The International Academy of Pathology
Hong Kong Division
2024 Scientific Congress

Immunohistochemistry in lung cancer

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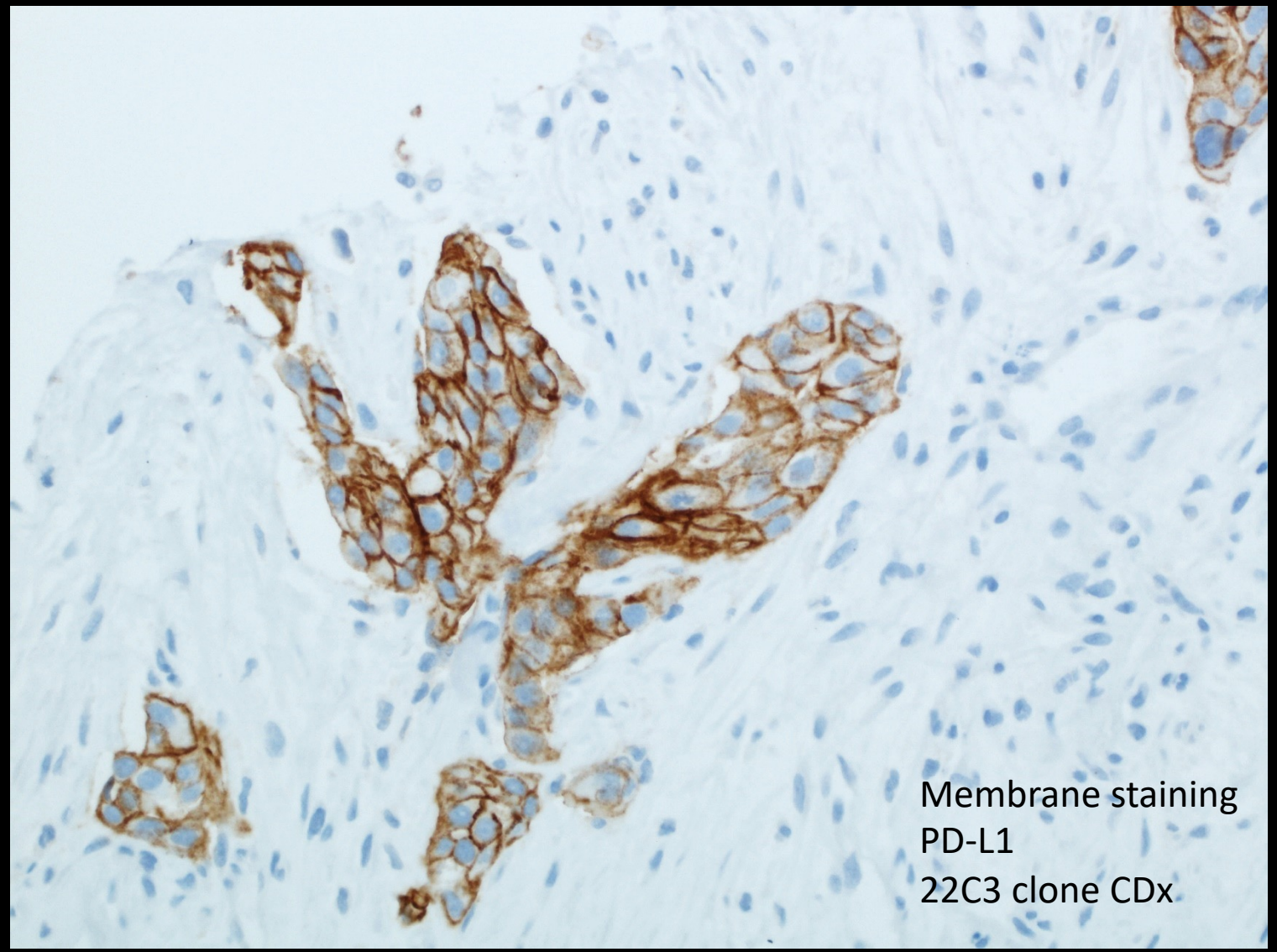
1 4 9 5
UNIVERSITY OF ABERDEEN

NHS Grampian



Immunohistochemistry

A Diagnostic tool

Tumour identification

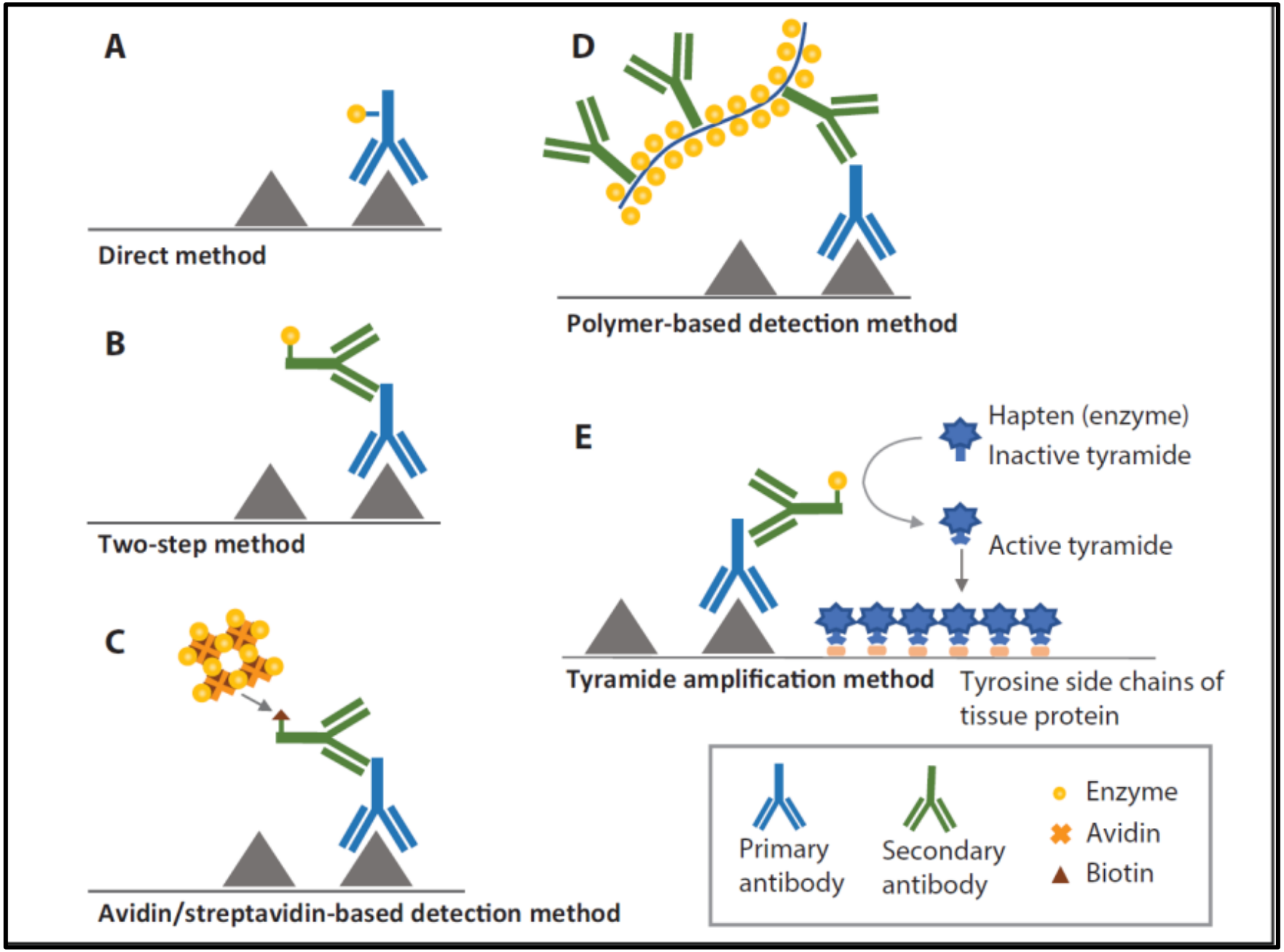
Tumour Classification

Biomarker Identification

Prognostic markers

Predictive markers

Immuno-Histochemistry Techniques

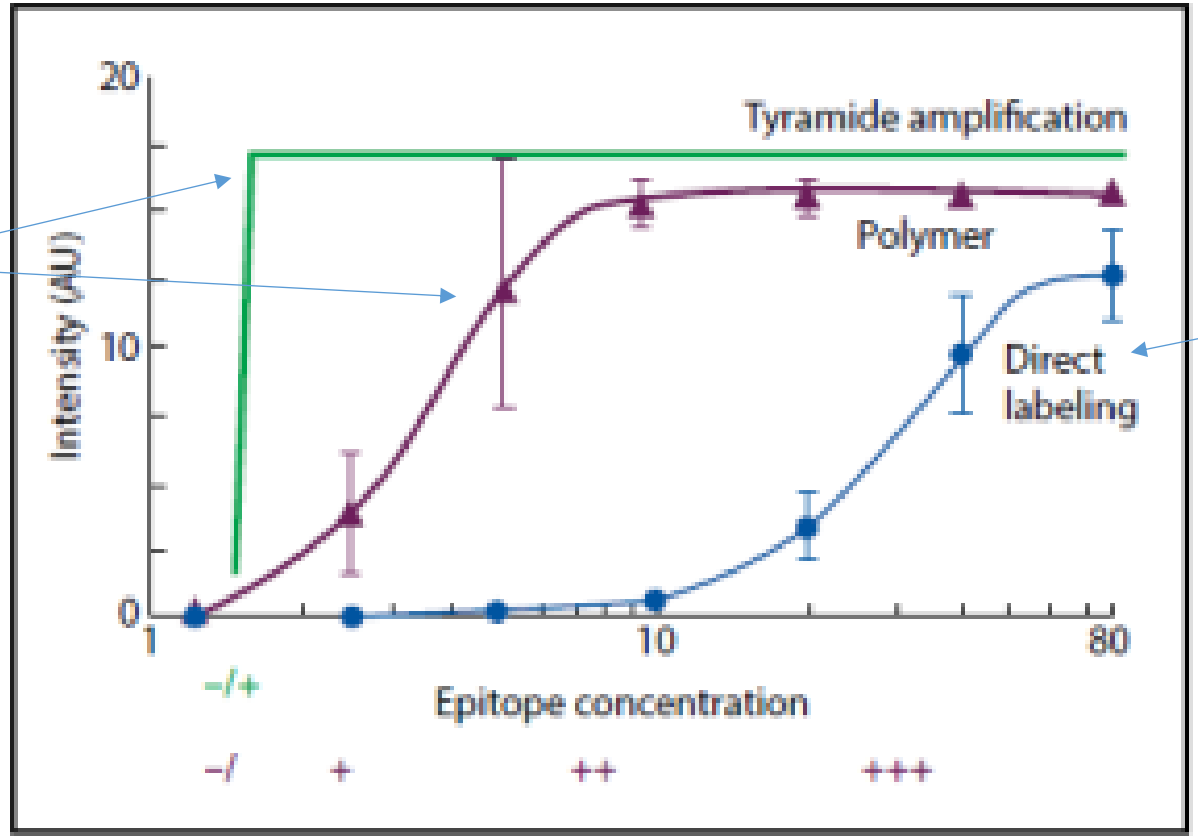


The enzyme (yellow) converts a chromogen into a coloured molecule (deposit on the tissue section)

Yatabe Y et al IASLC Atlas of Diagnostic Immunohistochemistry

‘Standard’ bright-field IHC techniques do not necessarily relate colour intensity to epitope concentration

Bright-field techniques



Immunofluorescent labelling technique

Figure 3-1. Relation between epitope concentration and signal enhancement in immunohistochemistry (IHC). AU = arbitrary unit. (Modified with permission from Prinsen et al 2003)

1+ 2+ 3+

Idea stolen from Dr Lukas Bubendorf, Basel ☺

Intensity (+, ++, +++)
Proportion staining
Localization

Combinations of the above

H-score=
(%1+x1)+(%2+x2)+(%3+x3)
Max possible score
100%x3 = 300

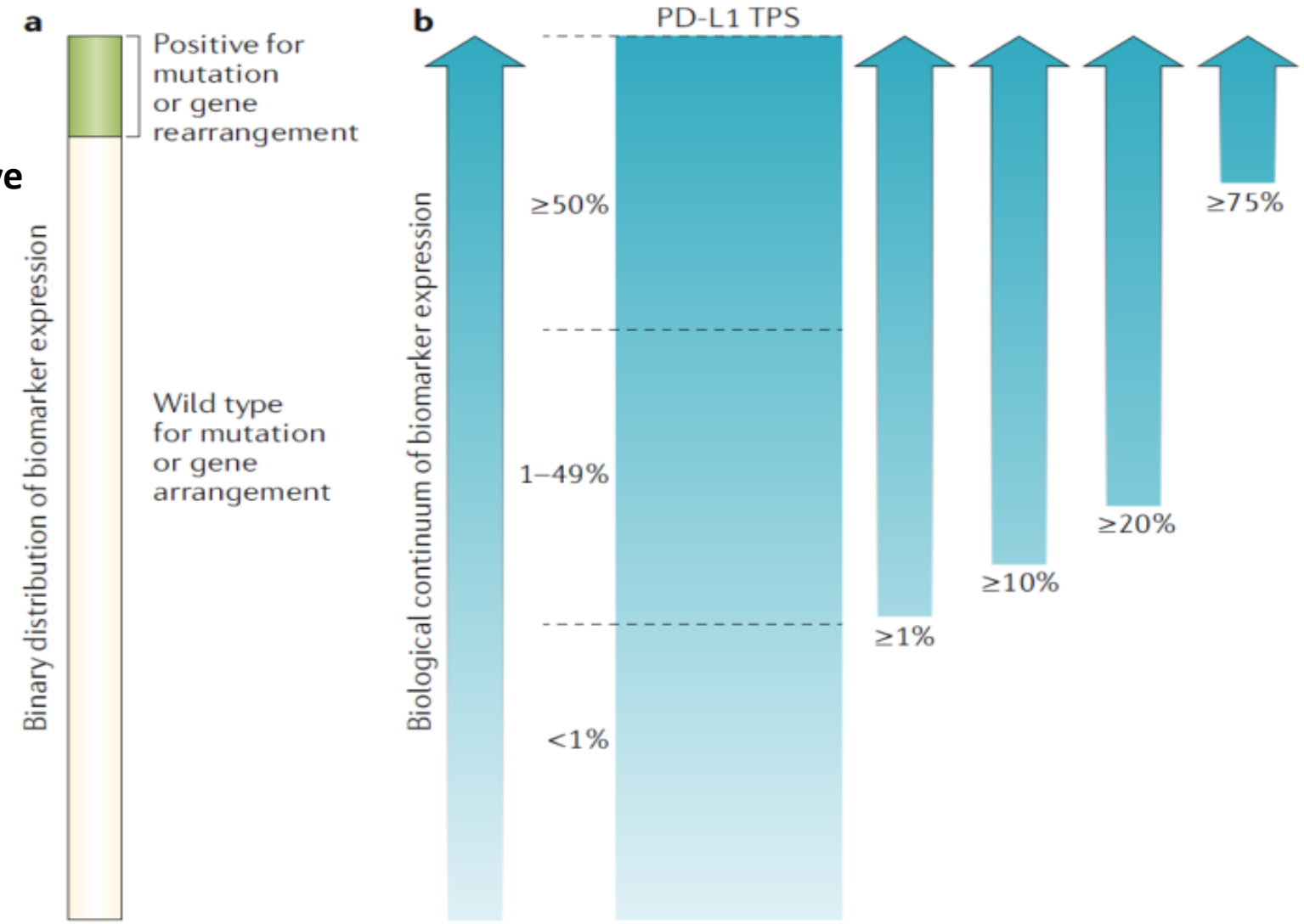
…….why Pathologists like IHC so much!!

Categorical versus Continuous Biological variables as predictive biomarkers of therapeutic benefit

Oncogene addictive Driver

Single dominant determinant?
More or less

Spatially and Temporally constant



Immunotherapy Biomarkers (PD-L1)

% of cells with ANY staining

Other IHC-based biomarkers

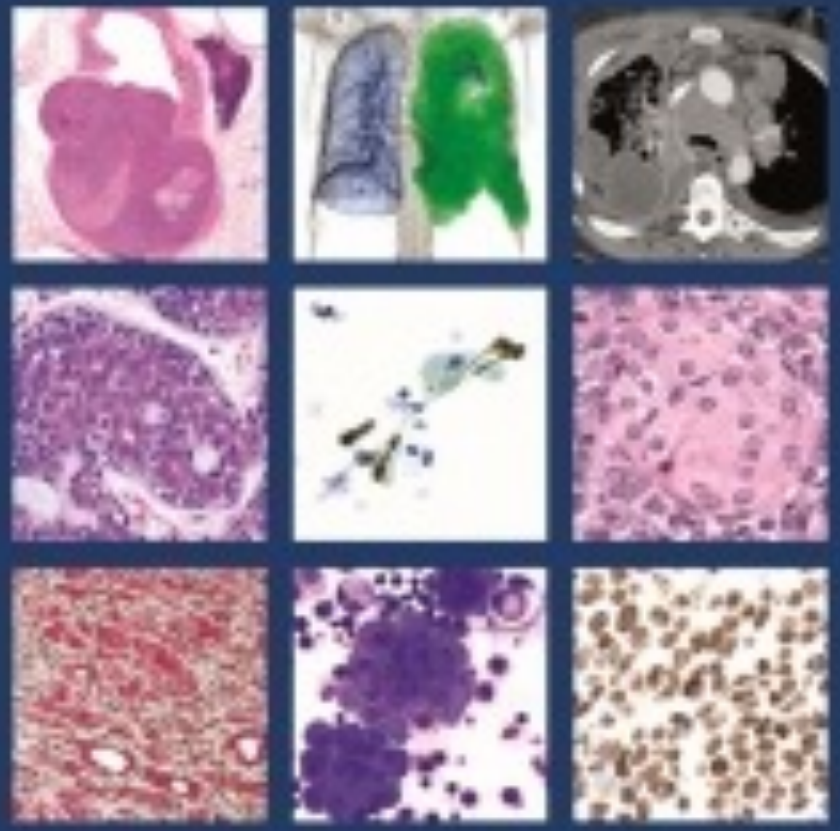
Range of H-scores is also a continuous variable

Camidge DR, Spiegel D, Kerr KM. Nat Rev Clin Oncol 2019

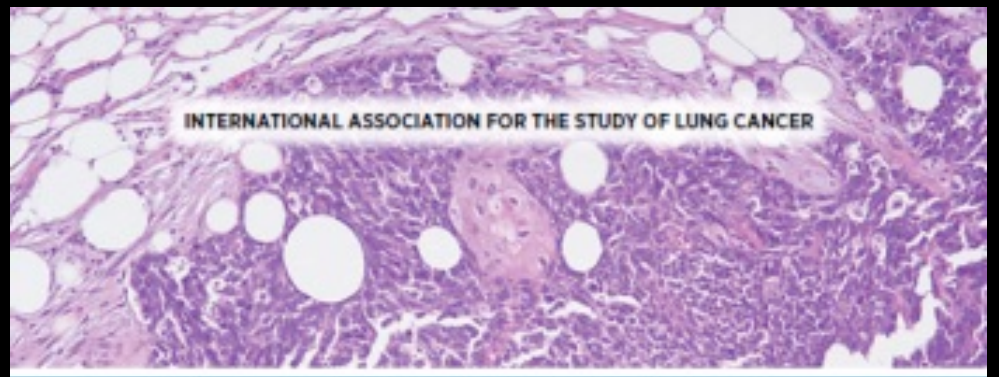
WHO Classification of Tumours • 5th Edition

Thoracic Tumours

Edited by the WHO Classification of Tumours Editorial Board



World Health Organization



INTERNATIONAL ASSOCIATION FOR THE STUDY OF LUNG CANCER

IASLC ATLAS OF DIAGNOSTIC IMMUNOHISTOCHEMISTRY

EDITED BY

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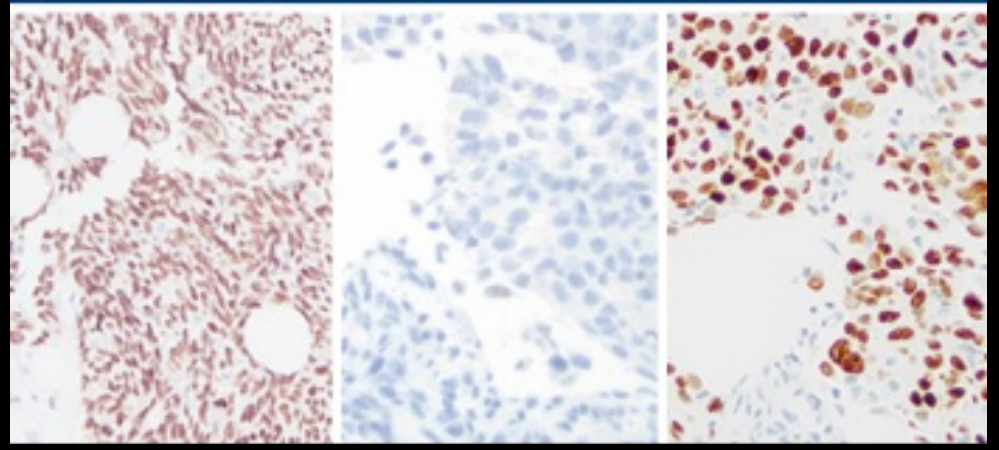
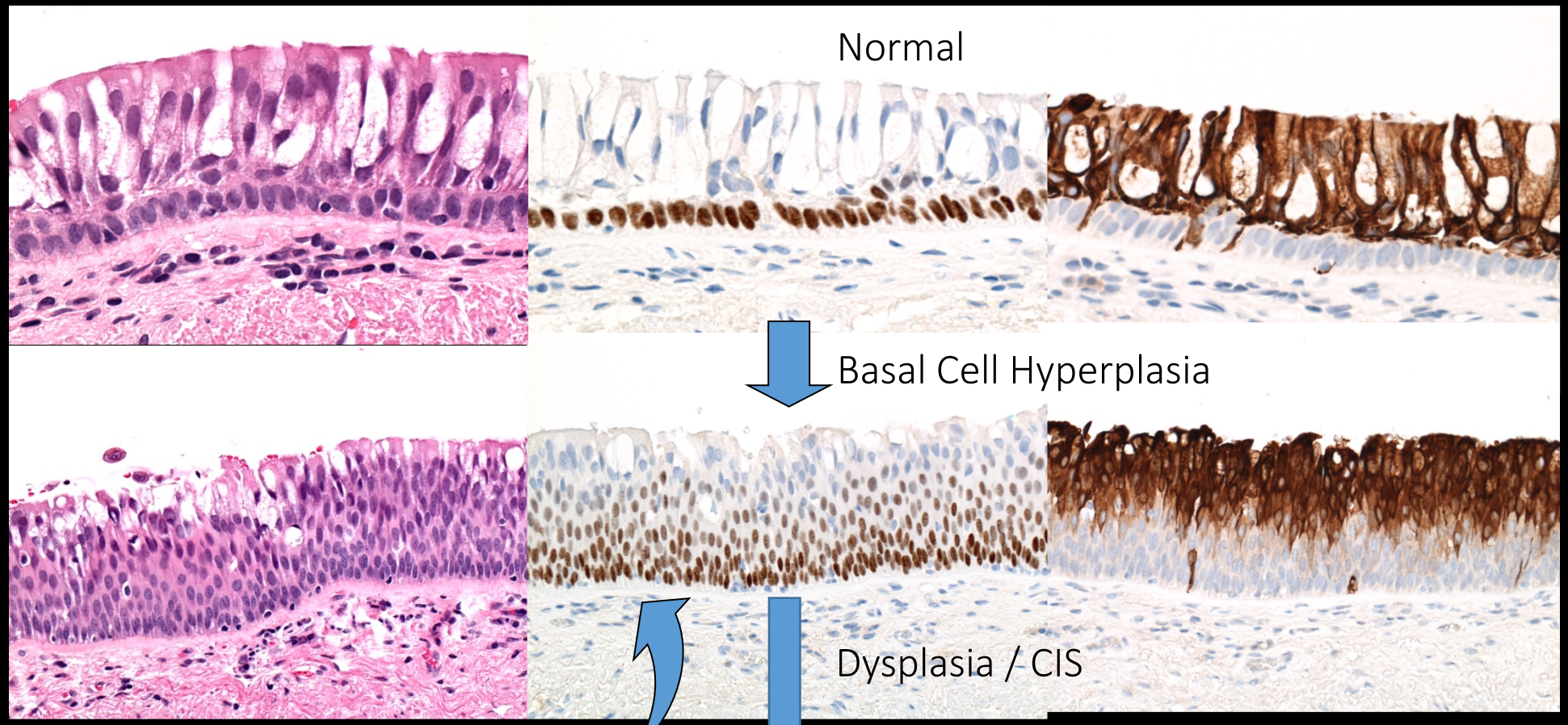
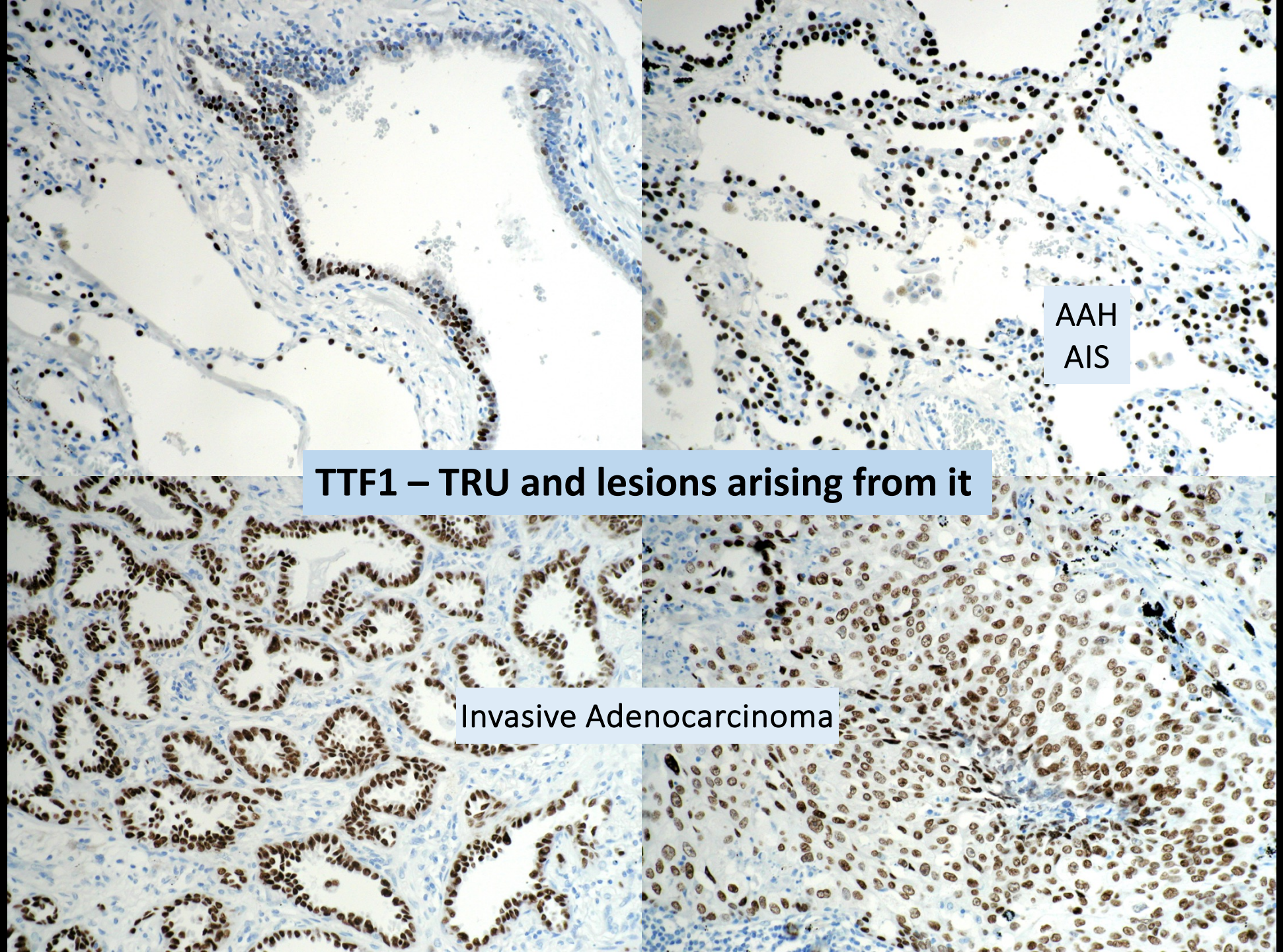
IASLC

Conquering Thoracic Cancers Worldwide




p40
CK7
Normal
Basal Cell Hyperplasia
Dysplasia / CIS
This population also expresses CK5/6 and p40
Invasive Squamous Cell Carcinoma

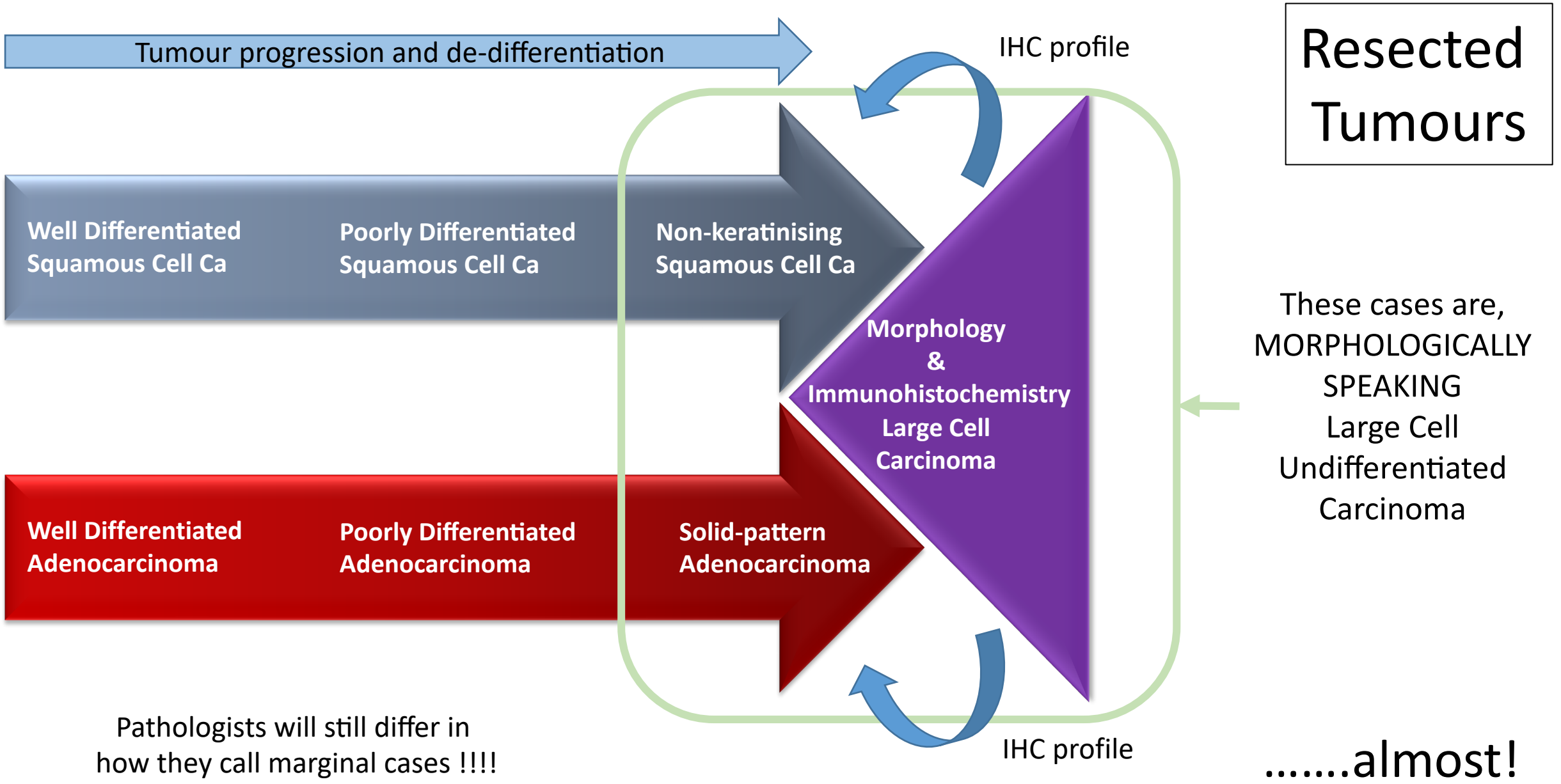


TTF1 – TRU and lesions arising from it

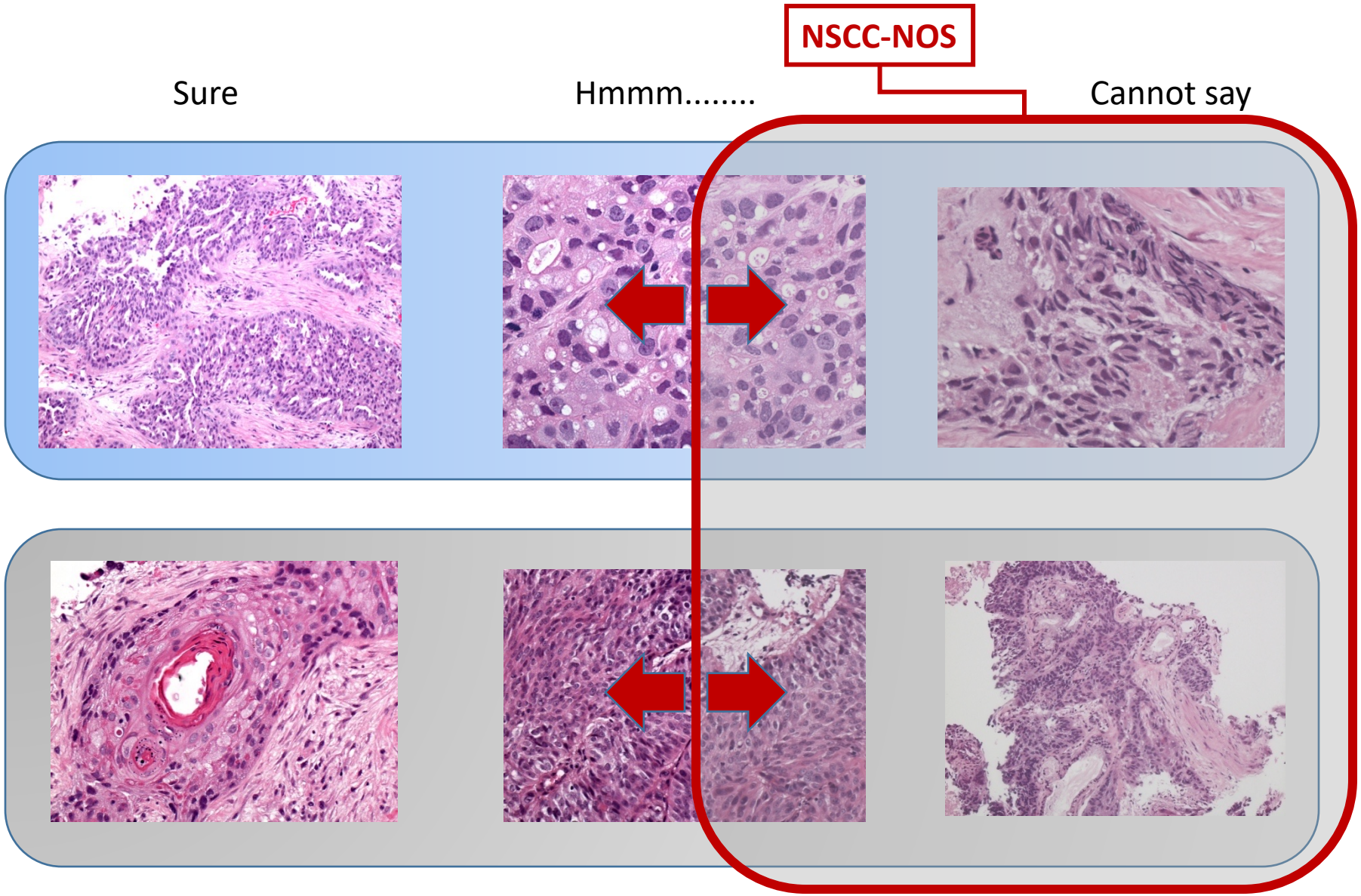
TRU
Terminal
Respiratory
Unit

TTF1:
Use
clone
8G7G3/1
!!!!!!!

