Biologic Subtypes of SCLC and their Clinical Significance

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Disclosure: Dr. Gazdar receives licensing fees for the cell lines he has established

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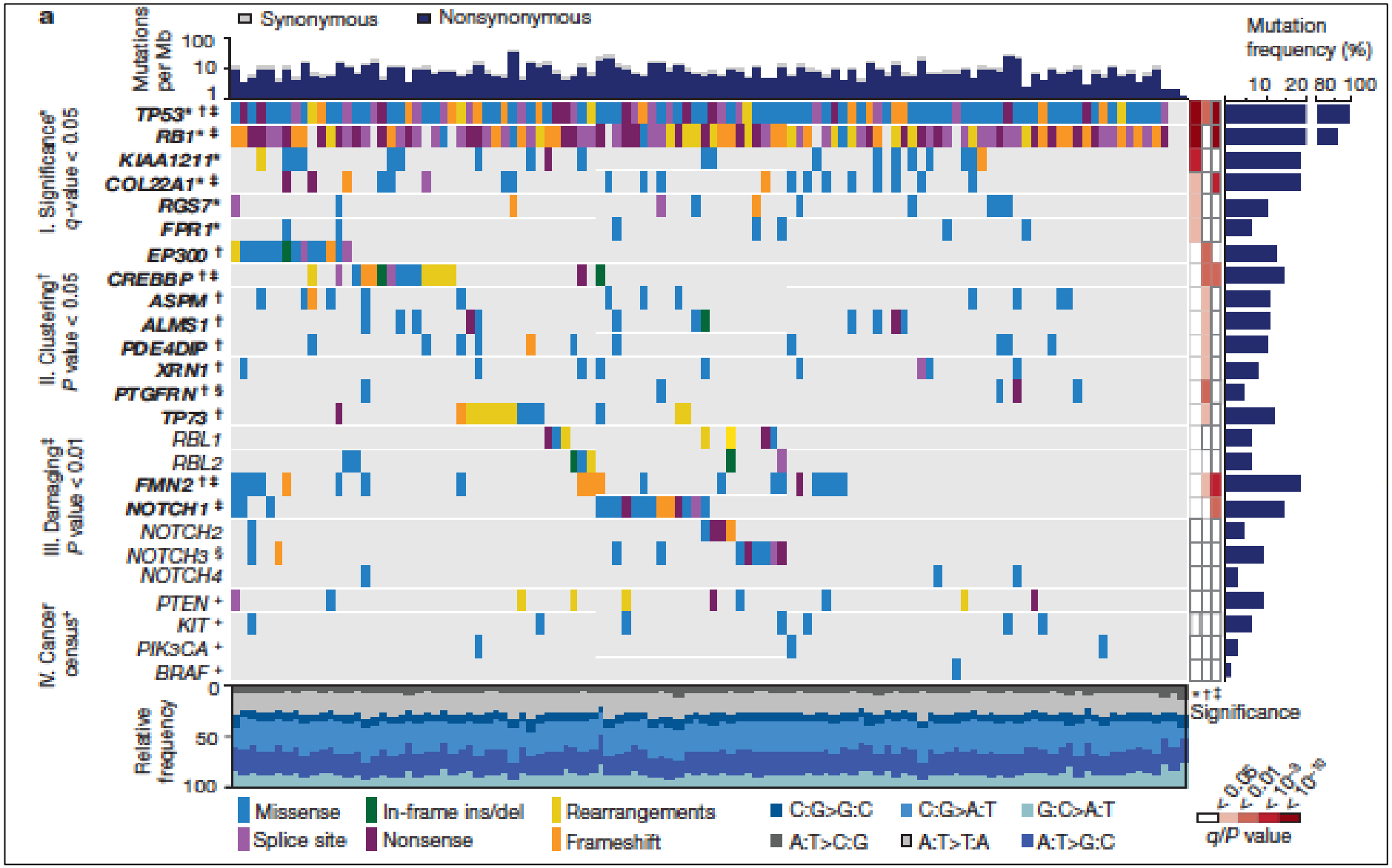
Mutated genes in SCLC tumors (n = 81)



Key Points

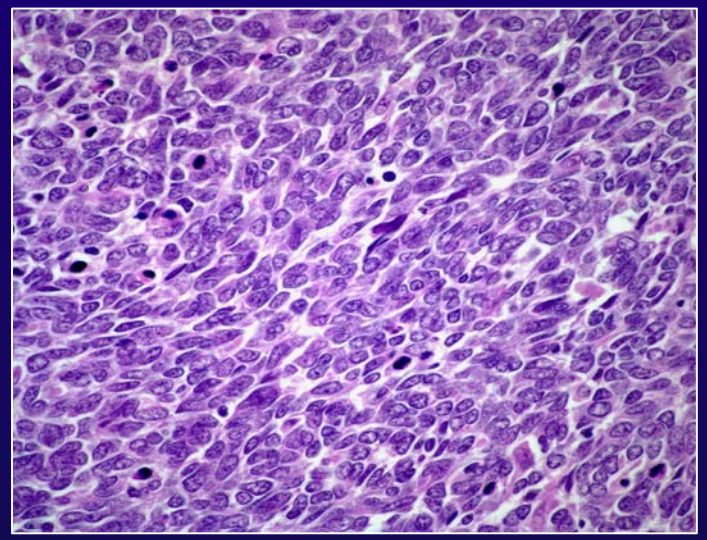
- TP53 and RB1 universally inactivated
- Histone acetyltransferase genes EP300 & CREBBP
- Notch genes
- FMN2 and COL22A1 involve in collagen and actin binding and cytoskeleton
- KIAA1211 - not much known

