Molecular and immune profiling of NSCLCs: present approaches and a look into the future

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Disclosures

Consultancy

- AbbVie, Amgen, AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Eli Lilly, Merck Serono, Merck Sharp & Dohme, Novartis, Pfizer, Roche, Ventana

Honoraria (speaker)

- AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Eli Lilly, Medscape, Merck Serono, Merck Sharp & Dohme, Novartis, Pfizer, Prime Oncology, Roche, Ventana

Biomarker Testing

Where are we now

- Evolution of biomarkers and the need for precision medicine
- Factors to consider in development of biomarkers
  - Nucleic acid extraction vs morphology
  - DNA, RNA, or protein
  - Categorical vs continuous
- Established genomic biomarkers in lung cancer
  - EGFR, ALK, ROS1, BRAF

Recent developments

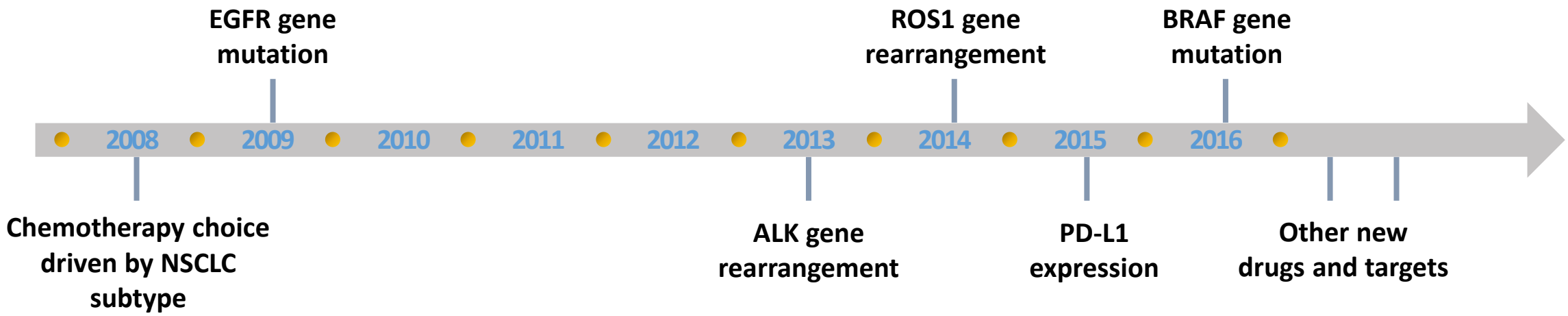
- Emerging Genomic Biomarkers in Lung Cancer
  - NTRK, RET, MET, KRAS, HER2, NRG1
- Immuno-Oncology Biomarkers
  - PD-L1, TMB, GEP

Remaining issues

- Resistance mechanism testing
- Utility of cfDNA samples
- Evolving I-O testing landscape
- Dealing with NGS data

Lung Cancer Diagnostics and Treatment of Lung Cancer

When the Molecular Revolution and Personalised, Precision Medicine for Lung Cancer Patients Took Off…



Virtually every new therapy introduced in the last 10 years is allied to biomarker-driven patient selection

1. Scagliotti et al. *J Clin Oncol.* 2008;26:3543; 2. Mok et al. *N Engl J Med.* 2009;361:947; 3. Shaw et al. *N Engl J Med.* 2013;368:2385; 4. Shaw et al. *N Engl J Med.* 2014:371(21):1963-71; 5. Garon et al. *N Engl J Med.* 2015;372:2018; 6. Planchard et al. *Lancet Oncol.* 2016;17(7):984-993.

Why Do We Need Biomarkers for Therapy?

Precision medicine is a reality for lung cancer

Not all patients respond to and benefit from all treatments

- Enrich the treatment population for benefit
- ***How many to treat, to get 1 response?***
- Benefit is relative to standard of care

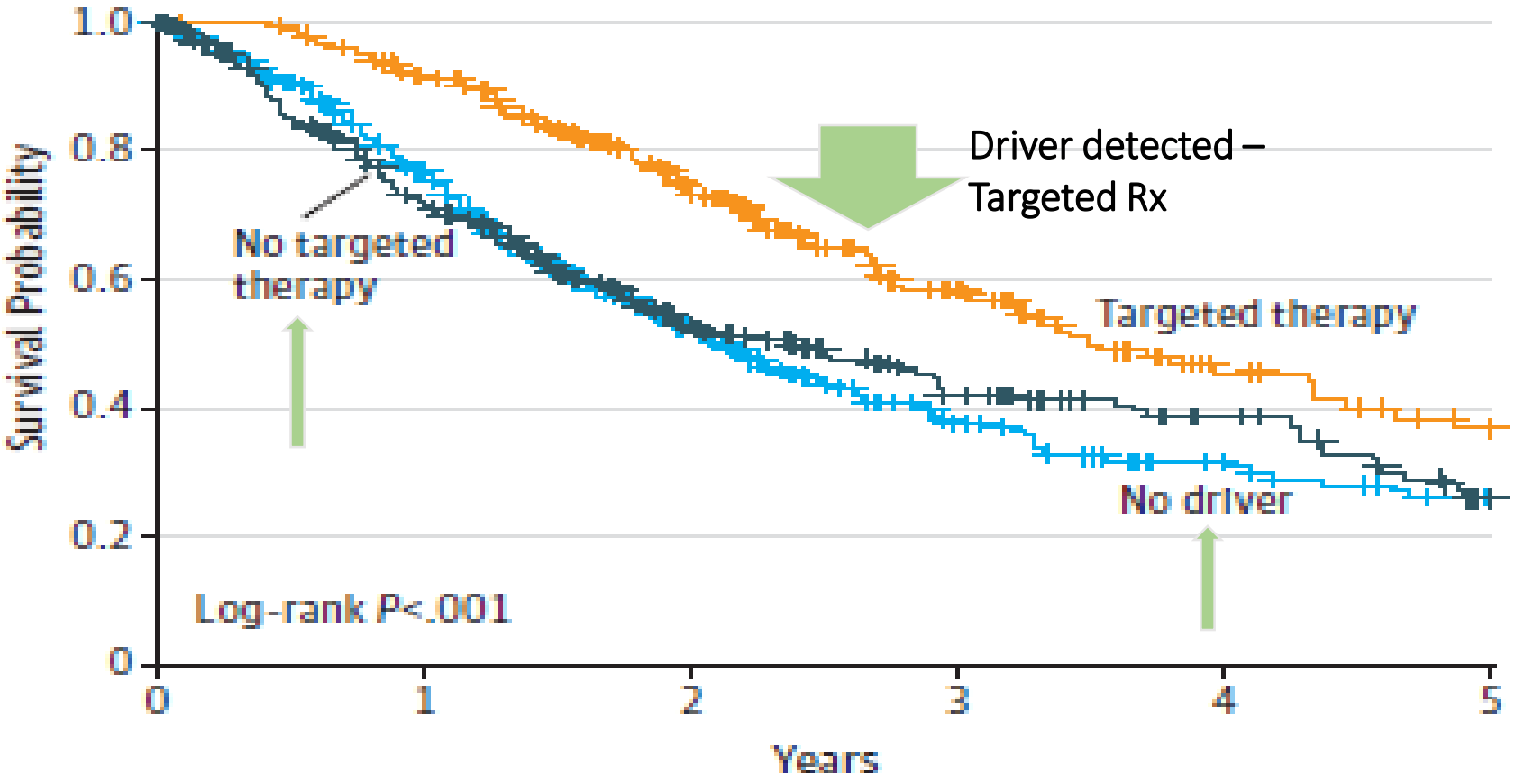
Avoidance of harm?

- There are toxicities from these drugs
- Is there a subgroup of patients who fair worse on targeted treatment?
- Alternative treatment would be better

Financial burden of expensive therapy

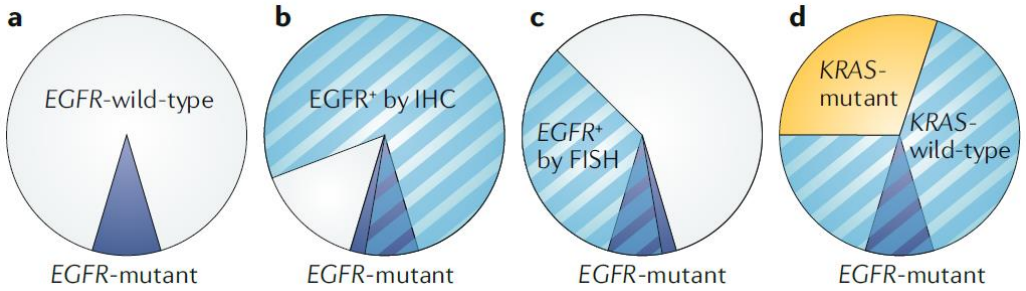
It Is Worthwhile Finding an Actionable Genetic Alteration in Lung Cancer

Using Multiplexed Assays of Oncogenic Drivers in Lung Cancers to Select Targeted Drugs



Kris et al. *JAMA*. 2014;311:1998.

Which Level to Test? How Will You Do It?



Camidge et al. *Nat Rev Clin Oncol.* 2019;16:341.

Change in DNA sequence?

- Mutation
- Rearrangement
- Gene copy number

Transcription

mRNA script

- Fusion gene transcripts
- Gene expression levels
- Gene expression profiles

Translation

Protein

- Protein expression

ALK data
PD-L1 data

Nucleic acid extraction or Morphology?

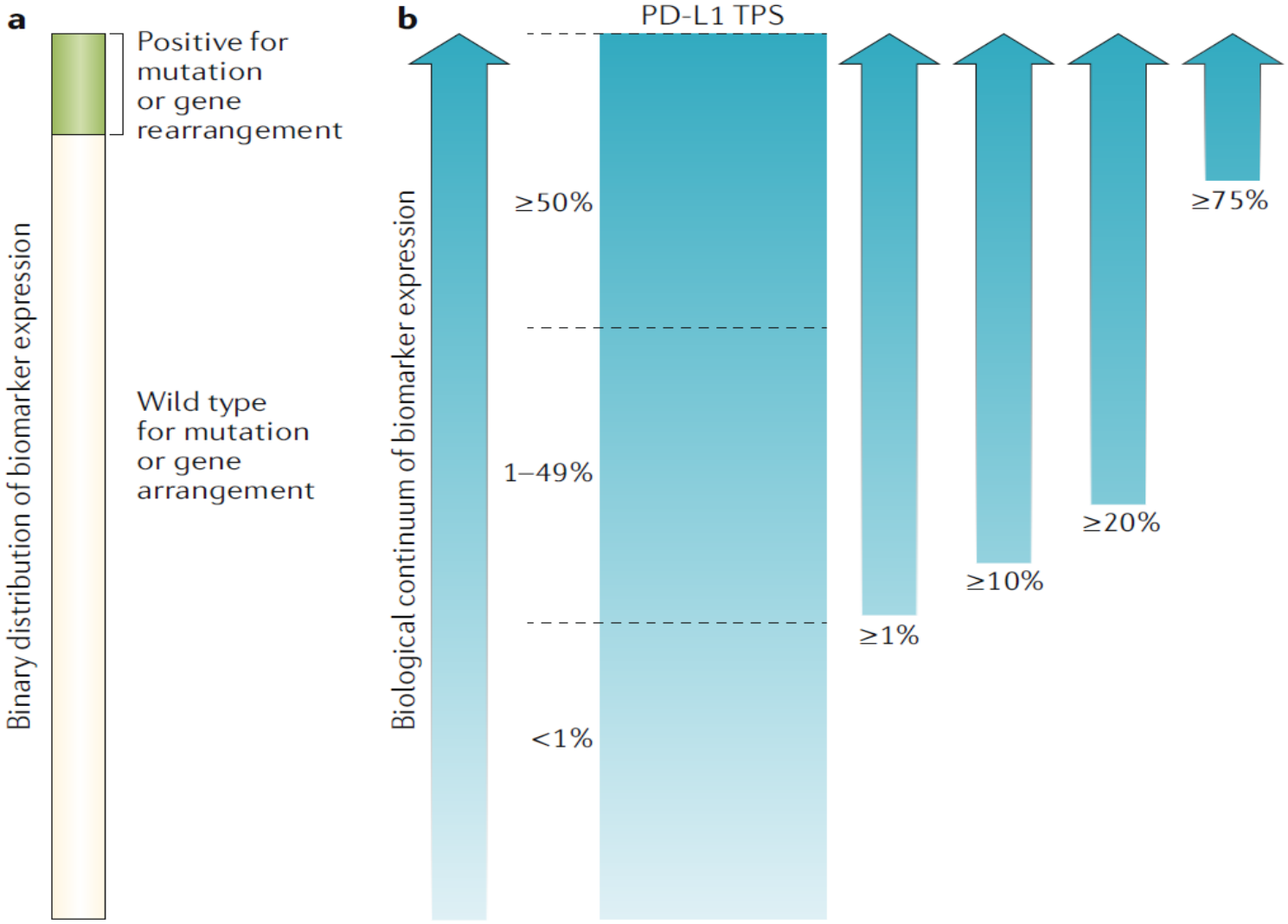
- Biological activity
- Oncogenesis
- Drug target

Categorical vs 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)

One of multiple determining factors

Spatially and Temporally Variable

Individual efficacy driven by biomarker quantity

Camidge et al. *Nat Rev Clin Oncol.* 2019;16:341.

