Optimizing the Biomarker Testing for NSCLC

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Stock shareholder:	None
Spouse / partner:	None
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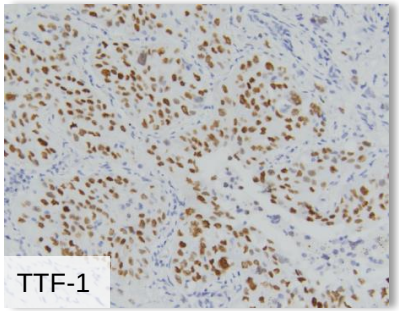
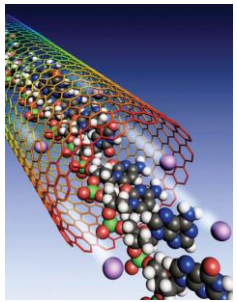
Biomarker Testing in NSCLC

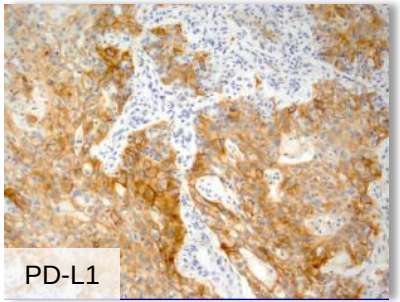
Diagnostic / subtyping



TTF-1, p40, etc.

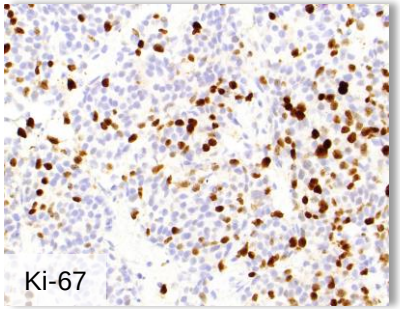
Predictive





NGS, FISH, PD-L1, etc.

Prognostic

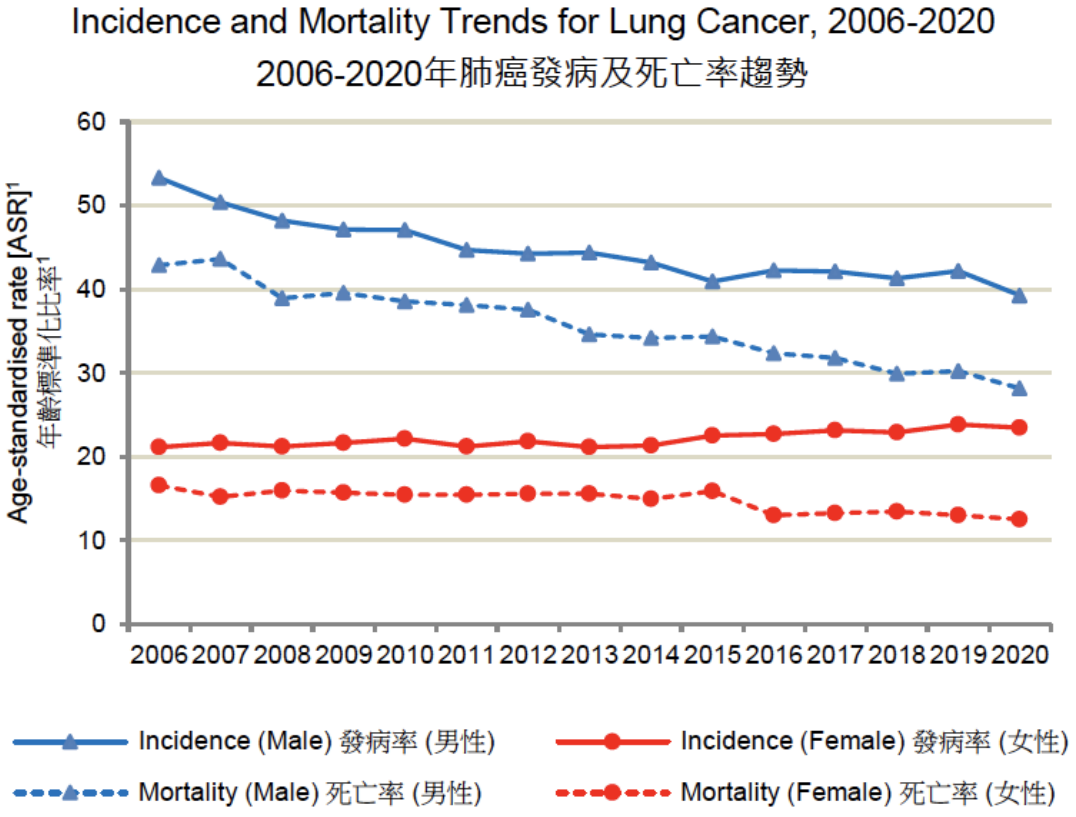


Ki-67, p53, etc.

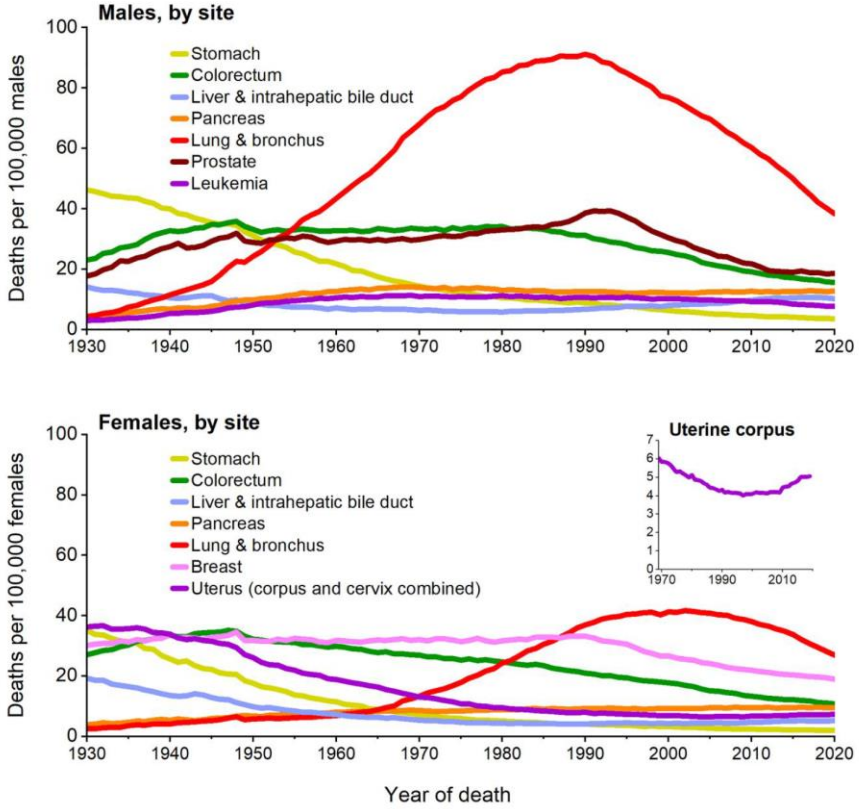
Outline

- Available targets (driver gene alterations and PD-L1) in first line treatment for NSCLC, their treatment options and clinical benefits
- A global perspective on predictive biomarker testing guidelines and practices
- Diagnostic and predictive biomarker testing with small biopsy / cytology samples and its practical approach
- PD-L1 IHC - analytical and pre-analytical issues

Improved Mortality from Lung Cancer in Hong Kong



Improved Mortality from Lung Cancer in US

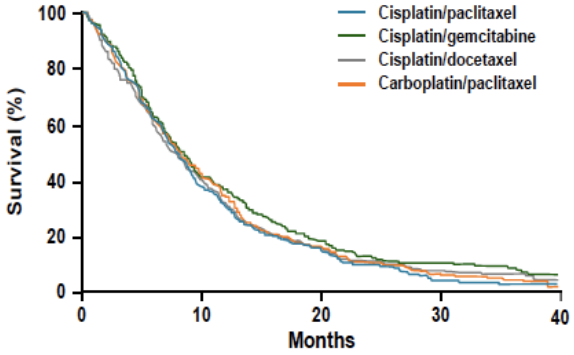


- Disease prevention
  - Tobacco cessation
  - Asbestos mitigation
- Early detection
  - Low-dose CT scan screening
- Improved management
  - Molecular targeted therapy
  - Immunotherapy
  - Neoadjuvant and adjuvant therapy for early-stage tumors

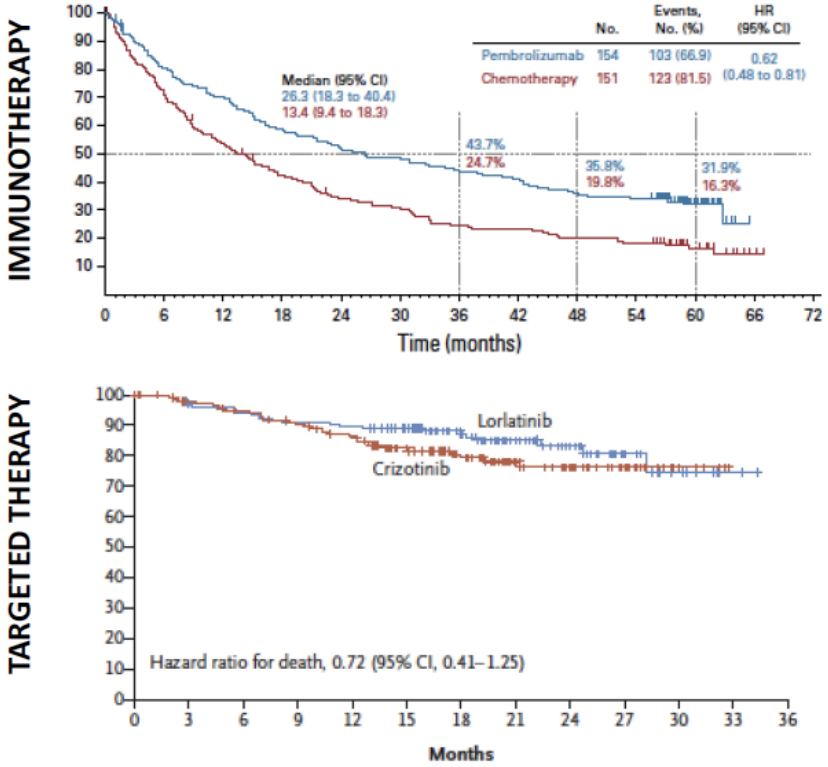
Siegel RL, at al. CA Cancer J Clin 2023;73:17-48

Treatment of Advanced NSCLC Then and Now

2002



2023

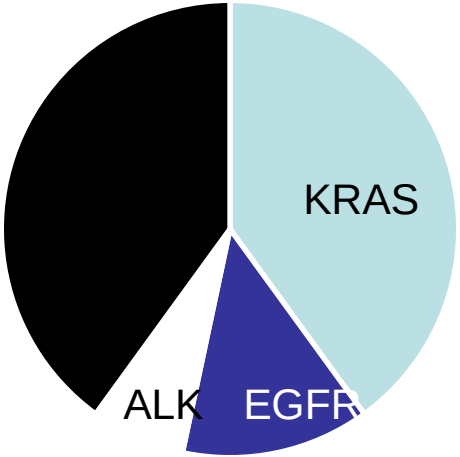


Schiller J, NEJM 2002
Reck M, JCO 2021.
Shaw AT, NEJM 2020.

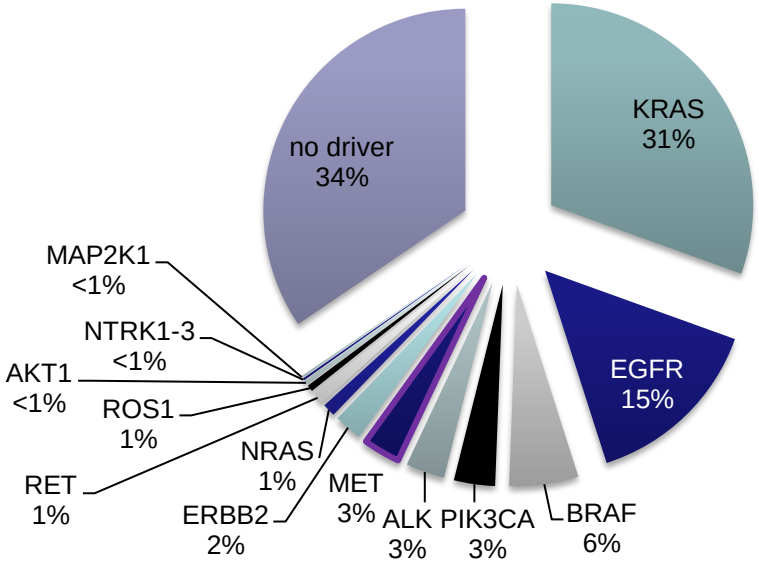
How did we get here?

Mutation Profiling in Lung Adenocarcinomas

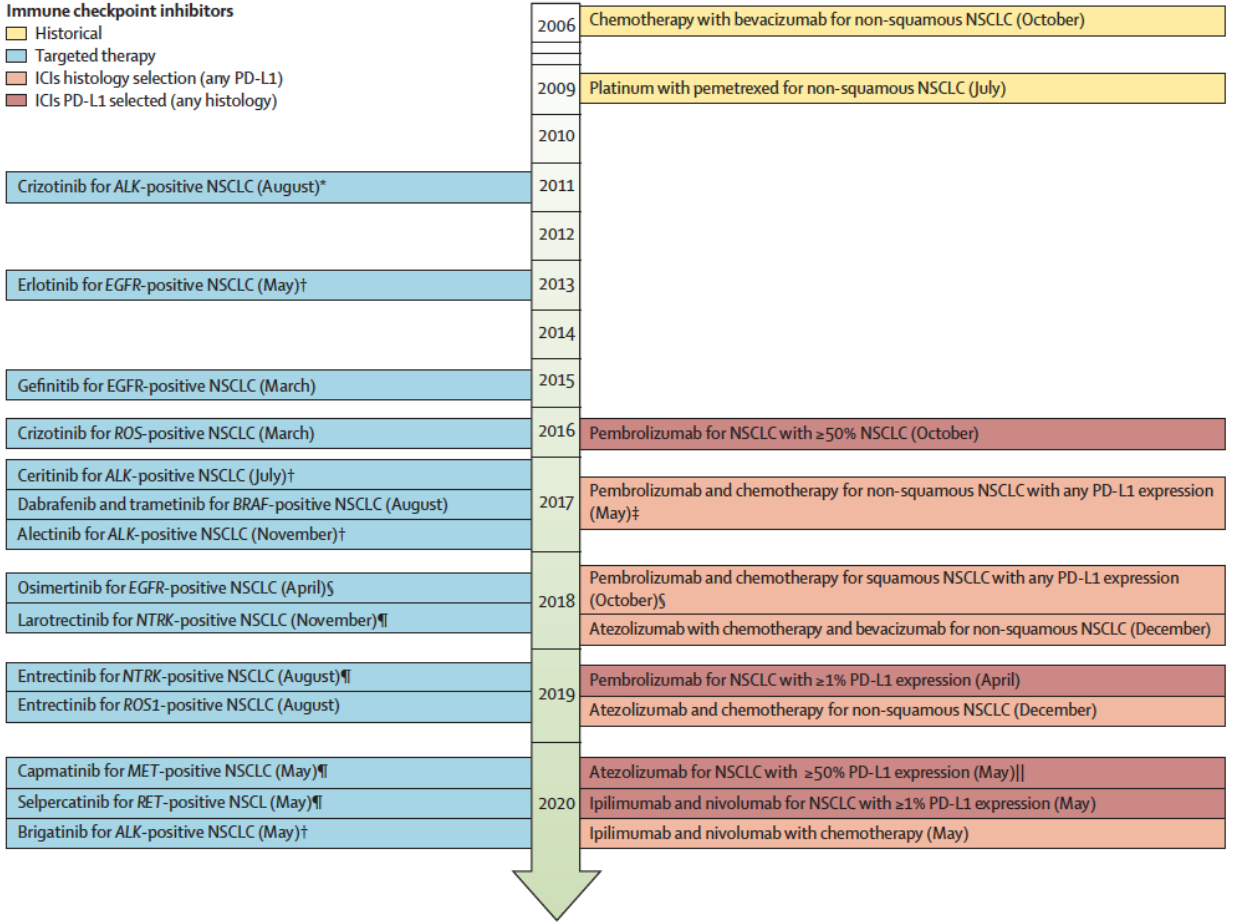
Early 2000's



2023



How did we get here?

