Current Topics in Lung Cancer Research

Mutations Biomarkers

RPN532: Oncology for Scientists II

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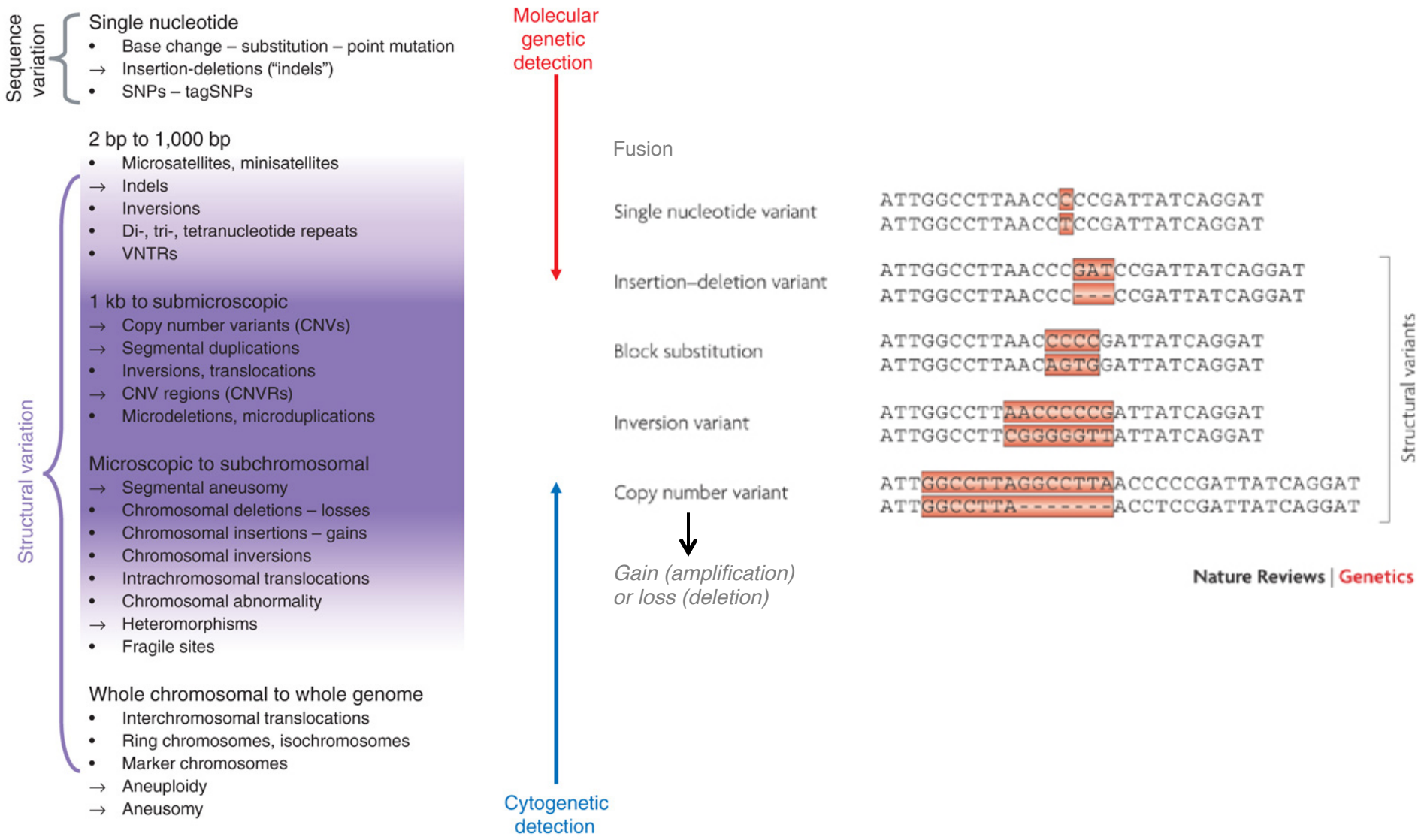
9 May 2017