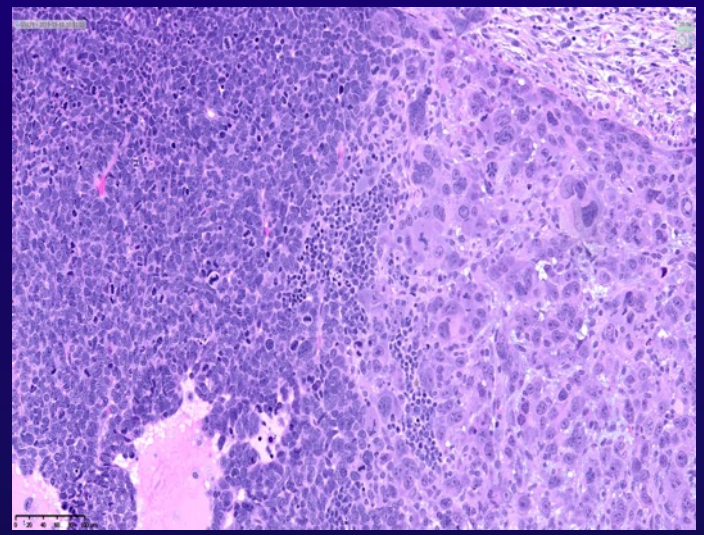
George et al, Nature, 2015

Is SCLC a Homogenous Cancer?

- Only one morphologic type recognized by the WHO Classification 2015, although it may be combined with non-SCLC elements
- Clinically it is treated uniformly, with adjustments for tumor extent/staging
- No approved targeted therapies

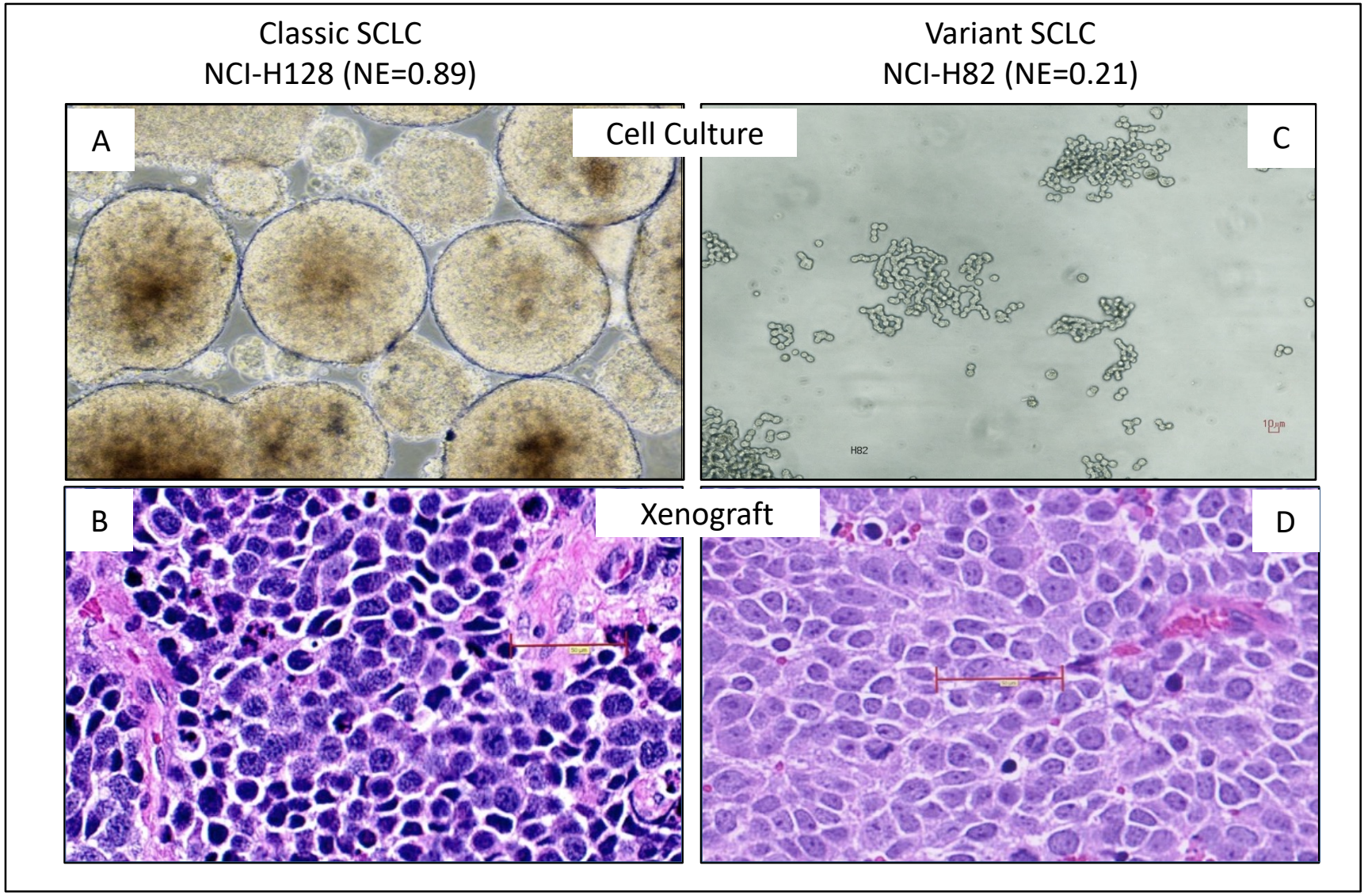
SCLC





Combined SCLC (>10% NSCLC)