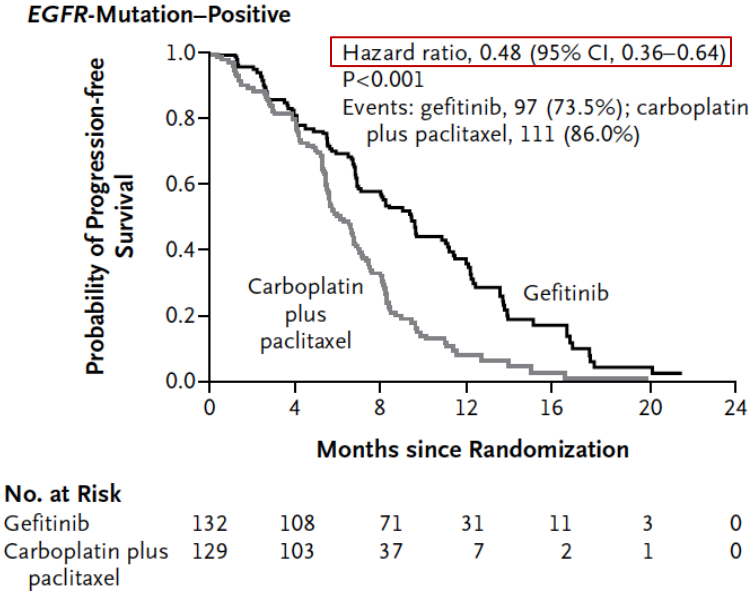


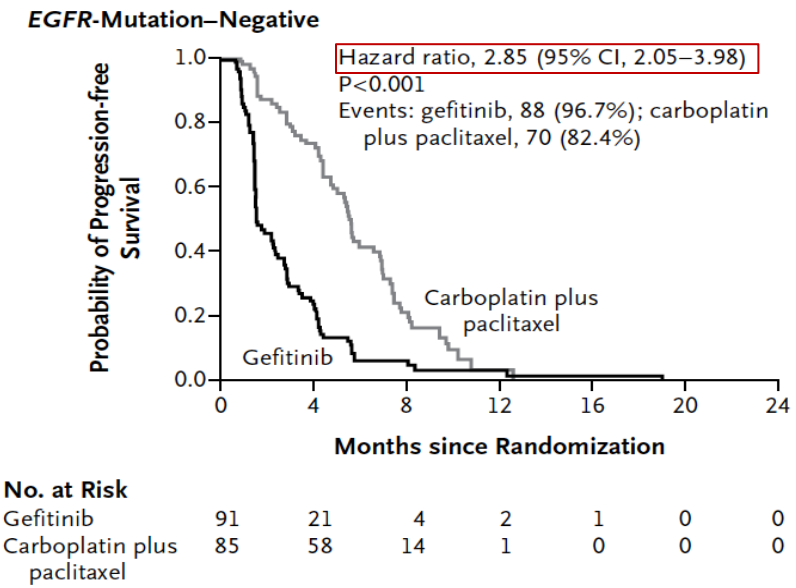
Timeline of selected US FDA drug approvals for patients with treatment-naive metastatic NSCLC

Thai AA, et al. Lancet 2021;398:535-54

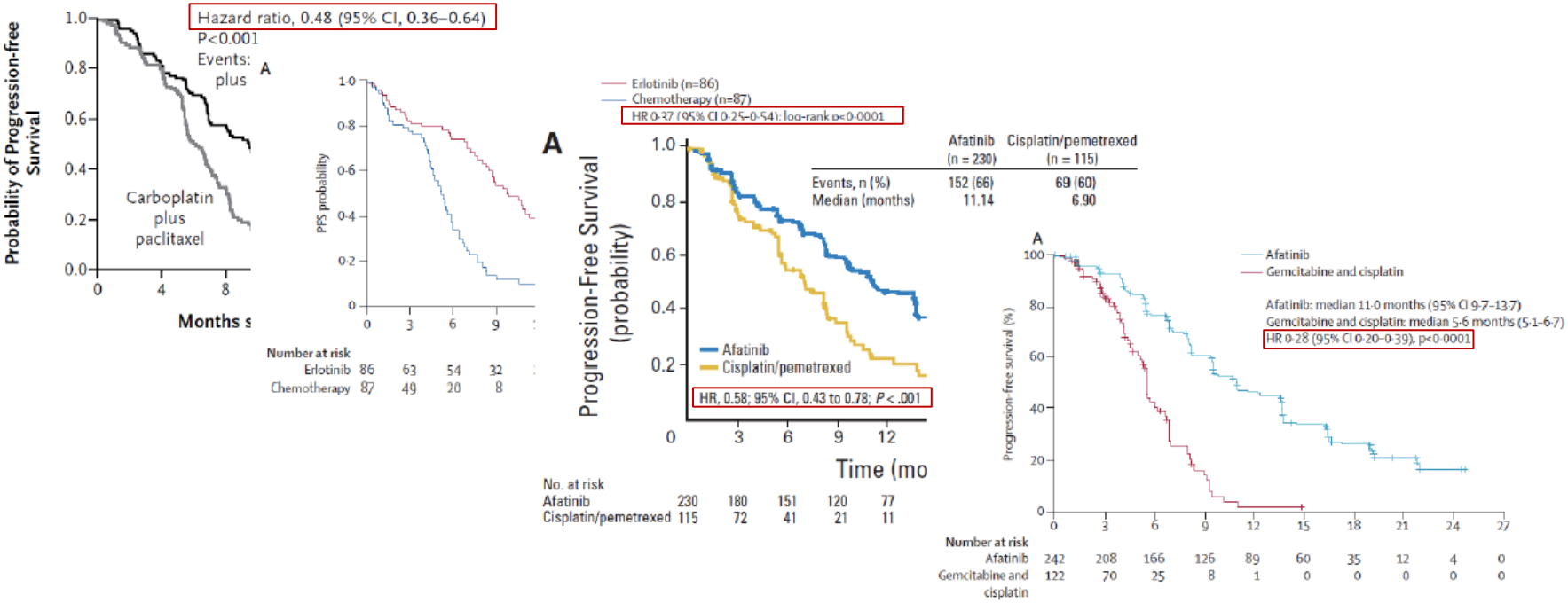
EGFR and Iressa Pan-Asian Study (IPASS)

East Asian patients with previously untreated, stage IIIB-IV lung adenocarcinoma never or former light smokers





1st/2nd Generation EGFR Inhibitors vs. Chemotherapy



Mok T, et al. NEJM 2009; Rosell R , et al. Lancet Oncol 2012; Sequist LV, et al. J Clin Oncol 2013; Wu YL, et al. Lancet Oncol 2014

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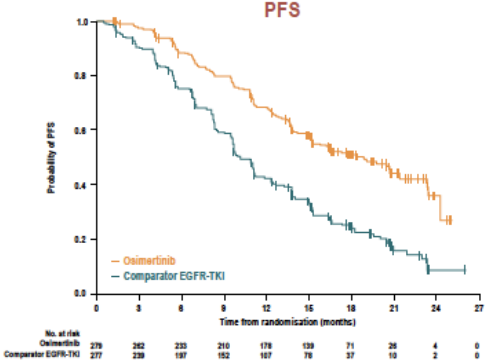
EGFR Mutant NSCLC

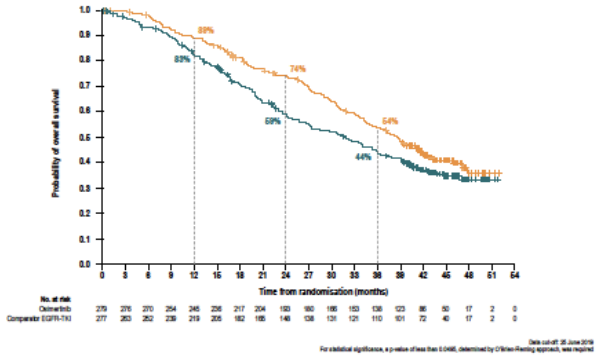
For patients with EGFR-mutant NSCLC, the FLAURA trial established osimertinib as the preferred first-line EGFR TKI.

PFS



	Median PFS, months (95% CI)	HR (95% CI)
Osimertinib	18.9 (15.2, 21.4)	0.46 (0.37, 0.57); p<0.001
Comparator EGFR-TKI	10.2 (9.6, 11.1)	

Overall Survival



	Median OS, months (95% CI)
– Osimertinib	38.6 (34.5, 41.8)
– Comparator EGFR-TKI	31.8 (26.6, 36.0)
HR (95.05% CI)	0.799 (0.641, 0.997); p=0.0462

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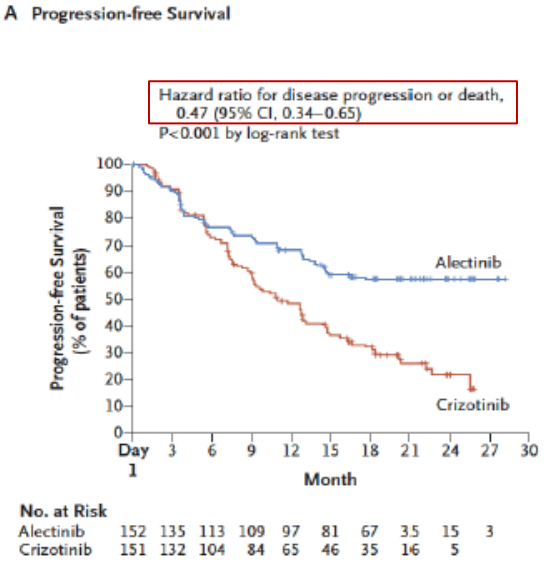
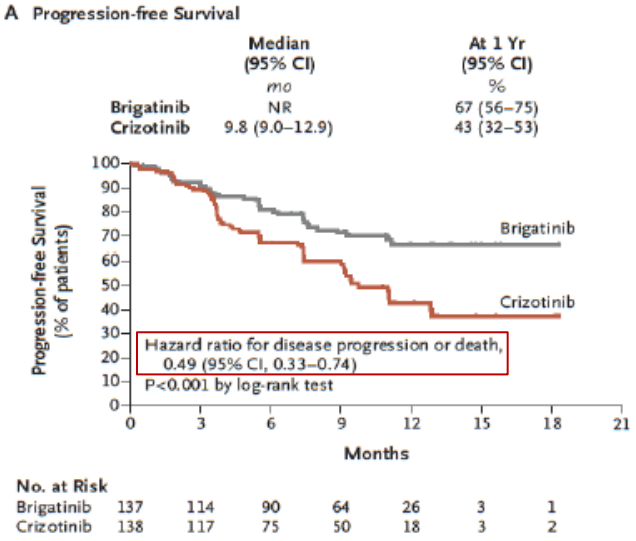
Ramalingam, ESMO 2017 and Ramalingam, NEJM 2019.

ALK positive Advanced NSCLC

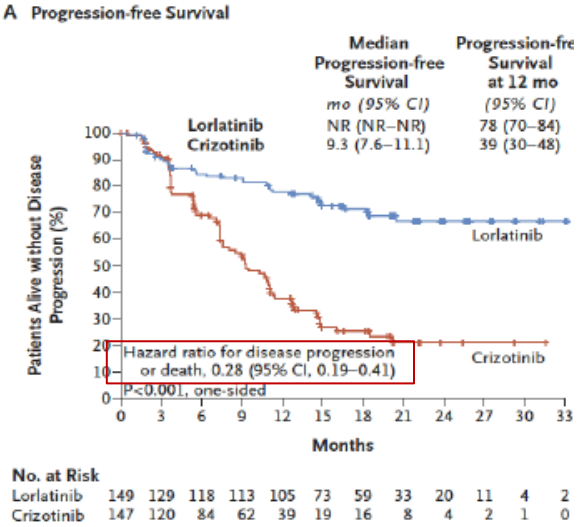
For patients with ALK+ NSCLC, 2nd or 3rd generation ALK inhibitors are the preferred first-line therapy.



Alectinib (ALEX)



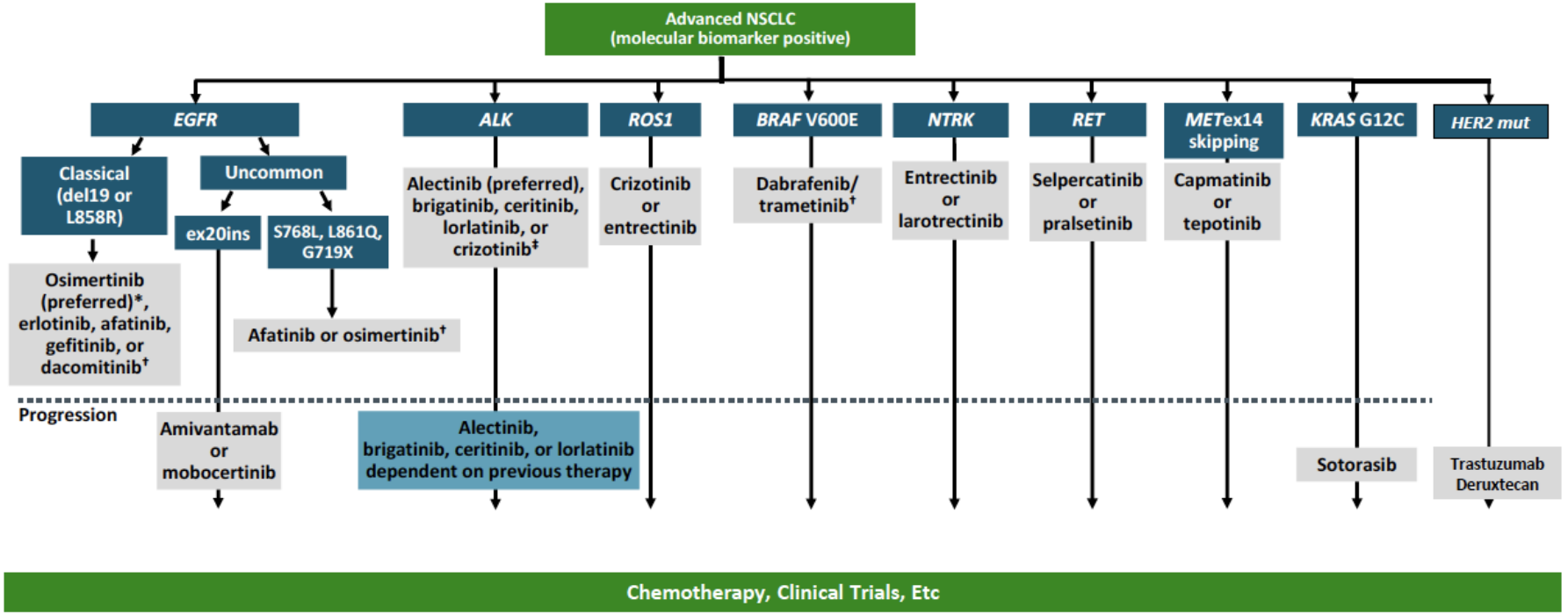
Brigatinib (ALTA-1L)



Lorlatinib (CROWN)

Peters S, NEJM 2017; Camidge DR, NEJM 2018; Shaw AT, NEJM 2019

Molecularly Targeted Therapies 2023 in the US



Approved targeted therapy for 1L treatment of Advanced NSCLC in Hong Kong

Approved 1L Tx in HK	Detection of driver alterations	Safety Net Coverage
EGFR Inhibitor		
Osimertinib, Gefitinib, Erlotinib, Afatinib, Dacomitinib	CDx/LDT	Osimertinib, Gefitinib, Afatinib
ALK Inhibitor		
Crizotinib, Ceritinib, Alectinib, Lorlatinib, Brigatinib	CDx/LDT	Crizotinib, Ceritinib, Alectinib, Lorlatinib, Brigatinib
ROS1 inhibitor		
Crizotinib	CDx/LDT	Crizotinib
BRAF/MEK inhibitor		
Dabrafenib/trametinib	CDx/LDT	No
MET inhibitor		
Capmatinib, Tepotinib	CDx/LDT	No
RET inhibitor		
Pralsetinib	CDx/LDT	No
NTRK inhibitor		
Entrectinib, Larotrectinib	CDx/LDT	No

CDx: companion diagnostic, LDT: laboratory developed test

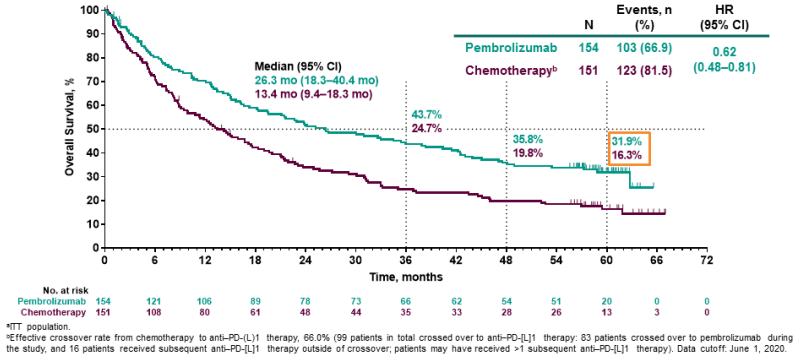
Clinical benefit of Pembrolizumab in advanced (Stage IV) NSCLC

- IO has improved OS in advanced NSCLC
- Durable and long-term survival benefit is observed in 5-year follow-up
  - **5-yr OS rate: ~20-30%**
  - **~30-40% ↓ in risk of death**

KEYNOTE-024 5-year follow up
Pembro in 1L NSCLC (PD-L1 ≥50%)

5-yr OS rate: 31.9%
HR 0.62

Overall Survival[a]



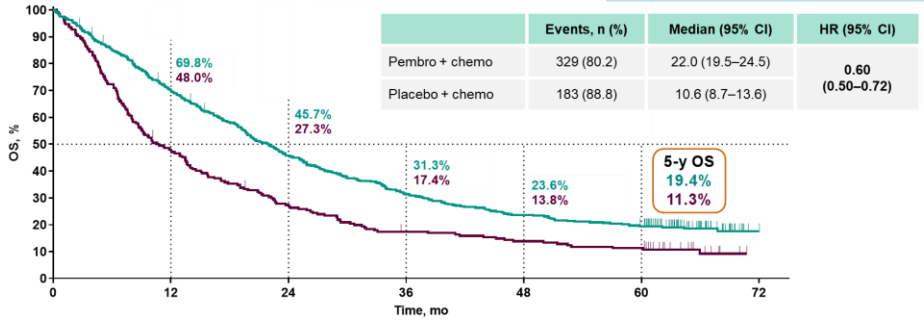
[a]ITT population.
[b]Effective crossover rate from chemotherapy to anti–PD-(L)1 therapy, 66.0% (99 patients in total crossed over to anti–PD-[L]1 therapy: 83 patients crossed over to pembrolizumab during the study, and 16 patients received subsequent anti–PD-[L]1 therapy outside of crossover; patients may have received >1 subsequent anti–PD-[L]1 therapy). Data cutoff: June 1, 2020.