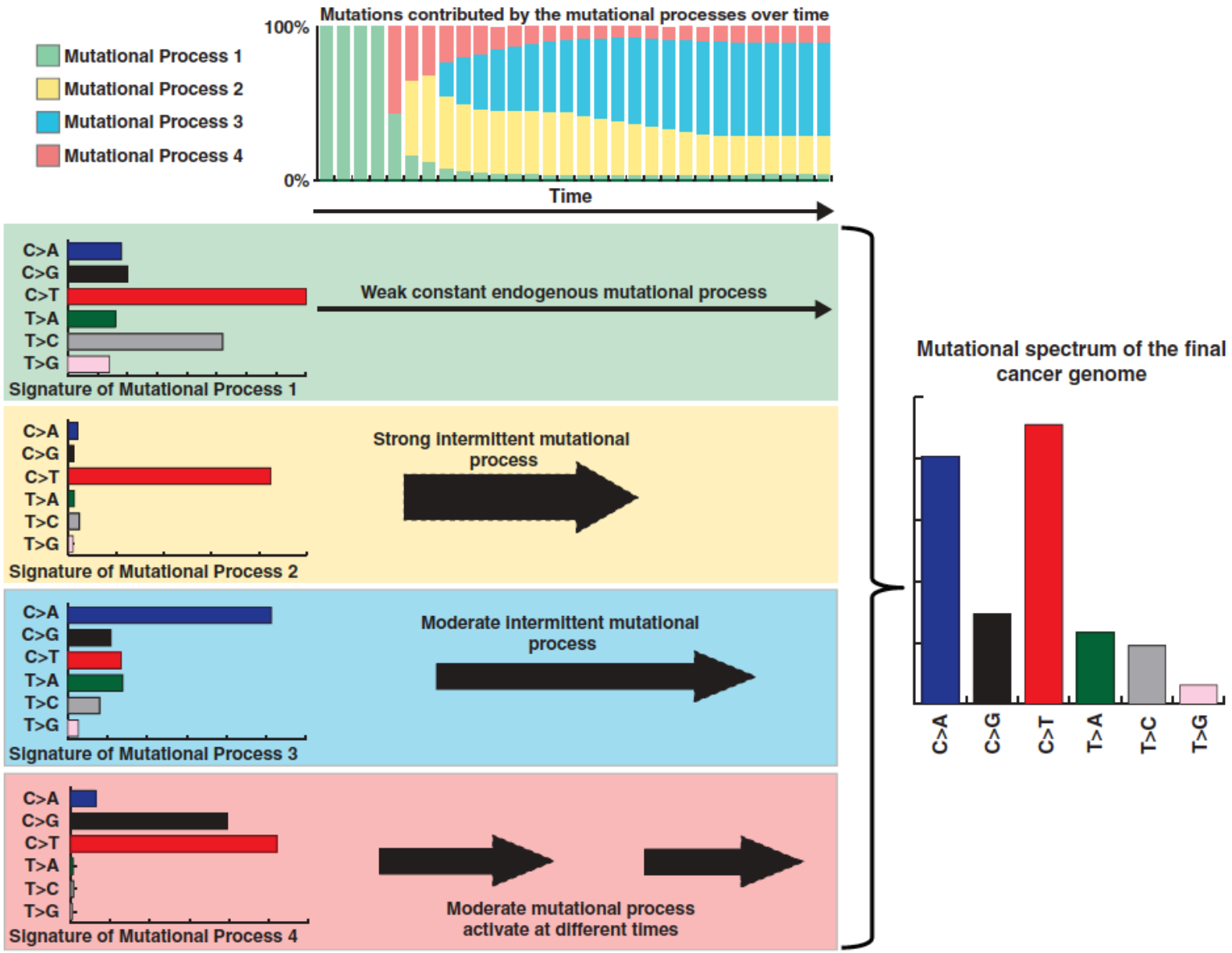
"Mutations" in lung cancer

Wide variety of genomic variations



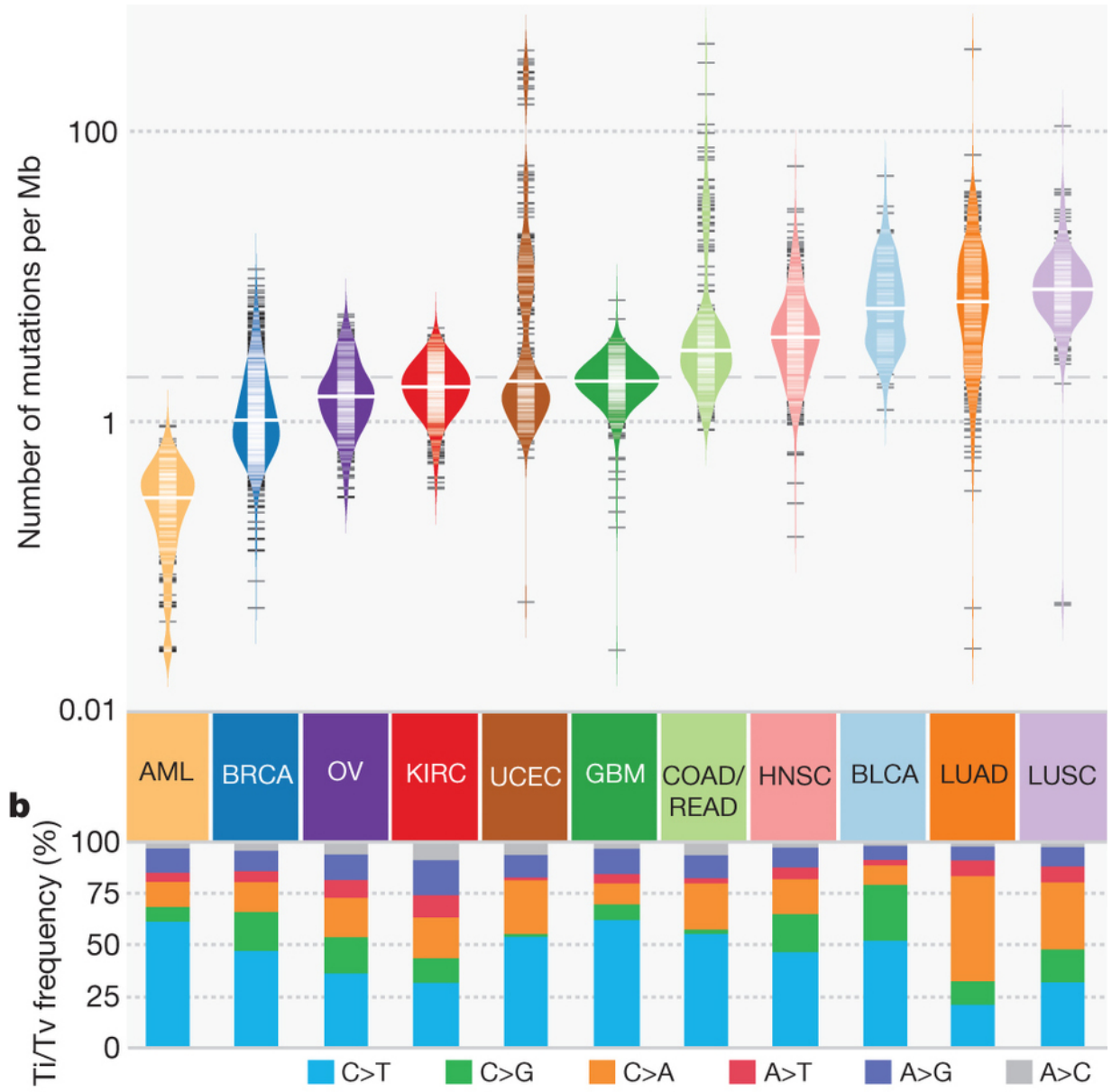
Nature Genetics, 39, S7, 2007

Multiple mutation processes in cancer



Current Opinion in Genetics & Development

Mutation landscape in 12 major cancers



The Cancer Genome Atlas (TCGA)

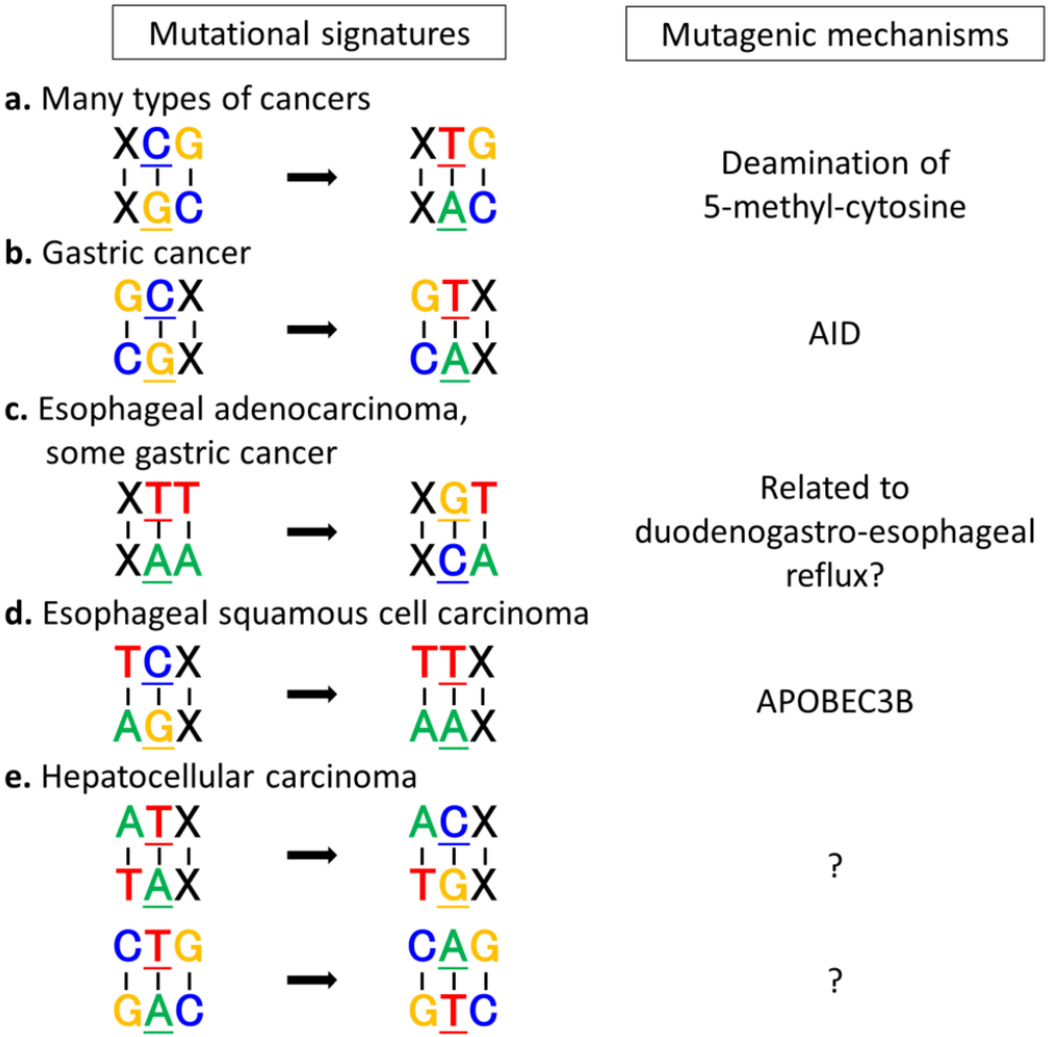
Lung adenocarcinoma (LUAD)
Lung squamous cell ca. (LUSC)
Colorectal adenoca. (COAD/READ)
Uterine corpus endometrial ca. (UCEC)

Higher C>A transversions in lung cancer

Nature, 502, 333, 2013

Mutation signatures

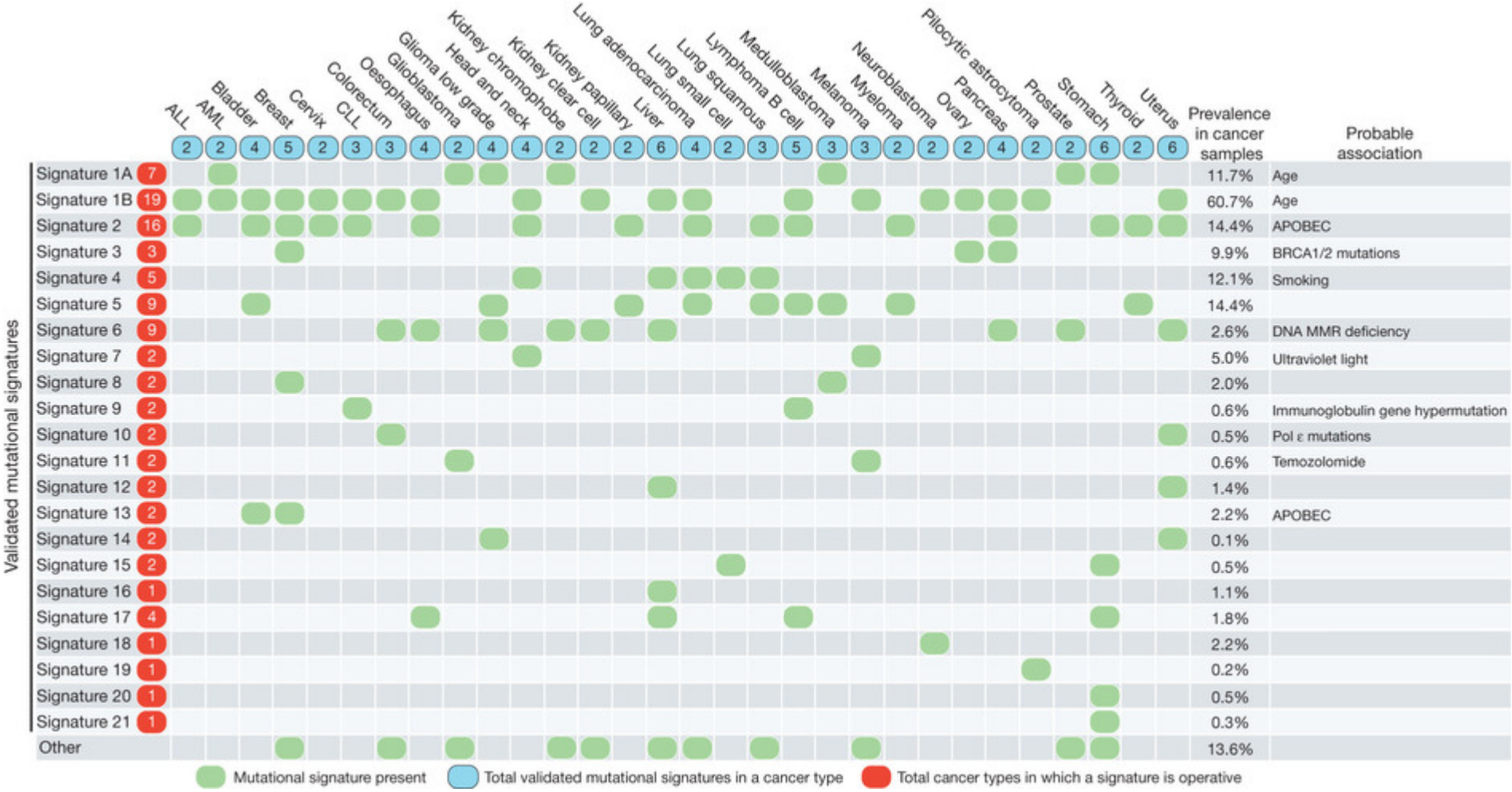
Mutation signature = Sequence context of a mutation