Did we classify Large Cell Undifferentiated Carcinoma out of existence?



Small biopsy/Cytology: Thresholds of ‘certainty’



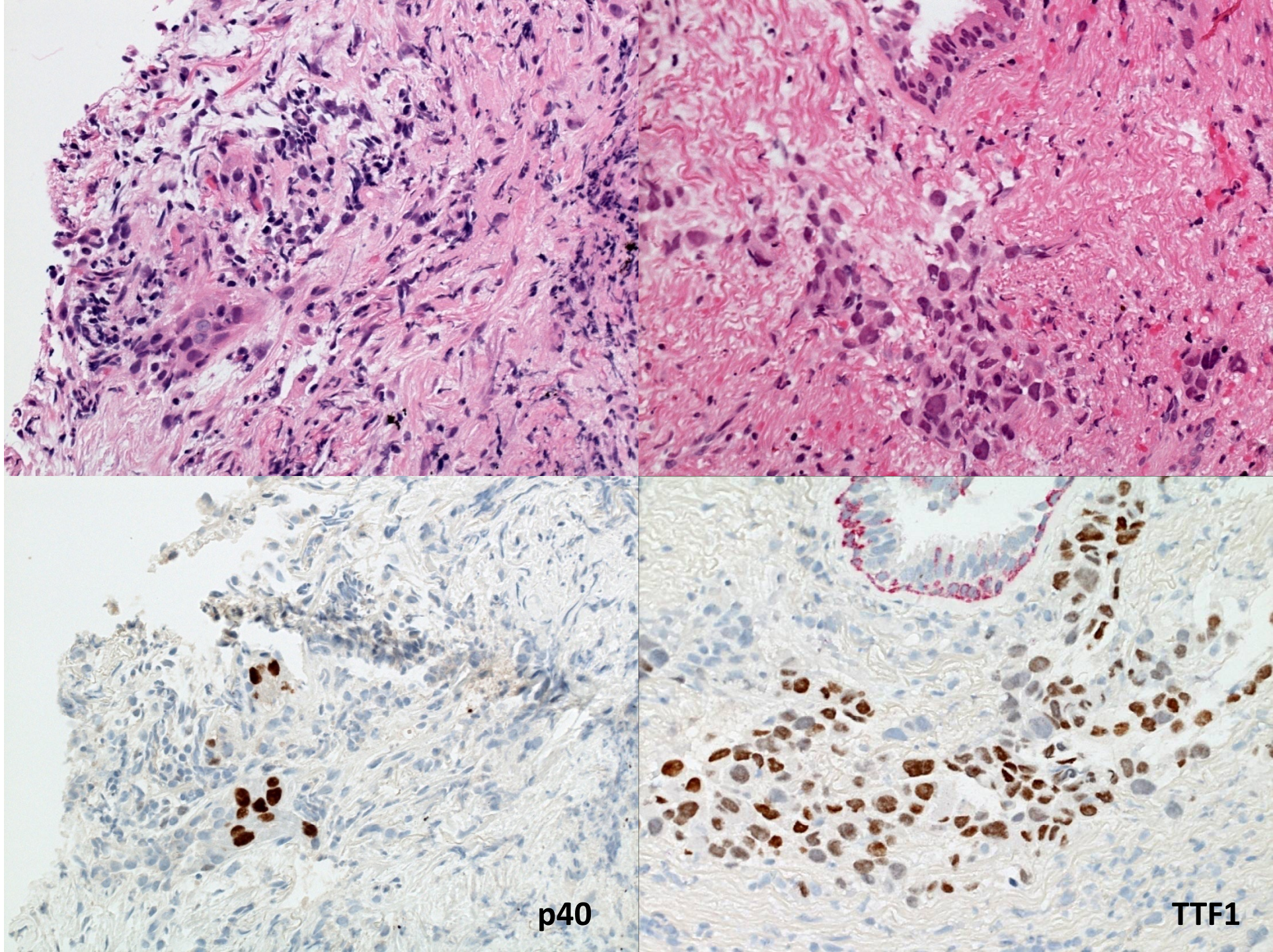
Non-small cell carcinoma
Not otherwise specified
25-40% cases
by H&E morphology alone

p40 & TTF1 IHC

Diagnosis of Probable
Squamous or
Adenocarcinoma
'Favoured'

Leaving <10% NOS

Loo PS et al. J Thorac Oncol 2010; 5, 442



Terminology for use with small samples and cytology

1. Small Cell carcinoma
2. ‘High grade NE carcinoma’
3. ‘possible LCNEC’

4. Squamous cell carcinoma
5. **NSCC – favour squamous**

6. Adenocarcinoma
7. **NSCC – favour adenocarcinoma**

8. **Non-small cell carcinoma (NSCC) – NOS**

9. ‘possible adenosquamous carcinoma’
10. ‘pleomorphic features’

Terminology for use with resection specimens

1. **Small Cell carcinoma**
2. **Large cell neuroendocrine carcinoma**

3. **Squamous cell carcinoma**
4. **Non-keratinising squamous cell carcinoma**

5. **Adenocarcinoma** (describe patterns predominance)
6. **Solid adenocarcinoma (by IHC)**

7. **Large cell carcinoma** – IHC must be inconclusive or null or not done

8. **Adenosquamous carcinoma** if each component comprises at least 10% of the tumour

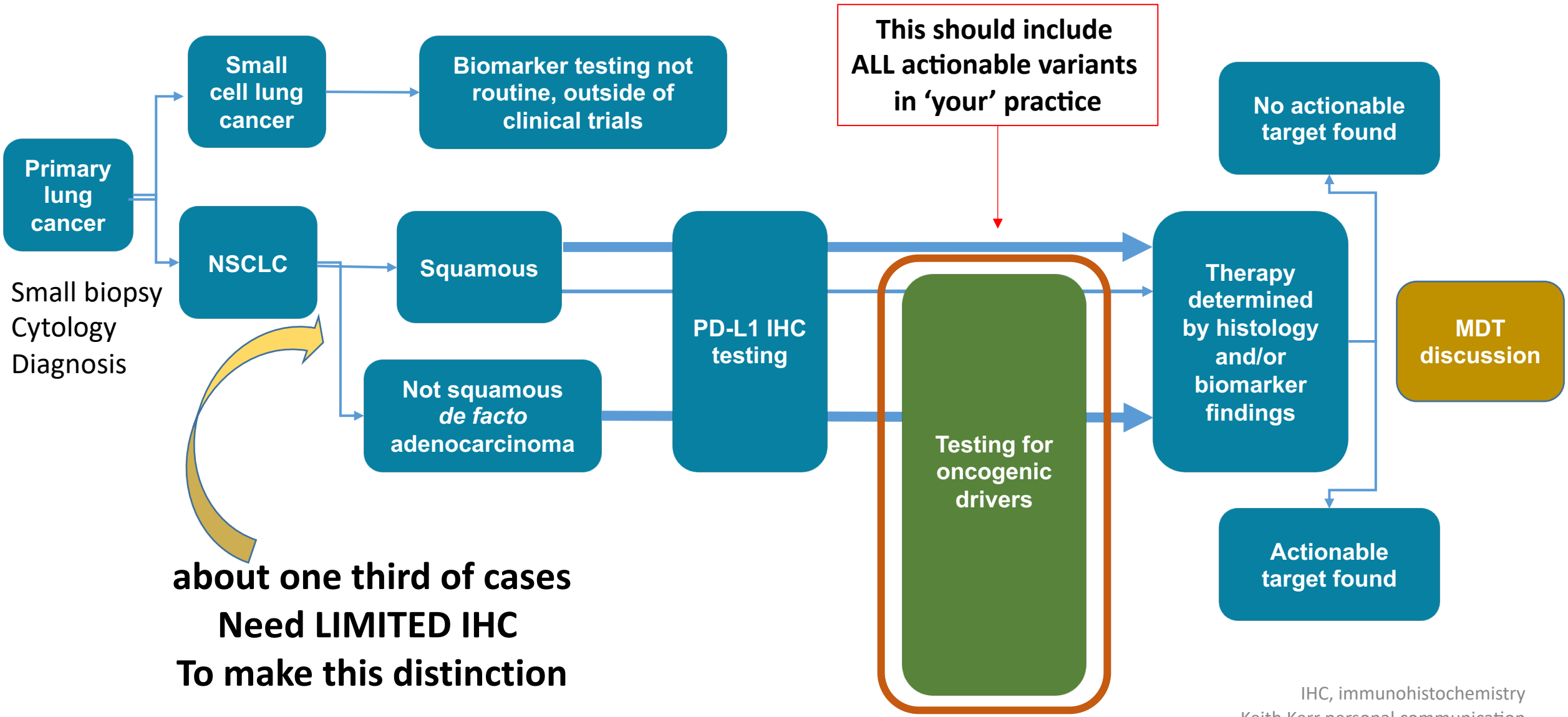
9. **Pleomorphic carcinoma** if at least 10% of the lesion is so – describe differentiated components if present

10. Et seq

Do’s and Don’ts……

- Diagnosing NSCC-NOS too often. Should be less than 10% of cases
- Indicate if IHC was used to make diagnosis
- Do not use the term non-squamous NSCC
- Reporting paired cytology and biopsy samples together
- Be aware of diagnoses you CANNOT make in small samples
  - Adenocarcinoma-in-situ, pleomorphic carcinoma, adenosquamous ca, large cell carcinoma
- Don’t overuse IHC
  - Adenocarcinoma
  - Neuroendocrine markers

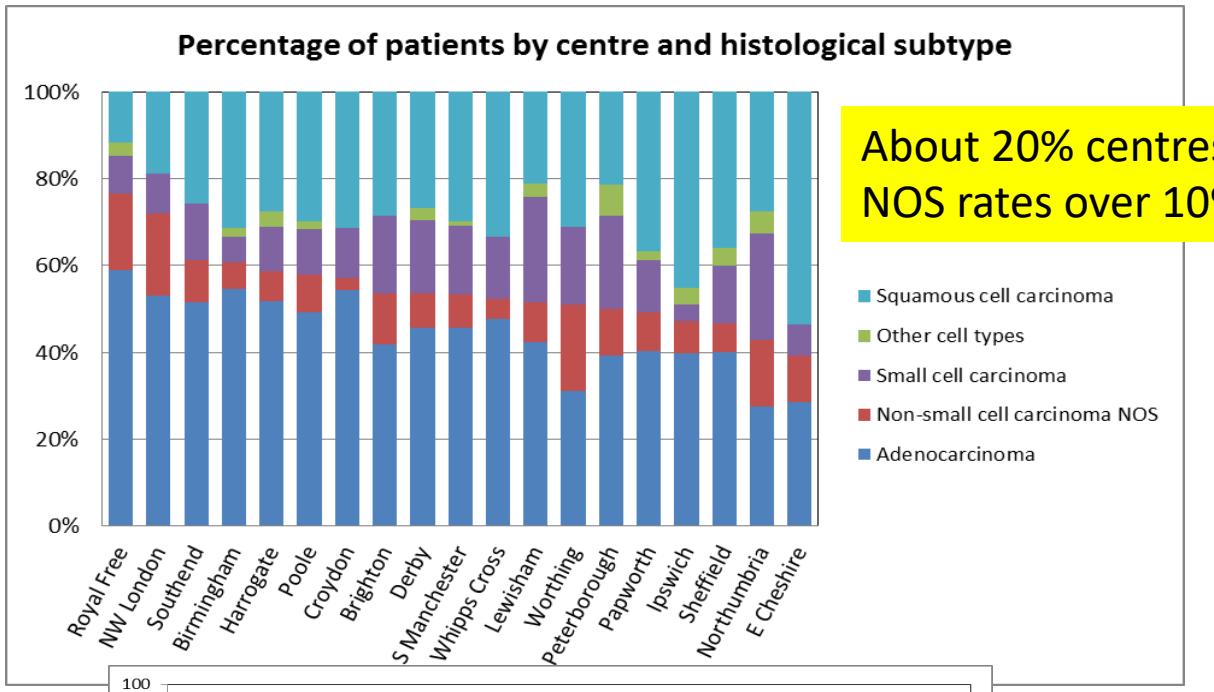
A Diagnostic flow chart……



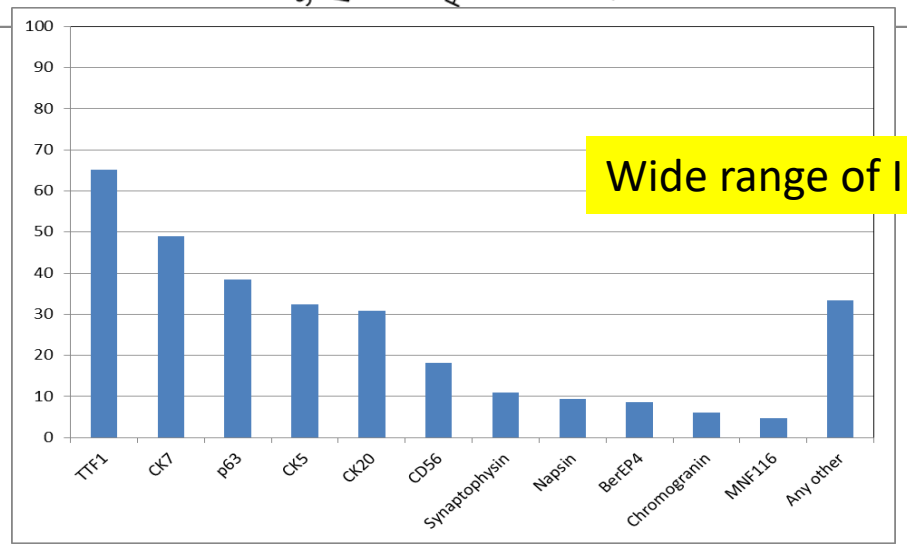
IHC, immunohistochemistry

Keith Kerr personal communication

Histology-IHC diagnostic practice UK



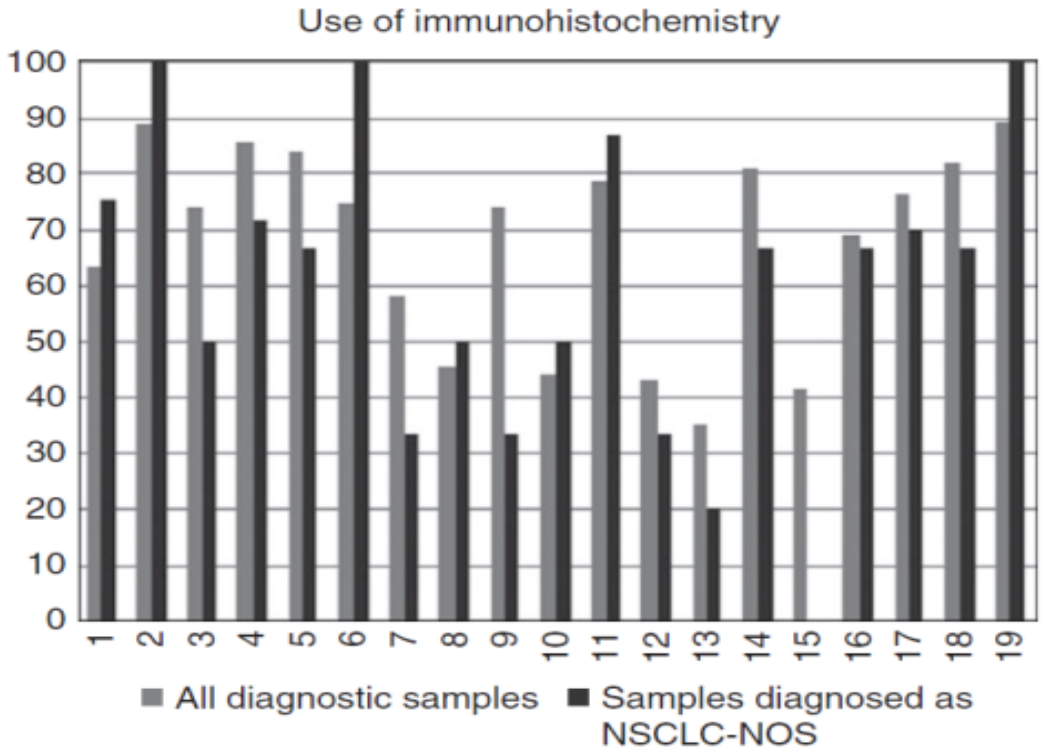
About 20% centres have NOS rates over 10%



Wide range of IHC used

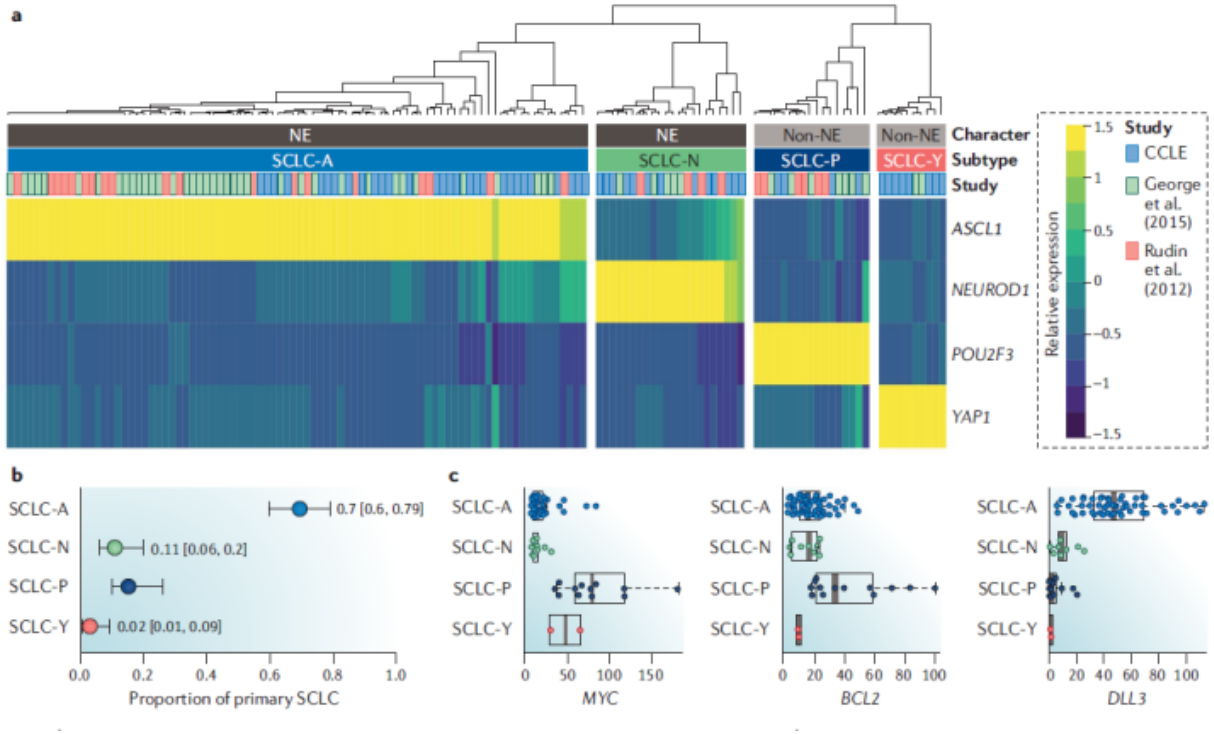
Cane P et al. Histopathology 2015

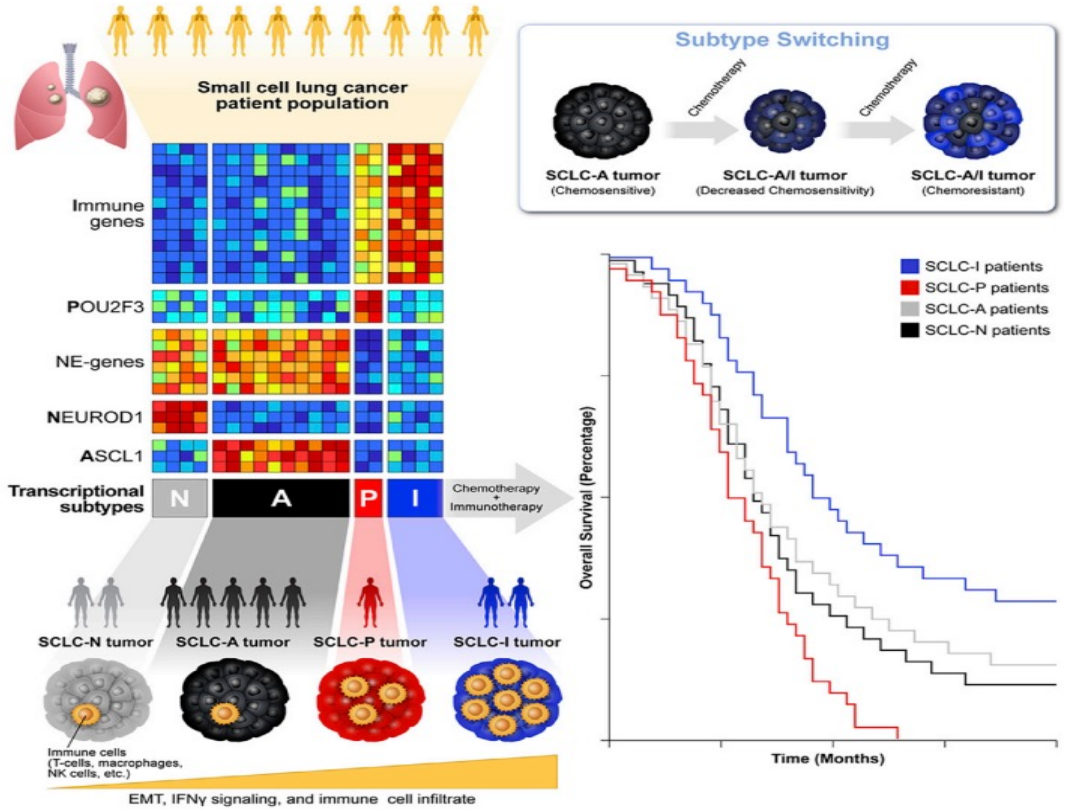
IHC over-used



Lung Pathology accounts for anything from
1 – 13++ hours per week per pathologist surveyed.

Small Cell Carcinoma of the Lung



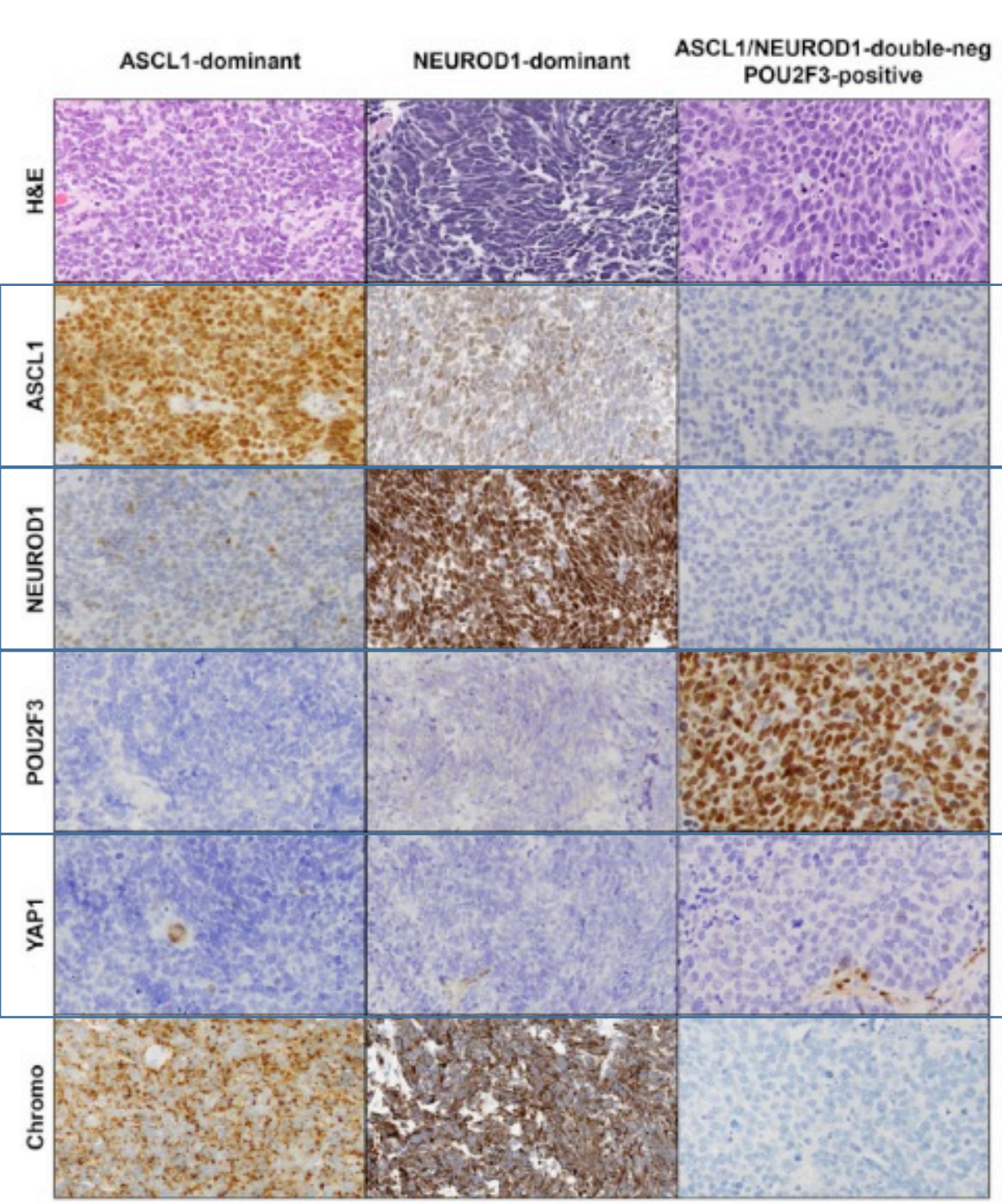


Gay CM, et al. *Cancer Cell.* 2021;39(3):346-360.e7.

Four molecularly defined groups
Potential for differential sensitivity to new drugs

Rudin CM, et al. *Nat Rev Cancer.* 2019;19(5):289-297.