"Classic" and "Variant" forms of SCLC



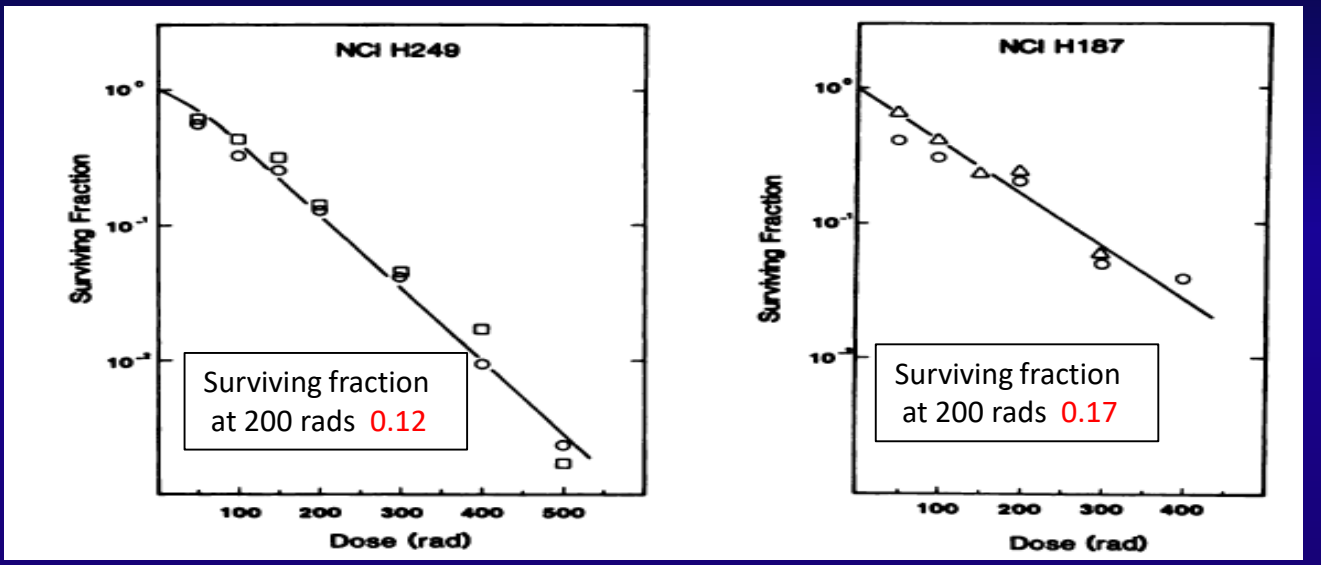
Properties of variant SCLC

- Morphology
- Substrate attachment
- Increased growth & cloning
- MYC overexpression
- Radioresistance
- Decreased NE properties

