Molecular Profiling in Small Cell Lung Cancer and Lung Neuroendocrine Tumors

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Disclosures

- Rebecca Feldman
  Employee – Caris Life Sciences
- Igor Astsaturov
  Consultant or Advisory Role – Caris Life Sciences
- Sherri Millis
  Employee – Caris Life Sciences
- Deepa Subramaniam
  Speakers' Bureau – Pfizer
- Stephen V. Liu
  Consultant or Advisory Role – Perthera

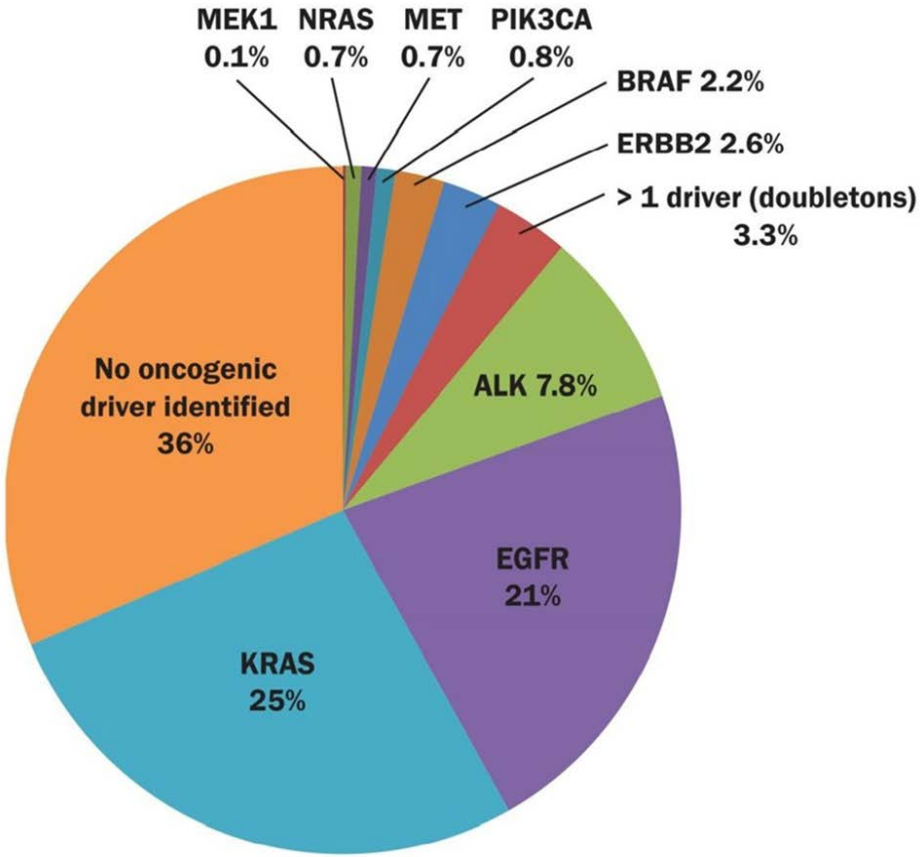
Introduction

- Lung cancer remains the leading cause of cancer death in the US and worldwide
  - Small cell lung cancer accounts for 13% of all cases
  - When considered independently, SCLC is the 5th leading cause of cancer mortality in the US
- Vast improvements over the past 10 years
  - Largely due to advances in molecular profiling
    - Identification of viable therapeutic targets
    - Primarily impacting adenocarcinoma