Cancers, 7, 1037, 2015

Mutation signatures

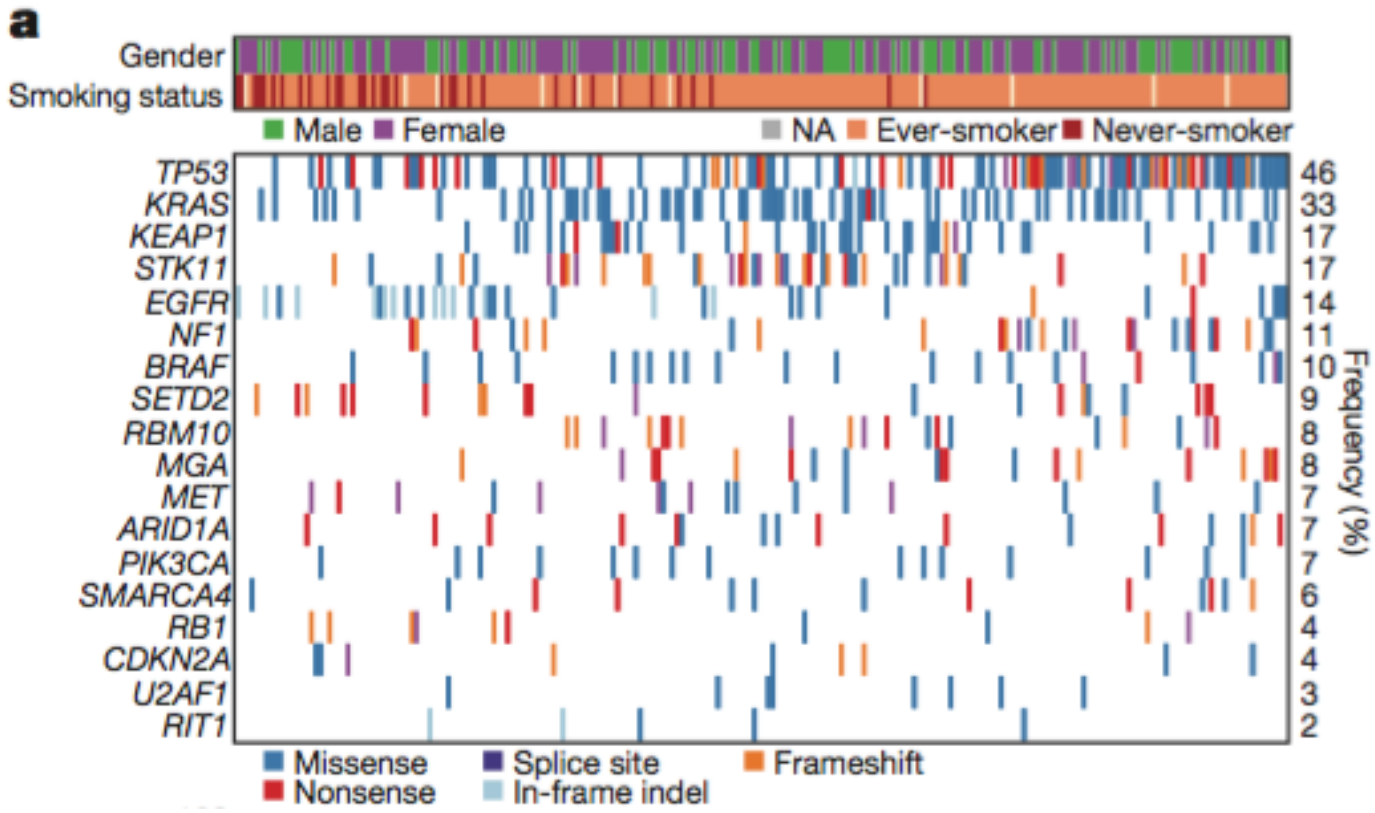
Distinct mutation signatures are seen in different cancers



Nature, 500, 415, 2013

Mutations

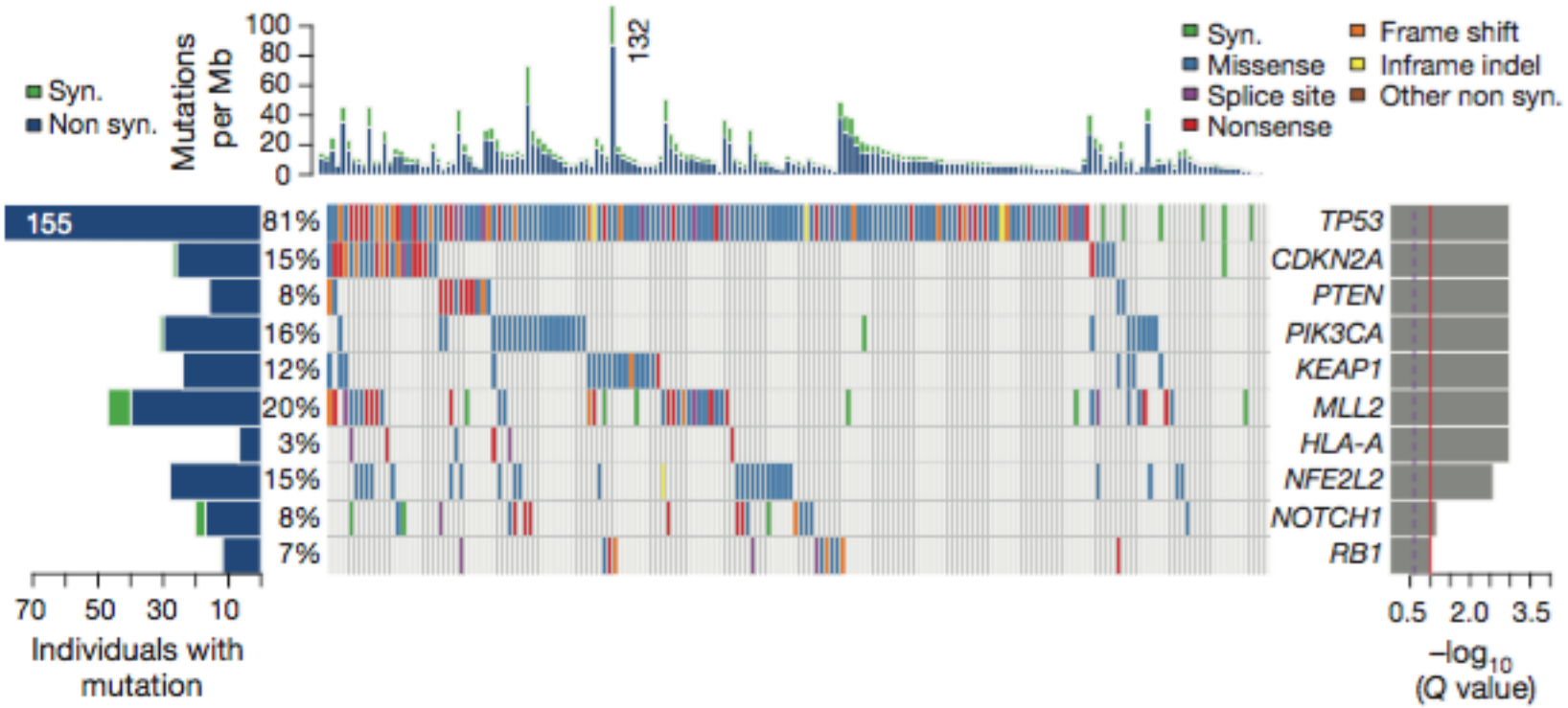
Common mutations in adenocarcinoma



TCGA project

Nature, 511, 543, 2014

Common mutations in squamous cell ca.



TCGA project

Nature, 498, 520, 2012

Mutations

Common mutations in lung cancer

	Adenocarcinoma	SCC	SCLC
Mutations	*TP53, KRAS, EGFR, NF1, BRAF, MET, RIT*	*TP53, CDKN2A, PIK3CA, NFE2L2, KEAP1, CUL3, PTEN, NF1, NOTCH1,2,* and *3, DDR2, EGFR*	*TP53, RB1, EP300, CREBBP, PTEN, SLIT2*
Fusions	*ALK, ROS1, RET*	*FGFRs*	..
SCNAs	Gains: *NKX2-1, TERT, EGFR, MET, KRAS, ERBB2, MDM2* Losses: *LRP1B, PTPRD, and CDKN2A*	Gains: Chr 3q 26 (*SOX2, PIK3CA, TP63* etc) Losses: *CDKN2A, PTEN*	Gains: *MYC, MYCN, MYCL1, SOX2, FGFR1, KIT* Losses: Chr 3p (*FHIT, FUS1, RASSF1A*)
Pathway alterations	RTK/RAS/RAF mTOR JAK-STAT DNA repair Cell cycle regulation Epigenetic deregulation	Squamous differentiation Oxidative stress response PIK3CA DNA repair Cell cycle regulation Epigenetic deregulation	Cell cycle regulation, epigenetic deregulation, Hedgehog, DNA repair, axonal guidance and neuroendocrine differentiation

SCNA=somatic copy number alteration.

SCC = lung squamous cell ca.
SCLC = small cell lung cancer

Lancet Oncol, 16, e342, 2015

Why examine mutations?