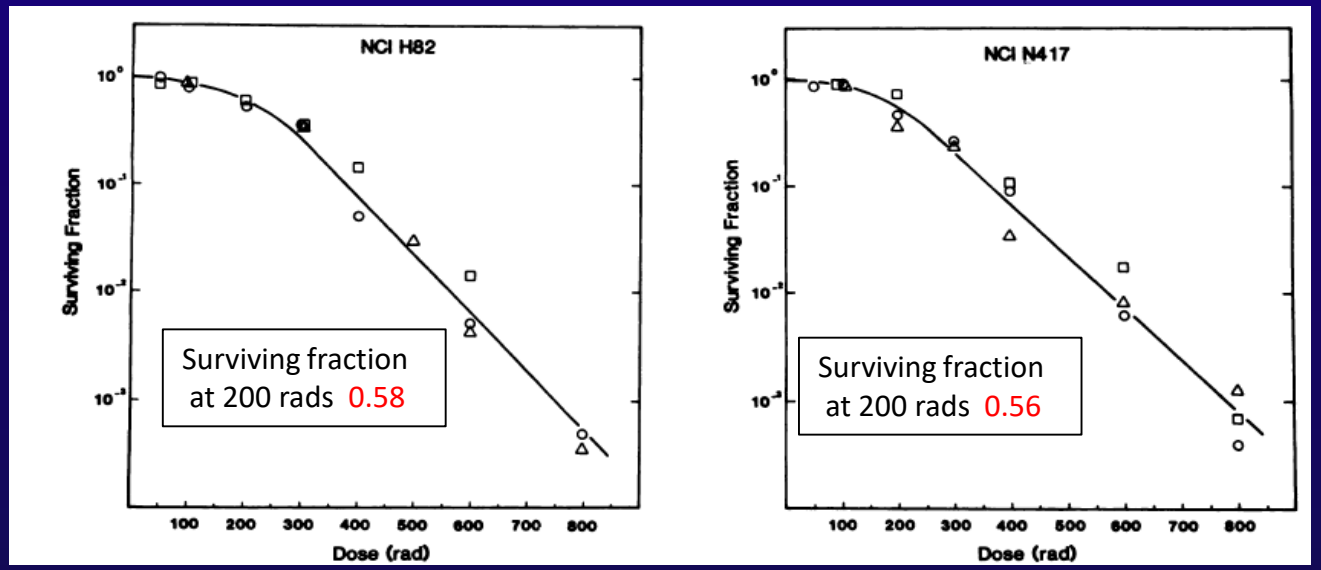
Gazdar et al, Cancer Res 1985

Radiation survival curves for classic and variant SCLC

Classic SCLC

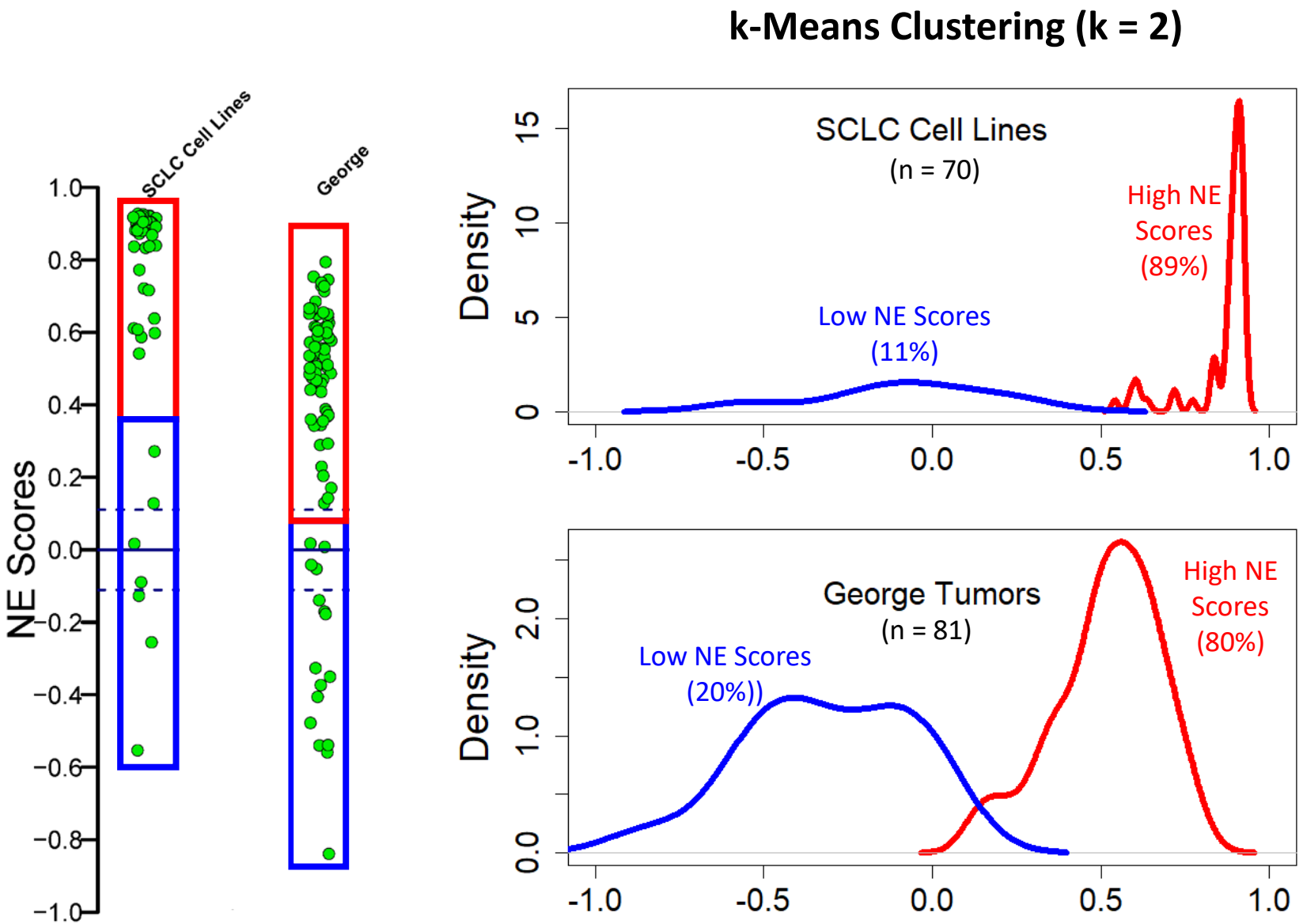


Variant SCLC
Myc family amplified



Carney, DN et al, Cancer Res, 1983

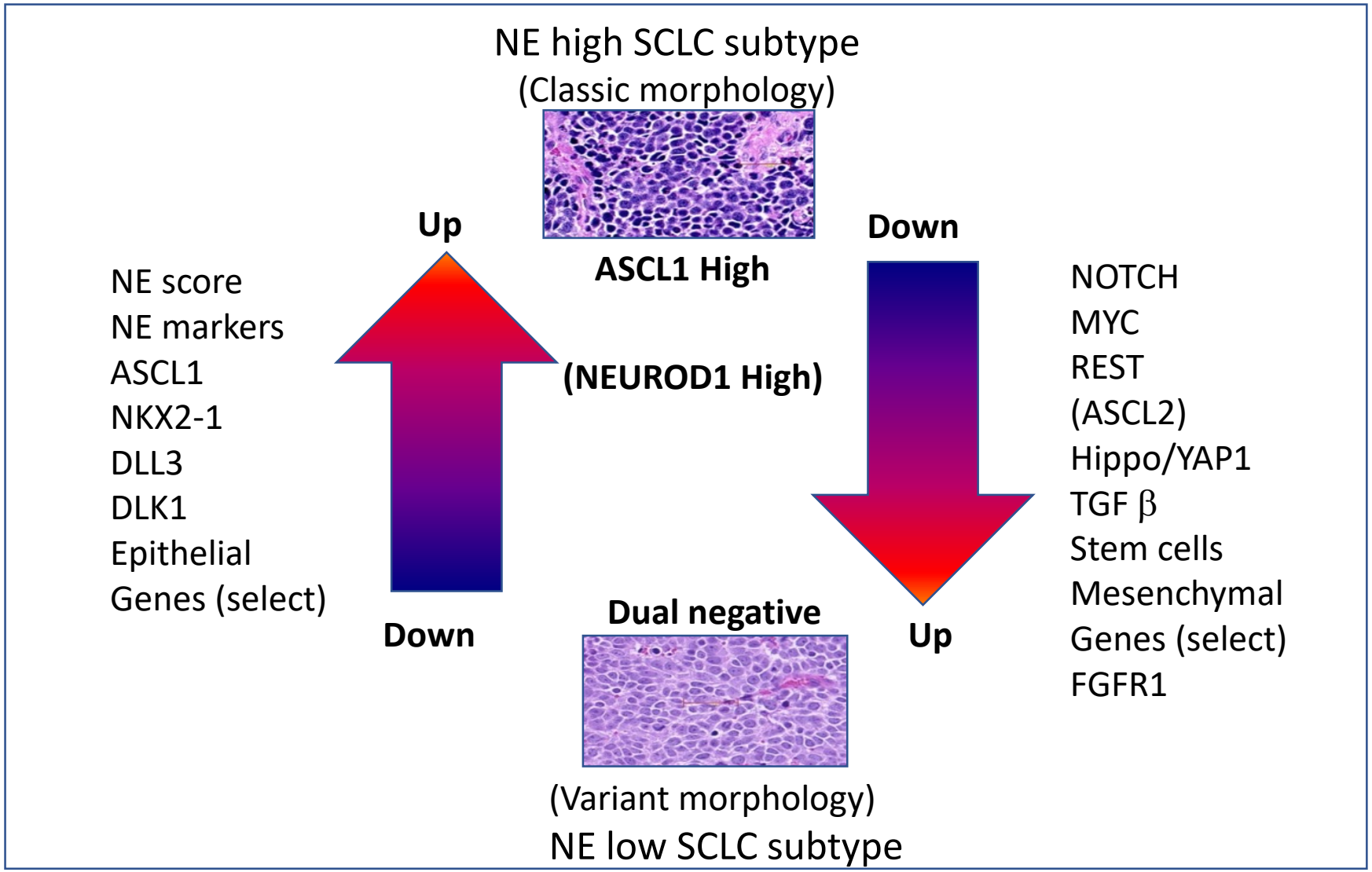
SCLC Cell Lines and Tumors Segregate in Low and High NE Subgroups