Courtesy of Dr. Jason Jen

KEYNOTE-189 5-year follow up *(Synergistic effect)*
Pembro + Chemo in 1L Non-sq NSCLC

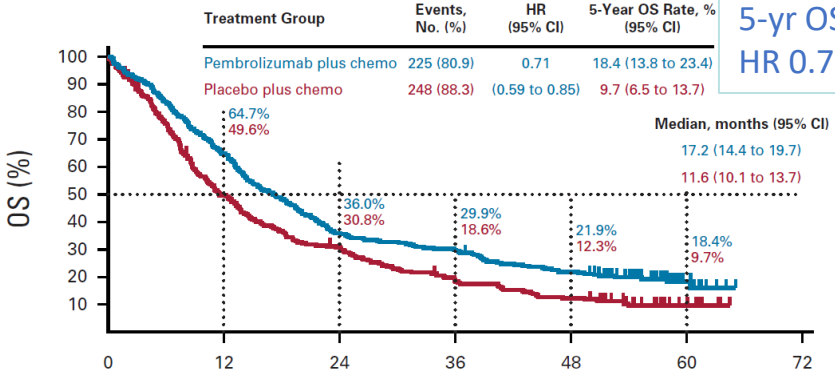
5-yr OS rate: 19.4%
HR 0.60

OS: ITT Population (ESMO 2022)



KEYNOTE-407 5-year follow up *(Synergistic effect)*
Pembro + Chemo in 1L sq NSCLC

5-yr OS rate: 18.4%
HR 0.71



First-line immunotherapy for metastatic and/or locally advanced NSCLC w/ no *EGFR* or *ALK* genomic tumor aberrations in the US

Agent	Mechanism	Indication
Pembrolizumab	anti PD-1 Ab	Metastatic (TPS ≥50%) or advanced (TPS ≥1%) NSCLC with PD-L1 expression
+ chemotherapy		Metastatic NSCLC irrespective of PD-L1 expression
Nivolumab + Ipilimumab		Metastatic NSCLC with PD-L1 expression (TPS ≥1%)
Cemiplimab-rwlc		Advanced NSCLC with PD-L1 expression (TPS ≥50%)
Atezolizumab	anti PD-L1 Ab	Metastatic NSCLC with PD-L1 expression (TC ≥50% or IC ≥ 10%)
+ chemotherapy & bevacizumab		Metastatic non-squamous NSCLC irrespective of PD-L1 expression

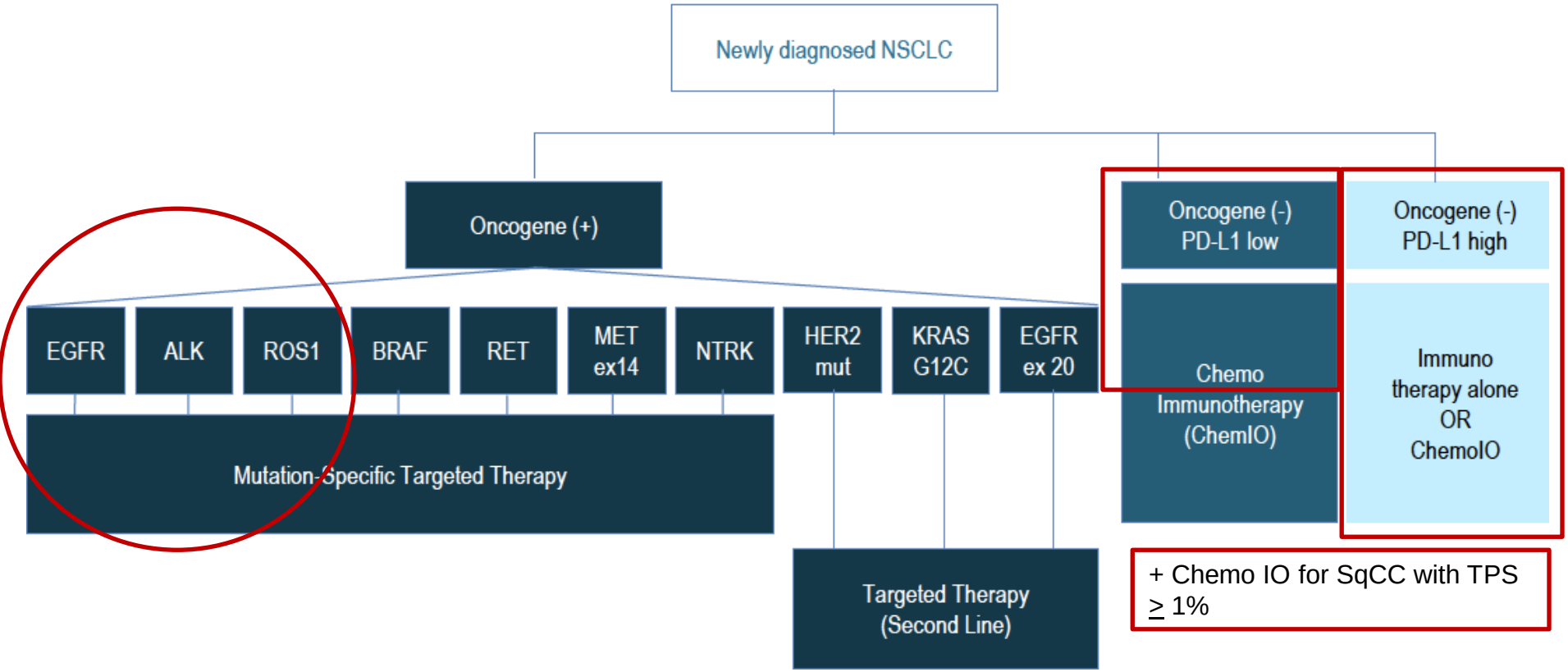
TPS: tumor proportion score, TC: tumor cells, IC: immune cell

Approved immunotherapy for 1L treatment of Advanced NSCLC in Hong Kong

Approved 1L Tx	CDx	Safety Net Coverage
Pembrolizumab Monotherapy	22C3 TPS≥1%	Yes, for TPS≥50%
Atezolizumab Monotherapy	SP142 TC≥50% or IC≥10%	Yes, for TC≥50% or IC≥10%
Pembrolizumab + chemotherapy	No	Yes, for squamous TPS≥1% and for non-squamous TPS≥50%
Nivolumab + ipilimumab + chemotherapy	No	No

CDx: companion diagnostic, TPS: tumor proportion score, TC: tumor cells, IC: immune cell

Treatment Paradigm for Advanced NSCLC



“Molecularly targeted therapy is dead.”
- Anonymous

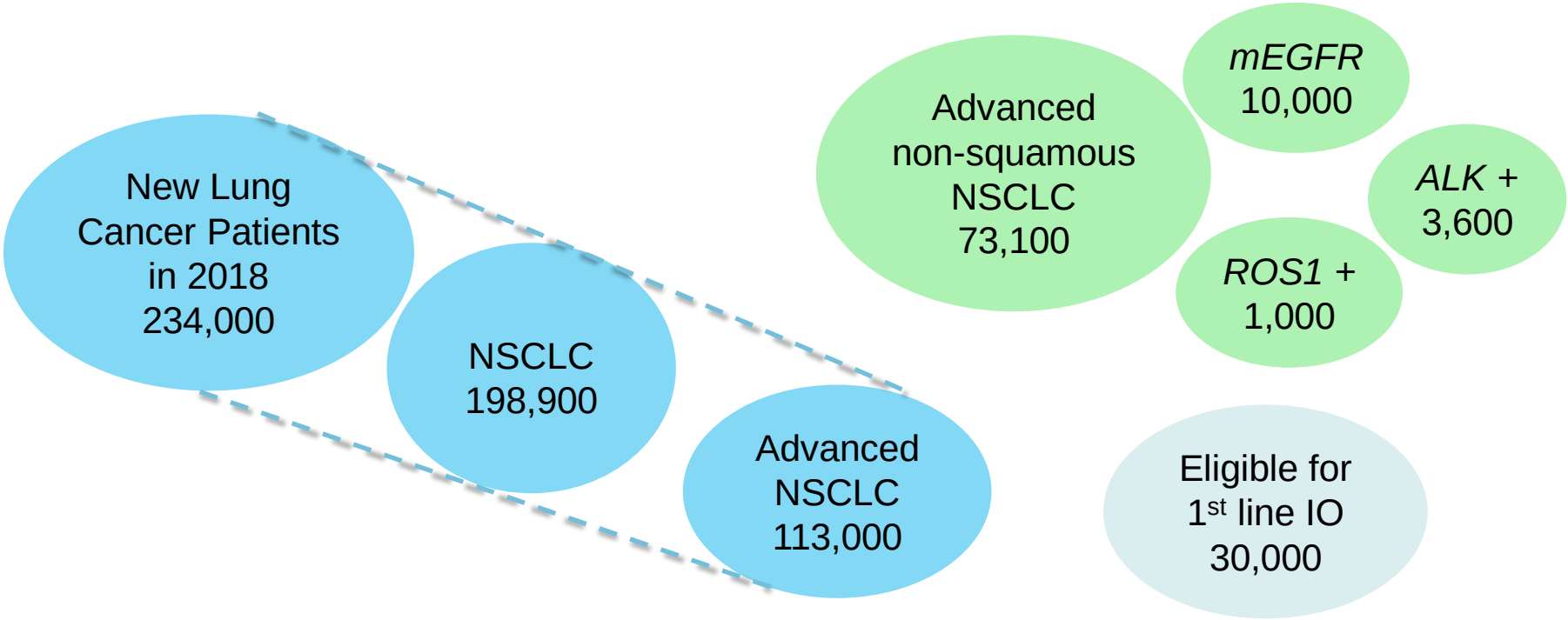
PD-1/PD-L1 inhibitors

- Applicable across many tumor types
- 20-30% response rate on average
- 18 month response durability on average

Mutation- targeted inhibitors

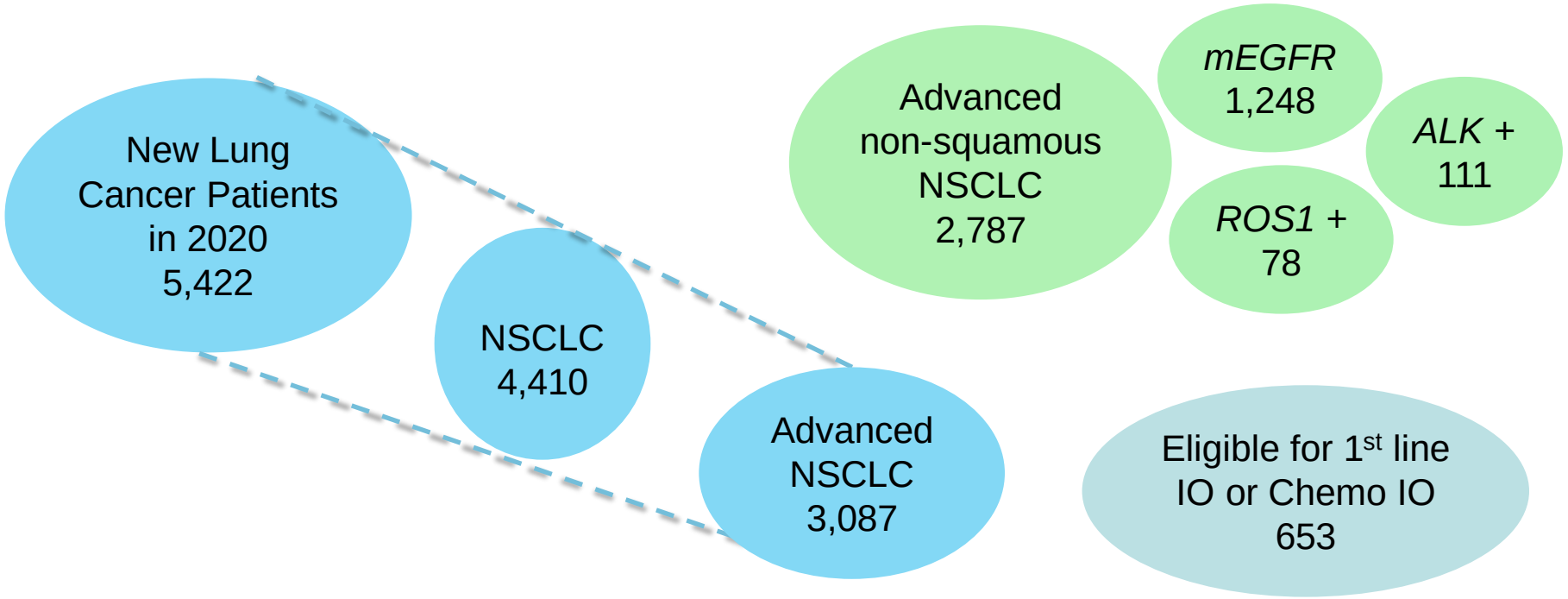
- Applicable to a minor subset of many tumors
- 50-70% response rate
- Response durability 7 months - over 2 years

How many NSCLC patients are eligible for 1st line PD-1/PD-L1 monotherapy (IO) in the US?



* Chemo + IO could be considered in the majority of the remaining advanced NSCLC patients

How many NSCLC patients are eligible for 1st line PD-1/PD-L1 therapy (IO + Chemo IO) in Hong Kong?



International Standard on Predictive Biomarker Testing Sequences

NCCN Guidelines

Population	Target	Method of testing
Newly diagnosed patients	EGFR (category 1)	Broad molecular profiling (NGS)
	ALK (category 1)	
	KRAS, ROS1, BRAF, NTRK1/2/3, MET exon 14 skipping, RET	
	PD-L1 (category 1)	IHC
Relapsed patients on targeted therapy	EGFR T790M and other genomic resistance mutations (cfDNA/tissue DNA)	Broad molecular profiling (NGS)

ESMO 2023

Population	Target	Method of testing
Newly diagnosed patients	EGFR	Any validated method to cover mutations in exon 18-21 (DNA NGS preferred)
	ALK	RNA NGS; IHC ± molecular confirmation (NGS, FISH)
	ROS1	RNA NGS; IHC screening, molecular confirmation essential (NGS, FISH)
	RET, MET, NTRK, ERBB2 (HER2), KRAS, BRAF	DNA/RNA NGS panel testing
	PD-L1	IHC
Relapsed patients on targeted therapy	EGFR T790M and other genomic resistance mutations (cfDNA/tissue DNA)	PCR/NGS/ISH

- European countries have a diverse range of health care systems and health economies impacting the delivery of tumor molecular profiling
- *EGFR*, *ALK* and PD-L1 testing is available in all countries; others vary

Predictive Biomarkers Tested in Different Countries on a World Map

Australia EGFR, ALK, MET, ROS1, PD-L1	**Austria** EGFR, ALK, ROS1, PD-L1, AKT1-3, BRAF, HER2, KRAS, MET, NTRK, PI3KCA, PTEN, RET, STK11/LKB1	**Belgium** EGFR, ALK, ROS1, PD-L1, BRAF, HER2, KRAS, MET, NTRK	**Brazil** EGFR, ALK, ROS1, PD-L1, BRAF, NTRK	**Canada** ALK, EGFR, ROS1, PD-L1 MULTIGENE PANELS	**China** EGFR, ALK, ROS1, RET, BRAF, MET exon 14, NTRK1	**Chile** EGFR, ALK , PD-L1, BRAF	**Colombia** EGFR, ALK, ROS1, PD-L1, BRAF, HER2, MET, NTRK	**France** EGFR, ALK, ROS1, PD-L1, BRAF, HER2, KRAS, NRG1, NTRK, PIK3CA, RET	**Georgia** EGFR, ALK, PD-L1, ROS1, BRAF		
Germany EGFR, ALK, ROS1, BRAF, HER2, KRAS, MET, NTRK, RET	**Greece** EGFR, ALK, ROS1, PD-L1, BRAF, HER2, KRAS, MET, NTRK, RET	**Hungary** EGFR, ALK, ROS1, PD-L1 BRAF, KRAS	**Italy** EGFR, ALK, ROS1, PD-L1	**Israel** EGFR, ALK, ROS1, PD-L1	**INDIA** EGFR, ALK, ROS1, PD-L1	**Japan** EGFR, ALK, ROS1, PD-L1, BRAF, NTRK, MET, RET	**Lithuania** EGFR, ALK, PD-L1	**MENA** EGFR, ALK, PD-L1	**Malaysia** EGFR, ALK, ROS1, BRAF, PD-L1	**Mexico** EGFR, ALK, ROS1, PD-L1, BRAF	**Netherlands** EGFR, ALK, ROS1, PD-L1, BRAF, HER2, KRAS, MET, NRG1, NTRK1-3, RET	**Nepal** EGFR, ALK, ROS1	**Peru** EGFR, ALK, PD-L1
Poland EGFR, ALK , ROS1, PD-L1, BRAF, MET, RET, HER2, and NTRK1-3,	**Pakistan** EGFR, ALK, PD-L1	**Rwanda** EGFR, ALK, ROS1 and PD-L1	**Sudan** No test Available	**South Africa** EGFR, ALK, ROS1, PD-L1, RAS, RAF	**South Korea** EGFR, ALK, ROS1, PD-L1, BRAF	**Serbia** EGFR, ALK, PD-L1	**Saudi Arabia** EGFR, ALK, PD-L1, Infreq ROS1, BRAF, HER2, MET, RET	**Slovenia** EGFR, ALK, PD-L1, BRAF, KRAS	**Singapore** EGFR, ALK, ROS1 and PD-L1	**Spain** PD-L1, BRAF	**Thailand** EGFR, ALK, PD-L1	**Turkey** EGFR, ALK, ROS1, PD-L1, KRAS, BRAF, NTRK	**UK** EGFR, ALK, ROS1, PD-L1	**Vietnam** EGFR, ALK, ROS1, PD-L1, BRAF, HER2, KRAS, MET, PIK3CA, V600E

