

ESMO ADVANCED COURSE ON BIOMARKERS FOR PRECISION MEDICINE:

Biomarkers for Immune Checkpoint Inhibitor Molecular Pathology

Paul Hofman, MD, PhD

Zürich, 28-29 November 2019

University Côte d'Azur and Inserm U1081 CNRS 7284, Nice, France



DISCLOSURE OF INTEREST



I herewith declare that,

I have the following potential conflict(s) of interest to report:

Receipt of grants/research supports:

Bristol-Myers Squibb

Receipt of honoraria or consultation fees:

Bristol-Myers Squibb, Thermo Fisher Scientific, Qiagen, Illumina, AstraZeneca, Roche, AbbVie, Pierre Fabre, Boehringer Ingelheim, Novartis

ROAD MAP



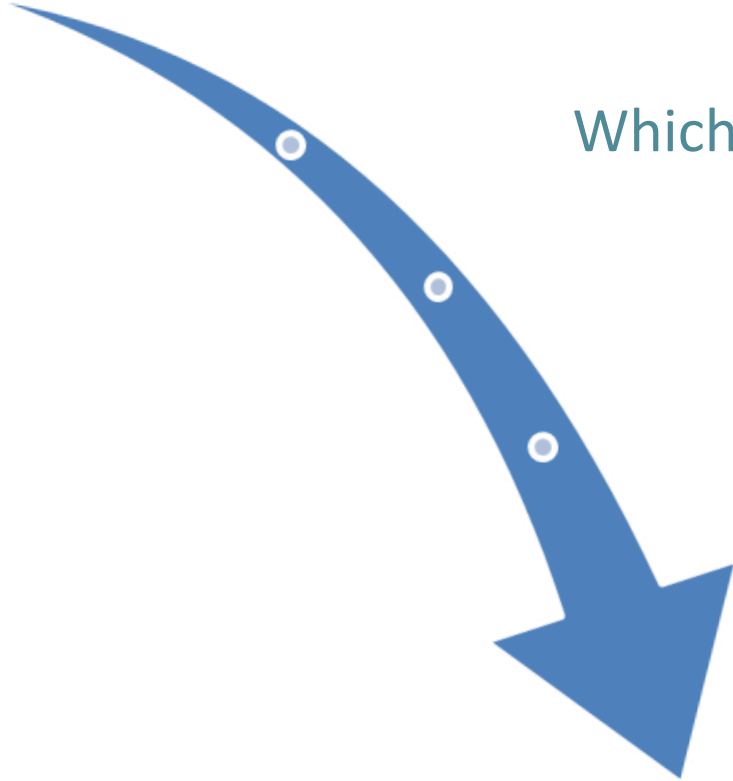
Brief background in thoracic oncology

Which samples and which limitations?

Which biomarkers?

Which technology ?

Take away message



ROAD MAP



Brief background in thoracic oncology

Which samples and which limitations?

Which biomarkers?

Which technology ?

Take away message

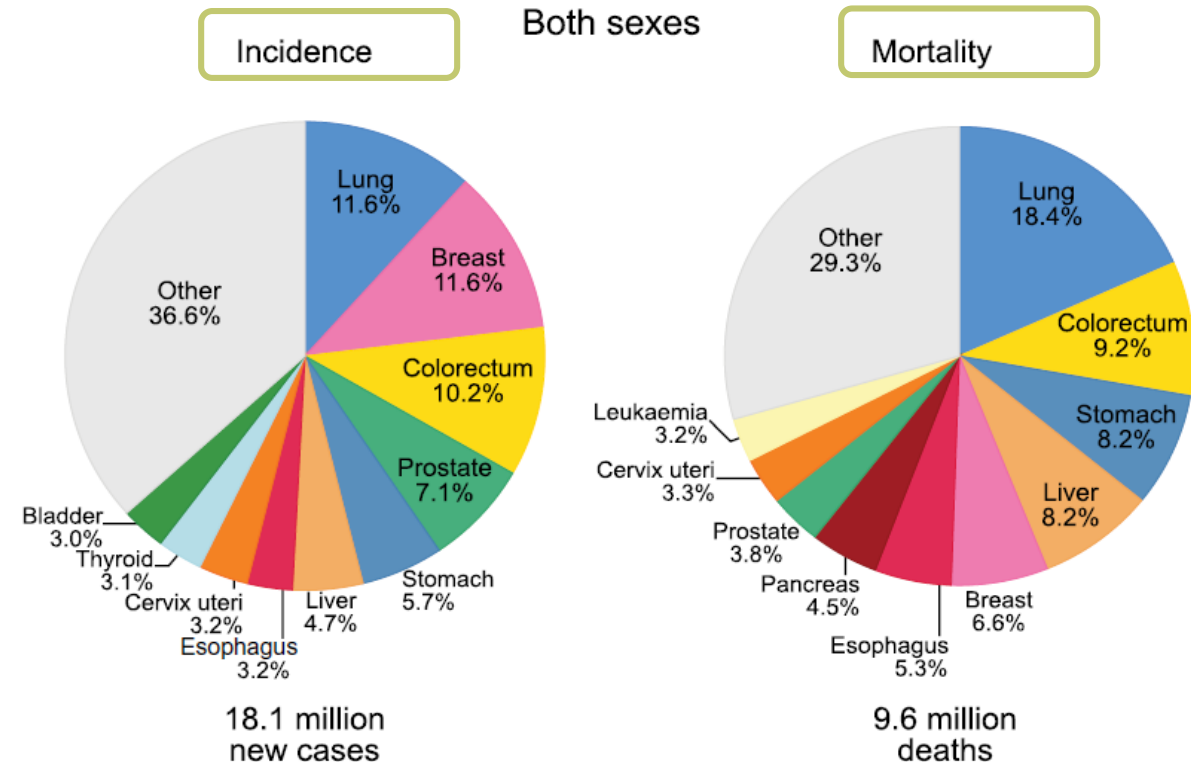
Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries

Freddie Bray, BSc, MSc, PhD¹; Jacques Ferlay, ME²; Isabelle Soerjomataram, MD, MSc, PhD³; Rebecca L. Siegel, MPH⁴; Lindsey A. Torre, MSPH⁵; Ahmedin Jemal, PhD, DVM⁶

Changing in lung cancer landscape?

Women/men
Aging
Pollutants

CANCER SITE	NO. OF NEW CASES (% OF ALL SITES)	NO. OF DEATHS (% OF ALL SITES)
Lung	2,093,876 (11.6)	1,761,007 (18.4)
Breast	2,088,849 (11.6)	626,679 (6.6)
Prostate	1,276,106 (7.1)	358,989 (3.8)
Colon	1,096,601 (6.1)	551,269 (5.8)
Nonmelanoma of skin	1,042,056 (5.8)	65,155 (0.7)
Stomach	1,033,701 (5.7)	782,685 (8.2)
Liver	841,080 (4.7)	781,631 (8.2)
Rectum	704,376 (3.9)	310,394 (3.2)
Esophagus	572,034 (3.2)	508,585 (5.3)
Cervix uteri	569,847 (3.2)	311,365 (3.3)
Thyroid	567,233 (3.1)	41,071 (0.4)
Bladder	549,393 (3.0)	199,922 (2.1)
Non-Hodgkin lymphoma	509,590 (2.8)	248,724 (2.6)



>2 million cases per year
>1.7 million deaths per year

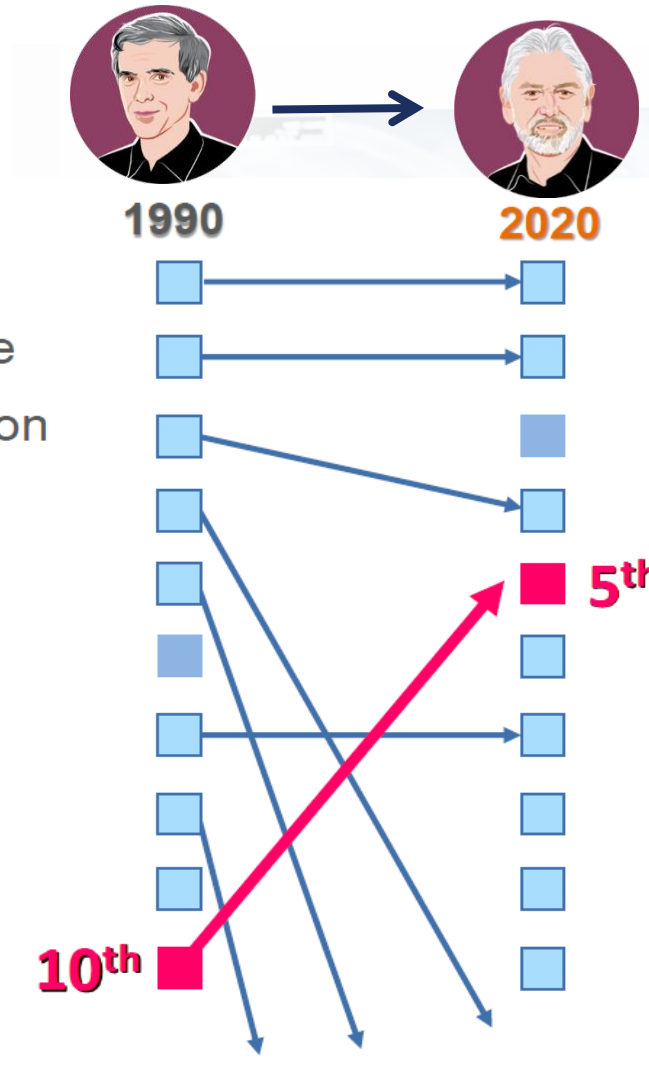
The main cancer killer

Aging and cancer
Two sides of the same coin



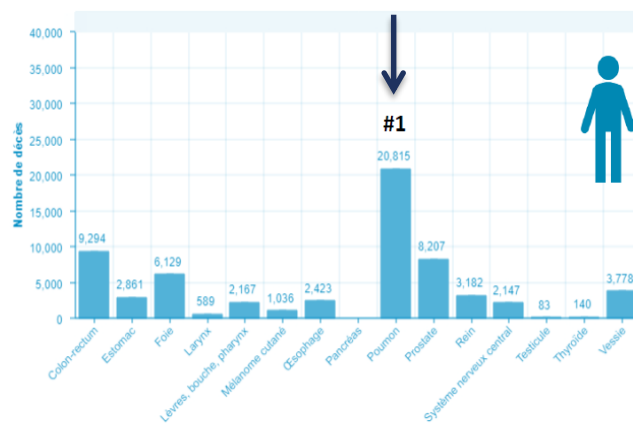
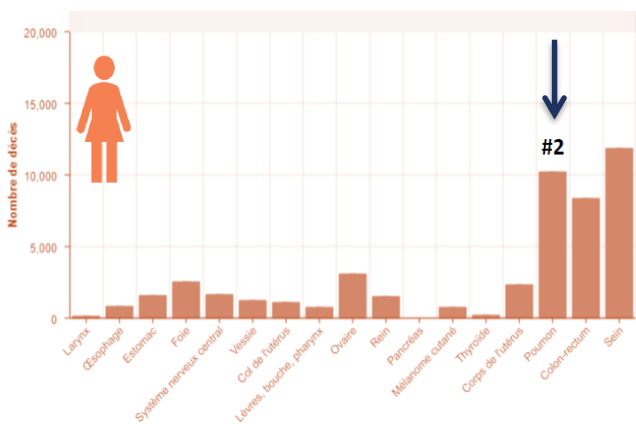
Ischemic heart disease
Cerebrovascular disease
Lower respiratory infection
Diarrheal disease
Perinatal disorders
COPD
Tuberculosis
Measles
Road traffic accidents

