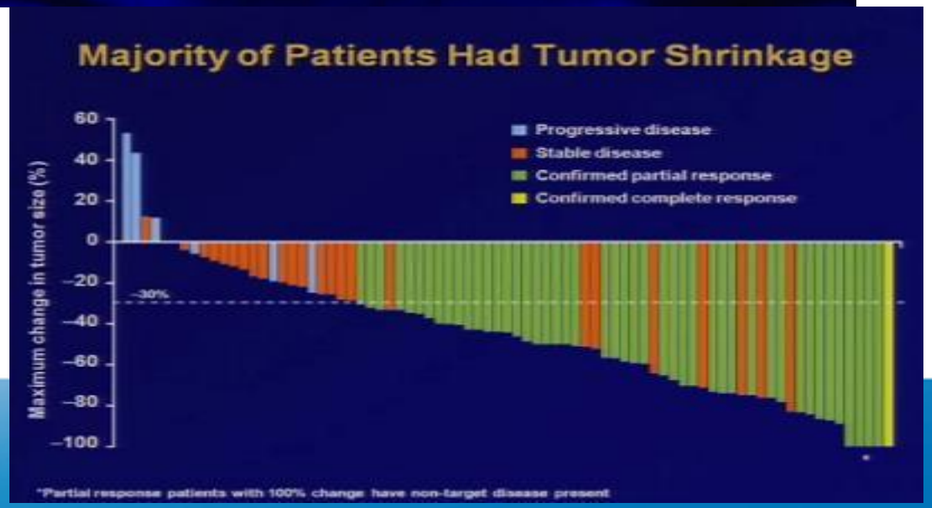
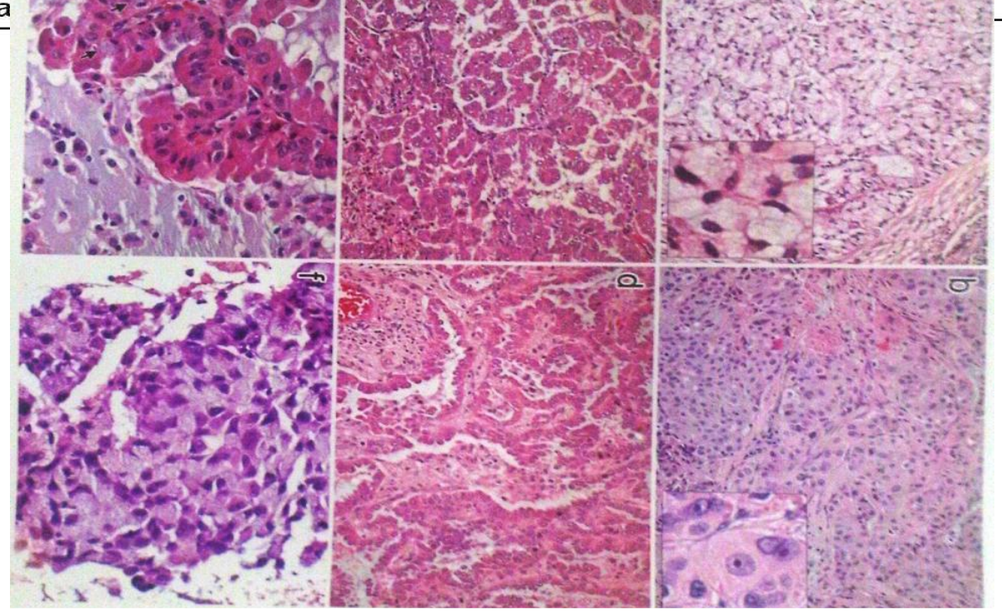