Understand cancer biology

Accurately sub-classify cancer

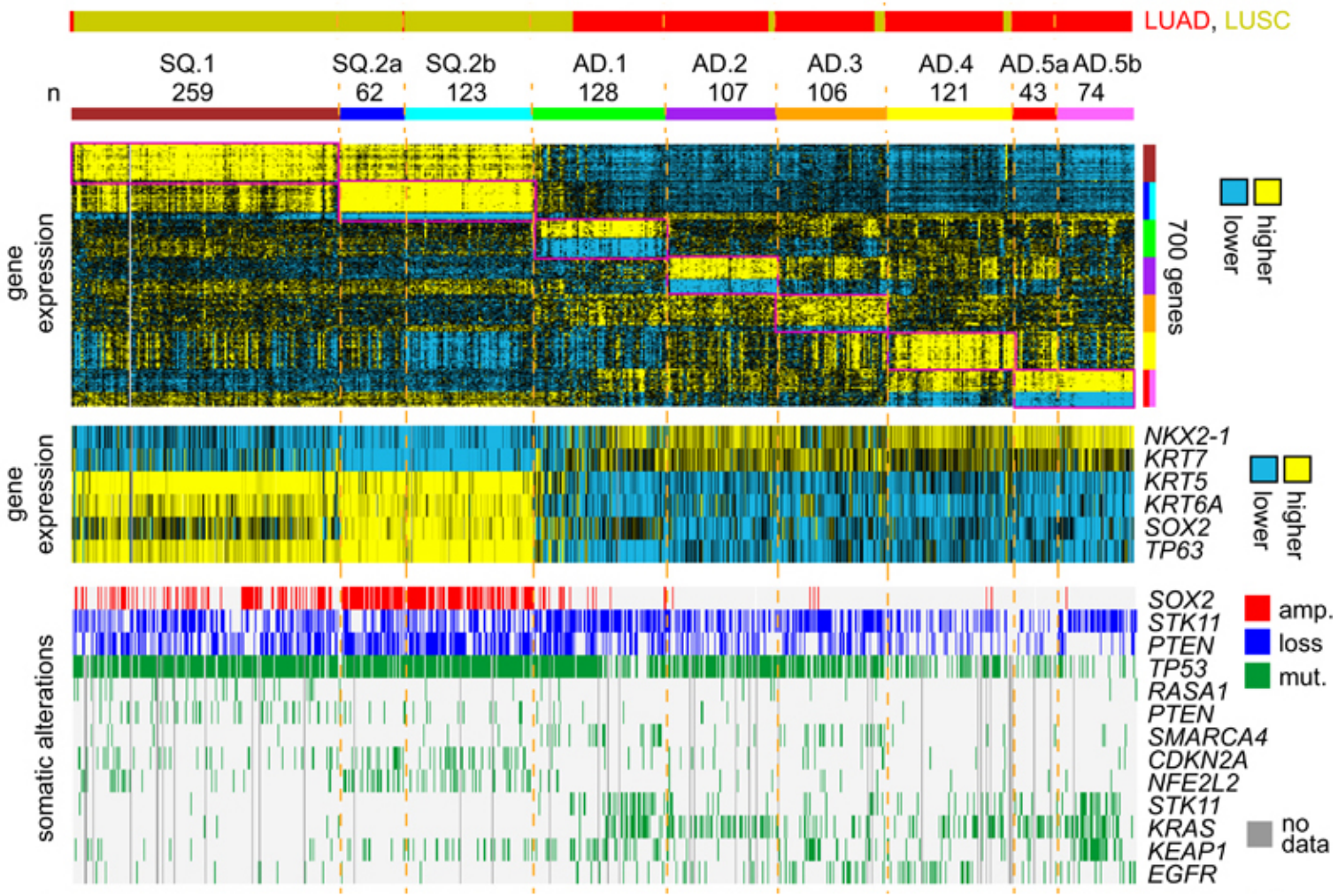
Target specific protein or pathway for drug treatment ("actionable mutation")

Design precision treatment

Utilize as biomarker

Accurately sub-classify cancer

Mutation data combined with other molecular information identifies
6 sub-types of adenocarcinoma (LUAD)
3 sub-types of squamous cell carcinoma (LUSC)



Oncogene, 36, 1384, 2017

Design precision treatment

50 y woman with 7 lung tumors (colorectal cancer metastases)

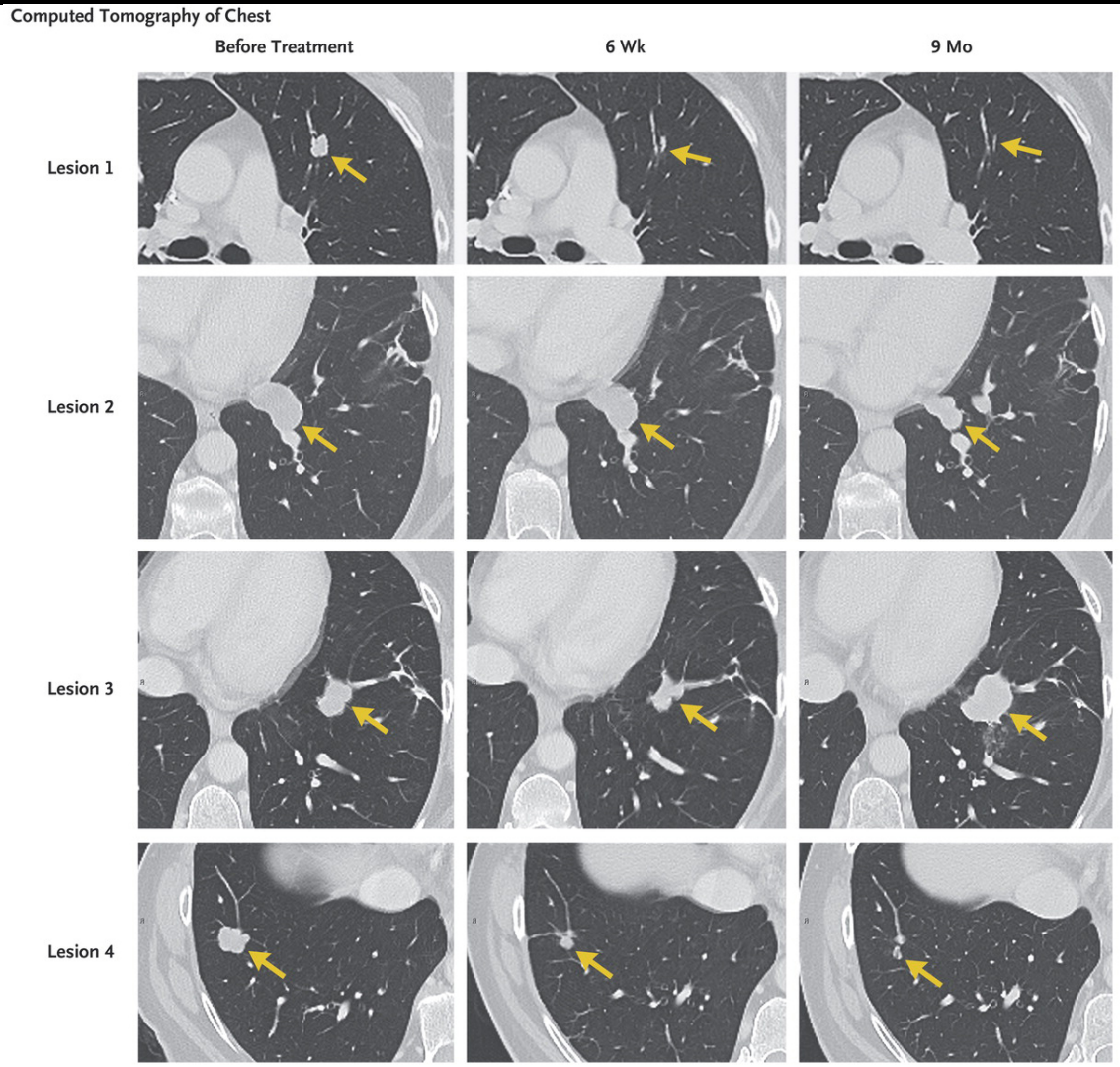
Exome DNA sequencing of 3 tumors => ~70 mutated genes

KRAS G12D mutation (27% freq.)

Biopsy => isolate CD8+ T cells

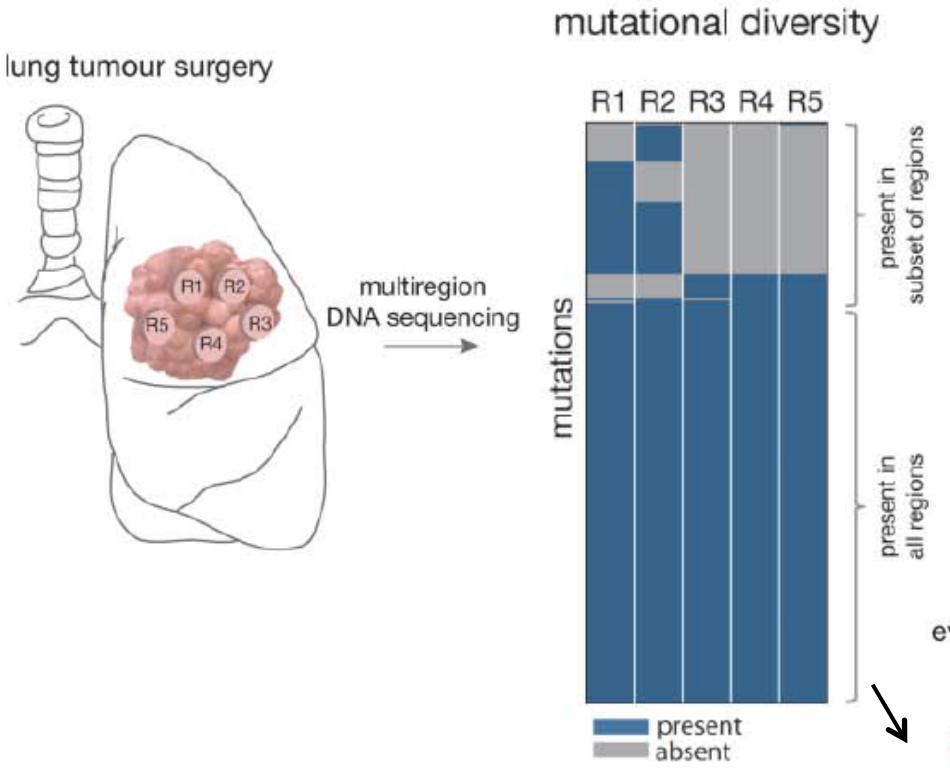
Expand KRAS G12D-reactive population => inject

