

# Gene Expression and Epigenetics of Lung Cancer

**Ming-Sound Tsao, MD, FRCPC**

**Princess Margaret Cancer Centre**

**University of Toronto**

26-29 March 2014, Geneva, Switzerland

**25 min**

Organisers

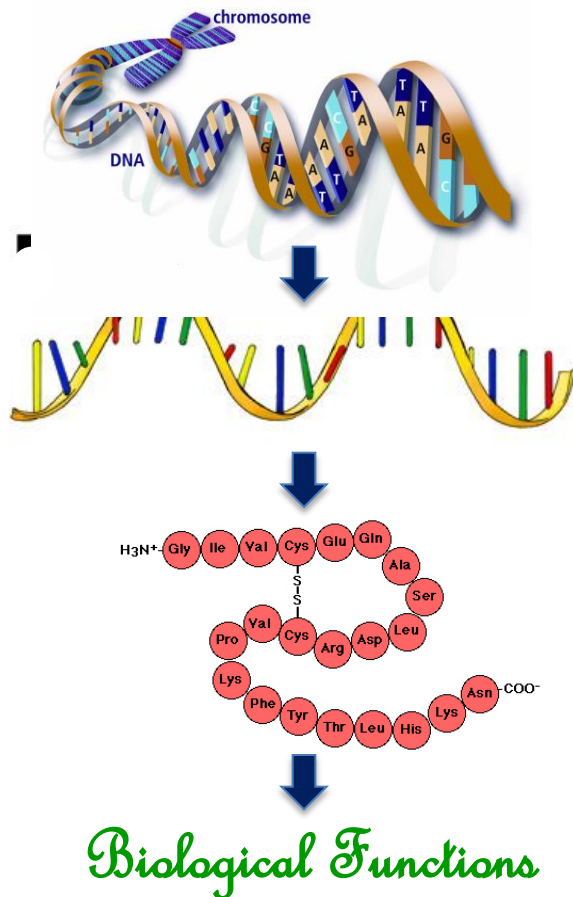
# Disclosure slide

- Patent holder on the 15-gene signature
- Honorarium from Precision Therapeutics

# Topics of Discussion

1. Central Dogma in Biology
2. Epigenetic regulation of gene expression
3. Lung cancer epigenome and microRNA
4. Gene expression in lung cancers
5. Prognostic gene expression signature

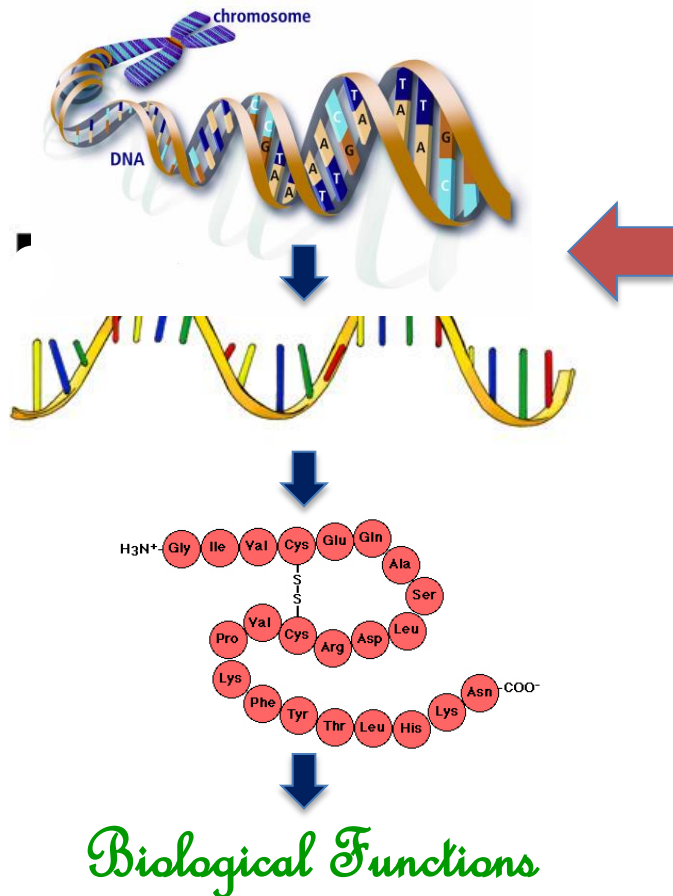
# Central Dogma in Biology



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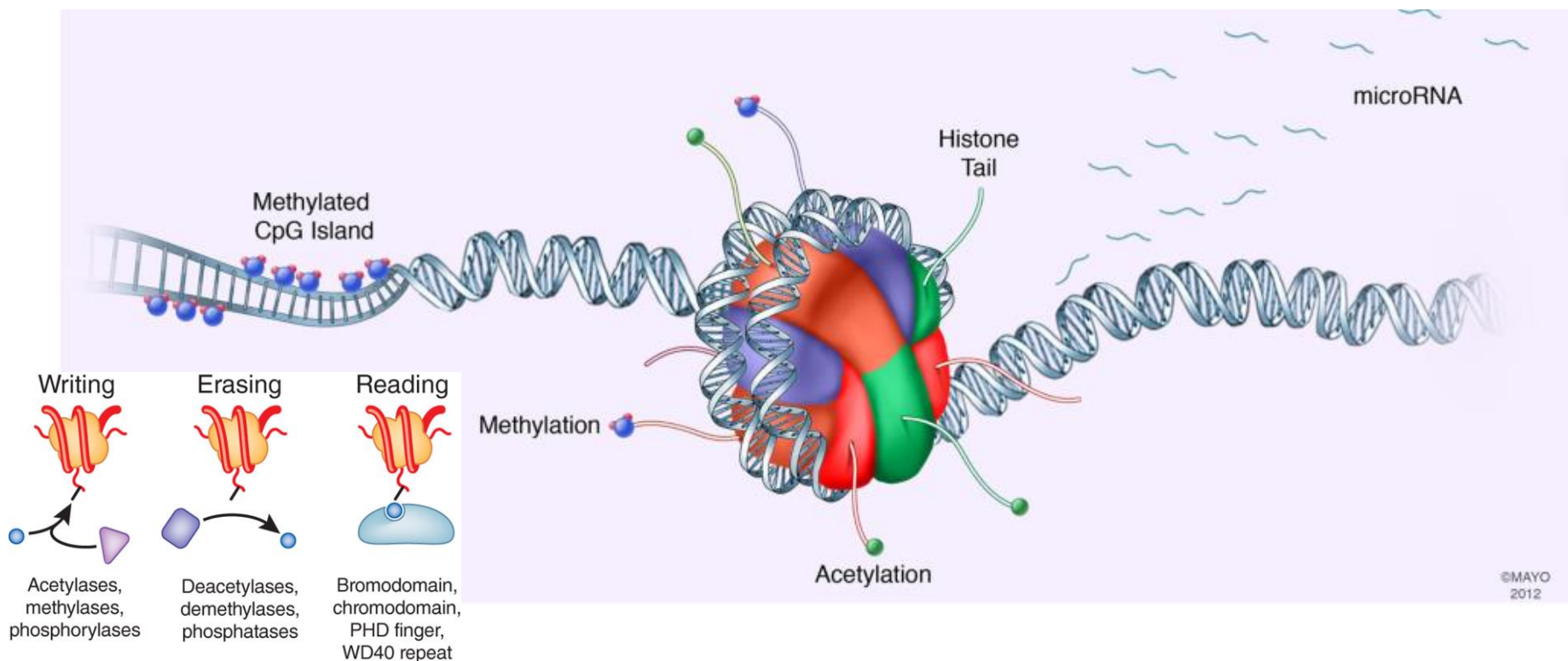
# Central Dogma in Biology



**Epigenetics: A mechanism for regulating gene activity independent of gene sequences that determine which genes are turned on and off in:**

- specific cell type
- different disease state
- response to specific physiological stimulus