Lung Cancer: Genomics and Targets

Adenocarcinoma

- Many genetically complex
  - High mutational burden
- Genetically simple cases
  - Oncogene addiction
  - NOT associated with tobacco carcinogenesis
  - Low mutational burden
  - 15%-20% of cases in the West
  - 55%-70% of cases in East Asian cohorts

Squamous cell carcinoma

- Vast majority genetically complex
  - High mutational burden
- Genetically simple cases
  - Very uncommon

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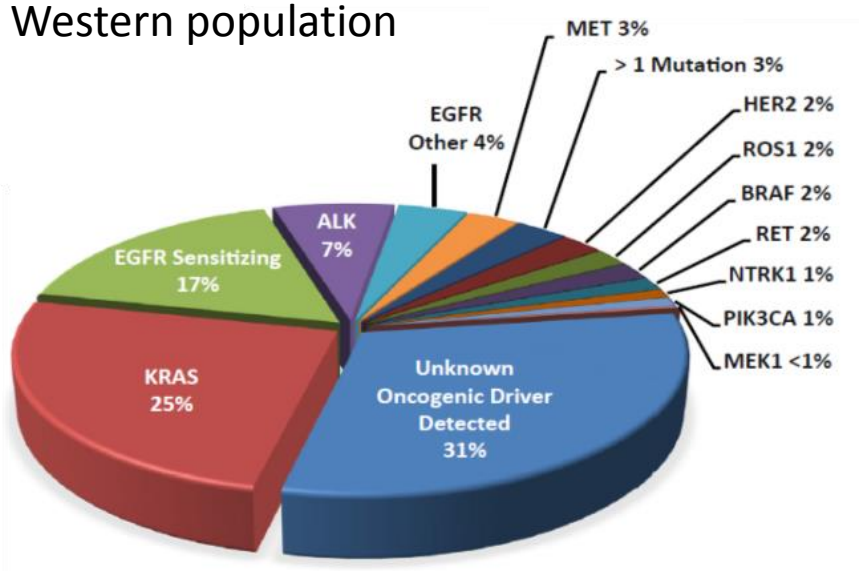
These cases present excellent drug targets and high-performance biomarkers

Adenocarcinomas, Targets, and Therapy

Only a minority are “genetically simple” with an addictive oncogenic driver; most are “genetically complex”

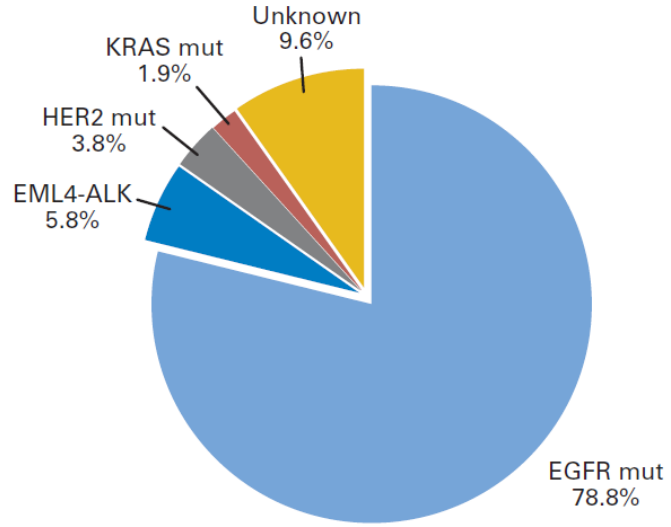
Western population



Only 5 of these targets have agents through approval by the FDA and EMA

- **EGFR mutation**
- **ALK rearrangement**
- **ROS1 rearrangement**
- **BRAF mutation**
- **NTRK rearrangement**

East Asian Never-smokers



Addictive oncogenic drivers make excellent drug targets and excellent biomarkers

1. Tsao et al. *J Thorac Oncol.* 2016;11:613; 2. Sun et al. *J Clin Oncol.* 2010;28:4616; 3. Odogwu et al. *Oncologist*. 2018;23(6):740; FDA website. https://bit.ly/2TDORX1. Accessed 22 Oct 2019.

Lung Cancer: Genomics and Targets

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Squamous cell carcinoma

- Vast majority genetically complex
  - High mutational burden
  - Loss of tumour suppressor genes
  - Very few ‘good’ targets
- Genetically simple cases
  - Very uncommon

Joint CAP/IASLC/AMP Guidelines: Published January 22, 2018

SPECIAL ARTICLE

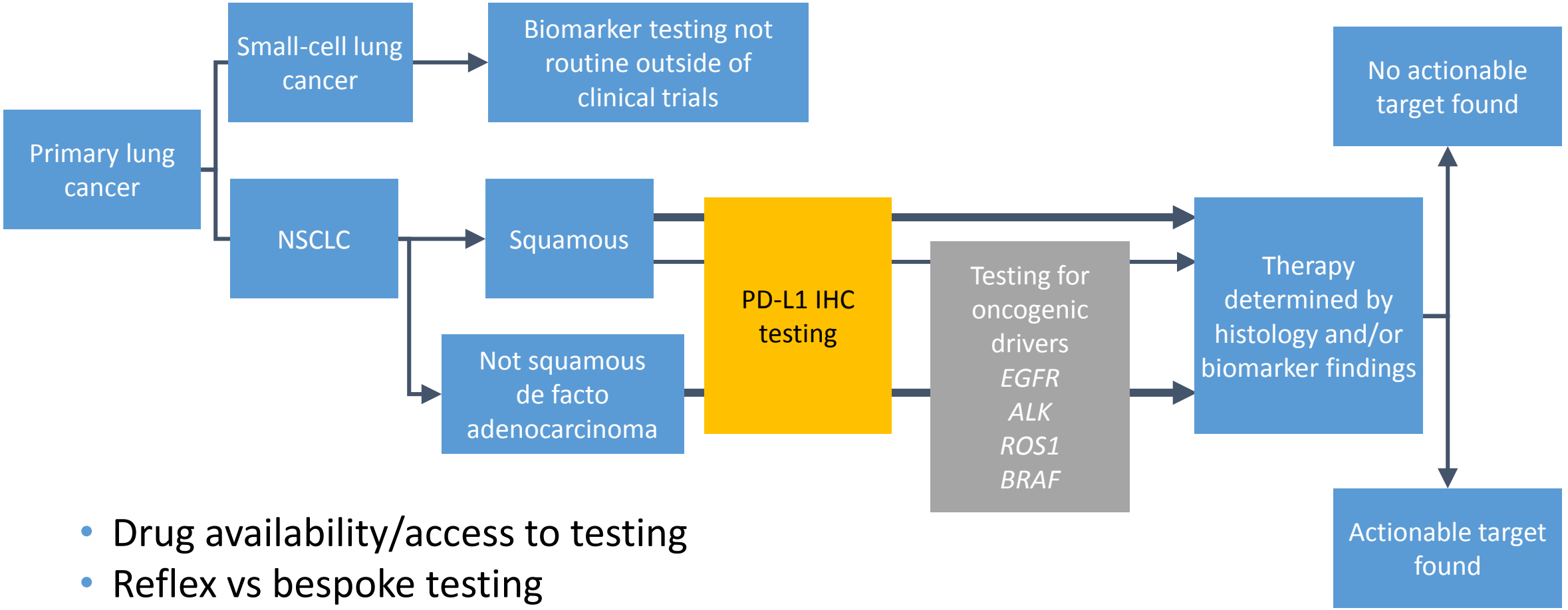
Updated Molecular Testing Guideline for the Selection of Lung Cancer Patients for Treatment With Targeted Tyrosine Kinase Inhibitors

Guideline From the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology

Neal I. Lindeman, MD,[a,*] Philip T. Cagle, MD,[c] Dara L. Aisner, MD, PhD,[d] **Maria E. Arcila, MD,**[e] Mary Beth Beasley, MD,[g] Eric Bernicker, MD,[h] Carol Colasacco, MLIS, SCT(ASCP),[i] Sanja Dacic, MD, PhD,[j] Fred R. Hirsch, MD, PhD,[k] Keith Kerr, MB, ChB,[l] David J. Kwiatkowski, MD, PhD,[b] Marc Ladanyi, MD,[f] Jan A. Nowak, MD, PhD,[m] Lynette Sholl, MD,[a] Robyn Temple-Smolkin, PhD,[n] Benjamin Solomon, MBBS, PhD,[o] Lesley H. Souter, PhD,[p] Erik Thunnissen, MD, PhD,[q] Ming S. Tsao, MD,[r] Christina B. Ventura, MPH, MT(ASCP),[i] Murry W. Wynes, PhD,[s] Yasushi Yatabe, MD, PhD[t]

1. Lindeman et al. *J Thorac Oncol.* 2018;13:323; 2. Lindeman et al. *Arch Pathol Lab Med.* 2018;142:321; 3. Lindeman et al. *J Mol Diagn.* 2018;20:129.

NSCLC Diagnosis: Tumour Subtype and Biomarker Testing in Routine Practice



- Drug availability/access to testing
- Reflex vs bespoke testing
  - Who orders the testing?
  - Pathologist or oncologist?

CAP/IASLC/AMP Guidelines 2013 and 2018

Table 3. Summary of the Updated Statements With Strength of Recommendations[a]

2013 Statement	2018 Statement
Expert consensus opinion: Cytologic samples are also suitable for EGFR and ALK testing, with cell blocks being preferred over smear preparations.	Recommendation: Pathologists may use either cell blocks or other cytologic preparations as suitable specimens for lung cancer biomarker molecular testing.
Expert consensus opinion: Laboratories should use EGFR test methods that are able to detect mutations in specimens with at least 50% cancer cell content, although laboratories are strongly encouraged to use (or have available at an external reference laboratory) more sensitive tests that are able to detect mutations in specimens with as little as 10% cancer cells.	Expert consensus opinion: Laboratories should use, or have available at an external reference laboratory, clinical lung cancer biomarker molecular testing assays that are able to detect molecular alterations in specimens with as little as 20% cancer cells.
Recommendation: Immunohistochemistry for total EGFR is not recommended for selection of EGFR TKI therapy.	Strong recommendation: Laboratories should not use total EGFR expression by IHC testing to select patients for EGFR-targeted TKI therapy.

Stronger statements on CYTOLOGY…it's OK, really, it is!
Assays need to work with 20% cancer cells…or FEWER!
IHC for EGFR mutation…NO THANK YOU!