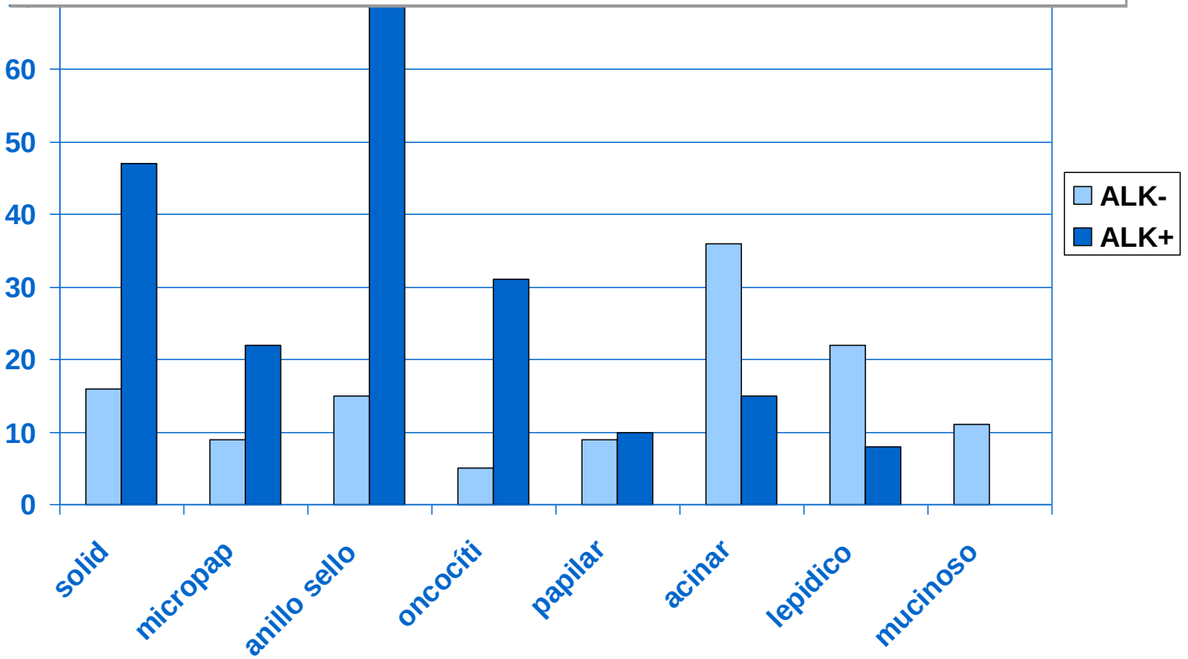
TABLE 5. Adenocarcinoma Histologic Subtypes, Molecula

Histological Subtype Predominant	Molecular Features
Nonmucinous AIS and MIA	TTF-1 + (100%)
	EGFR mutation never smokers: 10–30%
	KRAS mutation smokers: 10–30%
Lepidic (nonmucinous)	TTF-1 + (100%)
	EGFR mutation never smokers: 10–30%
	EGFR amplification: 20–50%
	KRAS mutation smokers: 10%
	BRAF mutations: 5%
Papillary	TTF-1 + (90–100%)
	EGFR mutation: 10–30%
	EGFR amplification: 20–50%
	KRAS mutation 3% (lack of *KRAS*)
	ERBB2 mutations: 3%
	p53 mutations: 30%
	BRAF mutations: 5%
Acinar	TTF-1 + or −
	KRAS mutation in smokers (20%)
	EGFR mutations <10% nonsmokers
	EGFR amplification: 10%
	EML4/ALK translocation: >5%
	P53 mutations: 40%
Micropapillary	*KRAS* mutations (33%)
	EGFR mutations (20%)
	BRAF mutations (20%)
Solid	TTF-1 (70%)
	MUC1 positive
	KRAS mutation smokers: 10–30%
	EGFR mutation never smokers: 10–30%
	EGFR amplification: 20–50%
	EML4/ALK translocation >5%
	p53 mutation: 50%
	LRP1B mutations
	INHBA mutations
Invasive mucinous adenocarcinoma	TTF-1 (0–33% positive)
	KRAS mutation: 80–100%
	No *EGFR* mutation
	MUC5+ MUC6+ MUC2+

AIS, adenocarcinoma in situ; MIA, minimally invasive adenocarcinoma;