These SCLC subtypes can be identified by IHC



SCLC subtype	Possible therapeutic relevance
SCLC-A ASCL1 IHC positive	DLL3 Histone deacetylase/demethylase inhibitors
SCLC-N NEUROD1 IHC positive	SVV oncolytic virus
SCLC-P POU2F3 IHC positive	IGF1R inhibitors BCL2, PARP, ATR, WEE1 Aurora kinase 1
SCLC-I IHC negative Immune subtype	Chemo-immunotherapy

In clinical samples - evidence of sub-clonal expression heterogeneity

Rudin CM et al 2019, Baine MK et al. 2020 Gay CM et al 2021

REVIEW

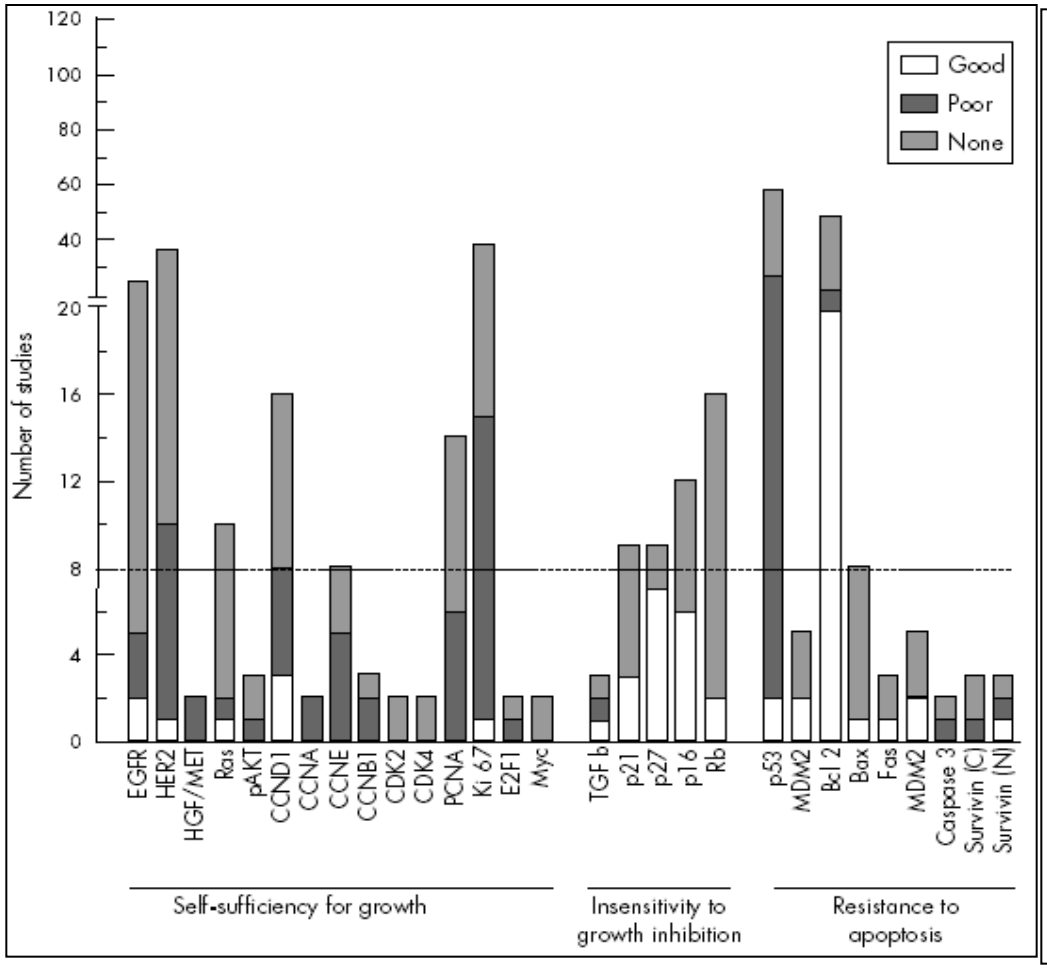
Immunohistochemical markers of prognosis in non-small cell lung cancer: a review and proposal for a multiphase approach to marker evaluation

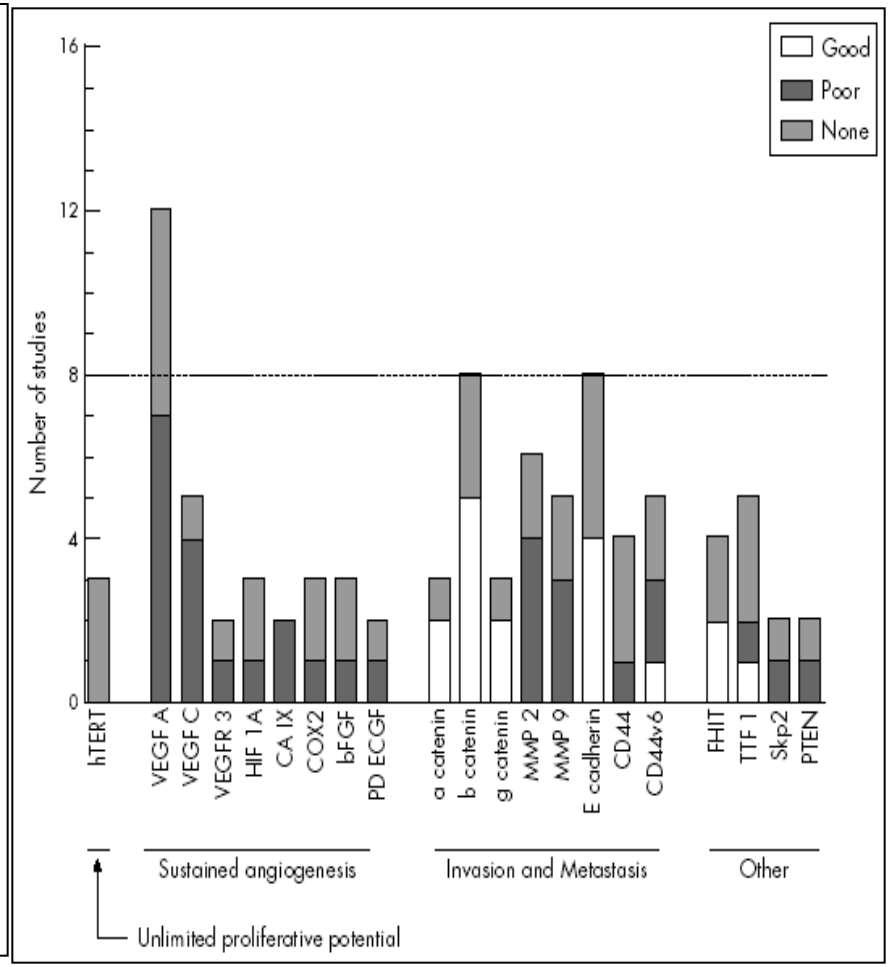
C-Q Zhu, W Shih, C-H Ling,* M-S Tsao

J Clin Pathol 2006;59:790–800. doi: 10.1136/jcp.2005.031351

J Clin Pathol 2006; 59,790-800

There is no strong case for any IHC marker as a clinically useful prognostic marker in NSCL



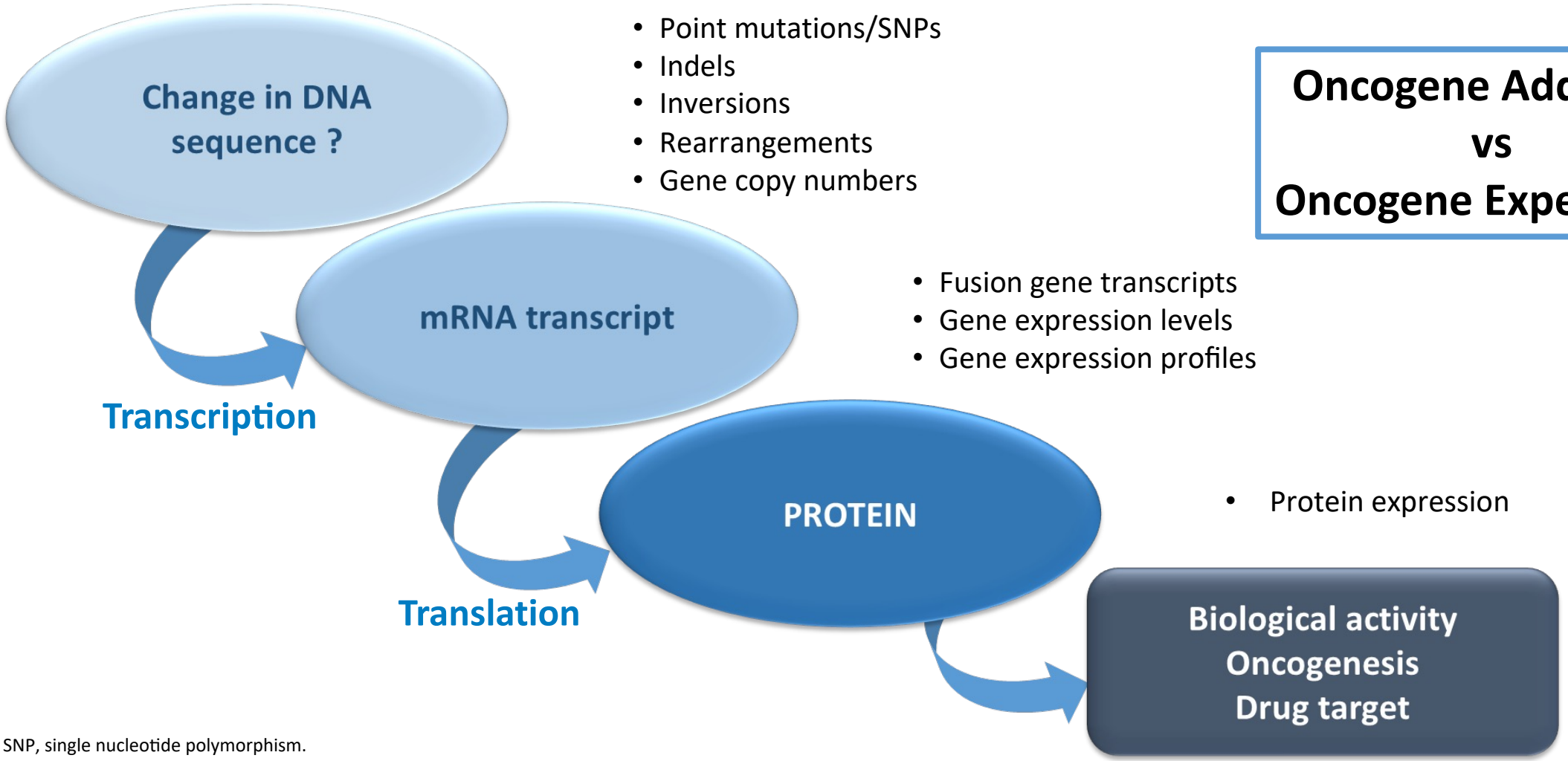


Immunohistochemistry for predictive biomarkers

Yesterday's technology? Still a valuable tool?

- Oncogenic drivers including mutations
- Protein elevation in tumours driven by fusion genes
  - Screening
  - Oncological validation of genomic findings
  - Proteomic or IHC expression associated with response in ALK, *MET*ex14, possibly NTRK patients
- Immunotherapy
  - PD-L1
  - IHC based identification of cell populations in the TME
- Antibody-Drug conjugate biomarkers
  - DLL3, MET, TROP2, HER2, HER3…….

Which Level to Test? How Will You Do It?



SNP, single nucleotide polymorphism.
Keith Kerr personal communication.

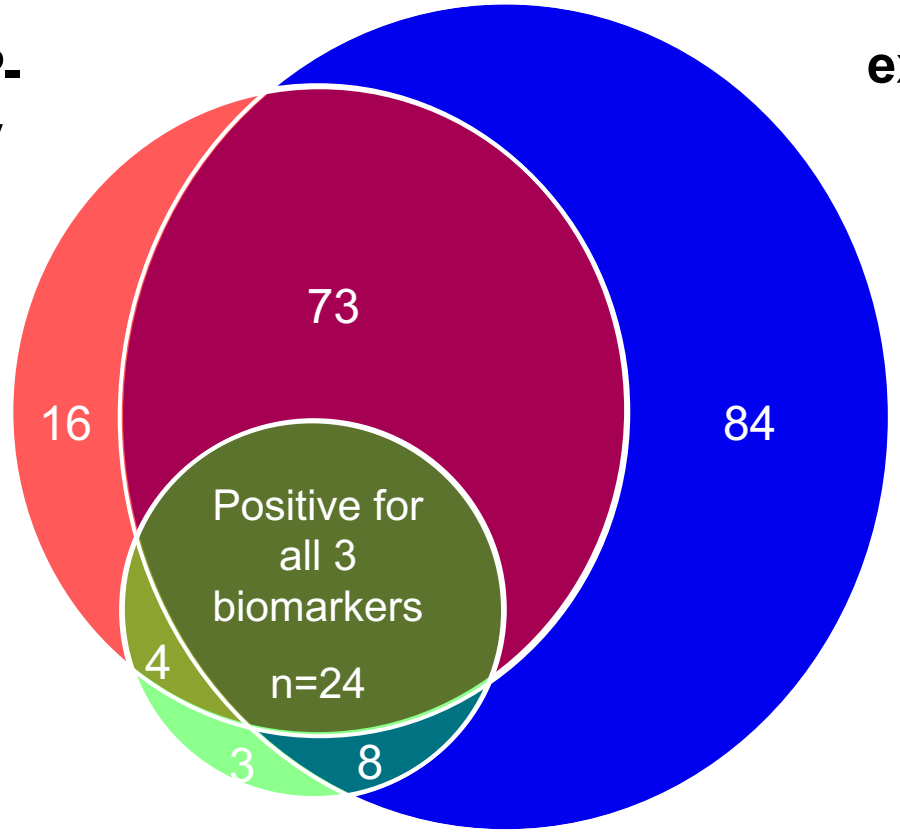
Overlap of EGFR biomarkers in NSCLC

High *EGFR*-gene-copy number

~ 47%

EGFR protein expression- IHC positive

~ 76%



***EGFR* mutation-positive**

~ 15%

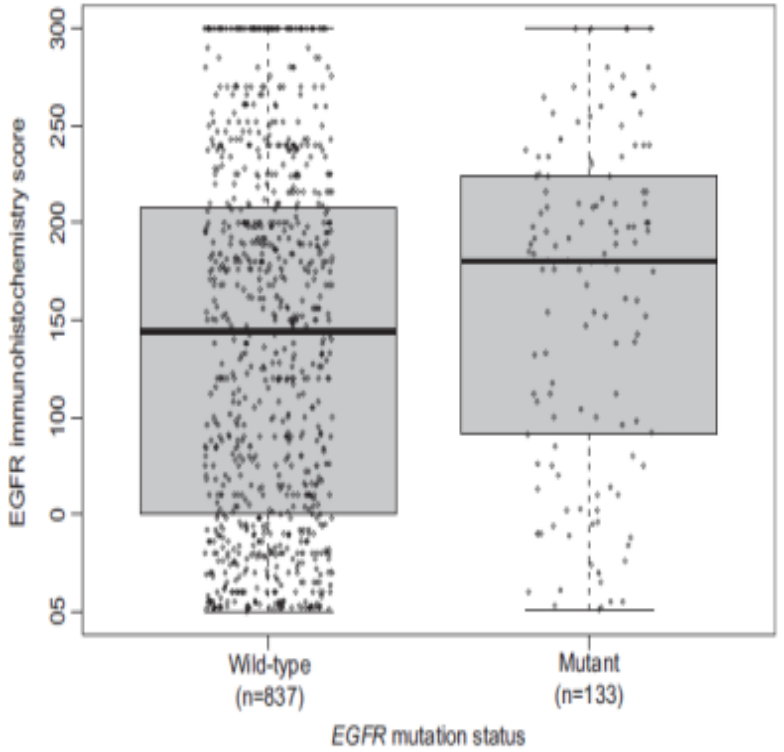
Figures for largely Caucasian population

Negative for all 3 biomarkers n=37

n=249 with known biomarker status for all 3 biomarkers

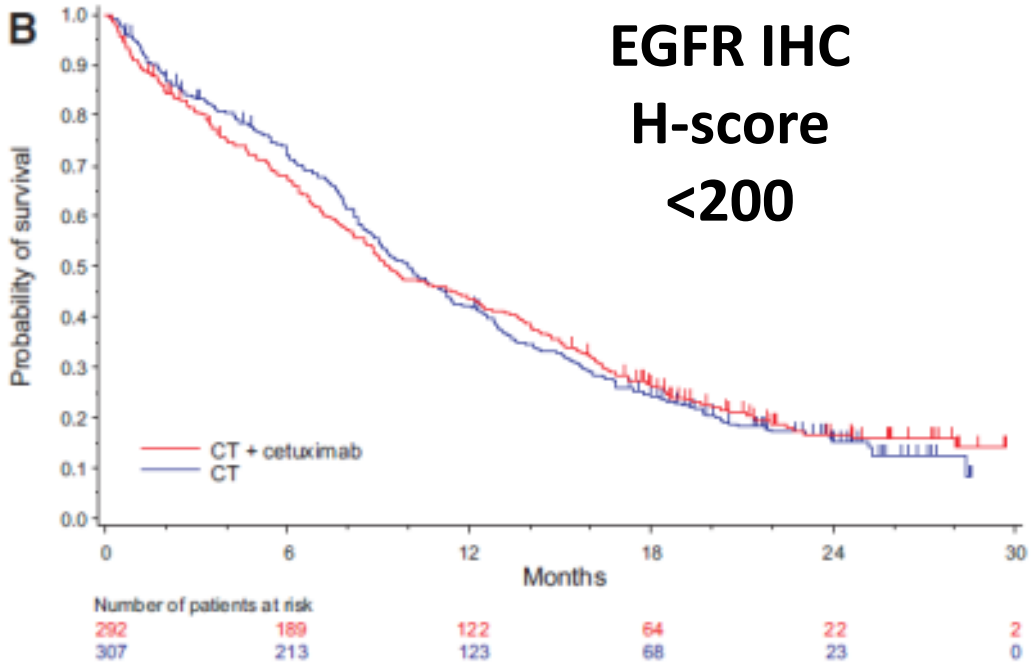
Adapted from Douillard et al. J Clin Oncol 2010

EGFR IHC levels NOT associated with EGFR mutation

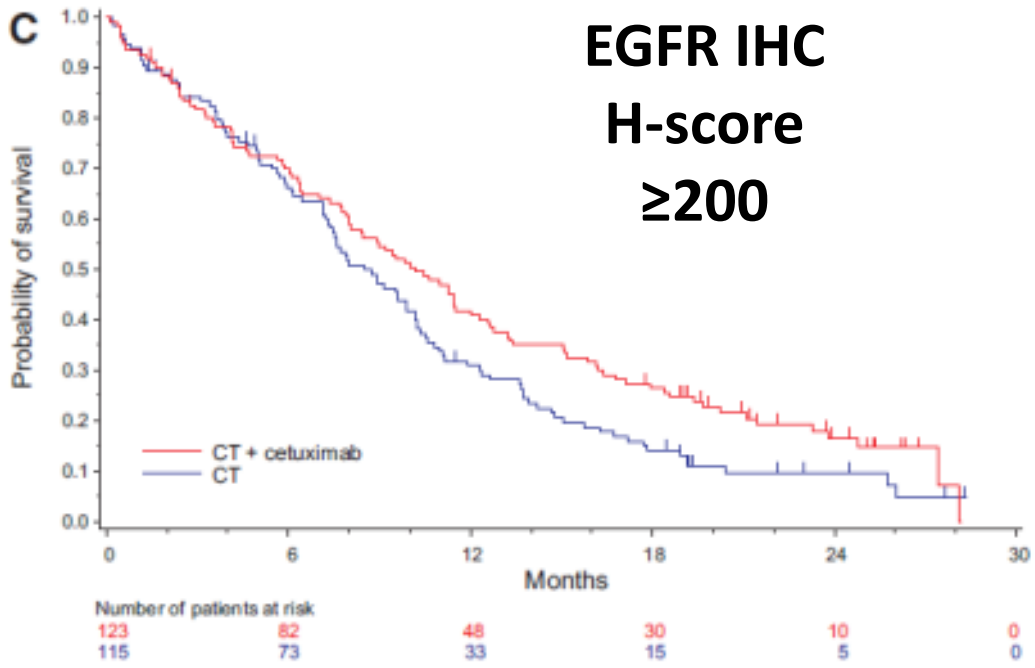


Douillard JY et al. J Thorac Oncol 2014

Relationship Between EGFR Expression, *EGFR* Mutation Status, and the Efficacy of Chemotherapy Plus Cetuximab in FLEX Study Patients with Advanced Non–Small-Cell Lung Cancer



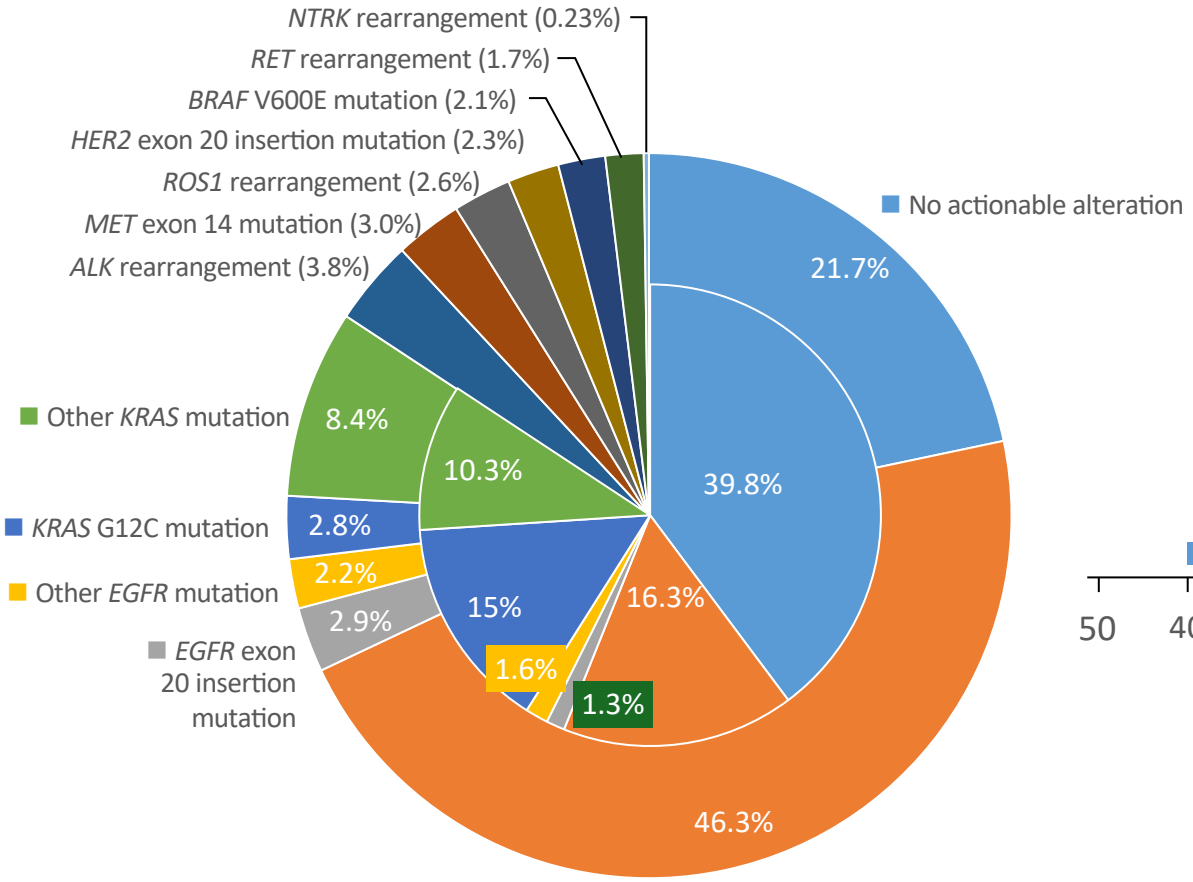
EGFR IHC H-score <200



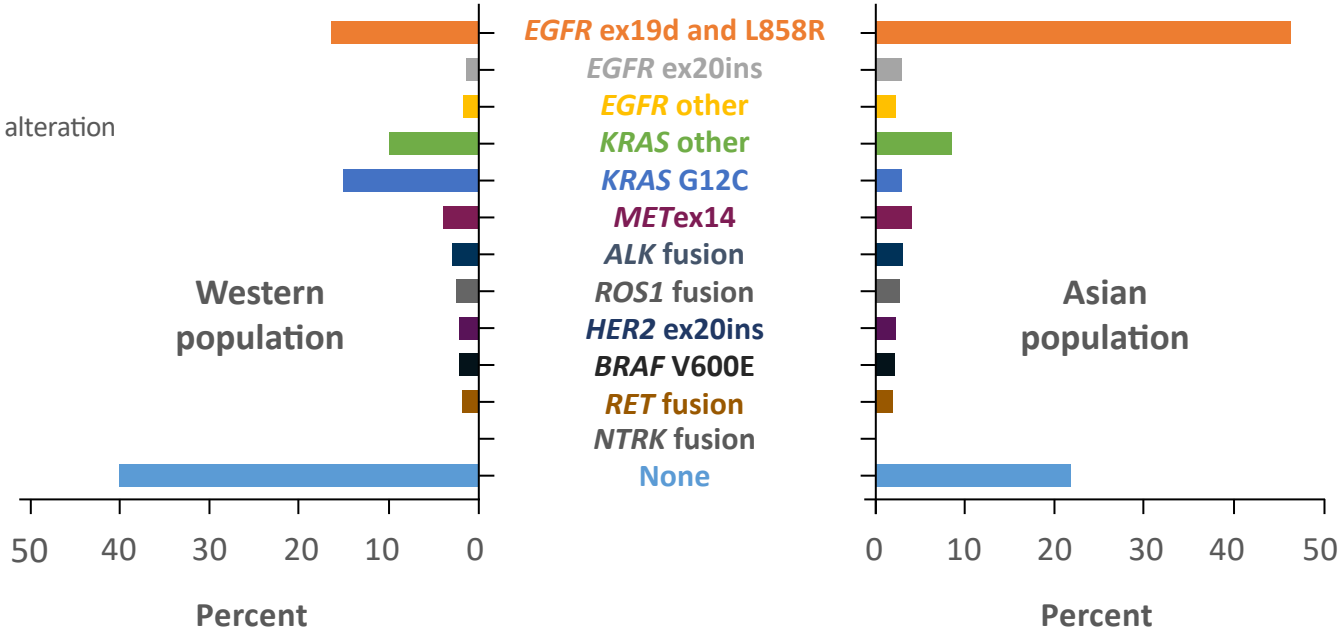
EGFR IHC H-score ≥200

Douillard JY et al. J Thorac Oncol 2014

Prevalence of genomic alterations



Outer circle: Asian populations
Inner circle: Western populations



Current approved drugs:
Five genes (range of mutations)
Four genes (range of fusions)

ex, exon; ex19d, exon 19 deletion; ins, insertion.
Adapted from: Tan AC, Tan DSW. *J Clin Oncol*. 2022;40(6):611-625.

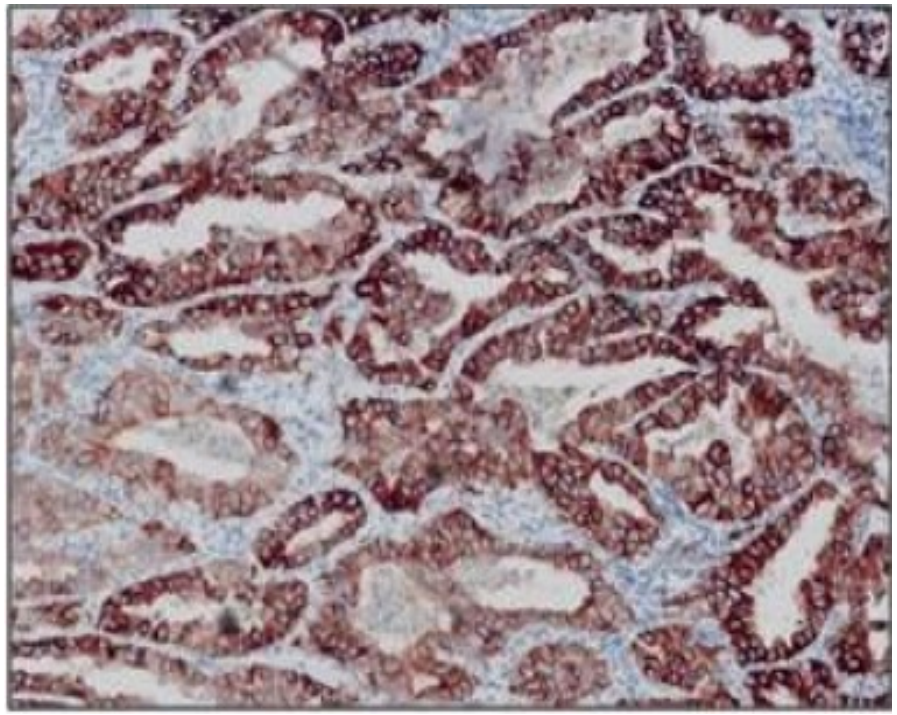
IHC antibodies against EGFR mutant proteins do exist

Meta-analysis

L858R (Sens 0.76; Spec 0.98)

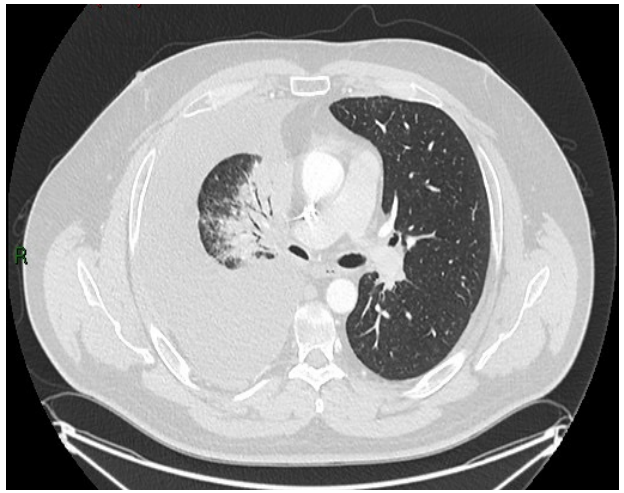
Exon19 E746-A750 (Sens 0.60; Spec 0.98)

Chen Z et al. PLoS one 2014



- Limited usage
- Rapid outcome
- Misses MANY EGFR mutations

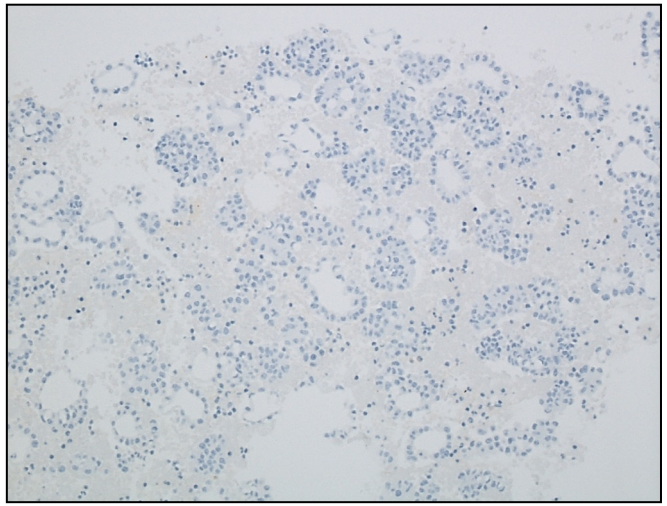
ALK fusion in Lung adenocarcinoma



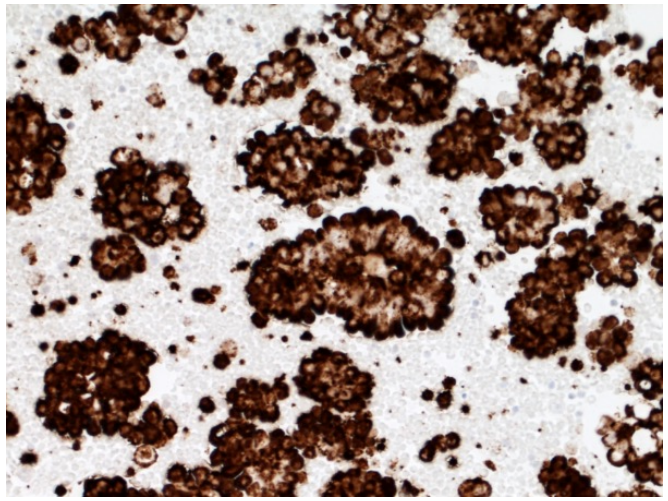
Pleural effusion

Gene fusion leads to activation of the protein kinase, and Upregulation (overexpression) of the protein

Effusion shows TTF1 positive Adenocarcinoma

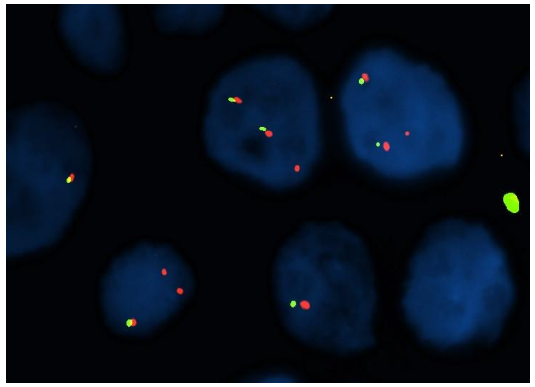


Tumour positive for ALK D5F3 CDx assay

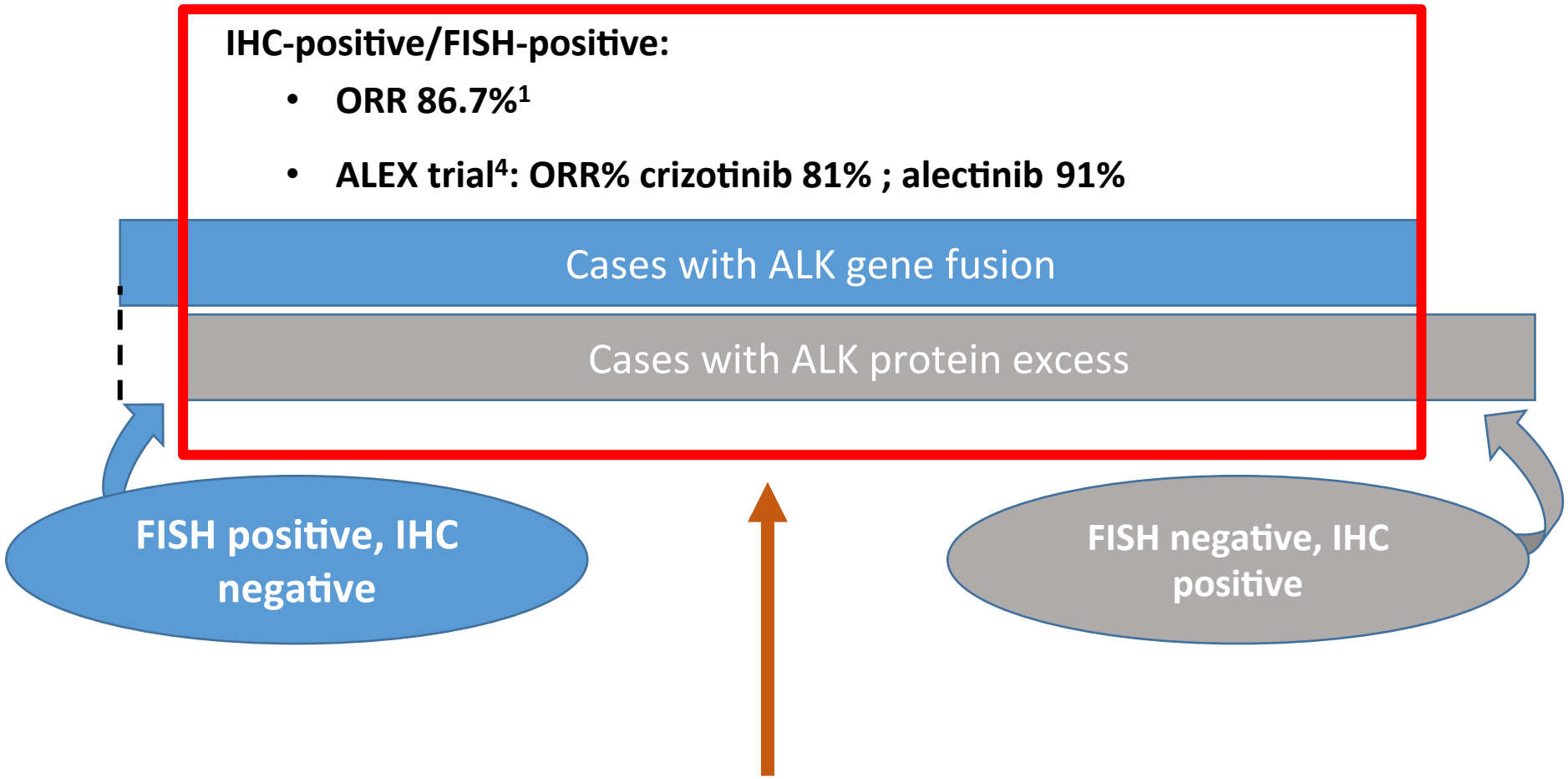


ALK FISH test is positive NGS for ALK fusion gene positive

?