Globocan, WHO 2012
Govindan, JCO 2006

Introduction

- Concerted efforts
  - LCMC
    - Adenocarcinoma



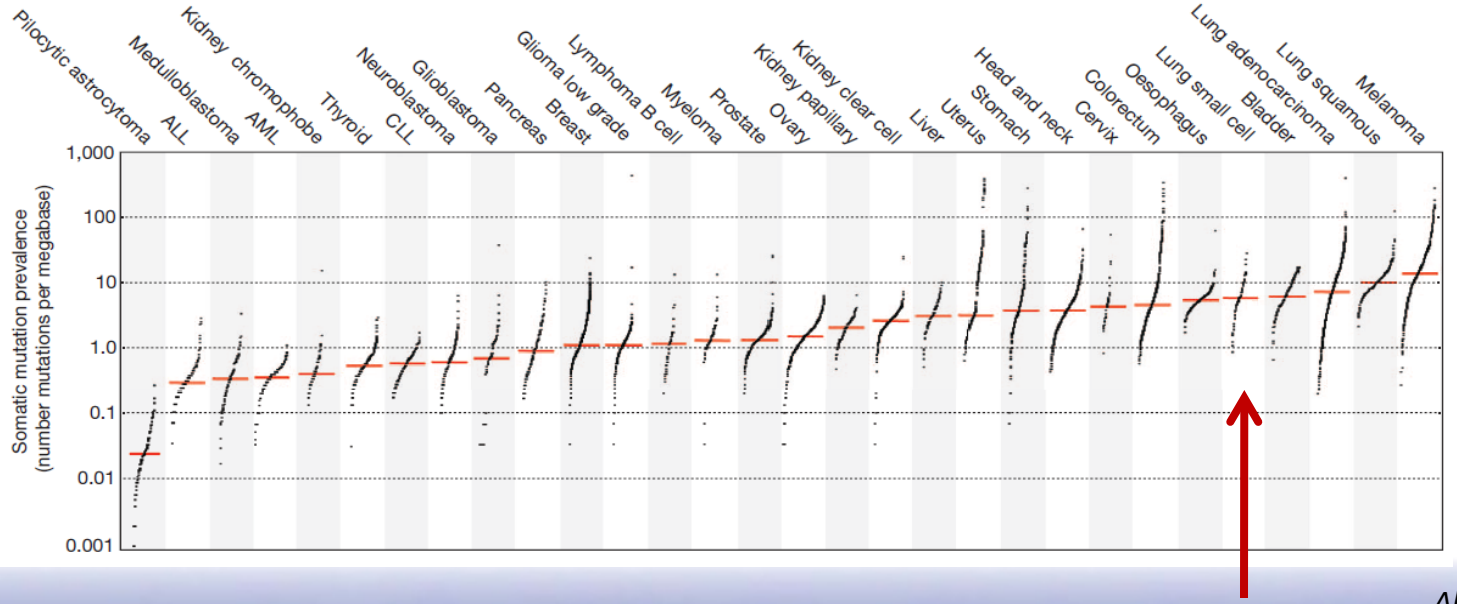
Aisner, ASCO 2014

Introduction

- Molecular profiling guides treatment
  - Adenocarcinoma is the current paradigm
    - Profiling is an established standard
  - Large efforts ongoing in squamous NSCLC
    - Including the NCI Lung-MAP
  - No clear role in small cell lung cancer

Molecular Profiling of SCLC

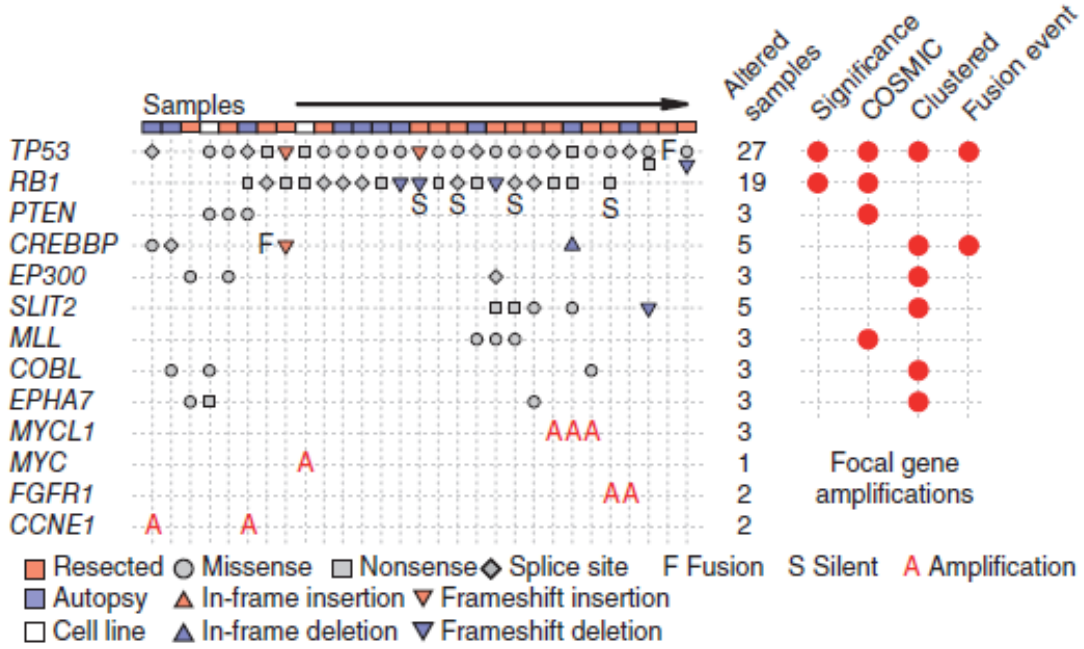
- SCLC is genomically complex
  - Heavy mutation burden consistent with tobacco-associated malignancy



Alexandrov, 2013

Molecular Profiling of SCLC

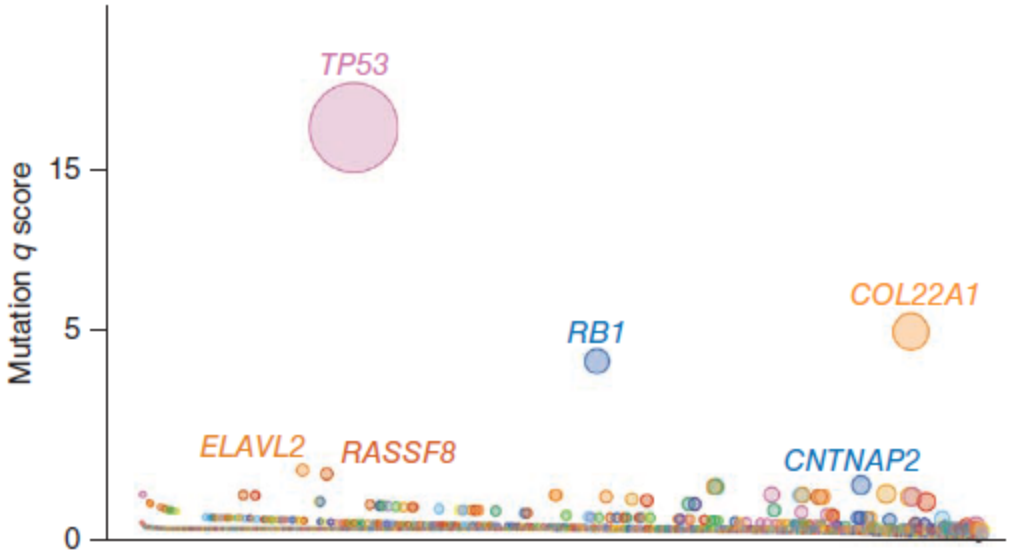
- Several groups have published genomic analyses of SCLC samples