2021 Histologic model for predicting ALK rearrangement



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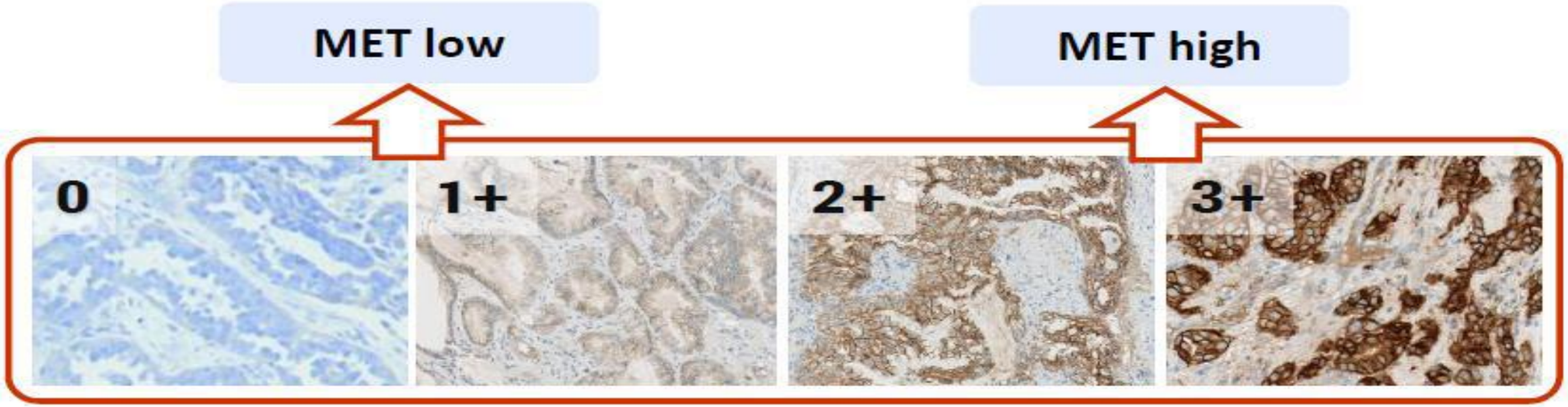
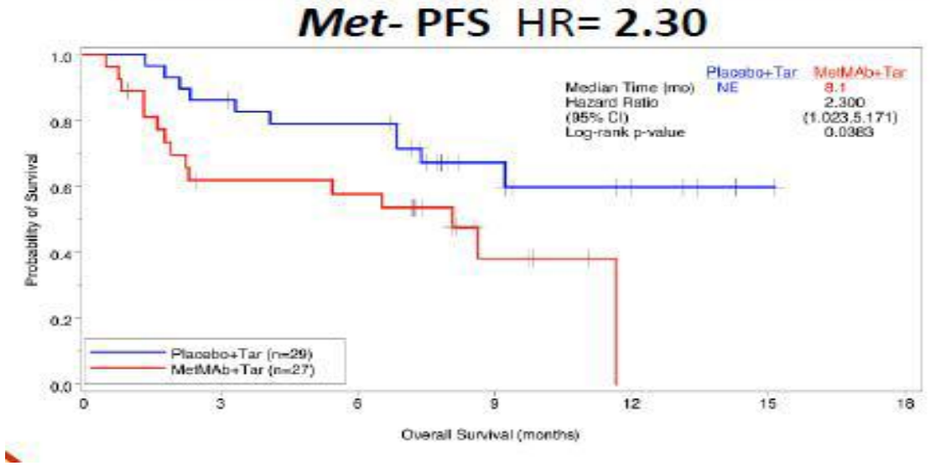
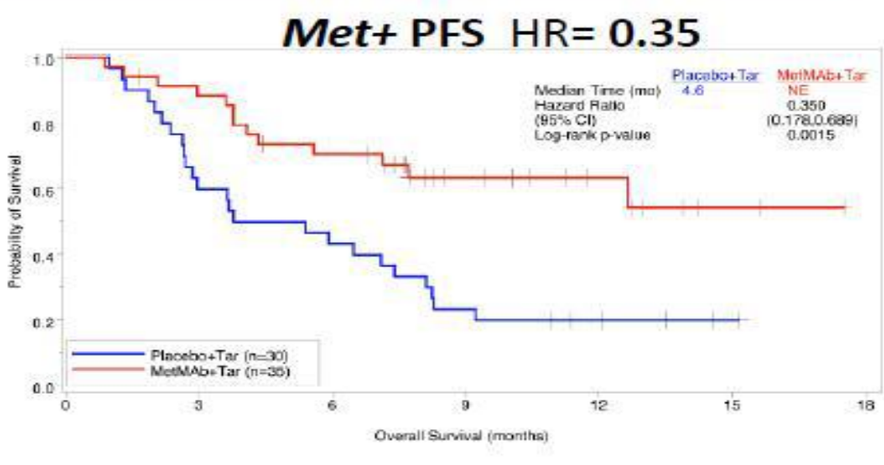
2001 Characterization and validation of an IHC assay for Met;