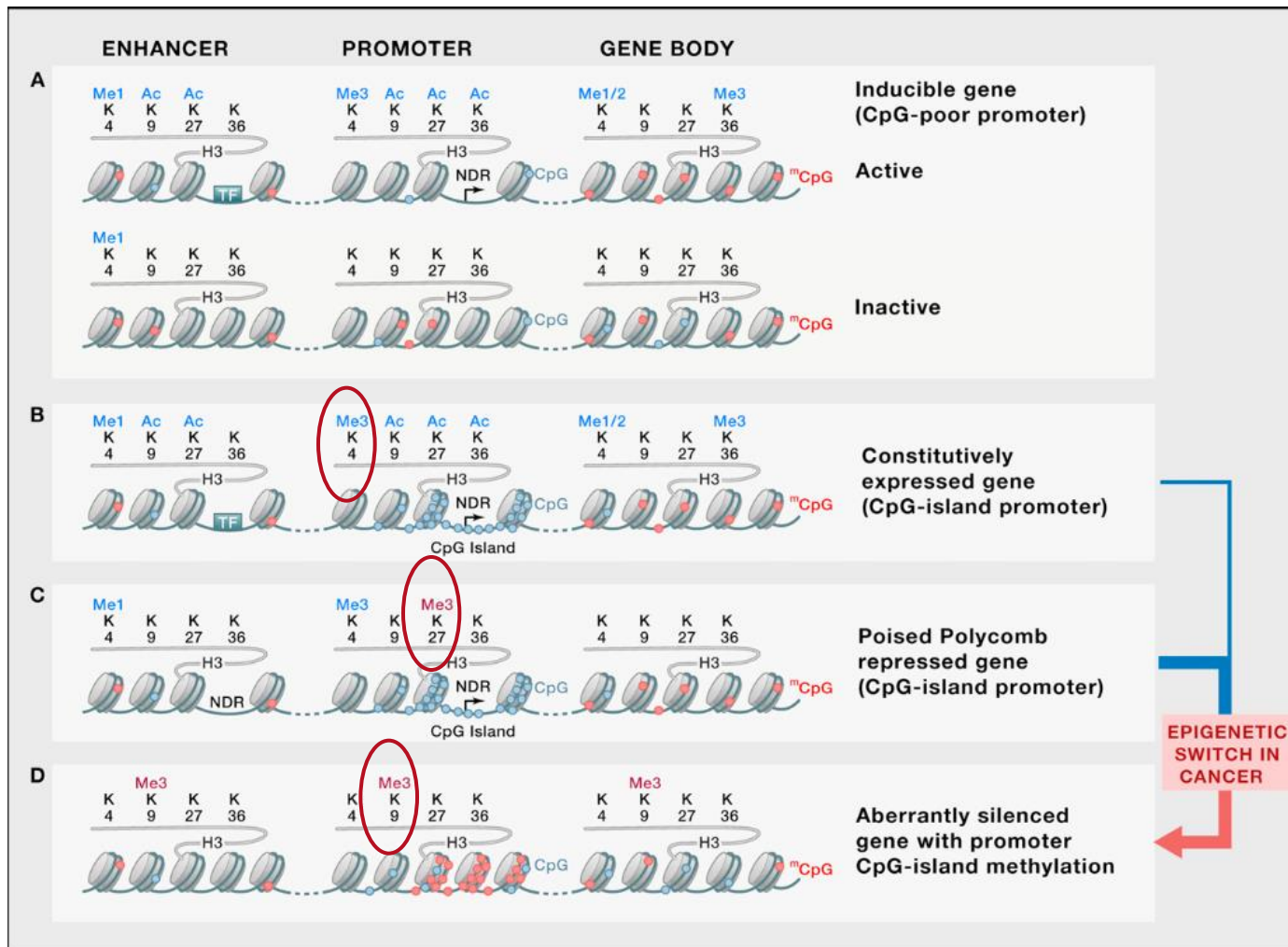
# Epigenetic Mechanisms



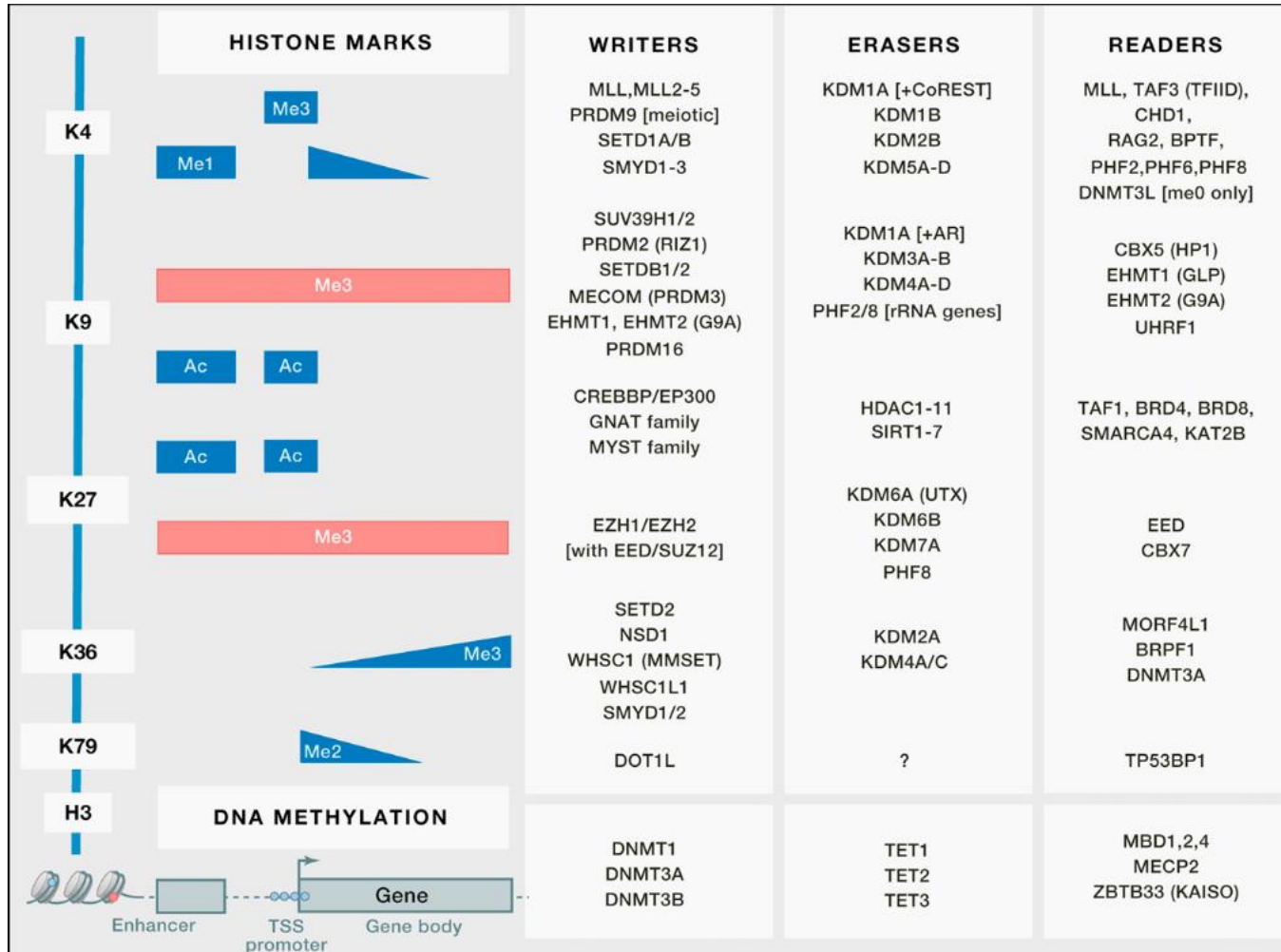
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# Epigenetic States



# Histone H3 (H3F3A)



**Writer:** establish the epigenetic marks

**Eraser:** remove the epigenetic marks

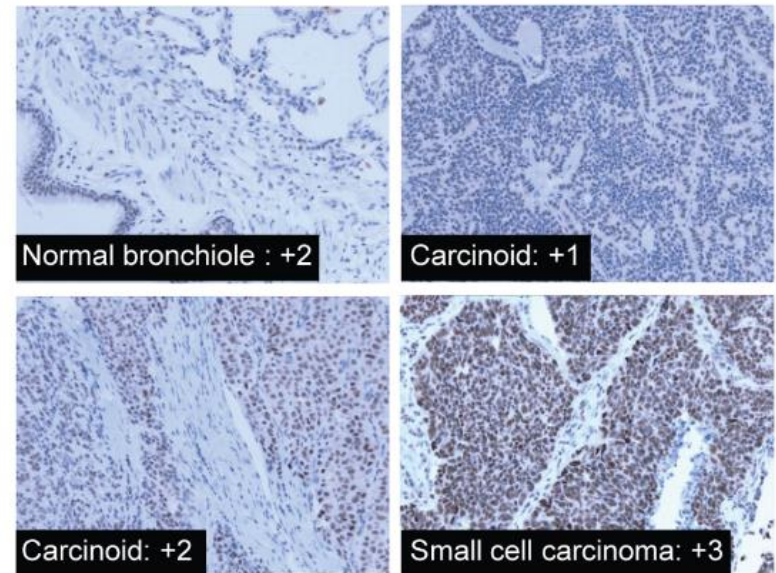
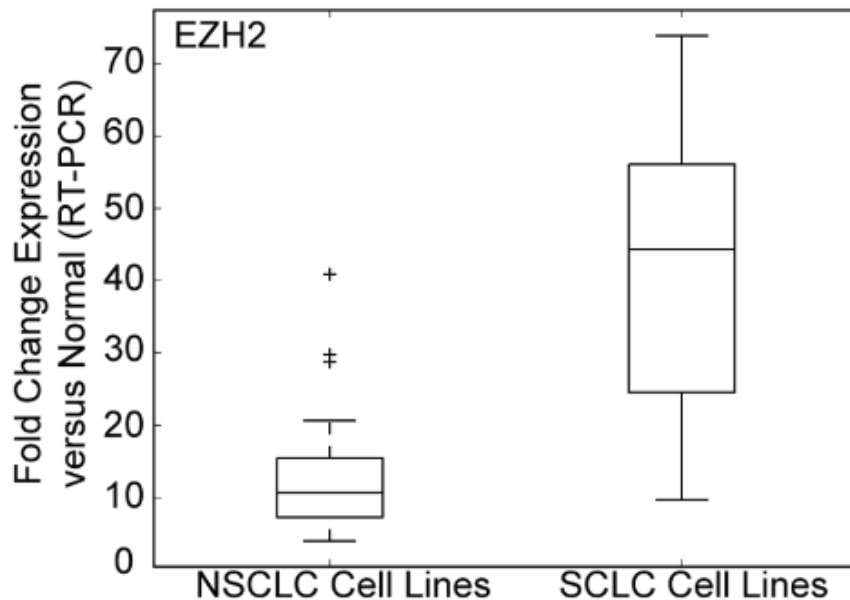
**Reader:** interpret the epigenetic marks

Martin Peifer<sup>1,2,57</sup>, Lynnette Fernández-Cuesta<sup>1,2,57</sup>, Martin L Sos<sup>1-4</sup>, Julie George<sup>1,2</sup>, Danila Seidel<sup>1,2,5</sup>, *et al*



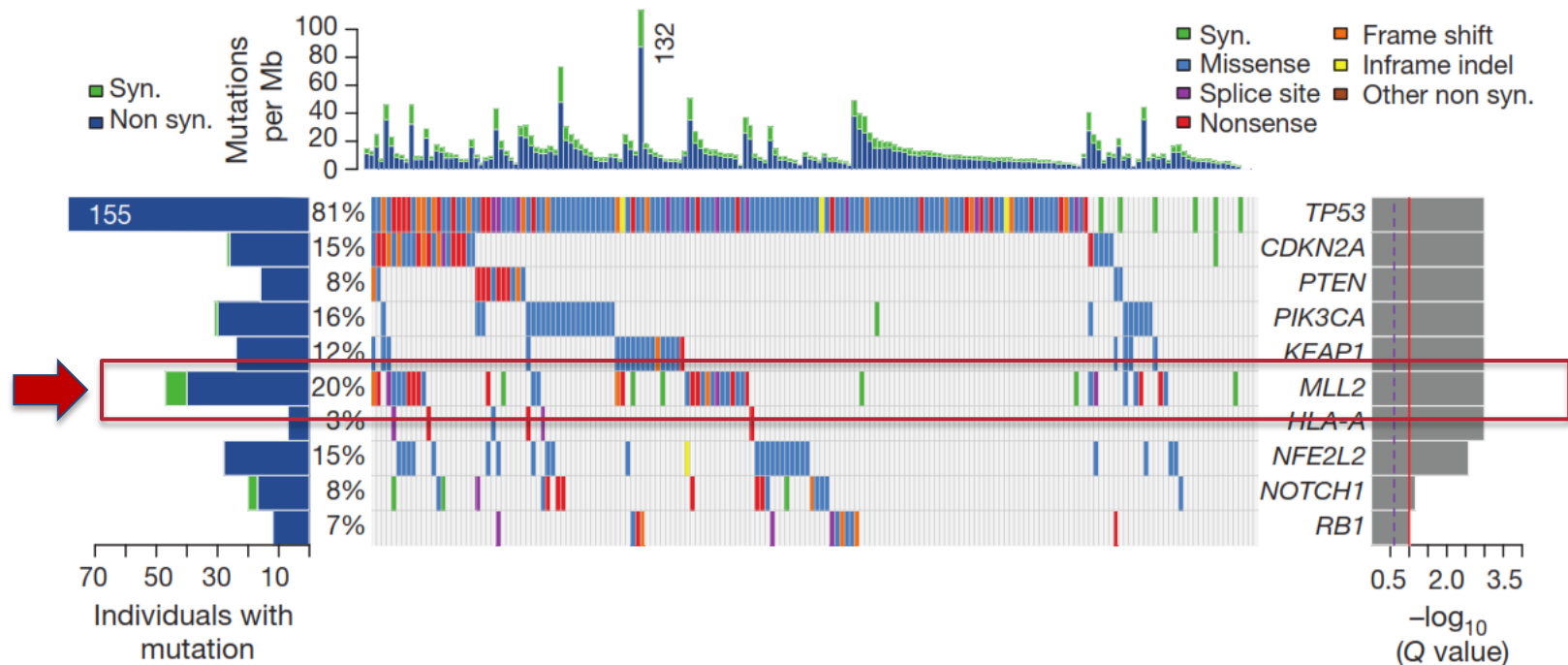
# Genomic Deregulation of the E2F/Rb Pathway Leads to Activation of the Oncogene EZH2 in Small Cell Lung Cancer

Bradley P. Coe<sup>1</sup>\*, Kelsie L. Thu<sup>1</sup>\*, Sarit Aviel-Ronen<sup>2</sup>, Emily A. Vucic<sup>1</sup>, Adi F. Gazdar<sup>3</sup>, Stephen Lam<sup>1</sup>, Ming-Sound Tsao<sup>4,5</sup>, Wan L. Lam<sup>1</sup>



# Comprehensive genomic characterization of squamous cell lung cancers

The Cancer Genome Atlas Research Network\*



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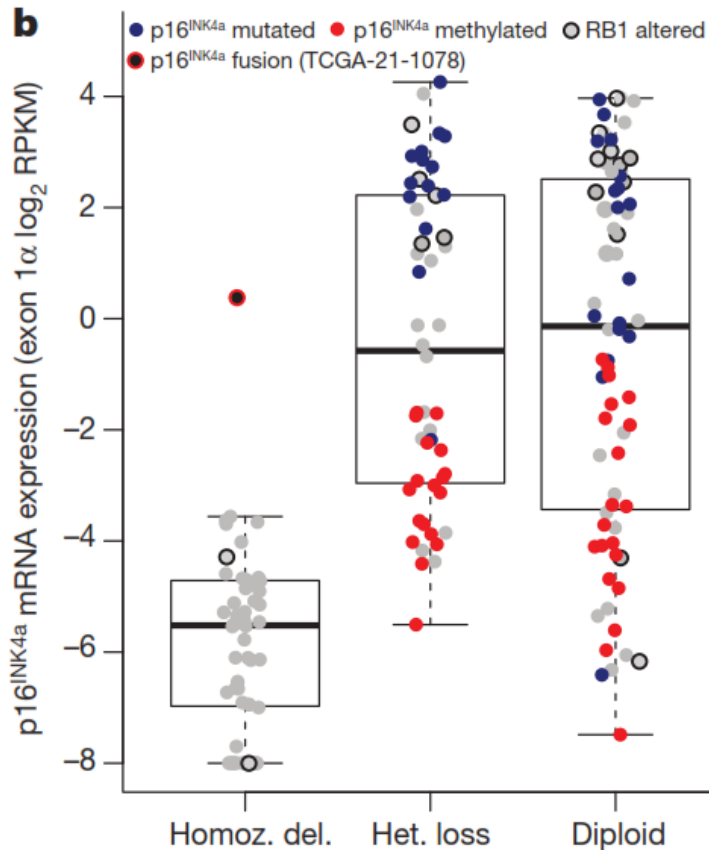


*Nature* 2012;489:519–525 (published on line September 9)