Peifer, 2012

Molecular Profiling of SCLC

- Several groups have published genomic analyses of SCLC samples



Rudin, 2012

Molecular Profiling of SCLC

- Complex genomic signature
  - Loss of tumor suppressor genes
  - Alterations in epigenetic regulators
  - Very few driver mutations
  - Has not led to improvements in therapy

Neuroendocrine tumors

- Heterogeneous group
  - Pulmonary carcinoid
  - Pulmonary neuroendocrine
- Biologically distinct from SCLC
- Less common than other subtypes
- Role of molecular profiling is unclear
  - Large scale efforts are lacking

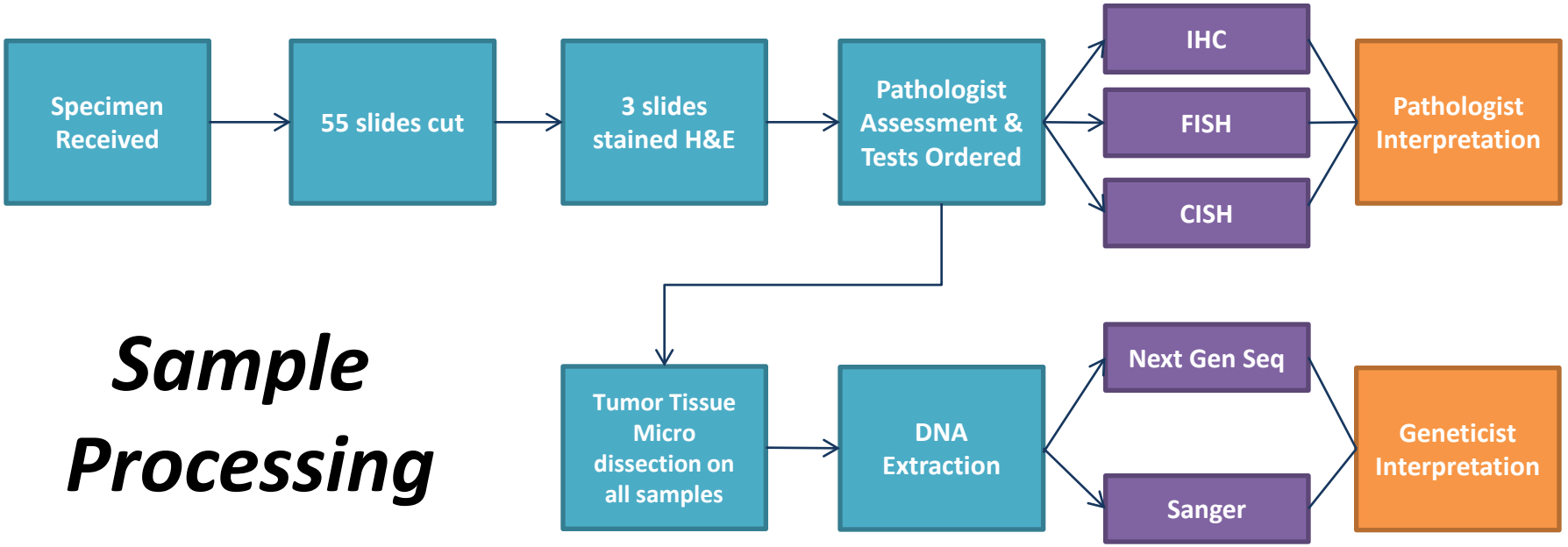
Molecular Profiling

- Many commercial assays now available
  - Genomic sequencing
  - Expression analyses
- Evaluated the database for one assay
  - Collected deidentified profiles for SCLC, pulmonary carcinoid and pulmonary neuroendocrine tumors previously submitted for analysis

Methods

- CLIA-certified, multiplatform profiling at Caris Life Sciences, CLIA certified, specimen reviewed by Board certified pathologists
  - DNA Sequencing (NGS or Sanger) for somatic mutations
    - Illumina MiSeq platform (Illumina TruSeq Amplicon Cancer Hotspot panel)
    - Up to 45 genes included in the panel
  - Fluorescence/Chromogenic *in situ hybridization* (FISH/CISH)
    - 6 gene panel
  - Immunohistochemistry using FFPE samples
    - 21 protein panel
    - Established thresholds specific to each antibody