Lung cancer

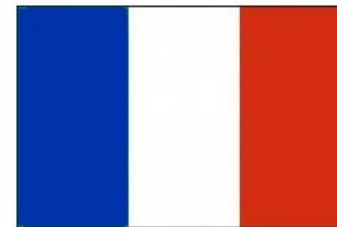




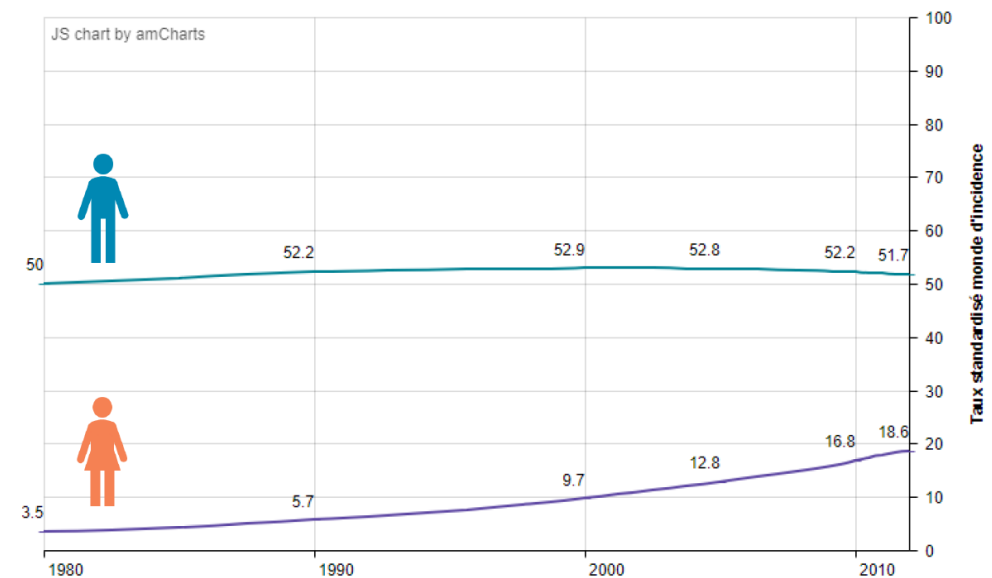
First cause of death cancer in France (> 35.000 deaths in 2018)



INCa, <http://lesdonnees.e-cancer.fr/> consulté le 24/01/2019



Global increase of incidence in France in 2018



INCa, <http://lesdonnees.e-cancer.fr/> consulté le 24/01/2019

Current therapeutic strategies in lung cancer

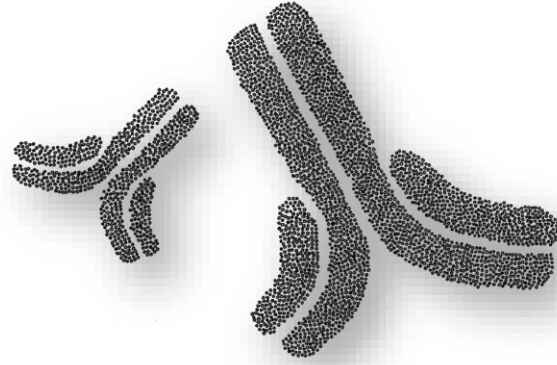
An urgent need to offer a personalized treatment for all patients



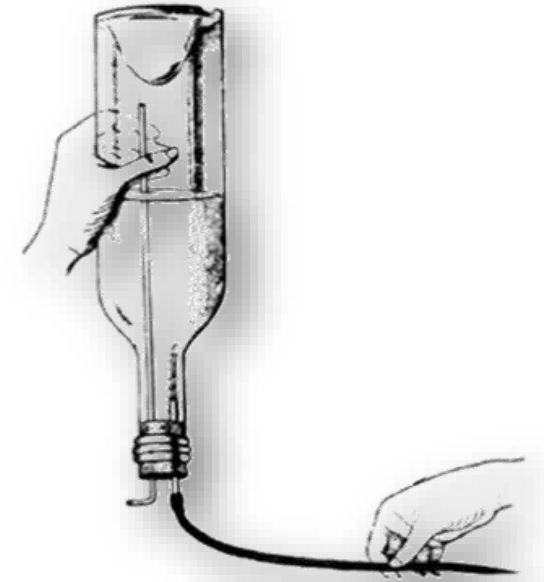
First line treatment in late stage NSCLC



Targeted therapy



Immunotherapy



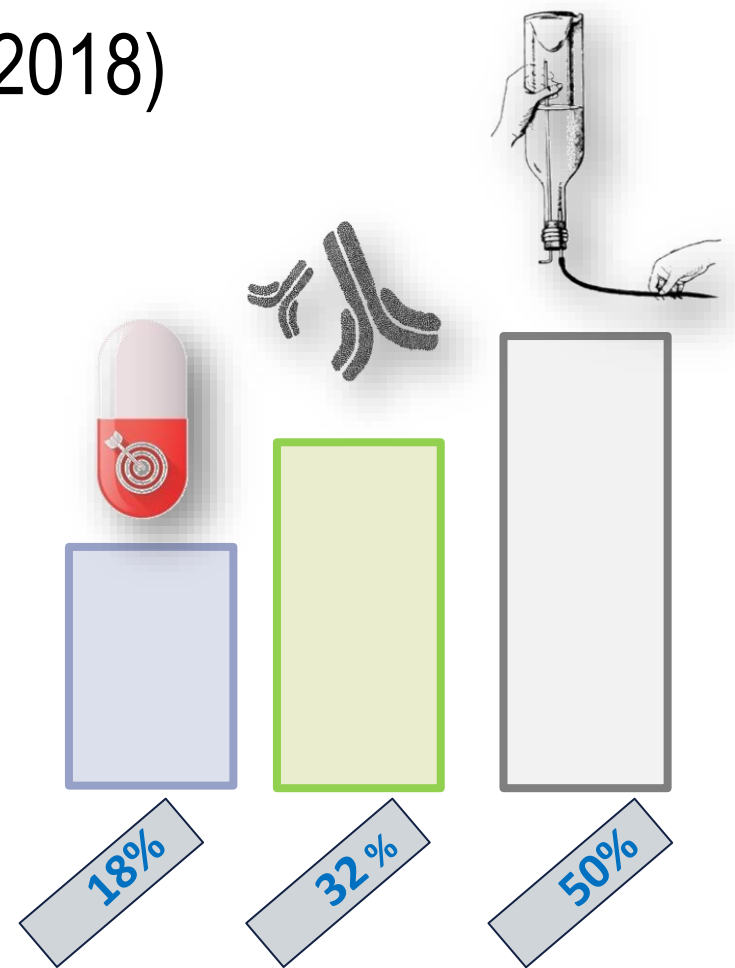
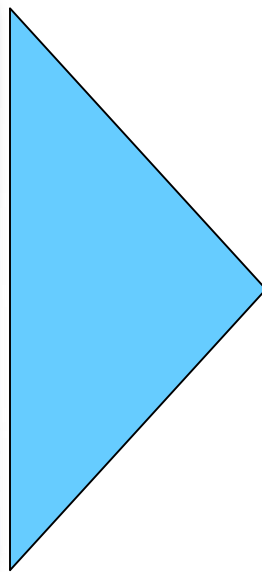
Chemotherapy

and/or

First line treatment in late stage NSCLC (2018)



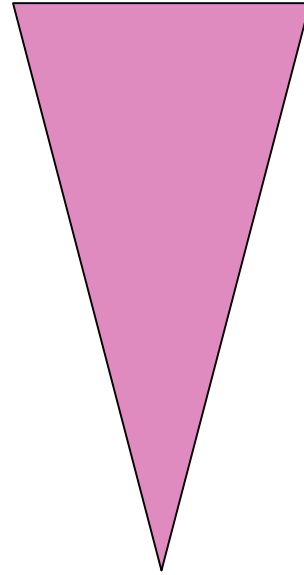
Challenge for precision
medicine



Less chemotherapy, more personalized treatment

Some patients (%?) do not get the « good » treatment due to:

- 1) **A long turnaround time** for getting the results and/or,
- 2) **A non appropriate testing** (then receive chemo in first line)



An urgent need for optimizing the process

Some patients (%?) do not receive the « good » treatment due to:

- 1) **A long turnaround time** for getting the results and/or,
- 2) **A non appropriate testing** (and receive chemo in first line)

In 2018 only 72 % of french patients with late stage lung adenocarcinoma
have been tested for EGFR

Urgent need for optimizing the process

ROAD MAP



Brief background in thoracic oncology

Which samples and which limitations?

Which biomarkers?

Which technology ?

Take away message

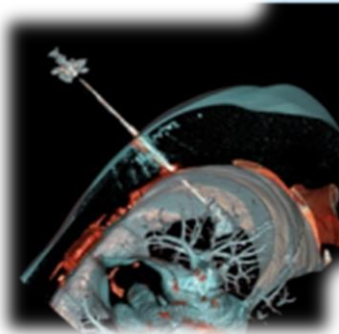
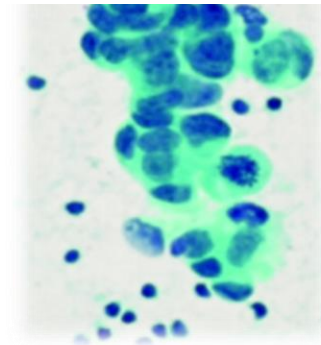