Genes of interest and their relationship to NE differentiation in SCLC tumors (n = 81)

Correlation with NE score	Gene	*r* value	P value
Positive Correlation	ASCL1	*r* = 0.68	1.6E-6
	NEUROD1	*r* = 0.31	0.02
	DLL3	*r* = 0.64	7.4E-08
	SOX2	*r* = 0.31	8.0E-03
Negative Correlation	REST	*r* = -0.85	2.8E-09
	Hippo pathway (Ajuba)	*r* = -0.81	4.5E-05
	NOTCH Pathway (NOTCH1)	*r* = -0.46	9.4E-07
	MYC	*r* = -0.66	1.8E-06
	TGF Pathway (TGFB1)	*r* = -0.47	9.9E-04
	Stem cells (ALDH1A3)	*r* = 0.72	9.9E-06
	FGFR1	*r* = -0.52	0.46
	JAK/STAT Signaling (JAK2)	*r* = -0.44	8.5E-07
No Correlation	SLFN11, NFIB, MYCL, MYCN, EZH2, DDR1, ATR, CDK7, KDM1A (LSD1A)		

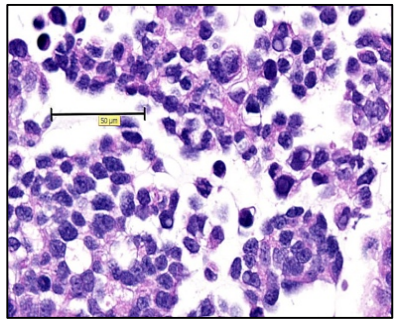
Molecular Changes Associated with Loss of NE Differentiation in SCLC



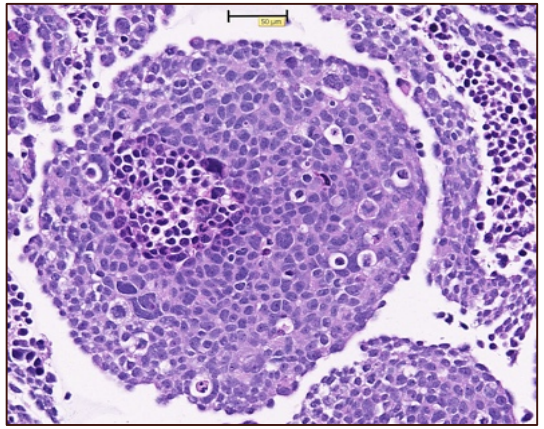
Zhang et al, Transl. Lung Cancer Res, On line 2018