Lung Cancer

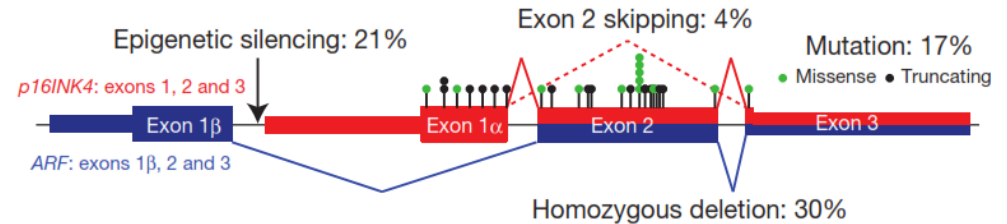
European Society for Medical Oncology

# CDKN2A Silencing in SqCC



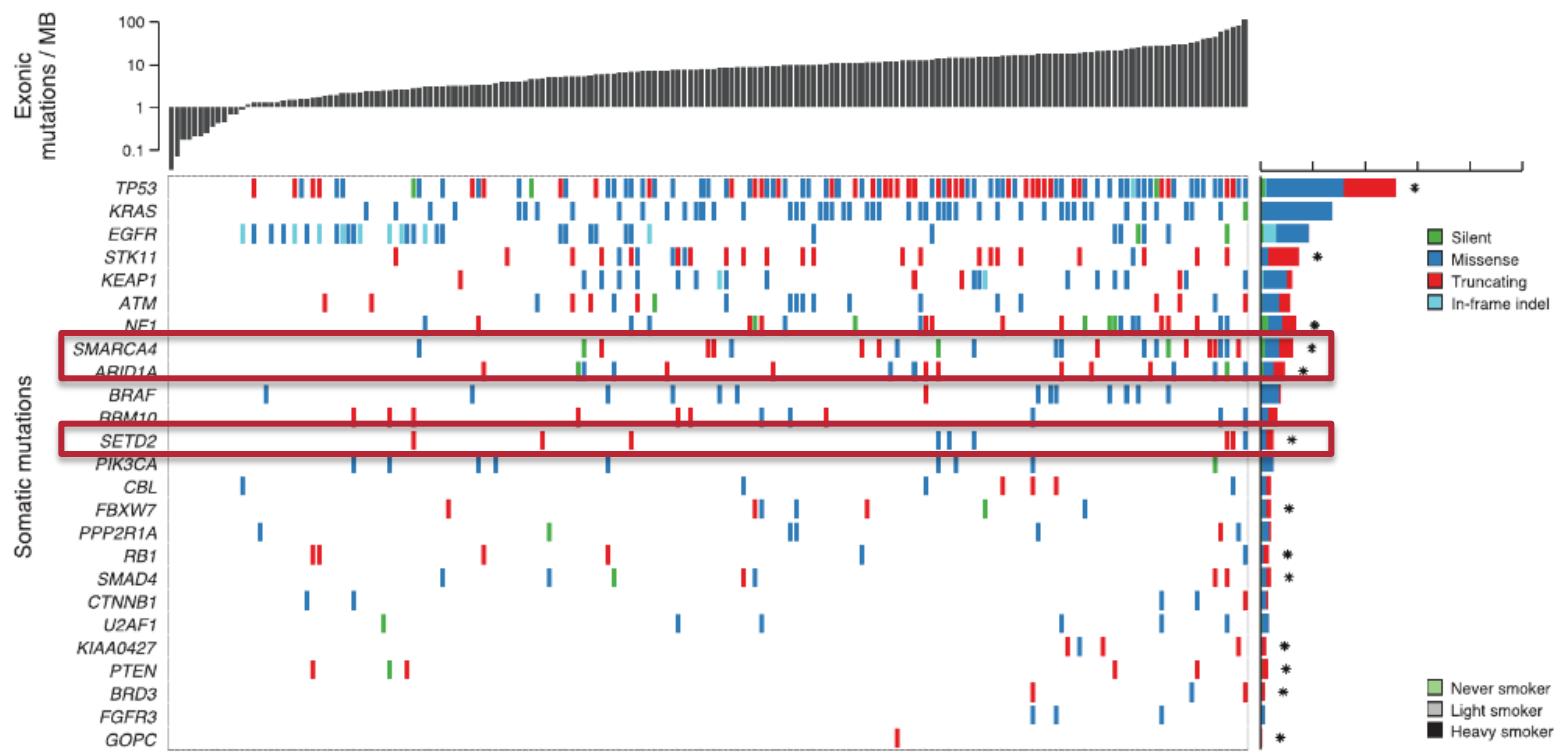
**a** CDKN2A locus

p16<sup>INK4a</sup> alteration rate: 72%



# Mapping the Hallmarks of Lung Adenocarcinoma with Massively Parallel Sequencing

Marcin Imielinski,<sup>1,2,3,5,18</sup> Alice H. Berger,<sup>1,5,18</sup> Peter S. Hammerman,<sup>1,5,18</sup> Bryan Hernandez,<sup>1,18</sup> Trevor J. Pugh,<sup>1,5,18</sup> *et al.*



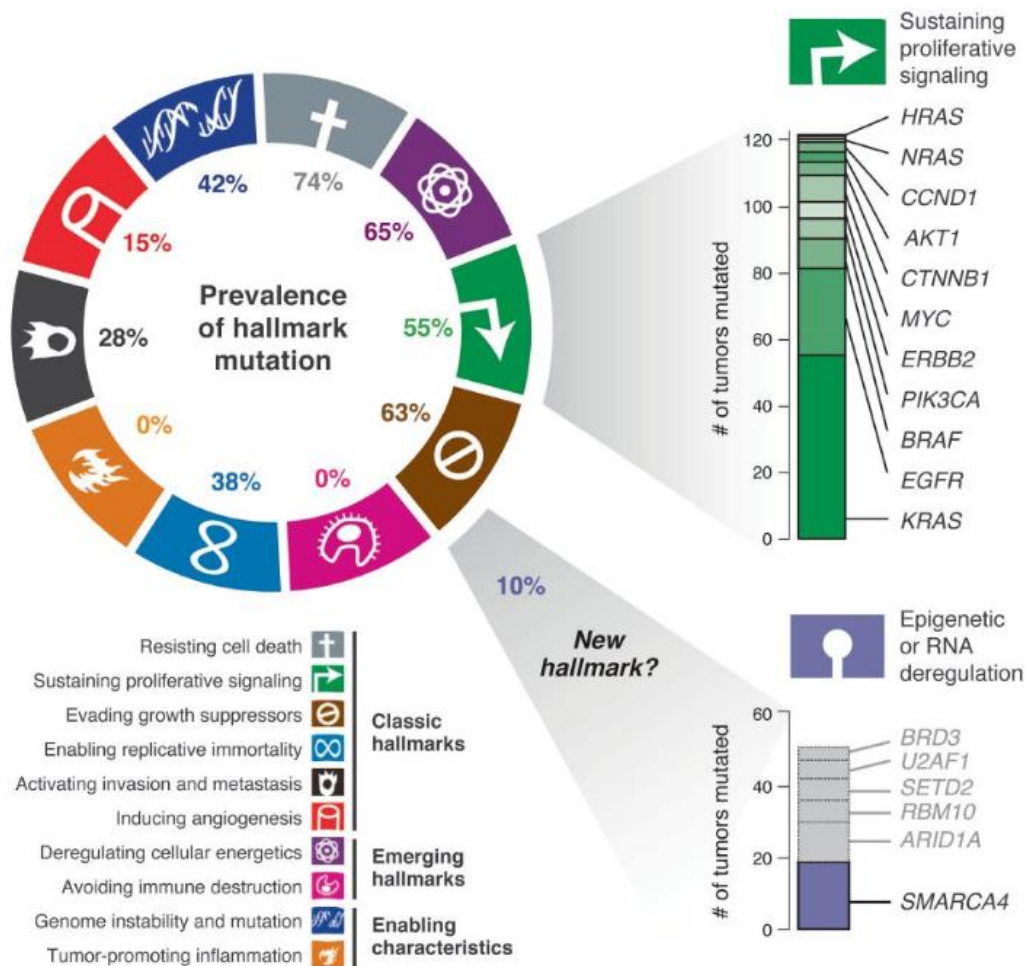
26-29 March 2014, Geneva, Switzerland

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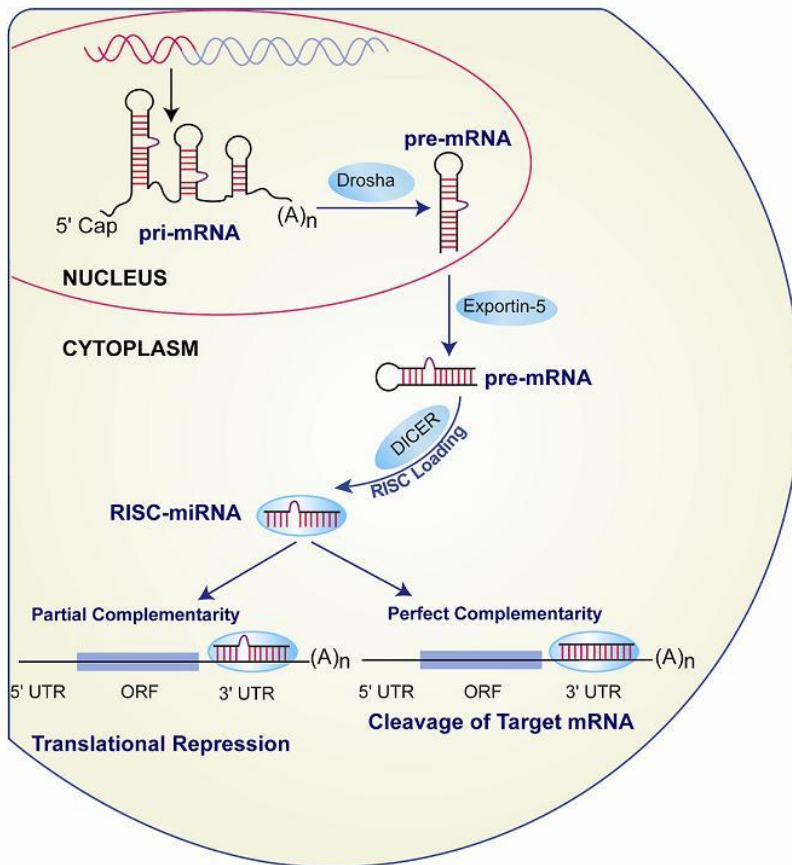
*Cell* 2012;150 (September), 1107–1120

# Hallmark of Lung Adenocarcinoma



26-29 March 2014, G

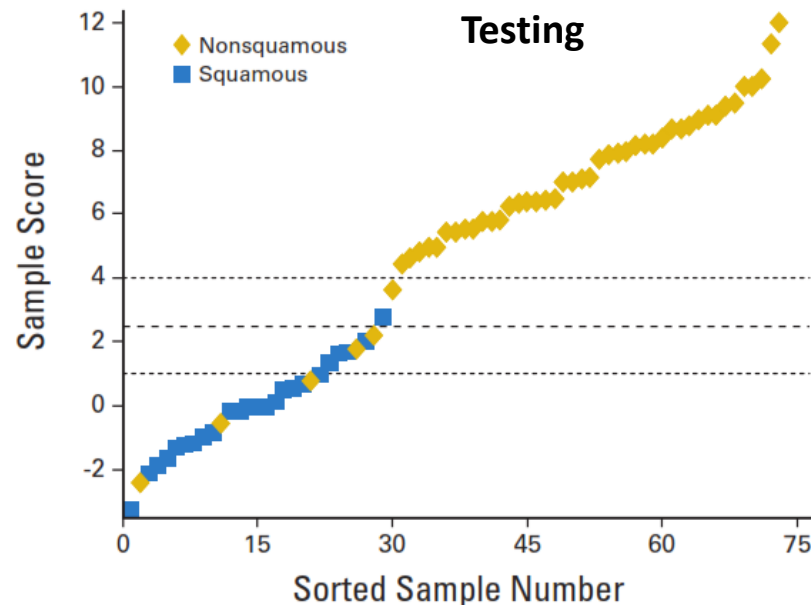
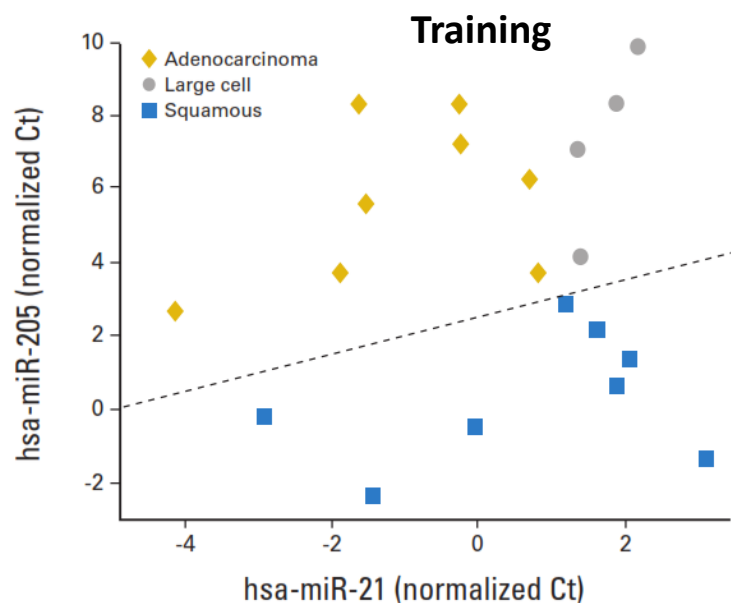
# Micro-RNA (miRNA)