Lindeman et al. *J Thorac Oncol.* 2018;13:323.

Table 4. Summary of 2018 Guideline Statements

Guideline Statements	Strength of Recommendation
Key Question 1: Which new genes should be tested for lung cancer patients?	
1. *ROS1* testing must be performed on all lung adenocarcinoma patients, irrespective of clinical characteristics.	Strong recommendation
2. ROS1 IHC may be used as a screening test in lung adenocarcinoma patients; however, positive ROS1 IHC results should be confirmed by a molecular or cytogenetic method.	Expert consensus opinion
3. *BRAF* molecular testing is currently not indicated as a routine stand-alone assay outside the context of a clinical trial. It is appropriate to include *BRAF* as part of larger testing panels performed either initially or when routine *EGFR*, *ALK*, and *ROS1* testing are negative.	Expert consensus opinion
4. *RET* molecular testing is not recommended as a routine stand-alone assay outside the context of a clinical trial. It is appropriate to include *RET* as part of larger testing panels performed either initially or when routine *EGFR*, *ALK*, and *ROS1* testing are negative.	Expert consensus opinion
5. *ERBB2* (*HER2*) molecular testing is not indicated as a routine stand-alone assay outside the context of a clinical trial. It is appropriate to include *ERBB2* (*HER2*) mutation analysis as part of a larger testing panel performed either initially or when routine *EGFR*, *ALK*, and *ROS1* testing are negative.	Expert consensus opinion
6. *KRAS* molecular testing is not indicated as a routine stand-alone assay as a sole determinant of targeted therapy. It is appropriate to include *KRAS* as part of larger testing panels performed either initially or when routine *EGFR*, *ALK*, and *ROS1* testing are negative.	Expert consensus opinion
7. *MET* molecular testing is not indicated as a routine stand-alone assay outside the context of a clinical trial. It is appropriate to include *MET* as part of larger testing panels performed either initially or when routine *EGFR*, *ALK*, and *ROS1* testing are negative.	Expert consensus opinion
Key Question 2: What methods should be used to perform molecular testing?	
8. IHC is an equivalent alternative to FISH for ALK testing.	Recommendation
9. Multiplexed genetic sequencing panels are preferred over multiple single-gene tests to identify other treatment options beyond *EGFR*, *ALK*, and *ROS1*.	Expert consensus opinion
10. Laboratories should ensure test results that are unexpected, discordant, equivocal, or otherwise of low confidence are confirmed or resolved using an alternative method or sample.	Expert consensus opinion
Key Question 3: Is molecular testing appropriate for lung cancers that do not have an adenocarcinoma component?	
11. Physicians may use molecular biomarker testing in tumors with histologies other than adenocarcinoma when clinical features indicate a higher probability of an oncogenic driver.	Expert consensus opinion
Key Question 4: What testing is indicated for patients with targetable mutations who have relapsed on targeted therapy?	
12. In lung adenocarcinoma patients who harbor sensitizing *EGFR* mutations and have progressed after treatment with an EGFR-targeted tyrosine kinase inhibitor, physicians must use *EGFR* T790M mutational testing when selecting patients for third-generation EGFR-targeted therapy.	Strong recommendation
13. Laboratories testing for *EGFR* T790M mutation in patients with secondary clinical resistance to EGFR-targeted kinase inhibitors should deploy assays capable of detecting *EGFR* T790M mutations in as little as 5% of viable cells.	Recommendation
14. There is currently insufficient evidence to support a recommendation for or against routine testing for *ALK* mutational status for lung adenocarcinoma patients with sensitizing *ALK* mutations who have progressed after treatment with an ALK-targeted tyrosine kinase inhibitor.	No recommendation
Key Question 5: What is the role of testing for circulating cell-free DNA for lung cancer patients?	
15. There is currently insufficient evidence to support the use of circulating cell-free plasma DNA molecular methods for the diagnosis of primary lung adenocarcinoma.	No recommendation
16. In some clinical settings in which tissue is limited and/or insufficient for molecular testing, physicians may use a cell-free plasma DNA assay to identify *EGFR* mutations.	Recommendation
17. Physicians may use cell-free plasma DNA methods to identify *EGFR* T790M mutations in lung adenocarcinoma patients with progression or secondary clinical resistance to EGFR-targeted tyrosine kinase inhibitors; testing of the tumor sample is recommended if the plasma result is negative.	Expert consensus opinion
18. There is currently insufficient evidence to support the use of circulating tumor cell molecular analysis for the diagnosis of primary lung adenocarcinoma, the identification of EGFR or other mutations, or the identification of *EGFR* T790M mutations at the time of EGFR TKI resistance.	No recommendation

Lindeman et al. *J Thorac Oncol.* 2018;13:323.

2018…

- ALK a mandatory test
  - IHC equivalent to FISH
- ROS1 a mandatory test
  - IHC as a screening tool
  - Requires confirmation
- BRAF (nearly) mandatory
- Others still investigational
- NGS preferred for extended panels
- Selection focuses on probability of oncogenic drivers
- Plasma cfDNA for EGFR mutation, including T790M in certain situations

More Molecular Targets on the Horizon (and Here the Concept of the Horizon Is Relative!)

Core testing 'now'

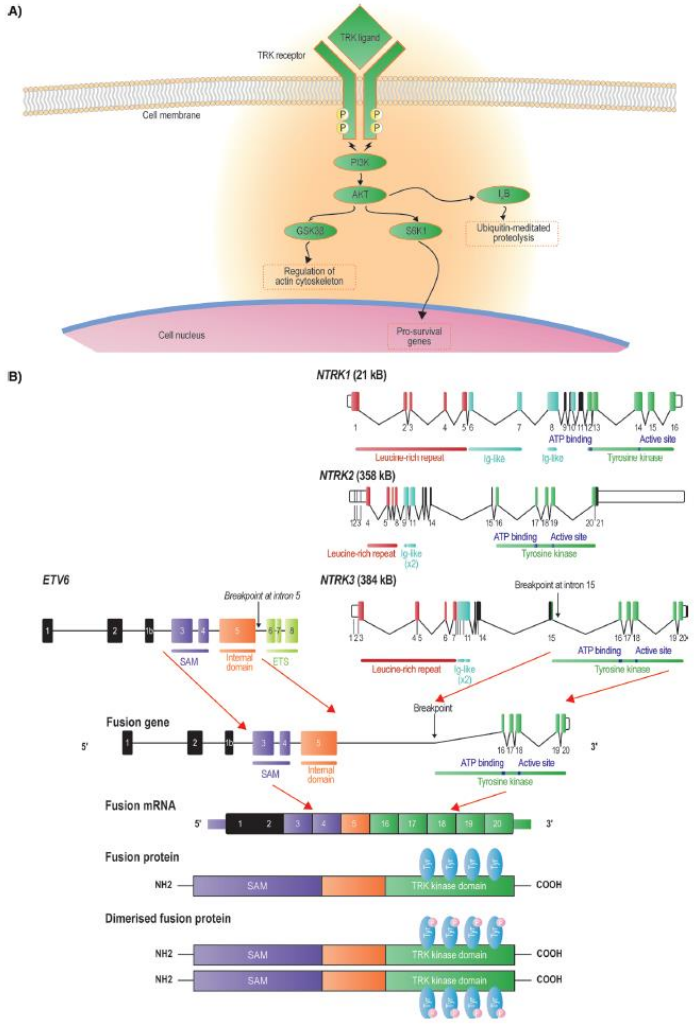
EGFR mutation
BRAF *V600* mutation

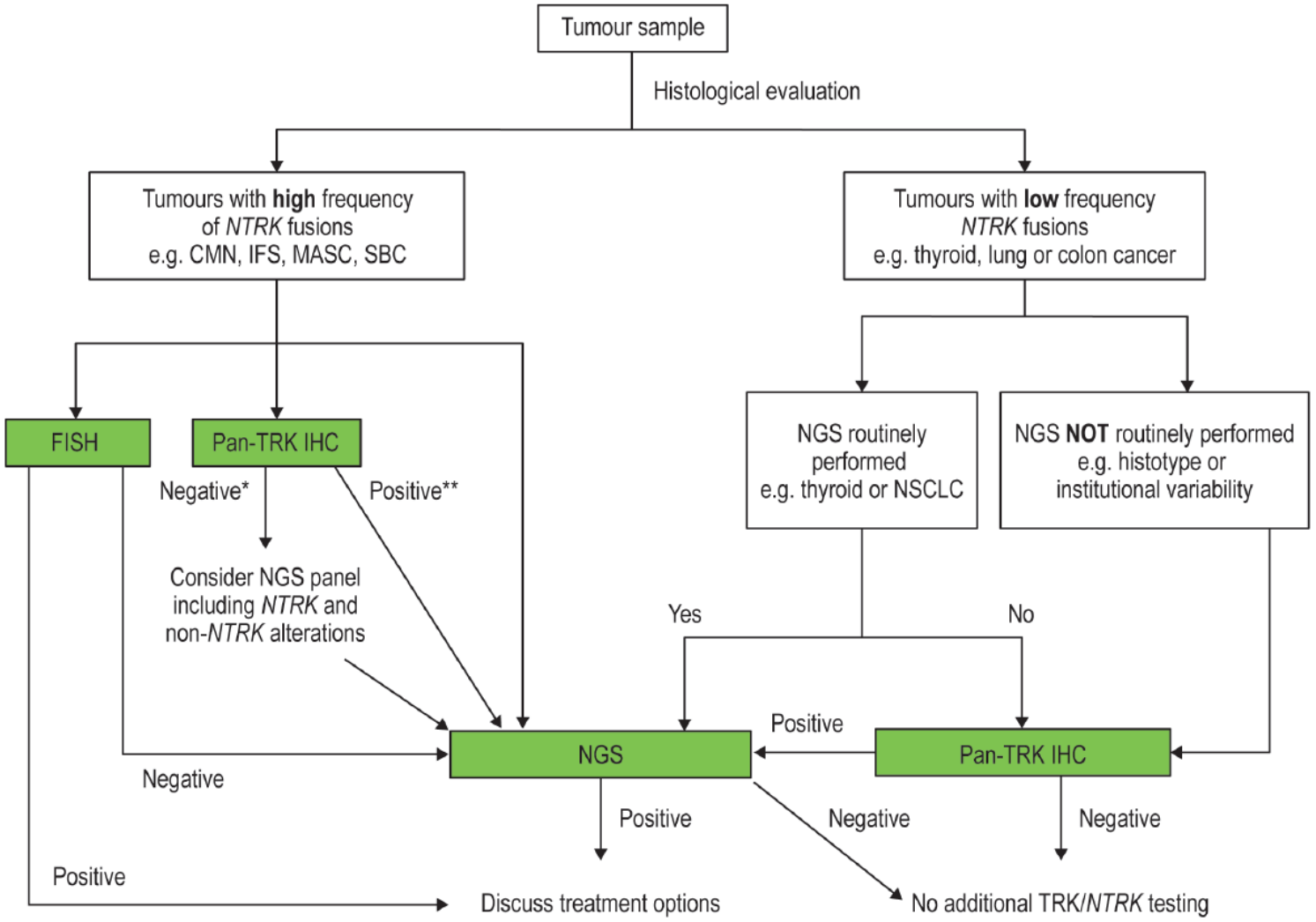
ALK rearrangement
ROS1 rearrangement

PD-L1 expression

- NTRK rearrangements
- RET rearrangements
- MET alterations
  - MET exon 14 skipping
  - Increased MET gene copy number
- KRAS *G12C* mutation
- HER2 mutations/fusions
- NRG1 rearrangements
- Etc…