Surgical specimen

BAL/CSF/pleural fluids



Bronchial aspirates



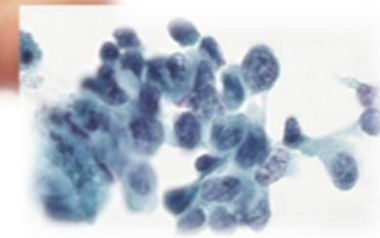
Bronchial biopsy/
Transthoracic Biopsy

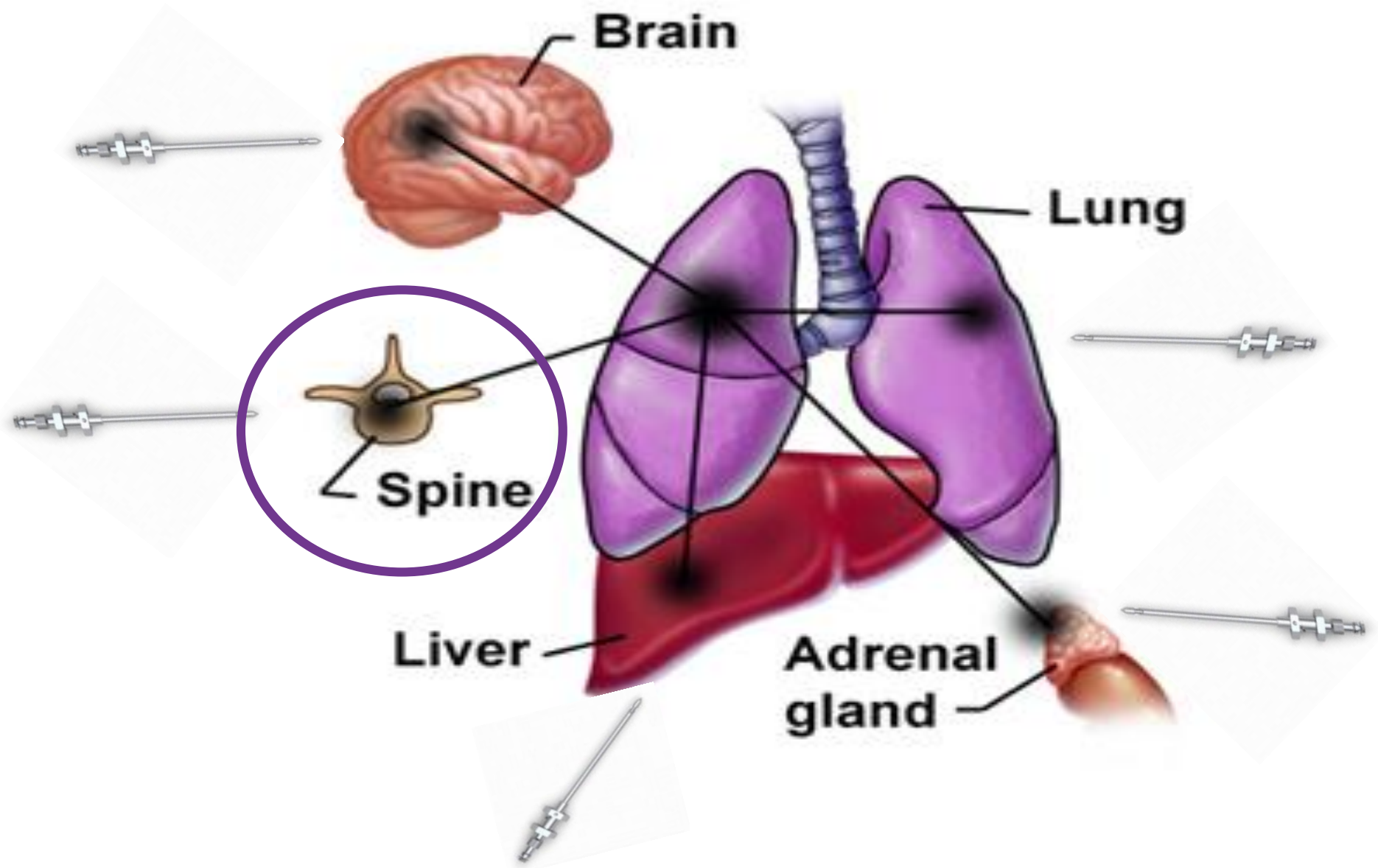


Blood



EBUS







The right drug

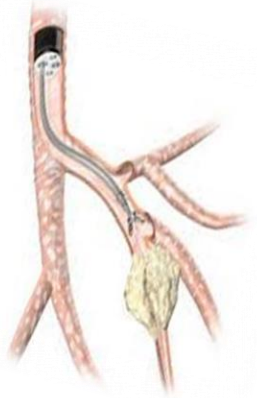
The right patient

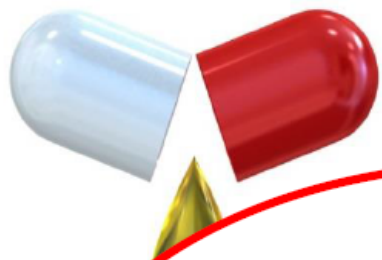
The right time

New therapies
in lung cancer patients

The right approach

The right cost





**The right turnaround
time**

t patient

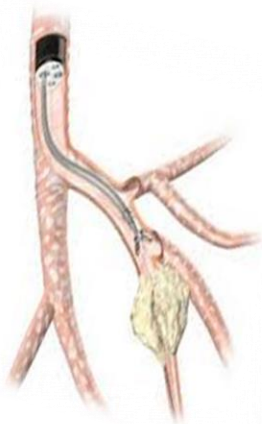
The right time

Stratified
in cancer

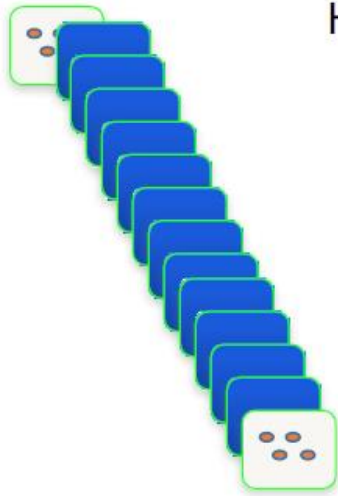
The right sample

The right approach

The right cost



More to do from small sample



HE first

Diagnostic stain TTF1

Diagnostic stain mucin

Diagnostic stain p63/p40

Predictive stain ALK IHC

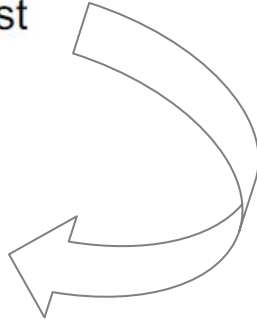
Predictive stain ROS1 IHC

Predictive stain PD-L1 IHC, etc

DNA isolation EGFR

DNA isolation

HE last



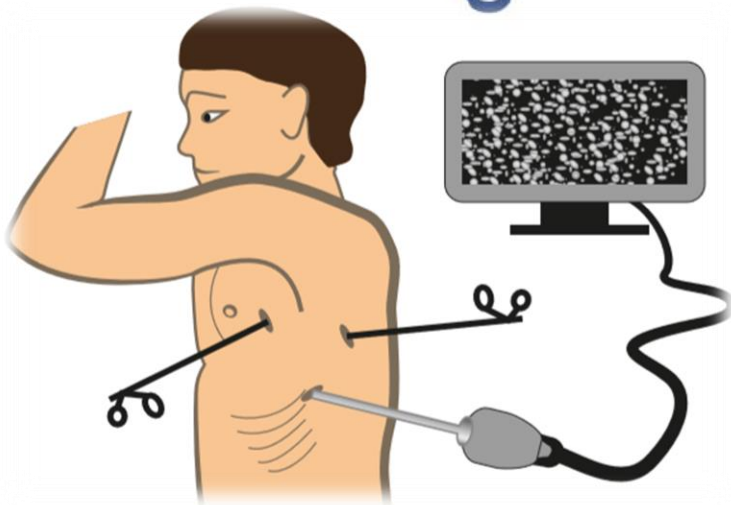
NGS

- miseq 40-250 ng
- Ion torrent 10 ng



Bioresource available

Number of biomarkers



Lung cancer and sampling management



Controversies on Lung Cancer: Pros and Cons

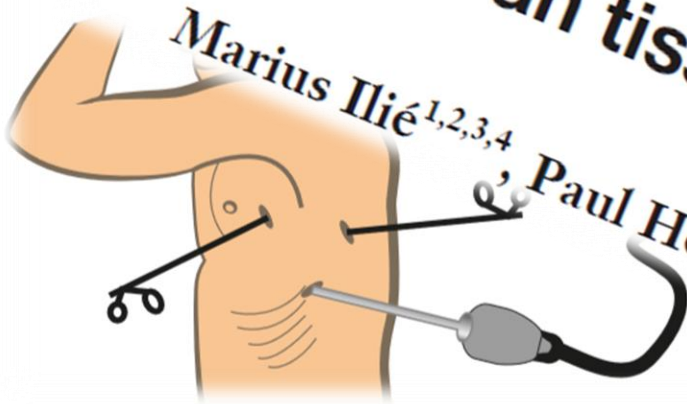
Pros: Can tissue biopsy be replaced by liquid biopsy?

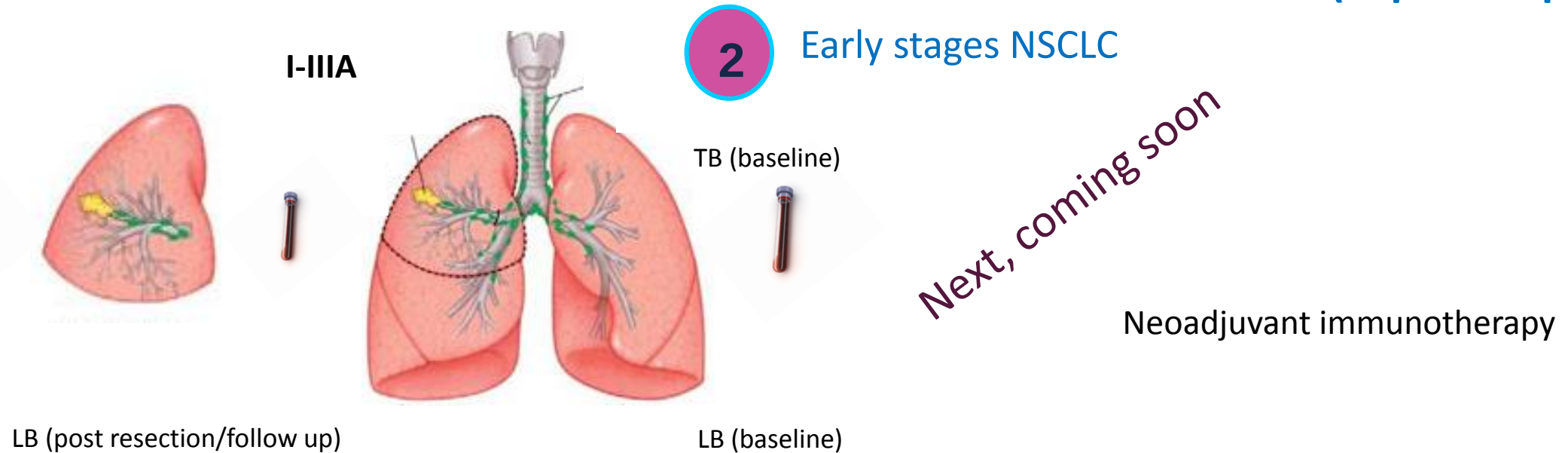
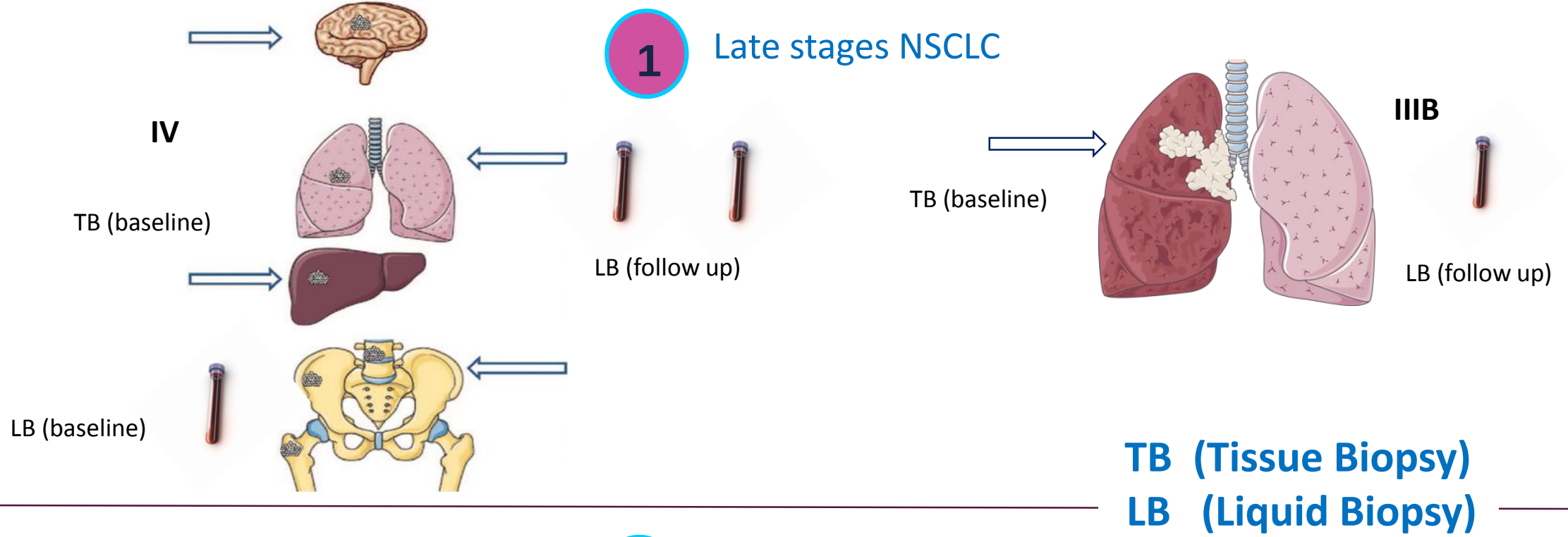
Marius Ilić^{1,2,3,4}, Paul Hofman^{1,2,3,4}

Transl Lung Cancer Res 2016;5(+):420-423

Lung cancer and

gement





ROAD MAP



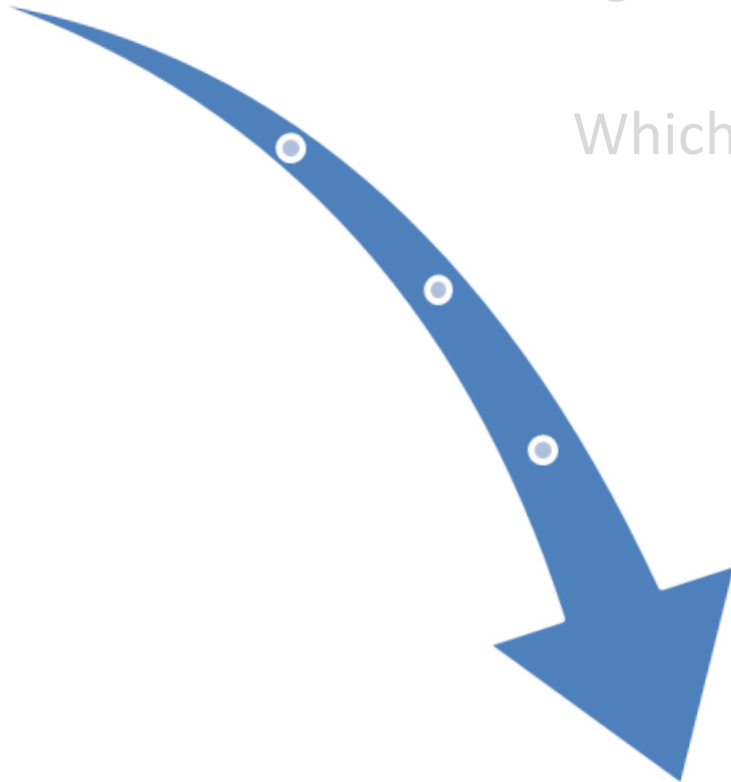
Brief background in thoracic oncology

Which samples and which limitations?

