Potential Biomarkers for Immune Checkpoint Inhibitors

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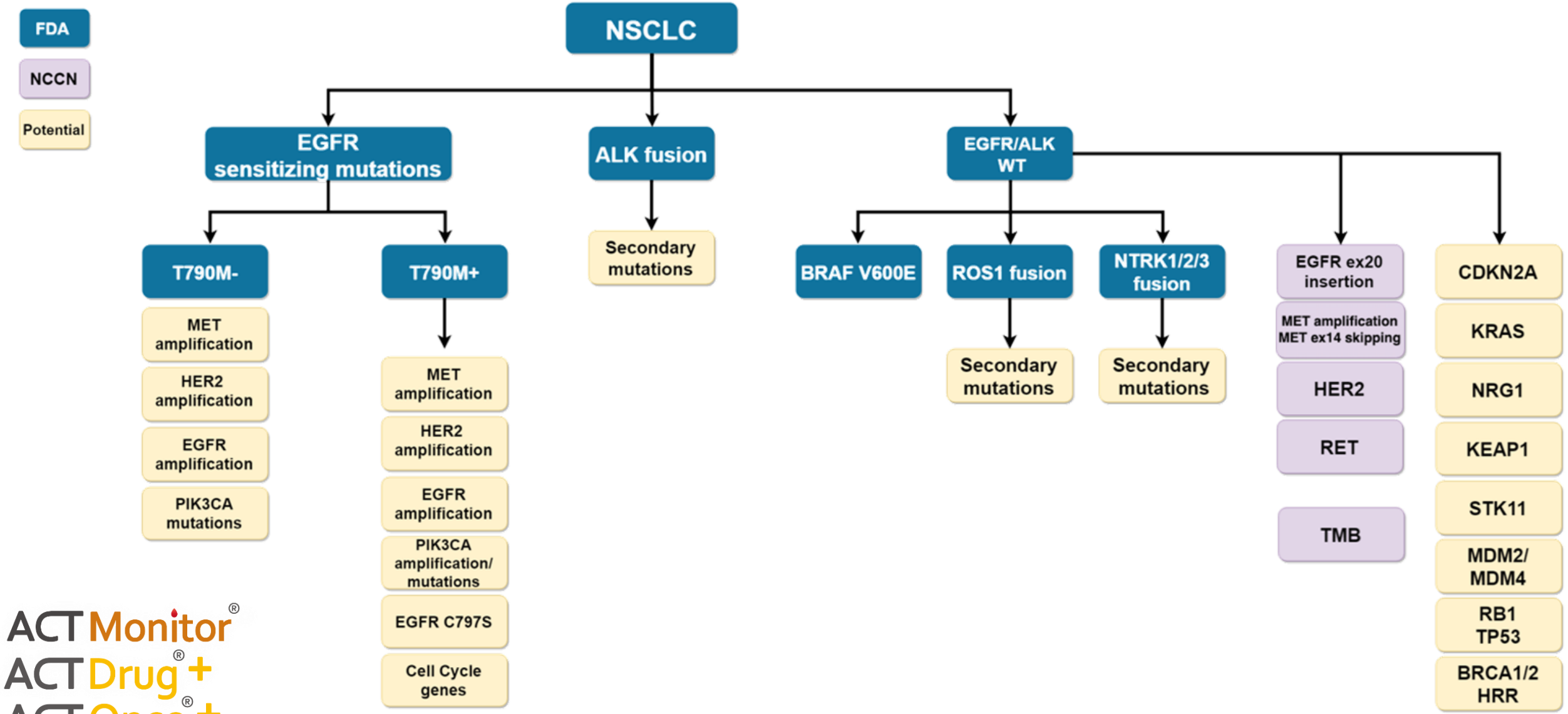
CSO, ACT Genomics

Disclosure

- I am an employee of ACT Genomics

The Potential Molecular Testing Guidelines for NSCLC



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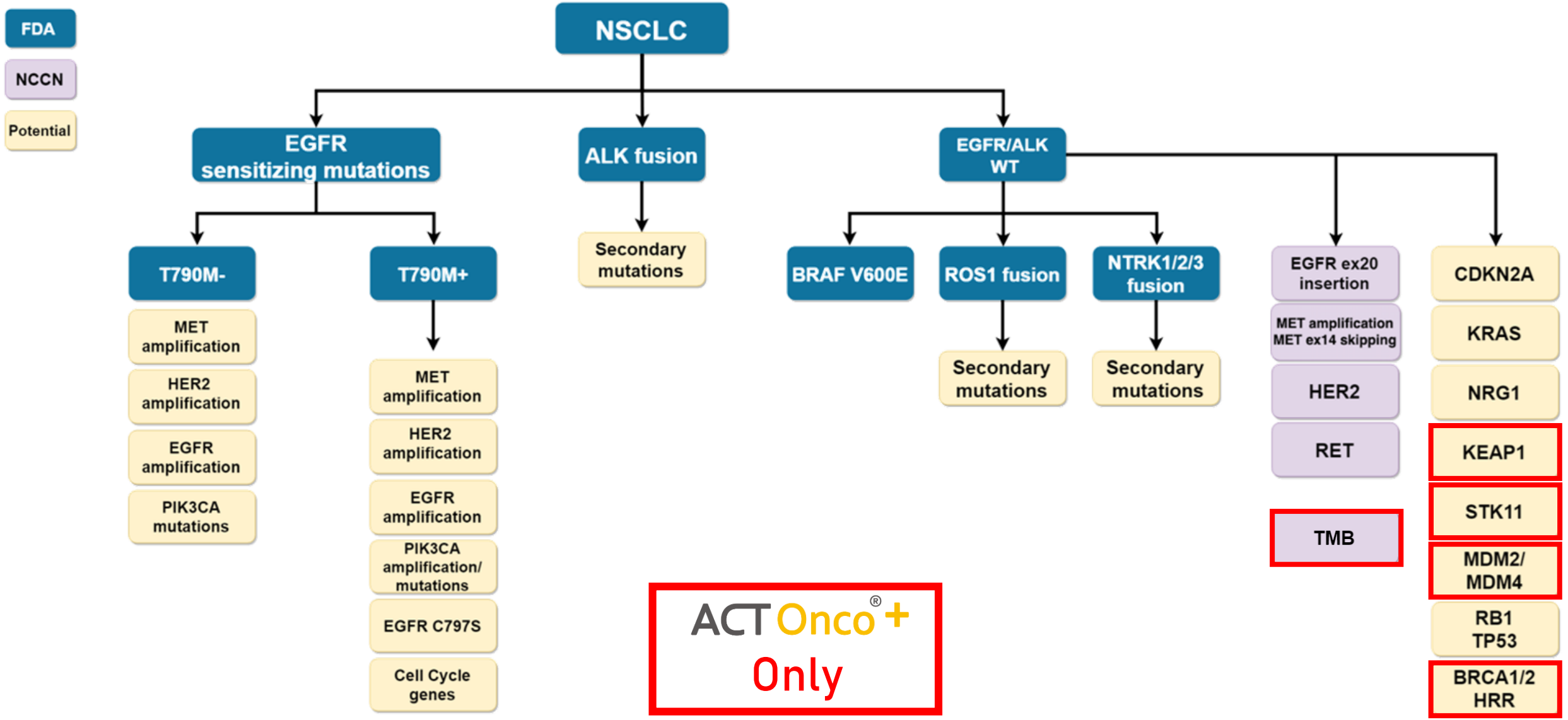
The Potential Molecular Testing Guidelines for NSCLC