- Small non-coding RNA (18-22 nucleotides long)
- Key regulators in many biological processes
- Negatively regulate gene expression

# Diagnostic Assay Based on hsa-miR-205 Expression Distinguishes Squamous From Nonsquamous Non-Small-Cell Lung Carcinoma

Danit Lebanony, Hila Benjamin, Shlomit Gilad, Meital Ezagouri, Avital Dov, Karin Ashkenazi, Nir Gefen, Shai Izraeli, Gideon Rechavi, Harvey Pass, Daisuke Nonaka, Junjie Li, Yael Spector, Nitzan Rosenfeld, Ayelet Chajut, Dalia Cohen, Ranit Aharonov, and Mahesh Mansukhani



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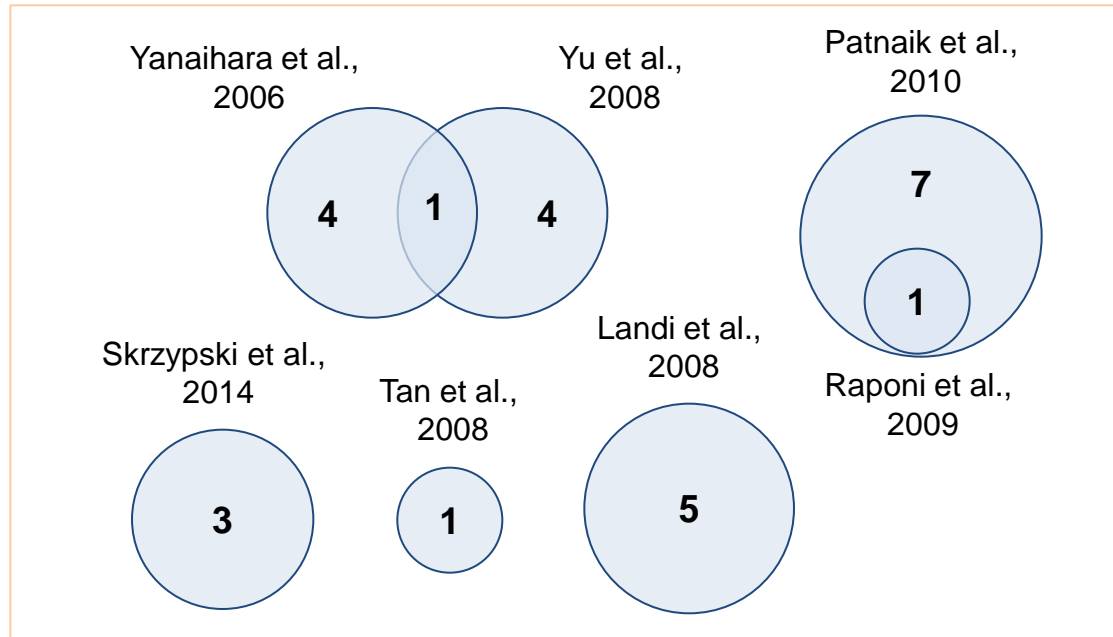
International Association for the Study of Lung Cancer



European Society for Medical Oncology

*J Clin Oncol* 2009;27:2030-2037.

# miRNA May Predict Prognosis of NSCL Patients



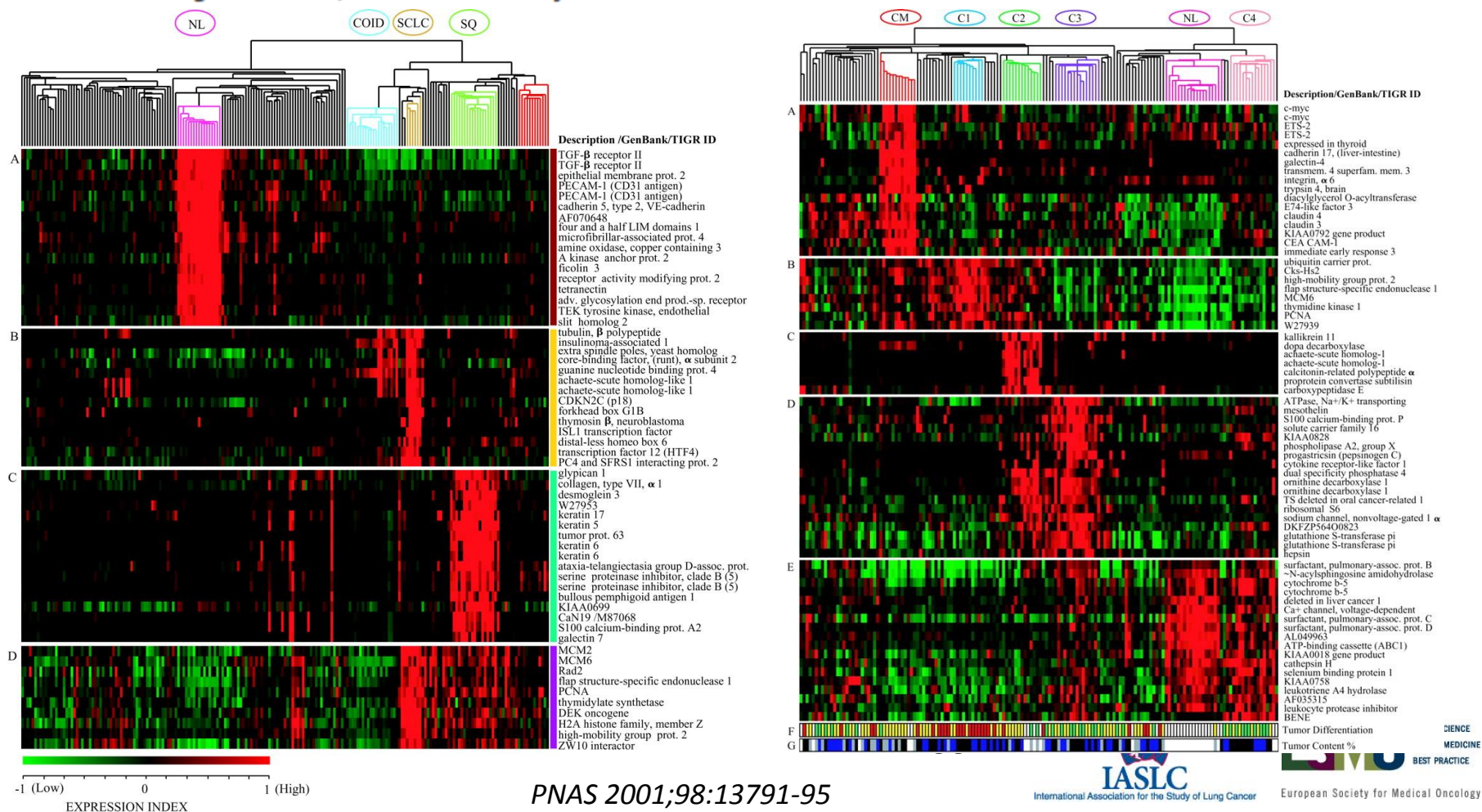
**Suggested to be superior biomarkers compared to mRNAs\!!!!;**

- **Lower complexity (~2,100 compared to ~30,000 coding mRNAs)**
- **Stable against enzymatic degradation and in FFPE specimen**

Landi MT, et al. 2010; Clin Cancer Res 16(2):430–441; Patnaik SK, et al. 2010; Cancer Res 70(1):36–45.; Tan X, et al. Clin Cancer Res. 2011; 17(21):6802–6811; Raponi M, et al. Cancer Res. 2009;69(14):5776–83.; Skrzypski M, et al. 2014; Br J Cancer 110:991–1000; Yanaihara N et al. 2006; Cancer Cell 9(3):189–198; Yu SL, et al. Cancer Cell. 2008;13(1):48–57.

# Classification of human lung carcinomas by mRNA expression profiling reveals distinct adenocarcinoma subclasses

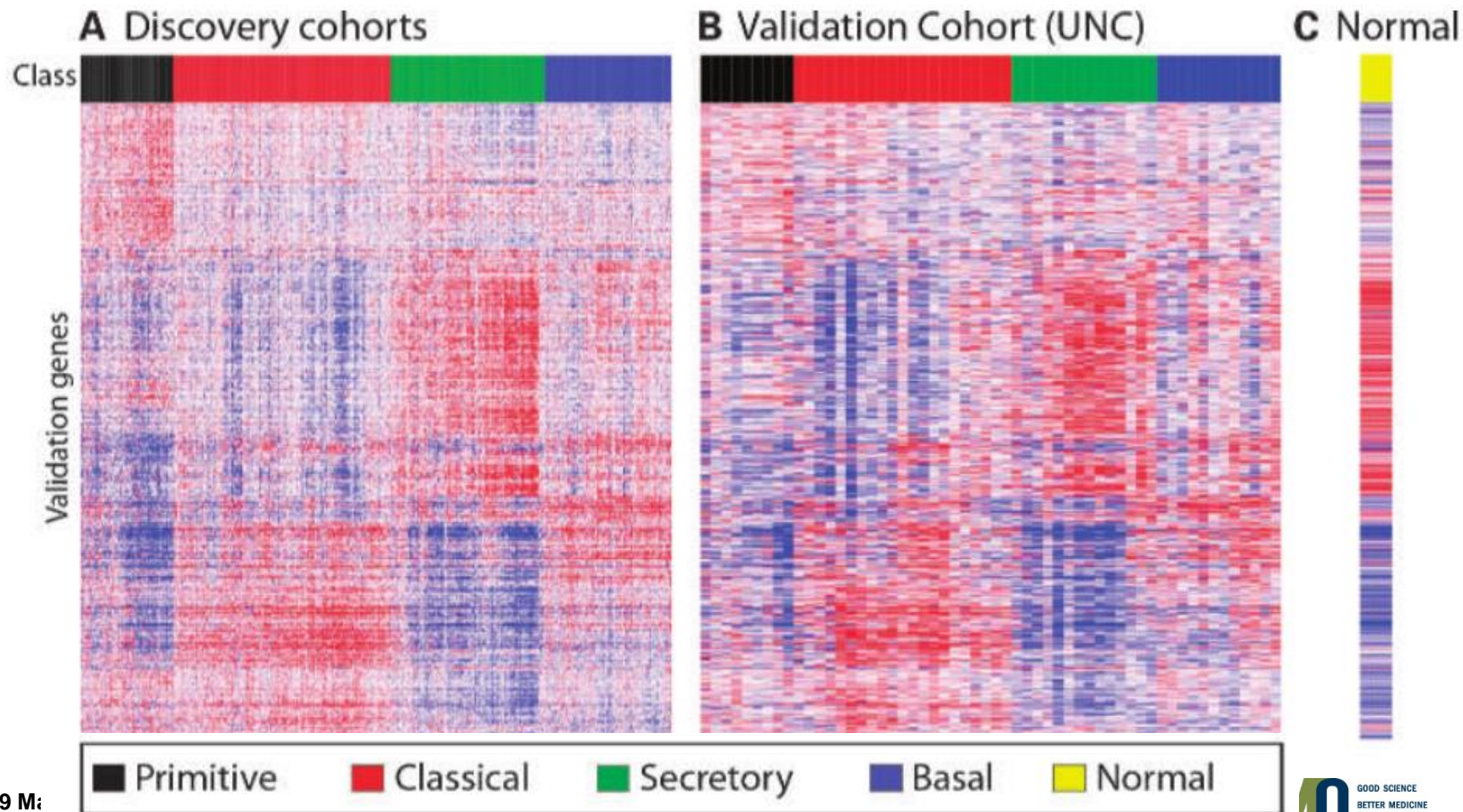
Arindam Bhattacharjee<sup>\*,†</sup>, William G. Richards<sup>\*,‡</sup>, Jane Staunton<sup>\*,¶</sup>, Cheng Li<sup>||</sup>, Stefano Monti<sup>||</sup>, Priya Vasa<sup>\*</sup>, Christine Ladd<sup>||</sup>, Javad Beheshti<sup>\*</sup>, Raphael Bueno<sup>‡</sup>, Michael Gillette<sup>||</sup>, Massimo Loda<sup>\*,\*\*</sup>, Griffin Weber<sup>\*</sup>, Eugene J. Mark<sup>††</sup>, Eric S. Lander<sup>||</sup>, Wing Wong<sup>||</sup>, Bruce E. Johnson<sup>\*</sup>, Todd R. Golub<sup>||,‡,§,¶,||</sup>, David J. Sugarbaker<sup>‡,¶,||</sup>, and Matthew Meyerson<sup>\*,§,¶,||</sup>



# Lung Squamous Cell Carcinoma mRNA Expression Subtypes Are Reproducible, Clinically Important, and Correspond to Normal Cell Types

Matthew D. Wilkerson, Xiaoying Yin, Katherine A. Hoadley, et al.