Which biomarkers?

Which technology ?

Take away message



Metastatic Non-Small-Cell Lung Cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up

Updated version published 18 September 2019 by the ESMO Guidelines Committee

[view details](#)



Published 18 September 2019 by the ESMO Guidelines Committee

[Download the current version from esmo.org \(PDF\)](#)

Published in 2018 – Ann Oncol (2018) 29 (suppl 4): iv192–iv237

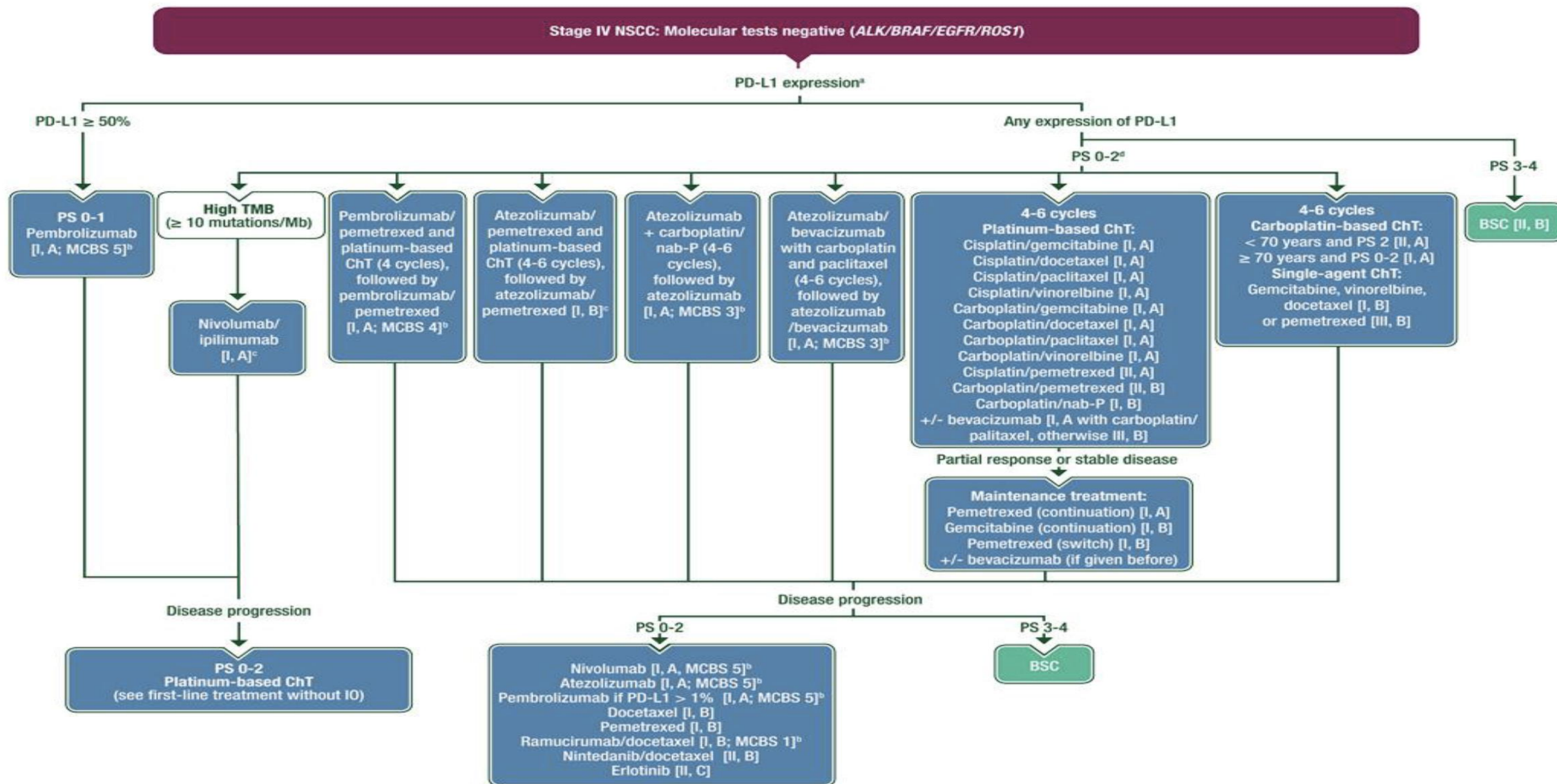
[Download the original version from Annals of Oncology \(PDF\)](#)

CLINICAL PRACTICE GUIDELINES

Metastatic non-small cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up[†]

Originally published in 2018 – Ann Oncol (2018) 29 (suppl 4): iv192–iv237

D. Planchard¹, S. Popat², K. Kerr³, S. Novello⁴, E. F. Smit⁵, C. Faivre-Finn⁶, T. S. Mok⁷, M. Reck⁸, P. E. Van Schil⁹, M. D. Hellmann¹⁰ & S. Peters¹¹, on behalf of the ESMO Guidelines Committee*



Molecular Testing Guideline for the Selection of Patients With Lung Cancer for Treatment With Targeted Tyrosine Kinase Inhibitors: American Society of Clinical Oncology Endorsement of the College of American Pathologists/ International Association for the Study of Lung Cancer/ Association for Molecular Pathology Clinical Practice Guideline Update

For advanced or metastatic lung adenocarcinoma: It is mandatory that all patients need, **1)** to have testing for **EGFR** and **BRAF** mutation, **ALK** and **ROS1** rearrangements, and **PD-L1** expression to predict response to **EGFR**, **ALK**, **ROS1** or **BRAF** targeted inhibitors or immunotherapy, respectively, and, **2)** to get results **in ten working days**

*Other biomarkers are under investigation as predictors of response to targeted therapies, in particular **Her2** mutation, **RET** and **NTRK** rearrangements and **MET** splice mutations and amplification.

For advanced or metastatic lung squamous carcinoma: **PD-L1** IHC is indicated to select patients for immunotherapy in the first line.

Stages IIIB/IV non squamous cell carcinoma of the lung



10 working days

Mandatory

PD-L1 IHC
ALK IHC and/or *ALK* FISH
ROS1 IHC then *ROS1* FISH
EGFR mutations
BRAFV600 mutations

Recommended

HER2 mutation
NTRK rearrangement
RET rearrangement
MET mutation
MET amplification

Large gene panel

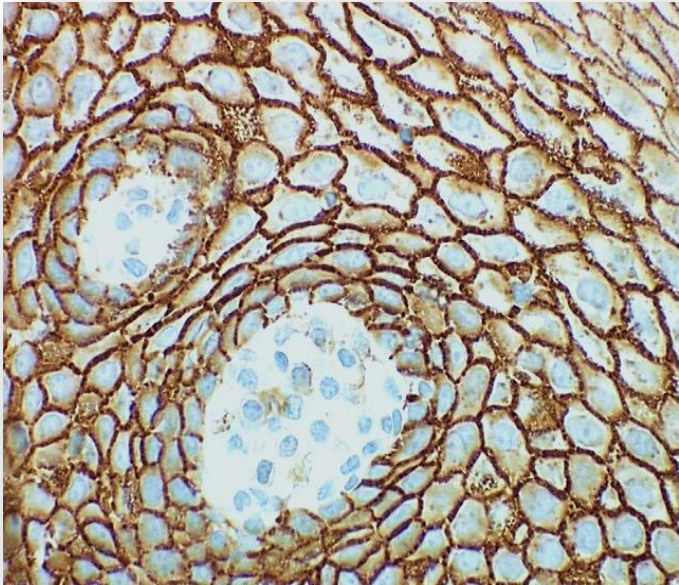
Stages IIIB/IV squamous cell carcinoma of the lung

PD-L1 IHC

IHC PD-L1

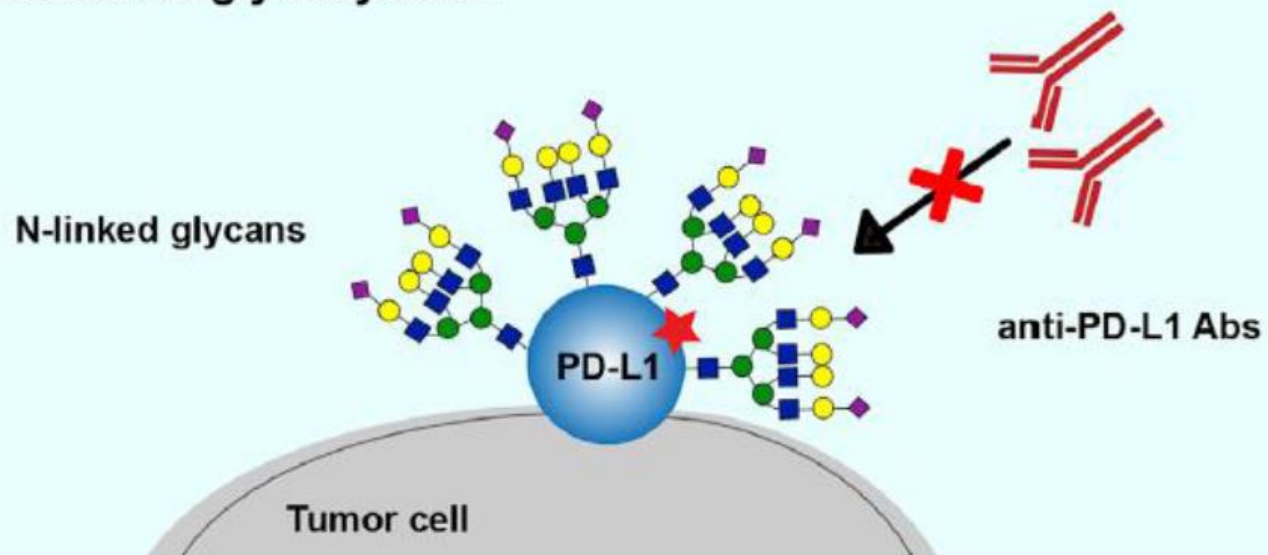
The **only validated test** as a predictive biomarker of I-O first line

Main issues

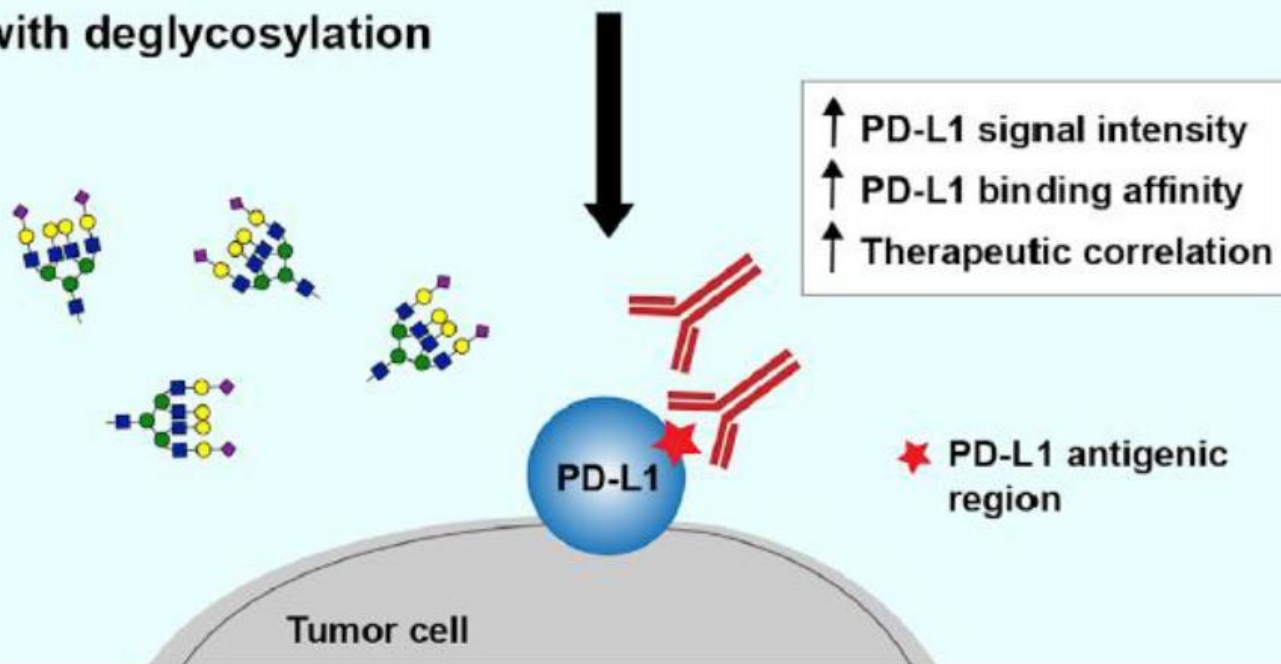


- Patients with 0% of PD-L1 TC positivity can be good responders
- Patients with $\geq 50\%$ of PD-L1 TC positivity can be non responders
- Heterogeneity of PD-L1 staining limits its interpretation in small biopsies
- Inter & intra operator variability in PD-L1 IHC assessment
- Many PD-L1 clones, different devices, different performances
- Many cut off ($>1\%$, $> 25\%$, $\geq 50\%$) according to the anti-PD1/PD-L1 trt
- Clinical value of positive immune cells for PD-L1 is debated in NSCLC
- N-linked glycosylation of PD-L1 hinders its recognition by PD-L1 antibodies

without deglycosylation



with deglycosylation



The beginning of the end?