The protein is the oncogenic driver *and* the target of the drug

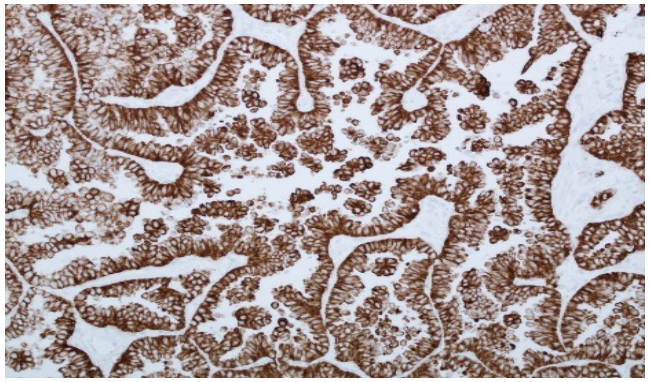


An ALK IHC positive cohort will be mostly (>95%) ALK fusion gene positiveprovided the IHC is performed adequately and validated for association with ALK gene rearrangement; therefore this cohort WILL respond (mostly) to ALK TKI

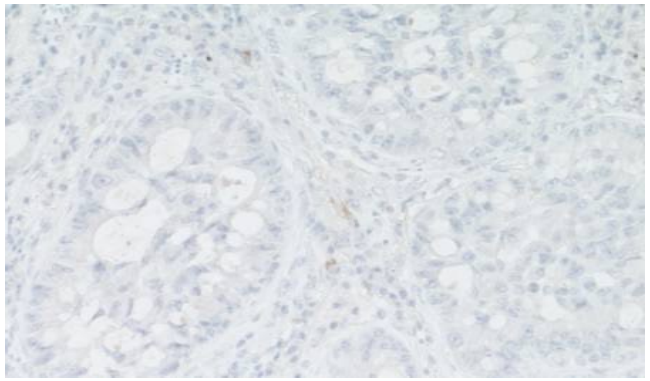
1. Wang, et al. J Thorac Oncol 2015; 2. Thorne-Nuzzo, et al. J Thorac Oncol 2017; 3. van der Wekken, et al. Clin Can Res 2017; 4 Mok T et al, WCLC 2017

ALK immunopositivity predicts ALK fusion gene?

D5F3 clone-based assay
Roche-Ventana CDX



POSITIVE

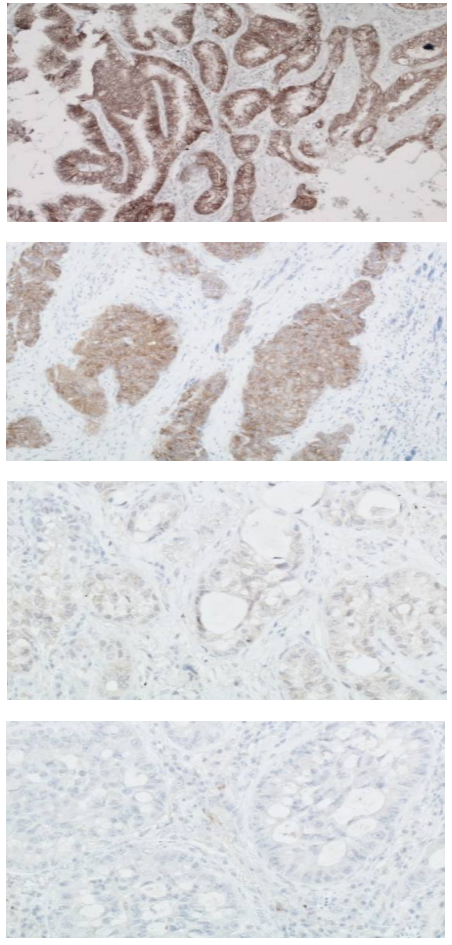


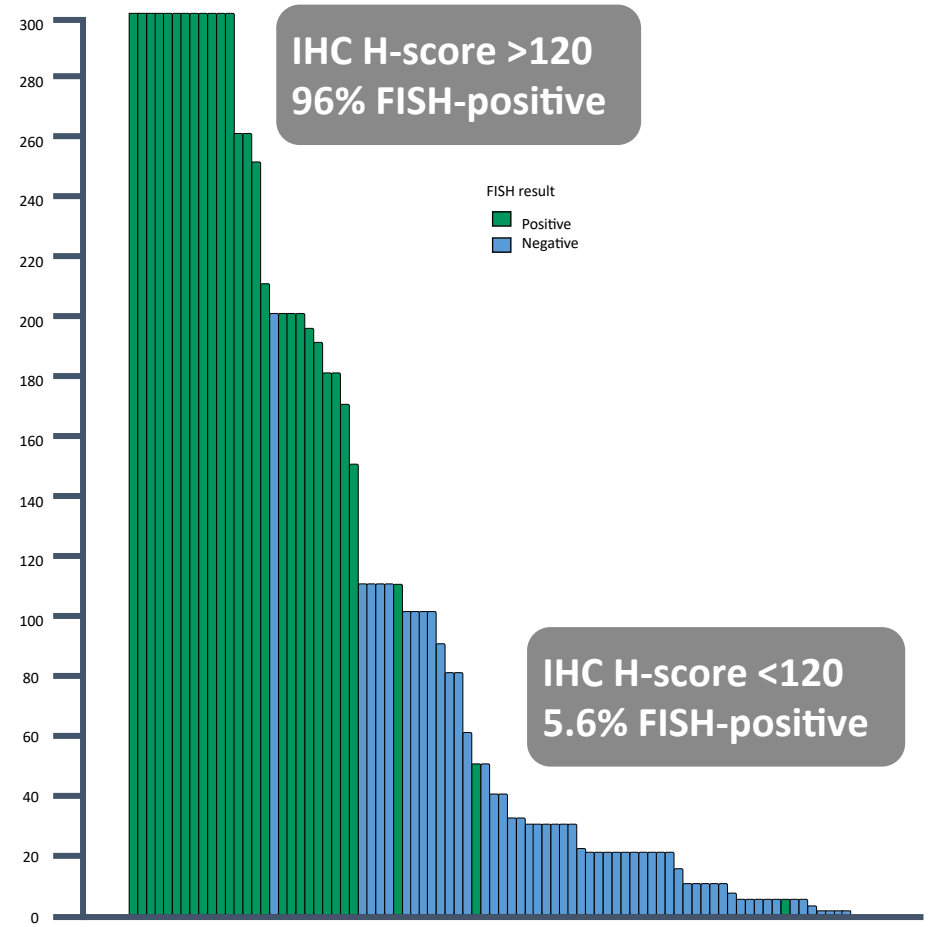
NEGATIVE

Defines two groups

Converts continuum to binary situation

5A4 clone-based Laboratory developed tests

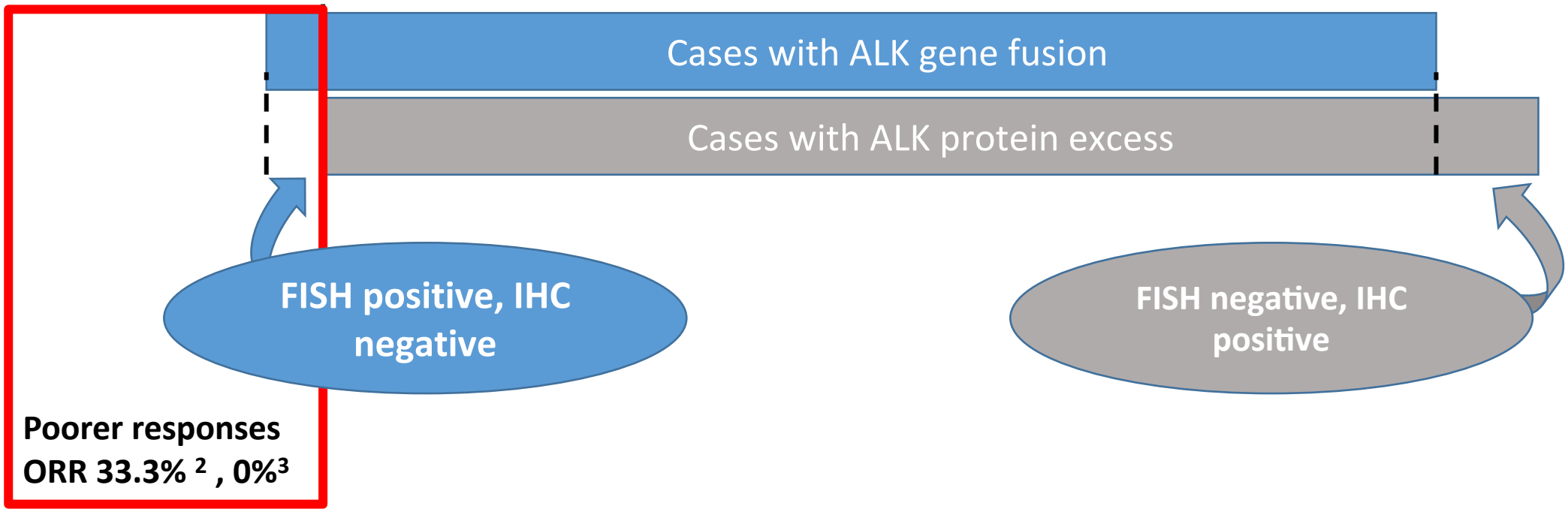




Dynamic range of 'standard' IHC staining 0, +, ++, +++

Blackhall F et al. J Clin Oncol 2014

The protein is the oncogenic driver *and* the target of the drug



An ALK IHC positive cohort will be mostly (>95%) ALK fusion gene positive provided the IHC is performed adequately and validated for association with ALK gene rearrangement; therefore this cohort WILL respond (mostly) to ALK TKI

1. Wang, et al. J Thorac Oncol 2015; 2. Thorne-Nuzzo, et al. J Thorac Oncol 2017; 3. van der Wekken, et al. Clin Can Res 2017; 4 Mok T et al, WCLC 2017

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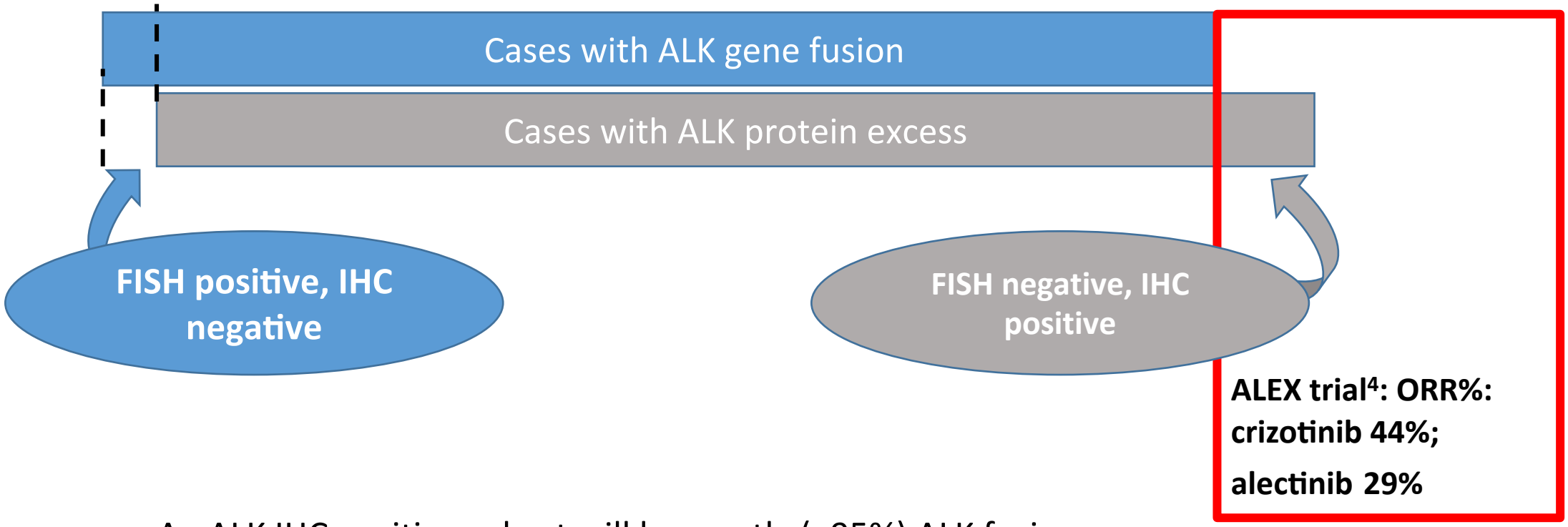

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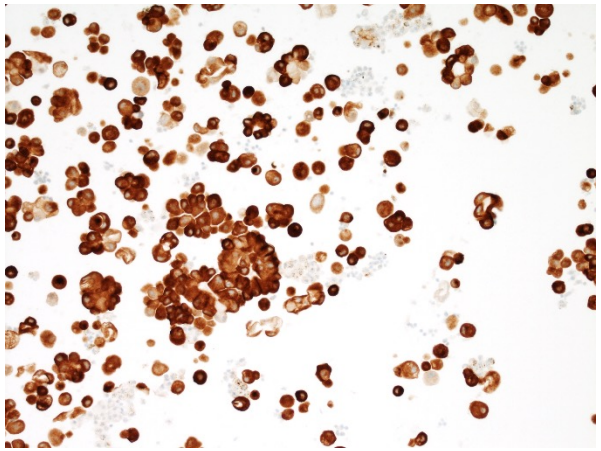
Issues with ALK for ALK fusion gene testing IHC

- Assay must be validated for high predictive power for fusion gene
- Be aware that false positives can occur
- When a fusion is present, the staining is almost always diffuse (and strong)
- Neuroendocrine tumours may be ALK IHC positive

ROS1 Gene Fusion in Lung adenocarcinoma

Another
Pleural effusion

ROS1 IHC positive



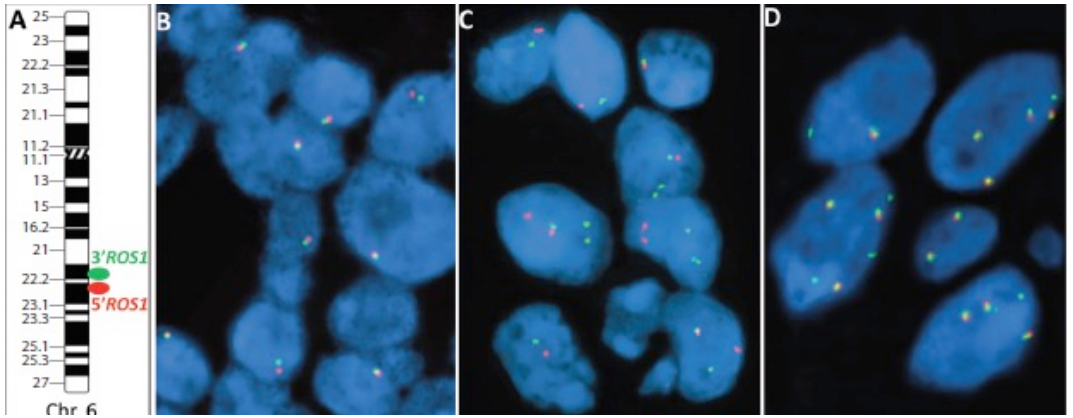
Confirmation of ROS1 gene

Rearrangement Is ESSENTIAL

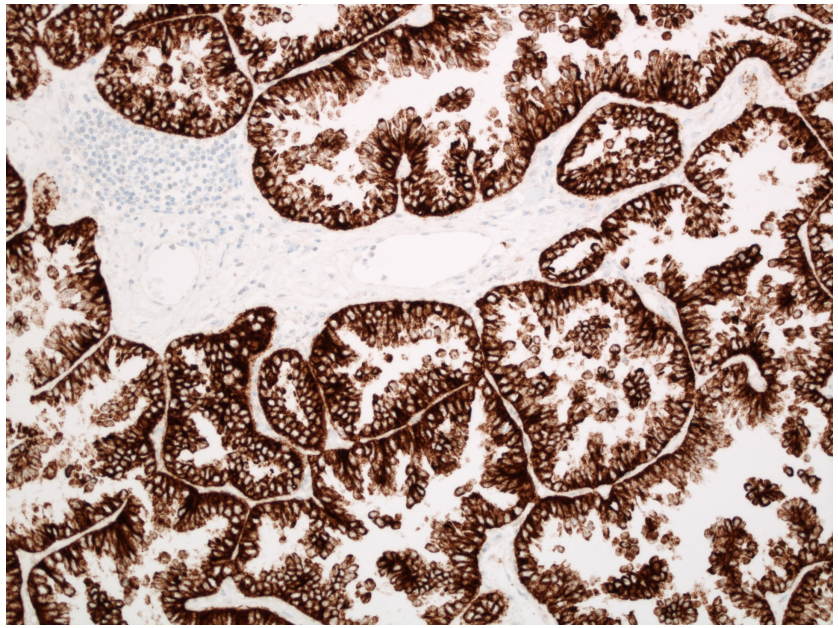
By FISH or by NGS

Up to 20% of IHC positive cases DO NOT have ROS1 fusion

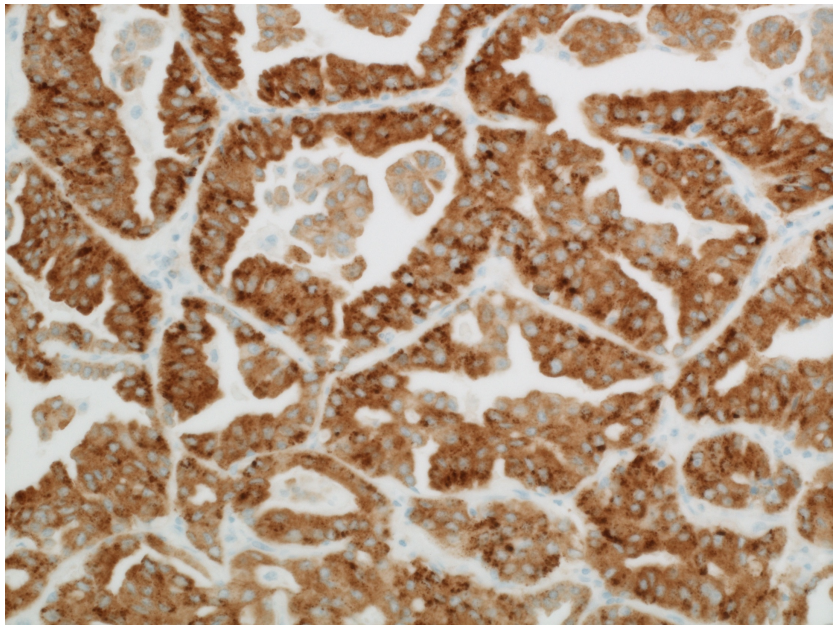
ROS1 fusion in IHC negative cases VERY rare



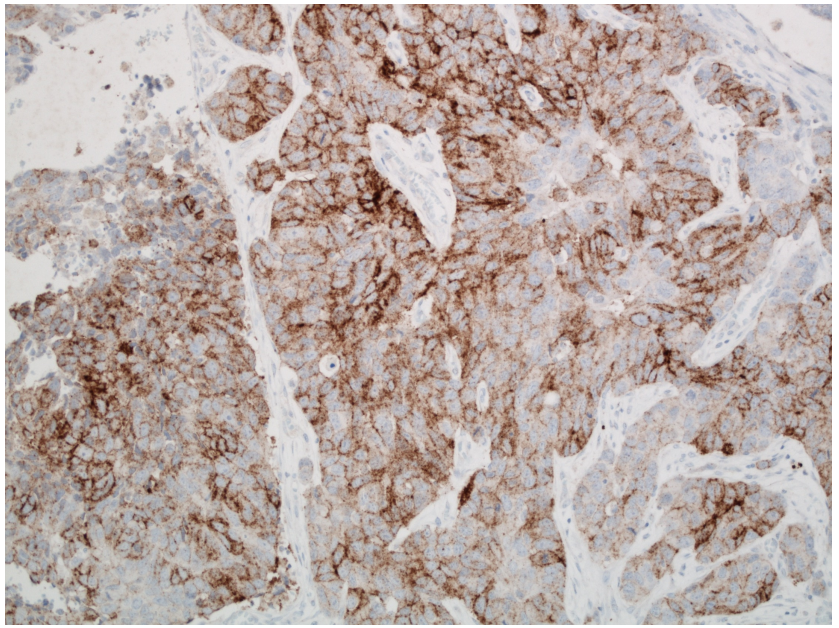
IHC for fusion gene products in NSCLC



ALK: D5F3 assay



ROS1: SP384 assay



panNTRK: EPR17341 LDT

- Screening tool to select cases for molecular confirmation
- Therapy-determining Companion diagnostic test (ALK D5F3 assay)
- Rapid turn-around: early warning to the molecular laboratory
- Validation tool to confirm molecular test results: translation has occurred

MET? WHAT DO YOU MEAN? MET… OR *MET?*

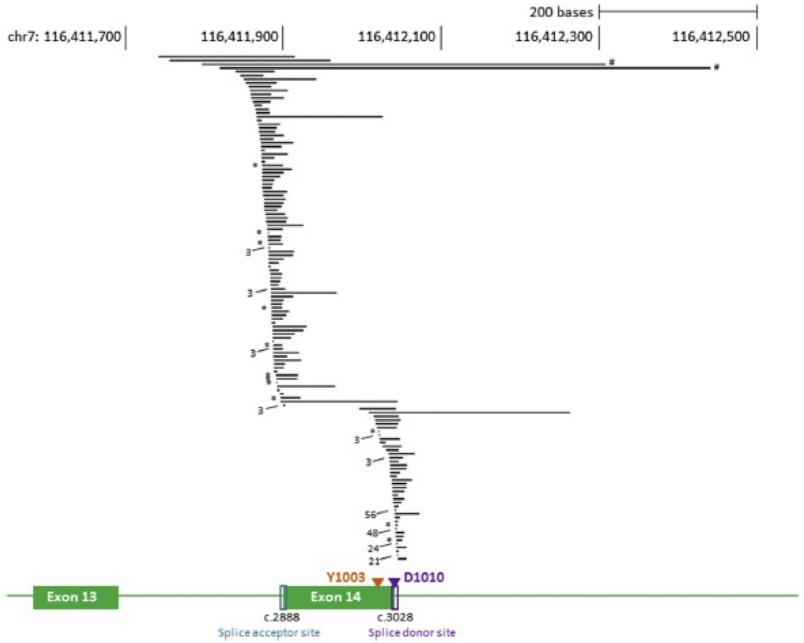
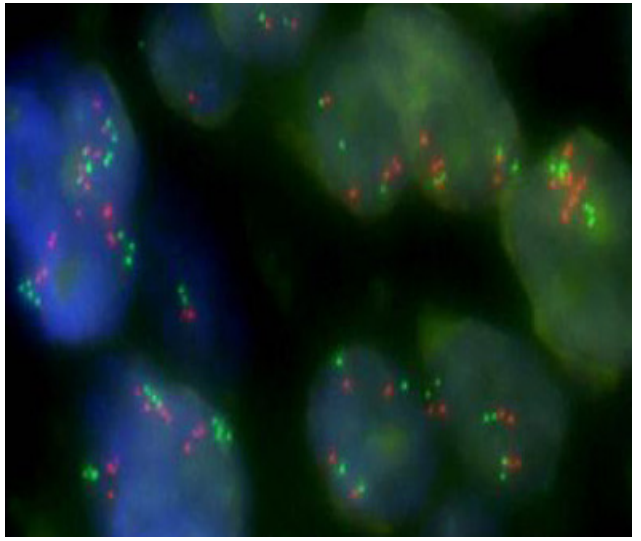


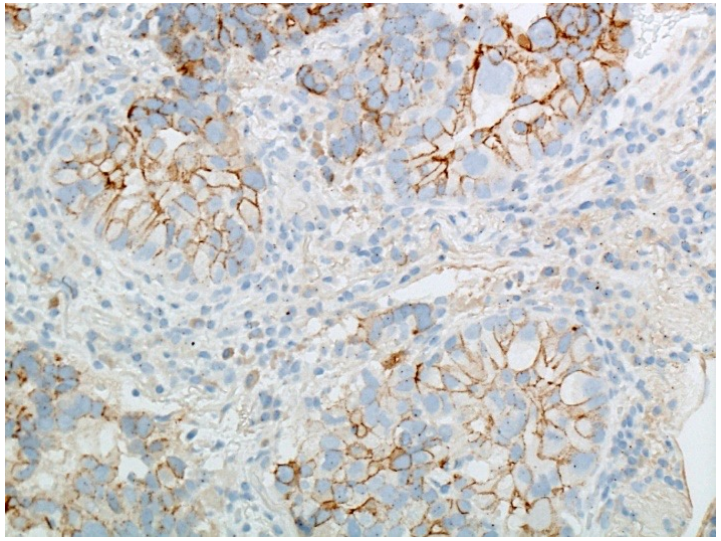
Image from Schrock AB *et al.* 2016 [Open access].[1]

- *MET*ex14 skipping mutations[1]
- ~4% of cases[2]
- Complex +++[1,3]
- Mutation behaves like a fusion gene[4]



Speaker's personal image.

- *MET* amplification[3]
- TKI resistance mechanism[5]
- Definitions variable[3]
- Testing confusing (confused)[3]



Speaker's personal image.

- MET protein IHC[3]
- Marker looking for a role?[3]
- May predict response in *MET*ex14 cases[4]

- IHC, immunohistochemistry; *MET,* mesenchymal epithelial transition factor; *MET*ex14, *MET* exon 14; TKI, tyrosine kinase inhibitor.

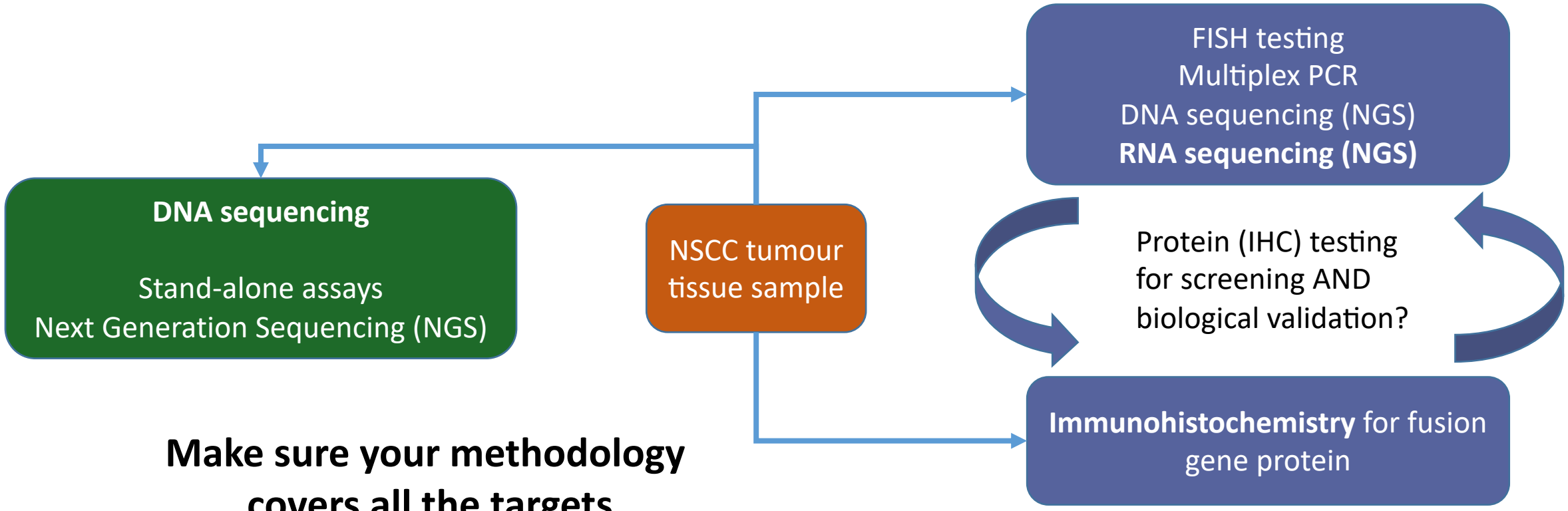
- 1. Schrock AB *et al. J Thorac Oncol* 2016; 11:1493–1502; 2. Benayed R *et al. Clin Cancer Res* 2019; 25:4712–4722; 3. Speaker's personal communications; 4. Guo R *et al. Clin Cancer Res* 2021;27:799–806; 5. Peng L-X *et al. Exp Hematol Oncol* 2021;10:52.

Multiplex Parallel (Simultaneous) testing of all required Biomarkers

Mutations
EGFR, KRAS, BRAF, HER2

MET exon14 mutations
MET amplification

Fusion genes
ALK, ROS1, NTRK, RET



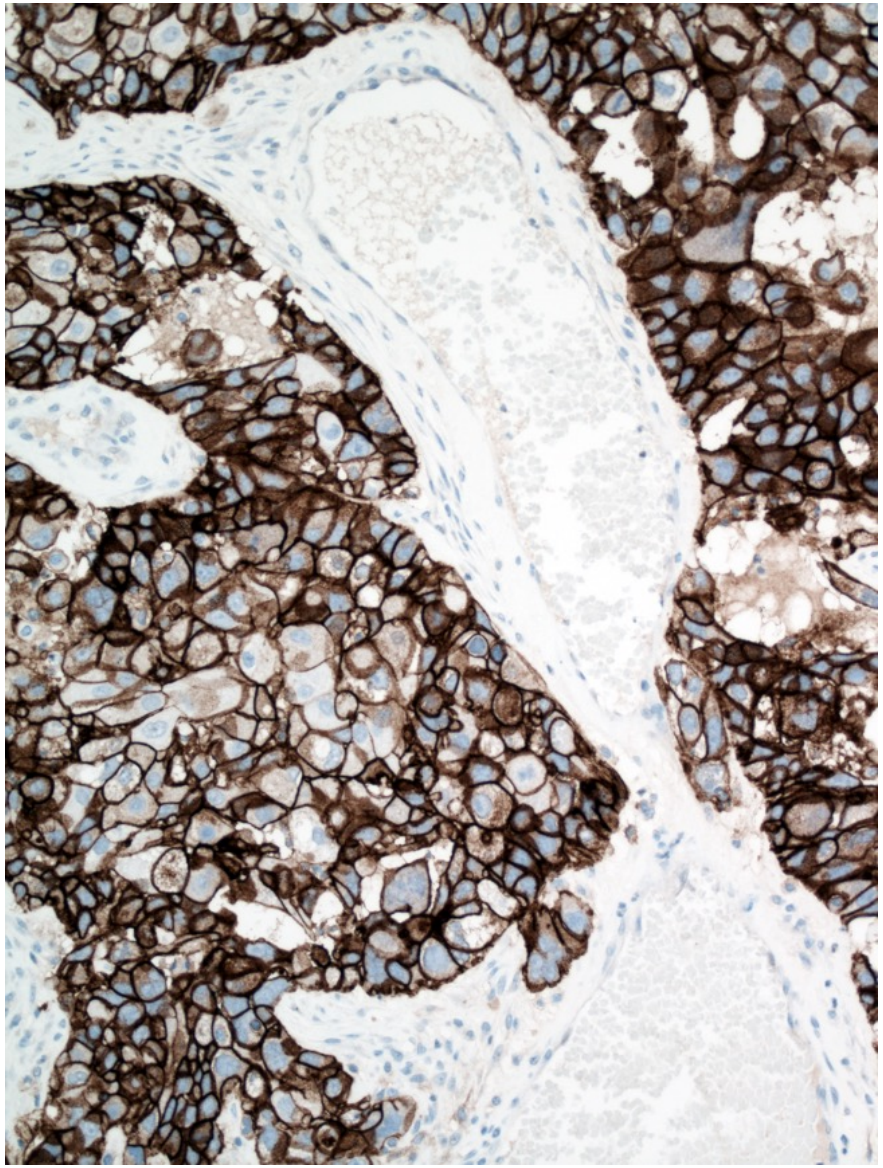
Make sure your methodology covers all the targets you need to find!