*Clin Cancer Res* 2010;16:4864-4875. Published OnlineFirst July 19, 2010.



26-29 M:

Functional:

Proliferation Xenobiotic/energy Immune resp. Cell adhesion

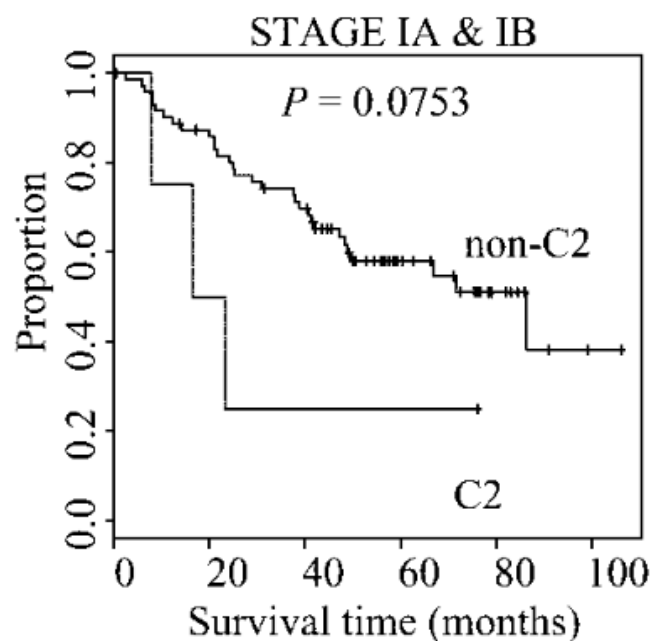
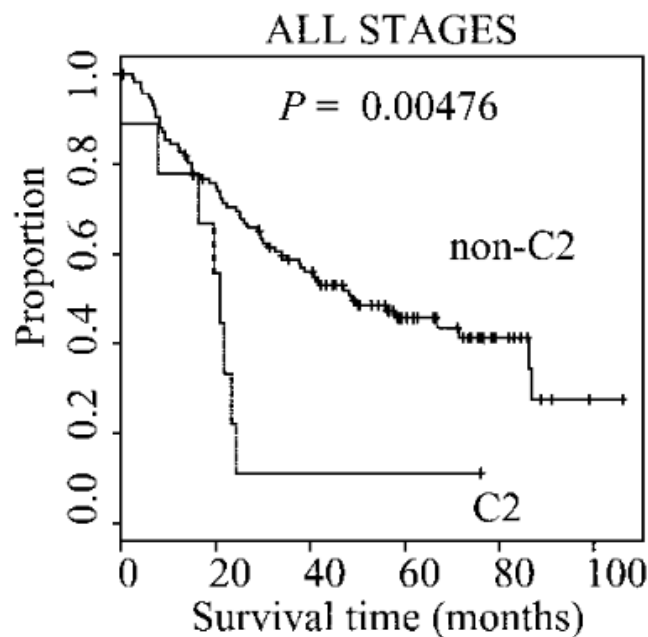
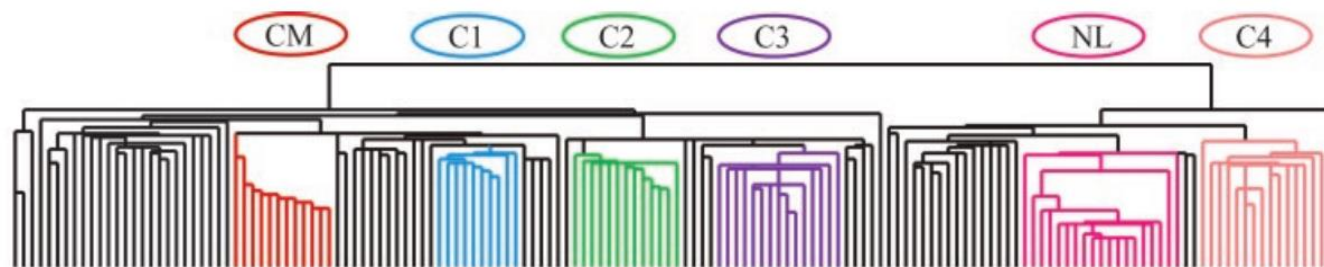
# Comprehensive genomic characterization of squamous cell lung cancers

The Cancer Genome Atlas Research Network\*

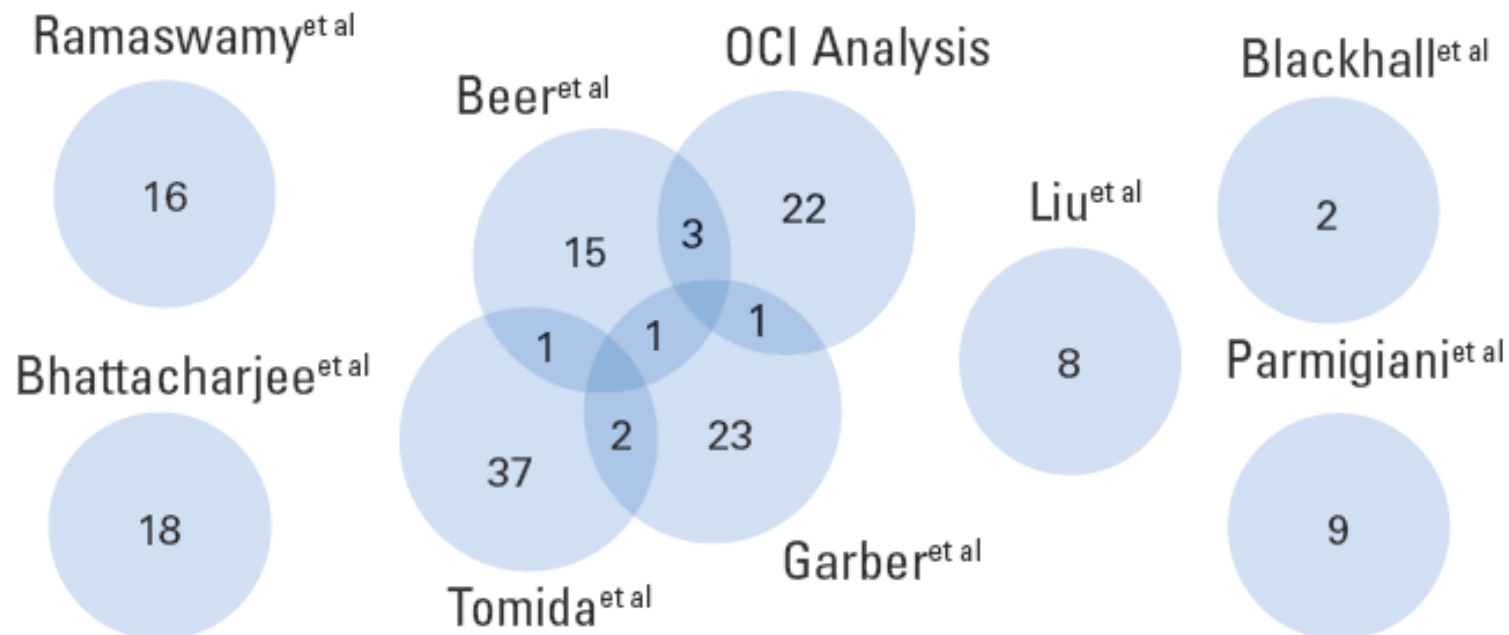


*Nature* 2012;489:519–525 (published on line September 9)

## Classification of human lung carcinomas by mRNA expression profiling reveals distinct adenocarcinoma subclasses



# Prognostic Gene Sets from Early Microarray Studies (2001-2004)



## Micro-array Prognostic Signatures (2005-07)

	Tumor Type	Sample Size	Gene Set	Validation
Raponi (2006)	SQC	129	50	Independent cohort (n=36; p=0.04)
Lu (2006)	NSCLC	197	64	MSKCC (n=63; stage I only p=1.5 x 10 <sup>-6</sup> ) Duke (n=64; stage I only p=6 x 10 <sup>-11</sup> )
Larsen (2007)	ADC	48	54	Independent cohort (n=55, p=0.039)
Larsen (2007)	SQC	51	111	Independent cohort (n=58; p=0.0008)
Raponi (2006)	ADC	86	47	Duke validation cohort (n=36; p=0.0008)

*Raponi M et al. Cancer Res 2006;66:7466-72. Lu Y, et al. PLOS Med 2006;3:e467. Larsen JE, et al. CCR 2007;13:2946; Carcinogenesis 2007;28:76*

# Gene expression–based survival prediction in lung adenocarcinoma: a multi-site, blinded validation study

Director's Challenge Consortium for the Molecular Classification of Lung Adenocarcinoma:<sup>\*1</sup>

Kerby Shedden<sup>2,3,17</sup>, Jeremy M G Taylor<sup>3,4,17</sup>, Steven A Enkemann<sup>5,17</sup>, Ming-Sound Tsao<sup>6,17</sup>, Timothy J Yeatman<sup>5,17</sup>, William L Gerald<sup>7,17</sup>, Steven Eschrich<sup>5,17</sup>, Igor Jurisica<sup>6,17</sup>, Thomas J Giordano<sup>8</sup>, David E Misek<sup>3,9</sup>, Andrew C Chang<sup>3,9</sup>, Chang Qi Zhu<sup>6</sup>, Daniel Strumpf<sup>6</sup>, Samir Hanash<sup>3</sup>, Frances A Shepherd<sup>6</sup>, Keyue Ding<sup>10</sup>, Lesley Seymour<sup>10</sup>, Katsuhiko Naoki<sup>11</sup>, Nathan Pennell<sup>11</sup>, Barbara Weir<sup>11</sup>, Roel Verhaak<sup>11</sup>, Christine Ladd-Acosta<sup>12</sup>, Todd Golub<sup>12</sup>, Michael Gruidl<sup>5</sup>, Anupama Sharma<sup>5</sup>, Janos Szoke<sup>7</sup>, Maureen Zakowski<sup>7</sup>, Valerie Rusch<sup>7</sup>, Mark Kris<sup>7</sup>, Agnes Viale<sup>7</sup>, Noriko Motoi<sup>7</sup>, William Travis<sup>7</sup>, Barbara Conley<sup>13</sup>, Venkatraman E Seshan<sup>14,17</sup>, Matthew Meyerson<sup>11,12,17</sup>, Rork Kuick<sup>3,17</sup>, Kevin K Dobbin<sup>15,17</sup>, Tracy Lively<sup>16,17</sup>, James W Jacobson<sup>16,17</sup> & David G Beer<sup>3,9,17</sup>

Nature Medicine 2008;14:822-827

**Provided the largest publicly available multi-institutional derived microarray dataset for future gene expression studies in lung adenocarcinoma**

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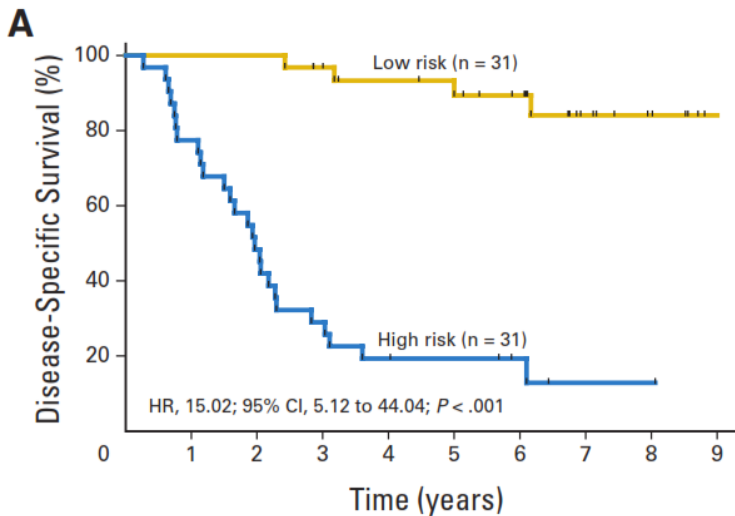
# Gene Signatures with Potential Predictiveness for Adjuvant Chemotherapy Benefit

1. Zhu CQ, *et al.* Prognostic and Predictive Gene Signature for Adjuvant Chemotherapy in Resected Non-Small Cell Lung cancer. J Clin Oncol. 2010;28(29):4417-24.
2. Kratz JR, *et al.* A practical molecular assay to predict survival in resected non-squamous, non-small-cell lung cancer: development and international validation studies. Lancet 2012;379:823-32.
3. Van Laar RKV. Genomic signatures for predicting survival and adjuvant chemotherapy benefit in patients with non-small cell lung cancer. BMC Medical Genomics 2012;5:30
4. Chen DT, *et al.* Prognostic and predictive value of a malignancy-risk gene signature in early-stage non-small cell lung cancer. J Natl Cancer Inst 2011;103:1859-70
5. Tang H, *et al.* A 12-gene set predicts survival benefits from adjuvant chemotherapy in non-small cell lung cancer patients. Clin Cancer Res 2013;19:1577-86.
6. Wistuba I, *et al.* Validation of a Proliferation-Based Expression Signature as Prognostic Marker in Early Stage Lung Adenocarcinoma. Clin Cancer Res 2013 19:6261-6271.