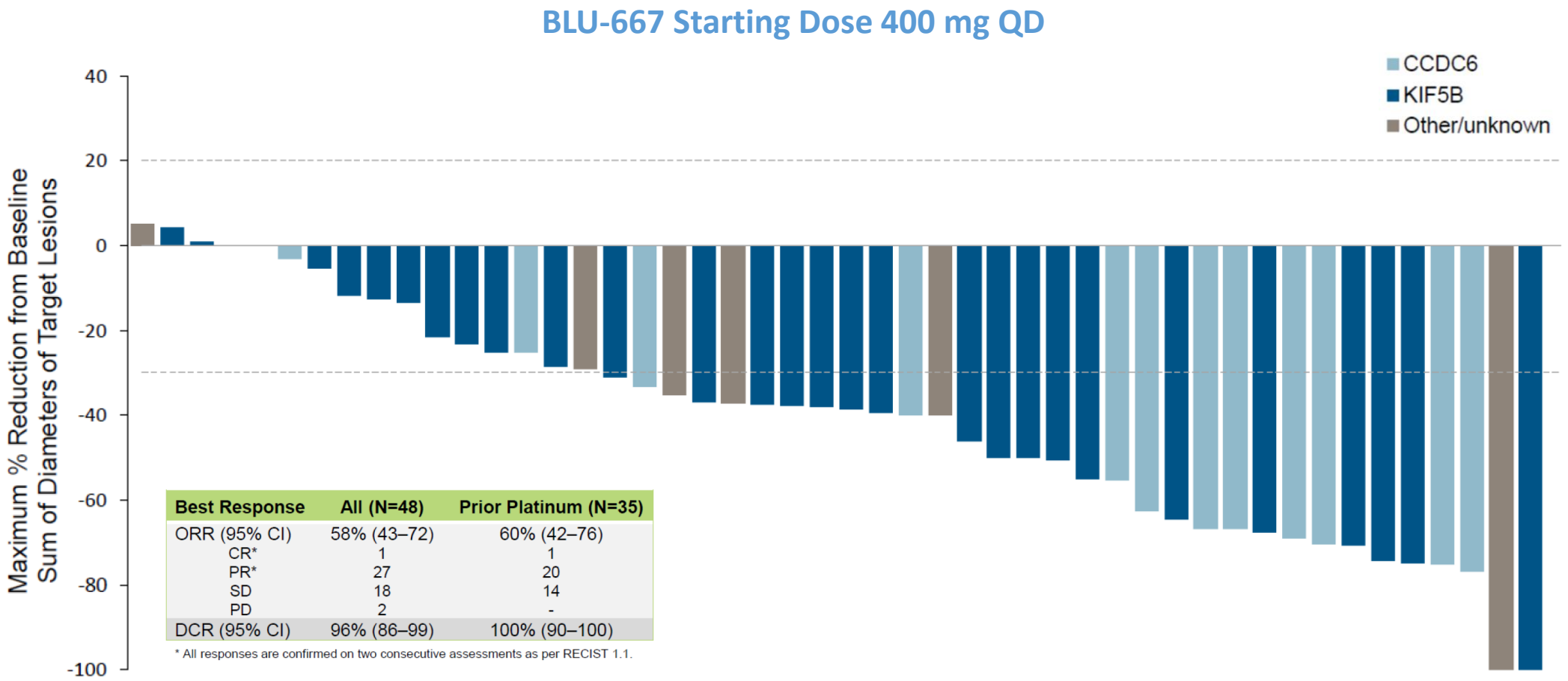
NTRK Fusion Genes: a Rare Target





Penault-Llorca et al. *J Clin Pathol.* 2019;72:460.

BLU-667 Is Active Across **RET Fusion** Genotypes



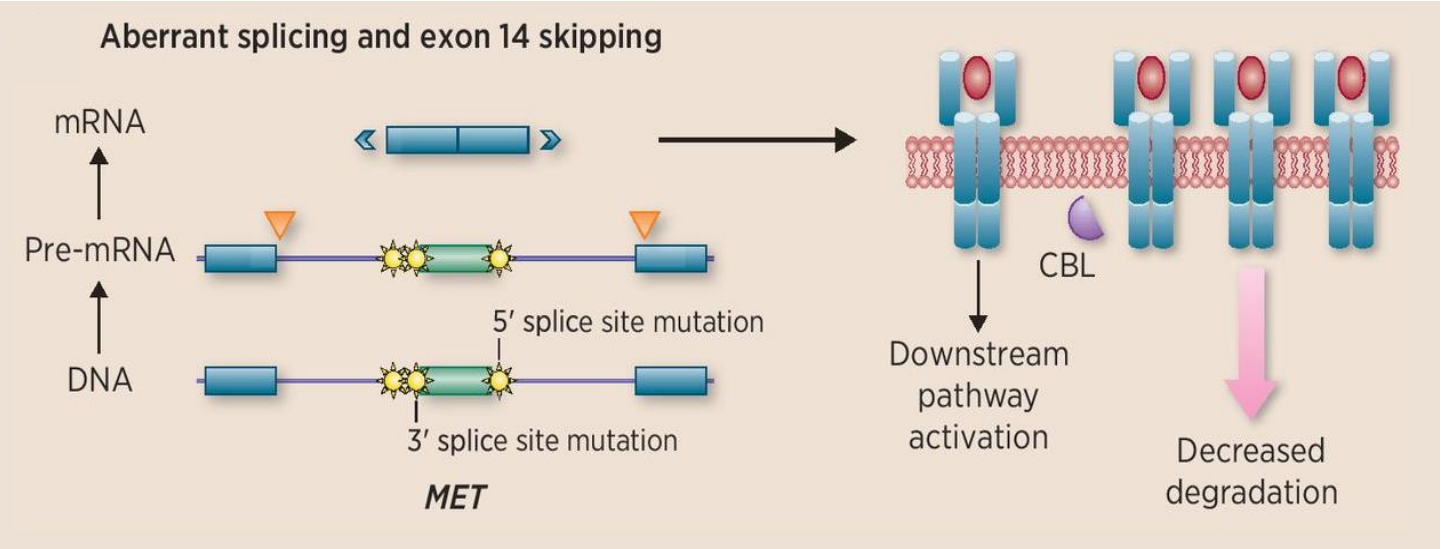
Best Response	All (N=48)	Prior Platinum (N=35)
ORR (95% CI)	58% (43–72)	60% (42–76)
CR*	1	1
PR*	27	20
SD	18	14
PD	2	-
DCR (95% CI)	96% (86–99)	100% (90–100)

* All responses are confirmed on two consecutive assessments as per RECIST 1.1.

Patients enrolled by 14 November 2018, data cut-off 28 April 2019.

Gainor et al. ASCO 2019. Abstract #9008.

MET Exon 14 Splice Site Alterations: Alternative Splicing Can Be Oncogenic



MET ex14 – How?
Testing methodology

- RNA-based approaches better than DNA
- Importance of gene copy number?
- Importance of MET protein expression (IHC)?

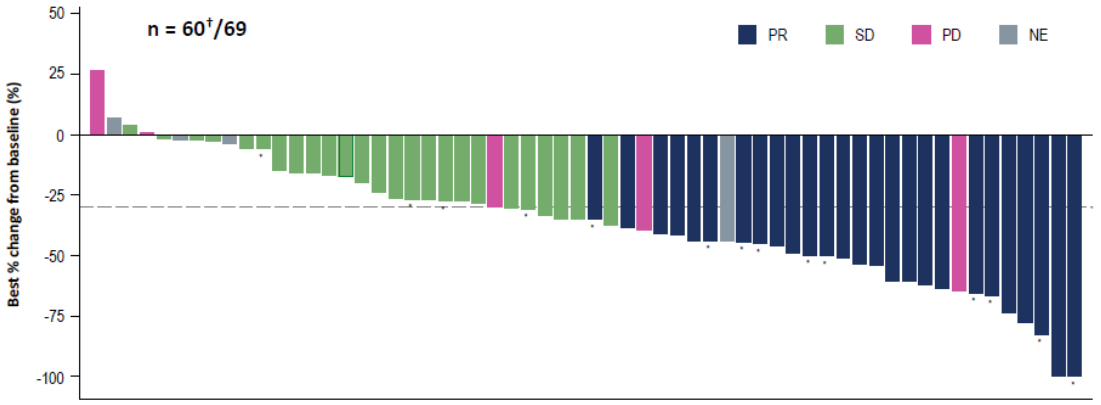
MET ex14 – Who?
Clinicopathological correlations

- Older patients
- Smoking not an issue
- Adenocarcinomas mainly
- Sarcomatoid carcinomas

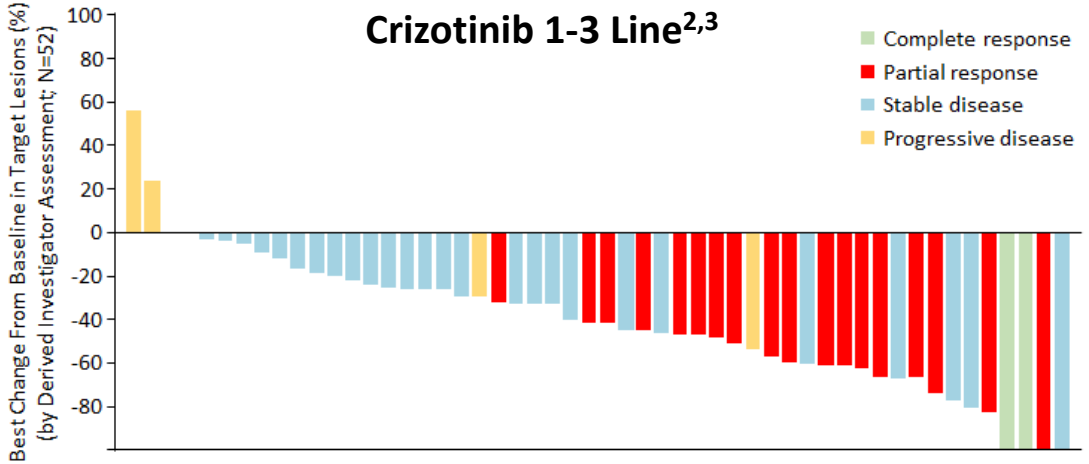
Awad et al. *J Clin* Oncol. 2016;34:721; Drilon et al. *Clin Cancer Res*. 2016;22:2832.

MET Exon 14 Splice Site Alterations: Clinical Benefit From MET Inhibitors

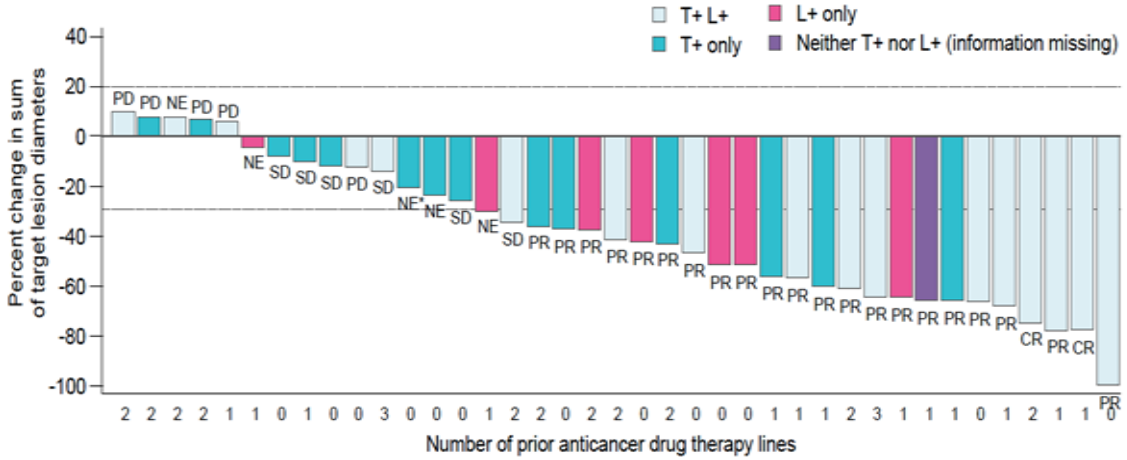
Capmatinib 2/3 Line[1]