PD-L1 IHC testing is now a routine standard biomarker for NSCLC

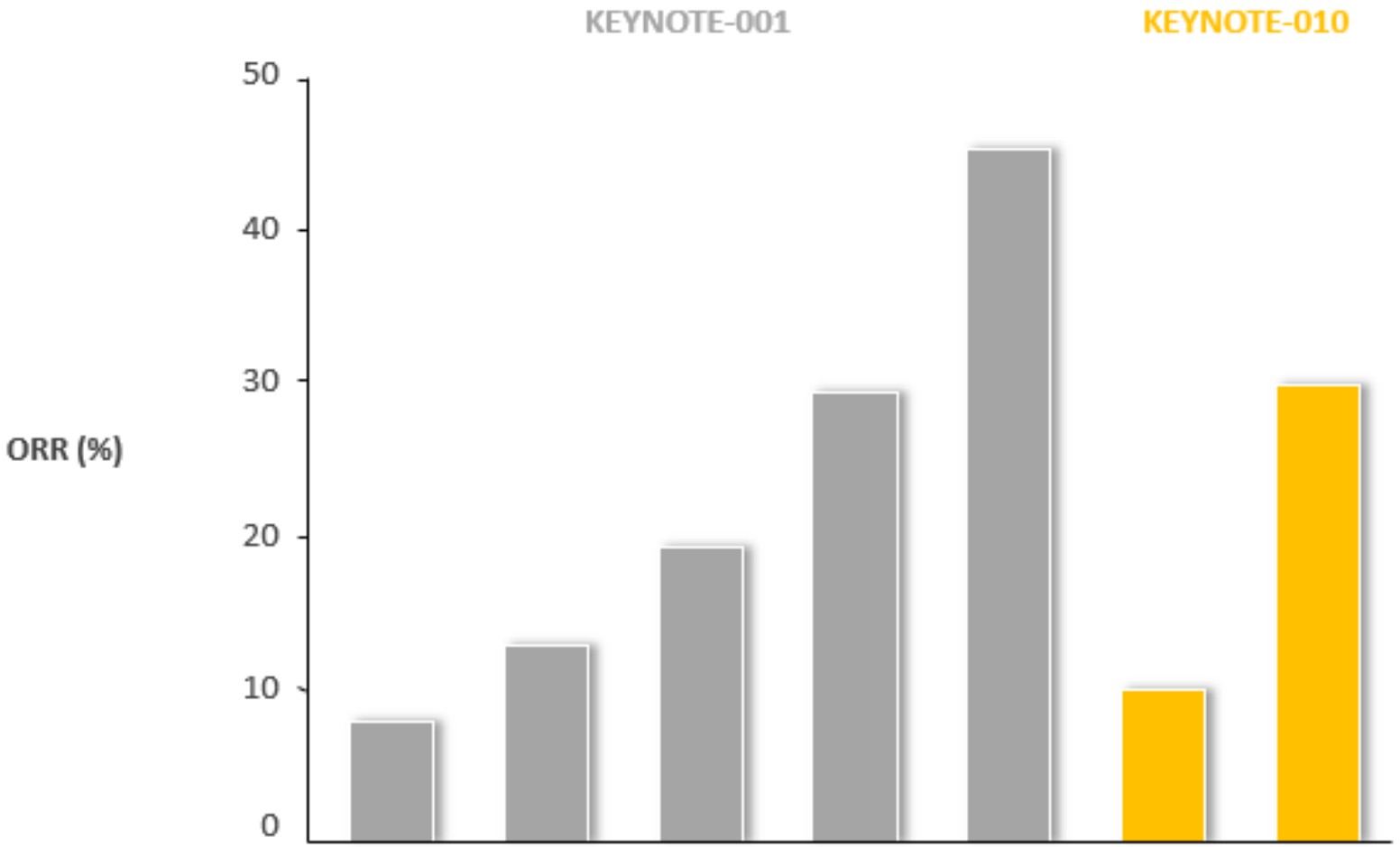
- Consistently enriches treatment populations for better outcomes from monotherapy
- Not required as a test for selection with all drugs/lines of therapy although the enrichment effect is fairly consistent
  - Companion vs complementary diagnostic tests

Approved usage

- First line pembrolizumab monotherapy – cut off 50% TPS (1% in US)
- Neoadjuvant or Adjuvant therapy – variable needs
- Stage 3 disease, CRT & durvalumab (EU only) – cut off 1% TPS
- All other usage would be complementary



Response to PD-L1 Inhibition Increases Proportionally With PD-L1 Expression



PD-L1 expression	<1%	1-24%	25-49%	50-74%	75-100%	1-49%	≥50%
Response rate	8.1	12.9	19.4	29.6	45.4	10.0	30.0
Prevalence	39.2	31.0	6.7	8.6	14.6	37.9	28.4

Grigg et al. *J Immunother Cancer*. 2016;4:48.

Other data with First line Monotherapy

1% cut off

Keynote 042
Mountzios G et al. Ann Oncol 2019

50% cut off

75% or 90% cut offs??

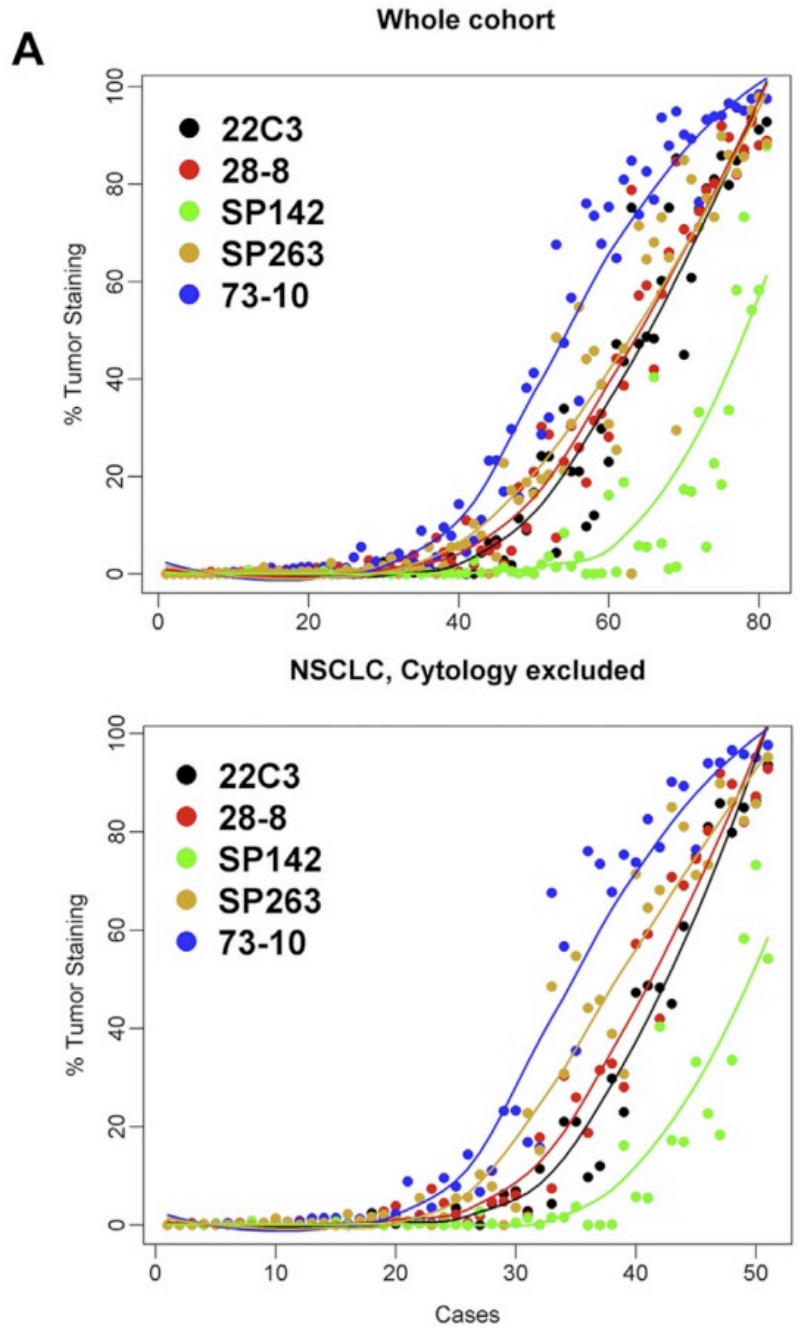
Aguilar EJ et al. Ann Oncol 2019

Impower 110 and the TC3/IC3 category

PD-L1 Immunohistochemistry Comparability Study in Real-Life Clinical Samples: Results of Blueprint Phase 2 Project

Ming Sound Tsao, MD,[a] Keith M. Kerr, MD,[b] Mark Kockx, MD, PhD,[c] Mary-Beth Beasley, MD,[d] Alain C. Borczuk, MD,[e] Johan Botling, MD,[f] Lukas Bubendorf, MD,[g] Lucian Chirieac, MD,[h] Gang Chen, MD,[i] Teh-Ying Chou, MD, PhD,[j] Jin-Haeng Chung, MD, PhD,[k] Sanja Dacic, MD, PhD,[l] Sylvie Lantuejoul, MD,[m] Mari Mino-Kenudson, MD,[n] Andre L. Moreira, MD,[o] Andrew G. Nicholson, DM,[p] Masayuki Noguchi, MD, PhD,[q] Giuseppe Pelosi, MD,[r] Claudia Poleri, MD,[s] Prudence A. Russell, MD,[t] Jennifer Sauter, MD,[u] Erik Thunnissen, MD, PhD,[v] Ignacio Wistuba, MD, PhD,[w] Hui Yu, MD, PhD,[x] Murry W. Wynes, PhD,[y] Melania Pintilie, MSc,[z] Yasushi Yatabe, MD, PhD,[aa] Fred R. Hirsch, MD, PhD[x,y,*]

- 81 lung cancer cases from routine clinical practice reflecting different sample and histological types
- 5 FDA-approved or clinical trial assays performed in CLIA-compliant laboratory
- Scored by 24 pathologists from 15 countries across 5 continents



Each circle represents the mean of all scores (by microscopy or digital image)

Tsao MS, et al. J Thorac Oncol. 2018;13:1302-1311

Tumour Cells, or Immune Cells or Both?

Tumour Cell (TC) or Tumour Proportion Score (TPS%): % of viable tumour cells in the sample expressing PDL1

22C3, SP263, SP142 assays

EXCELLENT interobserver correlation

Immune Cell (IC): the % of the AREA of viable tumour infiltrated by PD-L1 expressing immune cells

SP142 assay

POOR interobserver correlation

Combined Proportion Score (CPS%):

$$\frac{\text{Number of PD-L1 stained cells (tumour cells, lymphocytes and macrophages)}}{\text{Total number of viable tumour cells}} \times 100\%$$

22C3, SP263 assays

POOR interobserver correlation

Some observations on PD-L1 testing

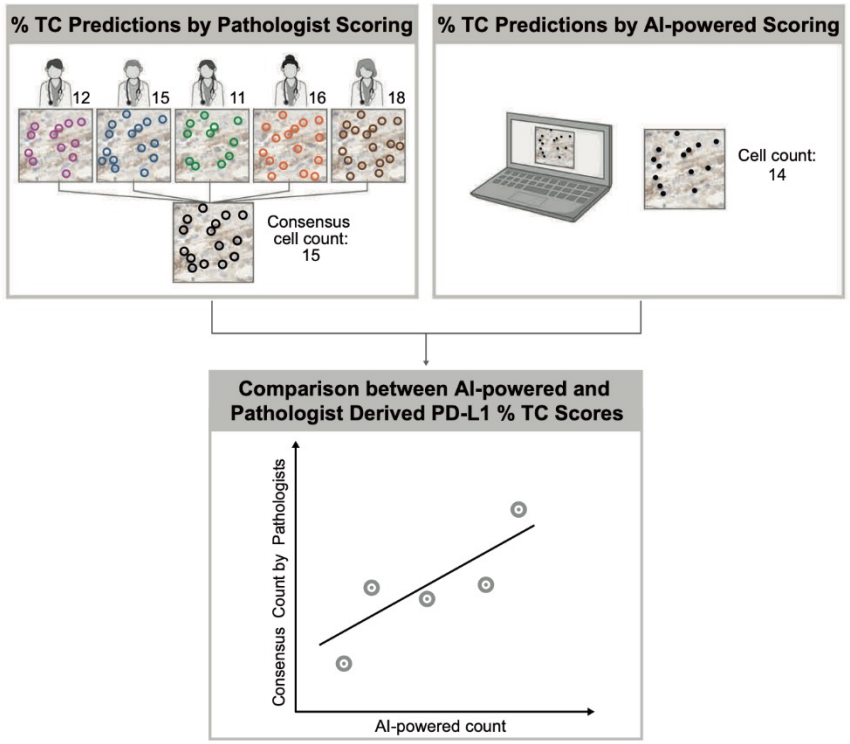
- Validation against a standard essential, especially if you are using an LDT
- Cytology type samples are OK if cell blocks are used
- 100 cells minimum for a TPS read
  - 9% cases inadequate over all
  - 12% cases inadequate for cytology samples
- Approximately one third of cases should fall in each score range:

<1%; 1-49%; ≥50%

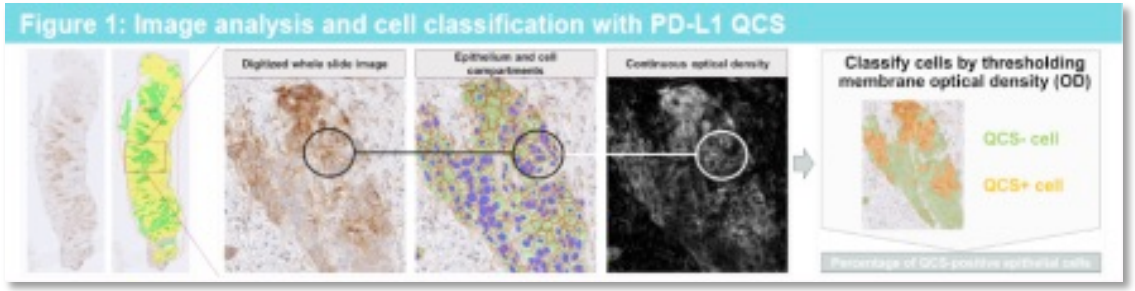
Tsao MS, et al. J Thorac Oncol. 2018;13:1302-1311
Gosney JG et al. J Clin Pathol 2024; 77, 135

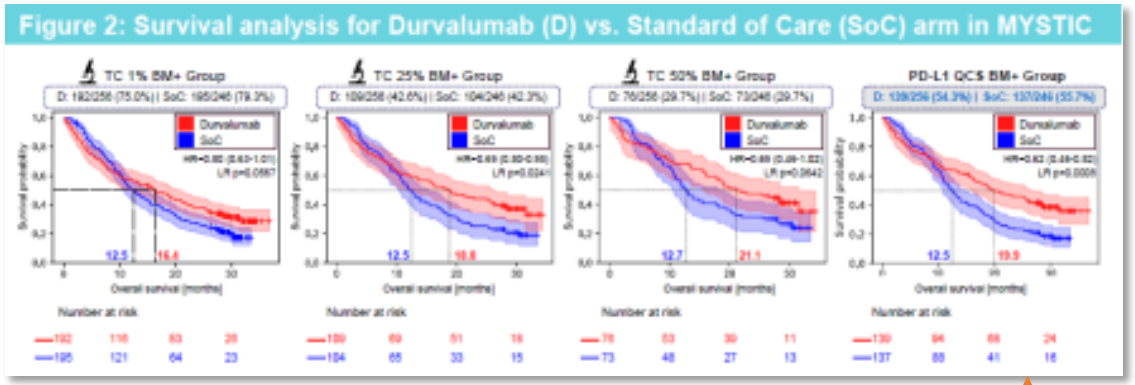
Computational pathology (AI) for PD-L1 assessment

Using AI to read PD-L1 TPS% in CheckMate trials



- AI-generated PD-L1 scores tended to be higher
- Clinical outcomes preserved





Alternate QCS methodology for assessment retains clinical benefit AND increases size of treatment group

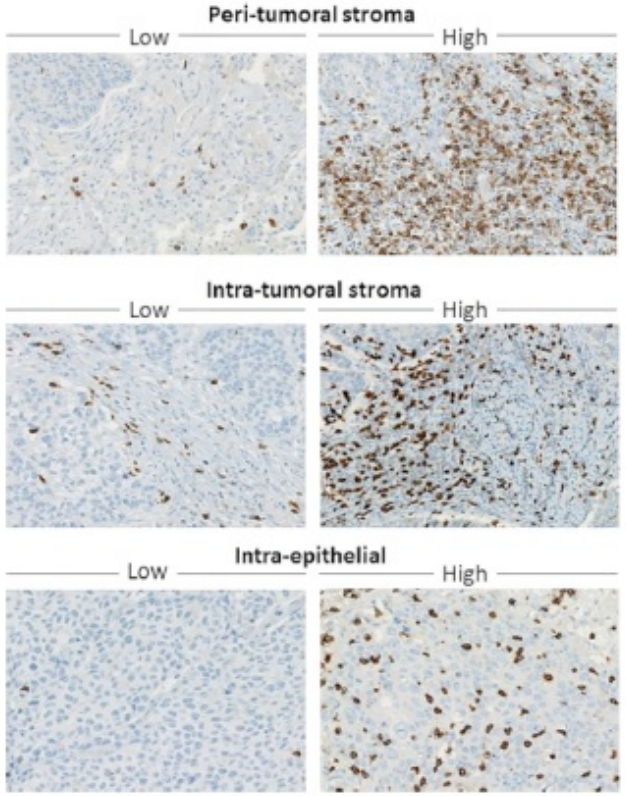
AI, artificial intelligence; BM, biomarker; QCS, quantitative continuous scoring; TC, tumour cell; TPS, tumour proportion score.

Baxi V, et al. *Mod Pathol*. 2022;35(11):1529-1539.

Lesniak J, et al. *Cancer Res*. 2024;84(Suppl 6):Abstract 7617 (AACR abstract).

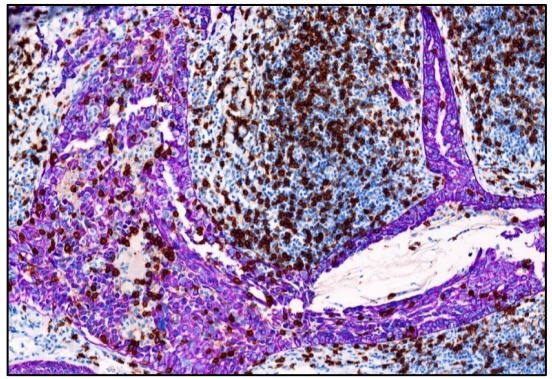
Morphological inflammation and Immunotherapy

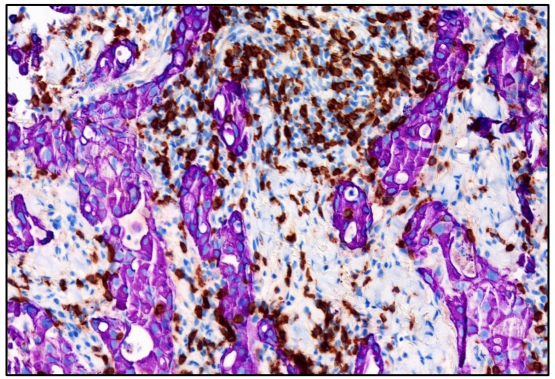
Which immune cells are present?
Where are the immune cells?
What are the cellular associations?
Assessment using Computational Pathology *

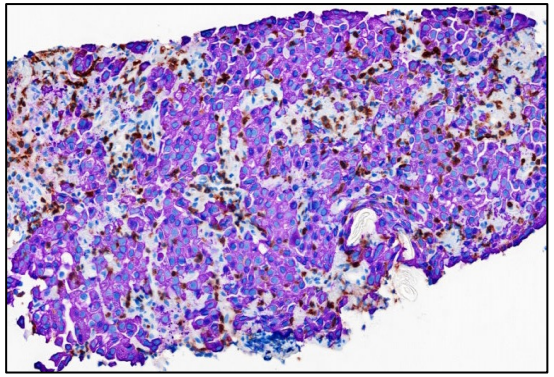


Conde E et al, Histopathol 2018

CD8
IHC







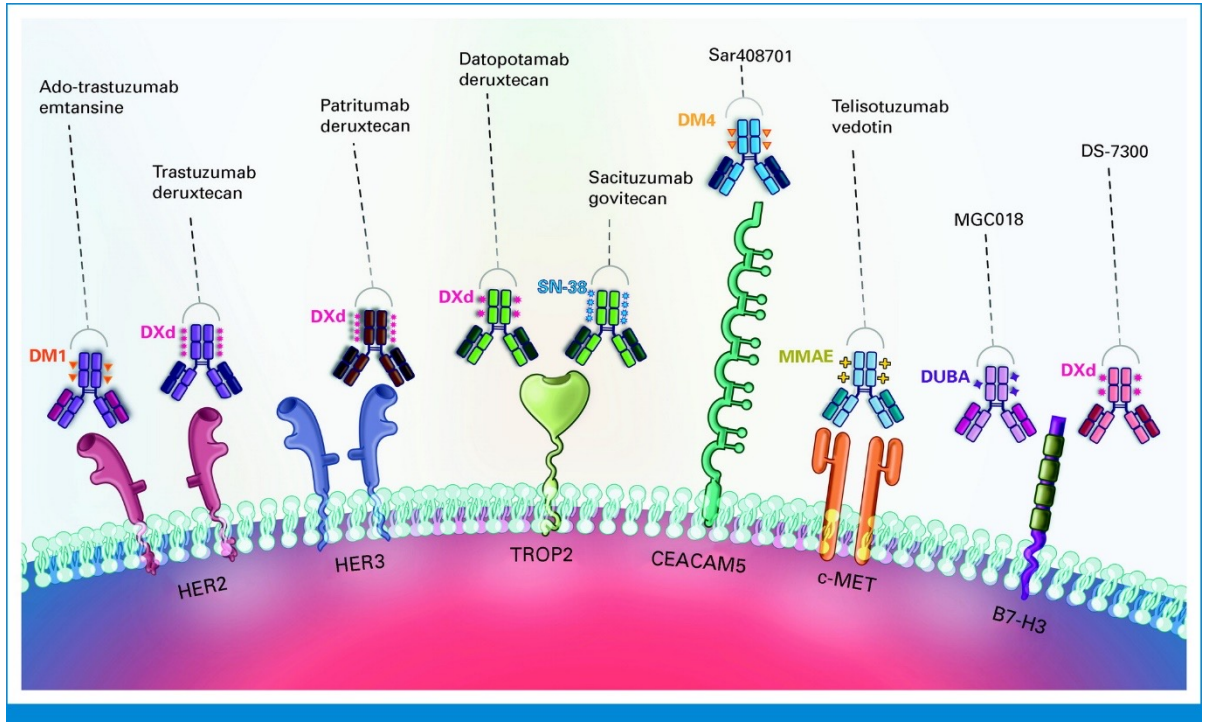
pan-CK/CD8 images
Courtesy of Mark Kockx
HistoGeneX,
Antwerp, Belgium

CD8
CD4
CD1a
CD68
CD163
FoxP3
etc

*Molero A et al. J Clin Pathol 2024

ADCs: Implications for diagnostics

Target	Drug	Payload
HER2	Trastuzumab-DM1	DM1
	Trastuzumab-DXd	Deruxtecan
HER3	Patritumab-DXd	Deruxtecan
TROP2	Datopotamab-DXd	Deruxtecan
	Sacituzumab govitecan	SN-38
CEACAM5	Tusamitamab ravtansine	DM4
c-MET	Telisotuzumab vedotin	MMAE
B7-H3	I-DXd (DS-7300a)	Deruxtecan
	MGC018	DUBA
CD56	Lorvotuzumab mertamsine	DM1
AXL	Enapotamab vedotin	MMAE
	Mecbotamab vedotin	MMAE
PK7	Cofetuzumab pelidotin	Auristatin-0101
PVRL4	Enfortumab vedotin	MMAE
TF	Tisotumab-vedotin	MMAE
EGFR	MRG003	MMAE
ROR2	Ozuriftamab vedotin	MMAE
NaPi2b	Upifitamab rilsodotin	AF-HPA
	Lifastuzumab vedotin	MMAE



Positivity Locality Quantity

The Pathologists' Conundrum

David L. Rimm, MD, PhD; Sanja Dacic, MD, PhD; Stuart J. Schnitt, MD

ADC, antibody-drug conjugate; AF-HPA, auristatin F- hydroxypropylamide; DUBA, deubiquitinating enzyme A; DXd, deruxtecan; I-DXd, ifinatamab deruxtecan; MMAE, monomethyl auristatin E.
Passaro A et al. J Clin Oncol. 2023;41(21):3747-3761.

Rimm DL, et al. *Arch Pathol Lab Med*. 2023;147(1):17-18.

Summary of SOME Biomarker data for ADCs in NSCLC

Target	Drugs	Response rates	Biomarker defined treatment group?	Biomarker used
HER2	Traztuzumab Deruxtecan	55% 20.8% and 28.2% 20% and 52.9%	YES YES YES	HER2 mutation HER2 IHC 2+ HER2 IHC 3+
HER3	Patritumab Deruxtecan	39% 26.9%-28.6%	NO	*EGFR mutation/TKI fail* *2L with or without other onco-driver*
cMET	Telisotuzumab Vedotin	52.2% 24.1% 11.1%	YES YES YES	cMET IHC HIGH (Non Squamous) cMET IHC Intermediate (NON-Squamous) cMET IHC 'positive' in Squamous
TROP2	Datopotamab Deruxtecan Sacituzumab Govitecan	21-25% 16.7% NSCLC 17.7% SCLC	NO NO NO	
CEACAM5	Tusamitamab Ravtansine	20.3% 7.1%	YES YES	CEACAM5 IHC* HIGH CEACAM5 IHC* MODERATE

* The MAb from the ADC also used in the IHC

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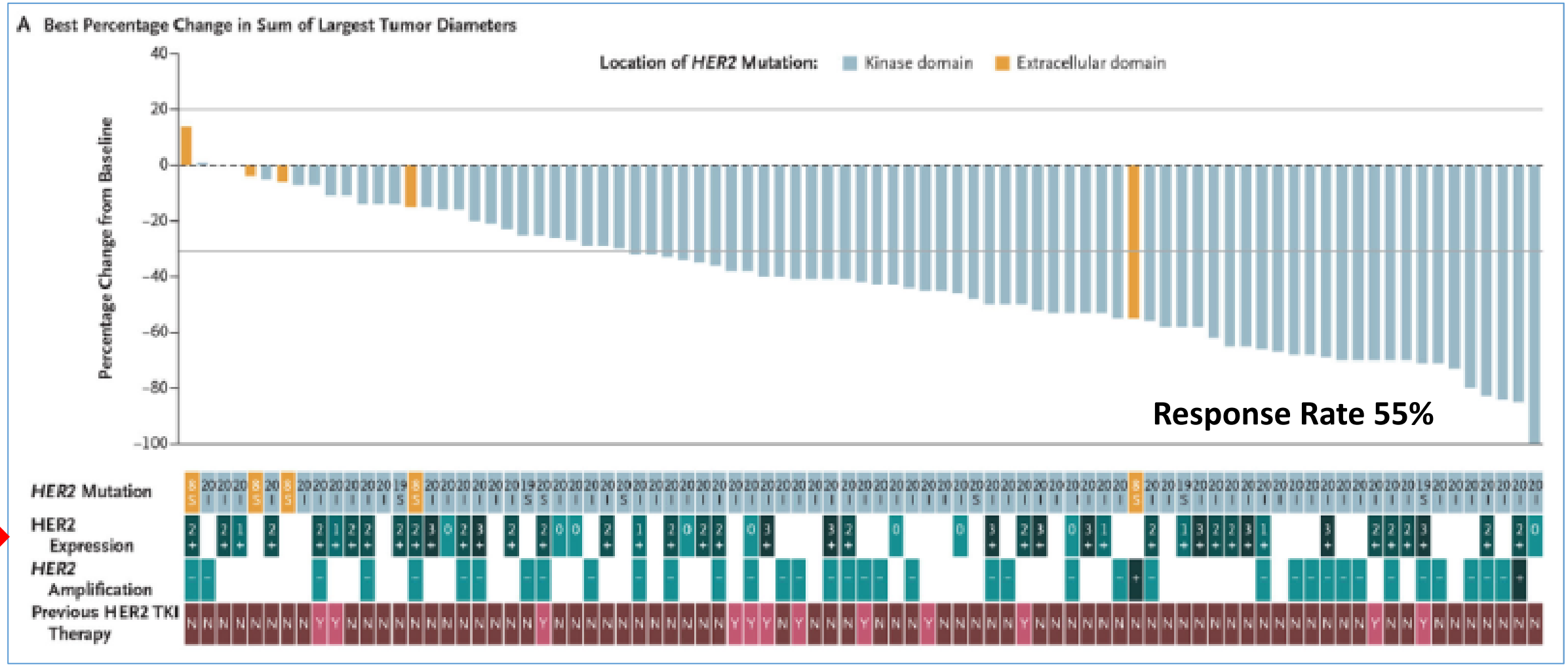
* The MAb from the ADC also used in the IHC

HER2 IHC Score	HER2 IHC Pattern in Surgical Specimen	HER2 IHC Pattern in Biopsy Specimen	HER2 Expression Assessment
0	No reactivity or membranous reactivity in <10% of cancer cells	No reactivity or no membranous reactivity in any cancer cell	**Negative by IHC**
1+	Faint or barely perceptible membranous reactivity in ≥10% of cancer cells; cells are reactive only in part of their membrane	Cancer cell cluster* with a faint or barely perceptible membranous reactivity **irrespective of percentage of cancer cells positive**	**Negative by IHC**
2+	Weak to moderate complete, basolateral or lateral membranous reactivity in ≥10% of tumor cells	Cancer cell cluster* with a weak to moderate complete, basolateral, or lateral membranous reactivity **irrespective of percentage of cancer cells positive**	**Equivocal by IHC**
3+	**Strong complete, basolateral or lateral membranous reactivity in ≥10% of cancer cells**	Cancer cell cluster* with a strong complete basolateral, or lateral membranous reactivity **irrespective of percentage of cancer cells positive**	**Positive**

College of American Pathologists
Version: GastricHER2Biomarkers 1.0.0.1 June 2017

*cancer cell cluster is ≥5 TC

DESTINY Lung01
Trastuzumab deruxtecan in **HER2 mutant** NSCLC

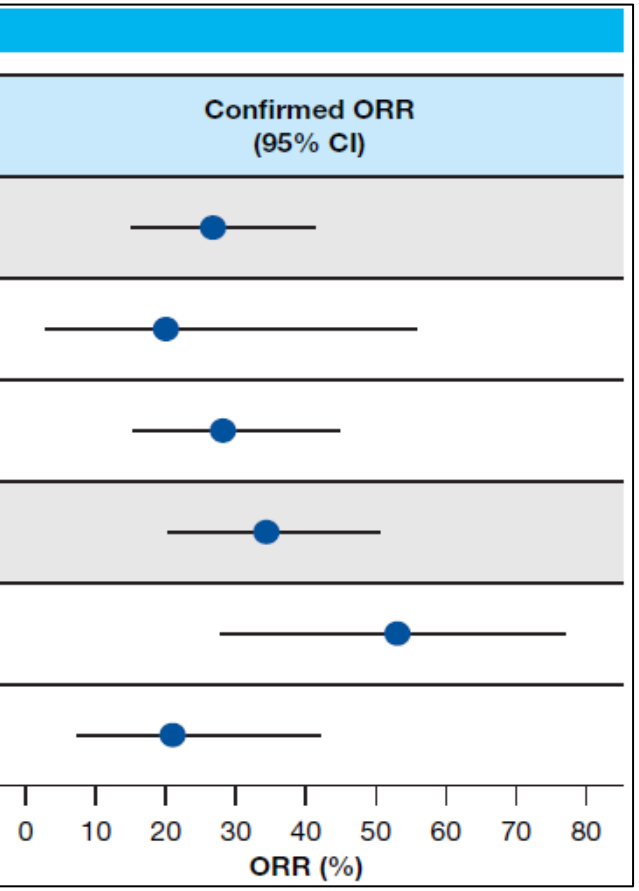


Li B et al. NEJM2022

HER2 ‘high’ IHC positive NSCLC without mutation: DESTINY Lung01

Figure 2. Response to T-DXd by HER2 IHC Status

	No. of responders	Confirmed ORR (95% CI)
Cohort 1 (all patients)	13/49	26.5 (15.0-41.1)
HER2 IHC 3+	2/10	20.0 (2.5-55.6)
HER2 IHC 2+	11/39	28.2 (15.0-44.9)
Cohort 1a (all patients)	14/41	34.1 (20.1-50.6)
HER2 IHC 3+	9/17	52.9 (27.8-77.0)
HER2 IHC 2+	5/24	20.8 (7.1-42.2)



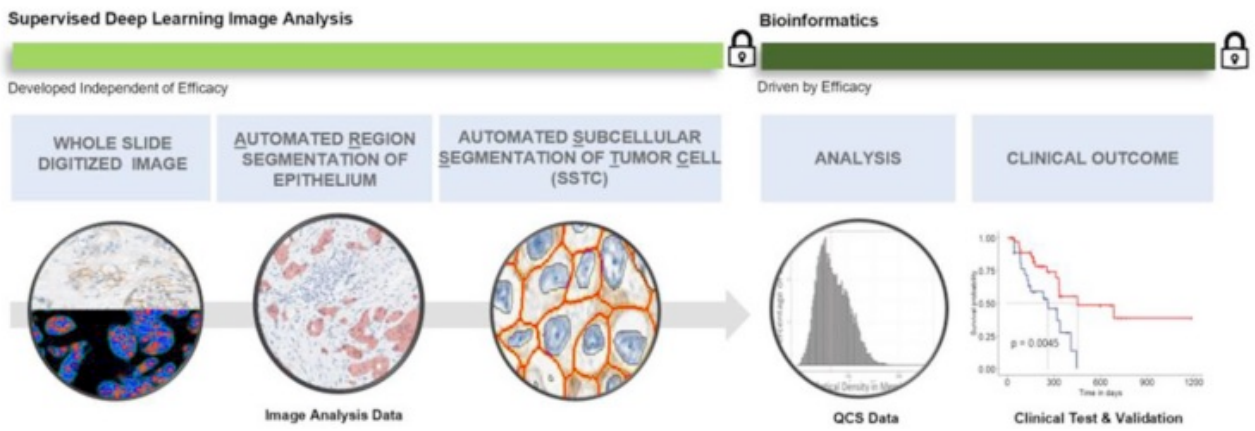
- ORR %
  - IHC 3+ 20% and 52.9%
  - IHC 2+ 28.2% and 20.8%
- ‘High’ HER2 expression (but is it?)
- Do we know what happens in patients with no or low HER2 expression?