# Prognostic and Predictive Gene Signature for Adjuvant Chemotherapy in Resected Non-Small-Cell Lung cancer

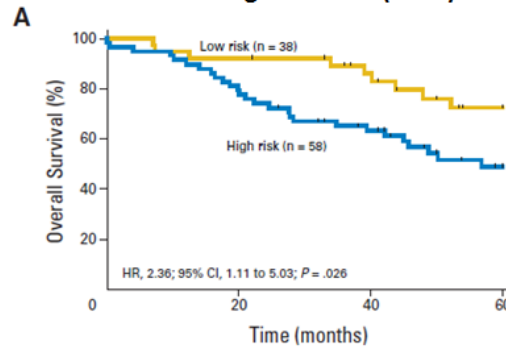
Chang-Qi Zhu, Keyue Ding, Dan Strumpf, Barbara A. Weir, Matthew Meyerson, Nathan Pennell, Roman K. Thomas, Katsuhiko Naoki, Christine Ladd-Acosta, Ni Liu, Melania Pintilie, Sandy Der, Lesley Seymour, Igor Jurisica, Frances A. Shepherd, and Ming-Sound Tsao

Training in JBR10

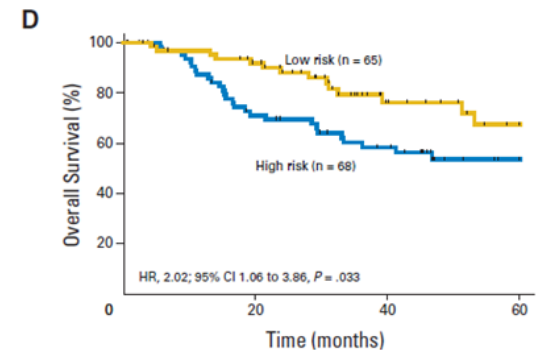
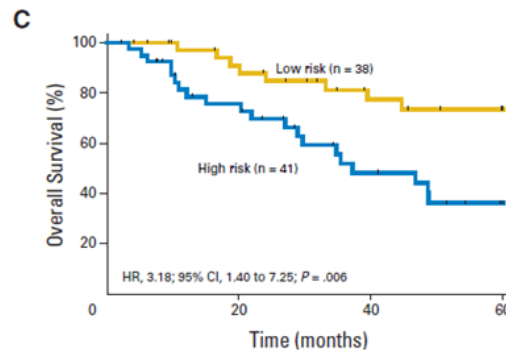
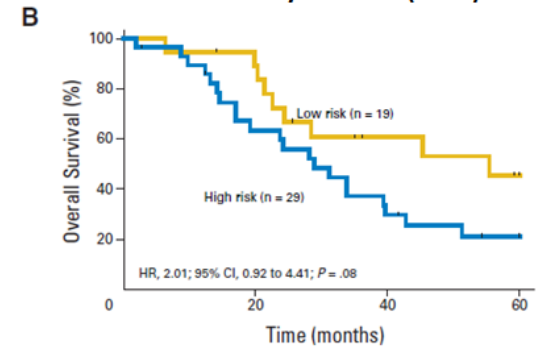


Testing in 4 other independent datasets

**Director's Challenge Patients (n=96)**



**Duke University Patients (n=48)**



**Univ. of Michigan Patients (n=79)**

**Netherland Cancer Institute (n=133)**

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Journal of Clinical Oncology September 7, 2010

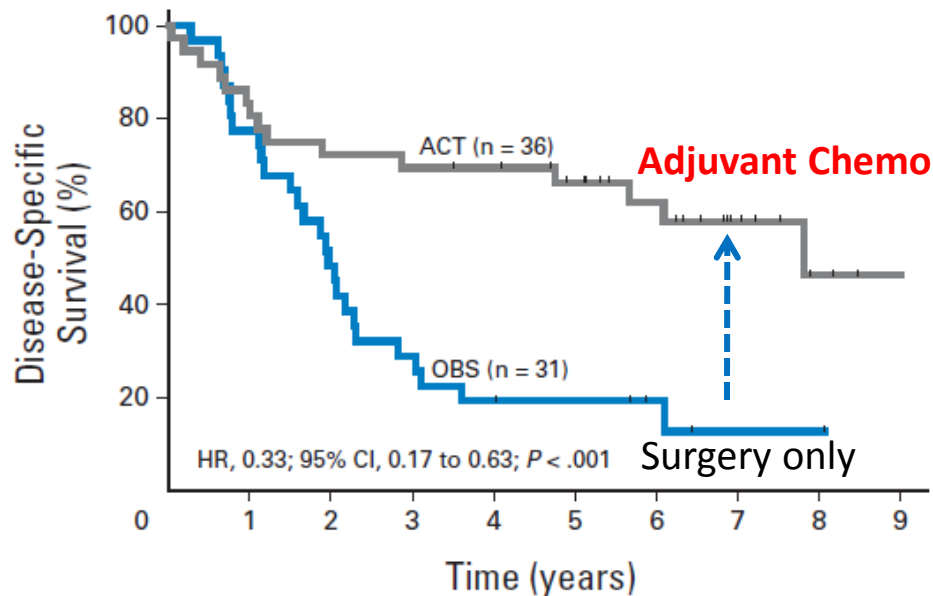
International Association for the Study of Lung Cancer

European Society for Medical Oncology

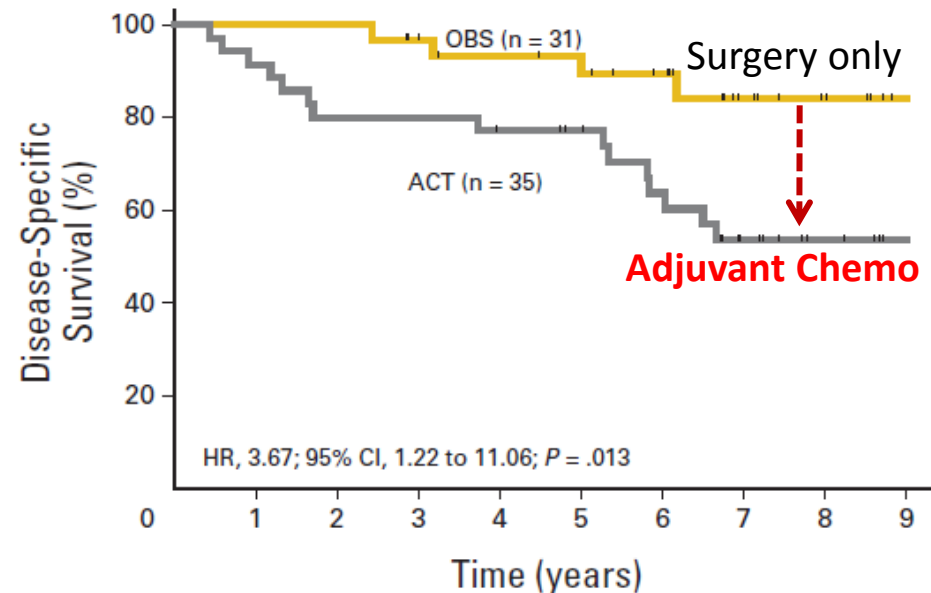
# Prognostic and Predictive Gene Signature for Adjuvant Chemotherapy in Resected Non-Small-Cell Lung cancer

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

Predicted as **High Risk (Poor Survival)**  
by Signature (Stage IB-II, n=67)



Predicted as **Low Risk (Good Survival)**  
by Signature (Stage IB-II, n=67)



**PREDICTIVENESS OF THE SIGNATURE STILL REQUIRES VALIDATION**

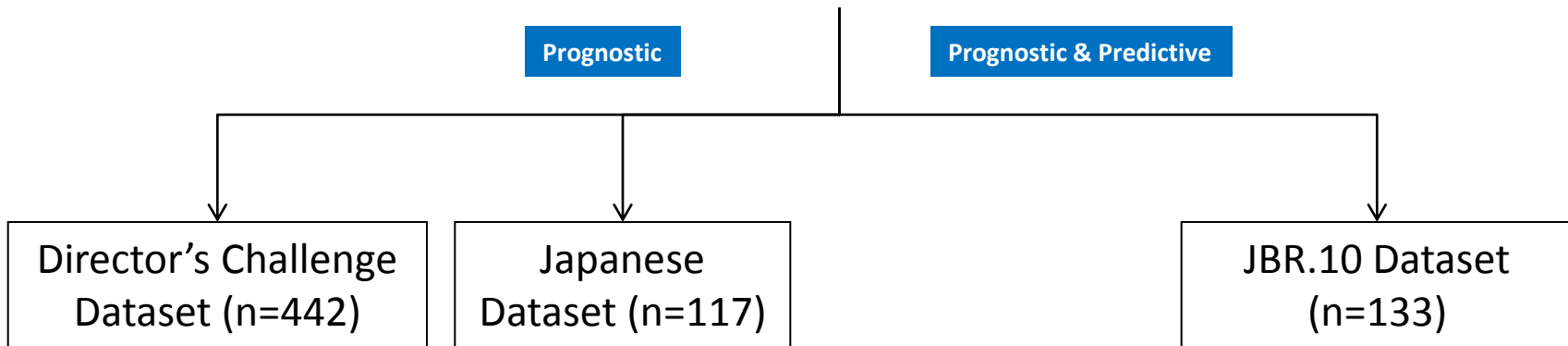
# Prognostic and Predictive Value of a Malignancy-Risk Gene Signature in Early-Stage Non-Small Cell Lung Cancer

Dung-Tsa Chen, Ying-Lin Hsu, William J. Fulp, Domenico Coppola, Eric B. Haura, Timothy J. Yeatman, W. Douglas Cress

**Moffitt Cancer Center and Research Institute**

## Malignancy-Risk Gene Signature

- Breast ca vs normal breast tissues
- 94 genes (102 probes) in Affymetrix U133A
- 56 (60%) are genes involved in proliferation



Gene expression–based survival prediction in lung adenocarcinoma: a multi-site, blinded validation study

Director's Challenge Consortium for the Molecular Classification of Lung Adenocarcinoma:<sup>\*1</sup>

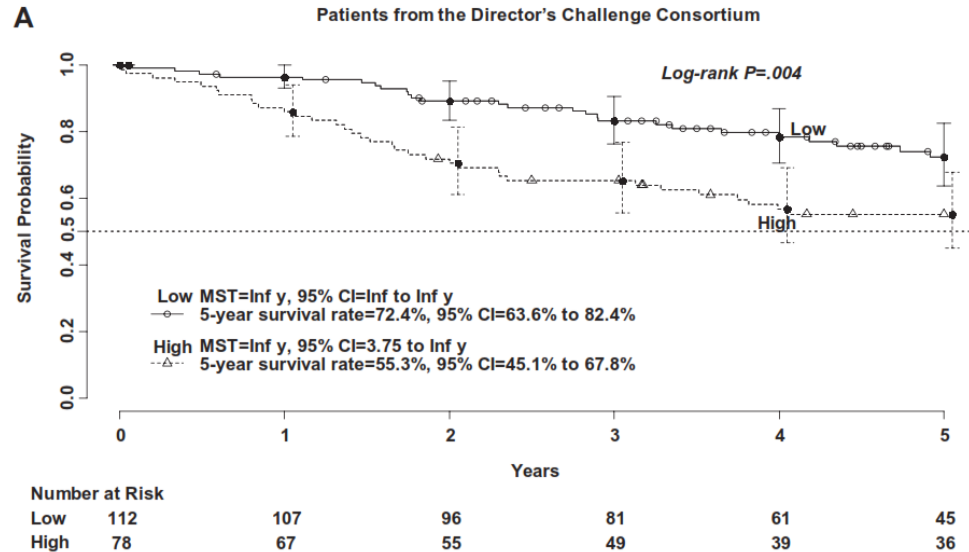
*Nature Medicine* 2008;14:822-827

**IASLC**  
International Association for the Study of Lung Cancer

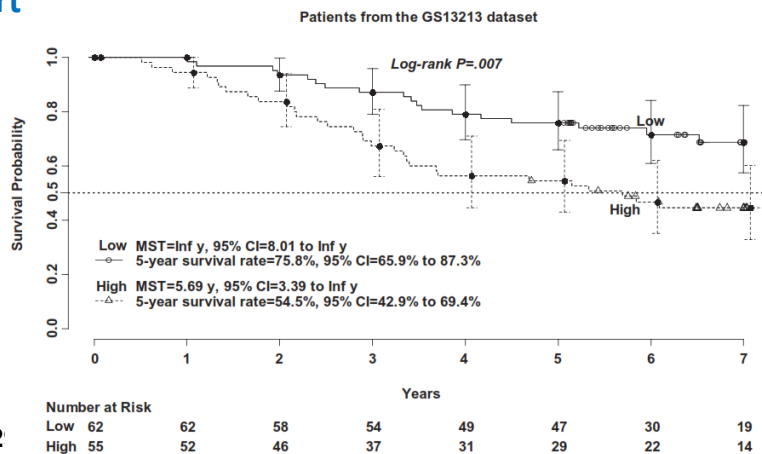
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# Prognostic Validation in Patients with no Adjuvant Chemotherapy