Methods



Methods

- Next-Generation Sequencing
  - Illumina MiSeq platform
    - Illumina TruSeq Amplicon Cancer Hotspot panel
  - Average depth of coverage > 1500X
  - Analysis of tumor tissue only
  - 45 gene panel

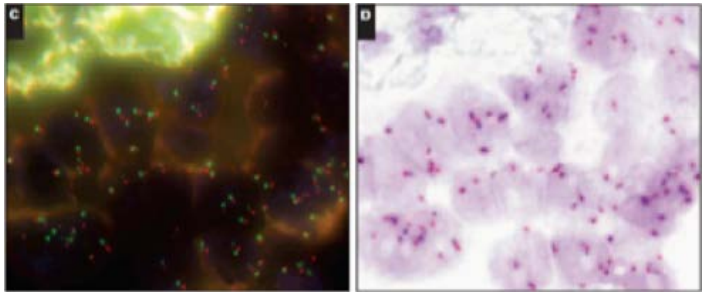
ABL1	CDH1	FBXW7	GNAS	KDR	NOTCH1	PTPN11	STK11
AKT1	CSF1R	FGFR1	HNF1A	cKIT*	NPM1	RB1	TP53
ALK	CTNNB1	FGFR2	HRAS	KRAS*	NRAS*	RET	VHL
APC	EGFR	FLT3	IDH1	cMET	PDGFRA	SMAD4	
ATM	ERBB2	GNA11	JAK2	MLH1	PIK3CA*	SMARCB1	
BRAF*	ERBB4	GNAQ	JAK3	MPL	PTEN	SMO	

*select genes with Sanger data included

Methods

- In Situ Hybridization
  - 6 gene panel
  - ALK and ROS1 – FISH break-apart probe tests for gene rearrangements
  - cMET, HER2, EGFR and TOPO2A – CISH/FISH tests for gene amplification

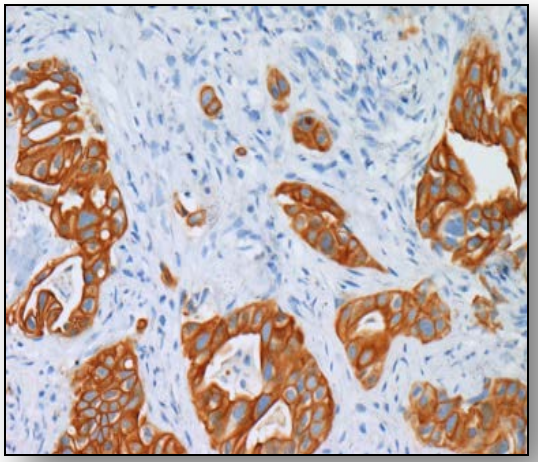
Anaplastic Lymphoma Kinase (ALK)
Proto-Oncogene Tyrosine-Protein Kinase (ROS1)
Human epidermal growth factor receptor 2 (HER2)
Epidermal growth factor receptor (EGFR)
Hepatocyte growth factor receptor (cMET)
Topoisomerase II-α (TOP2A)



*representative tumor samples

Methods

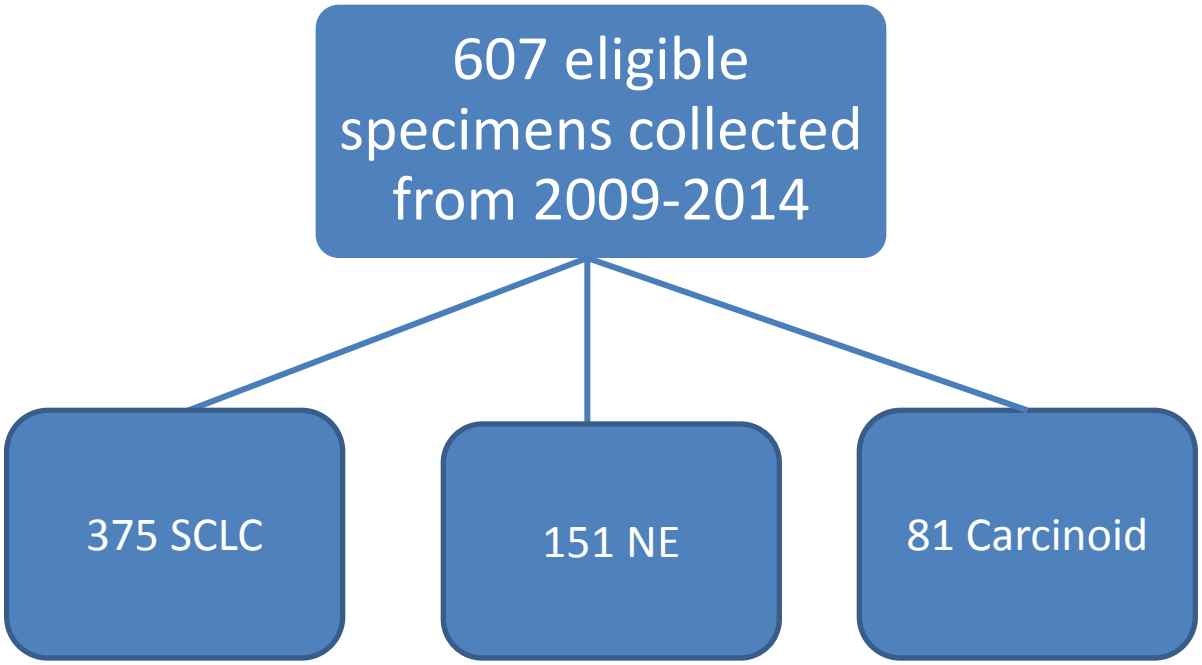
- Immunohistochemistry
  - 21 proteins tested
  - Ventana & Dako platforms
  - “overexpression” : intensity and percent staining exceeds predetermined cutoff