CellPress

Cancer Cell
Article

Removal of N-Linked Glycosylation Enhances PD-L1 Detection and Predicts Anti-PD-1/PD-L1 Therapeutic Efficacy

Heng-Huan Lee,^{1,14} Ying-Nai Wang,^{1,14} Weiya Xia,¹ Chia-Hung Chen,^{2,3} Kun-Ming Rau,^{4,5} Leiguang Ye,⁶ Yongkun Wei,¹ Chao-Kai Chou,¹ Shao-Chun Wang,⁷ Meisi Yan,^{1,8} Chih-Yen Tu,^{2,3} Te-Chun Hsia,^{2,3} Shu-Fen Chiang,⁹ K.S. Clifford Chao,⁹ Ignacio I. Wistuba,¹⁰ Jennifer L. Hsu,^{1,7,11} Gabriel N. Hortobagyi,¹² and Mien-Chie Hung^{1,7,11,13,15,*}

Cancer Cell 36, 168–178, August 12, 2019 © 2019 Elsevier Inc.

In the real world, how viable an option is first line immunotherapy in NSCLC according to the PD-L1 IHC status ?

- Patients with tumors $\geq 50\%$ PD-L1: 30% patients
- Patients with poor PS not eligible for treatment: 34% patients
- Patients with either *EGFR* mut or *ALK/ROS1* fusion: 18% patients
- Patients with preexisting autoimmune disease: 13% patients

Patients currently eligible for immunotherapy first line in clinical routine practice:

Around 10% of stage IIIB/IV NSCLC patients

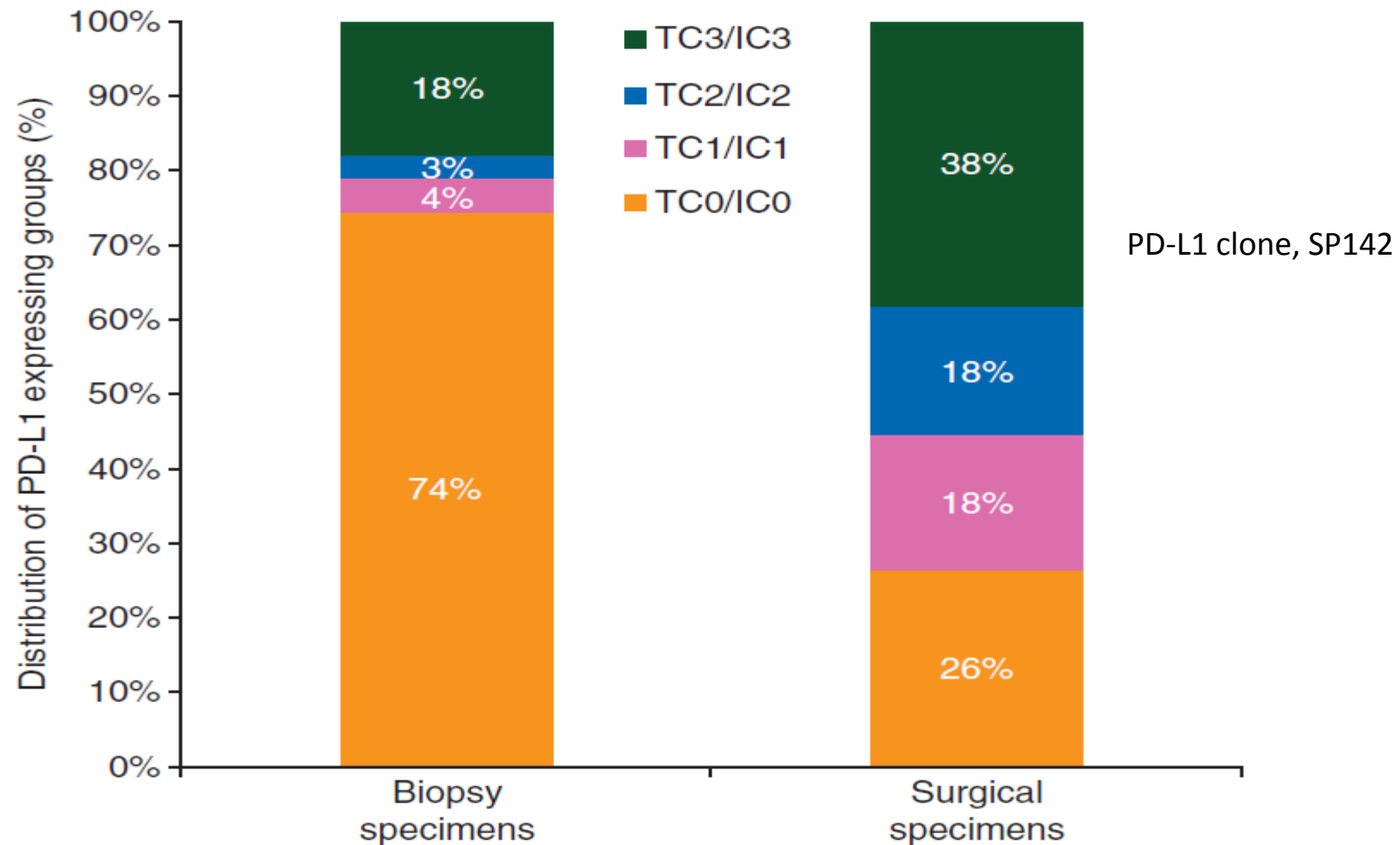
Thunnissen E et al. Programmed death-ligand 1 expression influenced by tissue sample size. Scoring based on tissue microarrays' and cross-validation with resections, in patients with, stage I-III, non-small cell lung carcinoma of the European Thoracic Oncology Platform Lungscope cohort. ***Mod Pathol.* 2019 Nov 18.**

Abstract

Oncology Platform Lungscope non-small cell lung carcinoma cohort to explore this issue. PD-L1 expression was assessed via immunohistochemistry on tissue microarrays (up to four cores per case), using the **DAKO 28-8** immunohistochemistry assay, following a two-round external quality assessment procedure. At the two external quality assessment rounds, **tissue microarray scoring agreement rates between pathologists were: 73% and 81%**. There were 2008 cases with valid immunohistochemistry tissue microarray results (50% all cores evaluable). Concordant cases at 1, 25, and 50% were: 85, 91, and 93%. Tissue microarray core results were identical for 70% of cases. **Sensitivity of the tissue microarray method for 1, 25, and 50% was: 80, 78, and 79% (specificity: 90, 95, 98%). Complete agreement between tissue microarrays and whole sections was achieved for 60% of the cases.** Highest sensitivity rates for 1% and 50% cutoffs were detected for higher number of cores. **Underestimation of PD-L1 expression on small samples is more common than overestimation.** **We demonstrated that classification of PD-L1 on small biopsy samples does not represent the overall expression of PD-L1 in all non-small cell cancer carcinoma cases**, although the majority of cases are 'correctly' classified. In future studies, sampling more and larger biopsies, recording the biopsy size and tumor load may permit further refinement, increasing predictive accuracy.

Comparative study of the PD-L1 status between surgically resected specimens and matched biopsies of NSCLC patients reveal major discordances: a potential issue for anti-PD-L1 therapeutic strategies

M. Ilie^{1,2}, E. Long-Mira^{1,2}, C. Bence¹, C. Butori¹, S. Lassalle^{1,2}, L. Bouhlel^{2,3}, L. Fazzalari², K. Zahaf¹, S. Lalvée¹, K. Washetine⁴, J. Mouroux^{2,5}, N. Vénissac⁵, M. Poudenx³, J. Otto⁶, J. C. Sabourin⁷, C. H. Marquette^{2,3}, V. Hofman^{1,2,4} & P. Hofman^{1,2,4*}



CDx
IVDs

Analytical validation
Clinical validation
(clinical trials)

This way

That way

LDTs

Available platforms
Less expensive
Harmonization across disease sites

I-O & Chemotherapy in first line for non squamous cell carcinoma of the lung

Patients wild type for *EGFR/ALK(&ROS1/BRAFV600)*

I-O in first line for non squamous cell carcinoma of the lung

Patients wild type for *EGFR/ALK/ROS1/BRAFV600*
and with > 50% of tumor cells positive for PD-L1

ROAD MAP



Brief background in thoracic oncology

Which samples and which limitations?

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Which technology ?

Take away message



IHC versus FISH (versus RNA seq)



***ALK* status:** ALK IHC +++ **stop**; ALK IHC + or ++ **go to ALK FISH**
***ROS1* status:** ROS1 + to +++ go to *ROS1* FISH



Targeted *versus* (large) panel sequencing?



Specific-based PCR *versus* Next Generation Sequencing

According to :
Urgent need
Technology available
Sample quantity/quality

Laboratory of Clinical and Experimental Pathology (Nice, Fr)

Targeted Sequencing

Pyromark24



COBAS 480



Rotor-Gene Q



Digital PCR



Idylla



Next Generation Sequencing

Thermo S5



Genereader (1.1)



Miseq



IHC + FISH



Tissue TAT < 3 days



Tissue TAT < 4 days

Targeted EGFR

COBAS 480



Idylla



Plasma TAT < 24h



Tissue TAT < 24h

NGS

Thermo S5



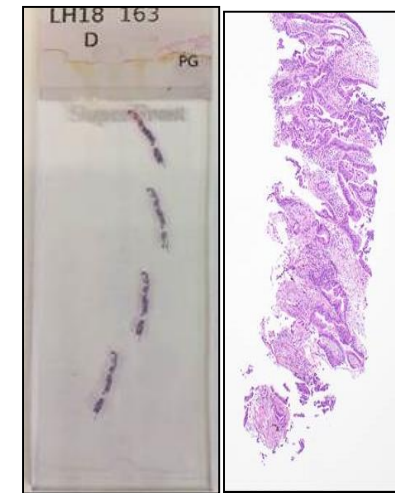
Genereader (1.1)