A Global Perspective on Molecular Testing Guidelines and Practices

6

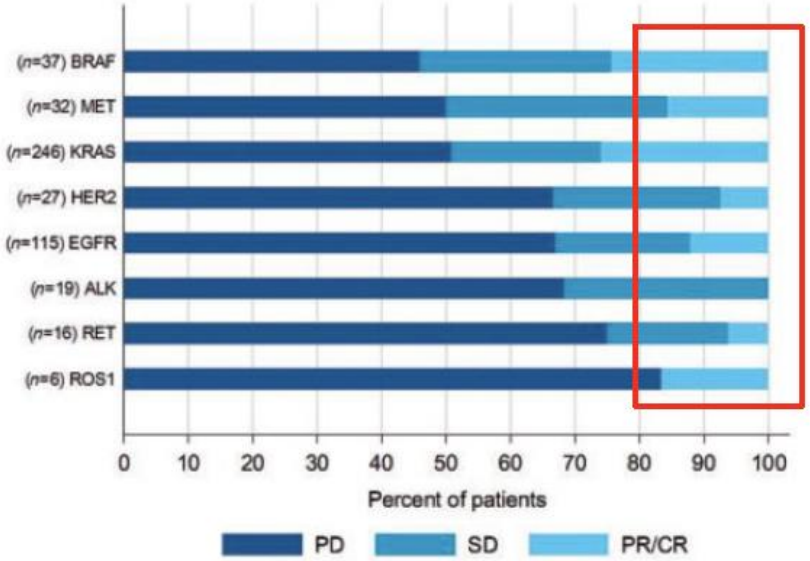
By Deepali Jain, Wendy A. Cooper, Lizza E. Hendriks, Fred R. Hirsch, Mehdi Karkouri, Keith M. Kerr, Dongmei Lin, Ming-Sound Tsao, and Yasushi Yatabe

Conclusion

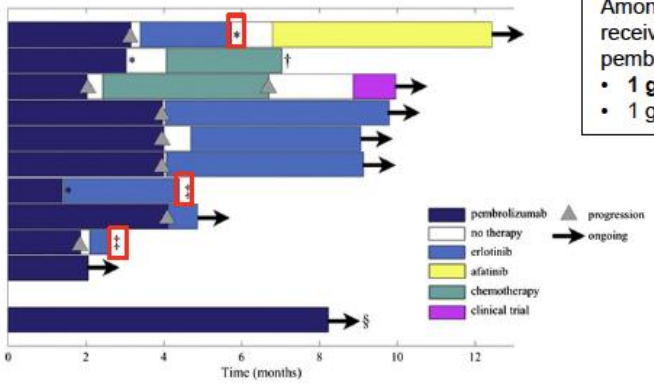
Global implementation of standard biomarker testing guidelines in lung cancer depends on country-specific local and regional factors that include availability of resources and infrastructure, affordability, reimbursement policies by government or private parties, access to testing assays, and drug approval mechanisms. Although the recommendations on the specific tests are not consistent across countries, testing for *EGFR*, *ALK*, *ROS1*, and PD-L1 is widely implemented. With increased availability of novel targeted therapies, we anticipate this list to expand, necessitating a shift toward multiplexed testing.

Molecular Profiling and PD-L1 Testing Should be Done at the Same Time

IMMUNOTarget Registry[1]



First-line Pembrolizumab in PDL1+ EGFR+ NSCLC[2]



Among 7 pts who received erlotinib post-pembro:

- **1 gr 5 pneumonitis**
- 1 gr 3 hepatitis

- Sequential use of critozinib following immunotherapy was also associated with a significantly increased risk of grade 3/4 hepatotoxicity[3]
- Data with newer MET, RET TKIs is not yet available, but all TKIs should be used with caution after immunotherapy.

Likelihood of Receiving Second Line Therapy for Advanced NSCLC

- 230 patients with stage IIIB (13%) or IV (87%) NSCLC
- First line therapy with carboplatin and paclitaxel
- Only 44% received second line therapy after progression
- An increased likelihood of receiving second line therapy was associated with:
  - High baseline performance status / female gender / non-squamous histology/ Two or more cycles of first line therapy

The most effective therapy should be used for the first line therapy – Appropriate assessment on available predictive biomarkers at the time of diagnosis is of paramount importance

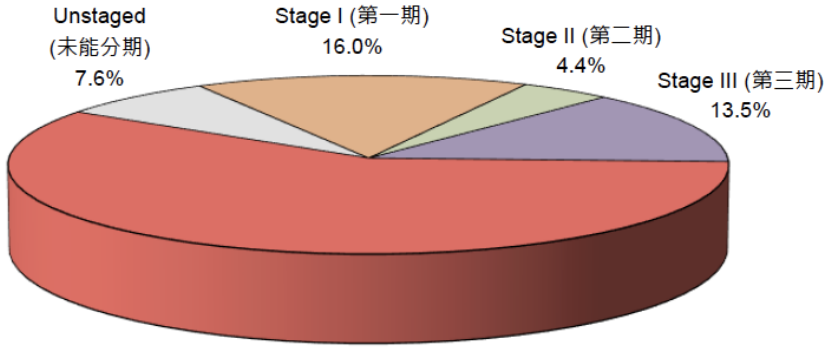
Biomarker Testing in NSCLC

- Diagnostic - TTF-1 / p40, etc.
- Predictive - Driver gene alterations, PD-L1, etc.
- Prognostic – Ki-67, p53, etc.

Practical Challenges for Pathologists

- NSCLC: 70% present at an advanced stage (stage III-IV)
- Tissue samples for the initial diagnosis from advanced stage NSCLC patients are typically small biopsies and cytology
- They need to be efficiently utilized for diagnosis, subtyping and molecular profiling, etc.
- Guidelines for good practice of small biopsies and cytological preparations (WHO 2021)

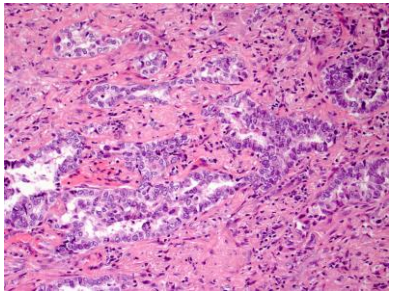
Stage Distribution of Lung Cancer in 2020
2020年肺癌期數分佈



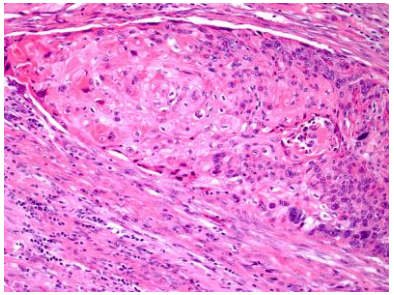
Staged according to the 8th edition of the AJCC system
按 AJCC 癌症分期手冊第八版分類

Histologic Subtyping of NSCLC

- Can be done based on HE in 70%
- IHC necessary to subtype poorly-differentiated non-small cell carcinoma (NSCC, NOS on HE) into solid ADC vs. non-keratinizing SqCC vs. others
- **Clinical implications**
  - Main targetable genetic alterations (*EGFR*, *ALK*, *ROS1*, etc.) are associated with ADC (non-squamous) histology
  - Histology-based agents: contraindicated for SqCC
    - Bevacizumab (Avastin)
    - Pemetrexed (Alimta)
  - Appropriate subtyping for future investigation?



Adenocarcinoma (ADC)

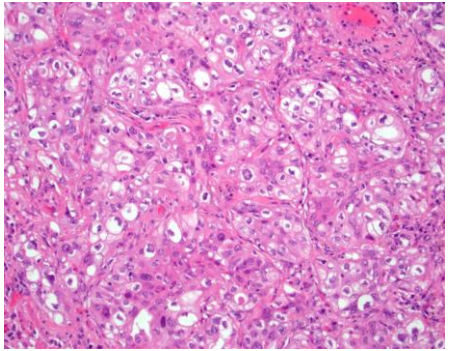


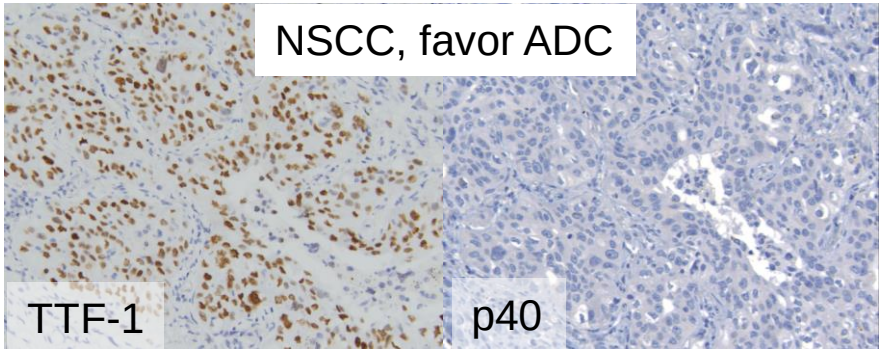
Squamous cell carcinoma (SqCC)

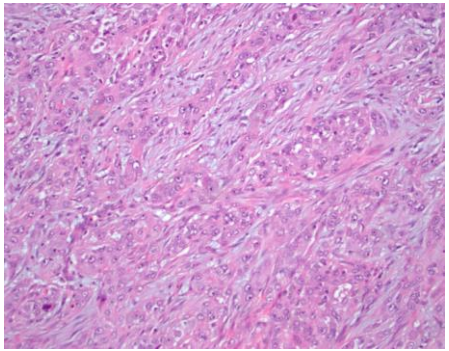
NSCC, NOS morphology on HE

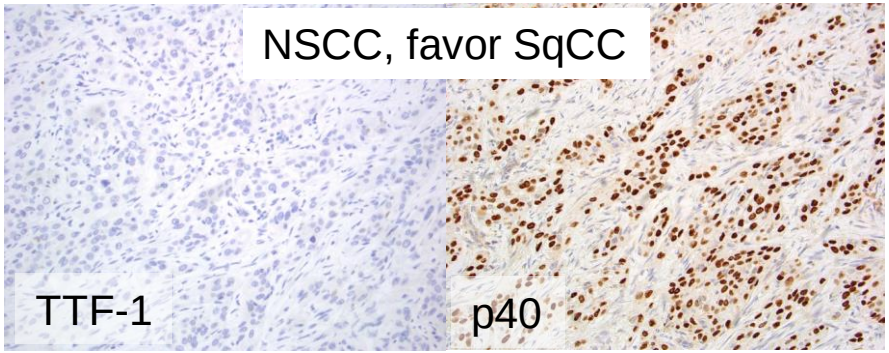
Minimal initial panel: TTF-1 and p40

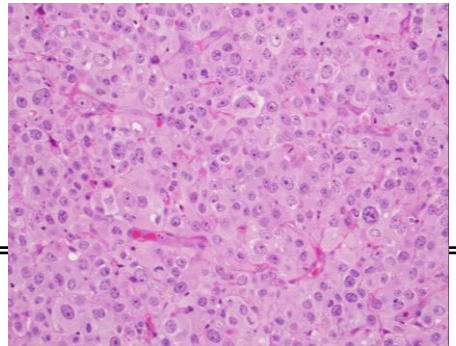
Additional markers, if the initial panel negative or unusual: Napsin A, CK5/6, etc.

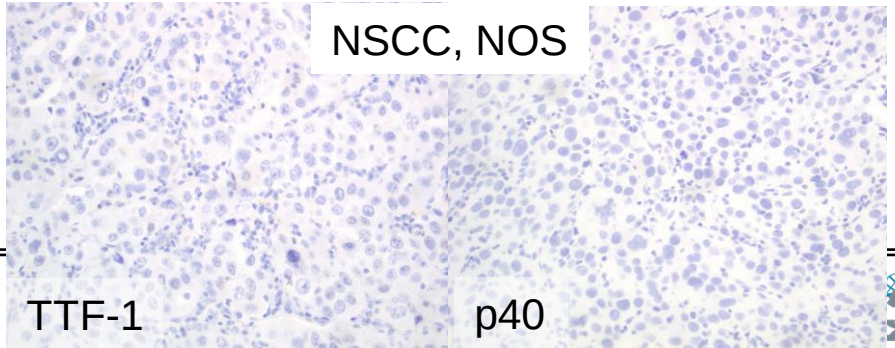










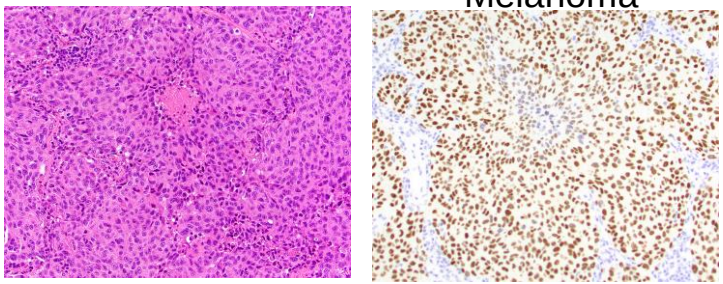


NSCLC subtyping with TTF-1 and p40

Presumable lung primary with poorly-differentiated NSCC morphology

IHC	TTF-1 (+) p40 (- or scattered cells +)	TTF-1 (-) p40 (+ in ≥50%)	TTF-1 (+) p40 (+) in different clones	TTF-1 (-) p40 (- or scattered cells +)	Unusual pattern Insufficient cellularity Technical failure
Subtype	NSCC, favor ADC	NSCC, favor SqCC	NSCC, NOS Possible adenosquamous	Napsin A and mucine stain (-) → NSCC, NOS	NSCC, NOS

Melanoma



Sox10

DDx.