*representative tumor sample

Androgen receptor (AR)	Phosophatase and Tensin Homolog (PTEN)
cKIT (CD117)	Ribonucleotide reductase M1 (RRM1)
Hepatocyte growth factor receptor (cMET)	Secreted protein, acidic, cysteine-rich (SPARC)
Estrogen receptor (ER)	Transducin-like enhancer of split 3 (TLE3)
Progesterone receptor (PR)	Topoisomerase II alpha (Topo2α)
Breast cancer resistance protein (BCRP)	Topoisomerase I (Topo1)
Human epidermal growth factor receptor 2 (HER2)	Thymydilate synthase (TS)
0(6)-methylguanine-methyltransferase (MGMT)	Class III member of beta-tubulin (TUBB3)
P-glycoprotein (PGP)	Excision-repair cross-complementation group 1 (ERCC1)
Platelet-derived growth factor receptor (PDGFRA)	Multidrug-resistance protein 1 (MRP1)
Epidermal growth factor receptor (EGFR)	

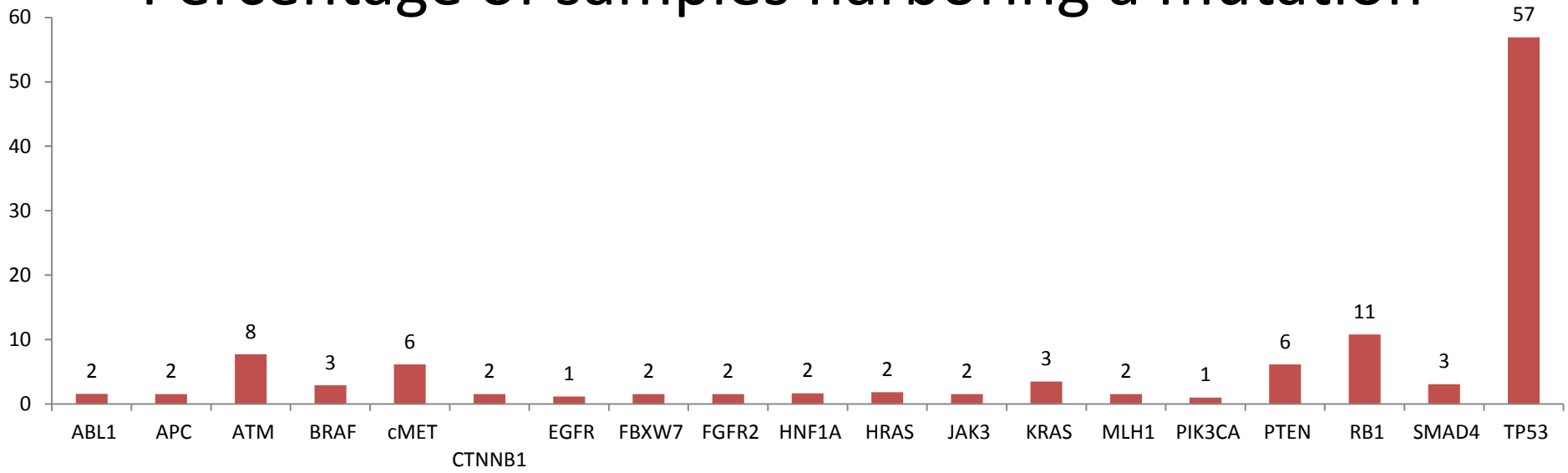
Results



Samples tested by platform			
	IHC: 340 ISH: 115 SEQ: 115	IHC: 140 ISH: 53 SEQ: 66	IHC: 77 ISH: 50 SEQ: 20