All 7 lung tumors regress



NEJM, 375, 2255, 2016

Understand cancer biology



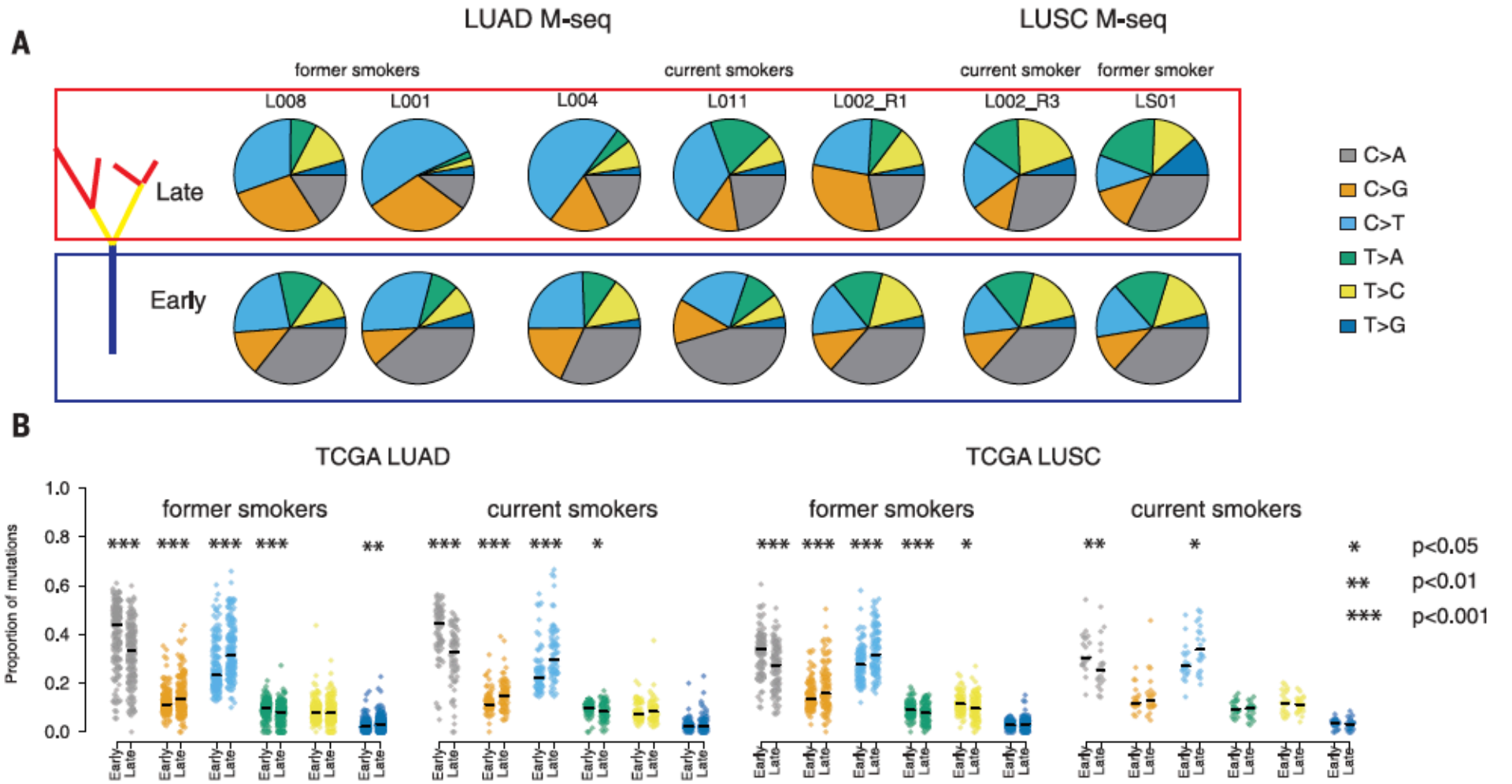
<u>APOBEC</u>
Family of cytidine deaminase enzymes
Deaminate DNA causing C>U (T) mutation

Mol Cell Oncol, 2, e985549, 2015

Understand cancer biology

APOBEC (C>T) mutation process
Occurs later in time (more "Late" mutations)
Persists after smoking cessation

Smoking-associated (C>A) mutation process
Starts early (more "Early" mutations)
Slows after smoking cessation



Science, 346, 253, 2014

Biomarkers

Biomarkers "mark" a specific "biological" state

Aspects of biomarkers

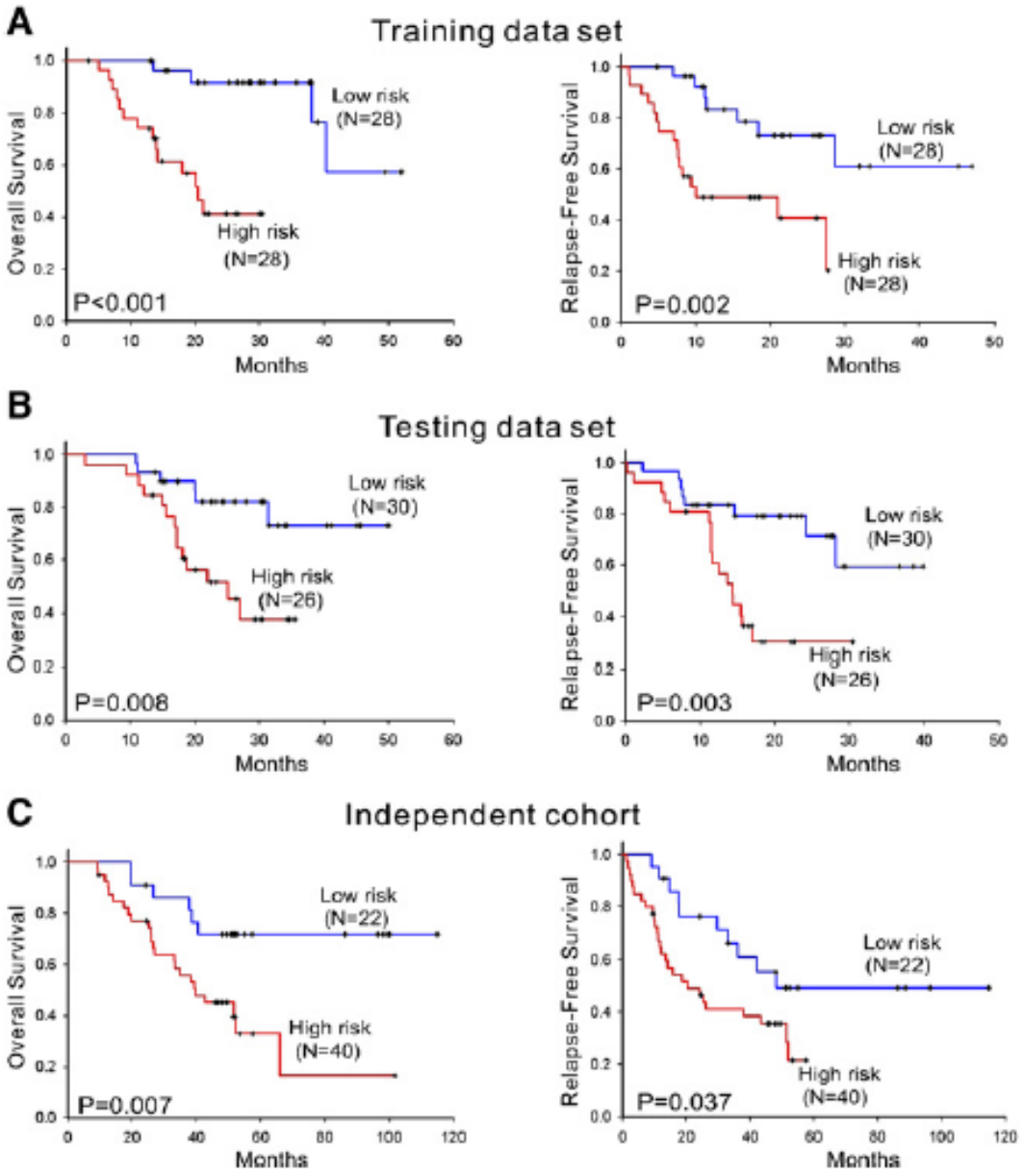
Purpose	Diagnostic, prognostic, etc. Biological state – specificity, sensitivity
Analyte	Type – gene, RNA, protein, cell number, small molecule, etc. Number – single analyte, or multiple
Measurement	Sensitivity of method Specificity of method
Sample	Diseased material (e.g., biopsy of tumor) Indirectly affected material (e.g., blood lymphocytes in lung cancer)
Sample acquisition	Invasive, non-invasive (blood, urine, etc.)