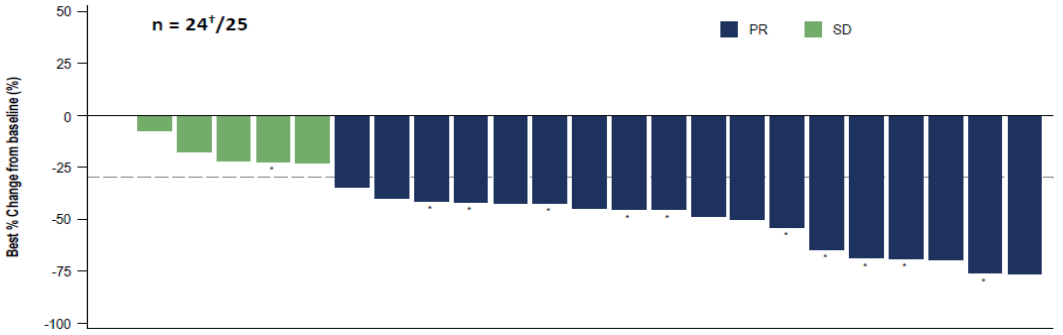
Crizotinib 1-3 Line[2,3]



Tepotinib 1-3 Line[4]



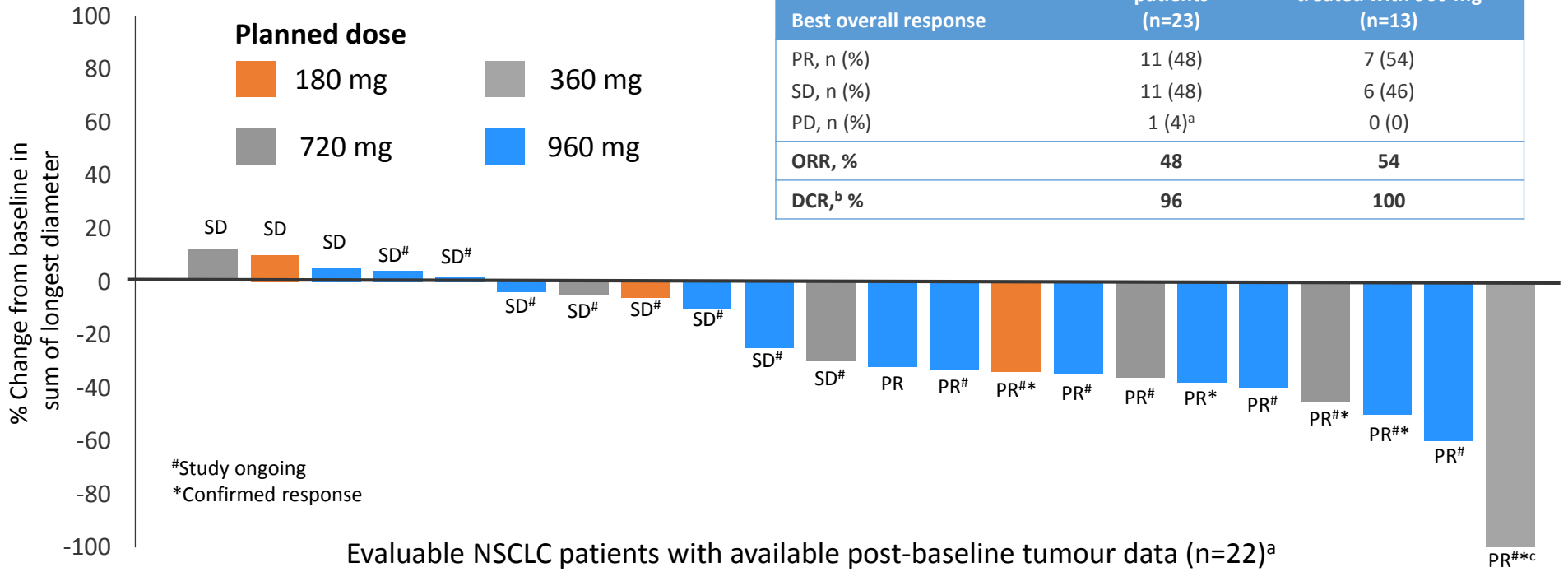
Capmatinib First-Line[1]



1. Wolf et al. *Ann Oncol.* 2018;29(suppl 8):abstract LBA52; 2. Drilon et al. *J Thorac Oncol.* 2018;10(13 suppl):abstract OA12.02; 3. Shaw et al. *N Engl J Med.* 2014;371:1963; 4. Felip et al. WCLC 2018. Abstract OA12.01.

OA02.02: Phase 1 Study of Safety, Tolerability, PK, and Efficacy of AMG 510, a Novel **KRAS**^{G12C} Inhibitor, Evaluated in NSCLC – Govindan R, et al WCLC 2019

Key results



Best overall response	All evaluable patients (n=23)	Evaluable patients treated with 960 mg (n=13)
PR, n (%)	11 (48)	7 (54)
SD, n (%)	11 (48)	6 (46)
PD, n (%)	1 (4)[a]	0 (0)
ORR, %	**48**	**54**
DCR,[b] %	**96**	**100**

Evaluable NSCLC patients with available post-baseline tumour data (n=22)[a]

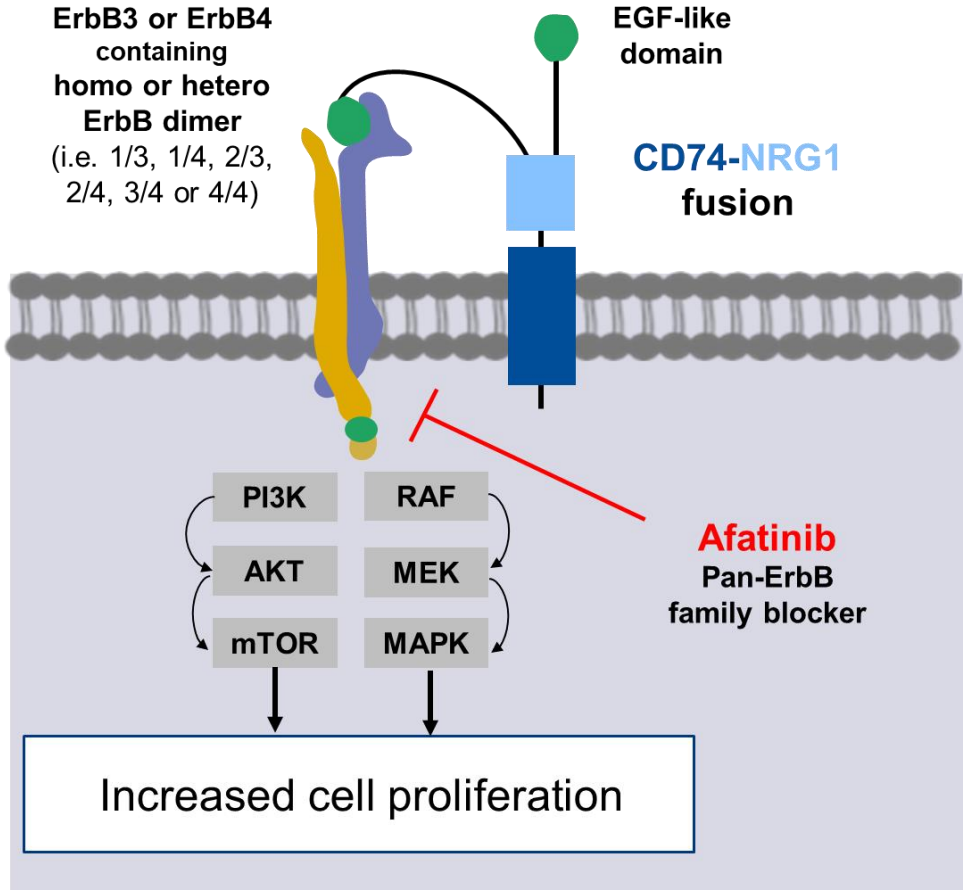
Conclusion

- In patients with **NSCLC** and a **KRAS *G12C*** mutation, AMG 510 demonstrated a manageable safety profile and provided encouraging responses

[a] Patient discontinued due to PD prior to first assessment; [b]PR or SD at week 6; [c]patient had CR.
Govindan et al. *J Thorac Oncol*. 2019;14(suppl):abstract OA02.02.

Oncogenic *NRG1* Gene Fusions

- NRG1+ NSCLC accounts for 0.3% of NSCLC cases
- Invasive mucinous adenocarcinoma (IMA) subtype, reported prevalence of 31% (Caucasian) to 61% (Asian) [5,6]



How to test?
Whom to test?

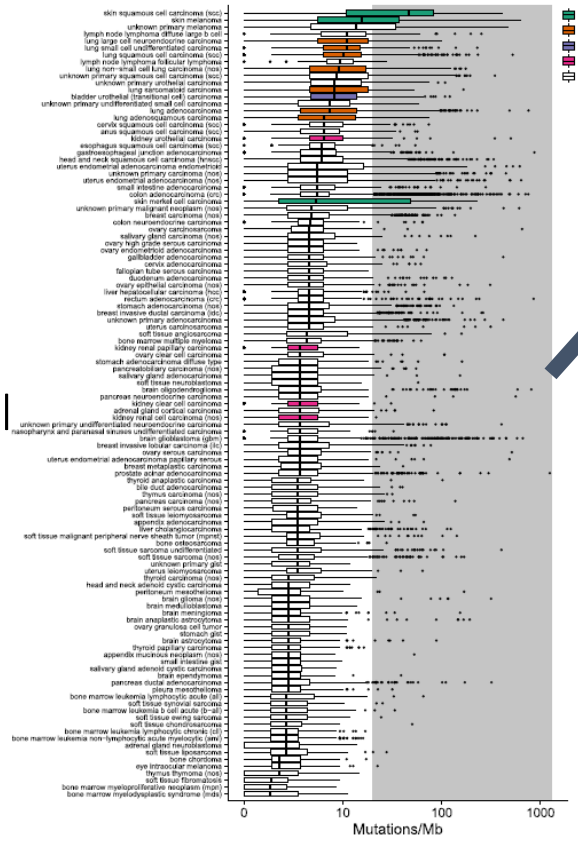
DNA?
RNA?
Protein?

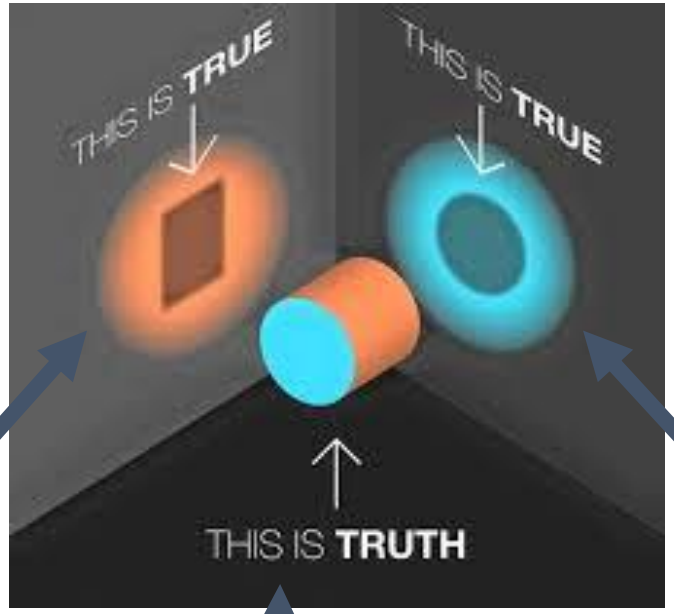
Fusion Partner	*NRG1* Fusion Breakpoint
CD74	CD74-NRG1 (C6, N6)
SLC3A2	SLC3A2-NRG1 (S5, N6)
SDC4-NRG1	SDC4-NRG1 (S4, N6)
RBPMS	RBPMS-NRG1 (R6, N6)
WRN	WRN-NRG1 (WRN 5'UTR, N1)
VAMP2	VAMP2-NRG1 (V4, N5)
KIF13B	KIF13B-NRG1 (K2, N2)
SMAD4	SMAD4-NRG1 (S1, N6)
THAP7	THAP7-NRG1 (T6, N6)
MDK	MDK-NRG1 (M4, N6)
TNC	TNC-NRG1 (T10, N6)
ATP1B1	ATP1B1-NRG1 (A2, N2)
DIP2B	DIP2B-NRG1 (D1, N2)
MRPL13	MRPL13-NRG1 (M2, N2)
PARP8	PARP8-NRG1 (P1, N2)
ROCK1	ROCK1-NRG1 (R1, N2)
DPYSL2	DPYSL2-NRG1 (D7, N5)

1. Drilon et al. *Cancer Discov.* 2018;8:686; 2. Duruisseaux et al. WCLC 2019. Poster P1.14-25; 3. Fernandez-Cuesta and Thomas. *Clin Cancer Res.* 2014;21:1989; 4. Jonna et al. *Clin Cancer Res*. 2019;25:4966; 5. Trombetta et al. *Oncotarget*. 2018;9:9661; 6. Nagasaka and Ou. *J. Thorac Oncol.* 2019;14:1354.