Sequencing – SCLC Samples

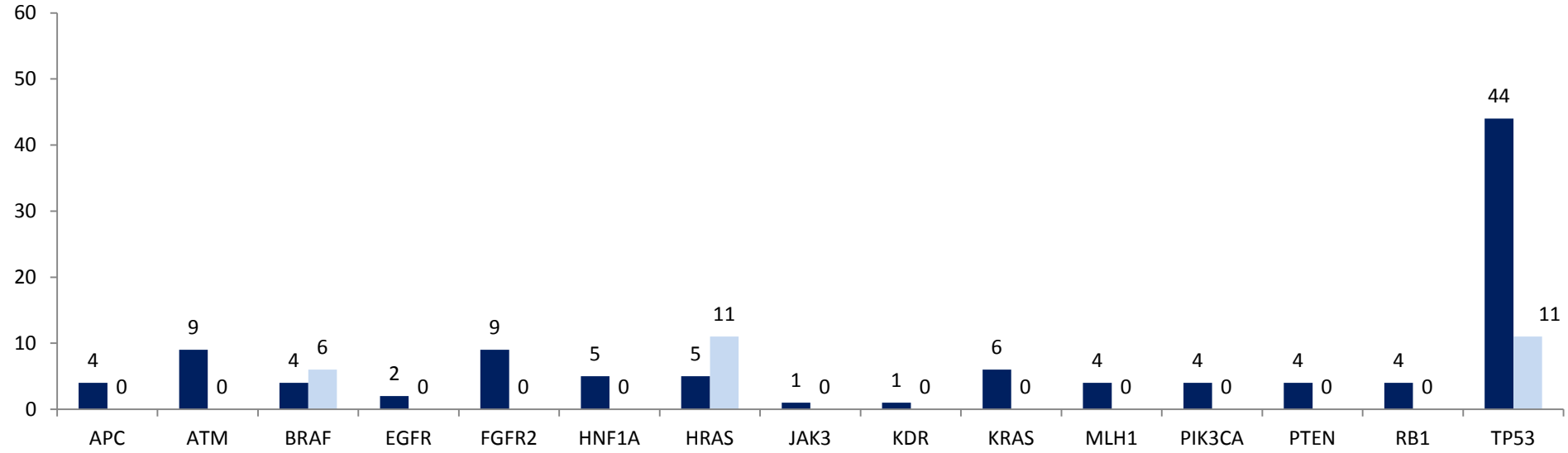
- Percentage of samples harboring a mutation



Genes tested without detection of variants : AKT, ALK, CDH1, cKIT, CSF1R, ERBB2, ERBB4, FGFR1, FLT3, GNA11, GNAQ/S, IDH1, JAK2, KDR, MPL, NOTCH1, NPM1, NRAS, PDGFRA, PTPN11, RET, SMARCB1, SMO, STK11, VHL

Sequencing – NE and Carcinoid

- Percentage of samples harboring a mutation



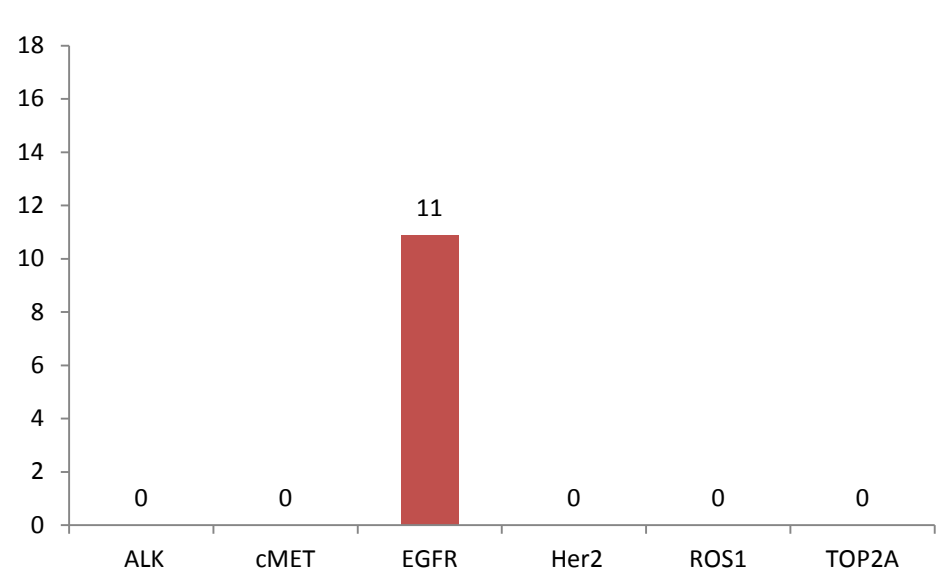
Genes tested without detection of variants: ABL, AKT, ALK, CDH1, cKIT, cMET, CTNNB1, CSF1R, ERBB2, ERBB4, FBXW7, FGFR1, FLT3, GNA11, GNAQ/S, IDH1, JAK2, MPL, NOTCH1, NPM1, NRAS, PDGFRA, PTPN11, RET, SMARCB1, SMAD4, SMO, STK11, VHL