When it comes to biomarker for checkpoint inhibitor,

we all know PD-L1 IHC alone is not enough …

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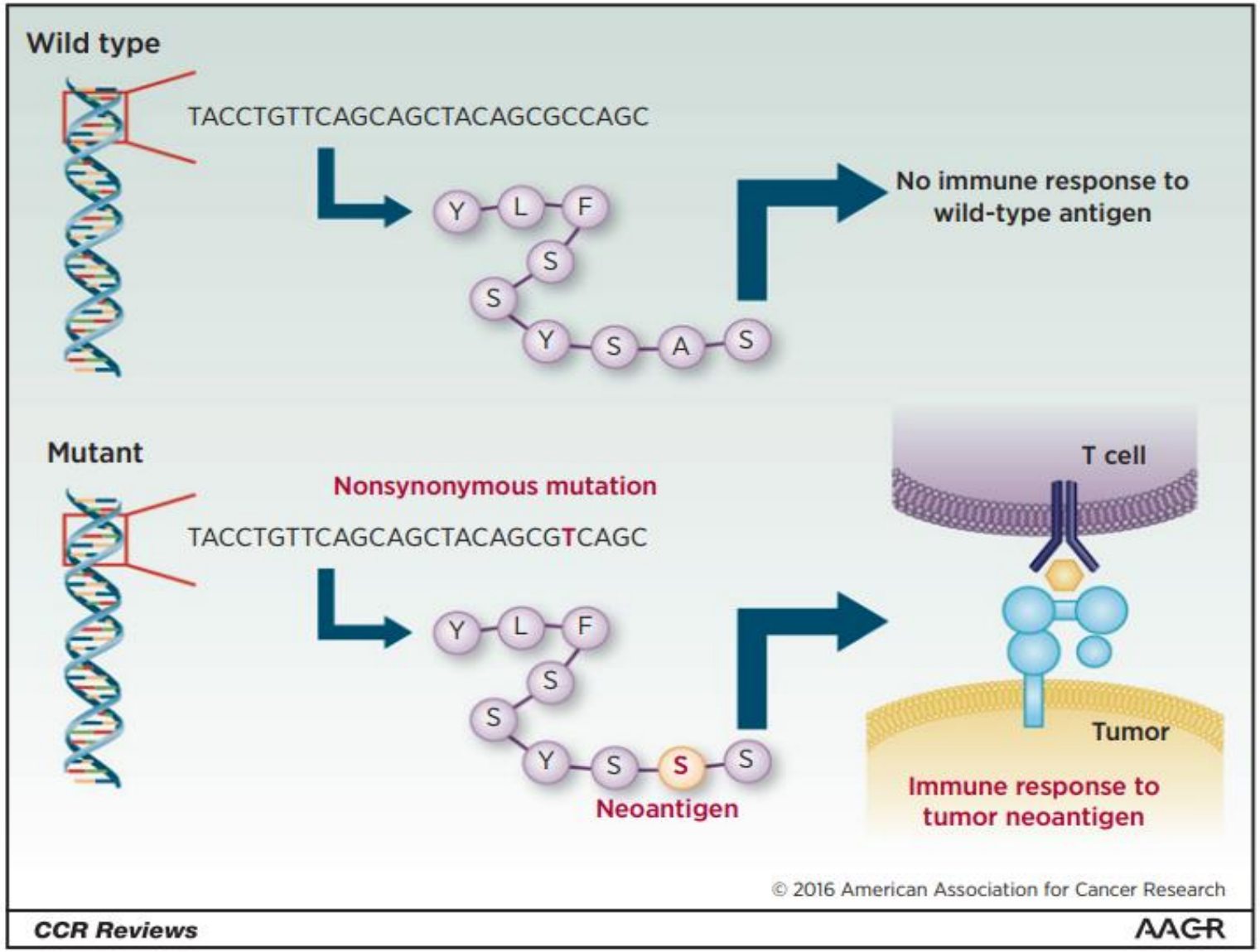
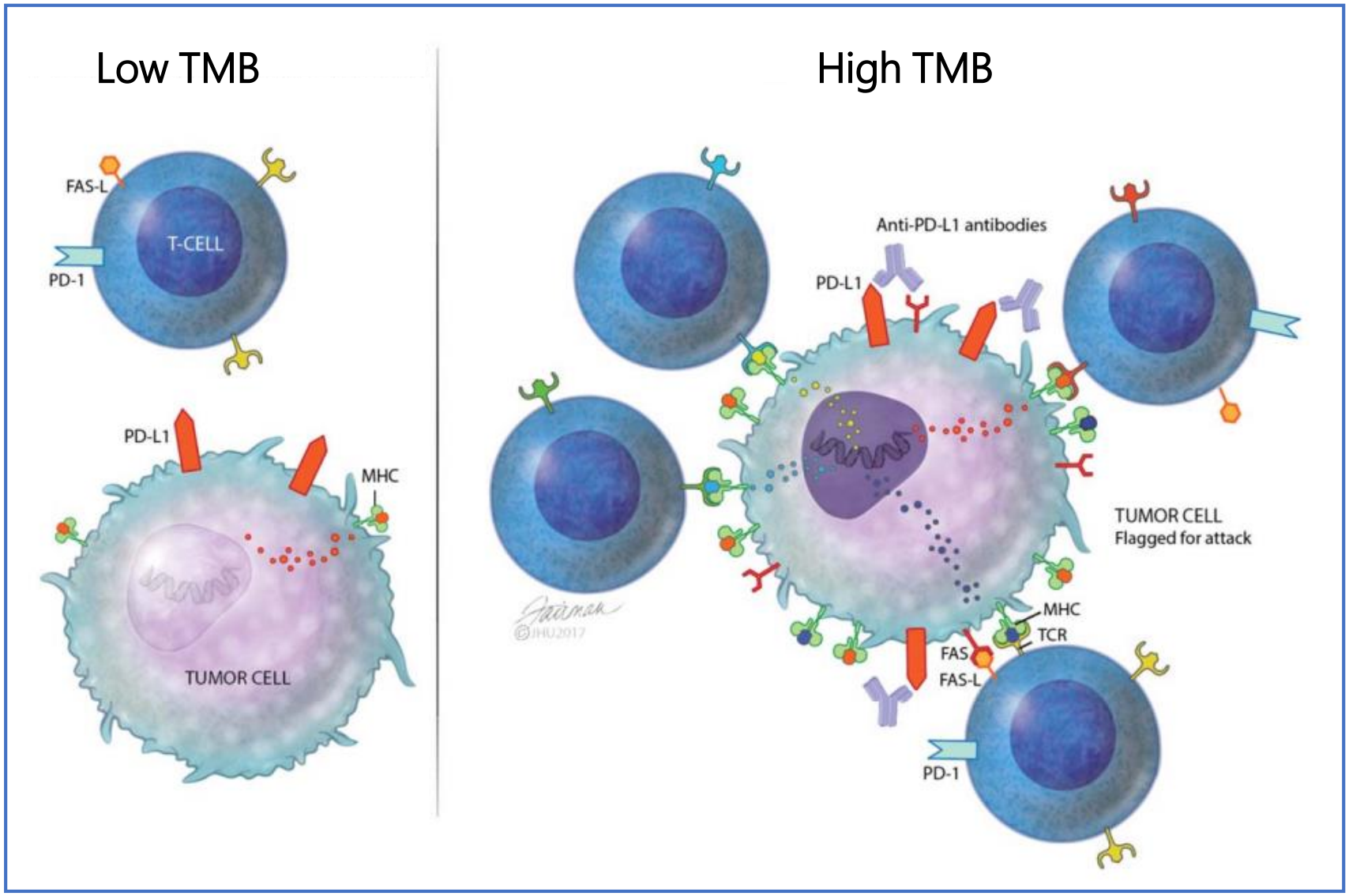
Mutation and Neoantigen Formation



- Wild-type antigens are recognized as “self”, and do not generate an immune response.
- Nonsynonymous mutations may lead to an altered peptide sequence that is ultimately presented on MHC molecules.
- This altered peptide sequence therefore produces a new or “neoantigen”, which may then be recognized by the host immune system, leading to an anti-tumor immune response.

Clin Can Res (2016) 22:5642

TMB and Immunotherapy Response



Greater mutation load **increases the likelihood** of recognition by neoantigen-reactive T cell, therefore, making the tumor **more immunogenic**.

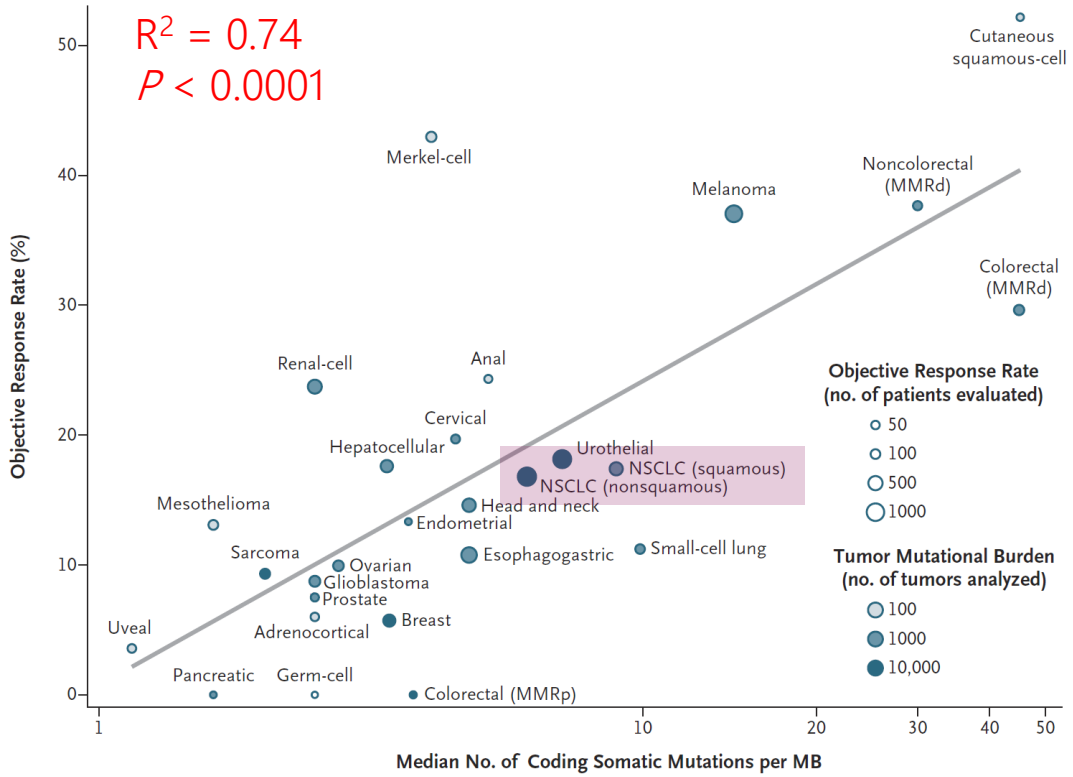
Oncologist (2017) 22:631

Cancers with high TMB tend to respond better to immunotherapy

The NEW ENGLAND JOURNAL of MEDICINE

Tumor Mutational Burden and Response Rate to PD-1 Inhibition

We observed a significant correlation between the tumor mutational burden and the objective response rate (P<0.001). The correlation coefficient of 0.74 suggests that 55% of the differences in the objective response rate across cancer types may be explained by the tumor mutational burden. Some cancer subtypes have a response to therapy that is better than would be predicted by the tumor mutational burden (e.g., Merkel-cell carcinoma), and some have a response that is worse than would be predicted (e.g., colorectal cancer with mismatch repair proficiency). The higher-than-anticipated objective response rates for Merkel-cell carcinoma and some other cancers that have been associated with viruses suggest that the presentation of viral antigens on certain tumor types may confer an increased response rate to anti–PD-1 therapy.[5]



NEJM (2017) 377:2500

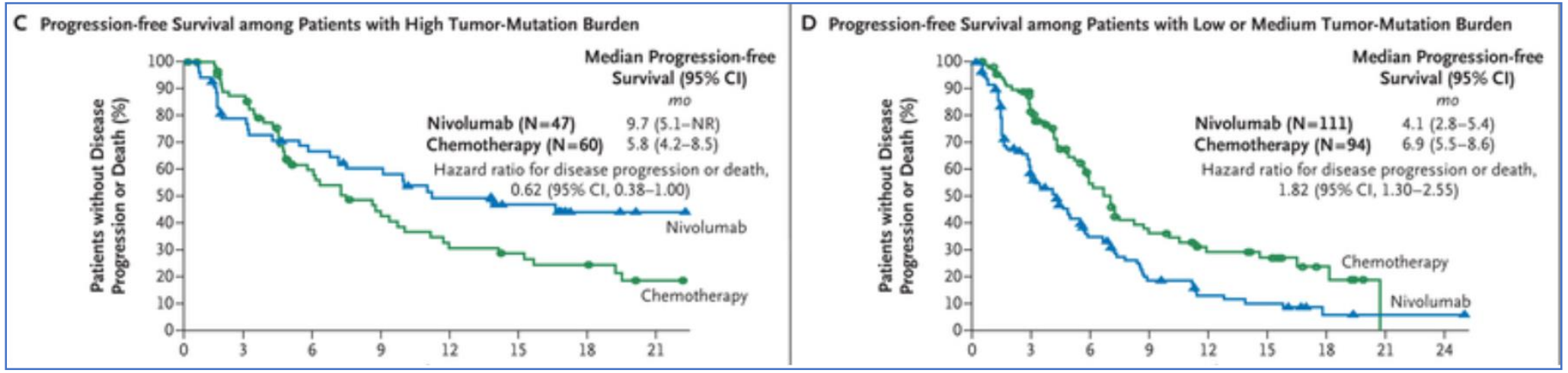
Key trials defining TMB for NSCLC checkpoint blockade benefit

Cancer	Trial and treatment	Method	Threshold defined	RR	PFS	OS	Ref.
NSCLC	KN 001 phase I/II Pembrolizumab	WES	200 mutations	59% versus 12%	NR versus 3.4 months		[40]
NSCLC	BIRCH, FIR phase II Atezolizumab	FM NGS	9.9 mut/Mb	25% versus 14%	HR 0.64	HR 0.87	[70]
NSCLC	POPLAR randomized phase II atezolizumab versus docetaxel	FM NGS	9.9 mut/Mb	20% versus 4%	7.3 versus 2.8 months	16.2 versus 8.3 months	[70]
NSCLC	MSKCC: various immunotherapies	MSKCC NGS	7.4 mut/Mb	38.6% versus 25%			[68]
NSCLC	CM 012 Nivolumab/ipilimumab	WES	158 mutations	51% versus 13%	17.1 versus 3.7 months		[62]
NSCLC	CM 568 Nivolumab/ipilimumab	FM NGS	10 mut/Mb	44% versus 12%	7.1 versus 2.6 months		[71]
NSCLC	CM 026 randomized phase III nivolumab versus chemotherapy	WES	>243 mutations	47% versus 23%	HR 0.62	HR 1.10	[42]
NSCLC	CM 227 randomized phase III nivolumab/ipilimumab versus chemotherapy	FM NGS	>10 mut/Mb	45.3% versus 24.6%	7.1 versus 3.2 months	NA	[77]