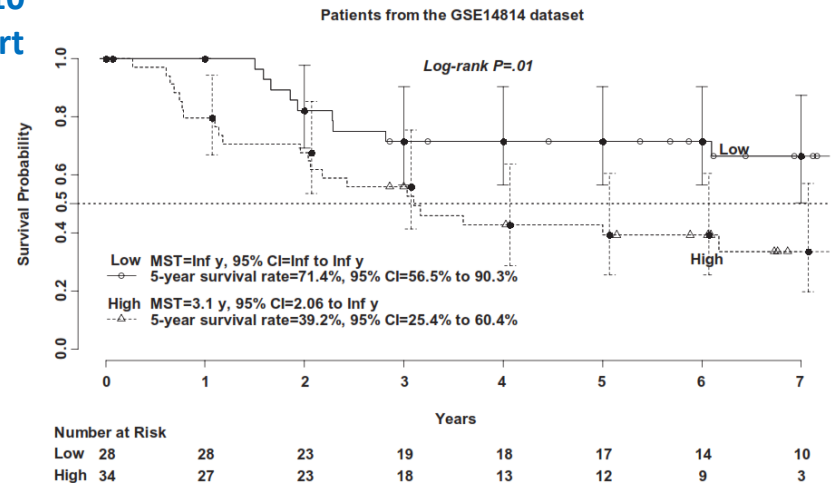
Director's  
Challenge  
consortium



Japanese  
cohort

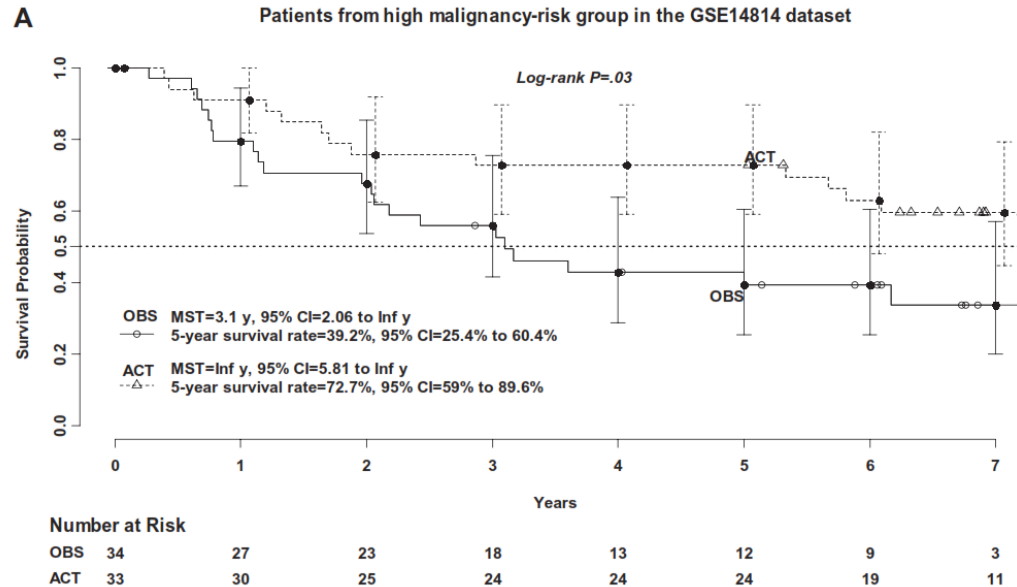


JBR.10  
cohort

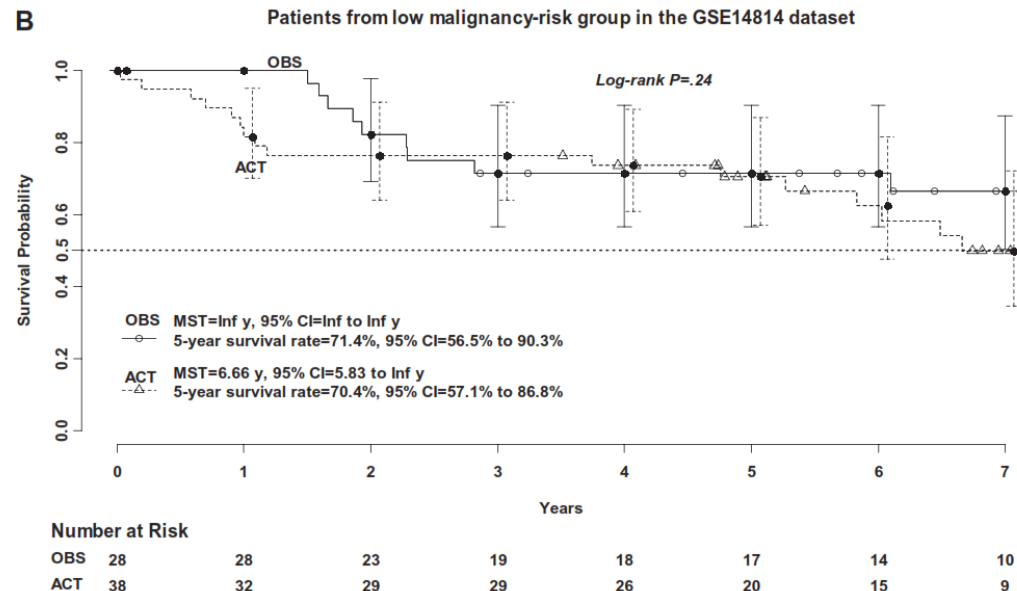


# Predictiveness Tested in JBR.10 Patients

High Risk  
Patients



Low Risk  
Patients



26-29 March 2014, Gene

## **insight review articles**

# Stromal fibroblasts in cancer initiation and progression

Neil A. Bhowmick<sup>1,2,3</sup>, Eric G. Neilson<sup>3,4</sup> & Harold L. Moses<sup>1,3,4</sup>

<sup>1</sup>Department of Cancer Biology, <sup>2</sup>Department of Urologic Surgery, <sup>3</sup>The Vanderbilt–Ingram Cancer Center and <sup>4</sup>Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee 37232, USA (e-mail: hal.moses@vanderbilt.edu)

It is widely accepted that the development of carcinoma — the most common form of human cancer — is due to the accumulation of somatic mutations in epithelial cells. The behaviour of carcinomas is also influenced by the tumour microenvironment, which includes extracellular matrix, blood vasculature, inflammatory cells and fibroblasts. Recent studies reveal that fibroblasts have a more profound influence on the development and progression of carcinomas than was previously appreciated. These new findings have important therapeutic implications.

***Nature 432 (18 November 2004): 332-337***

26-29 March 2014, Geneva, Switzerland

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International Association for the Study of Lung Cancer



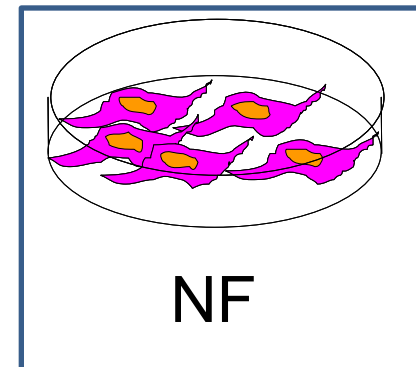
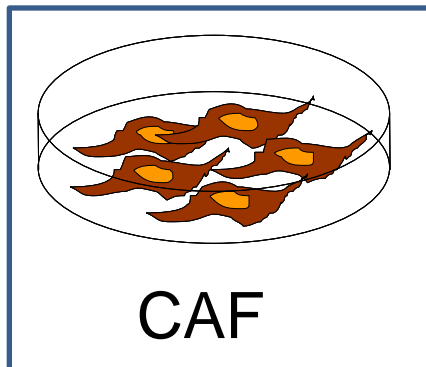
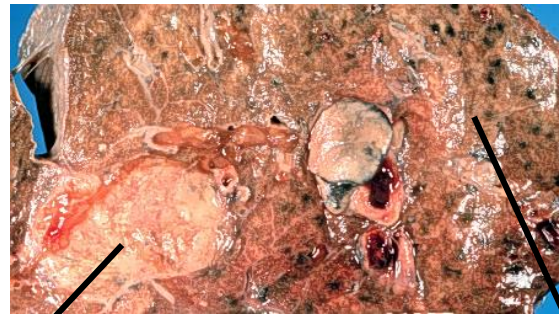
European Society for Medical Oncology

# A Molecular Signature of Metastasis in Primary Solid Tumors

**Table 1 • The 17-gene signature associated with metastasis**

Gene	Gene name	GenBank ID
Upregulated in metastases		
<i>SNRPF</i>	Small nuclear ribonucleoprotein F	AI032612
<i>EIF4EL3</i>	Elongation initiation factor 4E-like 3	AF038957
<i>HNRPAB</i>	Heterogeneous nuclear ribonucleoprotein A/B	M65028
<i>DHPS</i>	Deoxyhypusine synthase	U79262
<i>PTTG1</i>	Securin	AA203476
<i>COL1A1</i>	Type 1 collagen, $\alpha$ 1	Y15915
<i>COL1A2</i>	Type 1 collagen, $\alpha$ 2	J03464
<i>LMNB1</i>	Lamin B1	L37747
Downregulated in metastases		
<i>ACTG2</i>	Actin, $\gamma$ 2	D00654
<i>MYLK</i>	Myosin light chain kinase	U48959
<i>MYH11</i>	Myosin, heavy chain 11	AF001548
<i>CNN1</i>	Calponin 1	D17408
<i>HLA-DPB1</i>	MHC Class II, DP $\beta$ 1	M83664
<i>RUNX1</i>	Runt-related transcription factor 1	D43969
<i>MT3</i>	Metallothionein 3	S72043
<i>NR4A1</i>	Nuclear hormone receptor TR3	L13740
<i>RBM5</i>	RNA binding motif 5	AF091263

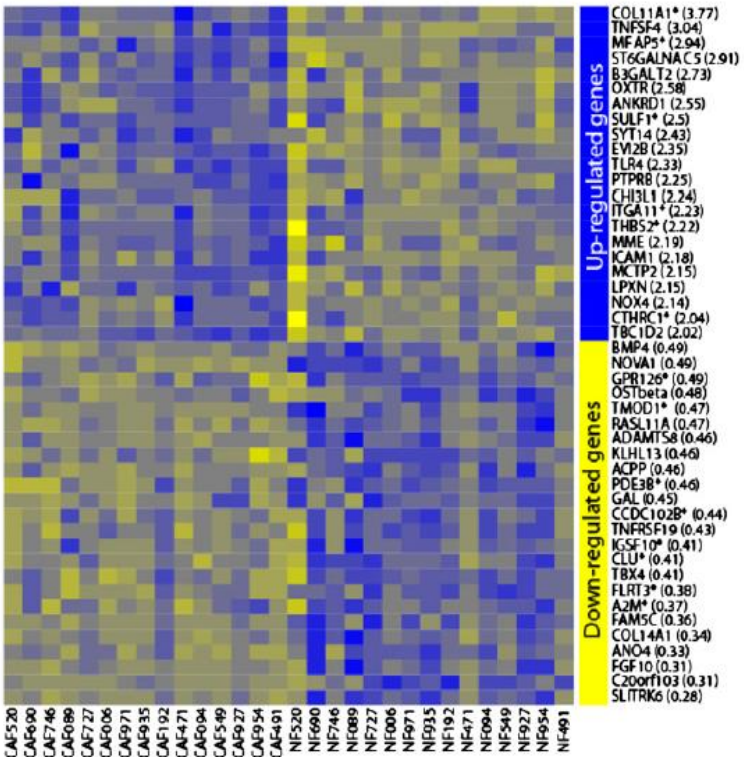
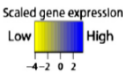
# Fibroblasts Cultured from Resected NSCLC and Corresponding Normal Lung