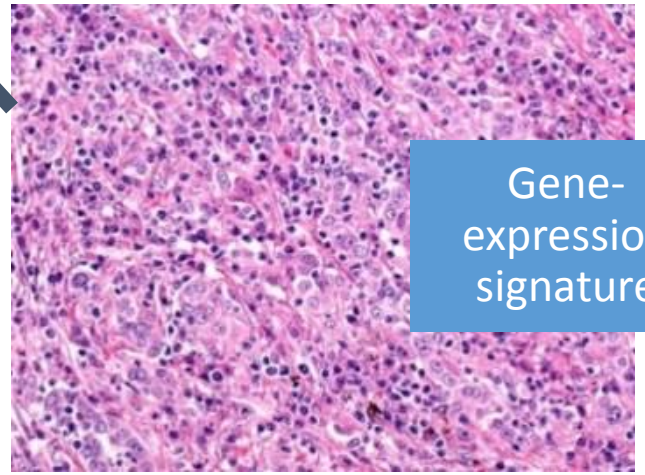
Immunotherapy Biomarkers

Tumour Mutational Burden



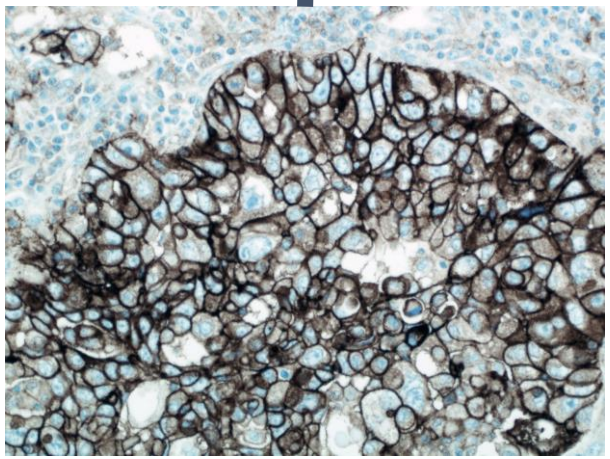


Different ways of looking at the same problem?



Gene-expression signature

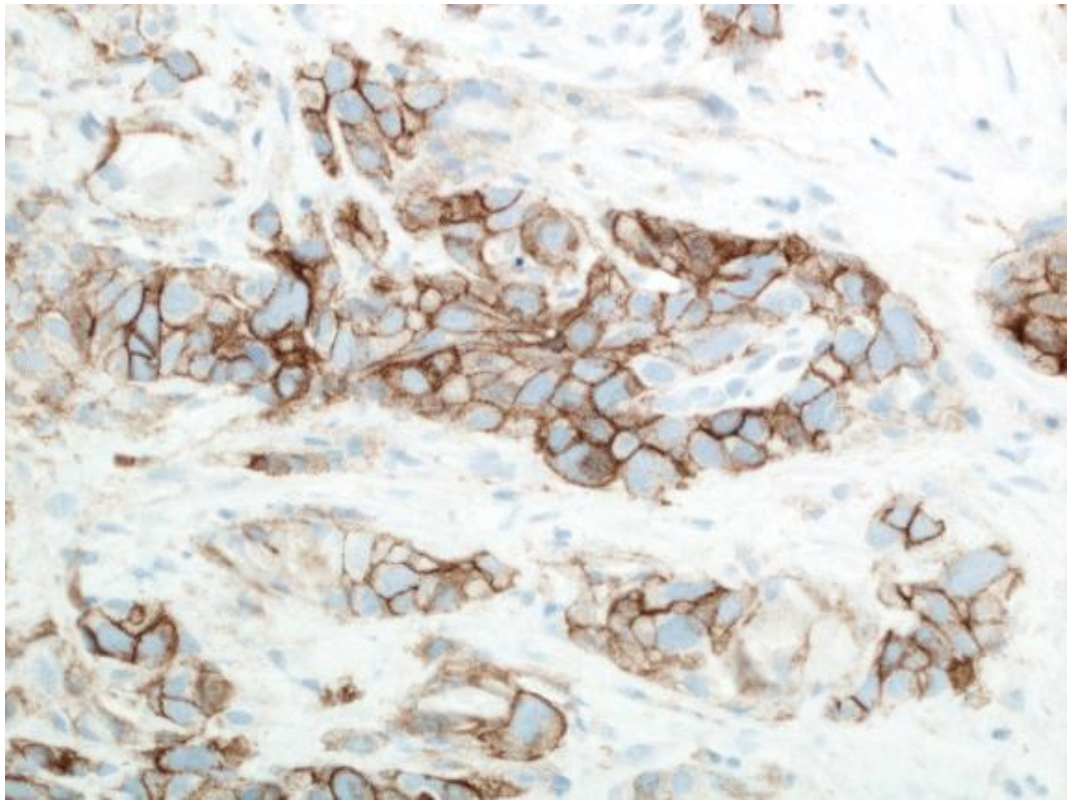
Tumour microenvironment
Tumour inflammation



PD-L1 IHC: a therapy-specific biomarker

Chalmers et al. *Genome Med*. 2017;9:34.

PD-L1 IHC is not going to disappear



- It works
- It is relatively simple, quick and cheap
- Discussions continue over cut points (assay dependant)
- 1% (Pacific, KN042 and pembro1L) [1, 2]
- 25%
- 50%
- >75% [3]
- **Reporting TPS is important**

✓ Cytology is OK
✓ We NEED 100 tumour cells minimum
✓ Choice of assay still crucial

Peters S et al. Ann Oncol 2019 [1] Mountzios G et al. Ann Oncol 2019 [2] ; Aguilar EJ et al. Ann Oncol 2019 [2]

Tumour mutation burden will be statistically associated with a greater chance of enhanced tumour immunogenicity **if**……

- There are **mutations** which code for ‘new’ proteins
- Which are clonal and not subclonal
- And which are ‘antigenic’ – act as neo-antigens
- And the tumour cells are able to process the neo-antigens appropriately
- And present them with MHC molecules

This determines **immunogenicity**

NGS assays for TMB assessment

Table 2 Examples of NGS gene panels in development or currently available to assess TMB

Status	Test name	Number of genes	Coverage (Mb)*	Gene variants	Sample type
FDA-approved or authorised diagnostic assays†	MSK-IMPACT[15 56 68]	468	1.5	SNVs, indels, rearrangements/ fusions, CNAs, parallel analysis of genomic signatures (eg, TMB and dMMR/MSI)	FFPE
	Foundation Medicine FoundationOne CDx[14 49]	324	0.8	SNVs, indels, CNAs, select rearrangements, parallel analysis of genomic signatures (eg, TMB and dMMR/MSI)	FFPE
Commercial assays for research use only	Caris Molecular Intelligence[132]	592	1.4	Somatic missense mutations	FFPE
	Illumina TruSight 500 gene panel[133]	500	2.0	SNVs and indels	FFPE
	Thermo Fisher Scientific Oncomine Tumor Mutation Load Assay[77]	409	1.7	SNVs	FFPE
	NEO New Oncology NEOplus v2 RUO[134]	>340	1.1	SNVs, indels, fusions, CNAs, parallel analysis of TMB, MSI, and driver mutations	FFPE
	Foundation Medicine FoundationOne[50]	315	1.1	SNVs, indels, CNAs, select gene rearrangements, genomic signatures for MSI and TMB	FFPE
	Foundation Medicine bTMB assay[88 122]	394	1.1	SNVs	Blood
	TruSight Tumor 170[135]	170	0.5	Fusions, splice variants, SNVs, indels, amplifications	FFPE
	QIAGEN GeneRead DNAseq Comprehensive Cancer Panel[97]	160	0.7	SNVs, CNAs, indels, and fusions	FFPE
	NEO New Oncology NEOplus[105 136]	94		SNVs, indels, CNAs, rearrangements, and fusions	FFPE

Buettner R et al. ESMO Open 2019

NGS assays for TMB assessment

- What is the denominator?
  - What is a mutation?
- Methodological variations, statistical assumptions and devices, and data filtering involved in generating a 'result'
- How much genome should be sequenced?
  - And which bits?
- Many technical challenges
  - Tissue availability - ~50% success in trials
  - Tissue requirements quite onerous for lung cancer samples
  - DNA input requirements vary
  - Blood vs Tissue debate…….
- Efforts are underway to 'harmonise' the process

Table 2 Examples of NGS gene panels in development or currently available to assess TMB

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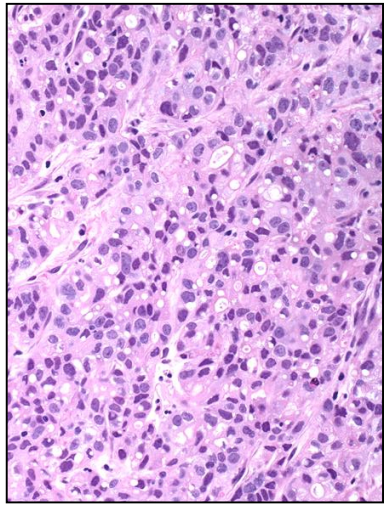
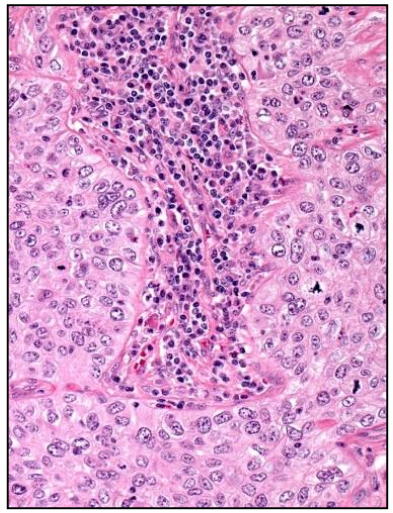
Buettner R et al. ESMO Open 2019