Plasma TAT ~ 3 days



Tissue TAT ~ 10 days

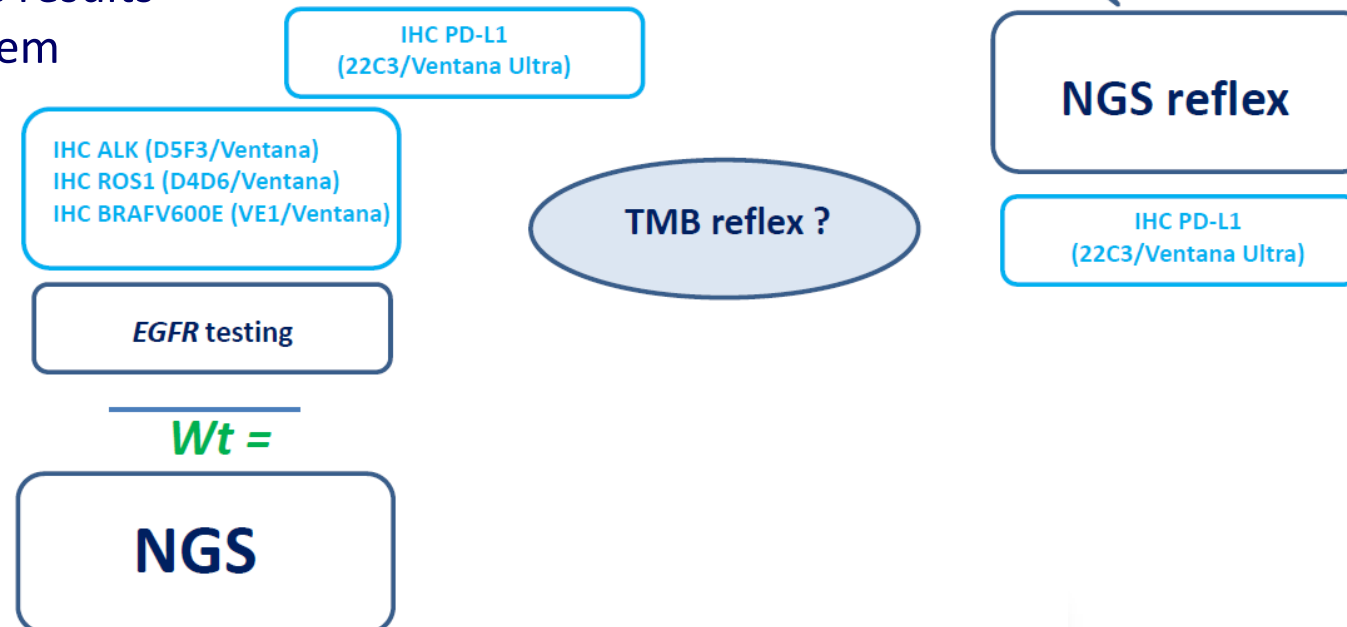


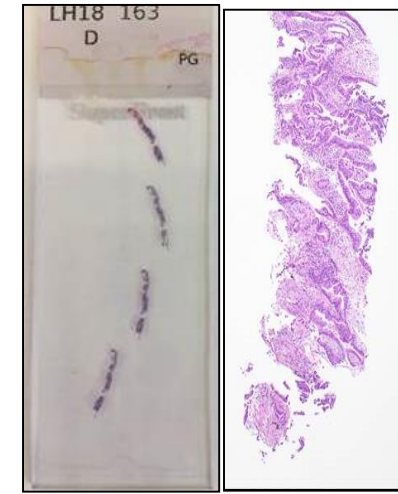
Different strategies according to:



- The « quality » of the biopsies
- The urgent need for getting the results
- The budget and economic system

Immuno-molecular testing algorithms



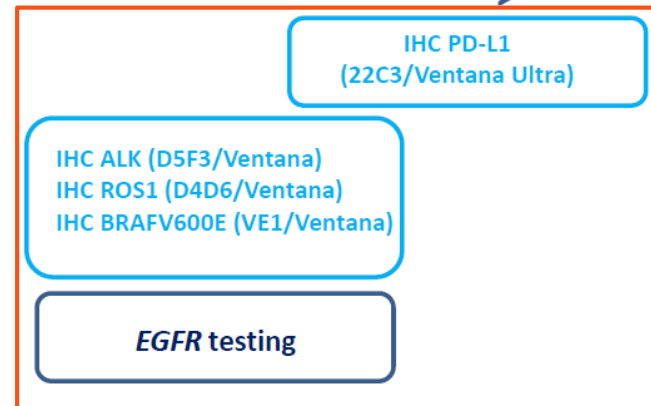


Different strategies according to:

- The quality of the biopsies
- The urgent need for getting the results
- The budget and economic system

Immuno test
Molecular testing

Immuno-molecular testing algorithms



How to optimize?

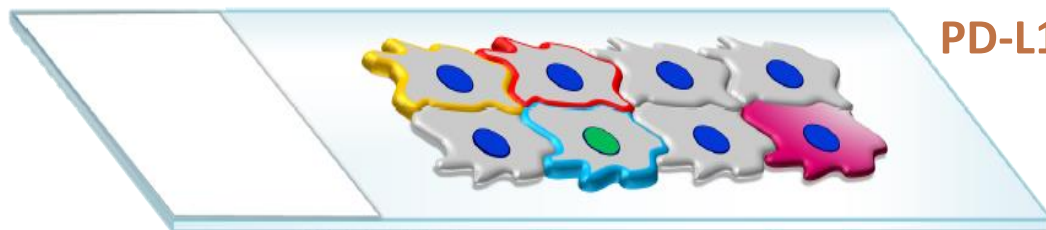
Multiplex IHC

Diagnosis
biomarkers

TTF1
P40
Keratin

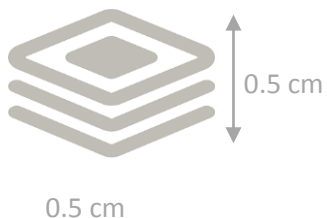
Predictive
biomarkers

ALK
ROS1
BRAF
PD-L1



From

bronchial
biopsy



7 slides

To
2 slides

Save 5 tissue sections

MIDI panel

TTF1

p40

PD-L1

Keratin

+

+

+

LADC

LSCC

CI MT

MIMP panel

ALK

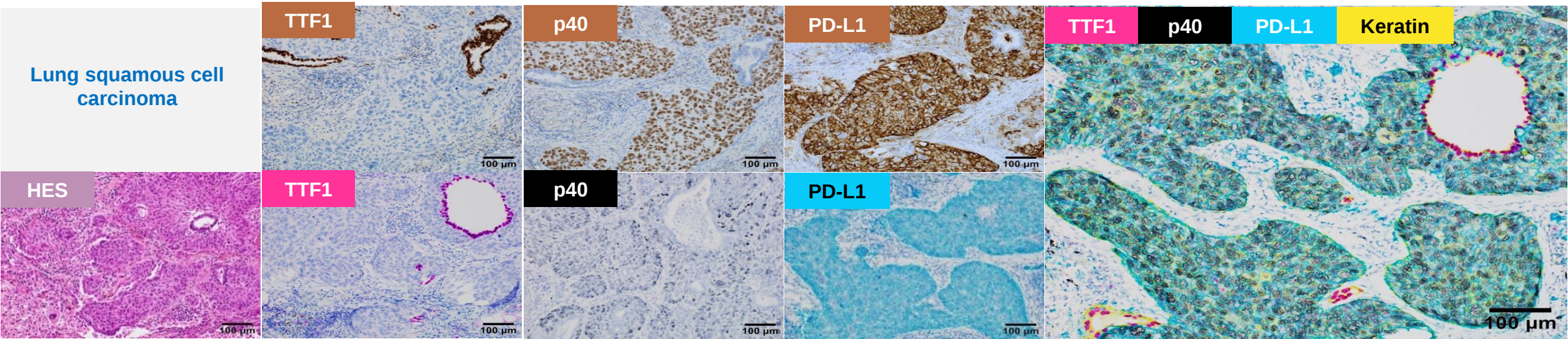
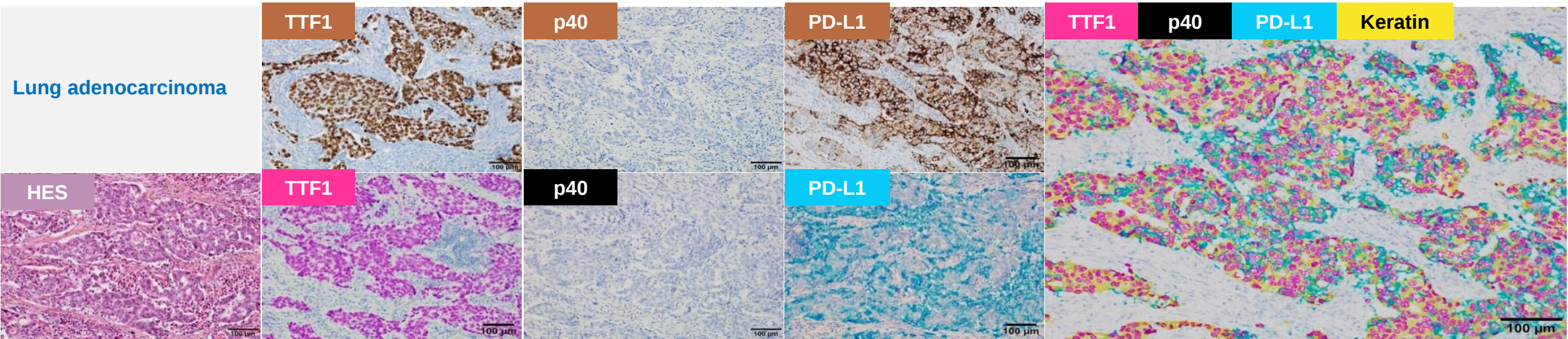
ROS1

BRAF

EGFR testing



5 mandatory biomarkers obtained
in 3 working days following the biopsy



ALK positive
lung adenocarcinoma

ALK

ROS1

BRAF

ALK

ROS

BRAF

HES

ALK

ROS1

BRAF

100 µm

ROS1 positive
lung adenocarcinoma

ALK

ROS1

BRAF

ALK

ROS

BRAF

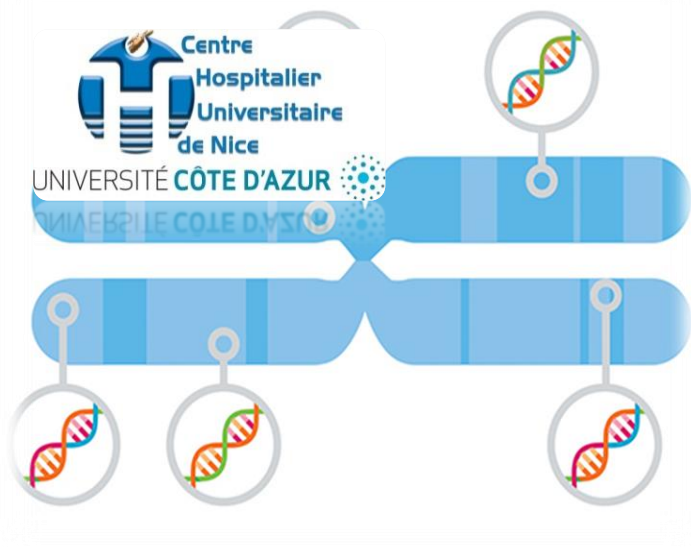
HES

ALK

ROS1

BRAF

100 µm



VOLUME 36 • NUMBER 30 • OCTOBER 20, 2018

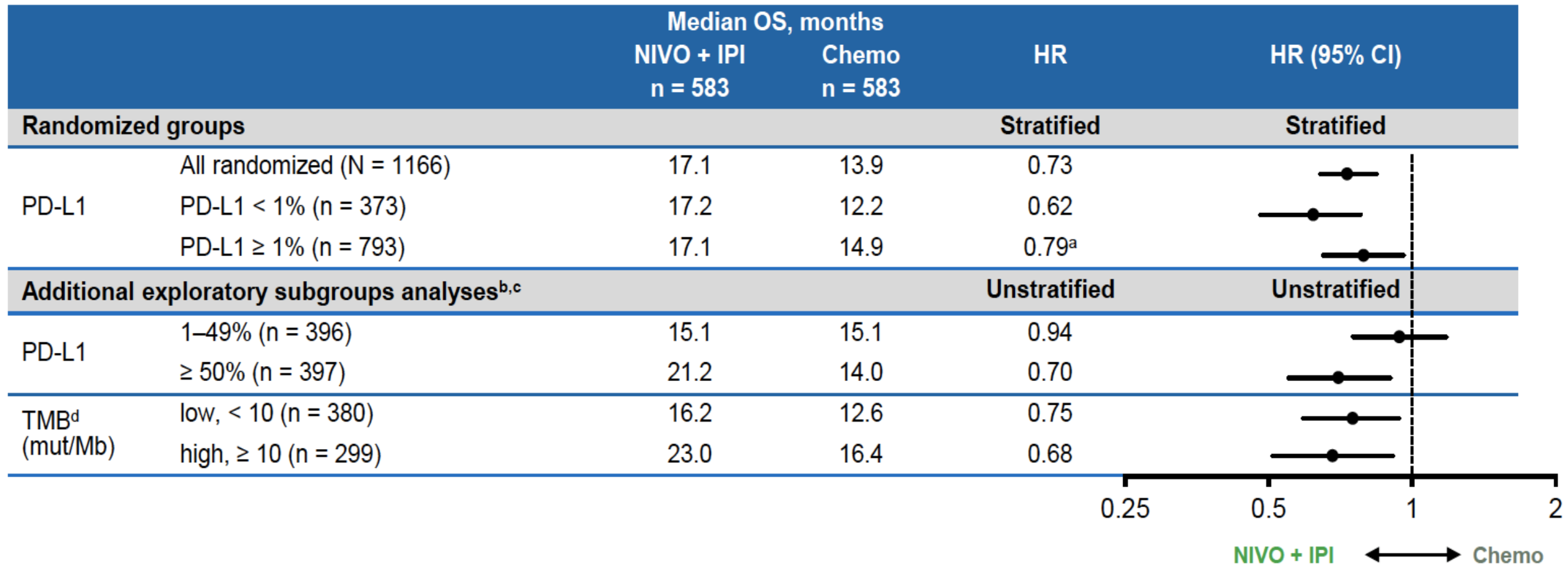
JOURNAL OF CLINICAL ONCOLOGY

EDITORIAL

Tumor Mutation Burden: Is It Ready for the Clinic?