Any epithelioid neoplasm
- Melanoma
- Mesothelioma
- Lymphoma
- Sarcoma

Metastatic carcinoma

Clinicoradiologic correlation
Other IHC markers

ADC: adenocarcinoma, SqCC: squamous cell carcinoma, NSCC: non-small cell carcinoma

Testing Workflows for Patients with Advanced (non-squamous) NSCLC

For molecular profiling, NGS is preferred, but if

Upfront NGS is not feasible, such as for the following reasons:

1. NGS is not approved/available (eg, reimbursement issues)
2. Immediate treatment intervention (eg, oncologic emergency)
3. The specimen is not optimal (eg, too small for NGS testing or low tumor cell content in the tissue)
4. Higher cost-benefit of an initial single testing for a particular gene alteration due to high prevalence in the region (eg, EGFR in Asian countries)

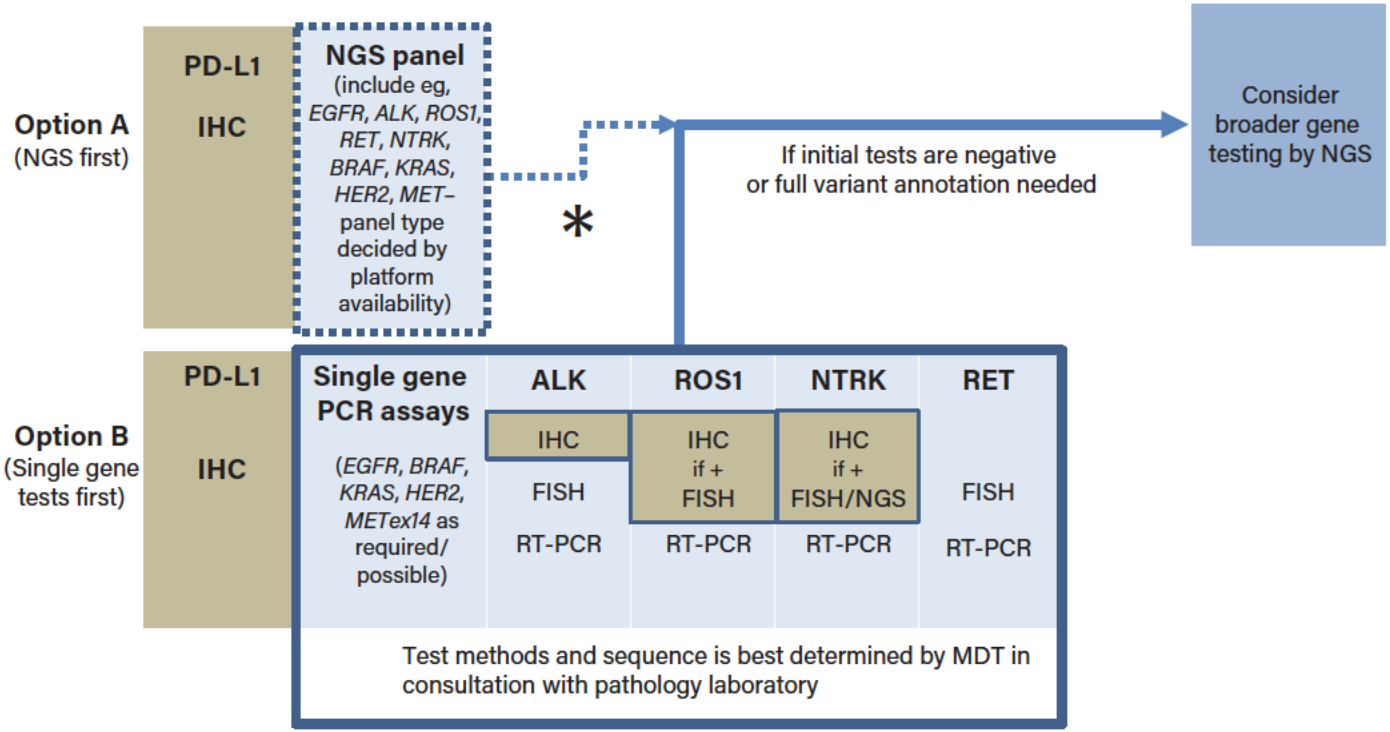
Multiple single gene testing simultaneous or sequenced

Examples of possible approaches to consider:

- PCR testing of EGFR, BRAF, and KRAS (single gene or rapid multi-gene assays)
- IHC for ALK (possibly ROS1 and NTRK)
- Rapid multiplex fusion gene assays

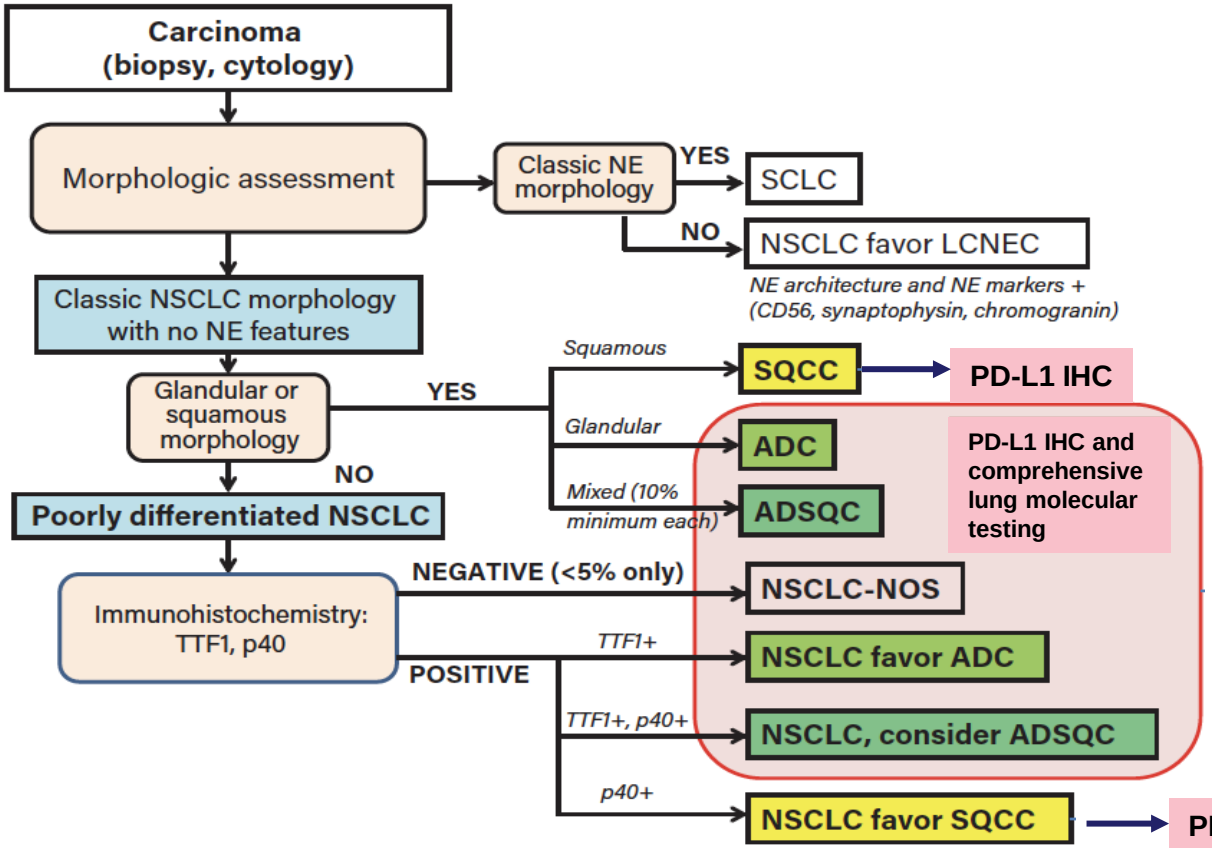
IASLC ATLAS of MOLECULAR TESTING for TARGETED THERAPY in LUNG CANCER

Testing Workflows for Patients with Advanced (non-squamous) NSCLC



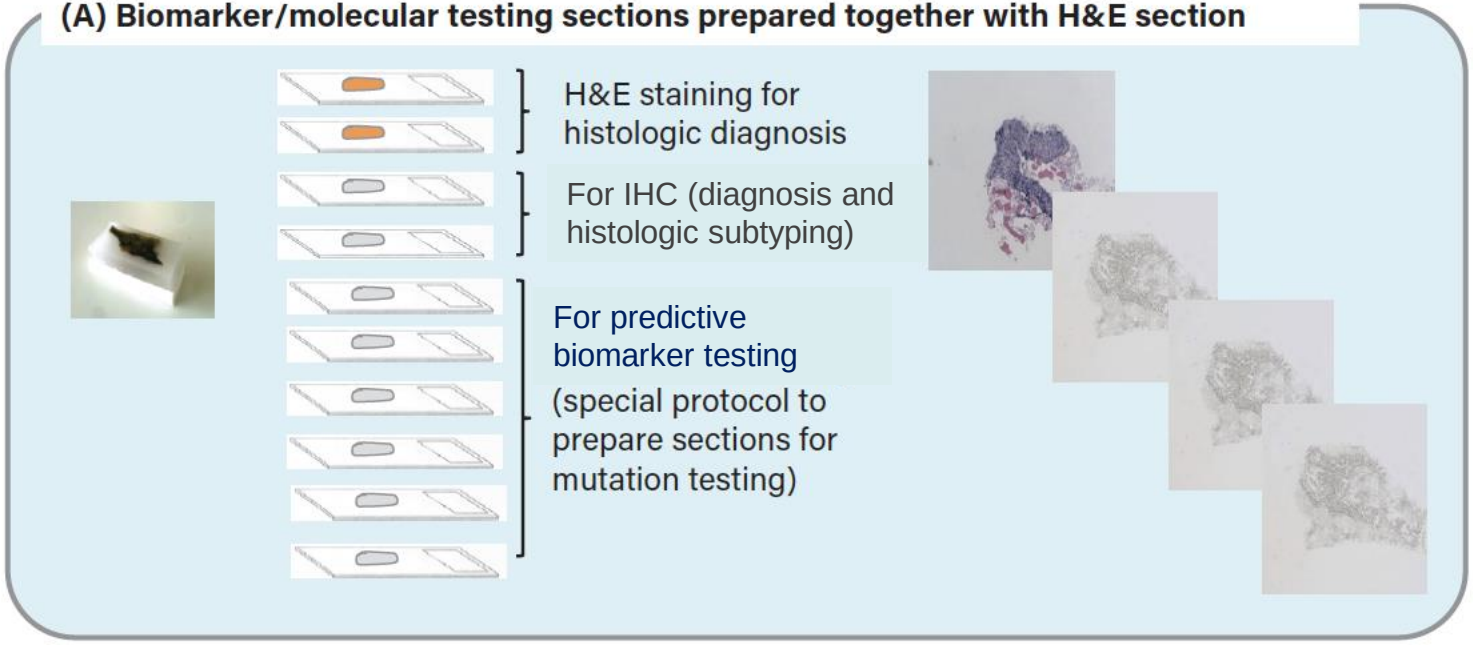
* A second larger NGS panel may also be considered, if the first NGS panel does not include additional genes/mutations with therapy available including in clinical trials

Algorithm for Biomarker Testing in Lung Cancer



IASLC ATLAS of MOLECULAR TESTING for TARGETED THERAPY in LUNG CANCER, modified

Strategy for Maximizing Tissue for Diagnosis, Subtyping, and predictive Biomarker Testing



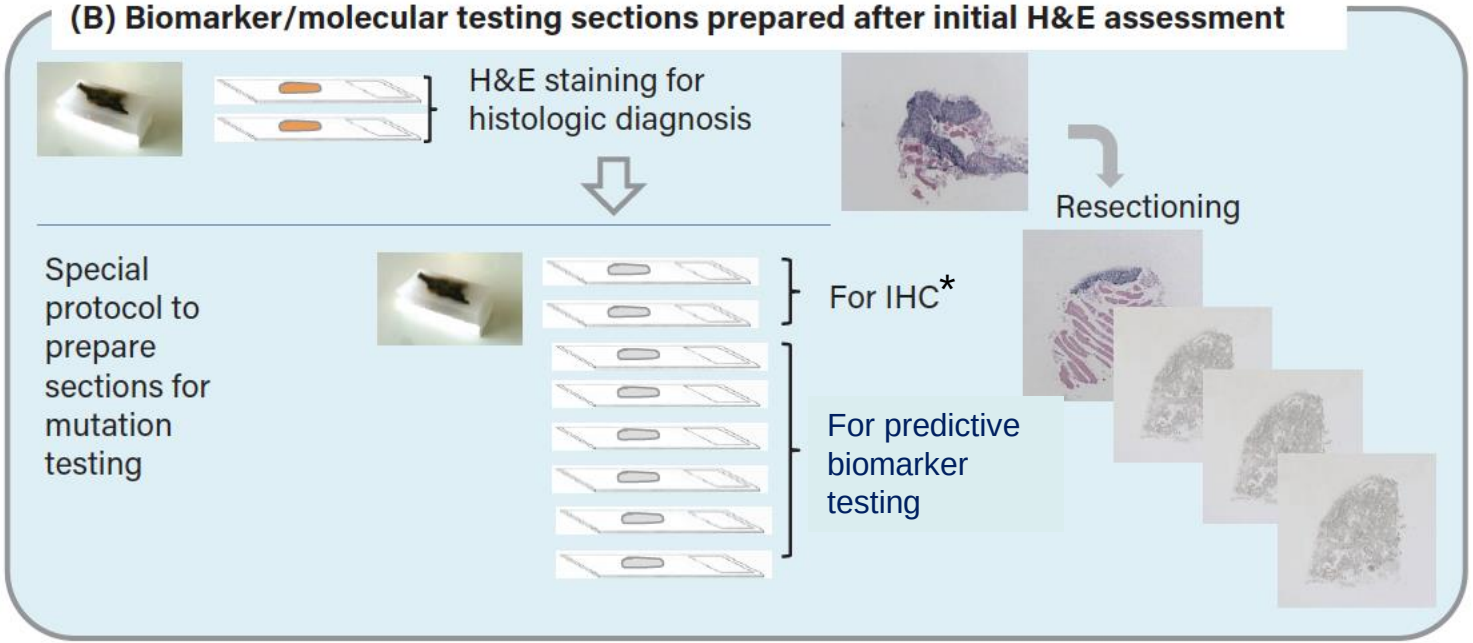
If subtyping can be done on HE alone, all the unstained slides will be used for predictive biomarker testing.

- # of unstained sections to be prepared is determined by the institutional/departmental strategy for optimal tissue use to reduce tissue sample loss and turnaround time
- Many laboratories cut sections for DNA/RNA extraction on a dedicated microtome to prevent cross-contamination

IASLC ATLAS of MOLECULAR TESTING for TARGETED THERAPY in LUNG CANCER, modified

MASSA GENER PATHO

Strategy for Maximizing Tissue for Diagnosis, Subtyping, and predictive Biomarker Testing

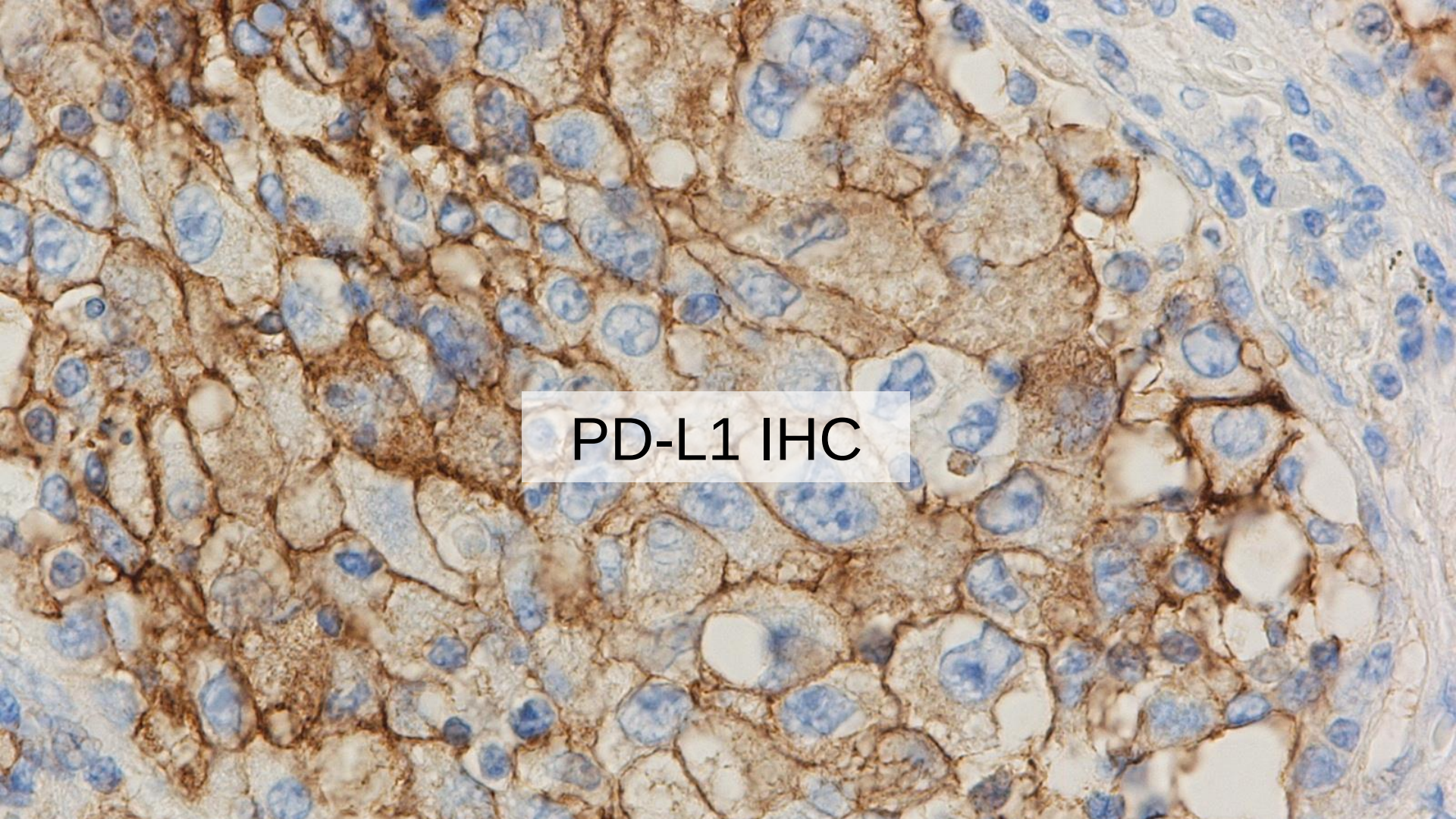


* For IHC (diagnosis and histologic subtyping)

If subtyping can be done on HE alone, unstained slides only for predictive biomarker testing are cut.

Tissue conservation is key.