Tumour inflammation & microenvironment

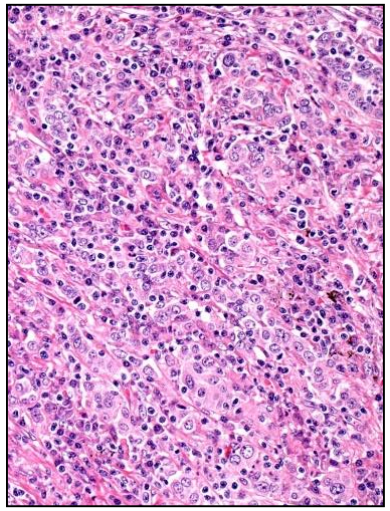
Immune desert

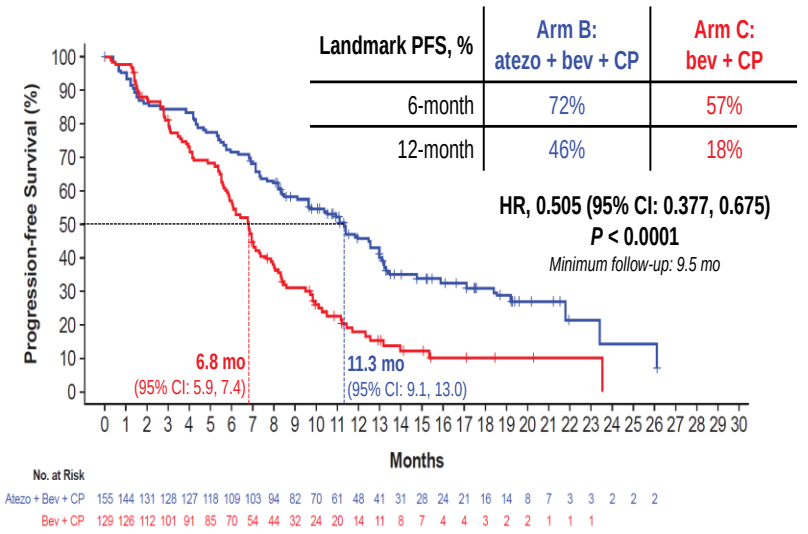


Immune excluded



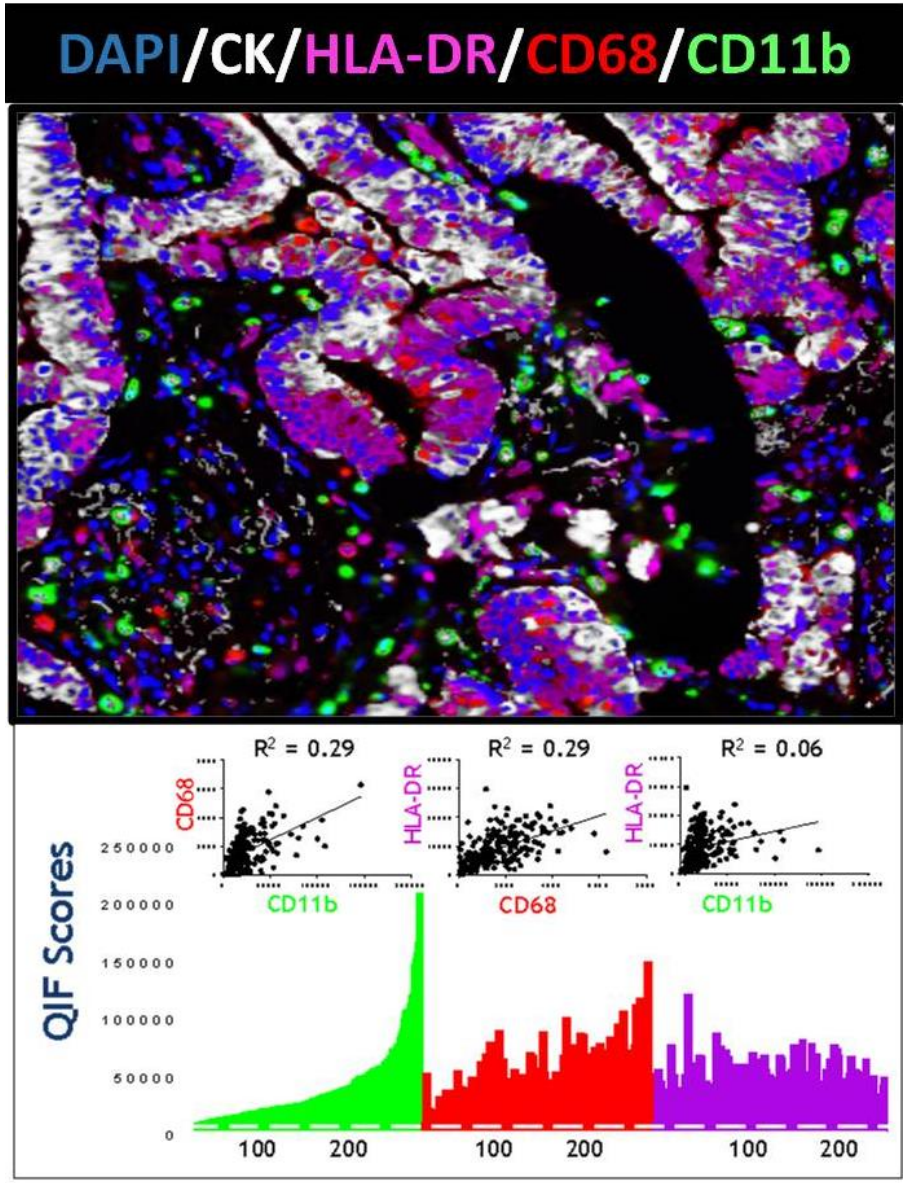
Immune infiltrated





Landmark PFS, %	Arm B: atezo + bev + CP	Arm C: bev + CP
6-month	72%	57%
12-month	46%	18%

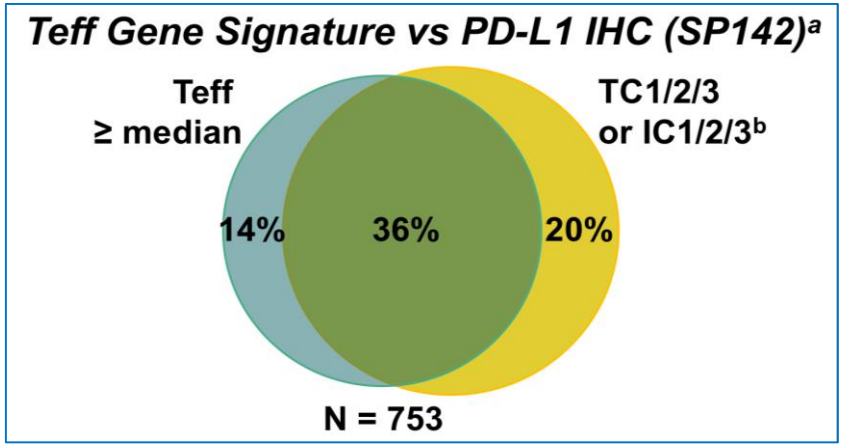
Teff Gene Signature	
PDL1	PD-L1 expression on TC and IC
IFNG	Pre-existing immunity
CXCL9	



Presented By Kurt Schalper at 2018 ASCO Annual Meeting

Our three main biomarkers are NOT co-incident

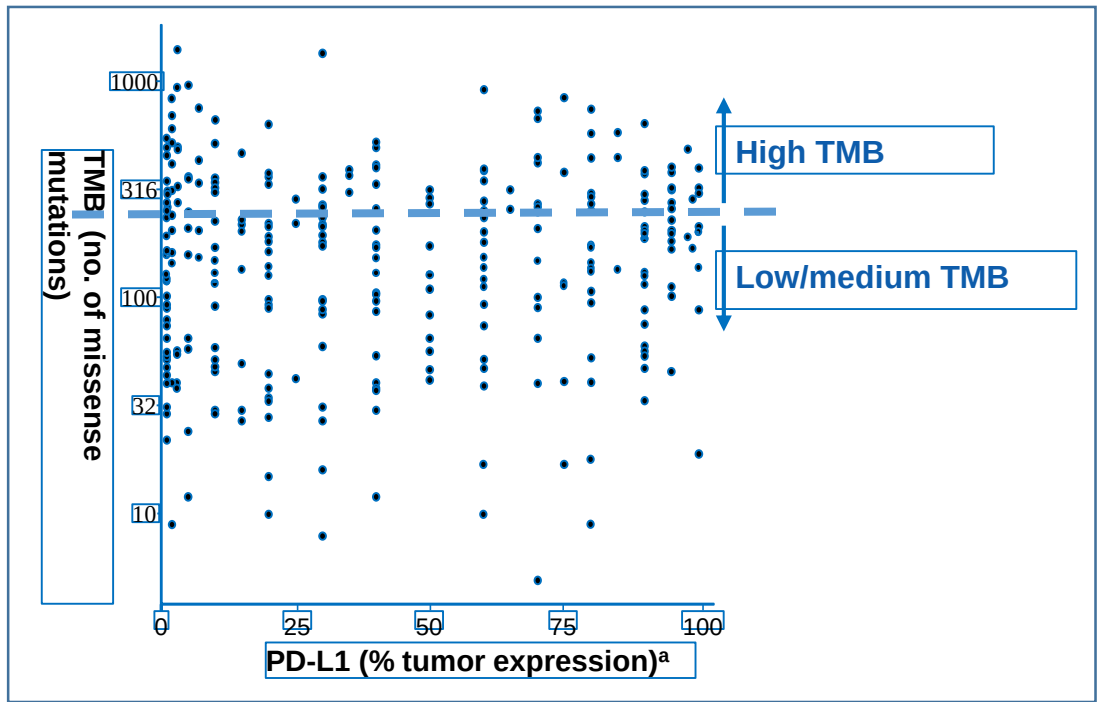
PD-L1 vs GES



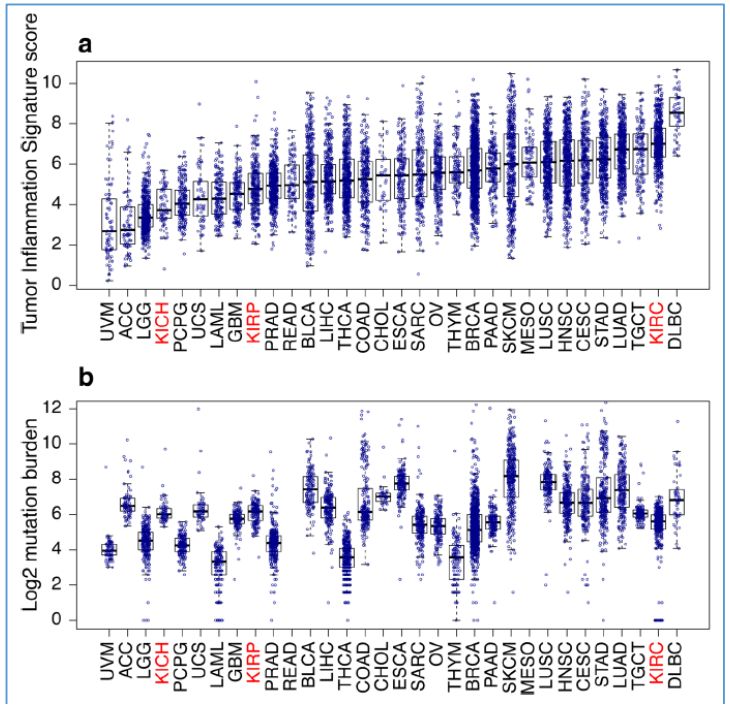
Kowanetz M et al. Presented at WCLC 2017.

GES – Inflammatory genomic signature
TMB – Tumour Mutational Burden

PD-L1 vs TMB



GES vs TMB



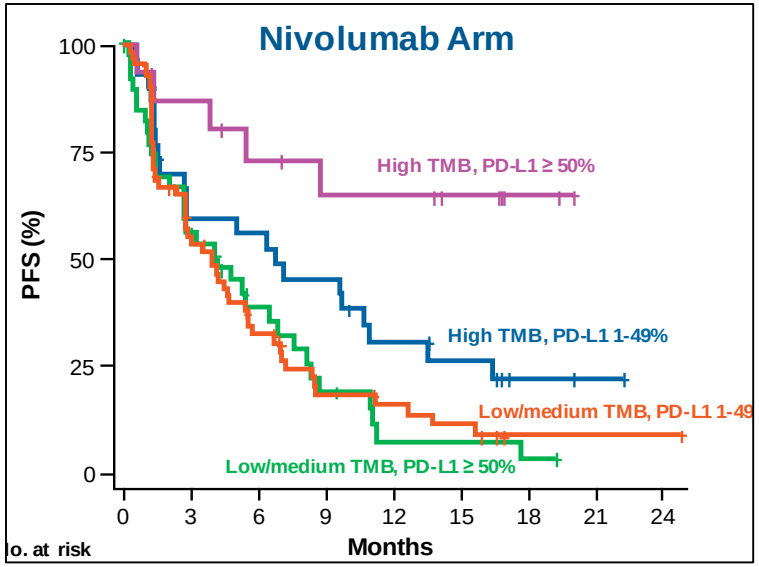
Tumour Inflammation Score

Tumour Mutational Burden

Danaher *et al. Journal for ImmunoTherapy of Cancer* (2018) 6:63

So far we have very little data on biomarker combinations or direct comparisons of different biomarkers in trials

- IMPower 150 trial: Immune gene expression signature (Teff) equivalent to (no better than) PD-L1 IHC
- TMB high and PD-L1 ≥25% subgroups had similar PFS and OS with Durvalumab plus Tremelimumab 2L NSCLC
- Checkmate 026: TMB plus High PD-L1 IHC better than either alone?