Heterogeneity and Plasticity in SCLC Cell Line NCI-H69

Early passage



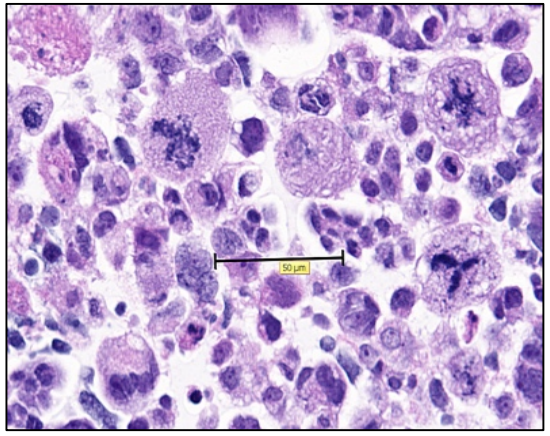
NE score 0.88



Spheroid Subline

NE score 0.87

- Morphology Classic
- ASCL1 High
- DLL3 High
- REST Absent
- MYC Low

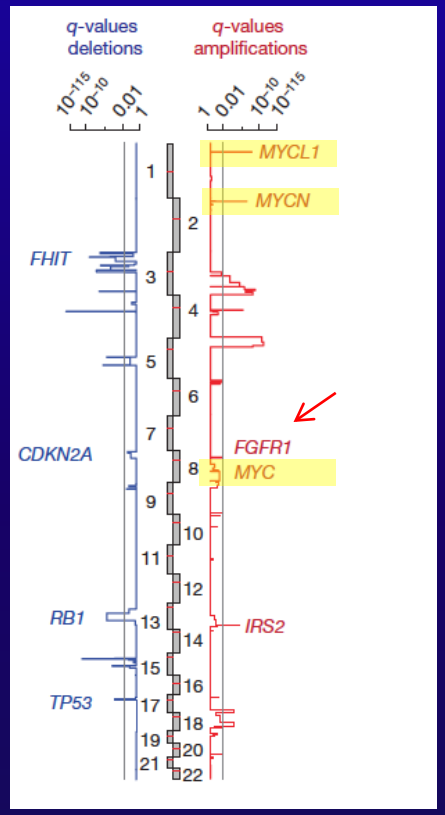


Attached Subline

NE score -0.2

- Morphology Different
- ASCL1 Low
- DLL3 Low
- REST High
- MYC High

MYC family and FGFR1 amplification in SCLC



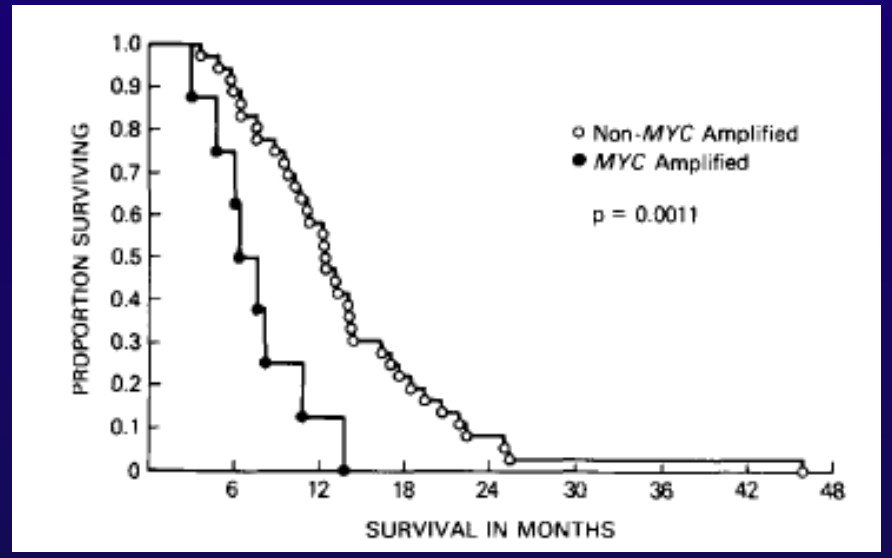
George et al, Nature, 2015

Frequency of MYC amplification

Pretherapy 7/52 (11%)

Post therapy 16/44 (36%)