Ann Oncol (2019) 30:44

CheckMate 026 PFS Result

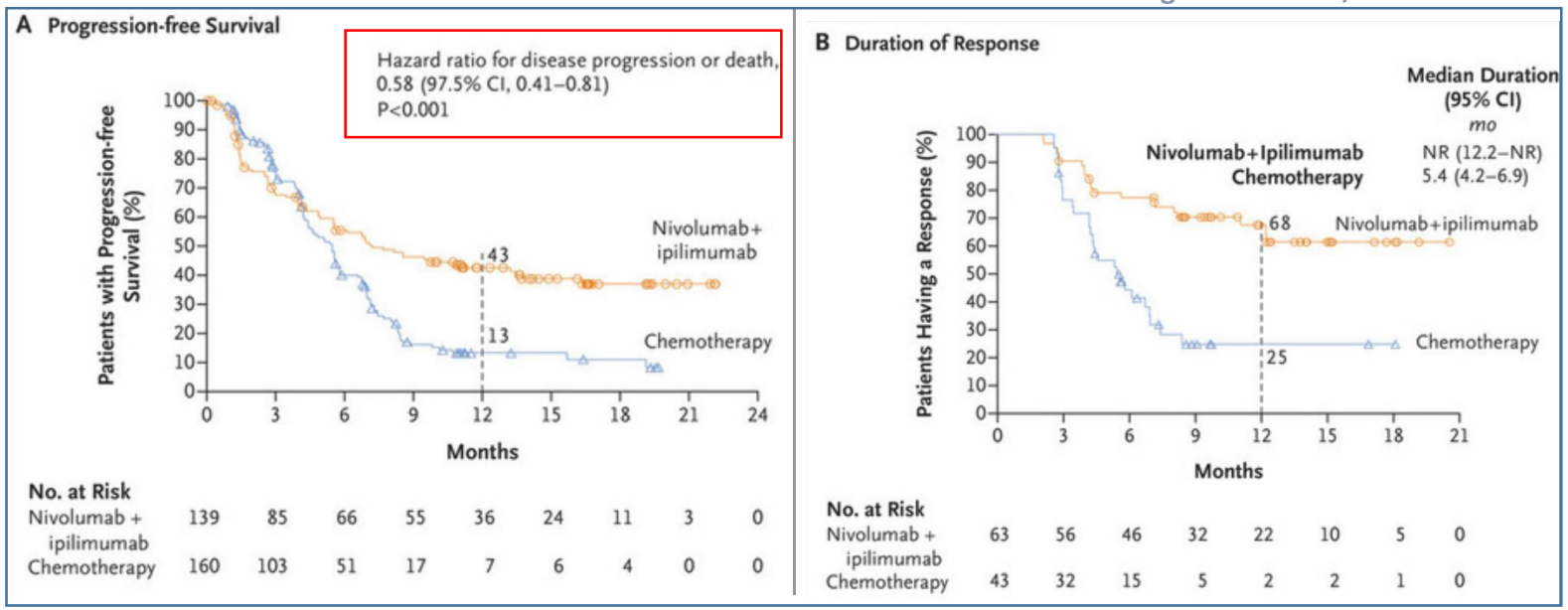
N Engl J Med (2017) 376:2415-2426



- **High tumor mutation burden: WES ≥ 243 mutations**
- **Treatment: nivolumab vs chemotherapy (1st line)**
- **Progression-free survival was significantly longer with first-line nivolumab than with chemotherapy among patients with NSCLC and a high tumor mutational burden, irrespective of PD-L1 expression level.**
- **The results validate the benefit of nivolumab in NSCLC and the role of tumor mutational burden as a biomarker for patient selection.**

CheckMate 227 PFS Result

N Engl J Med 2018; 378:2093-2104



- **Tumor mutation burden: FM1 (≥ 10 Mt/Mb)**
- **Treatment: nivolumab plus ipilimumab vs chemotherapy (1st line)**
- **Progression-free survival was significantly longer with first-line nivolumab plus ipilimumab than with chemotherapy among patients with NSCLC and a high tumor mutational burden, irrespective of PD-L1 expression level.**
- **The results validate the benefit of nivolumab plus ipilimumab in NSCLC and the role of tumor mutational burden as a biomarker for patient selection.**

TMB in the NCCN Guideline for NSCLC

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National Comprehensive Cancer Network®

NCCN Guidelines Version 2.2019
Non-Small Cell Lung Cancer

NCCN Guidelines Index
Table of Contents
Discussion

EMERGING BIOMARKERS TO IDENTIFY NOVEL THERAPIES FOR PATIENTS WITH METASTATIC NSCLC

Genetic Alteration (ie, Driver event)	Available Targeted Agents with Activity Against Driver Event in Lung Cancer
High-level MET amplification or MET exon 14 skipping mutation	Crizotinib[1-5]
RET rearrangements	Cabozantinib[6,7] Vandetanib[8]
Tumor mutational burden (TMB)*	Nivolumab + ipilimumab[10] Nivolumab[11]

*TMB is an evolving biomarker that may be helpful in selecting patients for immunotherapy. There is no consensus on how to measure TMB.

8001.
[3]Frampton GM, Ali SM, Rosenzweig M, et al. Activation of MET via diverse exon 14 splicing alterations occurs in multiple tumor types and confers clinical sensitivity to MET inhibitors. Cancer Discov 2015;5:850-859.
[4]Paik PK, Drilon A, Fan PD, et al. Response to MET inhibitors in patients with stage IV lung adenocarcinomas harboring MET mutations causing exon 14 skipping. Cancer Discov 2015;5:842-849.
[5]Awad MM, Oxnard GR, Jackman DM, et al. MET exon 14 mutations in non-small-cell lung cancer are associated with advanced age and stage-dependent MET genomic amplification and cMET overexpresion. J Clin Oncol 2016;34:721-730.
[6]Drilon A, Wang L, Hasanovic A, et al. Response to cabozantinib in patients with RET fusion-positive lung adenocarcinomas. Cancer Discov 2013; 3:630-635.
[7]Drilon A, Rekhtman N, Arcila M, et al. Cabozantinib in patients with advanced RET-rearranged non-small-cell lung cancer: an open-label, single-centre, phase 2, single-arm trial. Lancet Oncol 2016;17:1653-1660.
[8]Lee SH, Lee JK, Ahn MJ, et al. Vandetanib in pretreated patients with advanced non-small cell lung cancer-harboring RET rearrangement: a phase II clinical trial. Ann Oncol 2017;28:292-297.
[9]Li BT, Shen R, Buonocore D, et al. Ado-trastuzumab emtansine in patients with HER2 mutant lung cancers: Results from a phase II basket trial. J Clin Oncol 2018;36:2532-2537.
[10]Hellmann MD, Ciuleanu TE, Pluzanski A et al. Nivolumab plus ipilimumab in lung cancer with a high tumor mutational burden. N Engl J Med 2018; 378:2093-2104.
[11]Carbone DP, Reck M, Paz-Ares L et al. First-line nivolumab in stage IV or recurrent non-small-cell lung cancer. N Engl J Med 2017;376:2415–2426.

Note: All recommendations are category 2A unless otherwise indicated.
Clinical Trials: NCCN believes that the best management of any patient with cancer is in a clinical trial. Participation in clinical trials is especially encouraged.

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NSCL-H

Just when we think TMB is a smooth sail …

BMS withdraws Nivo/Ipi Application in TMB-High NSCLC

News >

BMS Withdraws Nivolumab/Ipilimumab Application in TMB-High NSCLC

Gina Columbus
Published Online:9:29 PM, Fri January 25, 2019

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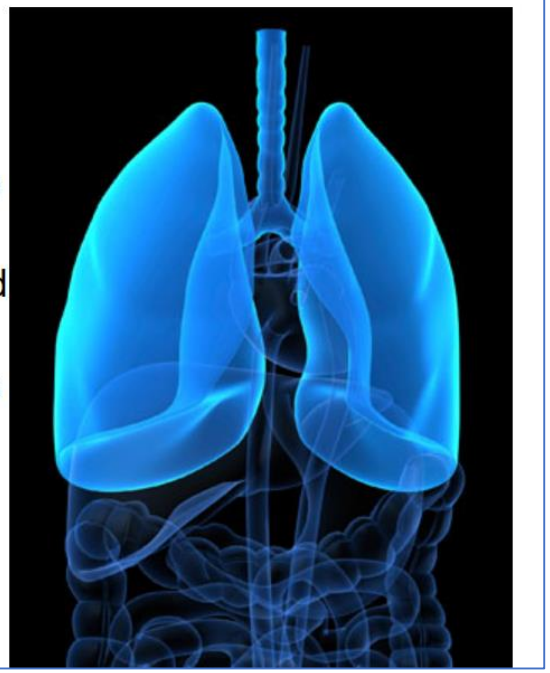
Bristol-Myers Squibb (BMS) has announced its decision to withdraw a supplemental biologics license application (sBLA) currently with the FDA seeking frontline approval for the combination of nivolumab (Opdivo) and ipilimumab (Yervoy) for patients with advanced non–small cell lung cancer (NSCLC) with tumor mutational burden (TMB) ≥10 mutations per megabase (mut/Mb).[1] The company withdrew its application following recent discussions with the FDA.

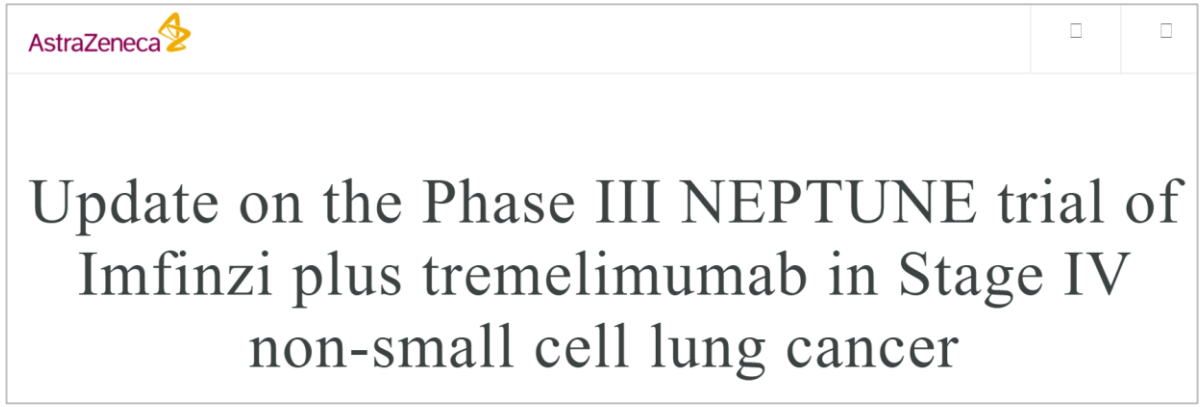


The application was initially accepted by the

Tissue TMB: F1CDx panel
Cutoff: 10 mut/Mb

Updated CheckMate 227 data did not show significant difference in overall survival benefit between TMB-High and TMB-Low groups.