Smit EF et al

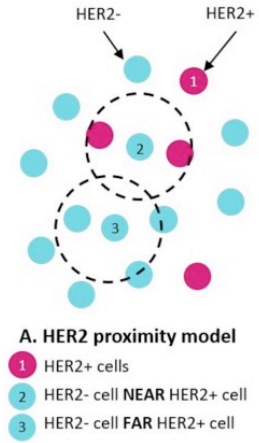
ABSTRACT| VOLUME 33, SUPPLEMENT 7, S994-S995, SEPTEMBER 2022

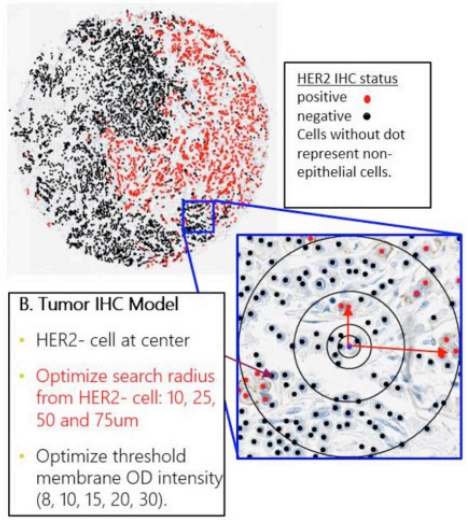
ESMO 2022 POSTER 975P

New methods of IHC quantification



HER2 ADC





Breast Cancer

Novel methodology
Better stratification than ‘manual’ scoring

ADC, antibody-drug conjugate; IHC, immunohistochemistry.
Kapil A, et al. *Sci Rep.* 2024;14(1):12129; Garassino MC, et al. Presented at: IASLC 2024 WCLC [Abstract PL02.11].

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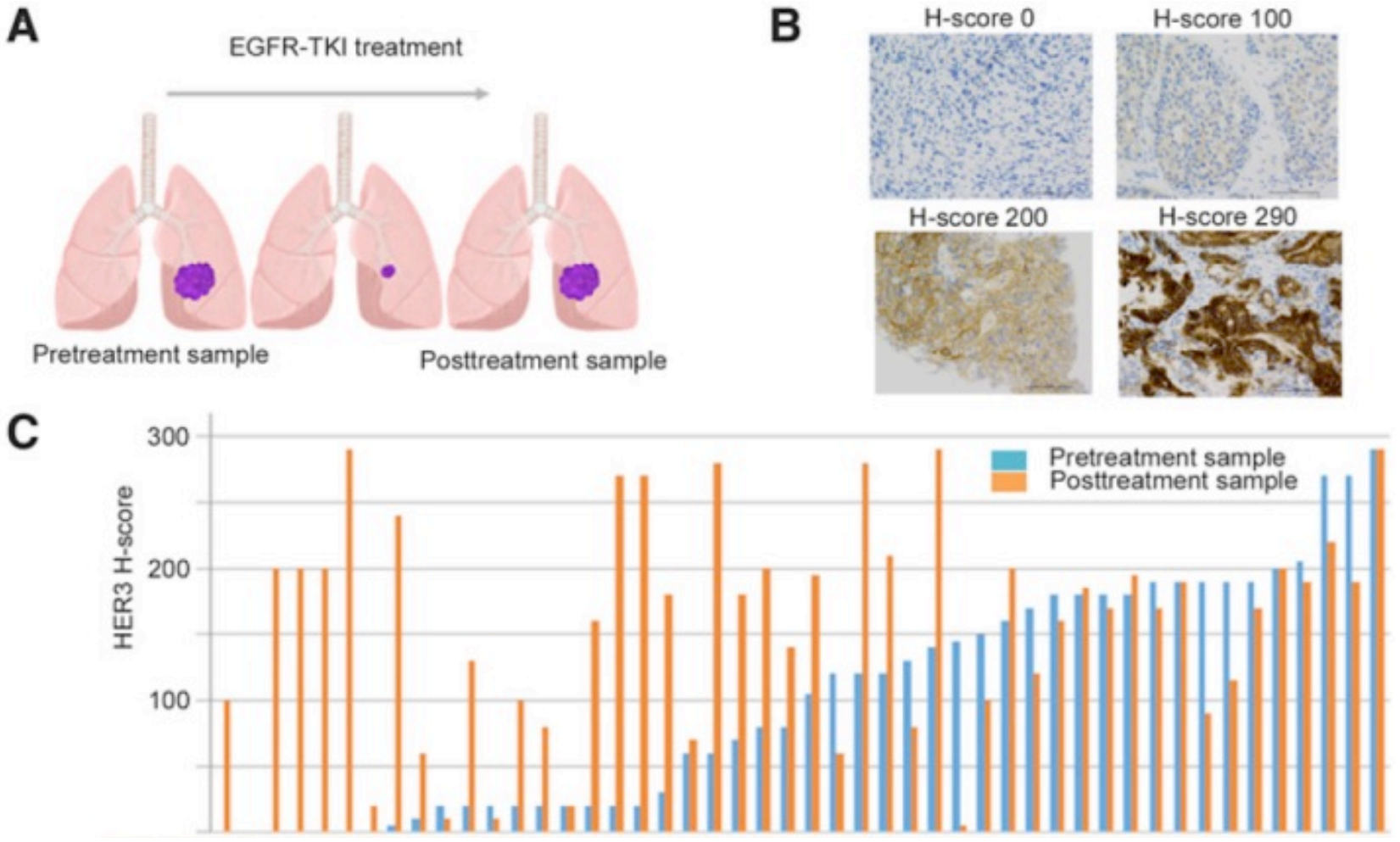
HER3	Patritumab Deruxtecan	39% 26.9%-28.6%	NO	*EGFR mutation/TKI fail* *2L with or without other onco-driver*
cMET	Telisotuzumab Vedotin	52.2% 24.1% 11.1%	YES YES YES	cMET IHC HIGH (Non Squamous) cMET IHC Intermediate (NON-Squamous) cMET IHC 'positive' in Squamous
TROP2	Datopotamab Deruxtecan Sacituzumab Govitecan	21-25% 16.7% NSCLC 17.7% SCLC	NO NO NO	
CEACAM5	Tusamitamab Ravtansine	20.3% 7.1%	YES YES	CEACAM5 IHC* HIGH CEACAM5 IHC* MODERATE

* The MAb from the ADC also used in the IHC

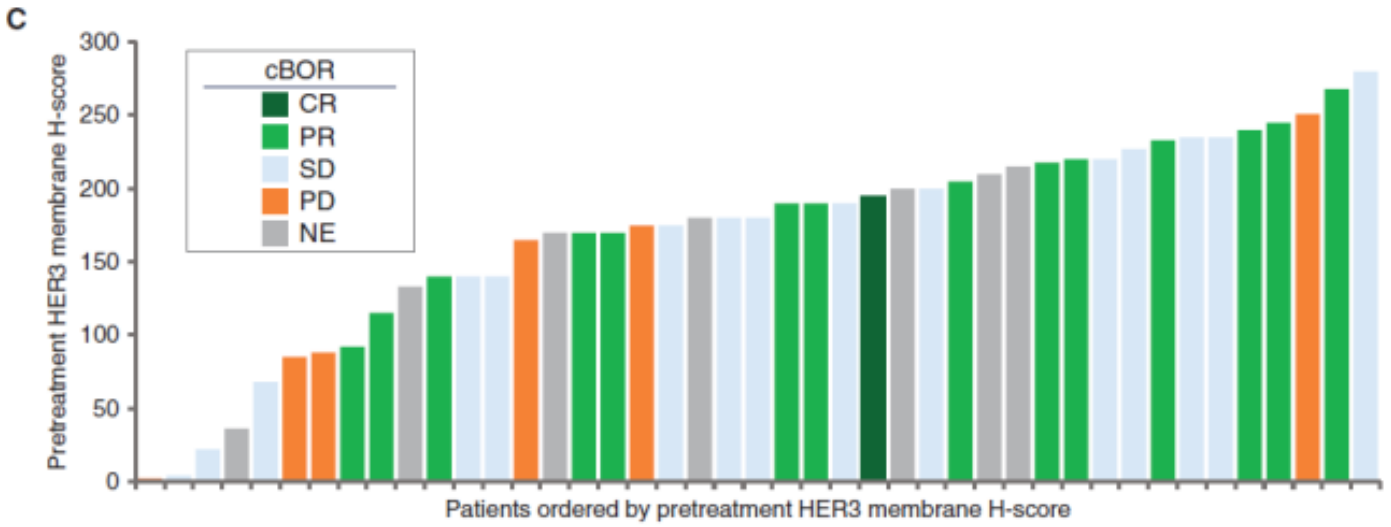
HER3 expression commonly increases after EGFR TKI therapy

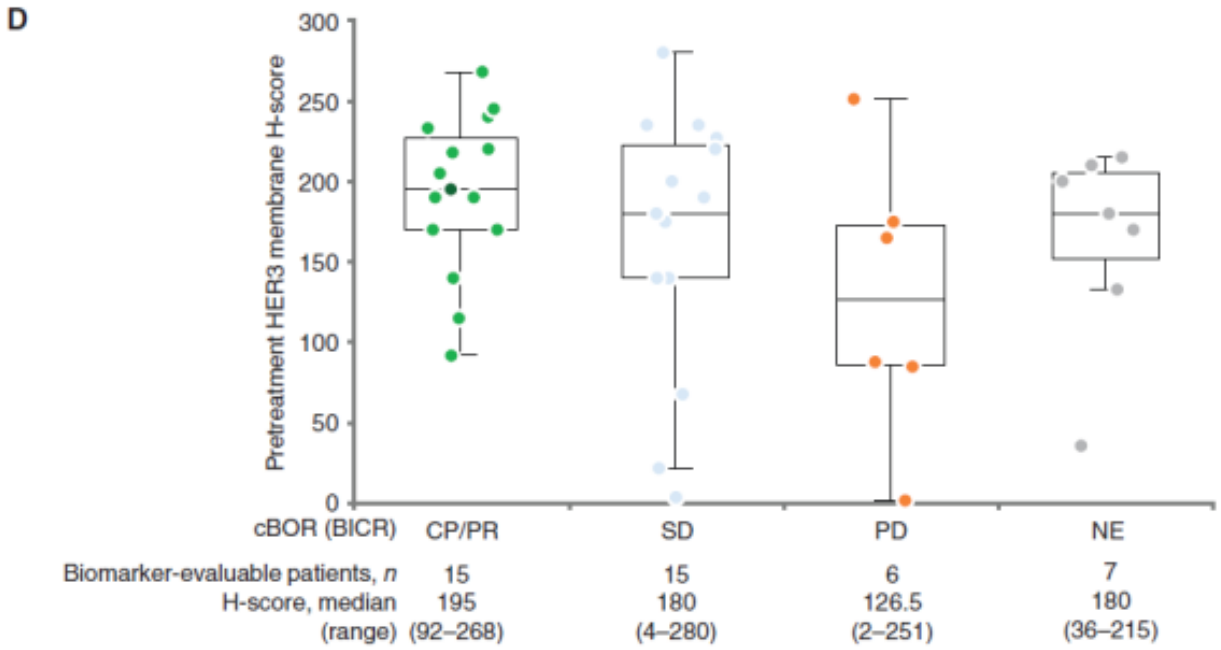
Effect may be driven by repression of the PI3K/AKT/mTOR pathway

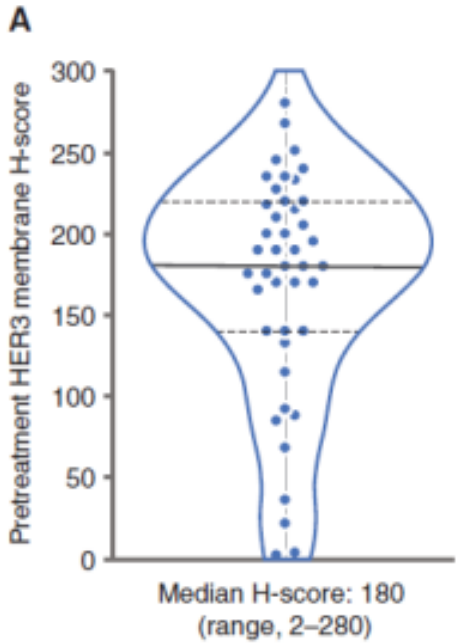


Yonesaka K et al. Clin Can Res 2022

HER3: Patritumab deruxtecan in EGFR TKI failed NSCLC







Response (ORR 39%) not related to IHC expression level?
or to
Molecular resistance mechanism

Janne PA et al. Can Discov 2022

Summary of SOME Biomarker data for ADCs in NSCLC

Target	Drugs	Response rates	Biomarker defined treatment group?	Biomarker used
HER2	Traztuzumab Deruxtecan	55% 20.8% and 28.2% 20% and 52.9%	YES YES YES	HER2 mutation HER2 IHC 2+ HER2 IHC 3+
HER3	Patritumab Deruxtecan	39% 26.9%-28.6%	NO	*EGFR mutation/TKI fail* *2L with or without other onco-driver*
cMET	Telisotuzumab Vedotin	52.2% 24.1% 11.1%	YES YES YES	cMET IHC HIGH (Non Squamous) cMET IHC Intermediate (NON-Squamous) cMET IHC 'positive' in Squamous
TROP2	Datopotamab Deruxtecan Sacituzumab Govitecan	21-25% 16.7% NSCLC 17.7% SCLC	NO NO NO	
CEACAM5	Tusamitamab Ravtansine	20.3% 7.1%	YES YES	CEACAM5 IHC* HIGH CEACAM5 IHC* MODERATE

* The MAb from the ADC also used in the IHC

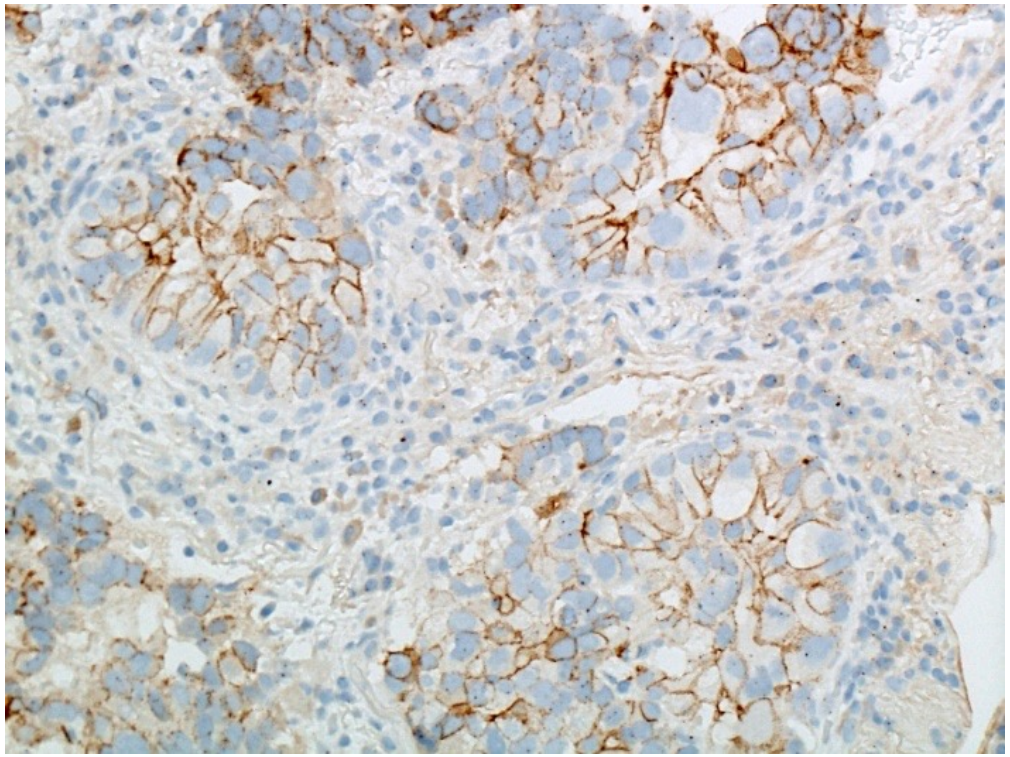
cMET IHC by Ventana SP44 assay (LUMINOSITY trial)

Non-Squamous

- Overexpression in at least 25% of tumor cells at 3+ intensity
  - **HIGH defined as ≥50% membrane staining at 3+ intensity**
  - **INTERMEDIATE defined as ≥25% to <50% membrane staining at 3+ intensity**

Squamous

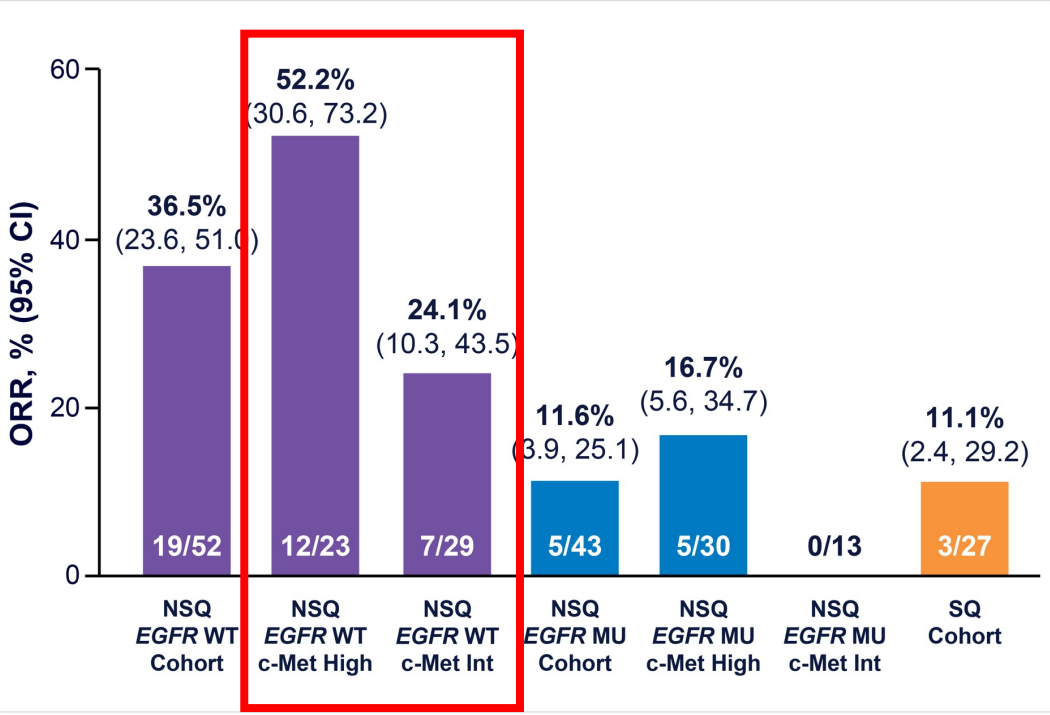
- ≥75% of tumor cells at 1+ intensity



Camidge DR, et al. J Clin Oncol 2022;40(suppl):Abstr 9016

Interim Efficacy

ORR per Central Review by Cohort/Group



CI, confidence interval; *EGFR*, epidermal growth factor receptor; Int, intermediate; MU, mutant; NSQ, non-squamous; ORR, overall response rate; SQ, squamous; WT, wild-type.

- The NSQ *EGFR* WT NSCLC cohort met protocol-specified criteria for expansion in Stage 2 at interim analysis 3. Updated data at the time of interim analysis 4 are shown
- The NSQ *EGFR* MU NSCLC cohort met protocol-specified criteria for futility at interim analysis 4. The SQ cohort met criteria for futility at the previous interim analysis; final data shown

DOR per Central Review by Cohort/Group

Cohort/Group	mDOR by ICR, No. of Events/No. of Responders, Months [95% CI]
NSQ *EGFR* WT	8/19, 6.9 [4.1, NR]
c-Met high	5/12, 6.9 [2.4, NR]
c-Met int	3/7, NR [4.1, NR]
NSQ *EGFR* MU	2/5, NR [3.0, NR]
c-Met high	2/5, NR [5.5, NR]
c-Met int	NA
SQ	2/3, 4.4 [3.0, NR]

CI, confidence interval; DOR, duration of response; *EGFR*, epidermal growth factor receptor; ICR, independent central review; int, intermediate; mDOR, median duration of response; MU, mutant; NA, not available; NR, not reached; NSQ, non-squamous; SQ, squamous; WT, wild-type.

Objective Response Rate per Central Review for Subgroups Defined by Prior Therapies: NSQ *EGFR* WT Cohort

Cohort/Group	Prior Platinum	Prior Platinum and Immune Checkpoint Inhibitor
NSQ *EGFR* WT	18/50 (36.0)	15/37 (40.5)
c-Met high	11/21 (52.4)	9/16 (56.3)
c-Met int	7/29 (24.1)	6/21 (28.6)

EGFR, epidermal growth factor receptor; int, intermediate; NSQ, non-squamous; WT, wild-type.

Molecular oncogene analyses in tumors of patients with available tissue are underway.

Slide courtesy of Ross Camidge, Colorado, USA

DOR, duration of response; EGFR, epidermal growth factor receptor; MU, mutant; NSCLC, non-small cell lung caner; NSQ, non-squamous; OE, overexpressing; ORR, overall response rate; SQ, squamous; Teliso-V, telisotuzumab vedotin; WT, wild-type.

Summary of SOME Biomarker data for ADCs in NSCLC

Target	Drugs	Response rates	Biomarker defined treatment group?	Biomarker used
HER2	Traztuzumab Deruxtecan	55% 20.8% and 28.2% 20% and 52.9%	YES YES YES	HER2 mutation HER2 IHC 2+ HER2 IHC 3+
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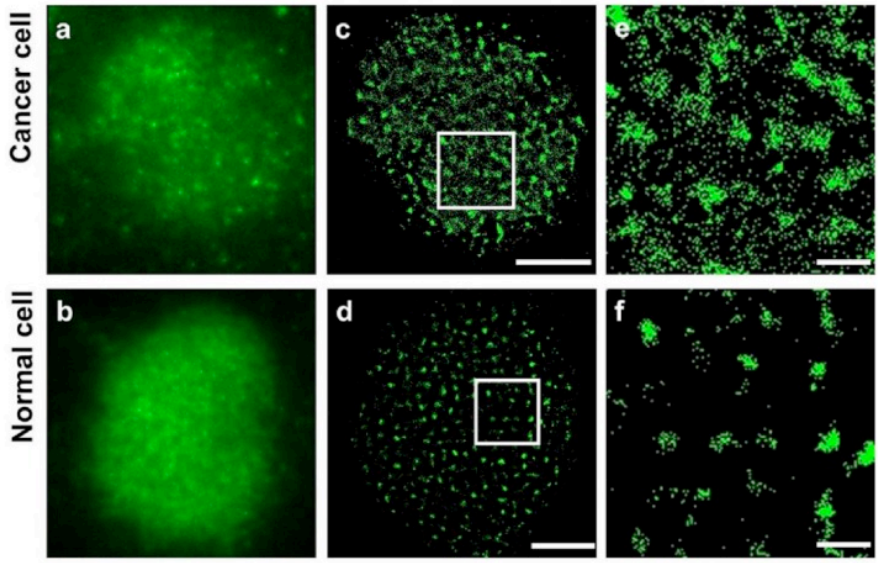
CEACAM5	Tusamitamab Ravtansine	20.3% 7.1%	YES YES	CEACAM5 IHC* HIGH CEACAM5 IHC* MODERATE

* The MAb from the ADC also used in the IHC

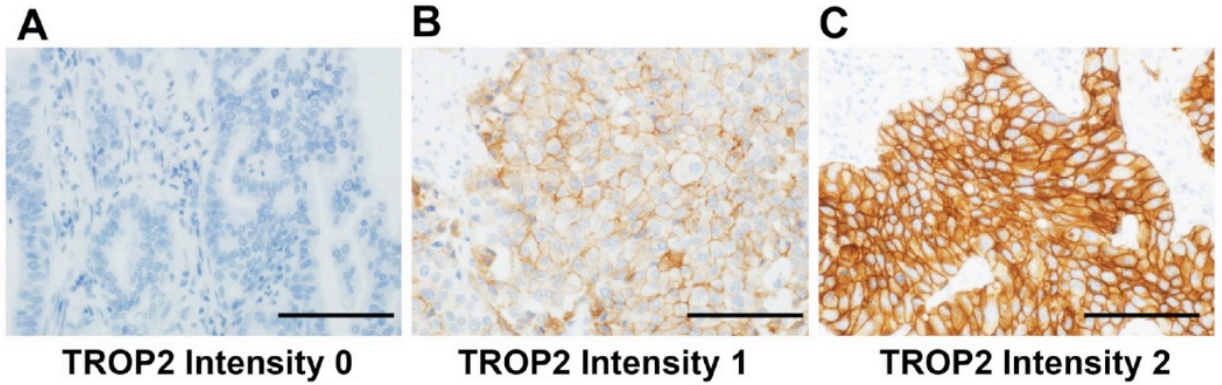
TROP2 is variably expressed in lung cancer



TROP2 is a pro-oncogenic transmembrane glycoprotein

Expression in cancer cells tends to exhibit molecular clustering

Fu Y et al. Talanta 2020



HIGH expression defined as **>50% @intensity1** OR **>10% @intensity2**

HIGH expression reported in	
Adenocarcinoma	**64%**
Squamous cell carcinoma	**75%**
HG NE carcinomas	**18%**

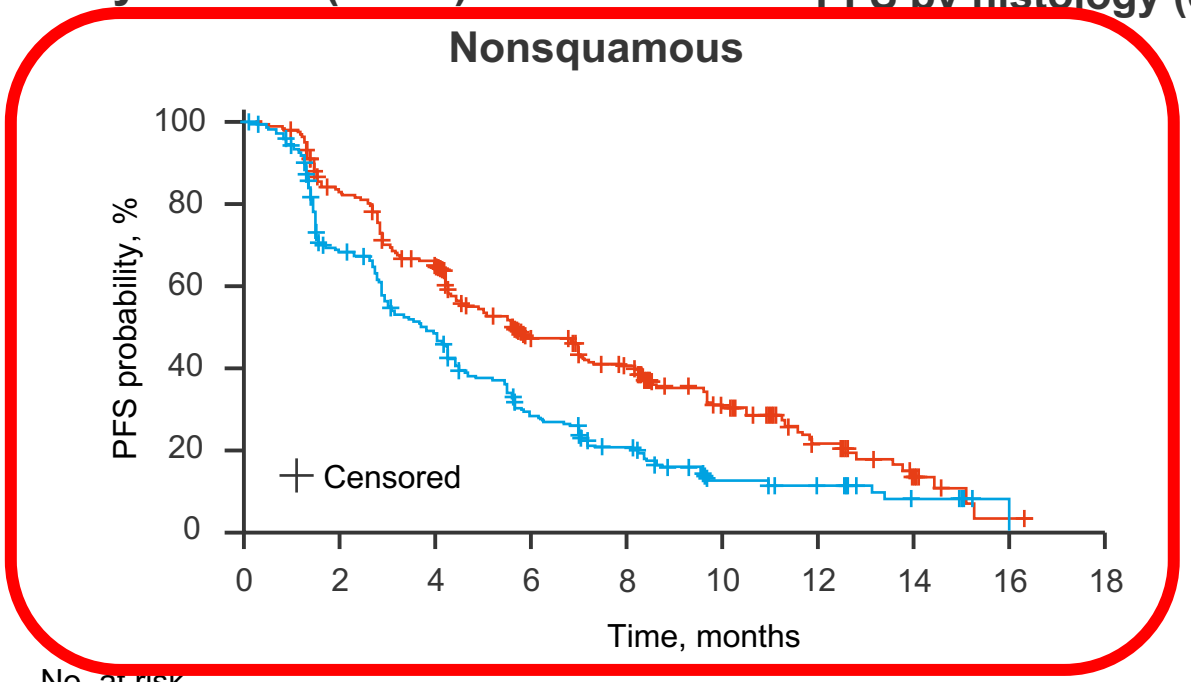
Inamura K et al. Oncotarget 2017

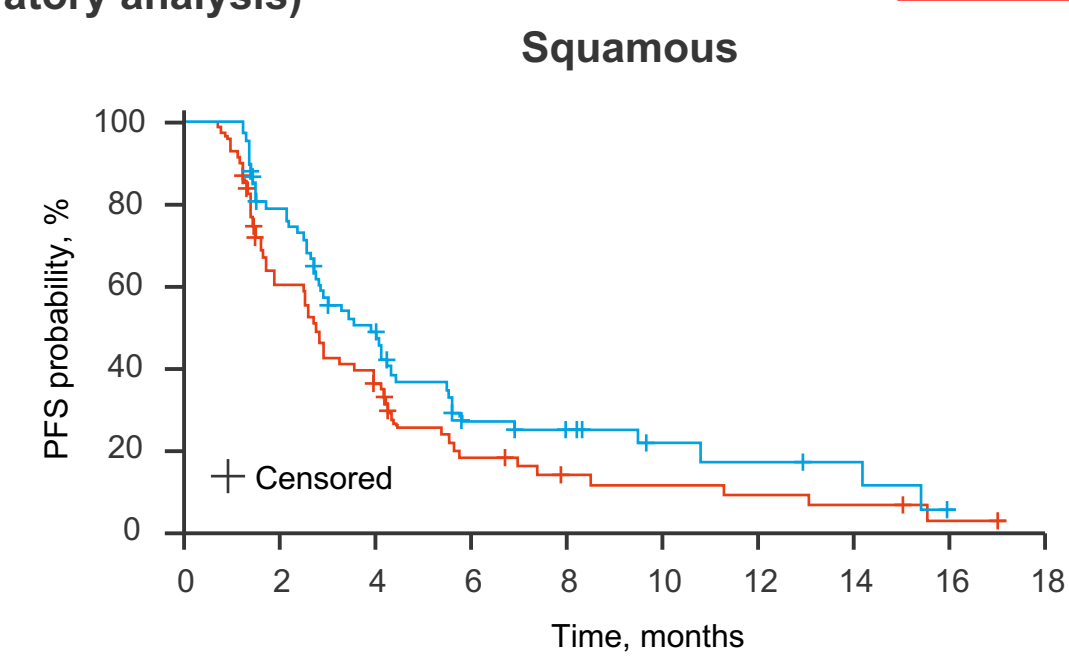
LBA12: Datopotamab deruxtecan (Dato-DXd) vs docetaxel in previously treated advanced/metastatic (adv/met) non-small cell lung cancer (NSCLC): results of the randomized phase 3 study TROPION-Lung01 – Lisberg AE, et al