Johnson, Gazdar, Minna et al, J Cell Biochem 1996

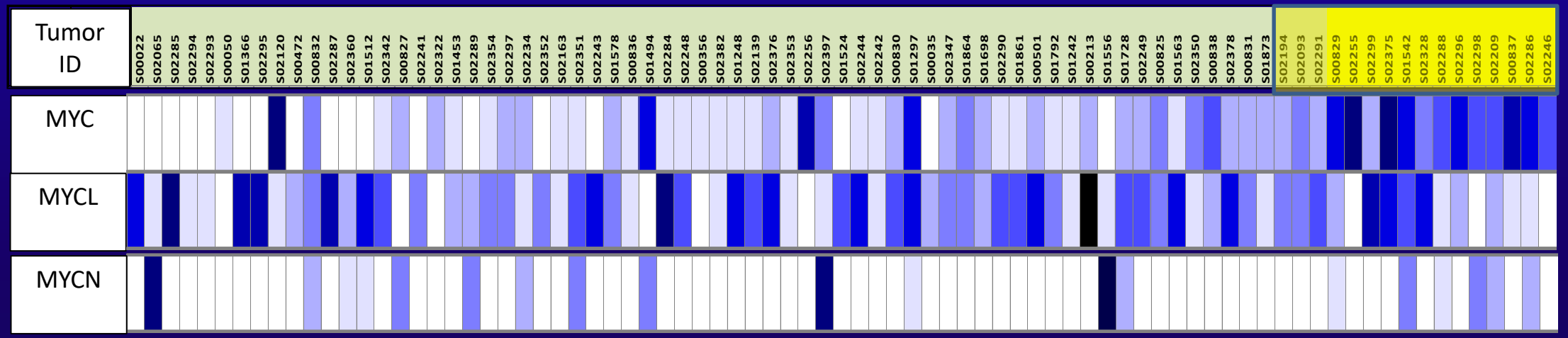


George, et al Nature, 2015

Myc Family Overexpression & Amplification

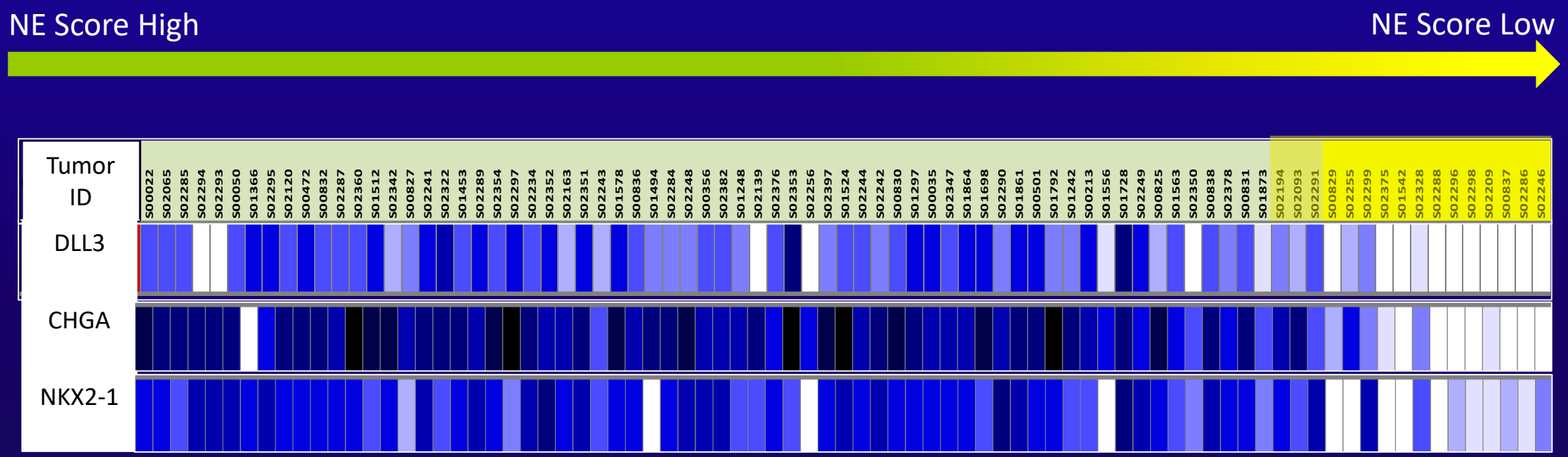
NE Score High

NE Score Low



MYC r = -0.66, p = 7.0E-05
MYCL r = 0.12, p = 0.5
MYCN r = -0.21, p = >0.5

Genes whose expression is positively correlated with NE differentiation SCLC Tumors (n = 81)



DLL3	r = 0.64,	p = 5.9E-06
CHGA	r = -0.77,	p = 6.9E-07
NKX2-1	r = 0.64,	p = 1.5E-04

Reports of NE high and low SCLC subtypes (some with responses to chemotherapy/targeted therapies)

First author (Year)	Specimens studied	Nomenclature for NE High	Nomenclature for NE low
Zhang (2018)	Tumors/Cell lines/GEMM	NE High	NE Low
George (2015)	Tumors/GEMM	NE High	NE Low
Cardnell (2017)	Tumors/Cell lines	High TTF1/Low cMYC	High cMYC/Low TTF1
Udyavar (2017)	Tumors/Cell lines	NE/epithelial	Non NE/mesenchymal
Ito (2016)	Cell lines	NE positive/YAP1 negative	NE negative/YAP1 positive
Mollaoglu (2017)	GEMM/Tumors/Cell lines	NE	NE low
Lim (2017)	GEMM	NE	Notch active, non-NE
Calbo (2011)	GEMM	Neuroendocrine profile	Mesenchymal profile
Stewart (2017)	Cell lines, PDXs	(High E-cadherin)	(EMT)

Adaptive and Innate Immunity

- Adaptive immunity
  Antigen specifc responses
  (3-5 days+)
- Innate immunity
  Non-specific defenses activated by the antigen
  (Within hours)

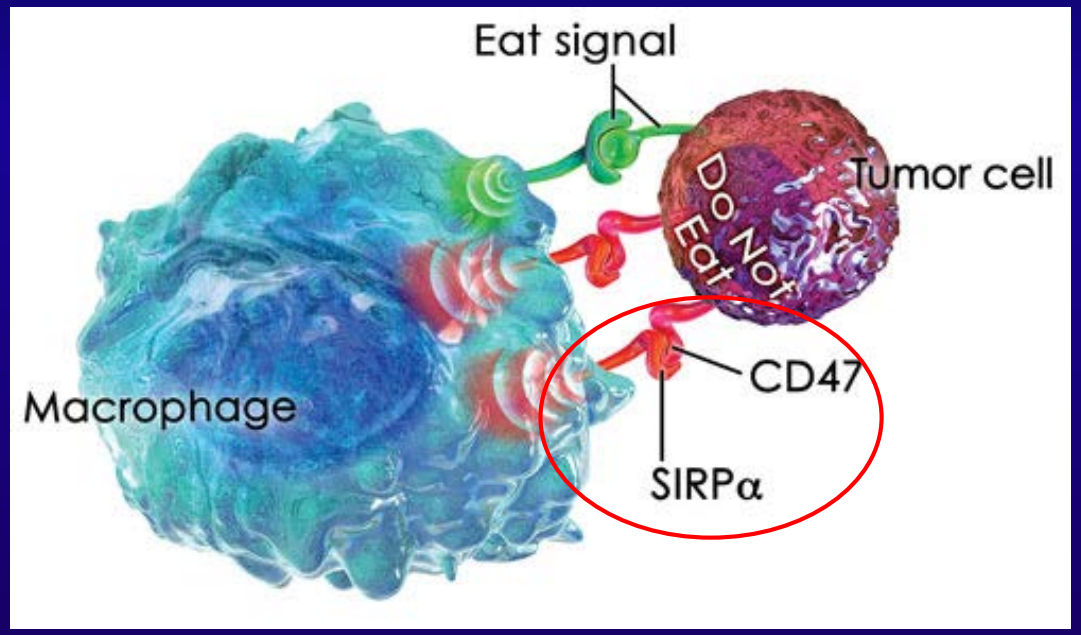
Components of Innate Immunity (Tumor Microenvironment is Different in Low and High NE SCLCs)

Punch line

- All of these components of the Innate Immune system (22 cell types, >200 genes) are highly up regulated in the low NE subtype of SCLC (p values 5.8E-03 to 2.6E-11)
- Cytokines signal through JAK/STAT, which is highly up regulated in the low NE subgroup

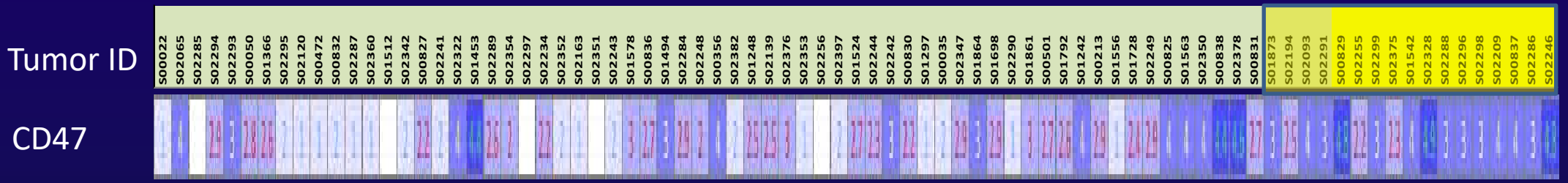
- Toll-like Receptors
- Cytokines (4 major families)
  - Interferon family including IFNγ
  - Interleukin family
  - Chemokine family
  - Tumor Necrosis Family
- Complement System
- Major Histocompatability Complex (HLA antigens)
- The Inflammasome
- Cellular responses – immune & Inflammatory cells
  - Neutrophils
  - Macrophages
  - NK cells
  - Dendritic cells
  - Eosinophils