MicroRNA-based biomarkers

Prediction of recurrence and survival in non-small cell lung cancer

Sample: Tumor
Analyte: Multiple microRNAs
Measurement method: RTPCR

Kaplan-Meier survival curves:



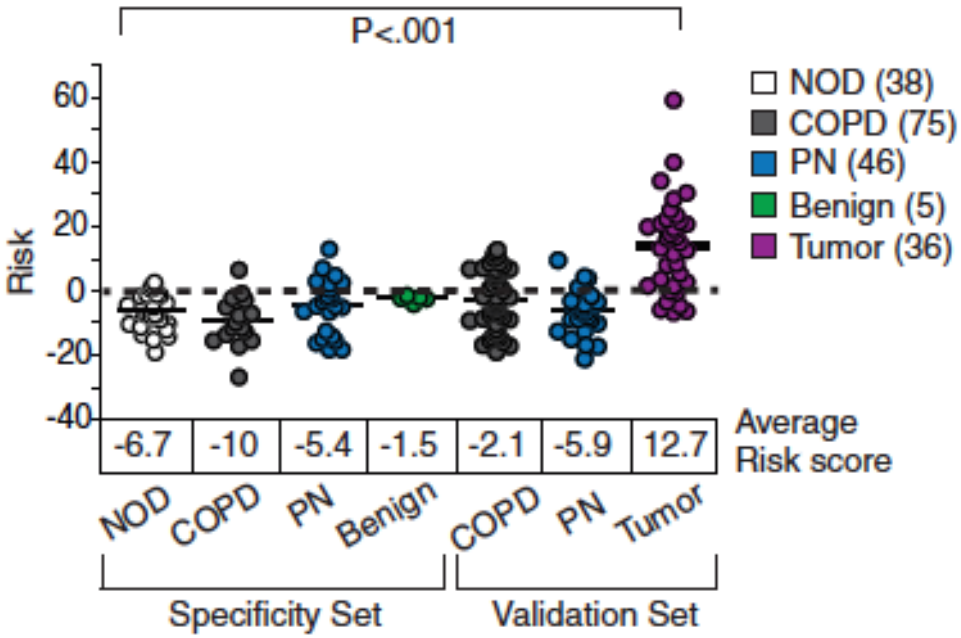
Yu et al. Mol Cell, 2008

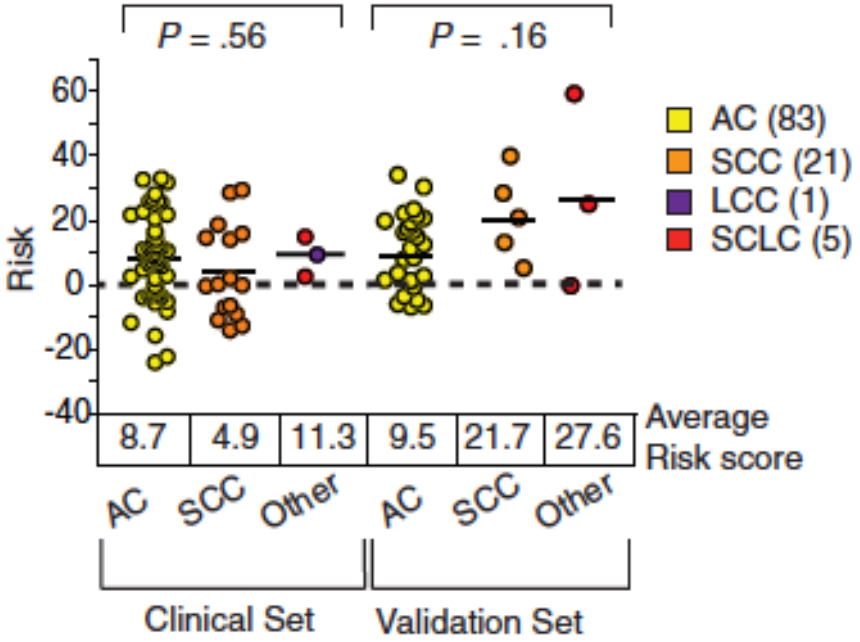
MicroRNA-based biomarkers

Diagnosing lung cancer in smokers

Sample: Serum
Analyte: 34 microRNAs
Measurement method: RTPCR

PN = pneumonia
COPD = chronic obstructive pulmonary disease
NOD = non-cancer lung nodule detected by CT
Tumor = lung cancer
LCC = large cell ca. of lung





JNCI, 107, djv063, 2015

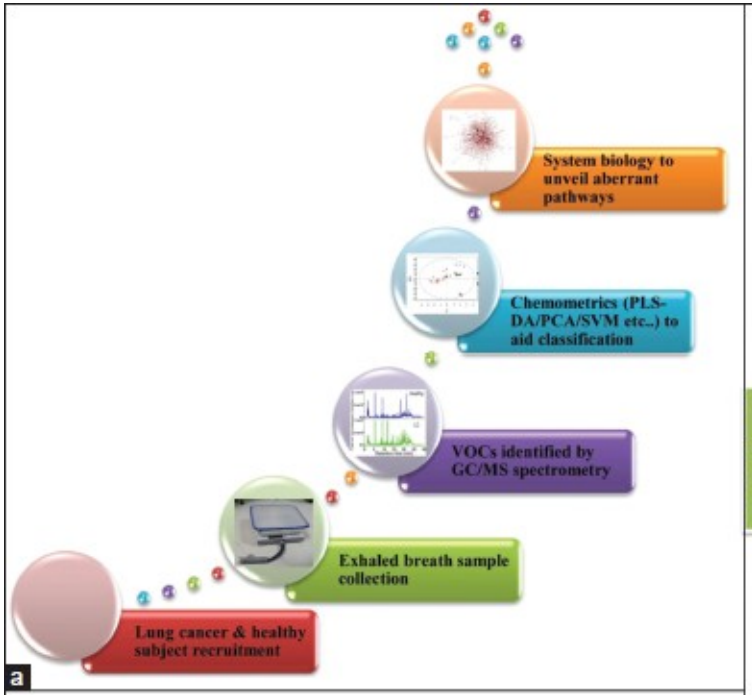
Biomarkers in breath

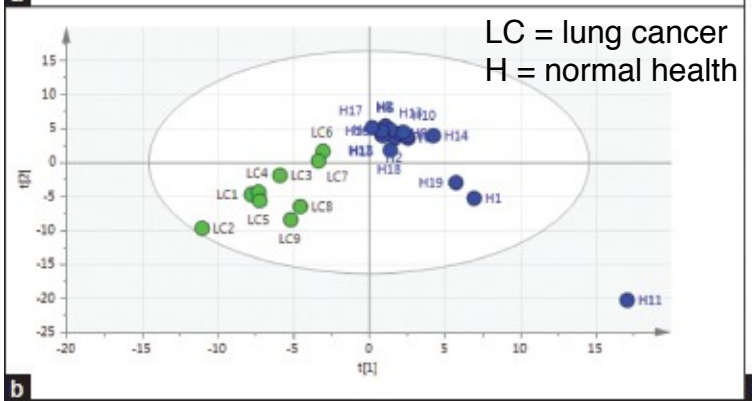
Diagnosing lung cancer by breath volatile organic compounds

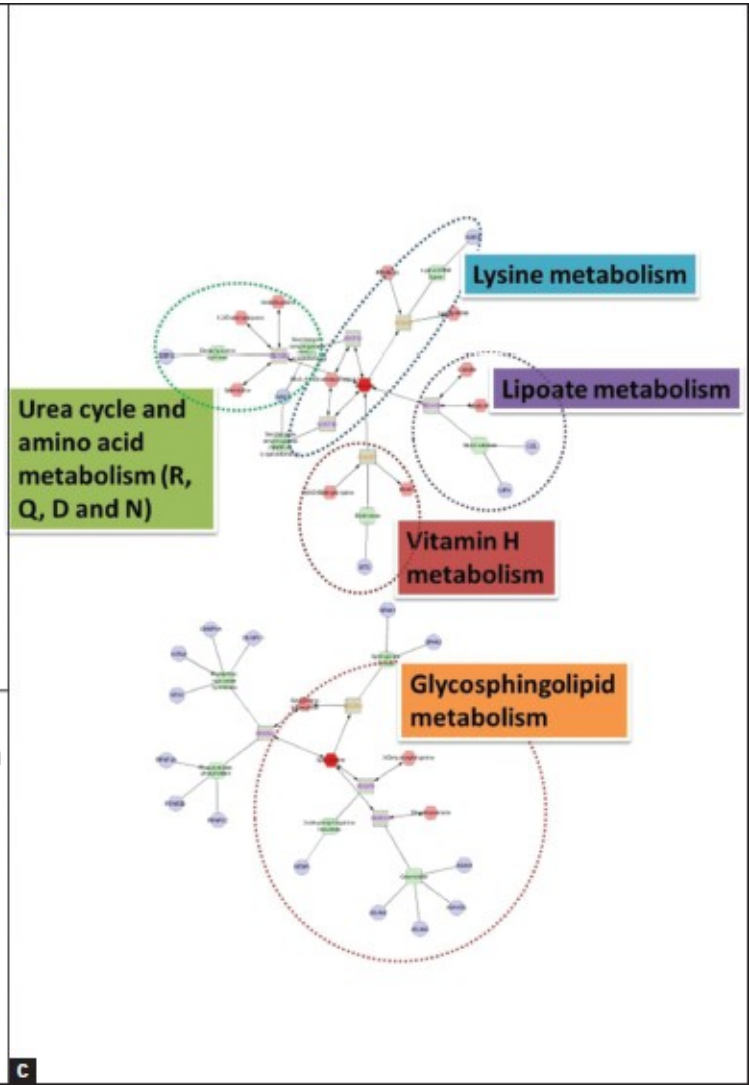
Sample: Breath

Analyte: Volatile organic compounds

Measurement method: Gas chromatography + mass spectrometry







J Carcinog, 12, 3, 2013

Gene mutation biomarkers

Examples that are clinically used

Biomarker	Treatment	Genomic aberration	Prevalence in NSCLC patients
EGFR	1. Tyrosine kinase inhibitors (e.g., gefitinib, erlotinib, and afatinib) 2. Monoclonal antibodies (e.g., cetuximab and necitumumab)	1. Activating mutation within intracellular catalytic domain of *EGFR* 2. Over-expression of extracellular part of EGFR	*EGFR* mutations (non-squamous histology) 1. ~15% in Caucasians 2. ~40% in Asians 3. ~75–80% in never-smoker Asians *EGFR* mutations (squamous histology) 1. ~5% EGFR over-expression 1. 39% in adenocarcinoma 2. 58% in squamous cell carcinoma 3. 38% in large-cell carcinoma
ALK	Tyrosine kinase inhibitors (e.g., crizotinib and ceritinib)	Chromosomal translocation and fusion of *ALK* gene	1. 3–5% in unselected NSCLC 2. ~10% in non-never-smokers 3. <1% in squamous carcinoma
MET	1. Tyrosine kinase inhibitors (e.g., tivantinib, cabozantinib, and crizotinib) 2. Monoclonal antibodies (onartuzumab, AMG 102, ficlatuzumab)	1. Increased *MET* copy number 2. Over-expression of extracellular part of MET receptor	1. 2–4% *MET* amplification (untreated) 2. 5–20% *MET* amplification in EGFR-TKI-resistant tumors 3. 25–75% over-expression of extracellular part of MET receptor
ROS-1	Tyrosine kinase inhibitor (crizotinib)	Chromosomal translocation and fusion of *ROS-1* gene	1–2% in unselected population
KRAS	Downstream pathway inhibitors (e.g., MEK inhibitors selumetinib and trametinib)	Activating mutation within catalytic *RAS* domain	1. *KRAS* are rare in never-smokers 2. ~25–30% in adenocarcinoma 3. ~5% in squamous cell carcinoma