PFS

- **Key results (cont.)**

PFS by histology (exploratory analysis)





No. at risk

Dato-DXd	229	178	134	86	68	41	20	7	1	0
Docetaxel	232	135	90	50	32	14	10	4	0	0

	Dato-DXd	Docetaxel
mPFS, mo (95%CI)	5.6 (4.4, 7.0)	3.7 (2.9, 4.2)
HR (95%CI)	0.63 (0.51, 0.78)	
ORR, %	31.2	12.8
DoR, mo	7.7	5.6

No. at risk

Dato-DXd	70	38	22	10	6	5	4	3	1	0
Docetaxel	73	51	30	13	10	5	4	3	0	0

	Dato-DXd	Docetaxel
mPFS, mo (95%CI)	2.8 (1.9, 4.0)	3.9 (2.8, 4.5)
HR (95%CI)	1.38 (0.94, 2.02)	
ORR, %	9.2	12.7
DoR, mo	5.9	8.1

Lisberg AE, et al. Ann Oncol 2023;34(suppl):Abstr LBA12

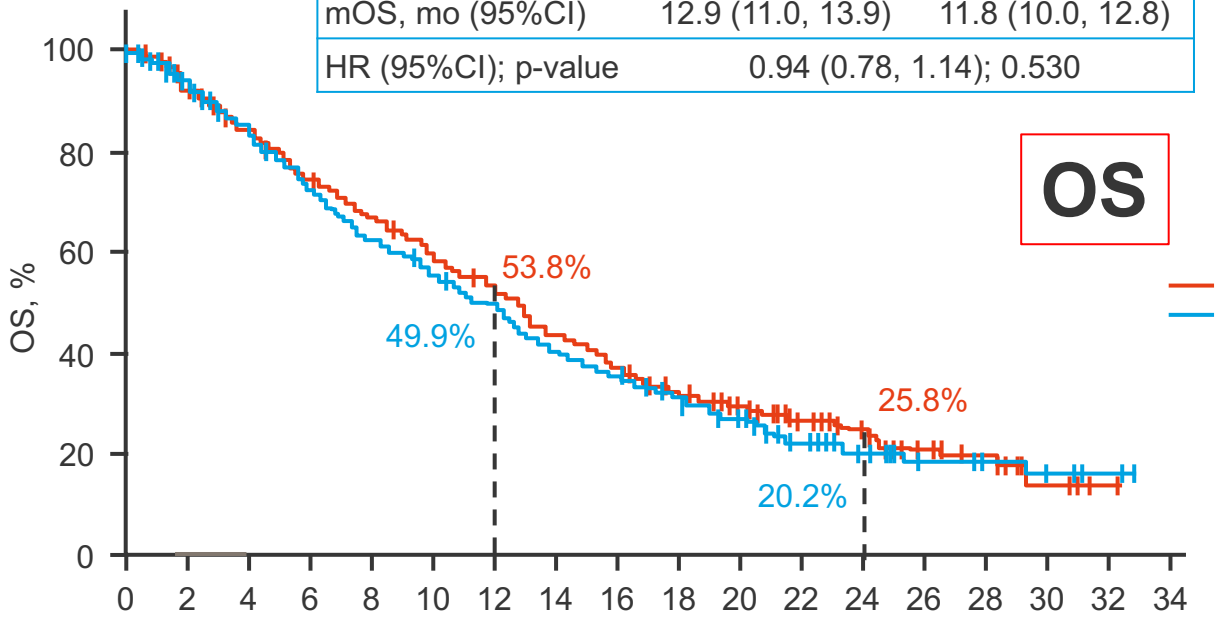
OA08.03: Datopotamab Deruxtecan Vs Docetaxel in Patients with Non-Small Cell Lung Cancer: Final Overall Survival from TROPION-Lung01 – Sands J, et al

- Key results

Overall survival

	Dato-DXd (n=299)	Docetaxel (n=305)
mOS, mo (95%CI)	12.9 (11.0, 13.9)	11.8 (10.0, 12.8)
HR (95%CI); p-value	0.94 (0.78, 1.14); 0.530	

OS

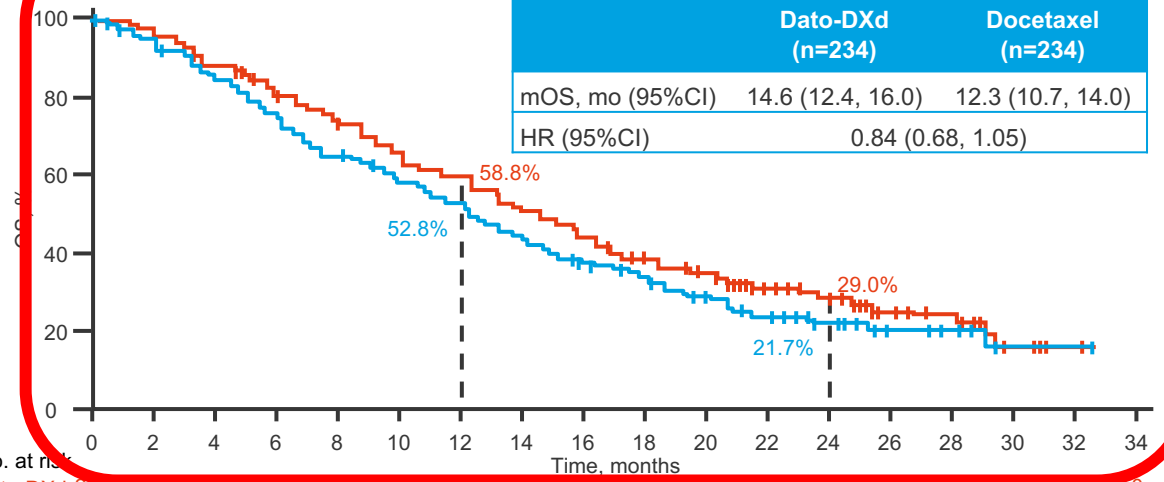


No. at risk

Dato-DXd	299	272	242	213	190	168	151	124	106	84	71	51	35	22	16	5	1	0
Docetaxel	305	273	239	205	175	157	138	112	98	81	63	41	26	15	11	4	2	0

Nonsquamous

	Dato-DXd (n=234)	Docetaxel (n=234)
mOS, mo (95%CI)	14.6 (12.4, 16.0)	12.3 (10.7, 14.0)
HR (95%CI)	0.84 (0.68, 1.05)	

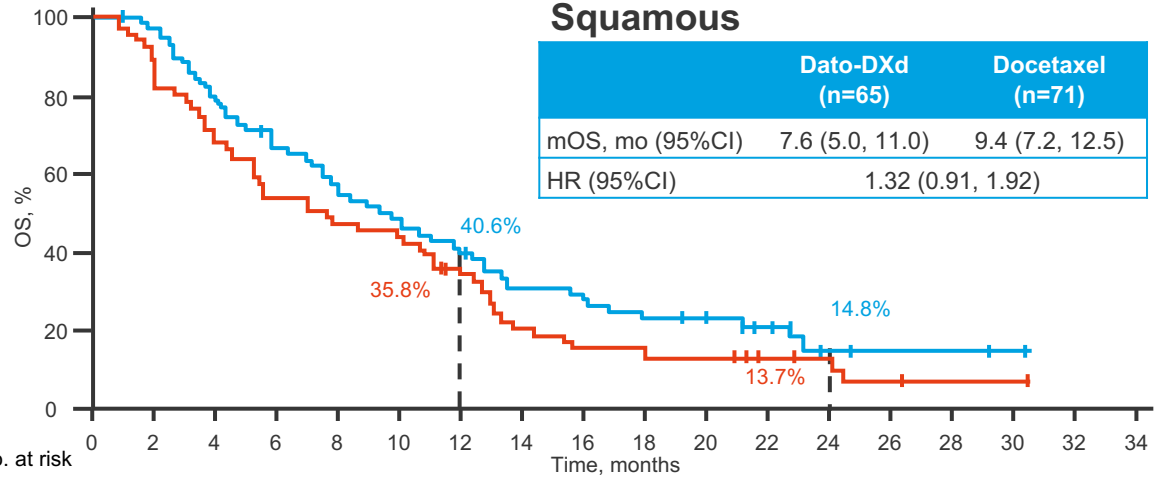


No. at risk

Dato-DXd	234	220	206	186	161	139	125	111	92	79	66	50	32	22	12	8	3	2	0
Docetaxel	234	206	186	161	139	125	111	92	79	66	50	32	22	12	8	3	2	0	

Squamous

	Dato-DXd (n=65)	Docetaxel (n=71)
mOS, mo (95%CI)	7.6 (5.0, 11.0)	9.4 (7.2, 12.5)
HR (95%CI)	1.32 (0.91, 1.92)	



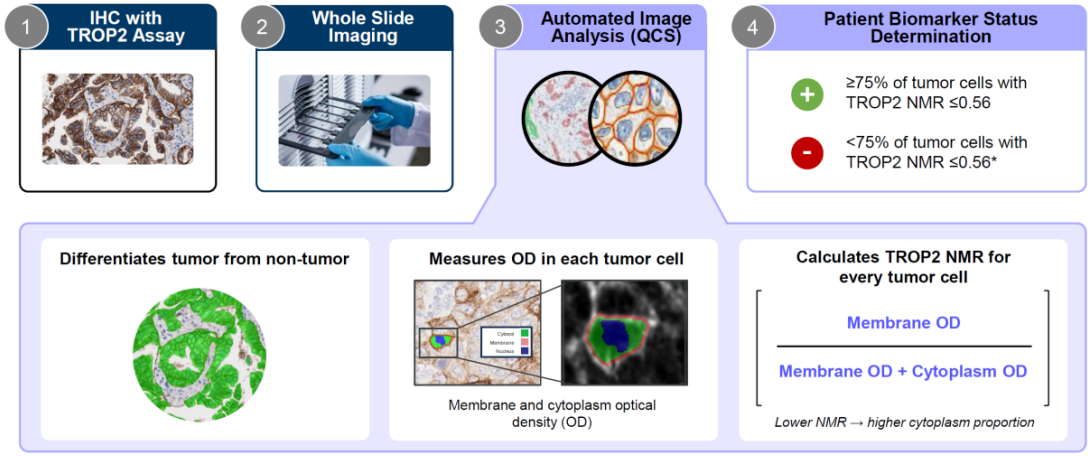
No. at risk

Dato-DXd	65	52	42	33	29	27	21	12	9	8	8	5	4	2	1	1	0	0
Docetaxel	71	67	53	44	36	32	27	20	19	15	13	9	4	3	3	1	0	0

New methods of IHC quantification

TROP2 Normalized Membrane Ratio (NMR) measured by Quantitative Continuous Scoring (QCS)

QCS is a novel, fully-supervised computational pathology approach that precisely quantifies and locates targets like TROP2

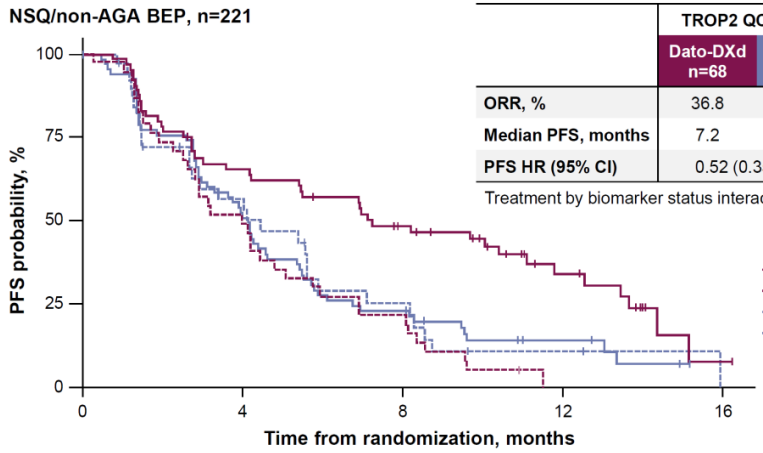


Dr Marina Chiara Garassino | Normalized Membrane Ratio of TROP2 by Quantitative Continuous Scoring is Predictive of Clinical Outcomes in TROPION-Lung01

OD, optical density (a measure of staining intensity).
*Or >25% of cells with an NMR >0.56

NSQ/non-AGA BEP: Efficacy by TROP2 QCS-NMR Status

TROP2 QCS-NMR positivity is predictive for longer PFS with Dato-DXd in the NSQ/non-AGA biomarker-evaluable population



	TROP2 QCS-NMR+		TROP2 QCS-NMR–	
	Dato-DXd n=68	Docetaxel n=72	Dato-DXd n=40	Docetaxel n=41
ORR, %	36.8	15.3	22.5	12.2
Median PFS, months	7.2	4.1	4.0	4.4
PFS HR (95% CI)	0.52 (0.35–0.78)		1.22 (0.74–2.00)	

Treatment by biomarker status interaction: p=0.0098

Dr Marina Chiara Garassino | Normalized Membrane Ratio of TROP2 by Quantitative Continuous Scoring is Predictive of Clinical Outcomes in TROPION-Lung01

Data cutoff: March 29 2023
PFS HR (95% CI) by TROP2 QCS-NMR status (+ vs -) within treatment: Dato-DXd: 0.40 [0.25-0.64]; Docetaxel:0.94 [0.60-1.49]

TROP2 ADC

ADC, antibody-drug conjugate; IHC, immunohistochemistry.
Garassino MC, et al. Presented at: IASLC 2024 WCLC [Abstract PL02.11].

Why might the IHC biomarker ‘not work’ for ADCs?

- Trials designed with no dynamic range of biomarker expression
  - HER2, HER3, TROP2
- Even the smallest amount of target is enough to have cell killing
  - Possibly below limit of detection of standard IHC
- Bystander effect offsets any difference in target expression
  - Dependant on the hydrophobic properties of the payload
  - Also related to DAR?
- Other active primary resistance mechanisms

Giugliano F et al. Curr Oncol Rep 2022
Garcia-Alonso S et al. Cancer Res 2018

Why might the IHC biomarker ‘not work’ for ADCs?

- The IHC assay used was poor
- The IHC worked but sensitivity not high enough
- IHC assays not truly quantitative
  - Fluorescent IHC might give better assessment
- The IHC assay identified a different epitope to the Ab in the drug
- Receptor ligands might interfere with drug binding to target

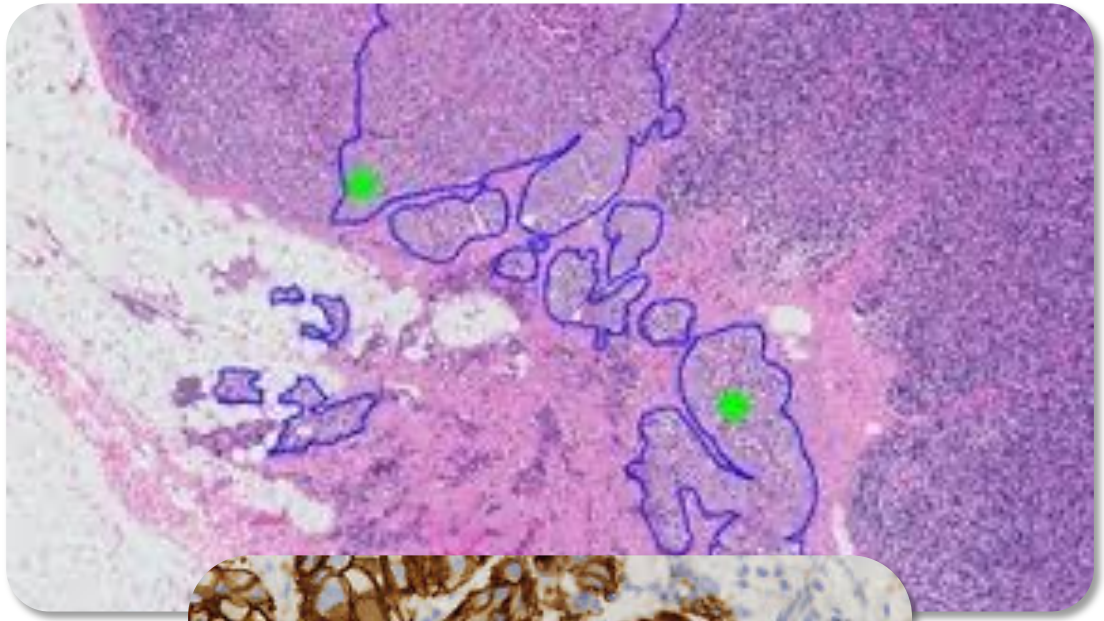
Issues in play with IHC biomarkers

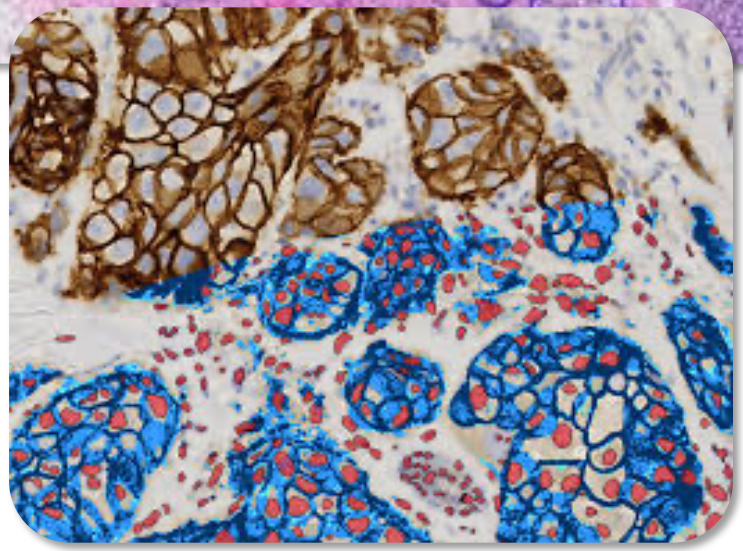
- Different definitions of ‘high’ or ‘positive’ expression
- Potential for different assays to be used
  - Also Companion Dx versus Laboratory developed test (LDT)
- Samples, pre-analytics, assessment
- 2L indication and timing of biopsy?

- Has enough work been done to reject the IHC biomarker?
- Is there actually interest in having the marker?
  - Is the marker needed?

} IHC for ADCs

Changing how we do things – digital pathology





Digital image analysis and multiplex IHC will become routine… but the possible permutations will be enormous

Analysis of heterogeneity

Tumour microenvironment

IHC, immunohistochemistry.

Words of caution

"There is nothing more dangerous (or expensive) than… making diagnoses on the basis of immunohistochemical profiles in disregard of the cytoarchitectural features of the lesion."

"Alas, this is true for any other special technique applied for diagnostic purposes to human tissue, molecular biology being the latest and most blatant example"

Rosai J. Theranostic and genomic applications. In: Dabbs DJ (ed). *Diagnostic Immunohistochemistry*. Elsevier; 2010.

Immunohistochemistry in lung cancer: a forgotten art?

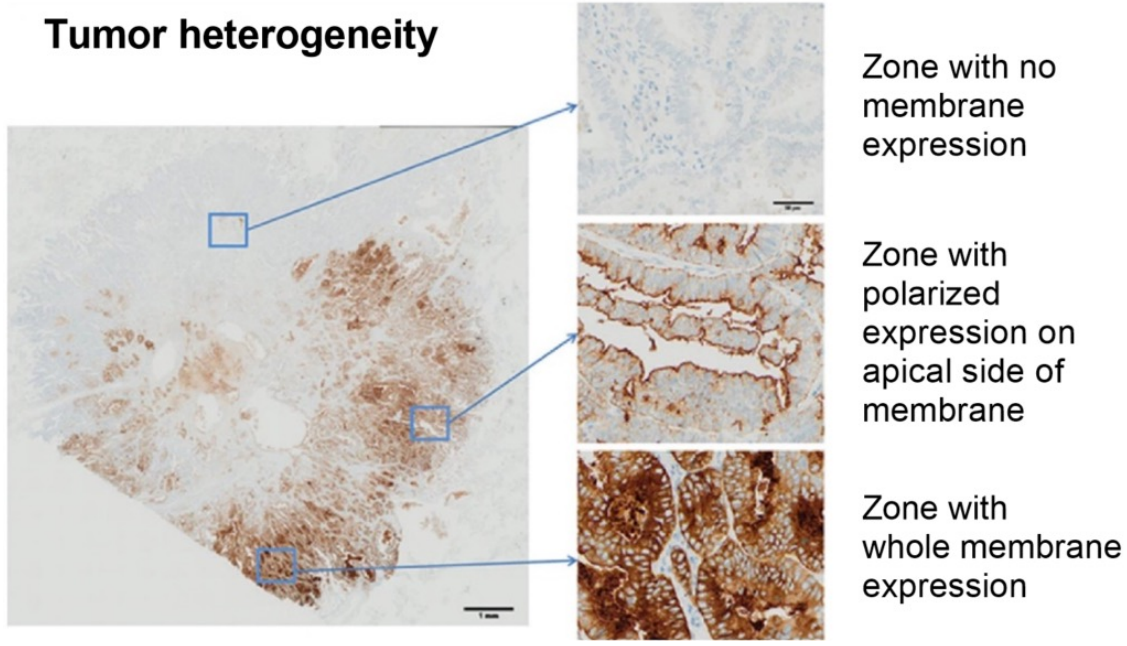
- Only forgotten by those who choose to forget, or never knew in the first place

- Hugely useful diagnostic tool
- Very much part of the diagnostic and biomarker landcape
- Set to become more important

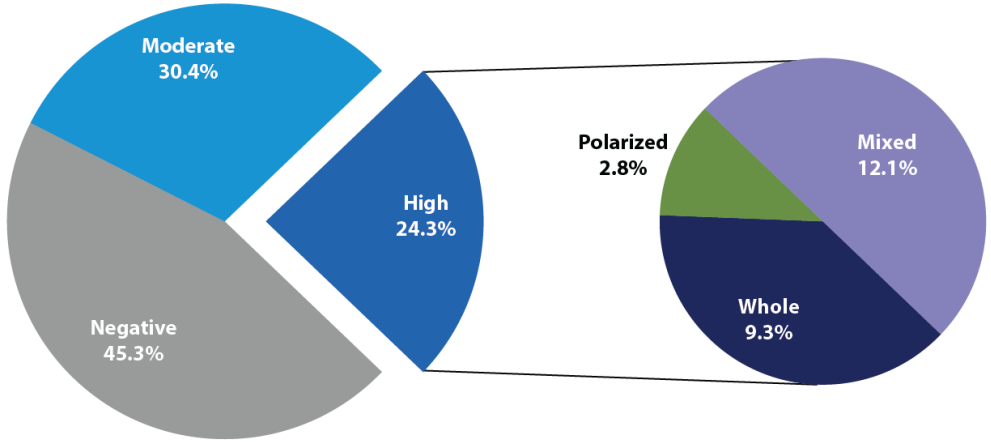
- Must be ‘Handled with Care’

Tusamitamab Ravtansine Anti-CEACAM5: CEACAM5 expression in NSQ NSCLC

CEACAM5 expression within NSQ NSCLC primary tumors



Prevalence of CEACAM5 expression in patients with NSQ NSCLC*



Intra-tumoral CEACAM5 expression was highly heterogeneous; therefore, it is recommended to use whole section formats for CEACAM5 IHC tumor assessment instead of tissue micro-arrays for prevalence/translational studies

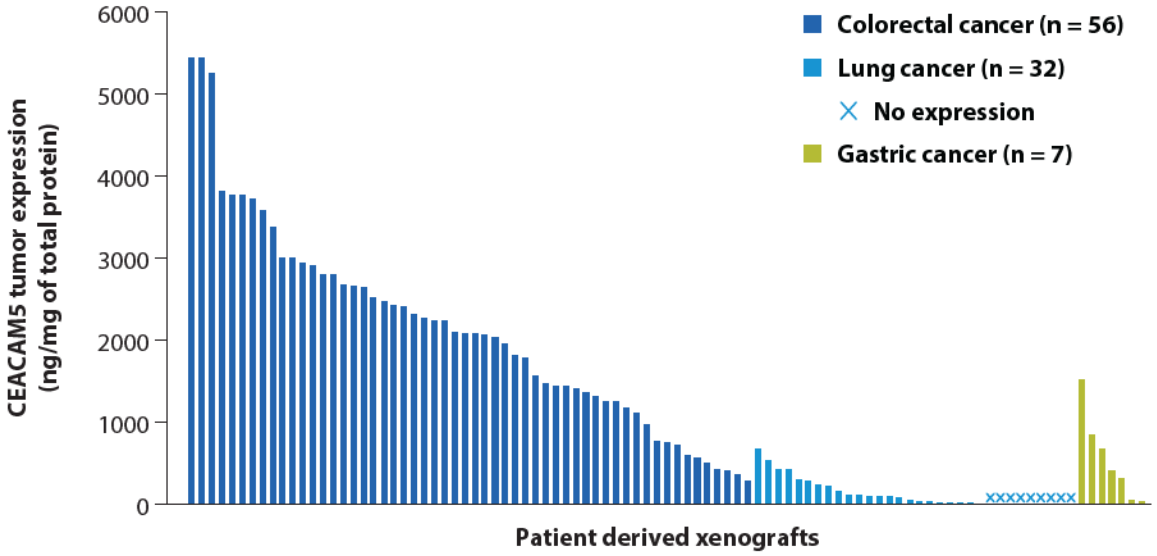
High expression: ≥50% of the tumor cells with CEACAM5-positive staining at ≥2+ intensity.

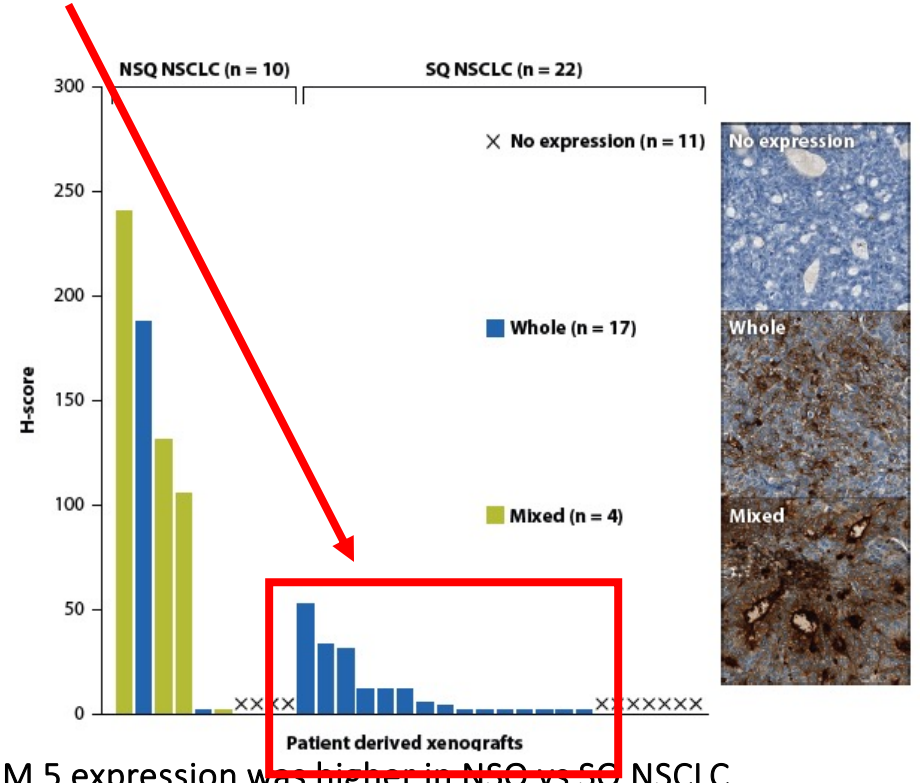
Moderate: >1% of tumor cells with CEACAM5 at +2 intensity.

Adam J, et al. Presented at: ESMO IO; Dec 8-11, 2021; Poster 19P.

Slide Courtesy of Max Schenk, Sanofi

Tusamitamab Ravtansine Anti-CEACAM5: Prevalence of CEACAM5 Expression is lower in Squamous Cell Carcinoma





CEACAM5 protein levels by ELISA were elevated to a lesser extent in lung versus colon PDX models

- Median values were 2071, 35, and 538 ng/mg of total protein for colon, lung, and gastric cancer PDX models, respectively.

CEACAM 5 expression was higher in NSQ vs SQ NSCLC

- Among 32 lung PDX models, 22 were SQ NSCLC and 10 were NSQ NSCLC (8 adenocarcinomas, 2 large-cell carcinomas)

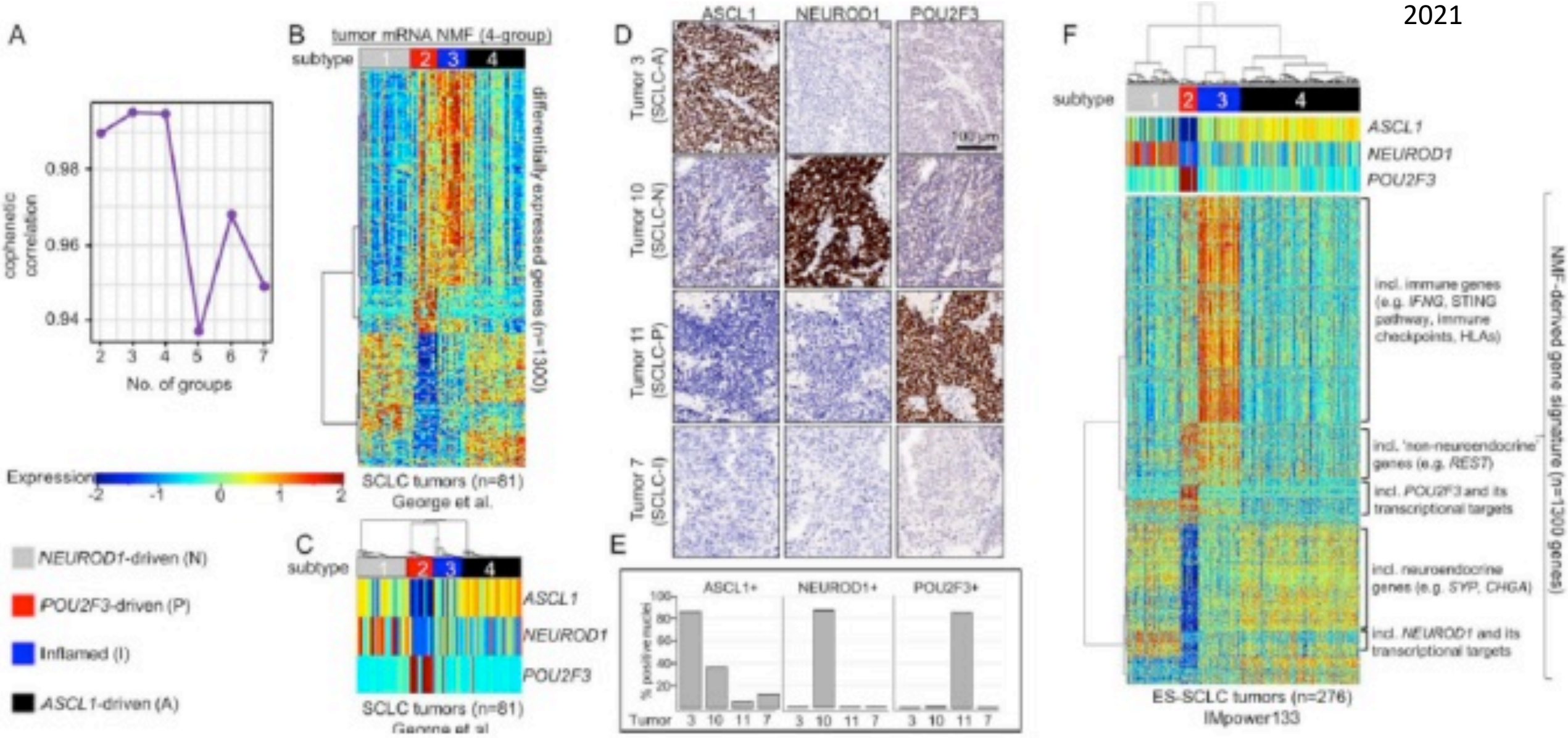
CEACAM5, carcinoembryonic antigen-related cell adhesion molecule 5; ELISA, enzyme-linked immunoassay; NSCLC, non-small cell lung cancer; NSQ, nonsquamous; PDX, patient derived xenograft; SQ, squamous.