- Biopsy protocols:
  - More than one core in the jar – submit one core per cassette -> HE
  - Only one core in the jar – HE & 6 unstained slides (kept in Histology/IHC lab) cut for routine diagnosis
  - Consider the following process upon ordering IHC for diagnosis in stage IIIB-IV NSCLC to **avoid refacing the block at multiple times**
    - IHC
    - A few unstained slides for potential additional stains (kept in Histology/IHC lab)
    - 1-2 USS for PD-L1 (+/- CD8)
    - 10 or 18 USS to molecular (CID) depending on tumor tissue size (run panel order “MOL” in CoPath)


PD-L1 IHC

FDA Approved IO for the First Line Treatment for NSCLC Patients with Required PD-L1 testing

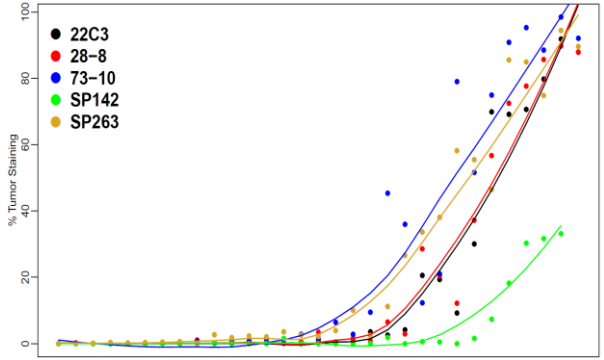
Immune checkpoint inhibitor	Companion Diagnostic*	IHC Platform	Scoring criteria
Pembrolizumab (anti PD-1)	22C3 pharmDx assay	Dako Autostainer Link 48	TPS $\geq$ 1%
Nivolumab (anti PD-1) + Ipilimumab (anti CTLA4)	28-8 pharmDx assay	Dako Autostainer Link 48	TPS $\geq$ 1%
Atezolizumab (anti PD-L1)	VENTANA PD-L1 (SP142) assay	Ventana BenchMark ULTRA	TC $\geq$ 50% or IC $\geq$ 10%
Cemiplimab-rwlc (anti PD-1)	22C3 pharmDx assay	Dako Autostainer Link 48	TPS $\geq$ 50%

*Each clinical trial/commercial IHC assay is determined by not only antibody clone, but also IHC platform and reagents

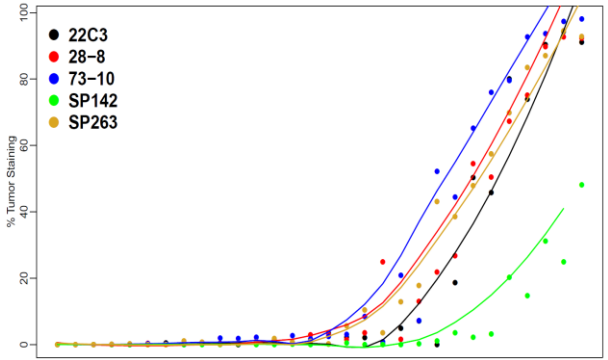
Blueprint phase 2A project

Compatibility of PD-L1 staining on tumor cells among 5 clinical trial (commercial) assays

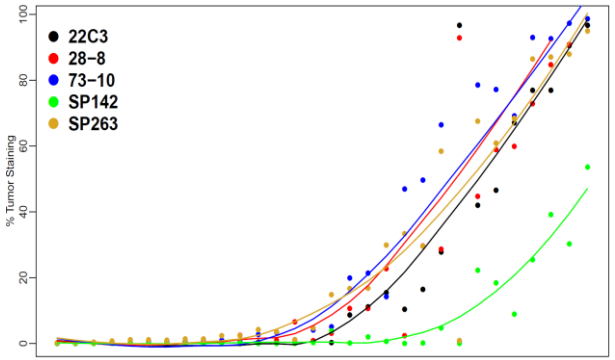
BIOPSY



ASPIRATE



SURGICAL RESECTION BLOCK

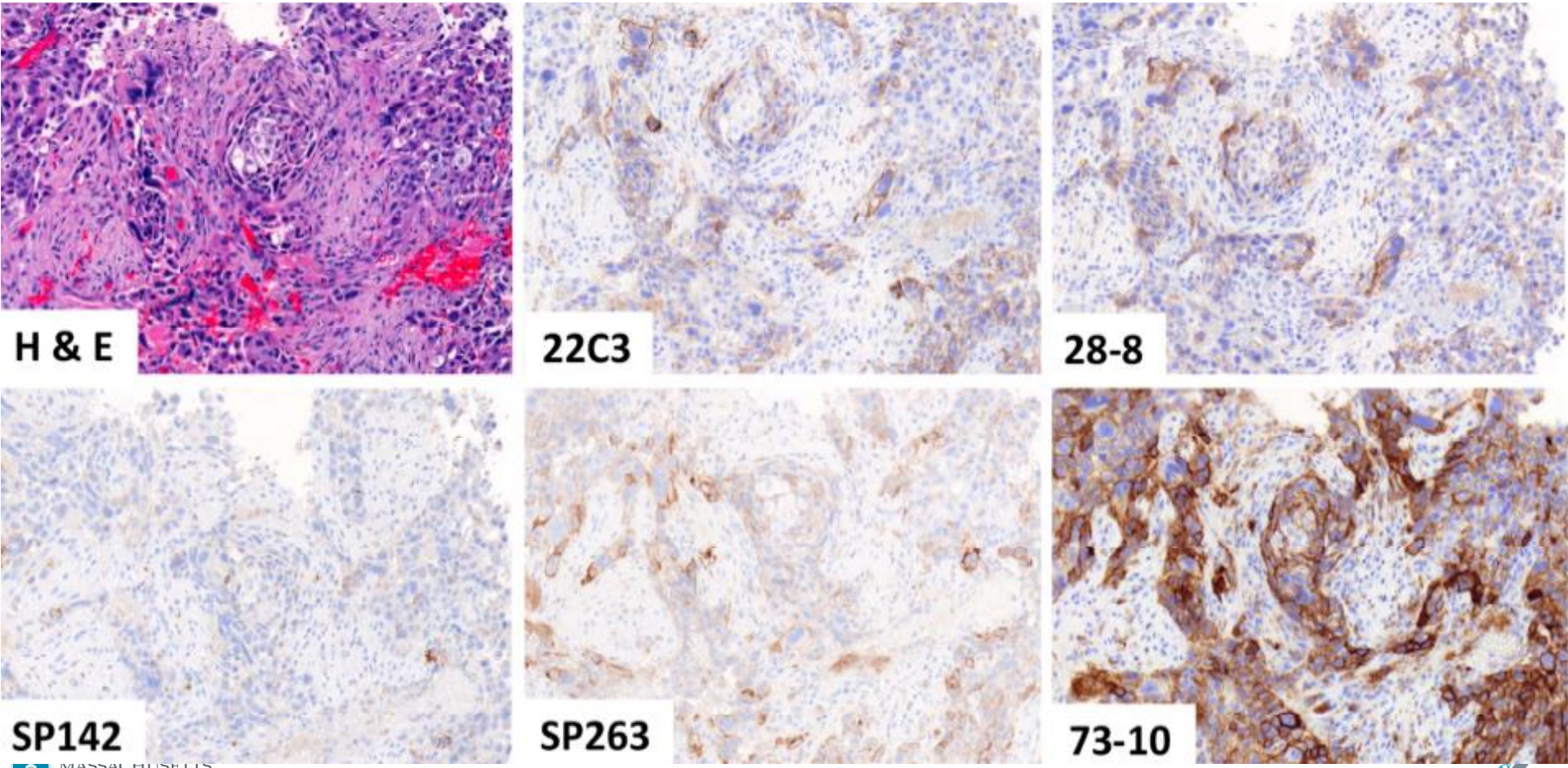


Data broadly similar to those found in previous studies

SP142 stains fewer tumor cells

73-10 stains, to variable degree, more tumor cells

SP263 may stain more tumor cells than 22C3 and 28-8



Tsao MS, et al. J Thorac Oncol 2018 Sep;13(9):1302-1311

Pre-analytical Issues

- Antigenicity may drop significantly in tissue blocks older than 3 years
- PD-L1 IHC assays have not been validated for decalcified tissue
- The use of cytology samples for PD-L1 IHC is currently not recommended, due to the lack of rigorous validation for this purpose

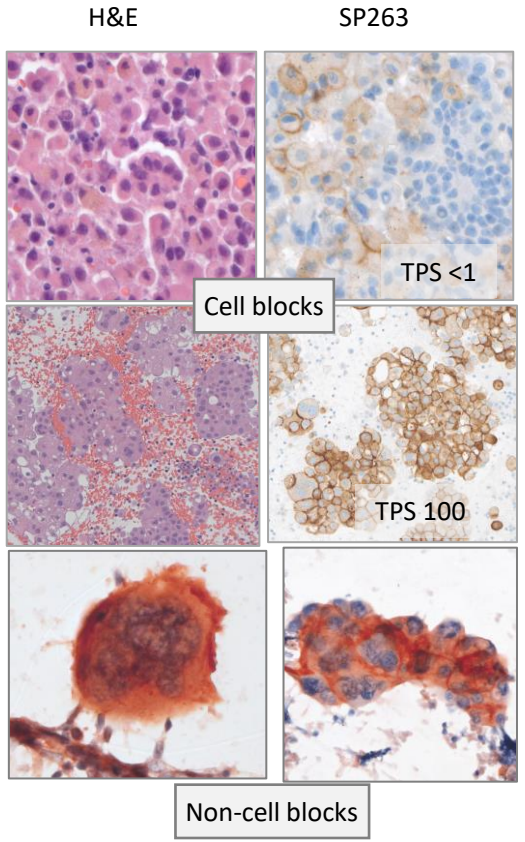
… but, cytology samples may be the only specimen available for PD-L1 testing in many advanced NSCLC patients

PD-L1 IHC in cytology *works*

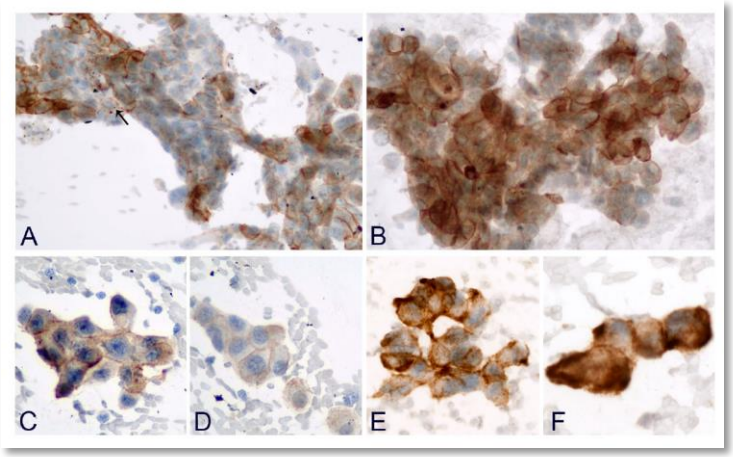
Publication (cell blocks)	n	Assay	Platform	Concordance TPS ≥ 50%
Skov BG	86	28-8pharmDx 22C3pharmDx	ASL48 (Dako)	90% 94%
Heymann J	23	22C3pharmDx	BMU (Ventana)	91%
Russel E	41	E1L3N LDT	ASL48 (Dako)	84%
Ilie M	70	22C3 LDTs	ASL48 (Dako) BM U (Ventana)	96%
Noll B	38	22C3pharmDx	ASL48 (Dako)	89%
Wang G	34	22C3pharmDx	ASL48 (Dako)	91%

Skov & Skov, Appl Immunohistochem Mol Morphol 2017;25:452, Heymann JJ et al, CCP 2017, Russel-Goldman E et al CCP 2018, Ilie M et al CCP 2018, , Noll B et al CCP 2018, Wang G et al, Lung Cancer 2019

Only for TCs *(not for ICs)*



PD-L1 IHC in ethanol-fixed cytology smears *works*



22C3 PharmDx SP263

FFPE samples	Direct Cytological Pap-Stained Specimens			
	TPS <1%	TPS ≥1%	TPS ≥50%	Total
TPS <1%	**56**	**1**	0	57
TPS ≥1%	**1**	**31**	0	32
TPS ≥50%	0	**1**	**23**	24
Total	57	33	23	113

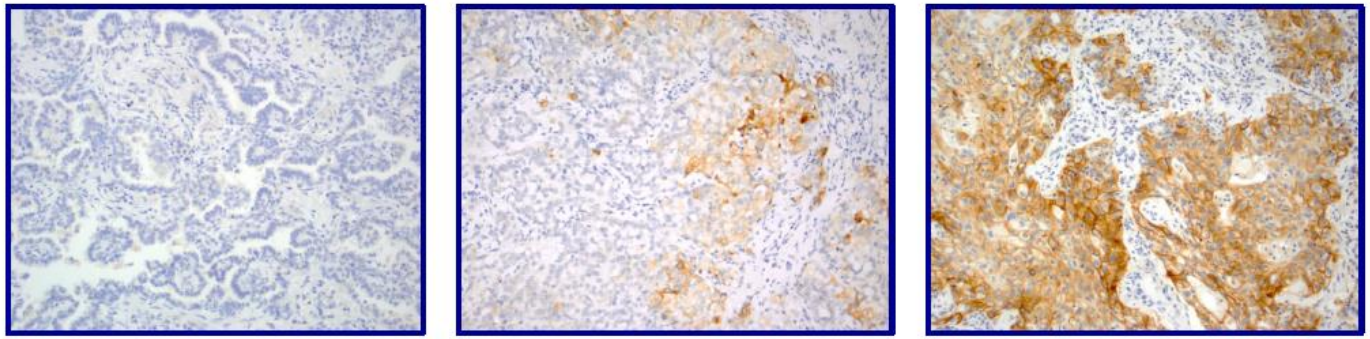
Noll et al (22C3 Pharm DX: Concordance TPS ≥ 50%:
PAP vs biopsy: 100% (37/37; 19 with TPS≥50)
CB vs biopsy: 89% (34/38; 14 with TPS≥50)

The quantification of PD-L1 expression on direct Papanicolaou-stained cytology smears is feasible and reliable for both assays (22C3 PharmDX & SP263 Assay).

Lozano MD et al, Cancer Cytopathol 2019, Noll B et al, Cancer Cytopathol 2018

Evaluation of PD-L1 IHC in NSCLC

Tumor Proportion Score



0% positive | 30% positive | 95% positive

- TPS (tumor proportion score) is typically classified into <1%, 1-49% or ≥50%
- Given that each anti-PD-1/PD-L1 agent is coupled to a specific cut-off(s), it would be ideal to measure % of PD-L1 positive tumor cells with 5-10% increments (<1%, 1-4%, 5-9%, then 5-10% increments)

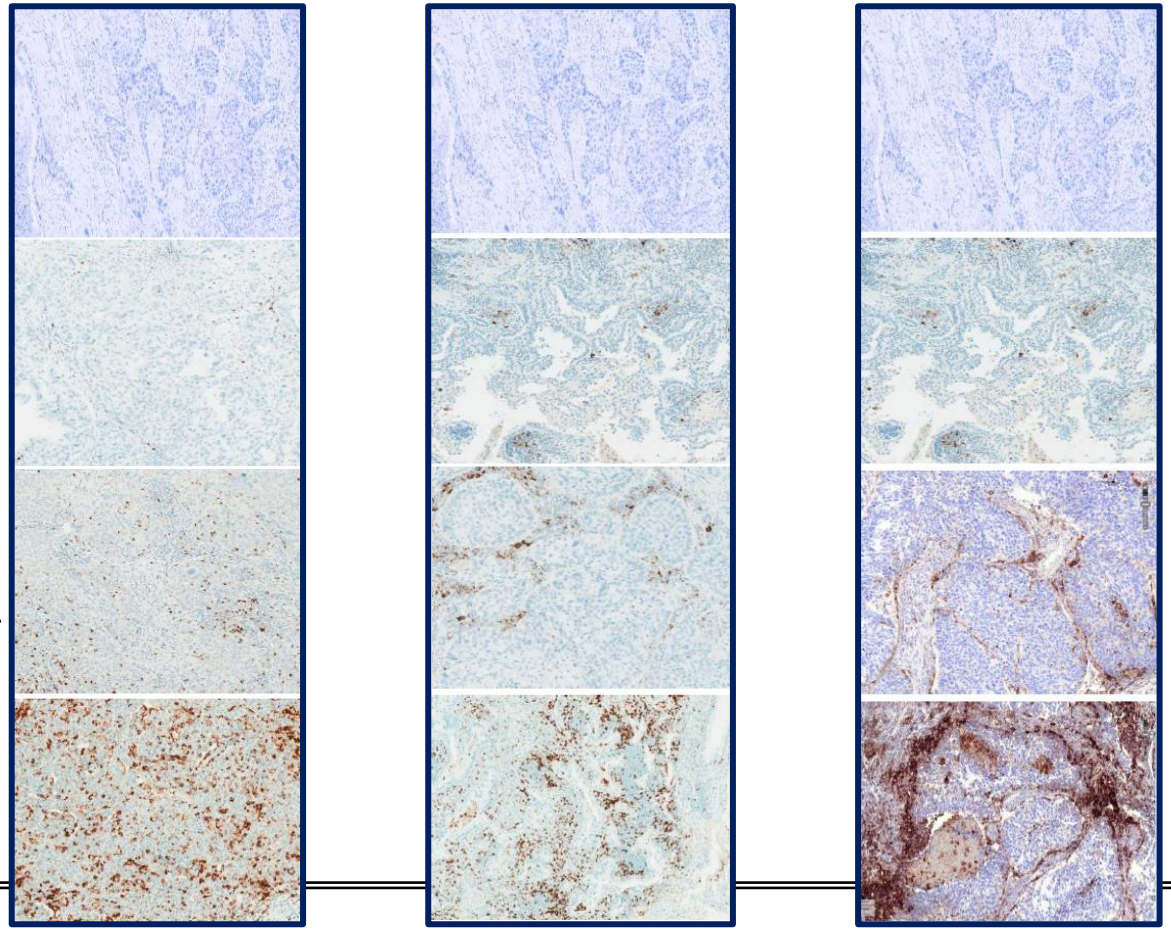
Tumor Infiltrating Immune Cell (IC) Scoring