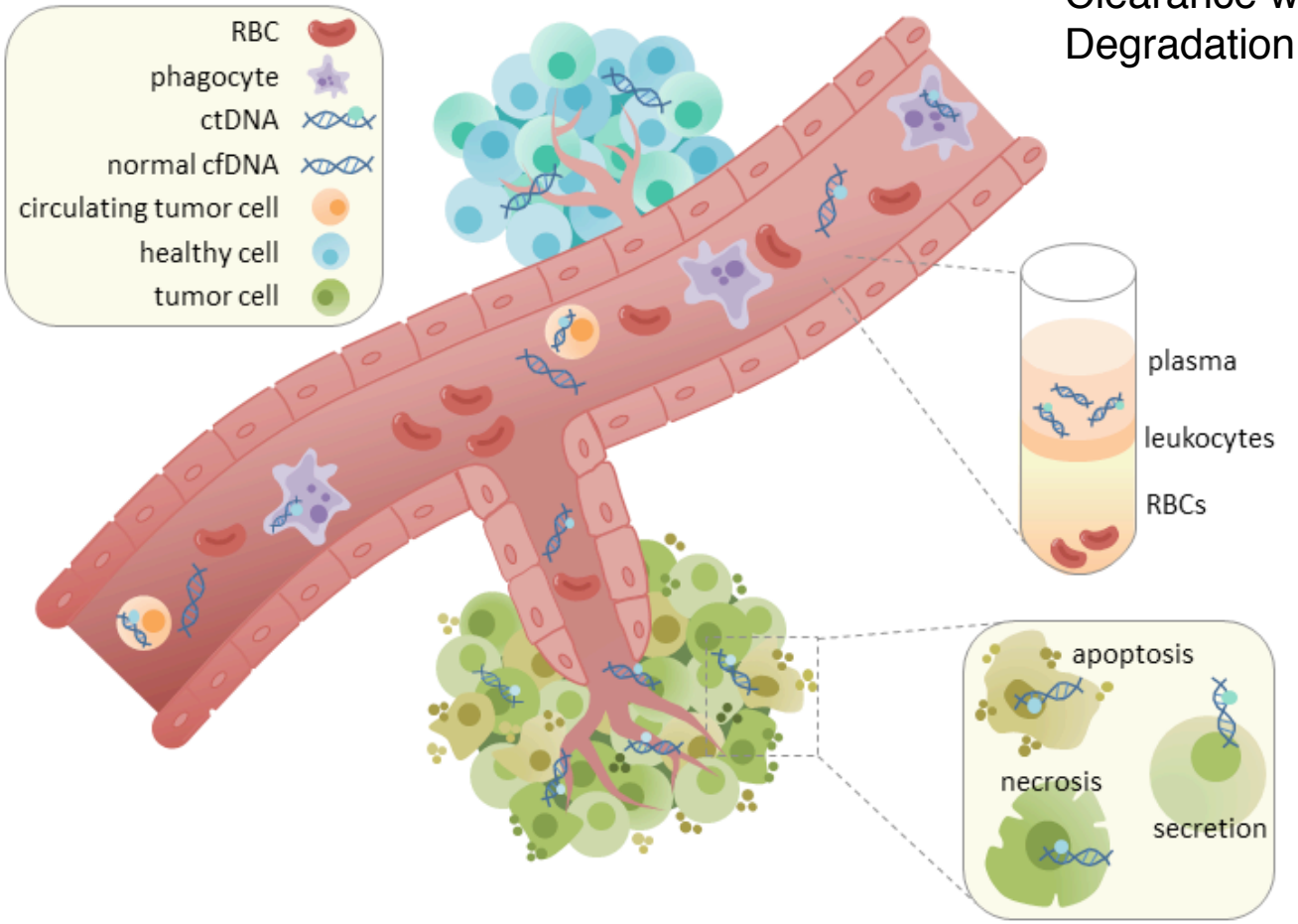
Front Oncol, 4, 204, 2014

Circulating DNA biomarkers

~50 ng circulating cfDNA per ml of plasma
= ~10,000 genome DNA

Clearance with half-life ~12 h
Degradation + clearance by spleen, etc.

ctDNA = circulating tumor DNA
cfDNA = cell-free DNA



Wikipedia

Biomarkers

Assigned ctDNA biomarker paper

nature

Accelerated Article Preview

ARTICLE

doi:10.1038/nature22364

Phylogenetic ctDNA analysis depicts early stage lung cancer evolution

Christopher Abbosh, Nicolai J. Birkbak, Gareth A. Wilson, Mariam Jamal-Hanjani, Tudor Constantin, Raheleh Salari, John Le Quesne, David A Moore, Selvaraju Veeriah, Rachel Rosenthal, Teresa Marafioti, Eser Kirkizlar, Thomas B K Watkins, Nicholas McGranahan, Sophia Ward, Luke Martinson, Joan Riley, Francesco Fraioli, Maise Al Bakir, Eva GrÖnroos, Francisco Zambrana, Raymondo Endozo, Wenya Linda Bi, Fiona M. Fennessy, Nicole Sponer, Diana Johnson, Joanne Laycock, Seema Shafi, Justyna Czyzewska-Khan, Andrew Rowan, Tim Chambers, Nik Matthews, Samra Turajlic, Crispin Hiley, Siow Ming Lee, Martin D. Forster, Tanya Ahmad, Mary Falzon, Elaine Borg, David Lawrence, Martin Hayward, Shyam Kolvekar, Nikolaos Panagiotopoulos, Sam M Janes, Ricky Thakrar, Asia Ahmed, Fiona Blackhall, Yvonne Summers, Dina Hafez, Ashwini Naik, Apratim Ganguly, Stephanie Kareht, Rajesh Shah, Leena Joseph, Anne Marie Quinn, Phil Crosbie, Babu Naidu, Gary Middleton, Gerald Langman, Simon Trotter, Marianne Nicolson, Hardy Remmen, Keith Kerr, Mahendran Chetty, Lesley Gomersall, Dean A. Fennell, Apostolos Nakas, Sridhar Rathinam, Girija Anand, Sajid Khan, Peter Russell, Veni Ezhil, Babikir Ismail, Melanie Irvin-sellers, Vineet Prakash, Jason F. Lester, Malgorzata Kornaszewska, Richard Attanoos, Haydn Adams, Helen Davies, Dahmane Oukrif, Ayse U Akarca, John A Hartley, Helen L Lowe, Sara Lock, Natasha Iles, Harriet Bell, Yenting Ngai, Greg Elgar, Zoltan Szallasi, Roland F Schwarz, Javier Herrero, Aengus Stewart, Sergio A Quezada, Peter Van Loo, Caroline Dive, C. Jimmy Lin, Matthew Rabinowitz, Hugo JWL Aerts, Allan Hackshaw, Jacqui A Shaw, Bernhard G. Zimmermann, the TRACERx consortium, the PEACE consortium & Charles Swanton