Koeppen et al, Ventana

N=127; 54%+





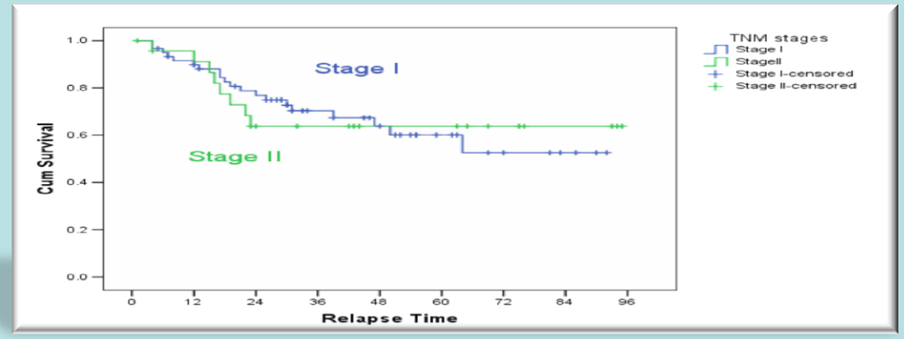


3.- Objetivo: CPNM temprano tras resección quirúrgica:

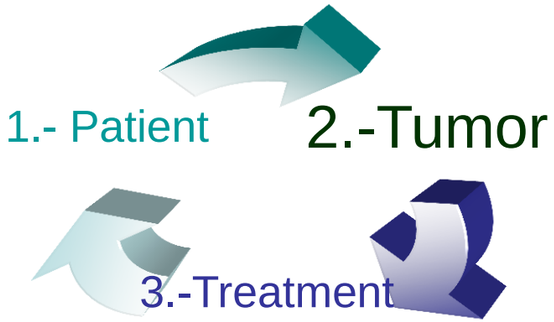
Aim 1: Prognostic tools, additional to TNM-staging

- - **5-y local recurrence rate by stage:**
  *Source: International Association for the Study of Lung Cancer
- IA: IB: IIA: IIB: IIIA:
- 16%, 23%, 37% 39% 30%

- Disease free survival in our series:

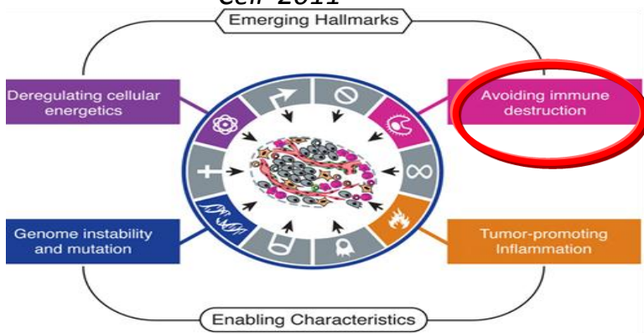


Aim 2: A useful immune score



Next generation hallmarks of cancer

Cell 2011

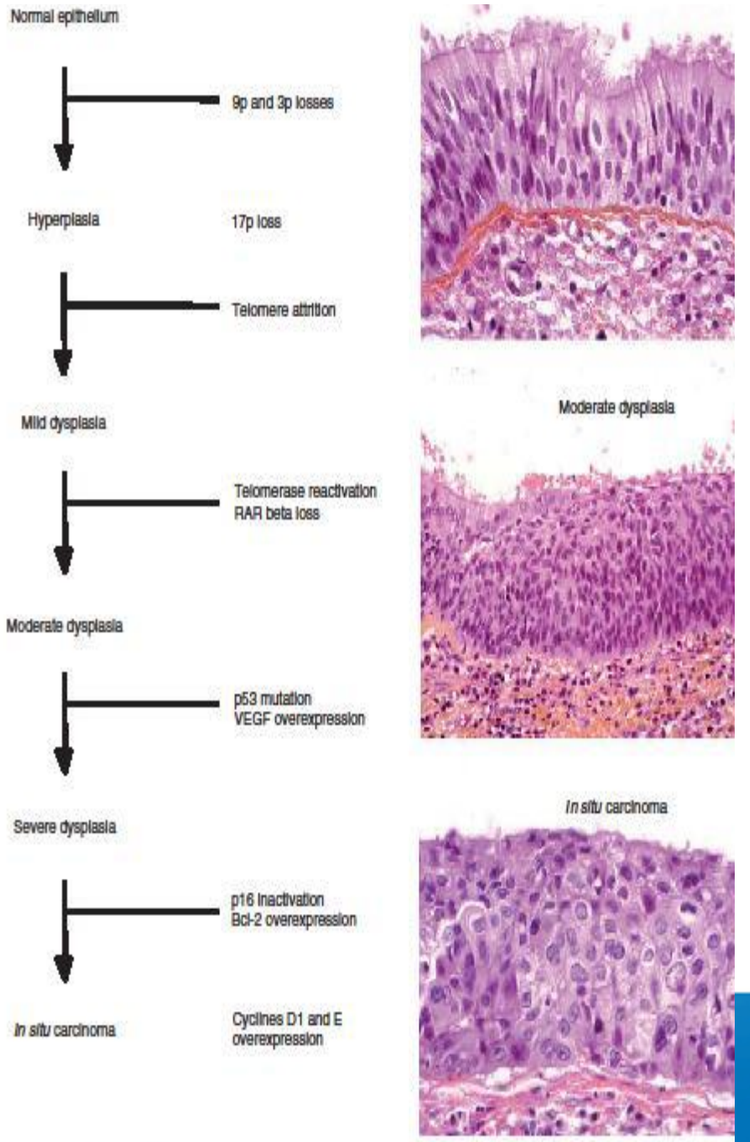


"..data collected from large cohorts of human cancers demonstrated that the immune-classification has a prognostic value that may be superior to TNM classification. Thus, it is imperative to begin incorporating immune scoring as a marker to classify cancers, as part of the routine…"

Galon et al: SITC; EATI, NCI consortium. J Tranlat Med 2012.

2030 Usefulness of miRNA as prognostic factors in early stage NSCLC, *Ramirez et al, Barcelona*

- Regulación de SOX2.
  - miR-145 regula su traslación.
  - SOX2 regula cluster miR302-367
- <miR302, >miR367 en SCC asociado con pronóstico
- 2043 SOX2 amplification in bronchial squamous dysplasia.



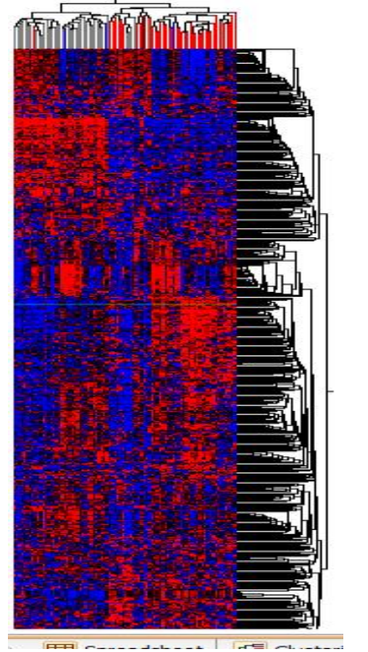
elcc
3RD EUROPEAN LUNG CANCER CONFERENCE
Geneva, Switzerland,
18-21 April 2012
ESMO
IASLC

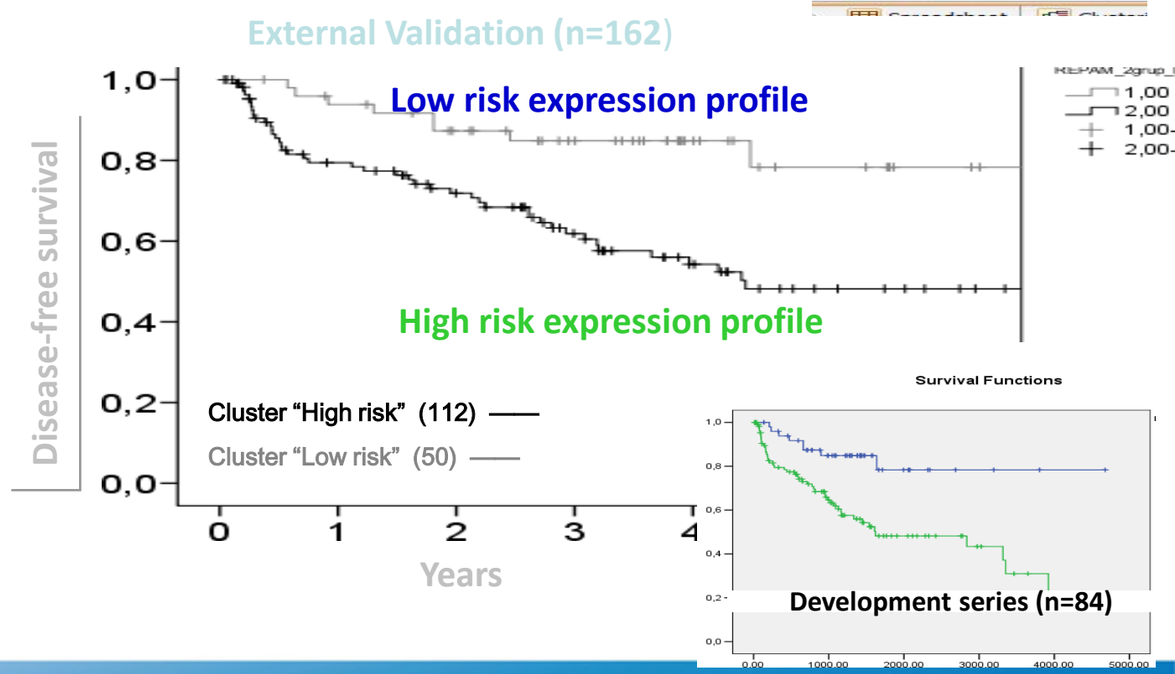
"A 50-gene expression profile classifies early-stage NSCLC (n=246) into low and high recurrence risk" *Sanz et al, HCSC*