Yasushi Goto, National Cancer Center Hospital, Tokyo, Japan

Tissue Tumor Mutation Burden 1L (CM227)



Does Tumor Mutational Burden dead...or not ?

Some similar challenges than for PD-L1 IHC evaluation:

- Needs for comparing the different available commercially panels (sensitivity & specificity)
- Needs for doing intra laboratory assessment
- Needs for doing inter laboratory assessment
- Needs to get an accreditation for using TMB in routine clinical practice
- Needs to evaluate the costs
- Needs to assess the turnaround time for getting results

But:

- Different panels (size, number and type of genes and mutations)
- Different approaches (hybrid capture, amplicon based sequencing)
- Different devices
- Different turnaround times according to the sequencing system
- Different costs

ROAD MAP



Brief background in thoracic oncology

Which samples and which limitations?

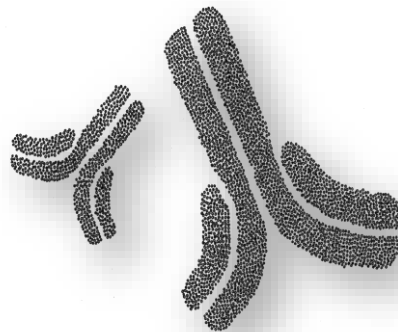
Which biomarkers?

Which technology ?

Take away message



Which perspectives?



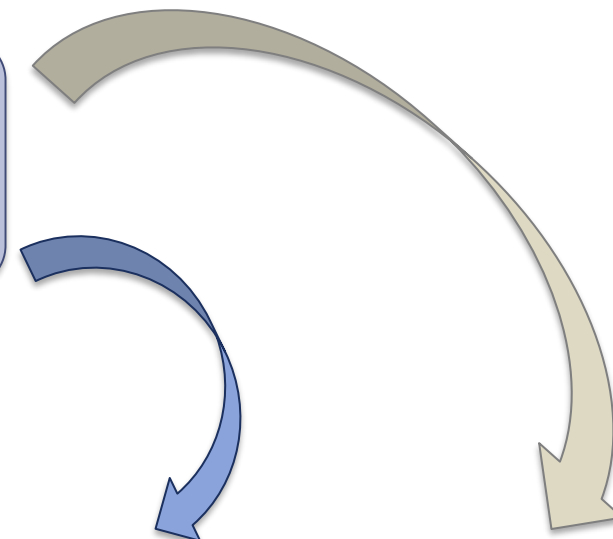
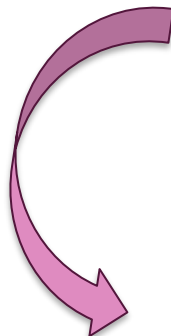
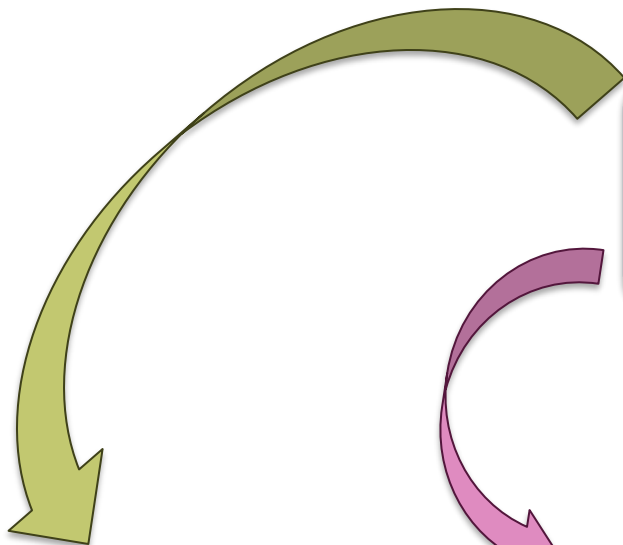
Biomarkers

Response

Resistance

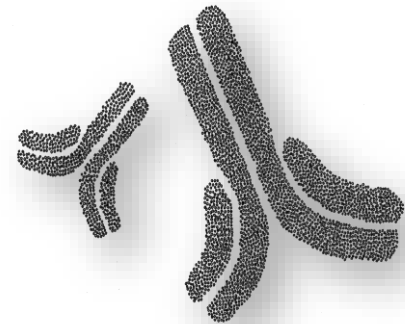
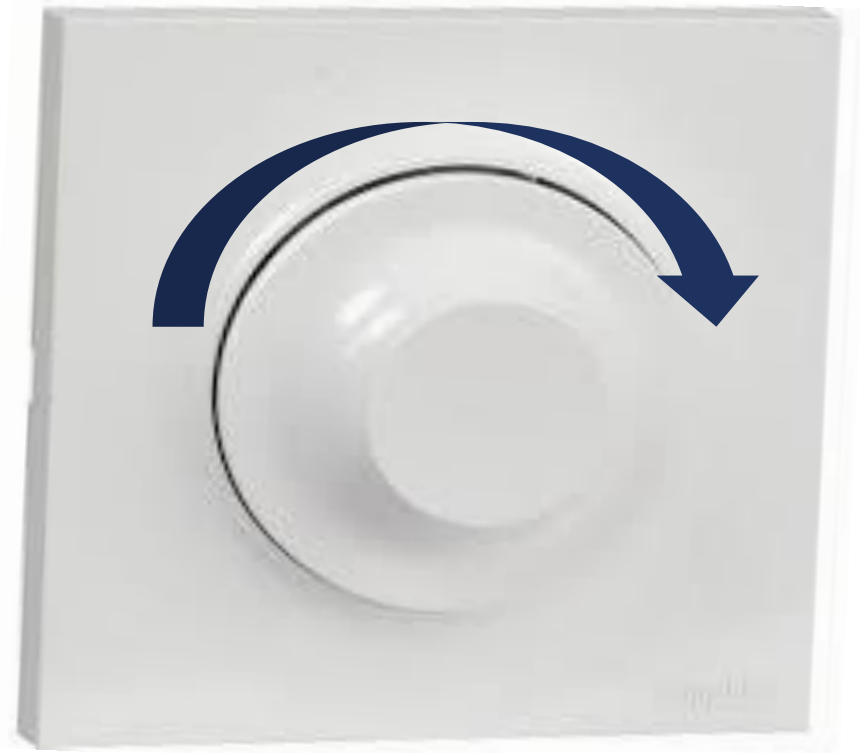
Toxicity