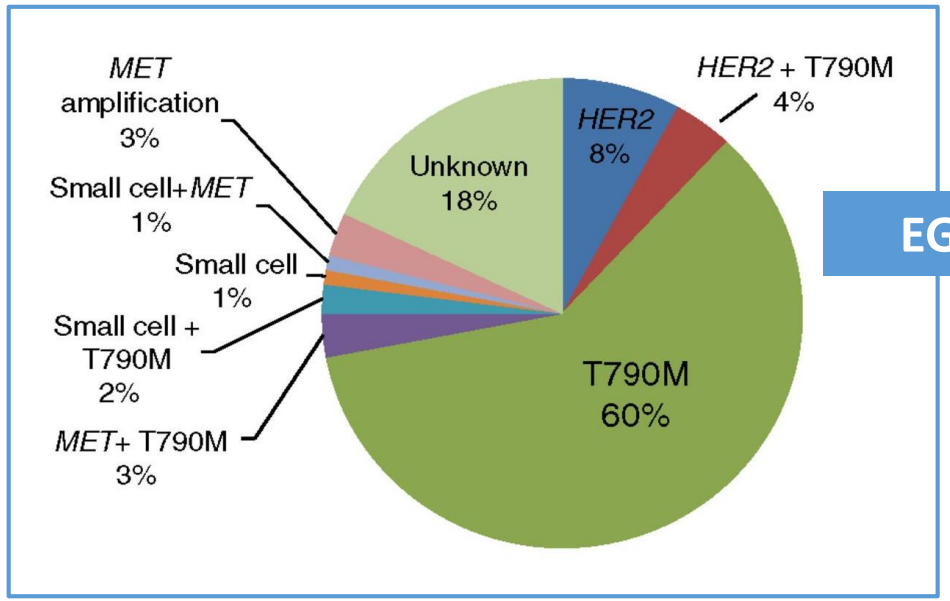


Peters S et al. ESMO-IO 2017; Higgs BW et al. CCR 2018; Carbone DP et al. *N Engl J Med.* 2017

Changes in Immunotherapy biomarker testing?

- PD-L1 IHC will not go away
  - We need to learn how to do this better
- Tumour Mutational Burden
  - Jury still out on a complex biomarker
  - Better ways of assessing neoantigenicity
- Assessing the inflammatory response
  - How best to do it?
- Understanding resistance to immunotherapy
  - Innate mechanisms in the TME (and the patient)
  - Acquired resistance mechanisms

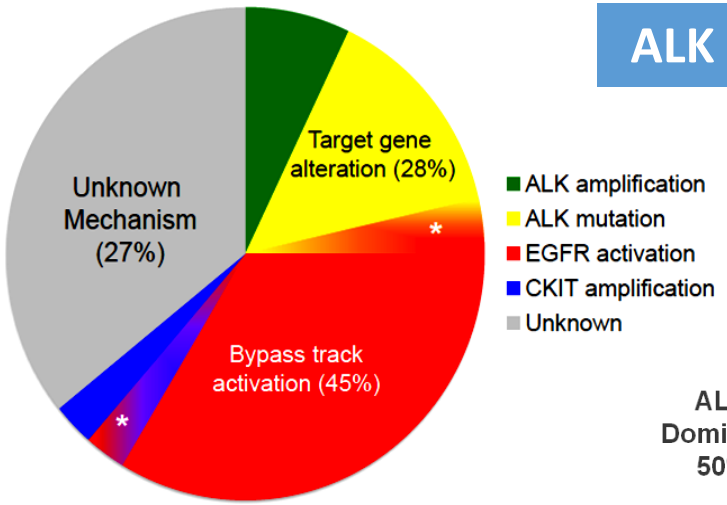
Resistance Mechanisms in TKI Recurrence have Implications for Biomarker Testing



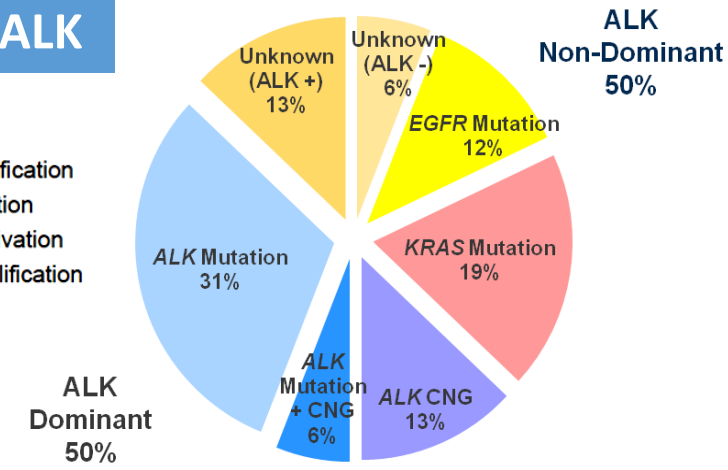
EGFR

Yu et al. *Clin Cancer Res*. 2013;19:2240.

1. Emergent clones resistant to therapy
2. Induction of resistance mutations
3. Adoption of bypass tracks



ALK



Left: Katayama et al. *Sci Transl Med*. 2012;4:120ra17.
Right: Doebele et al. Presentation at ASCO 2012. Abstract 7504.

4. Phenotypic alteration?

Small cell, squamous cell, or sarcomatoid carcinoma

Doebele et al. ASCO 2012. Abstract 7504; Katayama et al. *Sci Transl Med*. 2012;4:120ra17; Yu et al. *Clin Cancer Res*. 2013;19:2240.

ROS1, BRAF…

Testing for Resistance Mechanisms

- Driven by clinical need
  - Is there a clinical intervention predicated on the test results?
  - Is that intervention (drug) available in your practice?
- Samples for testing for resistance
  - Tissue testing
  - Plasma testing
- Shifting landscape
  - EGFR *T790M*
  - Osimertinib ……. ?

Use of cfDNA Assays to Identify EGFR *T790M*

- Plasma cfDNA may be used in adenocarcinoma **patients with progression** or secondary clinical resistance to EGFR-targeted TKIs
- cfDNA analysis has intermediate sensitivity (0.40–0.78) and high specificity
  - **Negative result does not rule out** the possibility of EGFR *T790M* mutation
  - **Positive** *T790M* finding in cfDNA is **equivalent to a tissue biopsy finding**
- If the plasma result is negative, tumour sample testing is recommended
- **Molecular** before **Radiological** before **Clinical** relapse
  - Would you intervene any earlier?

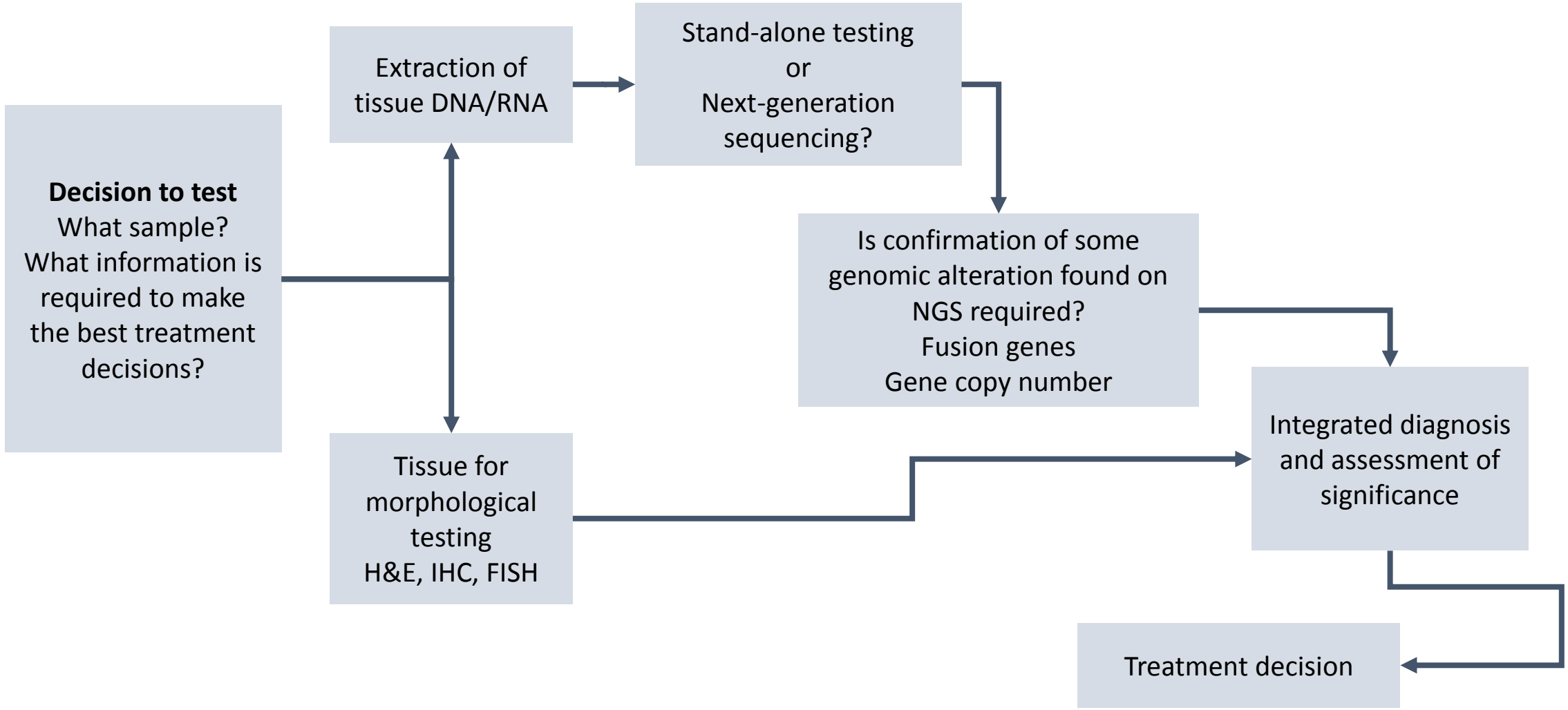
Lindeman et al. *J Thorac Oncol*. 2018;13:323.

Plasma Testing in a Wider Context

- Known target vs unknown profile
- Perhaps gives a more “holistic” view of the patient’s disease
- Monitoring response to therapy
- Technical issues
  - Sensitivity issues remain
  - Germline mutations
  - “False-positives”
  - Origin of mutation
  - Sampling and transport

Rolfo et al. *J Thorac Oncol.* 2018;13:1248.

Biomarker Testing Algorithm: A Dual Track?



Conclusions on Biomarker Testing in Lung Cancer

Improvements

- Established testing algorithms
- NGS becoming a standard
- New targets and biomarkers identified
- Better understanding of using cytology samples
- Better understanding of PD-L1 testing

Remaining Issues

- Dealing with evolving resistance issues
- Utility of cfDNA samples
- Best biomarker approach for immunotherapy
- Dealing with NGS data