Design:

- Check clinicopathological variables, K-ras, EGFR or bRaf mutations as **prognostic factors** for early NSCLC.
- **Research Quality:** Surgery, Biorepository Pathology, Microarray technology,….
- "Whole genome microarrays" (Agilent Tech™). **Non-supervised** hierarchical clustering and k-means. DFS analysis (Kaplan-Meier curves).
- **Validation:** external multicentric dataset
- Analysis of the **biological significance** of the gene signature.

Results:

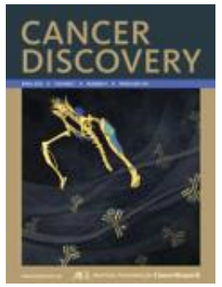




Hazard Ratio: 3.359, Log-Rank: P=0.001

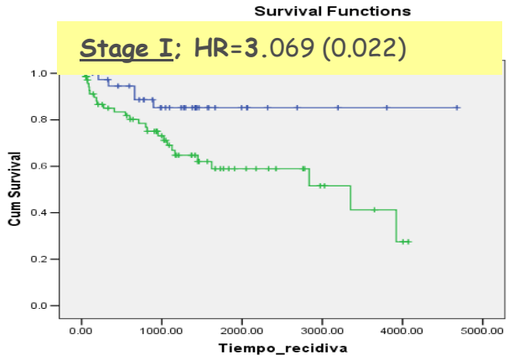
USCAP AACR HIGHLIGHTS 2012

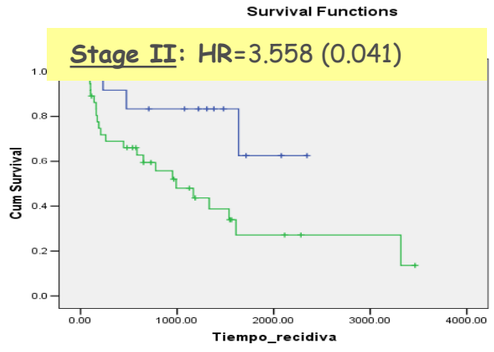
American Association for Cancer Research



"Gene Expression Signature Predicts Lung Cancer Relapse"

-Classifies **SCC and adenocarcinomas.**
-Classifies **only-Stage I** and **only-Stage II**.





-"Low risk of recurrence" profile in **1/3 of NSCLC**. It represents a specific **intratumoral immune response mediated by B/plasma cells.**

- *"::.there is a pressing need for both defining those patients at the greatest risk of relapse for trials of adjuvant therapy, but also potentially avoiding the toxicity of adjuvant therapy in patients with a low risk of relapse. This is a potentially extremely important classifier, which if independently validated, could be the basis of such a test …**David Carbone**; Vanderbilt-Ingram Cancer Center; Nashville, TN, EEUU*

Clinical implications:

- Prognostic factor additional to TNM for early NSCLC.
- Method to evaluate ("score") intratumoral immune response.
- Negative predictor of adjuvant chemotherapy for tumours with the "immune" signature.
- Biomarker predictive of response to certain immunotherapies or other personalized therapies.

USCAP & AACR HIGHLIGHTS 2012


Gracias….