Score 0
(<1%)*

Score 1
(≥1 <5%)*

Score 2
(≥5 <10%)*

Score 3
(≥10%)*



http://productlibrary.ventana.com/ventana_portal/OpenOverlayServlet?launchIndex=1&objectId=740-485910152 43US

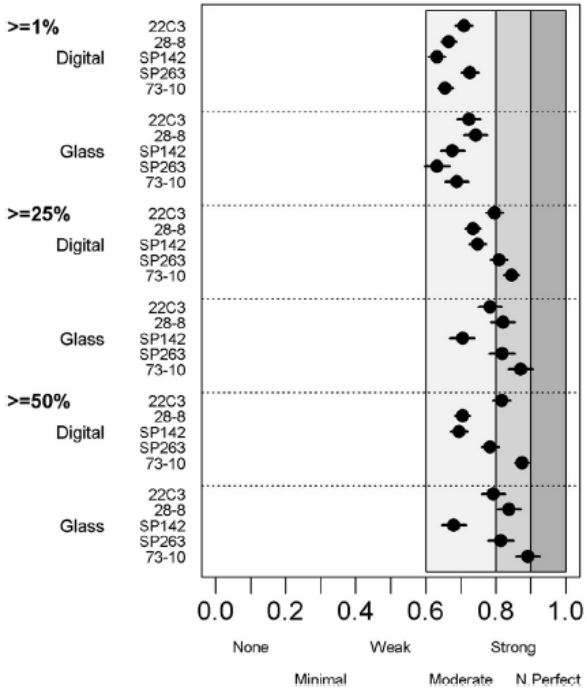
MASSACHUSETTS GENERAL HOSPITAL
PATHOLOGY

* % of tumor area with positive immune cells

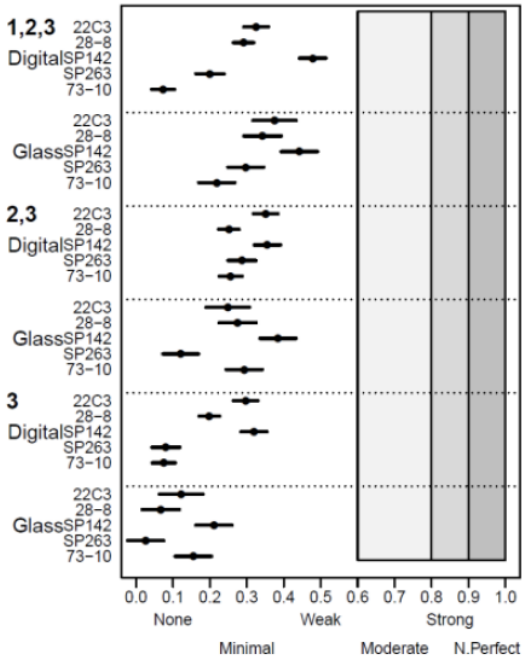
Advancing Diagnosis

Interobserver Agreement of Tumor Cells vs. Immune Cells PD-L1 expression

TC



IC



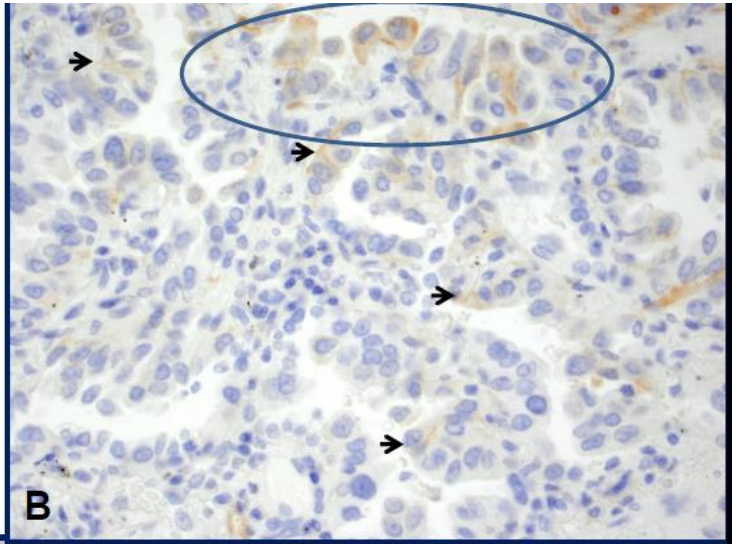
- Very good concordance in TC PD-L1 scoring with all assays (overall ICC 0.86-0.93)
- Poor concordance in IC PD-L1 scoring (overall ICC 0.18-0.19)

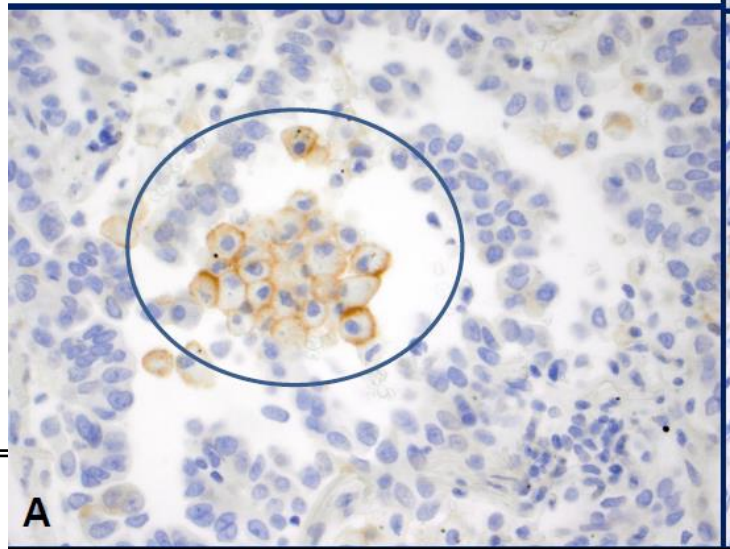
Sources of discordance

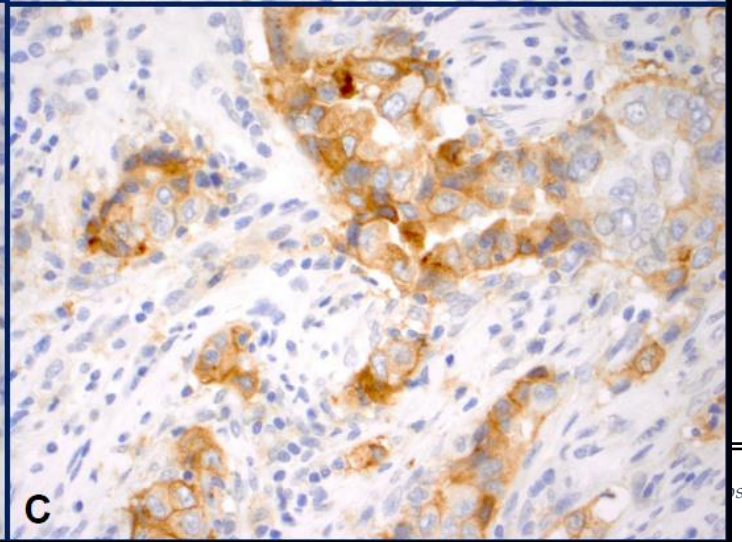
- PD-L1 expression in macrophages
- Low intensity membranous staining
- Heterogeneity of PD-L1 expression in tumor cells
- Cytology specimen

PD-L1 IHC

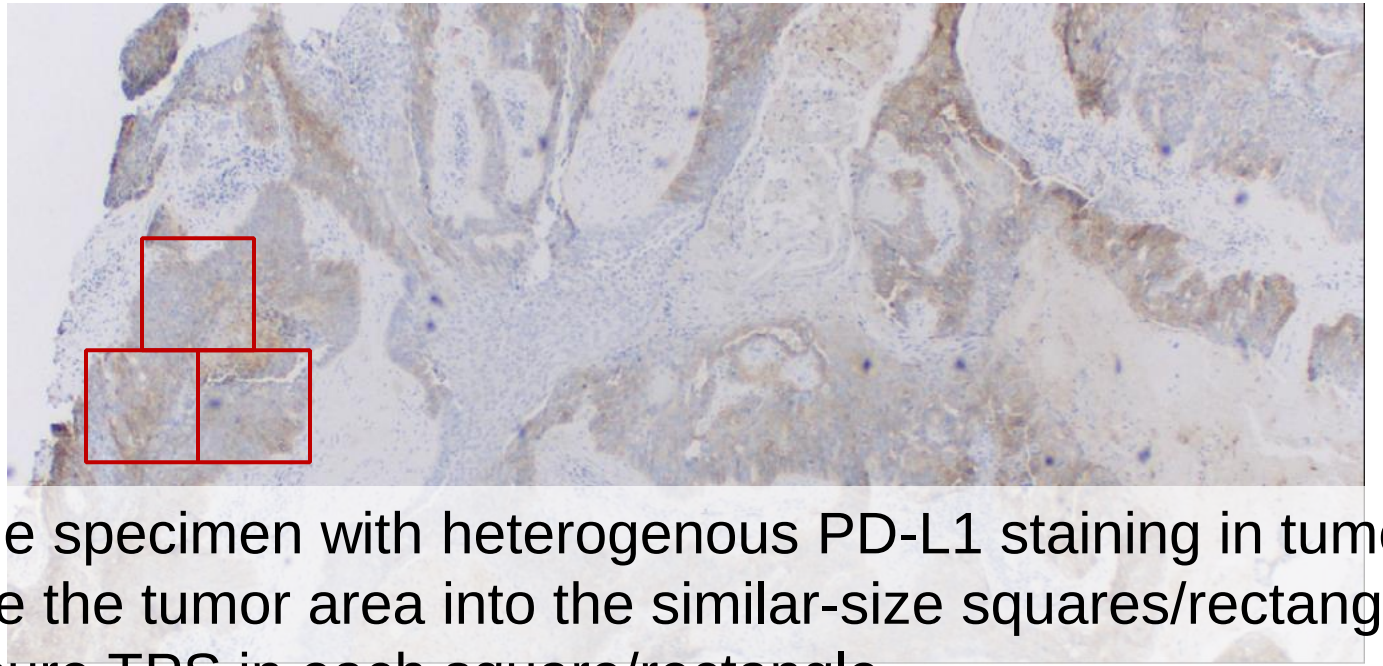
A. Alveolar macrophages exhibit membranous staining (circle), while tumor cells are negative
B. There are several tumor cells with weak, incomplete membranous staining (arrows), while several cells exhibit moderate membranous staining (circle) – both are considered positive for PD-L1 expression
C. An example of moderate to strong membranous staining of PD-L1





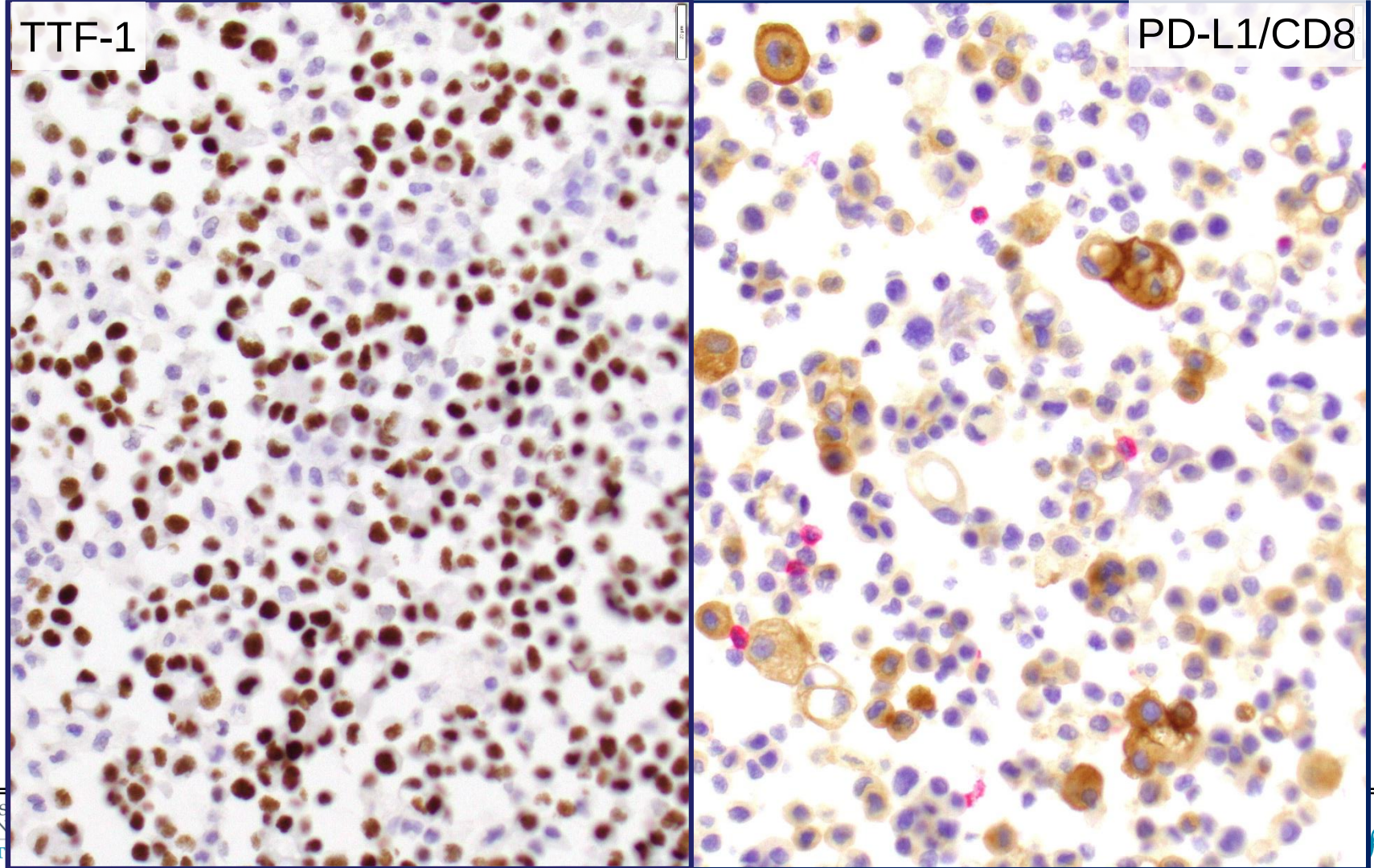


Intratumoral heterogeneity of PD-L1 expression



In a large specimen with heterogenous PD-L1 staining in tumor cells:

- Divide the tumor area into the similar-size squares/rectangles
- Measure TPS in each square/rectangle
- Calculate the mean of all TPSs


TTF-1
PD-L1/CD8

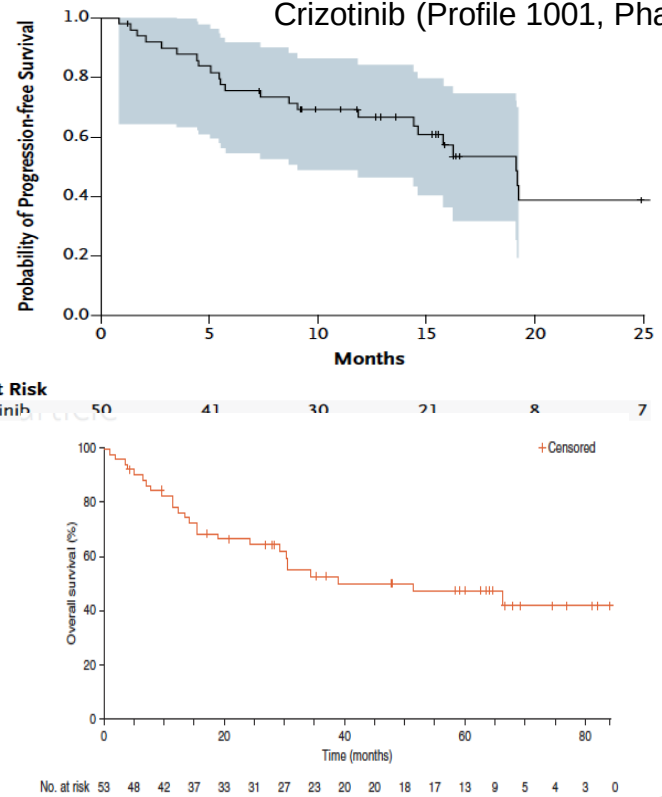
Points

- Multiple molecularly targeted therapies and immunotherapies have become available as 1st line treatment for advanced NSCLC patients
- Predictive biomarker testing during or following the diagnostic work-up has become an essential tool for the selection of patients for those therapies
- Optimizing the biomarker testing using often small tissue samples is of paramount importance for management of advanced NSCLC patients