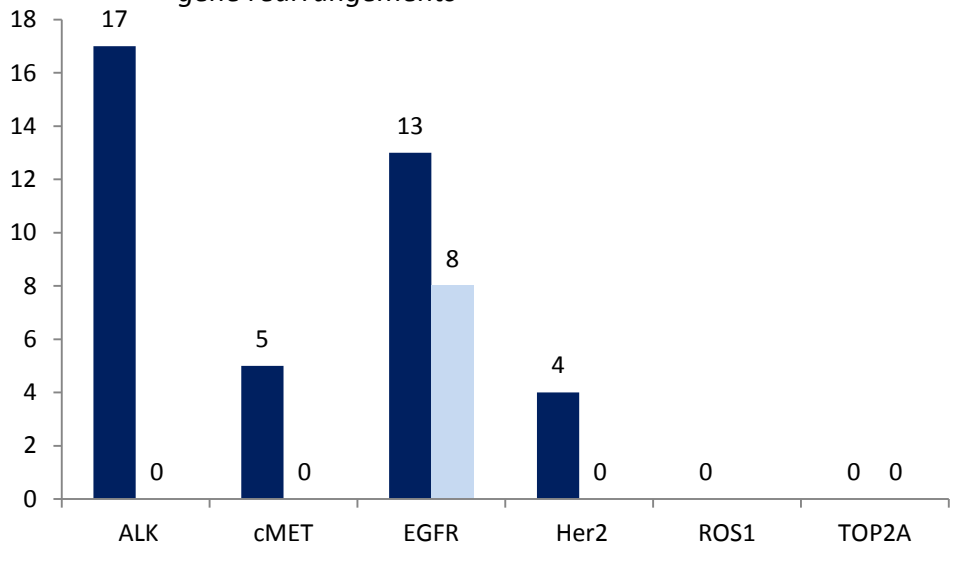
NE-NSCLC NE-Carcinoid

FISH/CISH

		ALK*	cMET	EGFR	Her2	ROS1*	TOP2A
SCLC	Positive	0	0	5	0	0	0
	Total	25	77	46	115	7	23
	% Altered	0%	0%	11%	0%	0%	0%
NE-NSCLC	Positive	1	1	7	1	0	0
	Total	6	19	53	25	1	8
	% Altered	17%	5%	13%	4%	0%	0%
NE-Carcinoid	Positive	0	0	4	0	0	0
	Total	3	11	50	17	0	5
	% Altered	0%	0%	8%	0%	---	0%