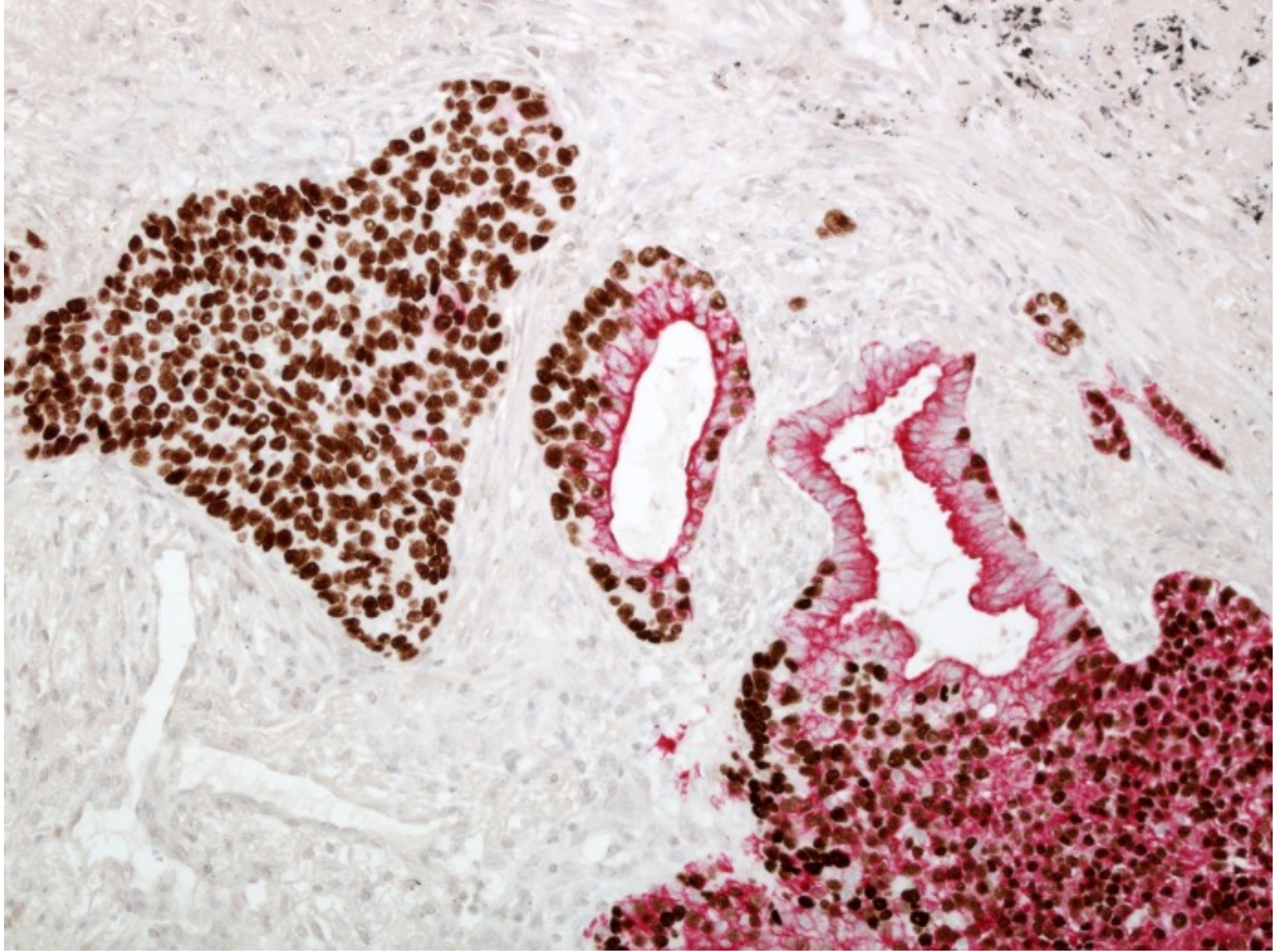
Adam J, et al. Presented at: ESMO IO; Dec 8-11, 2021; Poster 19P.

Slide Courtesy of Max Schenk, Sanofi

SCLC subtypes identified by Immunohistochemistry

Gay CM et al
Cancer Cell
2021





HER2 ‘high’ IHC positive NSCLC without mutation:

DESTINY Lung01

Table: 975P Efficacy and safety of T-DXd in pts with HER2-OE NSCLC

	Cohort 1 (6.4 mg/kg) N = 49	Cohort 1a (5.4 mg/kg) N = 41
Efficacy		
ORR by ICR, % (95% CI)	26.5 (15.0-41.1)	34.1 (20.1-50.6)
Complete response	0	4.9
Partial response	26.5	29.3
Stable disease	42.9	43.9
Progressive disease	22.4	9.8
Non-evaluable	8.2	12.2
ORR for HER2 IHC 3+/IHC 2+, % (n/N)	20.0 (2/10)/ 28.2 (11/39)	52.9 (9/17)/ 20.8 (5/24)
DCR, % (95% CI)	69.4 (54.6-81.8)	78.0 (62.4-89.4)
DOR, median (95% CI), mo	5.8 (4.3-NE)	6.2 (4.2-9.8)
Safety, %		
Drug-related TEAE	89.8	92.7
TEAE associated with drug discontinuation/dose reduction	26.5/36.7	17.1/17.1
TEAE associated with drug interruption	49.0	24.4
ILD, any G/G≥3	20.4/6.1	4.9/2.4

- ORR %
  - IHC 3+ 20% and 52.9%
  - IHC 2+ 28.2% and 20.8%
- ‘High’ HER2 expression
- Do we know what happens in patients with no or low HER2 expression?

Smit EF et al

ABSTRACT| VOLUME 33, SUPPLEMENT 7, S994-S995, SEPTEMBER 2022

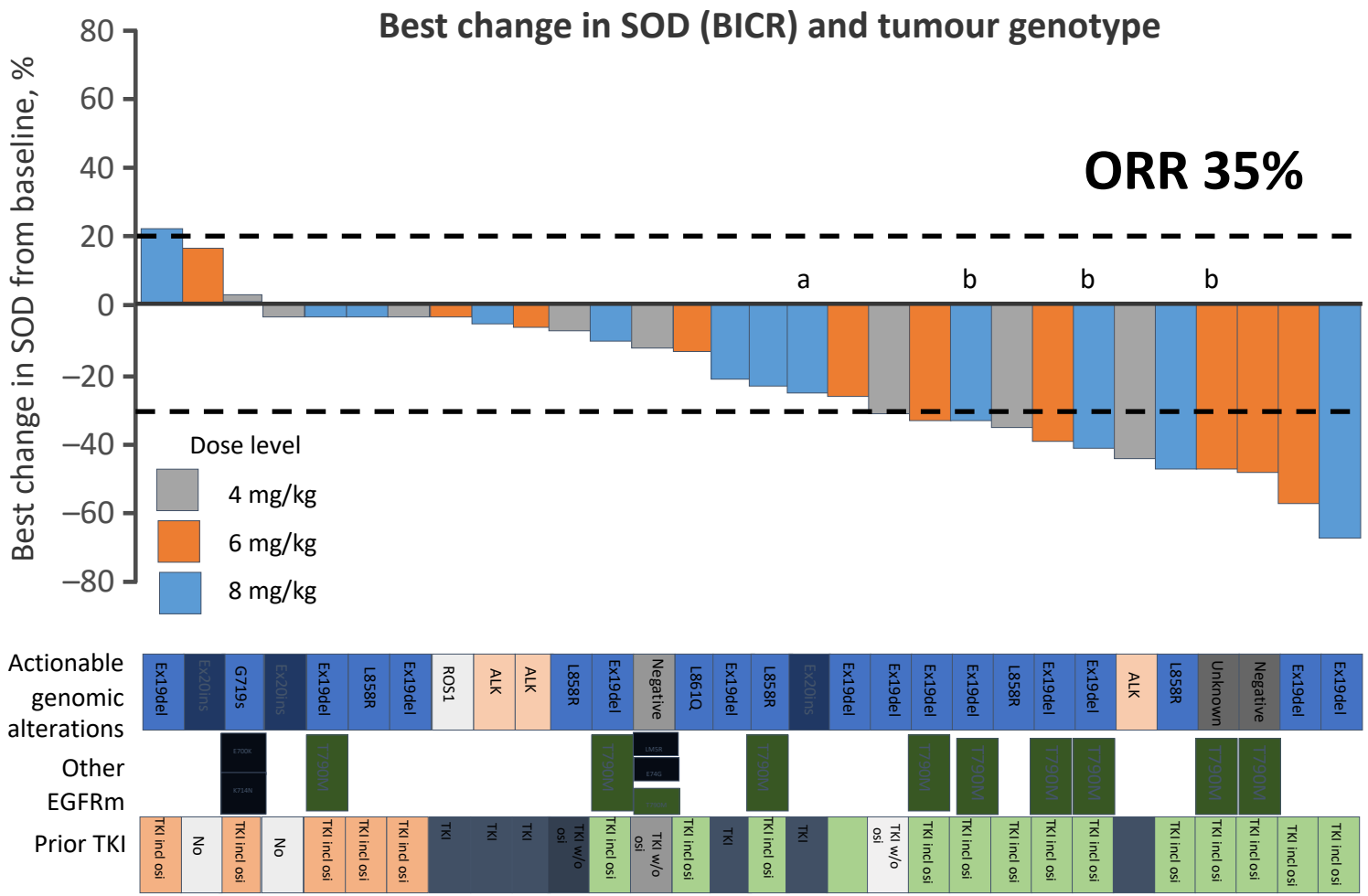
ESMO 2022

Efficacy of datopotamab deruxtecan (Dato-DXd) in patients (pts) with advanced/metastatic (adv/met) **non-small cell lung cancer (NSCLC) and actionable genomic alterations (AGAs):** preliminary results from the phase 1 TROPION-PanTumor01 study

- **Key results**

Patients	Data-Dxd (n=34)
ORR, n (%)	12 (35)
BOR, n (%)	
CR	0
PR	12 (35)
SD	14 (41)
Non-CR/PD	2 (6)
PD	2 (6)
NE	4 (12)
mDoR, mo (95%CI)	9.5 (3.3, NE)

No TROP2 IHC data



[a]Pateint NE; [b]patients with unconfirmed PR

Garon EB, et al. Ann Oncol 2021;32(suppl):Abstr LBA49

Tusamitamab Ravtansine Anti-CEACAM5: Best Overall Response in NSQ-NSCC

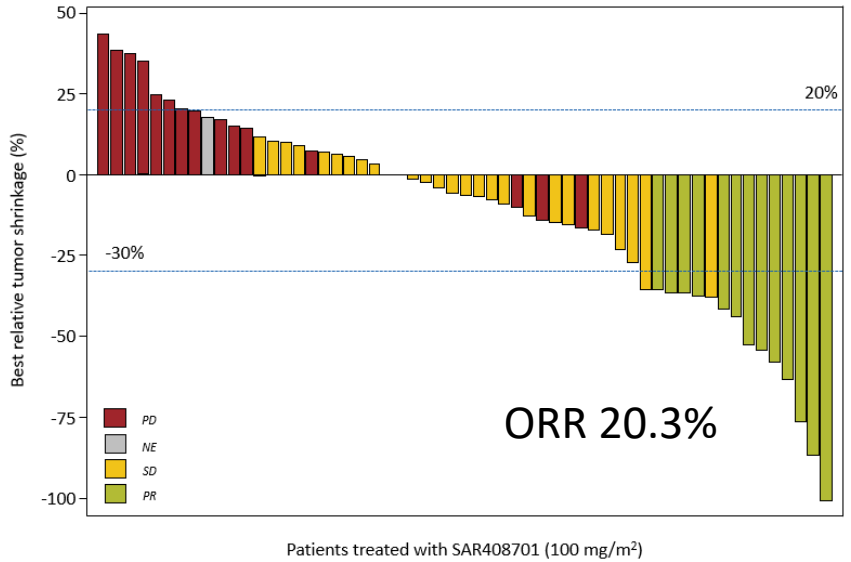
Overall Population

Response, n (%)	High expressors (n = 64)	Moderate expressors (n = 28)
ORR [95% CI]	13 (20.3%) [12.27-31.71]	2 (7.1%) [1.98-22.65]
Confirmed PR	13 (20.3%)	2 (7.1%)
SD	28 (43.8%)	15 (53.6%)
DCR	41 (64.1%)	17 (60.7%)
PD	21 (32.8%)	10 (35.7%)
NE	2 (3.1%)	1 (3.6%)

Best relative tumor shrinkage: Patients who had unconfirmed PR (>30% decrease) were counted as SD for BOR
BOR, best overall response; CI, confidence interval; DCR, disease control rate; NE, not evaluable; ORR, overall response rate; PD, progressive disease; PR, partial response; SD, stable disease.

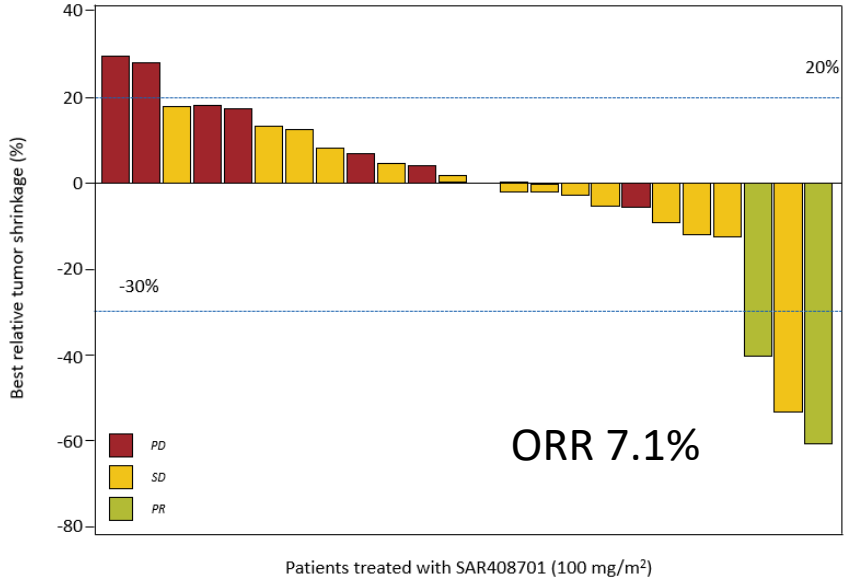
Best Relative Tumor Shrinkage – High Expressor Cohort

High Expressors



Best Relative Tumor Shrinkage – Moderate Expressor Cohort

Moderate Expressors



Slide Courtesy of Max Schenk, Sanofi

1+ 2+ 3+

Idea stolen from Dr Lukas Bubendorf, Basel ☺

H-score= (%1+x1)+(%2+x2)+(%3+x3)
Max possible score 100%x3 = 300

This will also place a premium on IHC standardisation, consistency and EQA

‘Standard’ bright-field IHC techniques do not necessarily relate colour intensity to epitope concentration

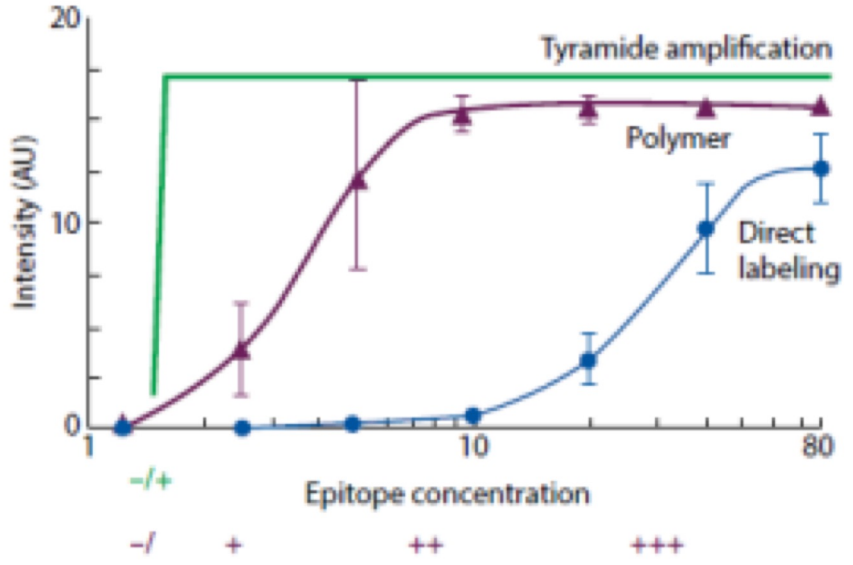


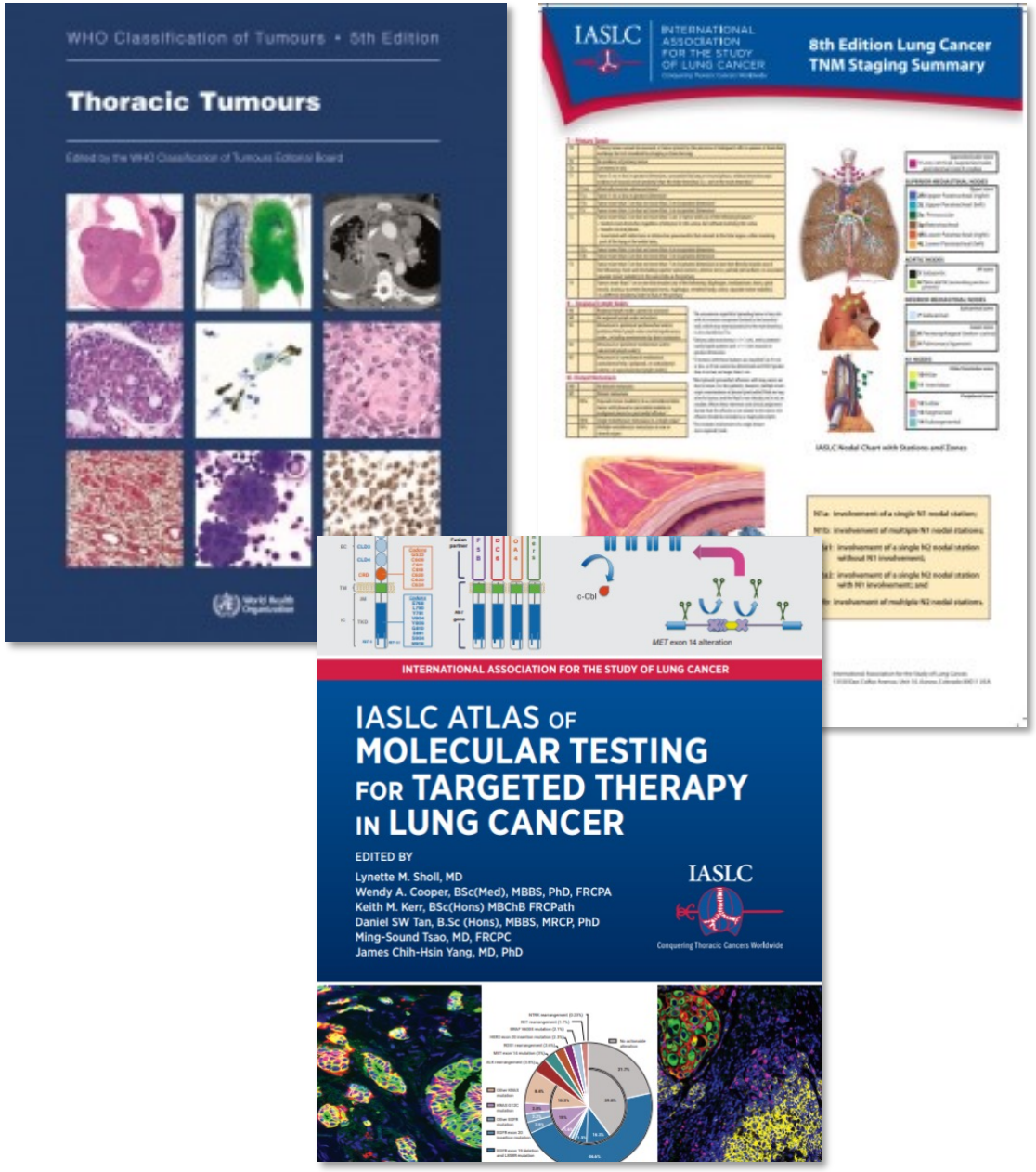
Figure 3-1. Relation between epitope concentration and signal enhancement in immunohistochemistry (IHC). AU = arbitrary unit. (Modified with permission from Prinsen et al 2003)

AU, arbitrary units; EQA, external quality assessment; IHC, immunohistochemistry.

1. World Health Organization. WHO Classification of Tumours Editorial Board. Thoracic Tumours. 5th ed. Lyon, France: International Agency for Research on Cancer; 2021; 2. Yatabe Y, et al. *J Thorac Oncol*. 2019;14(3):377-407.

Lung cancer diagnostics

- Diagnosis (identification) and classification of disease
- Prognostication
  - Anatomical location (stage)
  - Histopathology (morphology/microanatomy)
  - Molecular features
- Prediction of response to therapy
- Monitoring response to therapy



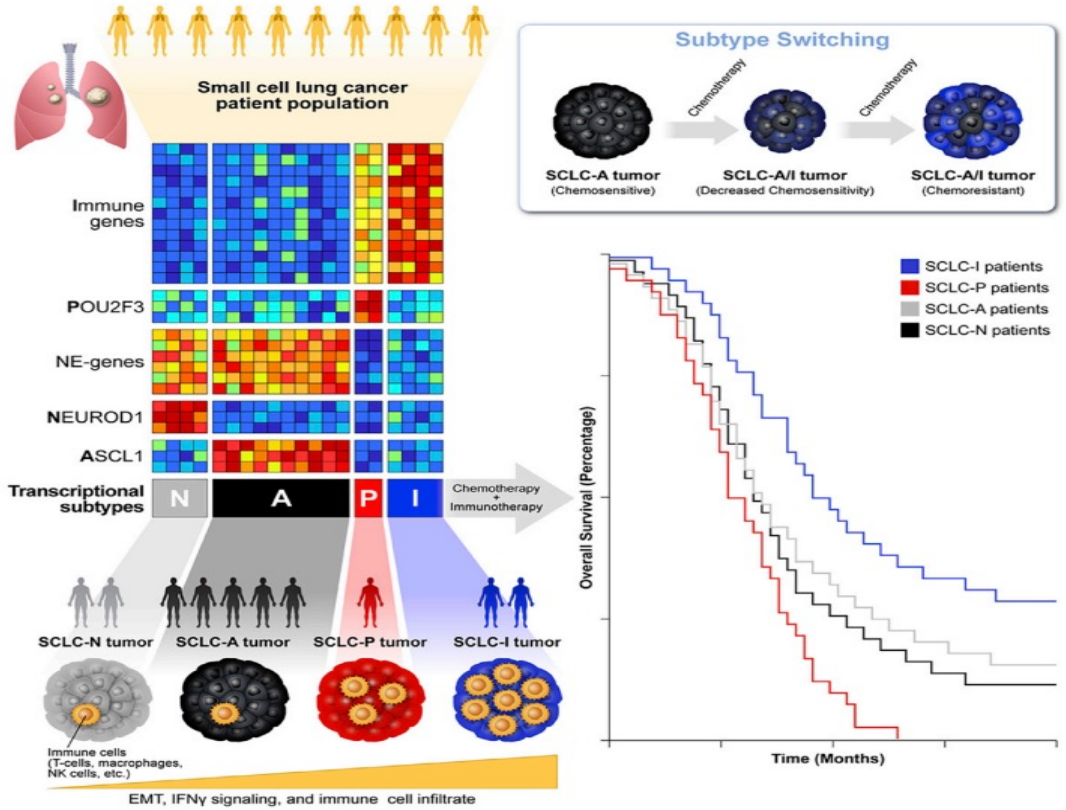
World Health Organization. WHO Classification of Tumours Editorial Board. *Thoracic Tumours.* 5th ed. Lyon, France: International Agency for Research on Cancer; 2021;
IASLC Atlas of Molecular Testing for Targeted Therapy in Lung Cancer. Published 2023. Accessed 16 October 2024. https://www.iaslc.org/iaslc-atlas-molecular-testing-targeted-therapy-lung-cancer

Disclosures

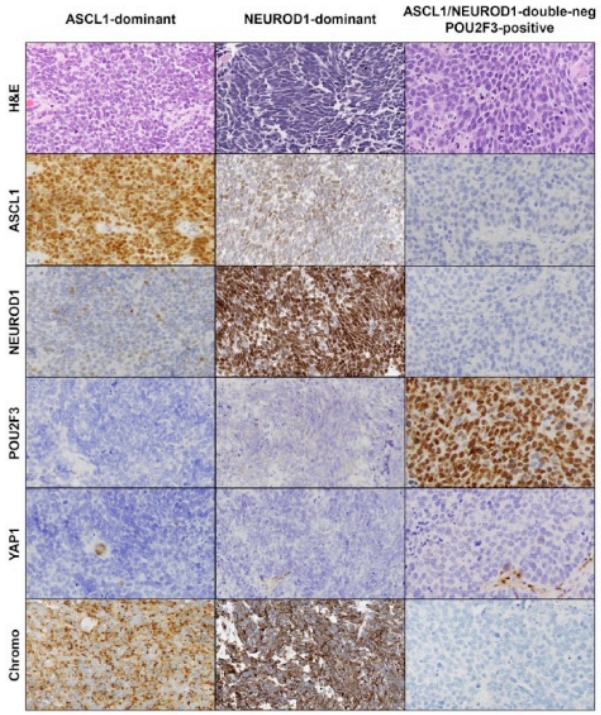
- **Consultancy**
  - AbbVie, Amgen, AstraZeneca, Bayer, Boehringer Ingelheim, Bristol-Myers Squibb, Daiichi Sankyo, Debiopharm, Diaceutics, Eli Lilly, Merck Serono, Merck Sharp & Dohme, Novartis, Pfizer, Regeneron, Roche, Roche Diagnostics/Ventana, Sanofi
- **Honoraria (speaker)**
  - AstraZeneca, Amgen, Boehringer Ingelheim, Bristol-Myers Squibb, Eli Lilly, Merck Serono, Merck Sharp & Dohme, Novartis, Pfizer, Roche, Roche Diagnostics/Ventana, Medscape, Prime Oncology

Small cell carcinoma of the lung

Four molecularly defined groups
Potential for differential sensitivity to new drugs



The four categories could be distinguished by IHC?



SCLC-A
ASCL1 IHC positive

SCLC-N
NEUROD1 IHC positive

SCLC-P
POU2F3 IHC positive

SCLC-I
IHC negative
Immune subtype

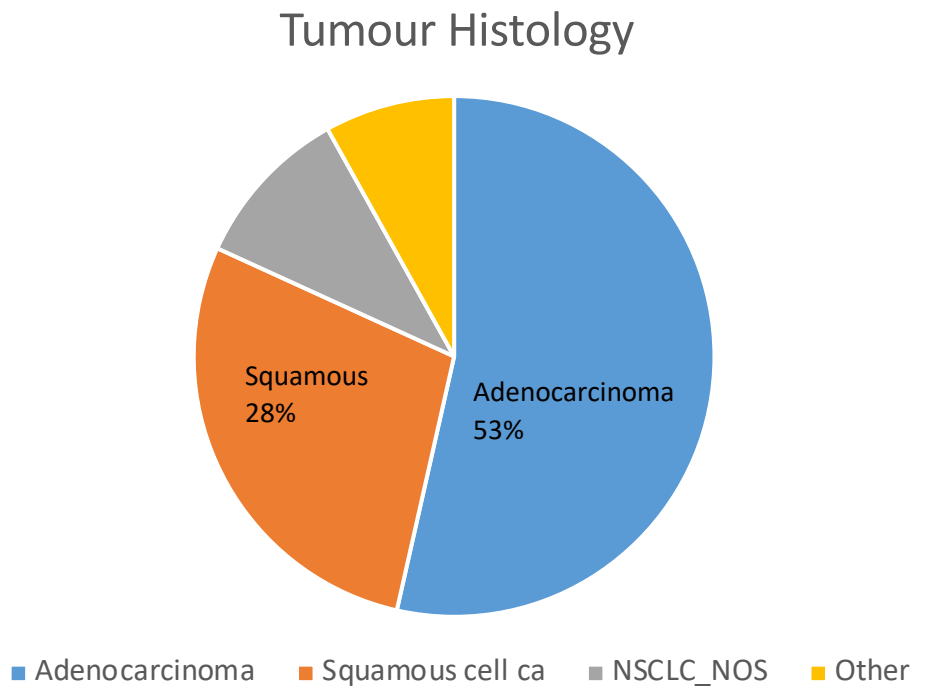
Triple negative SCLC ??

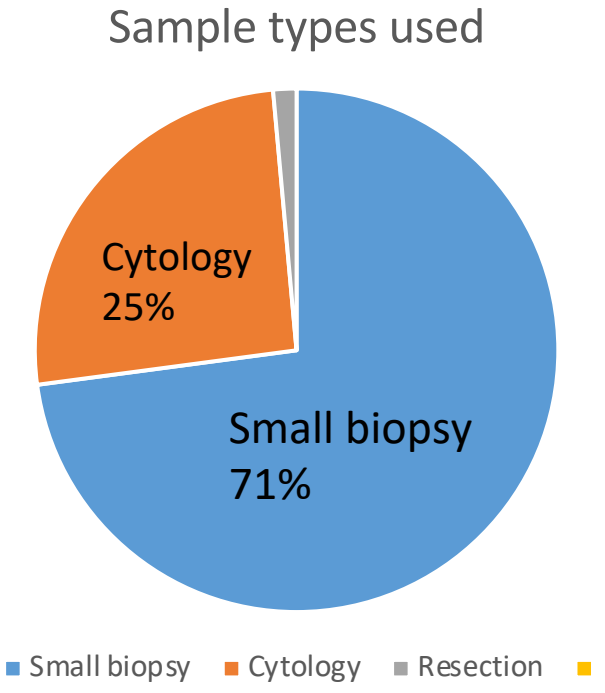
EMT, epithelial-to-mesenchymal transition; H&E, haematoxylin and eosin; IHC, immunohistochemistry; NK, natural killer; SCLC, small cell lung cancer.

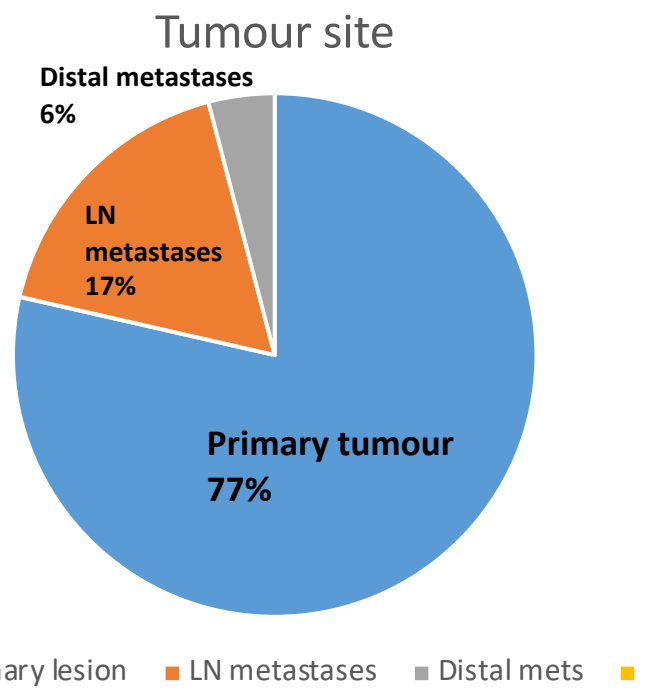
Gay CM, et al. *Cancer Cell.* 2021;39(3):346-360.e7.

Baine MK et al. *J Thorac Oncol*. 2020;15(12):1823-1835.

My first 1000 PD-L1 cases……







- **Sample inadequacy**, based on 100 cell threshold: **9%**
  - Inadequate biopsy samples 6%
  - Inadequate cytology samples 12% (identical data from Blueprint 2B study)