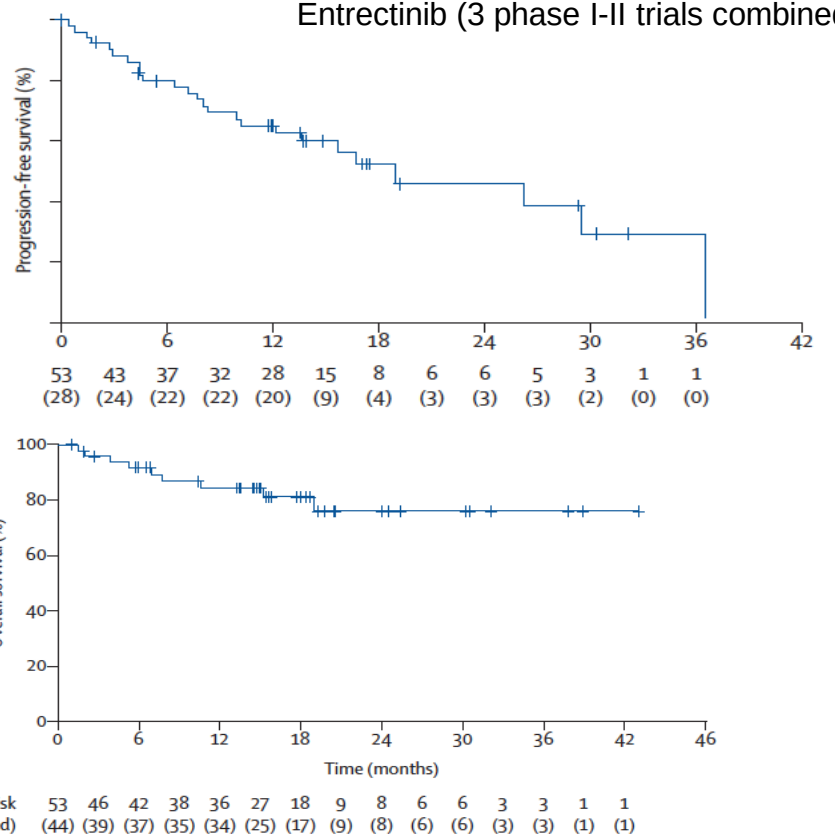
Thank you

ROS1 positive Advanced NSCLC

Crizotinib (Profile 1001, Phase I)

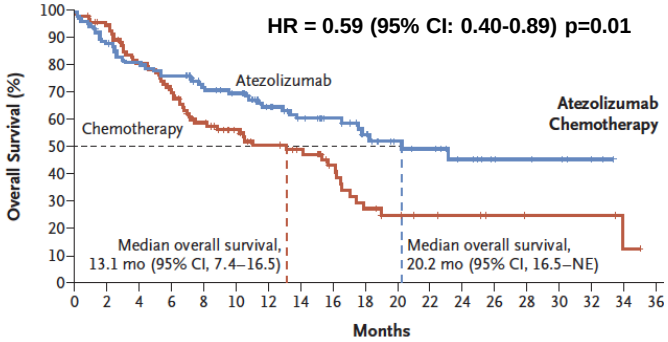


Entrectinib (3 phase I-II trials combined)



Clinical benefit of Atezolizumab in advanced (Stage IV) NSCLC

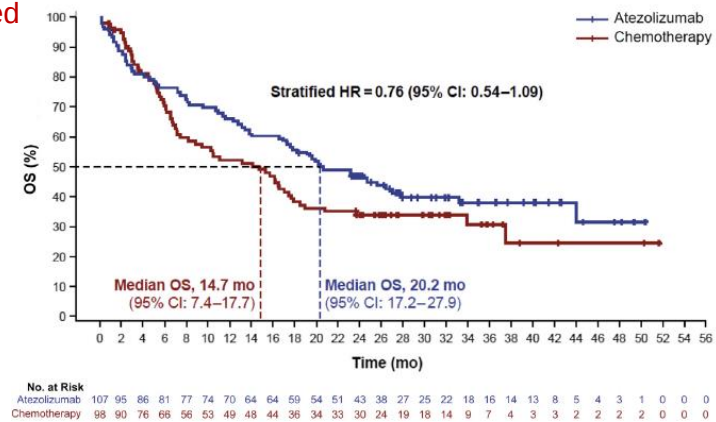
Impower110 PD-L1 high (TC3 or IC3) expression



No. at Risk

Atezolizumab	107	94	85	80	66	61	48	40	34	25	18	16	11	7	6	5	2
Chemotherapy	98	89	75	65	50	40	33	28	19	12	9	7	6	4	3	3	3	1

Updated



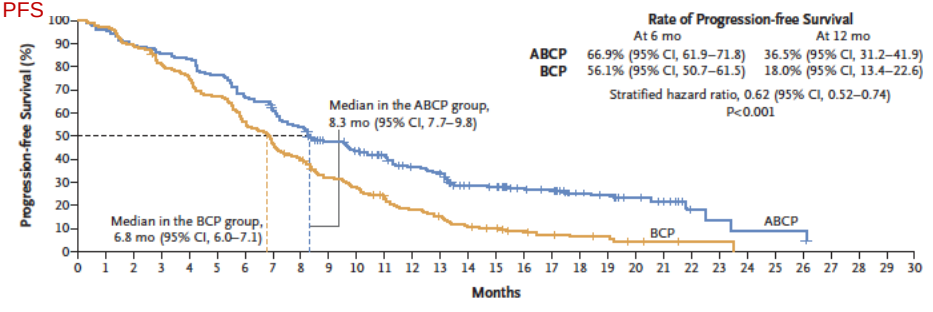
No. at Risk

Atezolizumab	107	95	86	81	77	74	70	64	64	59	54	51	43	38	27	25	22	18	16	14	13	8	5	4	3	1	0	0	0
Chemotherapy	98	90	76	66	56	53	49	48	44	36	34	33	30	24	19	18	14	9	7	4	3	3	2	2	2	2	0	0	0

Impower150 (+ Chemo) for non-squamous NSCLC

A: atezolizumab, B: bevacizumab, C: carboplatin, P: paclitaxel

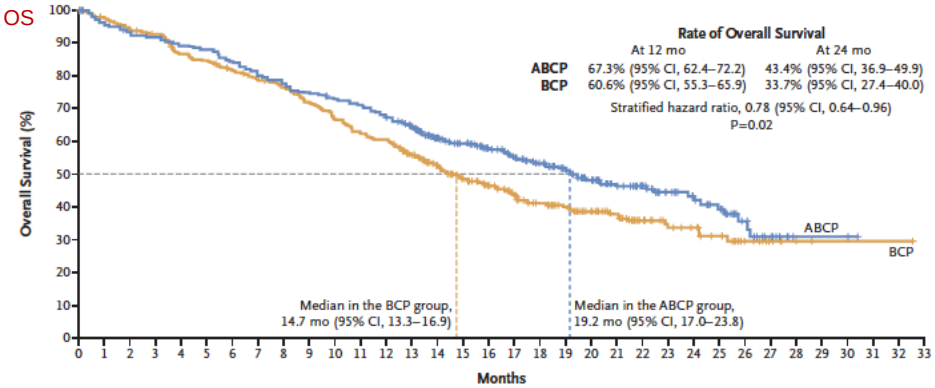
PFS



No. at Risk

ABCP	356	332	311	298	290	265	232	210	186	151	124	111	87	77	58	55	42	39	27	24	16	12	4	3	2	2	2
BCP	336	321	292	261	243	215	179	147	125	91	69	55	39	32	21	18	12	9	7	6	3	2	1	1			

OS

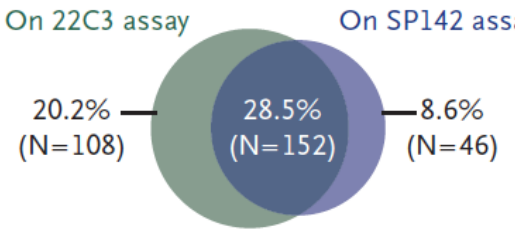


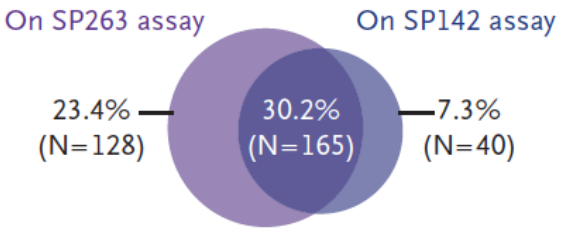
No. at Risk

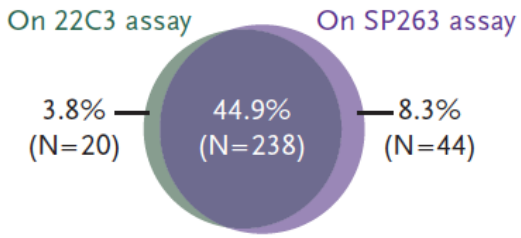
ABCP	359	339	328	323	314	310	296	284	273	264	256	250	235	218	188	167	147	133	119	103	84	66	57	41	34	28	16	9	2	2	2
BCP	337	326	315	308	287	280	268	255	247	233	216	203	196	174	152	129	115	101	87	77	66	56	40	32	29	22	13	6	3	1	1	1	1

China

- Approved therapeutic molecular targets: *EGFR* and *BRAF* mutations, *ALK*, *ROS1*, *RET*, and *NTRK* fusions, and *MET* exon 14 skipping mutations
- Testing methods
  - Mutations: PCR-based and NGS
  - Fusions: FISH, ARMS, PCR, NGS (RNA-based) and IHC (companion or screening)
- Molecular testing is routinely performed on surgical samples of NSCLC with non-squamous histology at stage IB or above
- PD-L1 detection is equally important and should be performed at the same time


On 22C3 assay
On SP142 assay
20.2%
(N=108)
28.5%
(N=152)
8.6%
(N=46)


On SP263 assay
On SP142 assay
23.4%
(N=128)
30.2%
(N=165)
7.3%
(N=40)


On 22C3 assay
On SP263 assay
3.8%
(N=20)
44.9%
(N=238)
8.3%
(N=44)

Consequences of False Positive and Negative Results

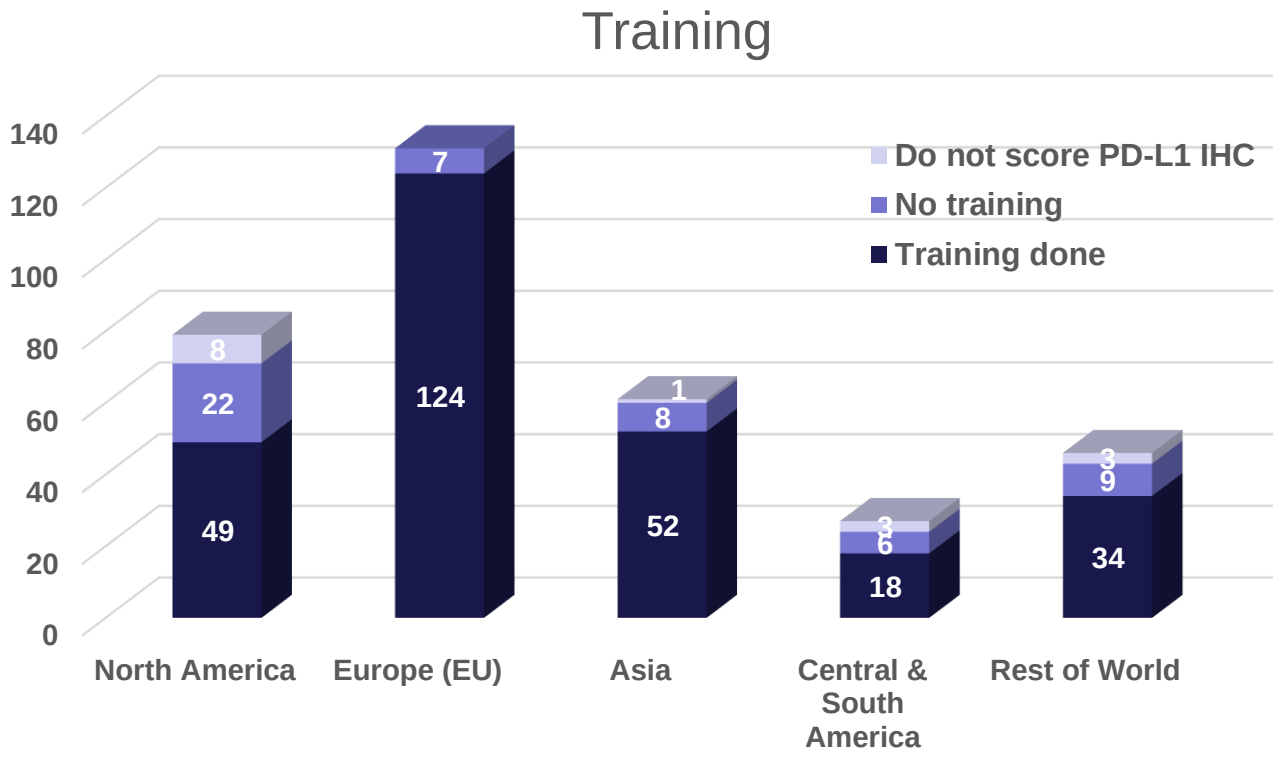
New lung cancer patients in 2018:
234,000
NSCLC: 198,900
Advanced: 113,000

KEYNOTE-001

TPS	<1%	1-24%	25-49%	50-74%	≥75%
Proportion	39%	31%	6.7%	8.6%	15%
ORR (1st line)	8.1%	12.9%	19.4%	29.6%	45.4%

- 10-20% of false positive results for the 50% cut-off could lead to treating 800 -1,500 patients with 1st line pembrolizumab only, for 80% of which additional chemotherapy might be helpful.
- 10-20% of false negative results for the 50% cut-off could lead to combination therapy in 1,000 - 2,000 patients, 30% of which would have responded to the 1st line pembrolizumab only (without having additional side effects due to chemotherapy).

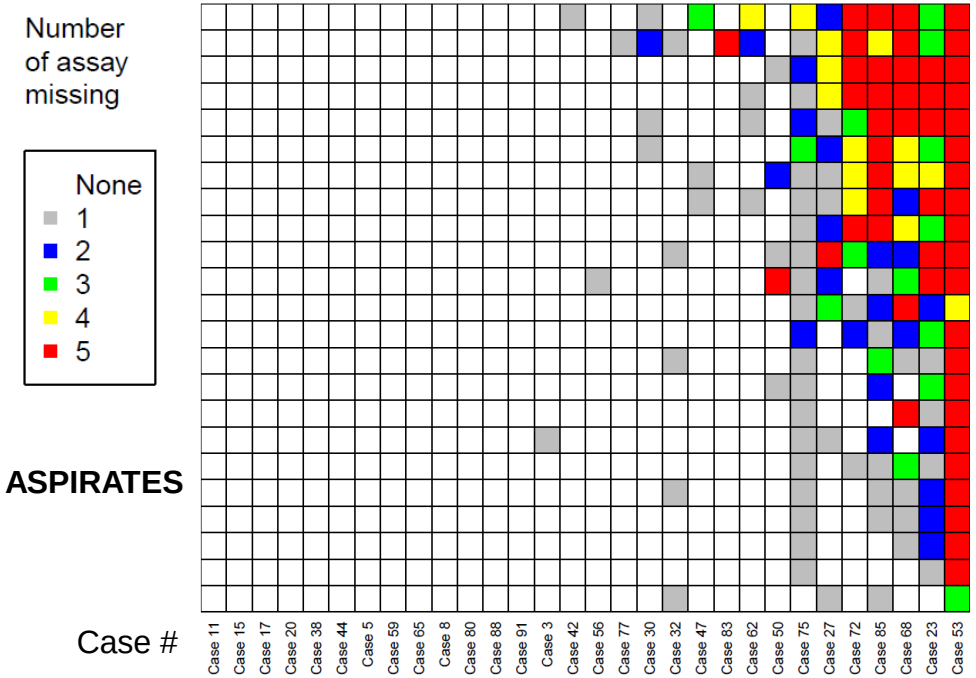
Garon, et al. N Engl J Med 2015;372:2018-28,
Reck M, et al. N Engl J Med 2016;375:1823-33



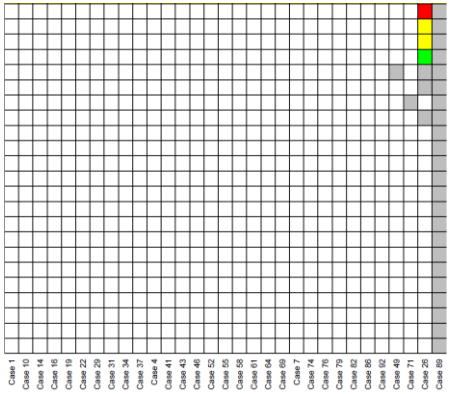
Training: 84% of participants have undergone some training.
The rate is lower in the North America (69.0%) and Central & South America (75.0%).

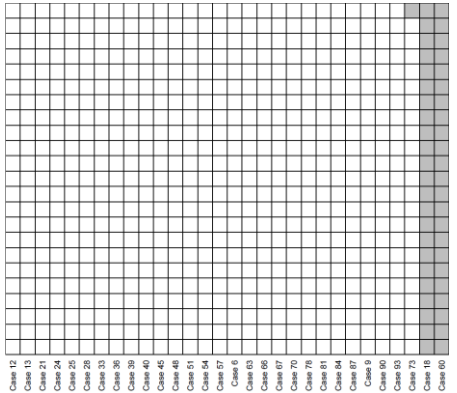
Blueprint phase 2A project

Cytology samples are often uninterpretable
(in particular, by non-cytopathologists)



Each row is one Reader n=23





About 13% of aspirate reads not possible; ≤1% of others

Courtesy of Dr. Keith Kerr