Hyperprogression





e.g. *EGFR*, *ALK*, *ROS1*



e.g. *IHC PD-L1*, *TMB*

2018

GEP

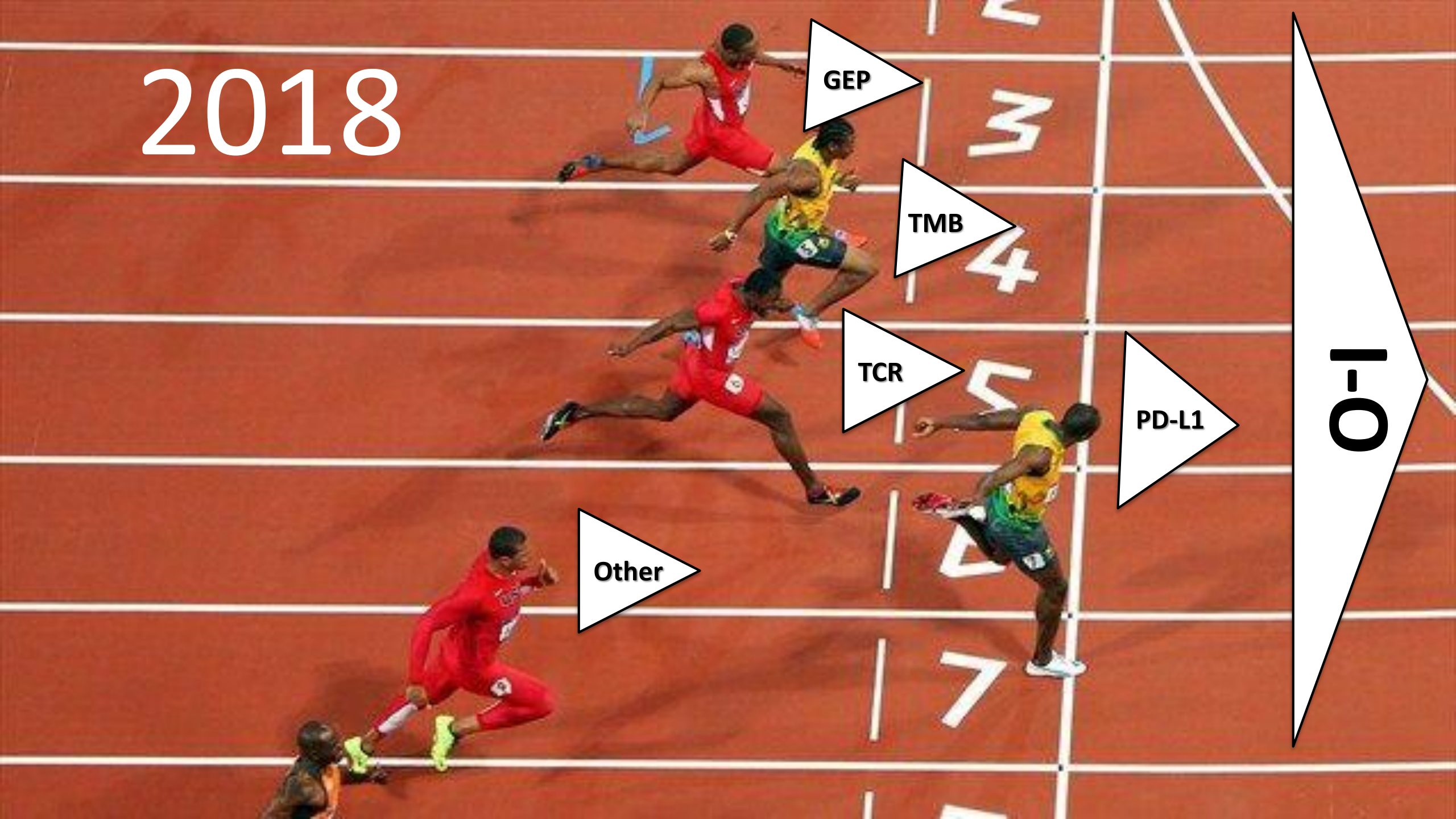
TMB

TCR

PD-L1

Other

10



2020

HLA

TCR

GEP/TIS

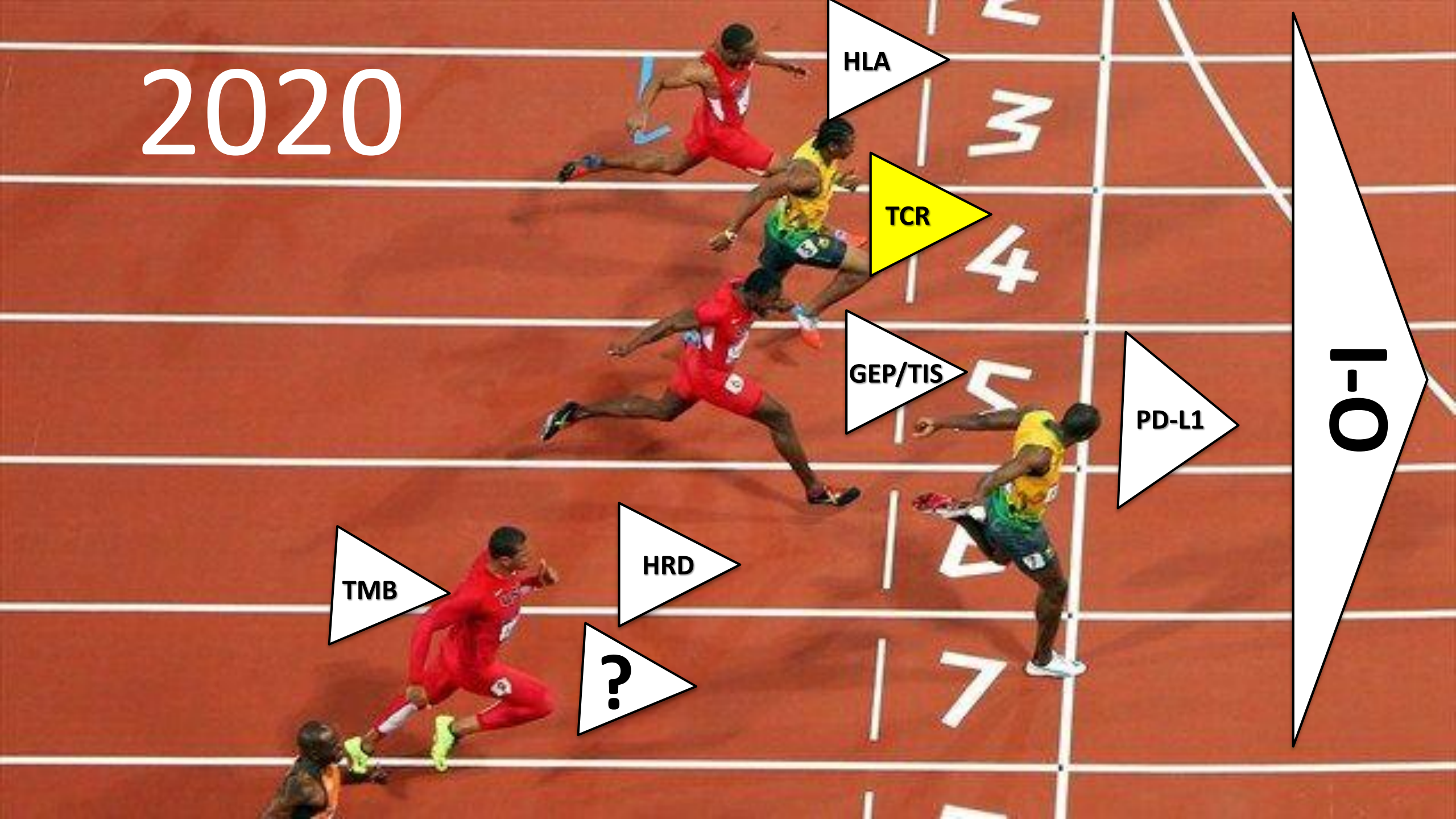
PD-L1

TMB

HRD

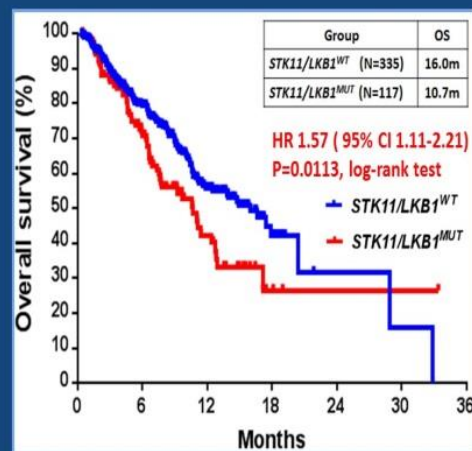
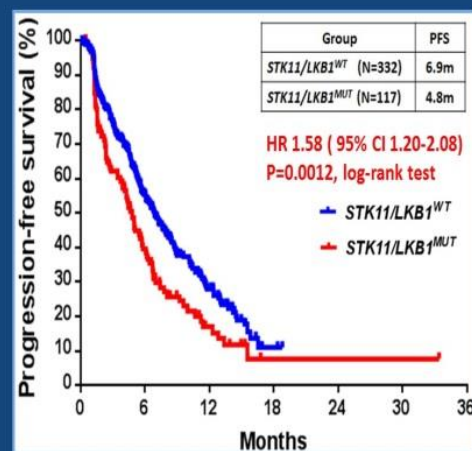
?

IO

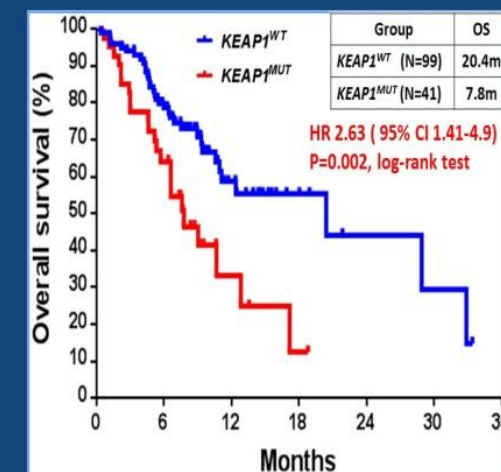
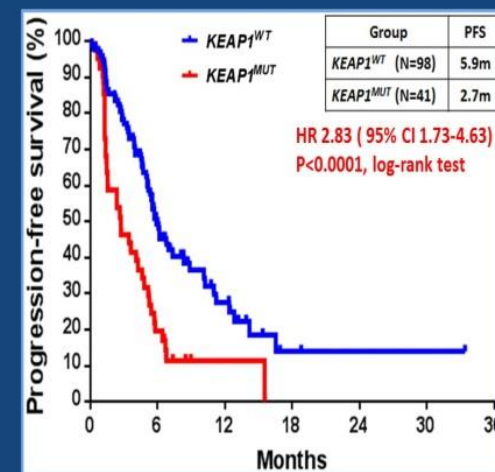


STK11/LKB1 & KEAP1 genomic alterations and I-O resistance in non squamous NSCLC

STK11 genomic alterations are associated with inferior clinical outcomes with PCP in non-squamous NSCLC



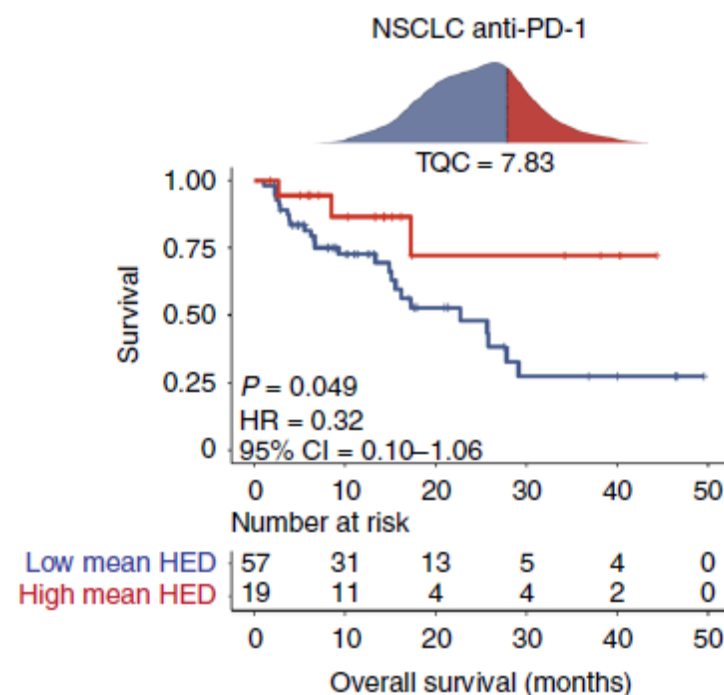
KEAP1 genomic alterations are associated with inferior clinical outcomes with PCP in non-squamous NSCLC



Evolutionary divergence of HLA class I genotype impacts efficacy of cancer immunotherapy

Diego Chowell^{1,2,9}, Chirag Krishna^{3,9}, Federica Pierini^{4,9}, Vladimir Makarov^{1,2}, Naiyer A. Rizvi⁵, Fengshen Kuo^{1b,2}, Luc G. T. Morris^{1b,2,6}, Nadeem Riaz^{2,7}, Tobias L. Lenz^{1b,4,10*} and Timothy A. Chan^{1b,1,2,7,8,10*}

NATURE MEDICINE | VOL 25 | NOVEMBER 2019 | 1715–1720 | www.nature.com/naturemedicine

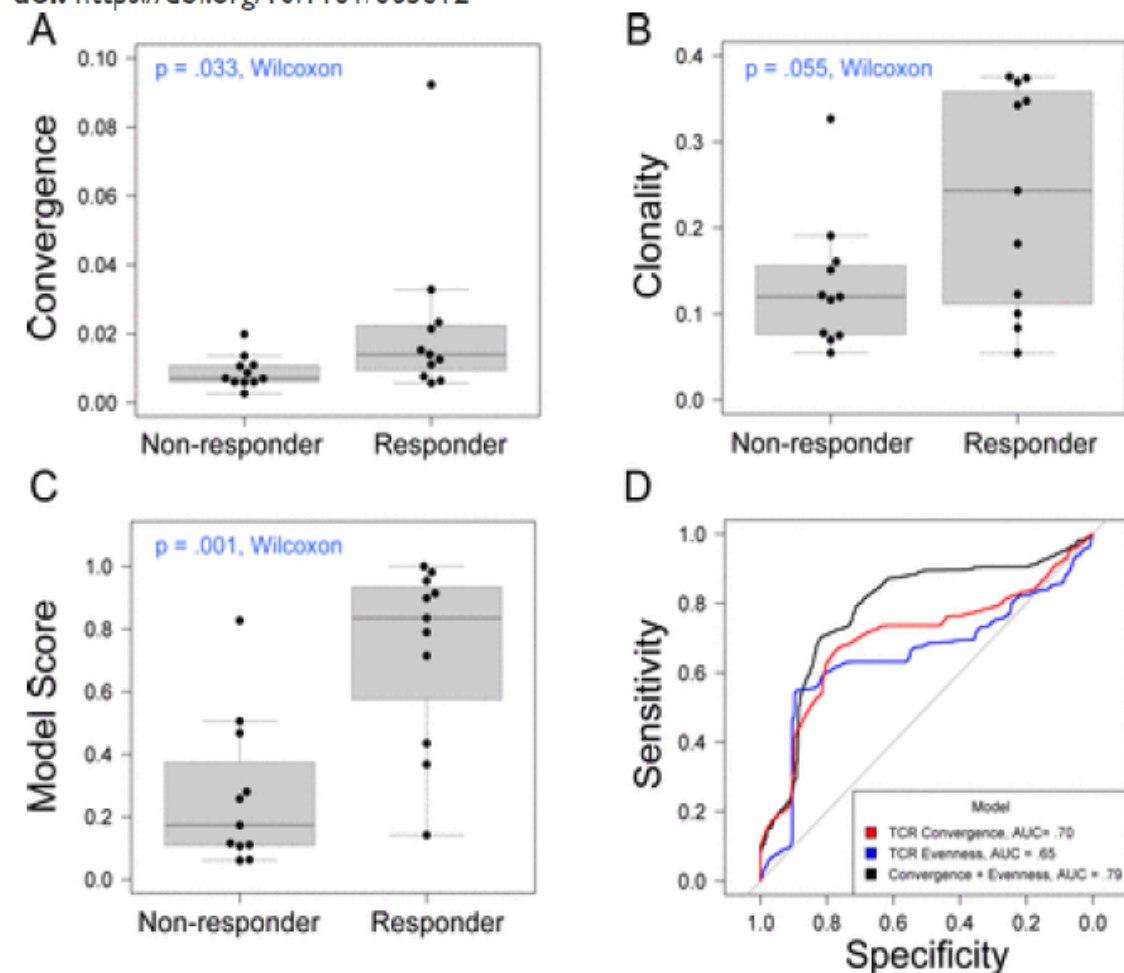


TCR Convergence, Evenness and CTL-4 – New Biomarker for I/O?

TCR convergence in individuals treated with immune checkpoint inhibition for cancer

Timothy Looney, Denise Topacio-Hall, Geoffrey Lowman, Jeffrey Conroy, Carl Morrison, David Oh, Lawrence Fong, Li Zhang

doi: <https://doi.org/10.1101/665612>



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Helen Diller Family Comprehensive Cancer Center,
University of California San Francisco

Category	Subdefinition	Responder	Non-responder
Cancer Type	Prostate	2	4
	Melanoma	7	6
	Adenocarcinoma	2	0
	Not Indicated	0	1
	Total	11	11
Repertoire Features	Clones Detected	32916 (5168-56231)	30015 (5894-58222)
	TCR Convergence	0.022 (.006-.092)	.008 (.002-.019)
	Clonality	.24 (.055-.376)	.133 (.055-.327)