AstraZeneca's NEPTUNE trial failed to meet primary endpoint


AstraZeneca

Update on the Phase III NEPTUNE trial of Imfinzi plus tremelimumab in Stage IV non-small cell lung cancer

PUBLISHED
21 August 2019

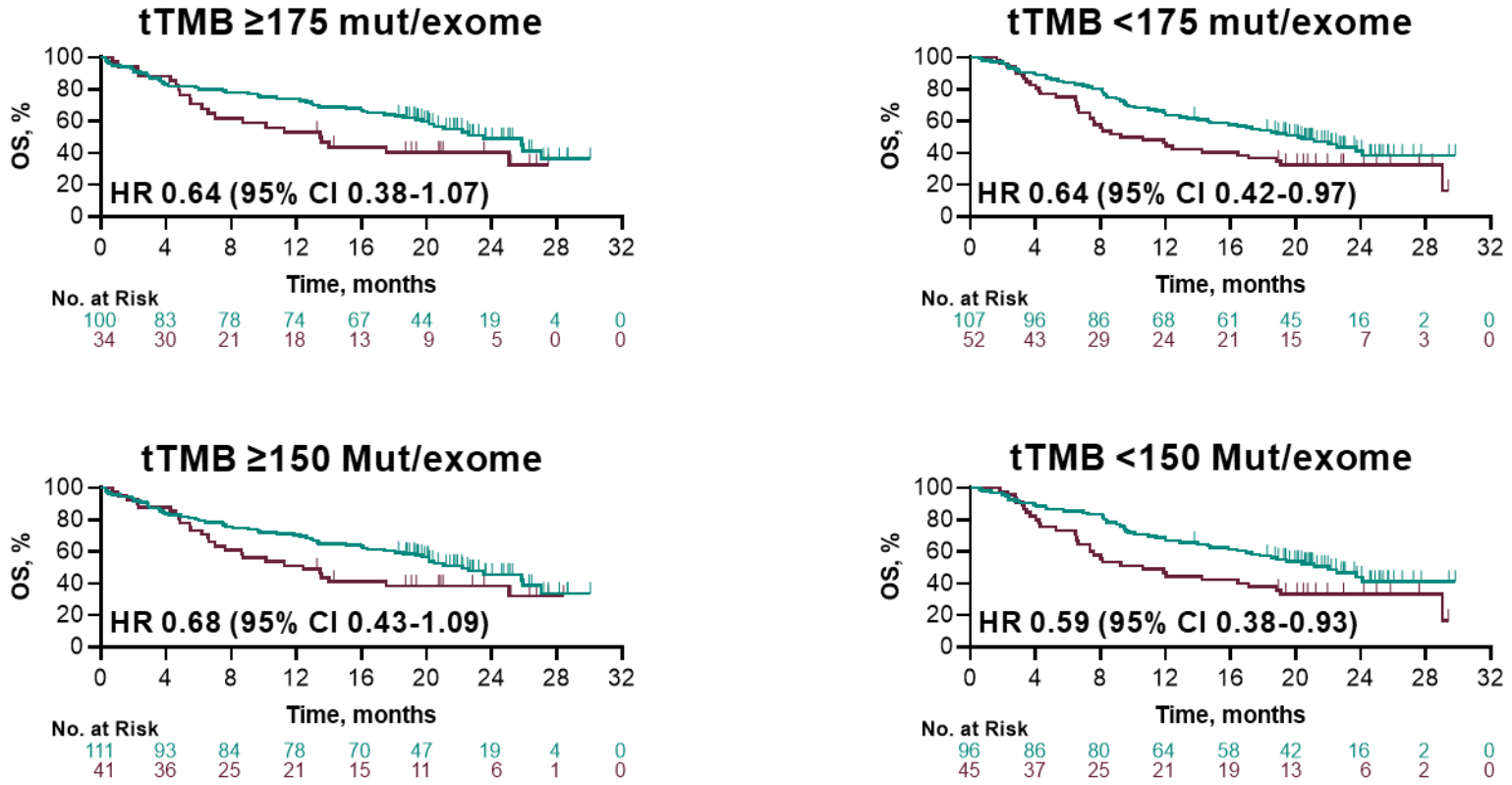
Blood TMB: GuardantOMNIpanel
Cutoff: 20 mut/Mb

AstraZeneca today announced final overall survival (OS) results from the Phase III NEPTUNE trial, a randomised, open-label, multi-centre, global trial of Imfinzi (durvalumab) in combination with tremelimumab, an anti-CTLA4 antibody, vs. standard-of-care (SoC) platinum-based chemotherapy in previously-untreated Stage IV (metastatic) non-small cell lung cancer NSCLC() patients. The trial was performed in an all-comers population, and the primary analysis population was patients with a high tumour mutational burden (TMB). TMB is a measurement of the number of mutations within the genome (DNA) of a tumour, and tumours with high levels of TMB may be more visible to the immune system.1,2

In the primary analysis population of patients whose blood TMB was 20 or more mutations per megabase (mut/Mb), the combination of Imfinzi and tremelimumab did not meet the primary endpoint of improving OS compared to SoC chemotherapy. The safety and tolerability profile for the combination of Imfinzi and tremelimumab was consistent with previous trials.

TMB failed to predict KEYNOTE-189 IO benefits

Clinical Utility for OS: tTMB Cutpoints of 175 and 150 mut/exome



Data cutoff date: Sep 21, 2018.

Tissue TMB: WES
Cutoff: 175 mut/exome

Adopted from Garassino WCLC 2019

No correlation between TMB and response in KN-021, 189 & 407

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Pembrolizumab Plus Platinum-Based Chemotherapy for Metastatic NSCLC: Tissue TMB (tTMB) and Outcomes in KEYNOTE-021, 189, and 407

Luis Paz-Ares,[1] Corey J. Langer,[2] Silvia Novello,[3] Balazs Halmos,[4] Ying Cheng,[5] Shirish M. Gadgeel,[6] Rina Hui,[7] Shunichi Sugawara,[8] Hossein Borghaei,[9] Razvan Cristescu,[10] Deepti Aurora-Garg,[10] Andrew Albright,[10] Andrey Loboda,[10] Julie Kobie,[10] Jared Lunceford,[10] Mark Ayers,[10] Gregory M. Lubiniecki,[10] M. Catherine Pietanza,[10] Bilal Piperdi,[10] Marina C. Garassino[11]

[1]Hospital Universitario 12 de Octubre, Spanish National Cancer Research Center, Universidad Complutense and Ciberonc, Madrid, Spain; [2]Abramson Cancer Center of the University of Pennsylvania, Philadelphia, PA, USA; [3]University of Turin, Orbassano, Italy; [4]Montefiore Medical Center/Albert Einstein College of Medicine, Bronx, NY, USA; [5]Jilin Cancer Hospital, Changchun, China; [6]Karmanos Cancer Institute, Detroit, MI, USA (currently at University of Michigan, Ann Arbor, MI, USA); [7]Westmead Hospital and University of Sydney, Sydney, NSW, Australia; [8]Sendai Kousei Hospital, Miyagi, Japan; [9]Fox Chase Cancer Center, Philadelphia, PA, USA; [10]Merck & Co., Inc., Kenilworth, NJ, USA; [11]Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy

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Tissue TMB: WES
Cutoff: 175 mut/exome

No Significant Association between tTMB and Efficacy

Paz-Ares KN021/189/407 TMB ESMO 2019

Association of tTMB (log_{10}) With Efficacy

Nominal *P* Value[a]	KEYNOTE-021 C and G		KEYNOTE-189		KEYNOTE-407	
	Pembro + Chemo (n = 44)	Chemo Alone (n = 26)	Pembro + Chemo (n = 207)	Placebo + Chemo (n = 86)	Pembro + Chemo (n = 143)	Placebo + Chemo (n = 169)
ORR	0.180	0.279	0.072	0.434	0.393	0.086
PFS	0.187	0.409	0.075	0.055	0.052	0.560
OS	0.081	0.475	0.174	0.856	0.160	0.818

No association between tTMB (continuous, log_{10}-transformed) and efficacy for pembrolizumab + chemotherapy or chemotherapy ± placebo in any study based on α = 0.05 significance level

[a]*P* were values calculated using the Wald test and are one-sided for pembro + chemo (a priori hypothesis that tTMB was positively associated with improved outcomes for pembro + chemo) and two-sided for chemo alone and placebo + chemo (no a priori hypothesis regarding direction of the association between tTMB and outcomes).
Data cutoff dates: Dec 1, 2017 (KEYNOTE-021); Sep 21, 2018 (KEYNOTE-189); May 9, 2019 (KEYNOTE-407).

Just when we think TMB is totally busted …

Positive association between tTMB in KN-010 and KN-042

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Association Between Tissue TMB and Clinical Outcomes with Pembrolizumab Monotherapy in PD-L1-Positive Advanced NSCLC in the KEYNOTE-010 and 042 Trials

Roy S. Herbst[1], Gilberto Lopes[2], Dariusz M. Kowalski[3], Makoto Nishio[4]; Yi-long Wu[5], Gilberto de Castro Jr[6], Paul Baas[7], Dong-Wan Kim[8], Matthew A. Gubens[9], Razvan Cristescu[10], Deepti Aurora-Garg[10], Andrew Albright[10], Mark Ayers[10], Andrey Loboda[10], Jared Lunceford[10], Julie Kobie[10], Gregory Lubiniecki[10], M. Catherine Pietanza[10], Bilal Piperdi[10], Tony SK Mok[11]

[1]Yale University School of Medicine, Yale Cancer Center, New Haven, CT, USA; [2]Sylvester Comprehensive Cancer Center at the University of Miami, Miami, FL, USA; [3]The Maria Sklodowska Curie Memorial Cancer Centre and Institute of Oncology, Warsaw, Poland; [4]Department of Thoracic Medical Oncology, The Cancer Institute Hospital of Japanese Foundation for Cancer Research, Tokyo, Japan; [5]Guandong Lung Cancer Institute, Guangdong General Hospital, and Guangdong Academy of Medical Sciences, Guangdong, China; [6]Instituto do Cancer do Estado de Sao Paulo, Sao Paulo, Brazil; [7]Netherlands Cancer Institute, Amsterdam, Netherlands; [8]Seoul National, University Hospital, Seoul, Republic of Korea; [9]University of California, San Francisco, CA, USA; [10]Merck &Co., Inc, Kenilworth, NJ, USA; [11]State Key Laboratory of Translational Oncology, Chinese University of Hong Kong, Shatin, Hong Kong, China

esmo.org

Tissue TMB: WES
Cutoff: 175 mut/exome

tTMB is associated with Efficacy of Pembro but not Chemo

Herbst KN010/042 ESMO 2019

Association of tTMB (log_{10}) With Efficacy (KEYNOTE-010[a])