Proposed SCLC Therapeutic Target: CD47 - The "Don't Eat Me!" Antigen (Upregulated in Low-NE Subtype)



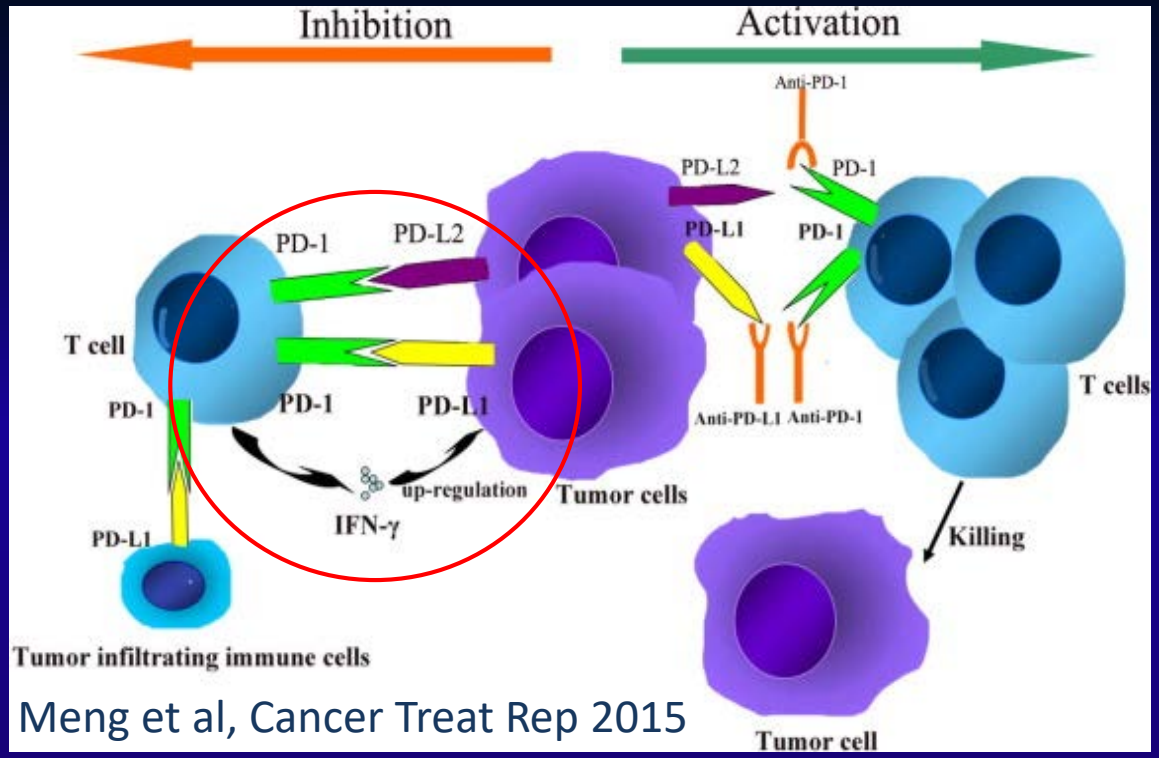
Trillium Therapeutics

- CD47 is expressed at variable levels on the surface of most cells, including SCLC
- It binds to the SIRPα receptor on macrophages sending a "Don't Eat Me!" signal
- Blocking this interaction results in greater cancer cell kill



(r = -0.54, p = 2.0E-05)

NE Differentiation and Checkpoint Inhibitor Immunotherapy



Meng et al, Cancer Treat Rep 2015

Higher in NE Low Subtype

Gene	Symbol	r	p
PD1	PDCD1	-0.4	6.0E-03
PD-L1	CD274	-0.30	0.09
PD-L2	PDCD1LG2	-0.51	0.9
Interferon γ (IFNγ)	IFNG	-0.26	6.0E-03
IFNγ Signature (18 gene)	NA	NA	4.9E-04
CD44	CD44	-0.52	7.5E-03
No correlation	CTLA4, CD133		

Predicted Therapeutic Responses of NE high and Low SCLC Subtypes

Therapy	Predicted Response NE High	Predicted response NE Low
Chemotherapy	Sensitive	Resistant
Radiotherapy	Sensitive	Resistant
PARP Inhibitors	Sensitive	Resistant
ROVA-T	Sensitive	Resistant
Topoisomerase inhibitors	Sensitive	Resistant
Polo-like kinase inhibitors	Resistant	Sensitive
HIPPO pathway Inhibition	Resistant	Sensitive
FGFR1 inhibitors	Resistant	Sensitive
JAK-STAT Inhibitors	Resistant	Sensitive
Immunotherapy (PD-1 blockage)	Resistant	Sensitive
CD47 Inhibitors	Resistant	Sensitive

Summary: SCLC - Heterogeneity and Clinical Response

- Recently, many new molecular targets have been identified for SCLC and several are in clinical trial
- SCLC shows considerable inter-tumor heterogeneity, especially for expression of NE properties
- Inter-tumor heterogeneity extends to the infiltrating immune & inflammatory cells
- This heterogeneity must be considered for selection of subgroups more likely to respond to specific therapies

What remains to be done?

- Heterogeneity observations must be extended to another large, fully characterized dataset
- Predictions for differential responses to targeted therapies need to be tested in prospective and retrospective trials
- Predictions for differential responses to PD-1 blockade must be confirmed in clinical trials