26-29 March 2014,

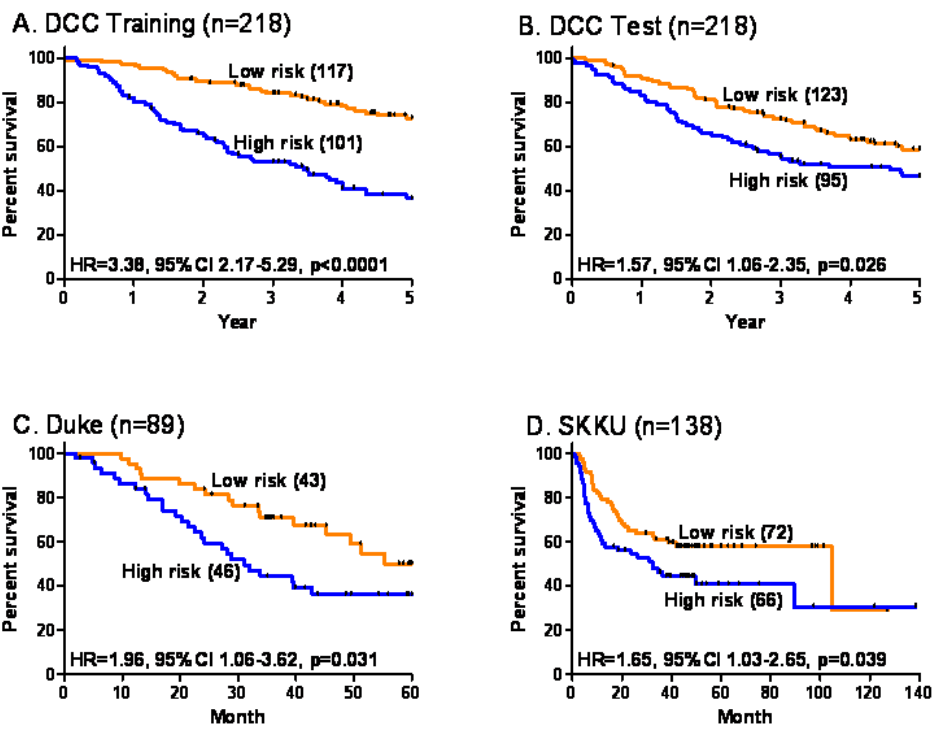
# Prognostic gene-expression signature of carcinoma-associated fibroblasts in non-small cell lung cancer

Roya Navab<sup>a,1</sup>, Dan Strumpf<sup>a,1</sup>, Bizhan Bandarchi<sup>a,1</sup>, Chang-Qi Zhu<sup>a,1</sup>, Melania Pintilie<sup>a</sup>, Varune Rohan Ramnarine<sup>a</sup>, Emin Ibrahimov<sup>a</sup>, Nikolina Radulovich<sup>a</sup>, Lisa Leung<sup>a</sup>, Malgorzata Barczyk<sup>a,b</sup>, Devang Panchal<sup>a</sup>, Christine To<sup>a</sup>, James J. Yun<sup>a</sup>, Sandy Der<sup>a</sup>, Frances A. Shepherd<sup>a,c</sup>, Igor Jurisica<sup>a,d,e</sup>, and Ming-Sound Tsao<sup>a,e,f,2</sup>



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## 11-gene prognostic signature

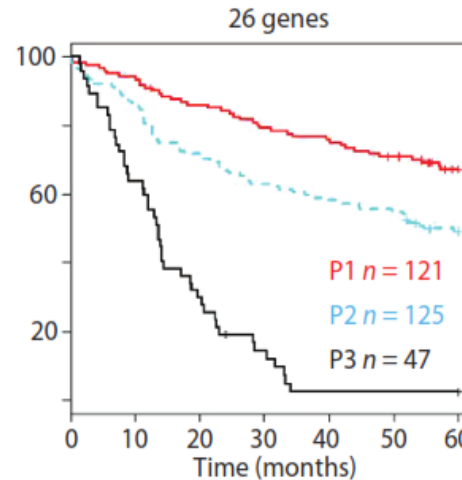
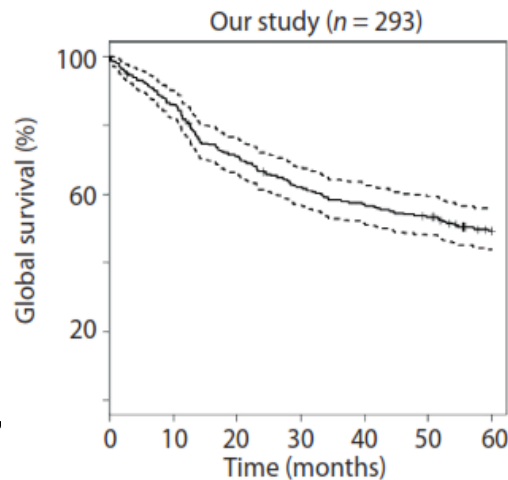
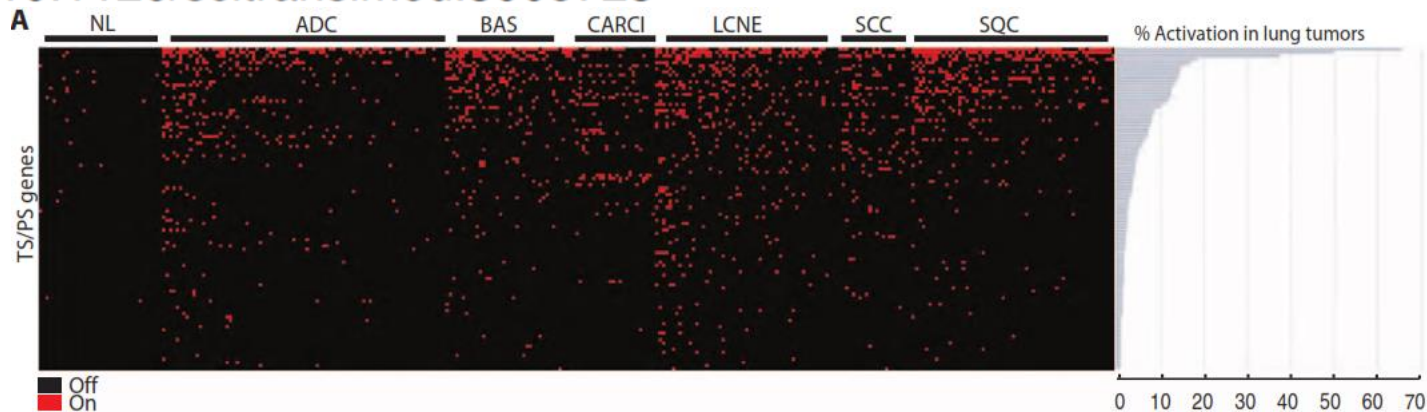


# Ectopic Activation of Germline and Placental Genes Identifies Aggressive Metastasis-Prone Lung Cancers

Sophie Rousseaux *et al.*

*Sci Transl Med* **5**, 186ra66 (2013);

DOI: 10.1126/scitranslmed.3005723



26-29 March 2014,

# A Gene Expression Signature Associated with “K-Ras Addiction” Reveals Regulators of EMT and Tumor Cell Survival

Anurag Singh,<sup>1</sup> Patricia Greninger,<sup>1</sup> Daniel Rhodes,<sup>2</sup> Louise Koopman,<sup>3</sup> Sheila Violette,<sup>4</sup> Nabeel Bardeesy,<sup>1</sup> and Jeff Settleman<sup>1,\*</sup>

*Cancer Cell 2009;15:489–500*

## An Epithelial–Mesenchymal Transition Gene Signature Predicts Resistance to EGFR and PI3K Inhibitors and Identifies Axl as a Therapeutic Target for Overcoming EGFR Inhibitor Resistance

Lauren Averett Byers, Lixia Diao, Jing Wang, et al.

*Clin Cancer Res 2013;19:279-290*

## A gene expression signature of RAS pathway dependence predicts response to PI3K and RAS pathway inhibitors and expands the population of RAS pathway activated tumors

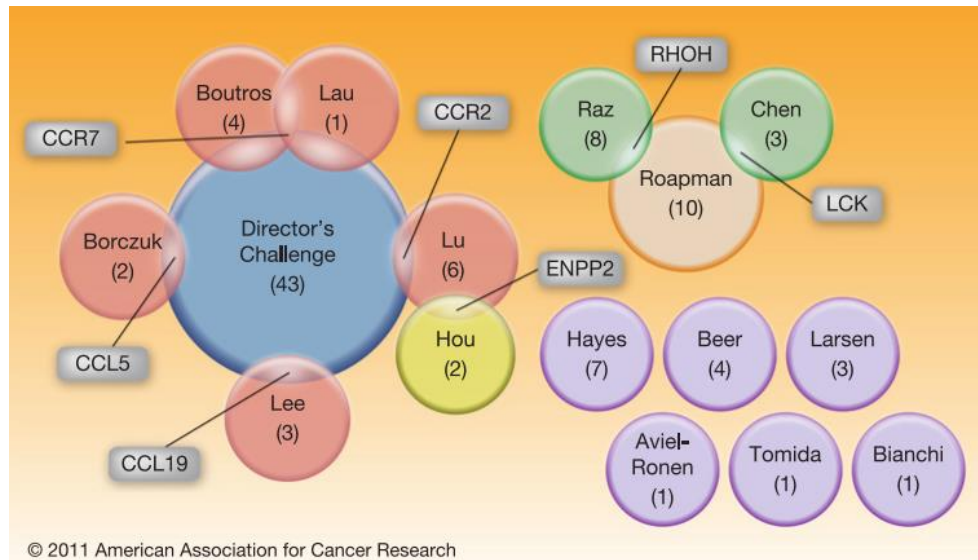
Andrey Loboda<sup>1</sup>, Michael Nebozhyn<sup>1</sup>, Rich Klinghoffer<sup>2</sup>, Jason Frazier<sup>2</sup>, Michael Chastain<sup>2</sup>, William Arthur<sup>2</sup>,

*BMC Medical Genomics 2010, 3:26*

# Prognostic Immune Markers in Non-Small Cell Lung Cancer

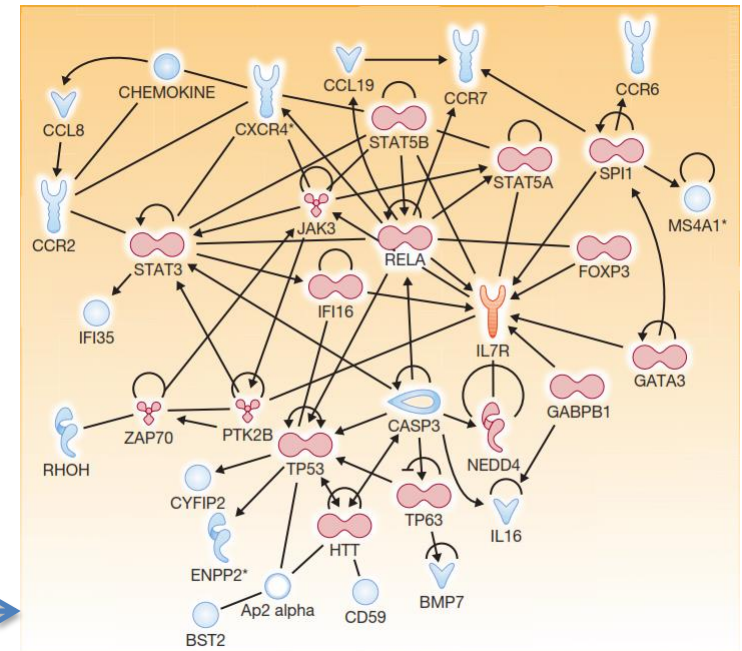
Kei Suzuki<sup>1</sup>, Stefan S. Kachala<sup>1</sup>, Kyuichi Kadota<sup>1,5</sup>, Ronglai Shen<sup>2</sup>, Qianxing Mo<sup>2</sup>, David G. Beer<sup>6</sup>, Valerie W. Rusch<sup>1</sup>, William D. Travis<sup>3</sup>, and Prasad S. Adusumilli<sup>1,4</sup>

*Clin Cancer Res; 17(16); 5247–56.*



Director's Challenge dataset

17 genes



## Predictive Gene Signature in MAGE-A3 Antigen-Specific Cancer Immunotherapy

Fernando Ulloa-Montoya, Jamila Louahed, Benjamin Dizier, Olivier Gruselle, Bart Spiessens, Frédéric F. Lehmann, Stefan Suci, Wim H.J. Kruit, Alexander M.M. Eggermont, Johan Vansteenkiste, and Vincent G. Brichard

*J Clin Oncol 2013;31:2388-2395*

# Conclusions

- **Epigenetic plays important role in lung cancer development, differentiation and prognosis, and specific aberrations may constitute therapeutic targets**
- **Gene expression signatures may define additional subtypes of major lung cancer histological types**
- **Expression signatures can define prognosis and predict benefit from adjuvant chemotherapy, yet routine clinical application still awaits further prospective validation**
- **Tissue or Pathway specific gene expression signatures may provide important biological insights into complexity of targeted therapies**