**gene rearrangements*

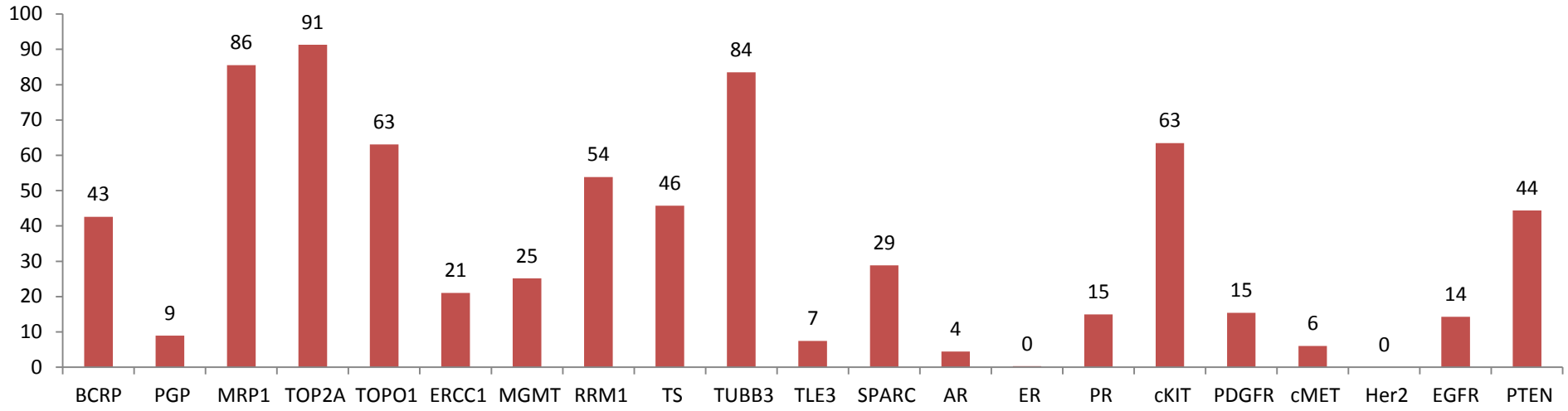




SCLC NE Carcinoid

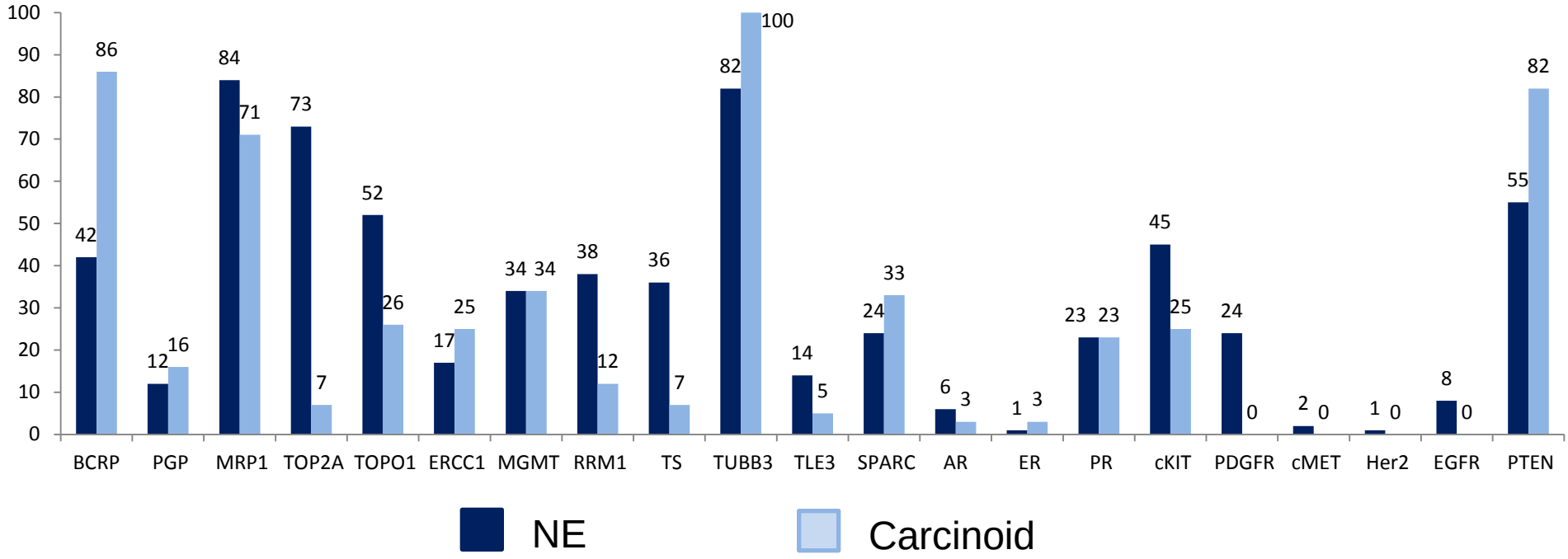
IHC – SCLC Samples

- Percentage of samples with overexpression



IHC – NE and Carcinoid

- Percentage of samples with overexpression



Conclusions

- SCLC, pulmonary neuroendocrine tumors and pulmonary carcinoid comprise a genomically heterogeneous population
- There were no consistent findings except p53 alterations
- Select driver mutations can be detected within these histologic subtypes

Conclusions

- Expression data are hypothesis generating but their clinical relevance must be established
- Large scale comprehensive molecular analysis remains an unmet need in these tumor types

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IHC Thresholds

Antibody (biomarker)	Threshold
Androgen receptor (AR)	0+ or <10% or ≥1+ and ≥10%
cKIT (CD117), PDGFRA	0+ and =100% or ≥2+ and ≥30%
Hepatocyte growth factor receptor (cMET)	<50% or <2+ or ≥2+ and ≥50%
Estrogen receptor (ER)	0+ or <10% or ≥1+ and ≥10%
Progesterone receptor (PR)	0+ or <10% or ≥1+ and ≥10%
Excision Repair Cross Complementation group 1 (ERCC1)	<2+ or ≤3+ and <10% or =2+ and <50% or ≥3+ and ≥10% or ≥2+ and ≥50%
Epidermal growth factor receptor (EGFR)	2+ and ≥10%
Human epidermal growth factor receptor 2 (HER2)	≤1+ or =2+ and ≤10% or ≥3+ and >10%
0(6)-methylguanine-methyltransferase (MGMT)	0+ or ≤35% or ≥1+ and >35%
P-glycoprotein (PGP), Multidrug Resistance Protein (MRP1) Breast Cancer Resistance Protein (BCRP)	0+ or <10% or ≥1+ and ≥10%
Phosophatase and Tensin Homolog (PTEN)	0+ or ≤50% or ≥1+ and >50%
Ribonucleotide reductase M1 (RRM1)	0+ or <50% or <2+ or ≥2+ and ≥50%
Secreted protein, acidic, cysteine-rich (SPARC)	<30% or <2+ or ≥2+ and ≥30%
Transducin-like enhancer of split 3 (TLE3)	<30% or <2+ or ≥2+ and ≥30%
Topoisomerase II alpha (Topo2α)	0+ or <10% or ≥1+ and ≥10%
Topoisomerase I (Topo1)	0+ or <30% or <2+ or ≥2+ and ≥30%
Thymidylate synthase (TS)	0+ or ≤3+ and <10% or ≥1+ and ≥10%
Class III member of beta-tubulin (TUBB3)	<30% or <2+ or ≥2+ and ≥30%

ISH Thresholds

Antibody (biomarker)	Threshold
HER2 FISH	HER2/Neu:CEP 17 signal ratio of >=2.0 is amplified and <2.0 is not amplified per Abbott (Pathvysion) and Herceptin package inserts. Per ASCO CAP guidelines, FISH amplification is >2.2 and non-amplification is <1.8. Please note, the range 1.8-2.2 is equivocal.
HER2 CISH	Her2/Neu:CEP 17 signal ratio of >= 2.0; and non-amplification as <2.0 per Ventana INFORM HER2 CISH Package insert.
EGFR FISH	
cMET CISH	Positivity for increased gene copy number by FISH has been defined as >= 5 copies in lung tumor cells. The gene copy number threshold for other tumor types has not been determined.
TOP2A CISH	In breast cancer, FISH amplification has been established as a TOP2:CEP17 signal ratio of >=2.0.
ALK	Positivity for ALK rearrangement is defined as >25 positive cells out of the 50 cells analyzed. A sample is considered negative if <5 positive cells are present out of the 50 cells analyzed. In cases where 5-25 cells are positive, the sample is considered equivocal, and an additional 50 cells are analyzed by a second technologist. From this expanded analysis, if ≥15 cells out of the 100 cells analyzed are positive for ALK rearrangement, the sample is considered positive. If <15 positive cells are observed out of the 100 analyzed, the sample is considered negative.
ROS1	Positivity for ROS1 rearrangement is defined as the presence of >15% positive cells out of the population of cells analyzed.