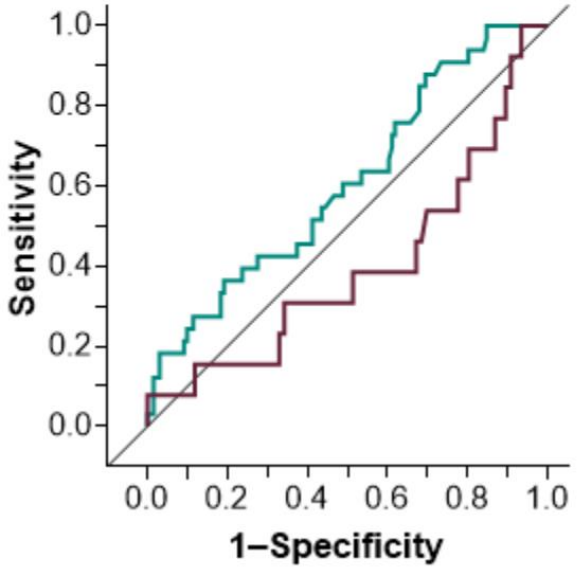
Nominal *P* Value[b]	Pembro (n = 164)	Chemo (n = 89)
OS	0.006 (one-sided)	0.410 (two-sided)
PFS	0.001 (one-sided)	0.579 (two-sided)
ORR	0.009 (one-sided)	0.330 (two-sided)

tTMB was associated with outcomes for pembro as a continuous variable but not with chemo based on α = 0.05 significance level and AUROC analysis

[a]All patients were PD-L1-positive (TPS ≥1%). [b]Wald test. *P* values are one-sided for pembro as the a priori hypothesis was that tTMB was positively associated with improved outcomes of pembro. *P* values are two-sided for placebo because there was no a priori hypothesis regarding the direction of the association between tTMB and outcomes of chemo. TMB was assessed as a continuous, log_{10}-transformed variable.
Data cutoff date: Mar 16, 2018.

ROC Curves of ORR for tTMB

	AUROC (95% CI)
Pembro	0.61 (0.50-0.71)
Chemo	0.40 (0.21-0.58)



tTMB is associated with Efficacy of Pembro but not Chemo

Herbst KN010/042 ESMO 2019

Association of tTMB (log_{10}) With Efficacy (KEYNOTE-042[a])

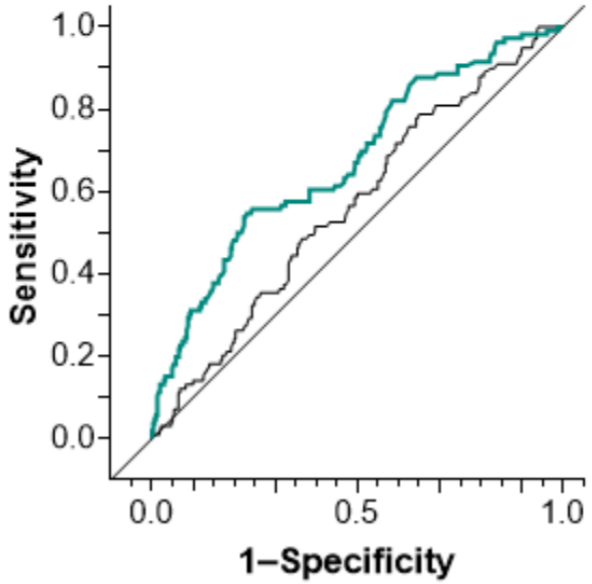
Nominal *P* Value[b]	Pembro (n = 414)	Chemo (n = 379)
OS	<0.001 (one-sided)	0.060 (two-sided)[c]
PFS	<0.001 (one-sided)	0.174 (two-sided)[c]
ORR	<0.001 (one-sided)	0.035 (two-sided)

tTMB was associated with outcomes for pembro as a continuous variable but not chemo in general, based on α = 0.05 significance level and AUROC

[a]All patients were PD-L1-positive (TPS ≥1%). [b]Wald test. *P* values are one-sided for pembro as the a priori hypothesis was that tTMB was positively associated with improved outcomes of pembro. *P* values are two-sided for placebo as there was no a priori hypothesis regarding the direction of association between tTMB and outcomes of chemo. TMB was assessed as a continuous, log_{10}-transformed variable. [c]tTMB showed negative directions of association with OS and PFS in the chemo arm.
Data cutoff date: Sep 4, 2018.

ROC Curves of ORR for tTMB

	AUROC (95% CI)
Pembro	0.67 (0.61-0.73)
Chemo	0.57 (0.50-0.63)



Association of tTMB with Pembro Efficacy in KN-158

BARCELONA 2019 ESMO congress

Association of Tumor Mutational Burden with Outcomes in Patients with Select Advanced Solid Tumors Treated with Pembrolizumab in KEYNOTE-158

Aurélien Marabelle,[1] Marwan Fakih,[2] Juanita Lopez,[3] Manisha Shah,[4] Ronnie Shapira-Frommer,[5] Kazuhiko Nakagawa,[6] Hyun Cheol Chung,[7] Hedy Kindler,[8] Jose A. Lopez-Martin,[9] Wilson H. Miller, Jr.,[10] Antoine Italiano,[11] Steven Kao,[12] Sarina Piha-Paul,[13] Jean-Pierre Delord,[14] Robert McWilliams,[15] Deepti Aurora-Garg,[16] Menghui Chen,[16] Fan Jin,[16] Kevin Norwood,[16] Yung-Jue Bang[17]

[1]Gustave Roussy, INSERM U1015, Villejuif, France; [2]City of Hope Comprehensive Cancer Center, Duarte, CA, USA; [3]The Royal Marsden Foundation Trust and the Institute of Cancer Research, London, UK; [4]Ohio State University Comprehensive Cancer Center, Columbus, OH, USA; [5]Sheba Medical Center, Ramat Gan, Israel; [6]Kindai University Faculty of Medicine, Osaka, Japan; [7]Yonsei Cancer Center and Yonsei University College of Medicine, Seoul, South Korea; [8]University of Chicago, Chicago, IL, USA; [9]12 de Octubre University Hospital & Research Institute (i+12), Madrid, Spain; [10]Jewish General Hospital and McGill University, Montréal, QC, Canada; [11]Institut Bergonié, Bordeaux, France; [12]Chris O'Brien Lifehouse, Sydney, NSW, Australia; [13]University of Texas MD Anderson Cancer Center, Houston, TX, USA; [14]Institut Claudius Regaud IUCT-Oncopole, Toulouse, France; [15]Mayo Clinic, Rochester, MN, USA; [16]Merck & Co., Inc., Kenilworth, NJ, USA; [17]Seoul National University College of Medicine, Seoul, South Korea

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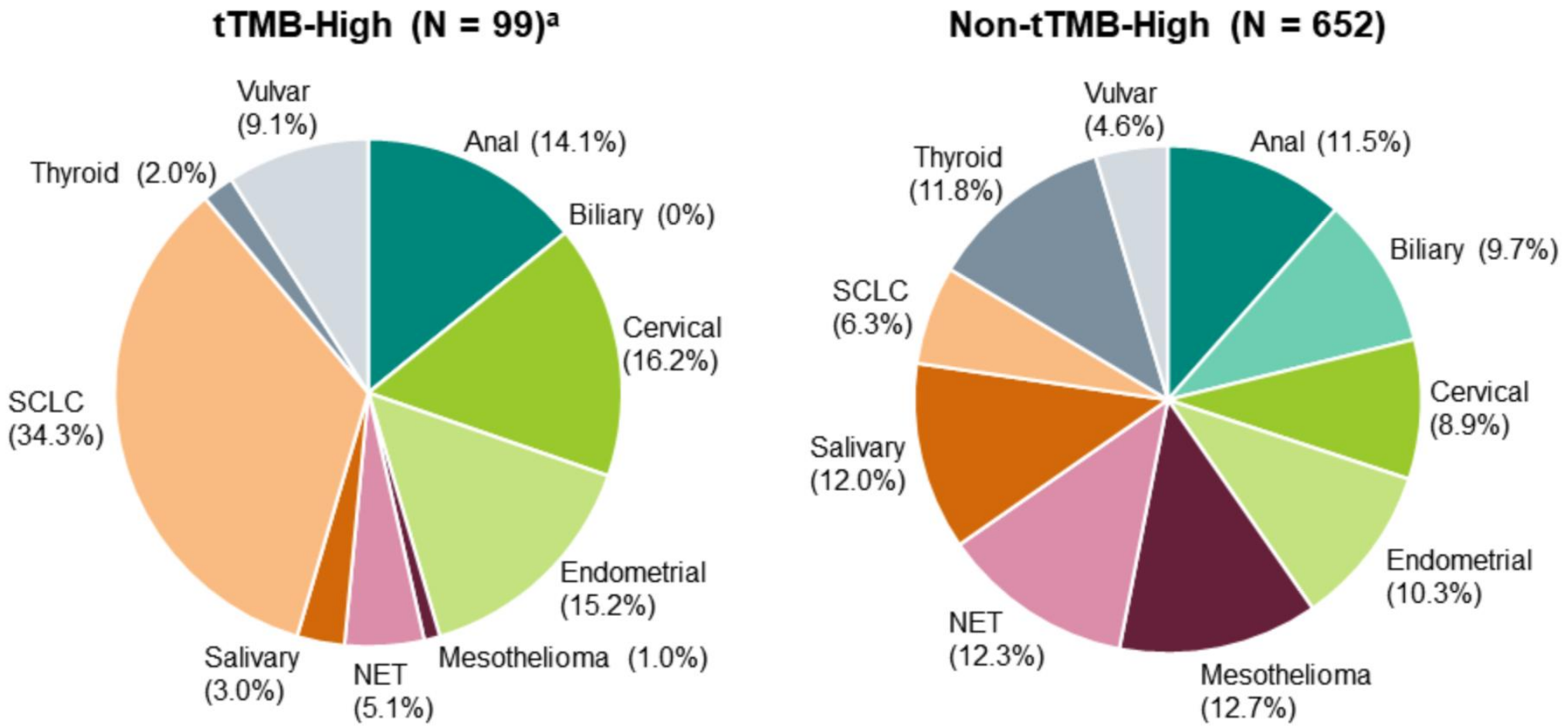
Tissue TMB: WES
Cutoff: 175 mut/exome