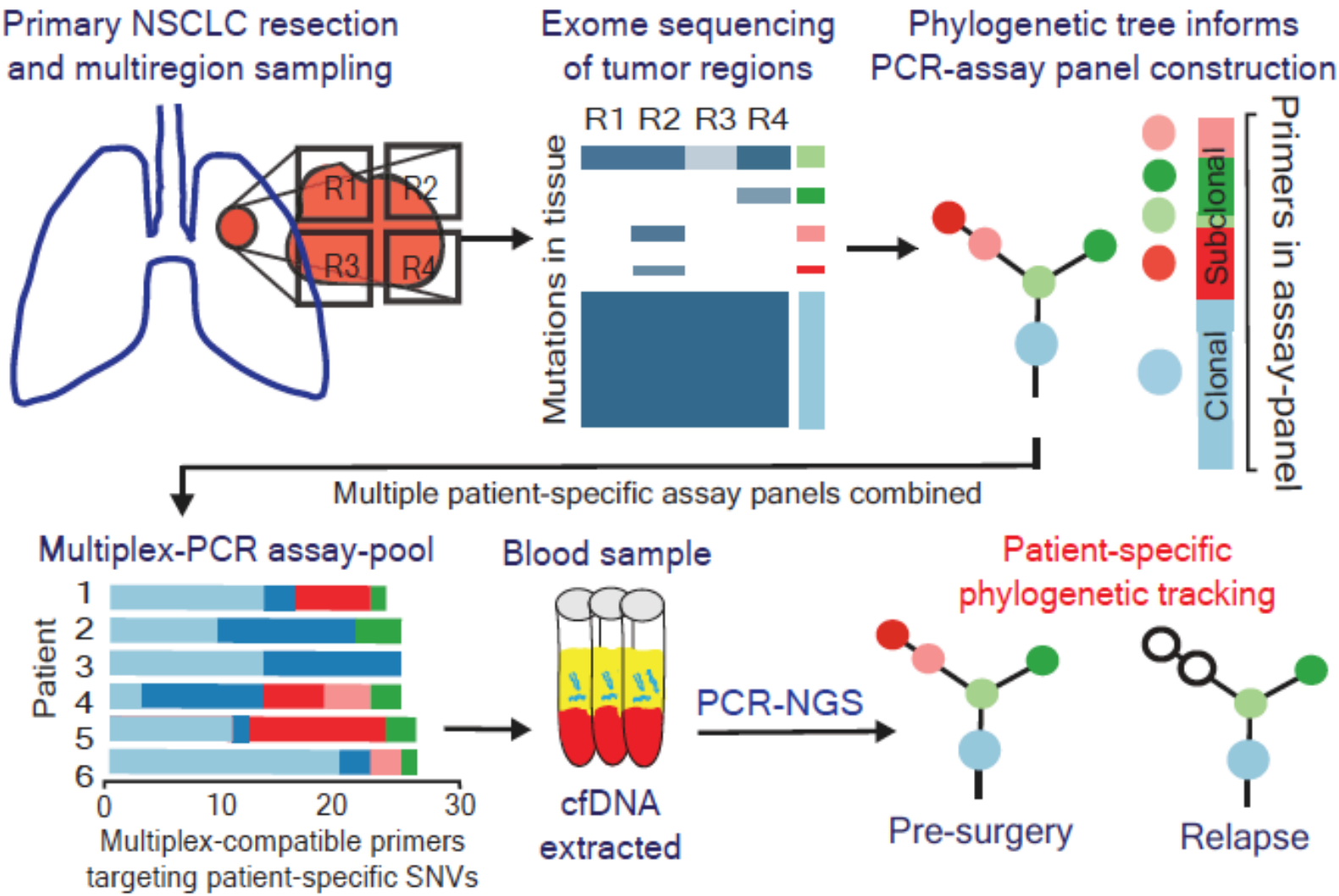
Study design

TRACERx
Sequencing
NSCLC blood and samples
~900 patients
Diagnosis to 5 y later
Cancer Research UK
2013–

First 100 enrollees for this study

Tissue microarray with duplicates of primaries – Ki67 staining
Histopathology – subtype, %necrosis, lymphovascular invasion
Radiology (CT, PET) – SUV, tumor volume, TBR
Exome DNA sequencing – blood, tumor (primary, metastasis): 200 ng DNA per sample
Circulating free DNA – PCR for 18 (median) patient-specific SNVs (mutations) -> sequencing to find mutation frequency

Study design

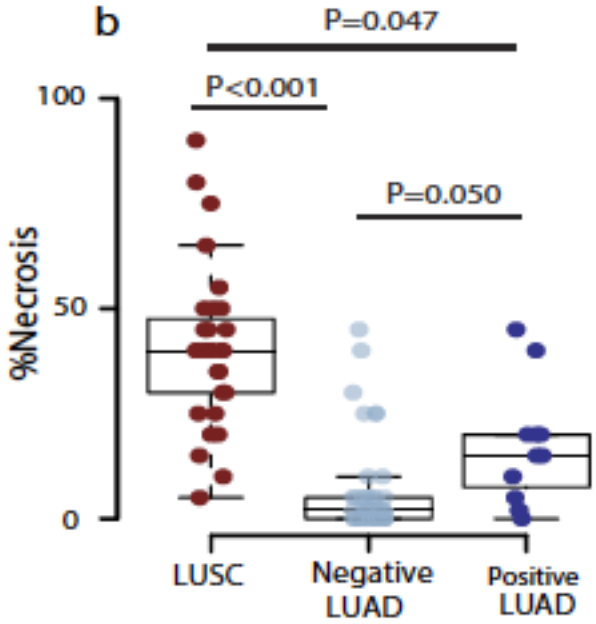


Tumor necrosis improves ctDNA detection

a

Histological subtype			TNM Stage I	II	III	Total
LUAD	ctDNA detected	Yes	5	2	4	11
		No	34	7	6	47
LUSC	ctDNA detected	Yes	16	12	2	30
		No	1	0	0	1
Other	ctDNA detected	Yes	1	2	2	5
		No	2	0	0	2

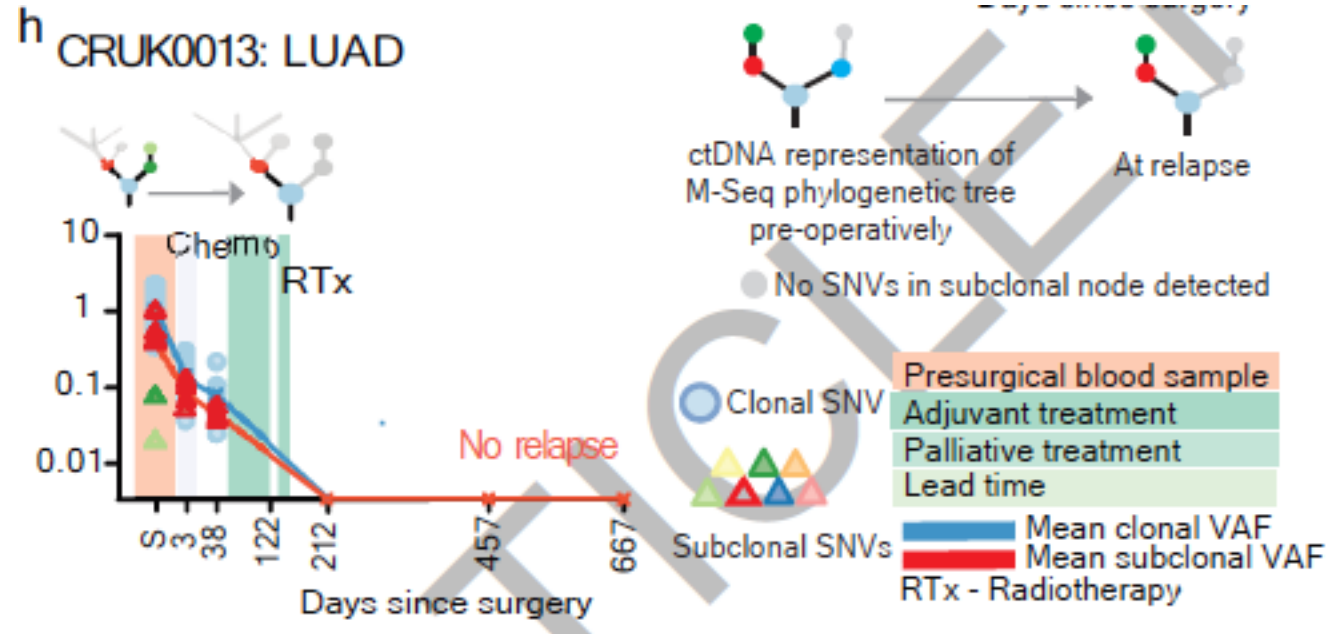
b

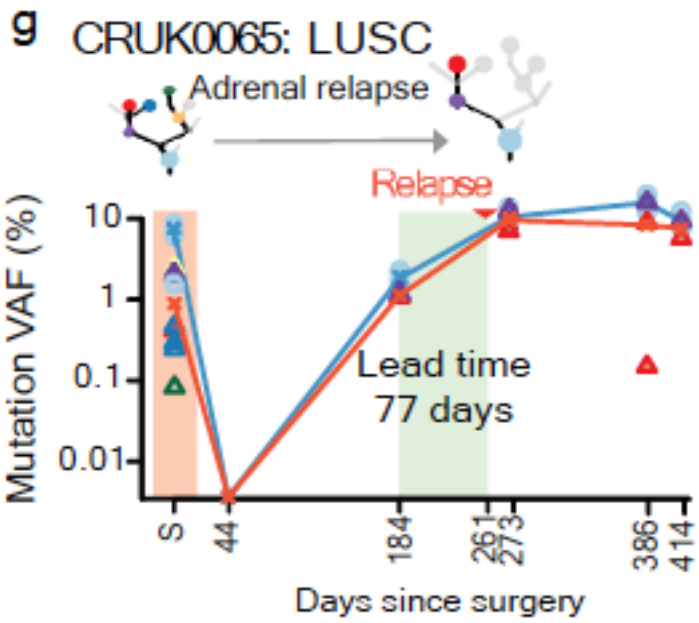


Predictors of ctDNA detection by multiplex-PCR NGS in early stage NSCLC

	Univariable analysis		Multivariable analysis	
	OR (95% CI)	P-value	OR (95% CI)	P-value
Clinicopathological variables				
Non-adenocarcinoma histology	49.85 (12.93 -192.19)	<0.001	40.76 (4.55 - 365.14)	0.001
%Ki67+ cells (10% increase)	1.72 (1.40 - 2.12)	<0.001	1.40 (1.05 - 1.84)	0.022
Lympho-vascular invasion	2.53 (1.10 - 5.80)	0.028	5.84 (1.07 - 32.03)	0.042
Necrosis (10% increase)	2.16 (1.58 - 2.97)	<0.001	1.04 (0.64 - 1.71)	0.862
Path tumor size (10mm increase)	1.45 (1.13 - 1.86)	0.004	1.32 (0.91-1.91)	0.134
Lymph-node involvement	3.60 (1.33 - 9.77)	0.012	3.82 (0.61 - 23.99)	0.153
Male gender	1.80 (0.78 - 4.16)	0.172	1.06 (0.21-5.39)	0.941
Age (years)	0.96 (0.92 - 1.01)	0.115	0.99 (0.92-1.07)	0.820

ctDNA biomarkers predict cancer relapse





Questions?

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