Tumor type and TMB Distribution in KN-158

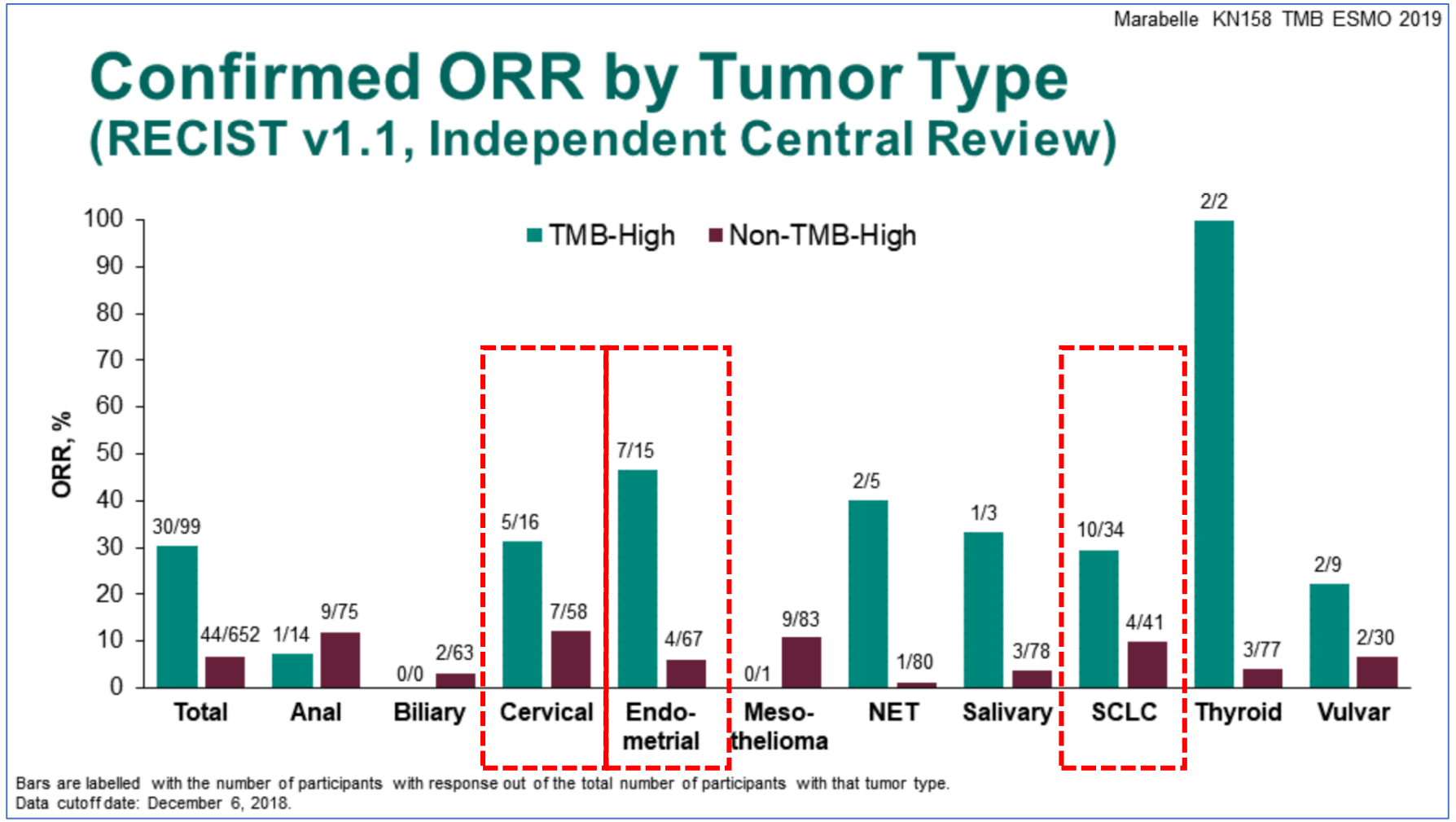
Marabelle KN158 TMB ESMO 2019

Representation of Tumor Types



[a]The 14 MSI-H tumors were endometrial (n = 10), cervical (n = 2), thyroid (n = 1), and salivary (n = 1). Data cutoff date: December 6, 2018.

Distribution of TMB-High Population in Each Cancer Type



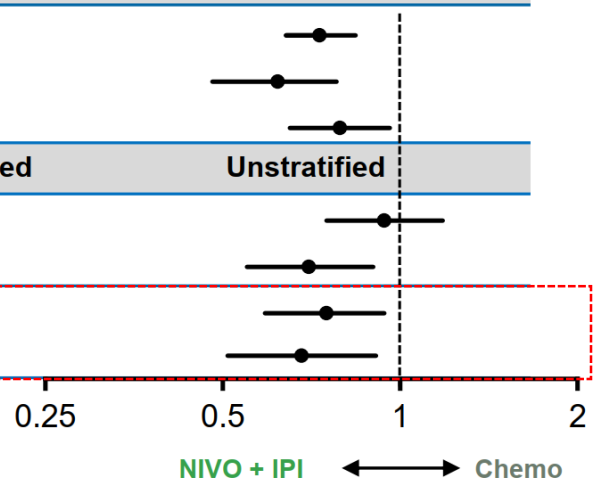
Tissue TMB: WES
Cutoff: 175 mut/exome

Sample size
Total: 751
TMB-High: 99 (13.2%)
Non-TMB-High: 652 (86.8%)

Response rate
Total: 9.8%
TMB-H group: 30%
Non-TMB-High: 6.7%

OS for NIVO + IPI vs Chemo by Tumor PD-L1 Expression, TMB Status, and Combined Subgroups in All Randomized Patients

		Median OS, months NIVO + IPI n = 583	Chemo n = 583	HR	HR (95% CI)
Randomized groups				**Stratified**	**Stratified**
PD-L1	All randomized (N = 1166)	17.1	13.9	0.73	
	PD-L1 < 1% (n = 373)	17.2	12.2	0.62	
	PD-L1 ≥ 1% (n = 793)	17.1	14.9	0.79[a]	
Additional exploratory subgroups analyses[b,c]				**Unstratified**	**Unstratified**
PD-L1	1–49% (n = 396)	15.1	15.1	0.94	
	≥ 50% (n = 397)	21.2	14.0	0.70	
TMB[d] (mut/Mb)	low, < 10 (n = 380)	16.2	12.6	0.75	
	high, ≥ 10 (n = 299)	23.0	16.4	0.68	



- No consistent correlation was observed between survival outcomes with NIVO + IPI vs chemo and PD-L1 or TMB alone or in combination[1]

[a]Stratified HR (97.72% CI); [b]Patients were not stratified by TMB or PD-L1 ≥ or < 50% – subgroup analyses therefore may be impacted by imbalances and should be interpreted with caution; [c]Not controlled by randomization; [d]Unstratified HR for NIVO + IPI vs chemo in TMB-evaluable (n = 679) and non-evaluable (n = 487) patients was 0.74 (95% CI, 0.61–0.88) and 0.74 (95% CI, 0.60–0.92), respectively.

[1]Hellmann MD, et al. N Engl J Med 2019. doi: www.nejm.org/doi/full/10.1056/NEJMoa1910231. 2019 Sept 28 [Epub ahead of print].

What’s going on with TMB?

Not all TMB assays have the same performance

Partners of TMB Harmonization Program

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Patient Advocacy Organization, Washington, DC

Partners:

Diagnostic
- ACT Genomics Company, Ltd
- Caris Life Sciences, Inc
- Foundation Medicine, Inc
- Guardant Health, Inc
- Illumina, Inc
- NeoGenomics Laboratories, Inc
- OmniSeq, Inc
- Personal Genome Diagnostics, Inc
- QIAGEN, NV
- Thermo Fisher Scientific, Inc

Academic
- Columbia University, NY
- Johns Hopkins University, MD
- Memorial Sloan Kettering Cancer Center, NY

Pharmaceutical
- AstraZeneca, LP
- Bristol-Myers Squibb Company, Inc
- EMD Serono, Inc
- Genentech, Inc
- Merck & Company, Inc
- Pfizer, Inc
- Regeneron Pharmaceuticals, Inc

Other
- NIH National Cancer Institute
- precisionFDA
- SeraCare Life Sciences, Inc
- US Food and Drug Administration

Friends and QuIP TMB Standardization and Harmonization Initiative Objectives

- Identify variation between TMB assessed by WES and by targeted gene panels
- Create TMB reference standards using WES to facilitate alignment of various targeted gene panels
- Assess interassay and interlaboratory variability and identify sources of this observed variation
- Develop recommendations to minimize, or account for, variation in methods of TMB estimation and reporting, and for TMB cutoff values, that will inform and advise best practices for prospective clinical studies

QuIP Quality in Pathology

Quality Assessment Service for Pathology, Berlin, Germany

Partners:

Diagnostic
- Foundation Medicine, Inc
- Illumina, Inc
- NEO New Oncology, AG
- QIAGEN, NV
- Thermo Fisher Scientific, Inc

Academic
- Charité Berlin
- LMU Munich
- Technical University Munich
- University Hospital Cologne
- University Hospital Dresden
- University Hospital Erlangen
- University Hospital Halle (Saale)
- University Hospital Heidelberg
- University Hospital Regensburg
- University Hospital Zurich

Pharmaceutical
- Bristol-Myers Squibb Company, Inc
- F. Hoffmann-La Roche, AG
- Merck Sharp & Dohme, Ltd

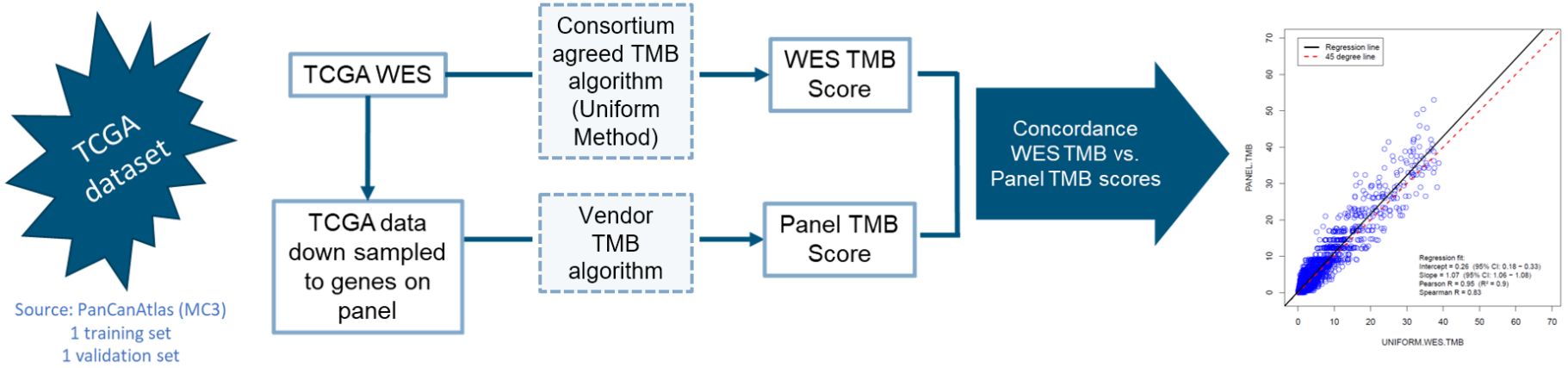
Other
- German Cancer Consortium (DKTK)
- Institute for Hematopathology, Hamburg

Modified from Stenzinger et al, 2019

Partners and Panels in TMB Harmonization Program

Diagnostic Partner	Panel Name	Gene No	Size (Mb)	Status
ACT Genomics	ACTOnco	440	1.12	LDT
AstraZeneca	AZ600	607	1.72	LDT
Caris Life Sciences	SureSelect XT	592	1.40	LDT
Foundation Medicine	FoundationOne CDx	324	0.80	IVD -CDX
Guardant Health	GuardantOMNI	500	1.00	LDT
Illumina	TSO500	523	1.33	LDT
MSKCC	MSK-IMPACT	468	1.14	IVD
NeoGenomics	NeoTYPE	372	1.03	LDT
Personal Genome Diagnostics	PGDx elio	507	1.33	LDT
QIAGEN	QIAseq TMB	486	1.33	LDT
Thermo Fisher Scientific	Oncomine TML	409	1.20	LDT

Phase 1: In silico analysis



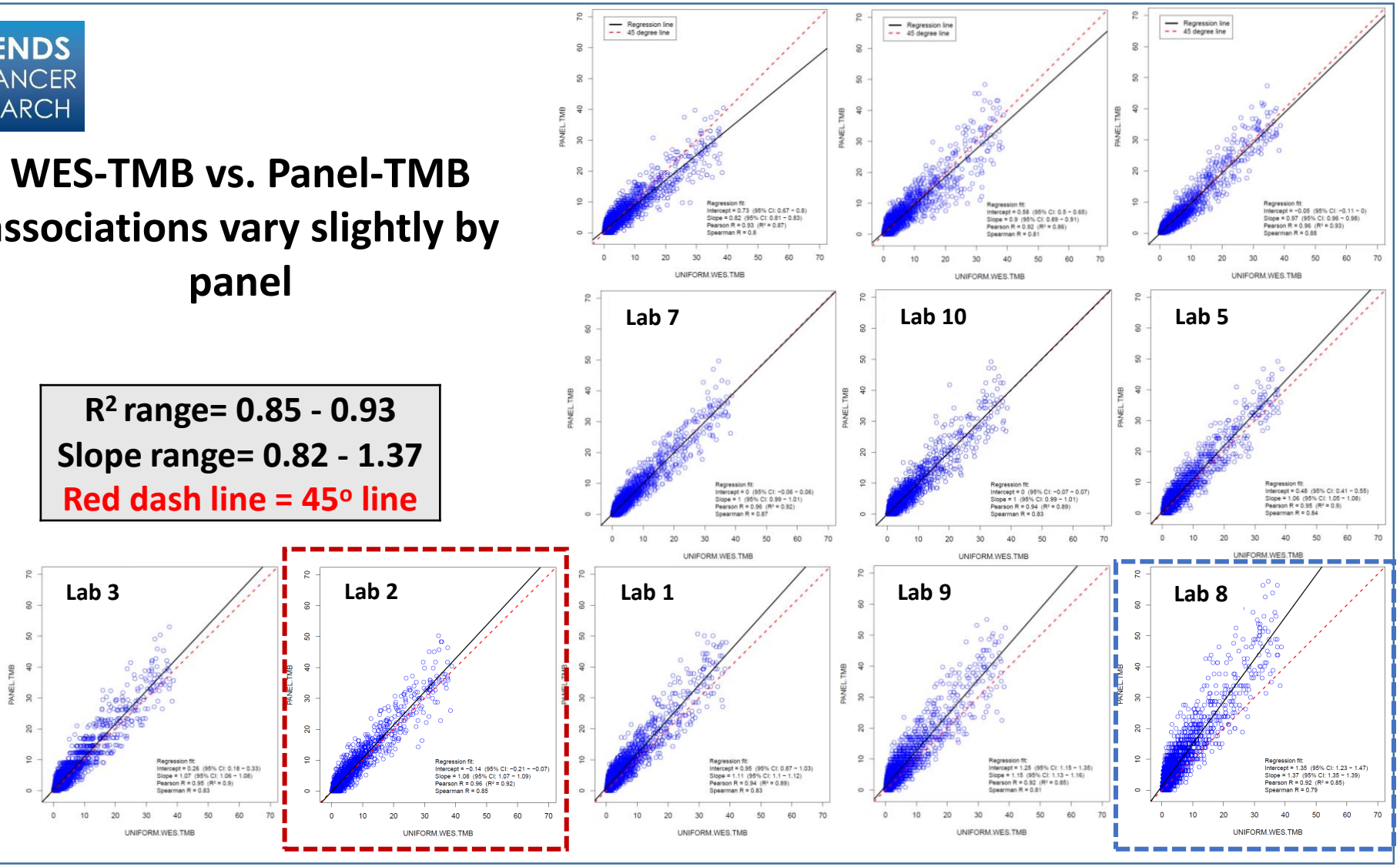
- **Data set: TCGA pan-cancer data set (MC3)**
- **WES of 4134 samples from 32 cancer types**
- **WES TMB determined by uniformed method**
- **Each diagnostic partner uses their gene panel and analysis algorithm to predict TMB for each sample**
- **Compare the panel-derived TMB to WES-derived TMB for correlation**
- **Calculate panel-derived TMB data for sensitivity, specificity & precision for TMB cut-off at 8, 10, 12, 14, 16, 18 & 20**
- **Blinded data analyzed by NCI**

Association between WES-TMB and Panel-TMB

FRIENDS of CANCER RESEARCH

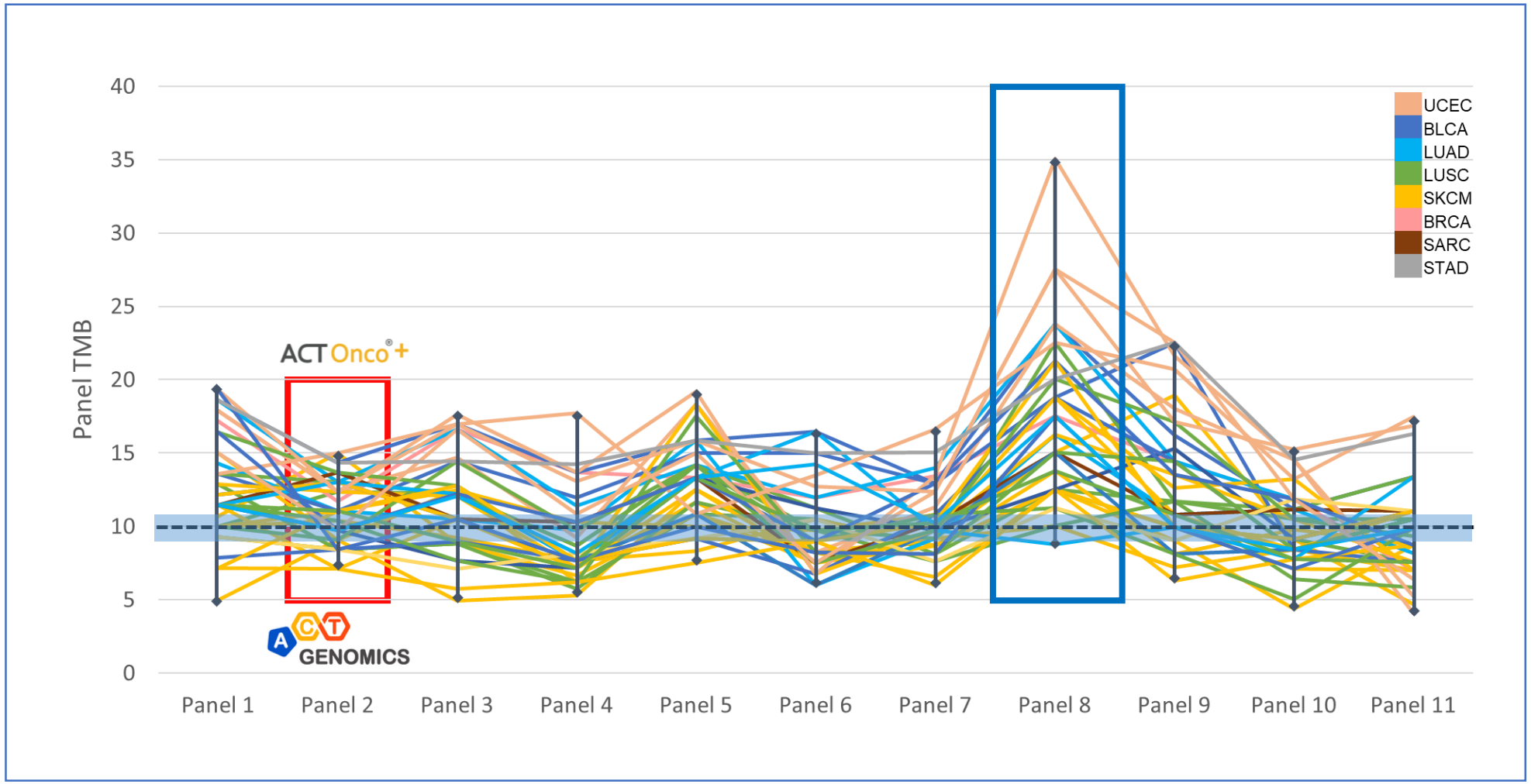
WES-TMB vs. Panel-TMB associations vary slightly by panel

R^2 range= 0.85 - 0.93
Slope range= 0.82 - 1.37
Red dash line = 45° line



*Data presented in Society for Immunotherapy of Cancer 2018
In silico assessment of variation in TMB quantification across diagnostic platforms:
Phase 1 of the Friends of Cancer Research Harmonization Project,

Variability of Panel-TMB vs WES-TMB

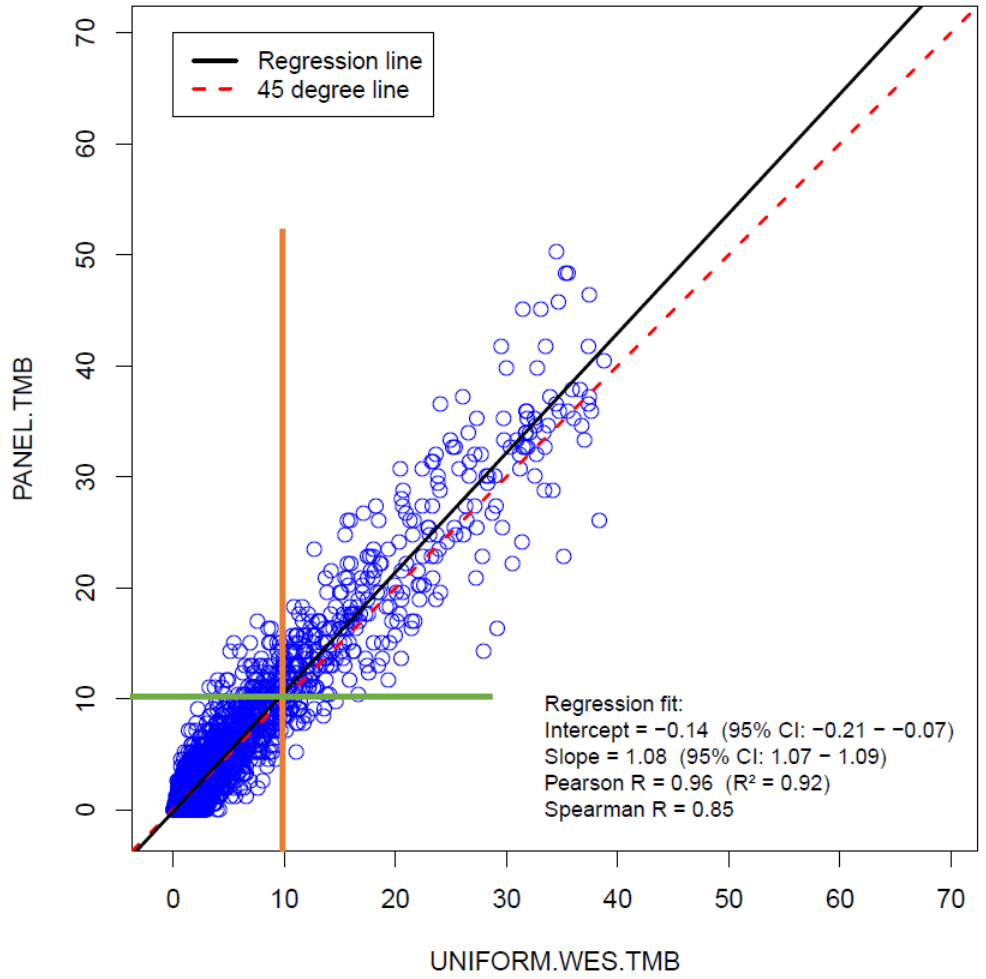


*Data presented in Society for Immunotherapy of Cancer 2018

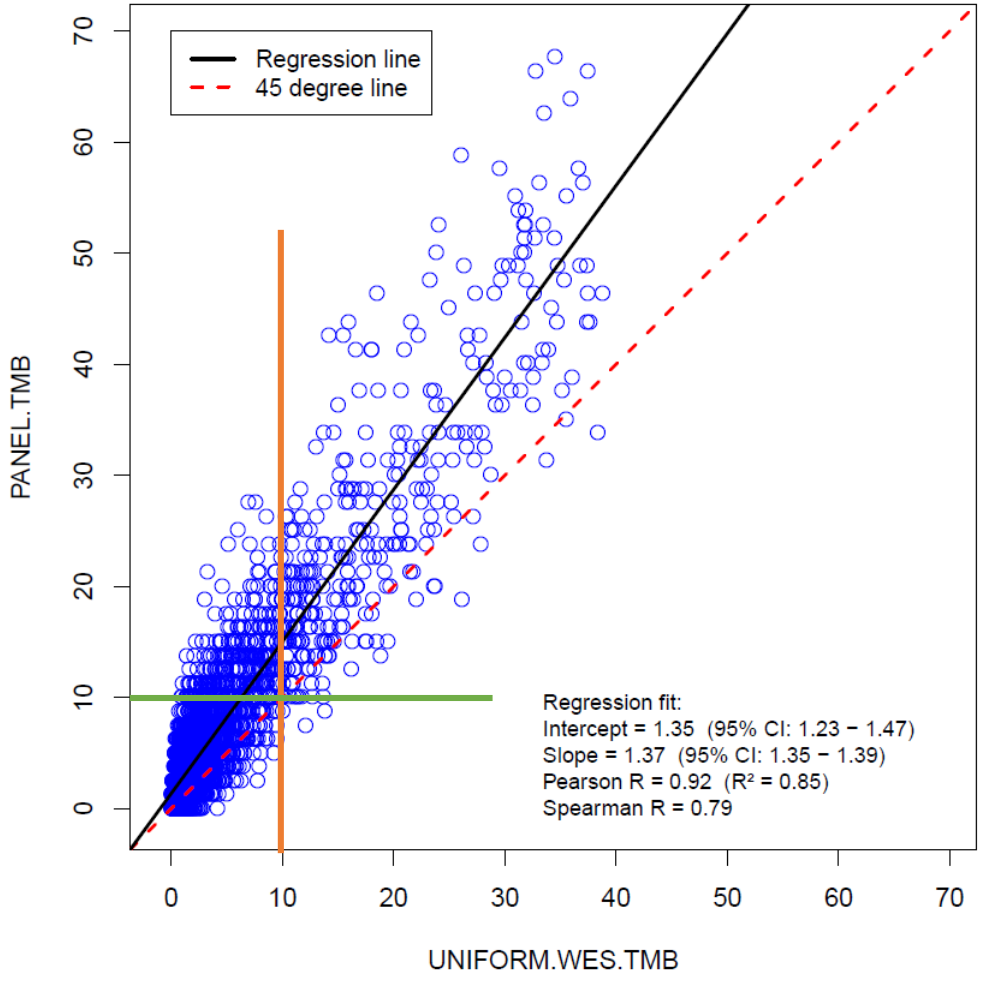
In silico assessment of variation in TMB quantification across diagnostic platforms: Phase 1 of the Friends of Cancer Research Harmonization Project,

Impact of Panel-TMB Performance on cutoff

Panel 2 WES-TMB 10 = Panel-TMB 10

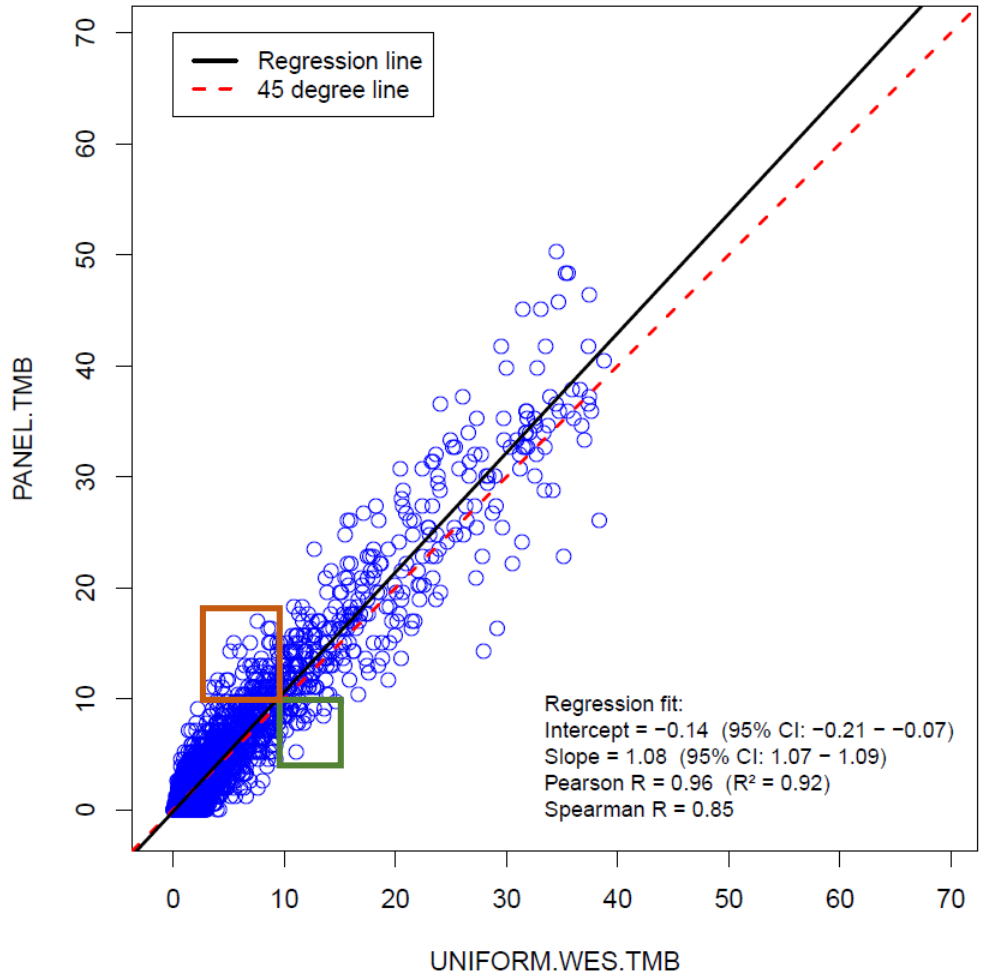


Panel 8 WES-TMB 10 = Panel-TMB 15

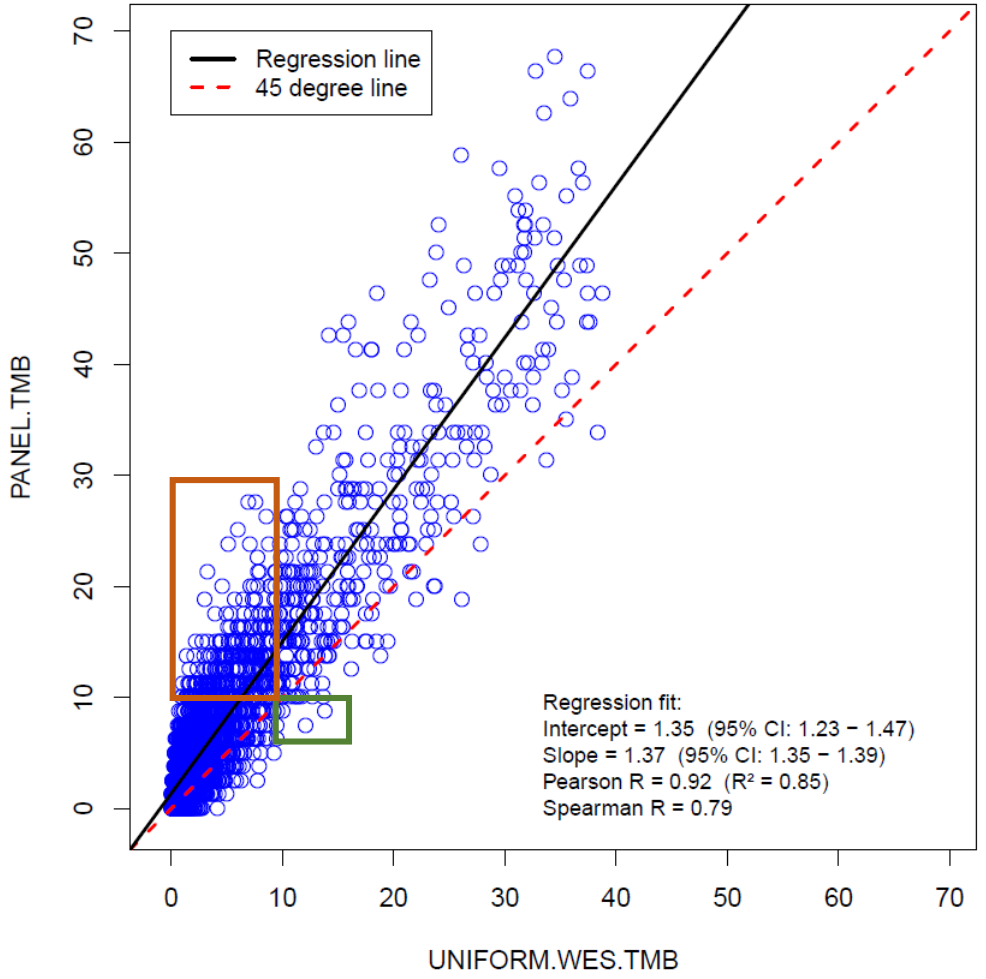


Impact of Panel-TMB Performance

Panel 2



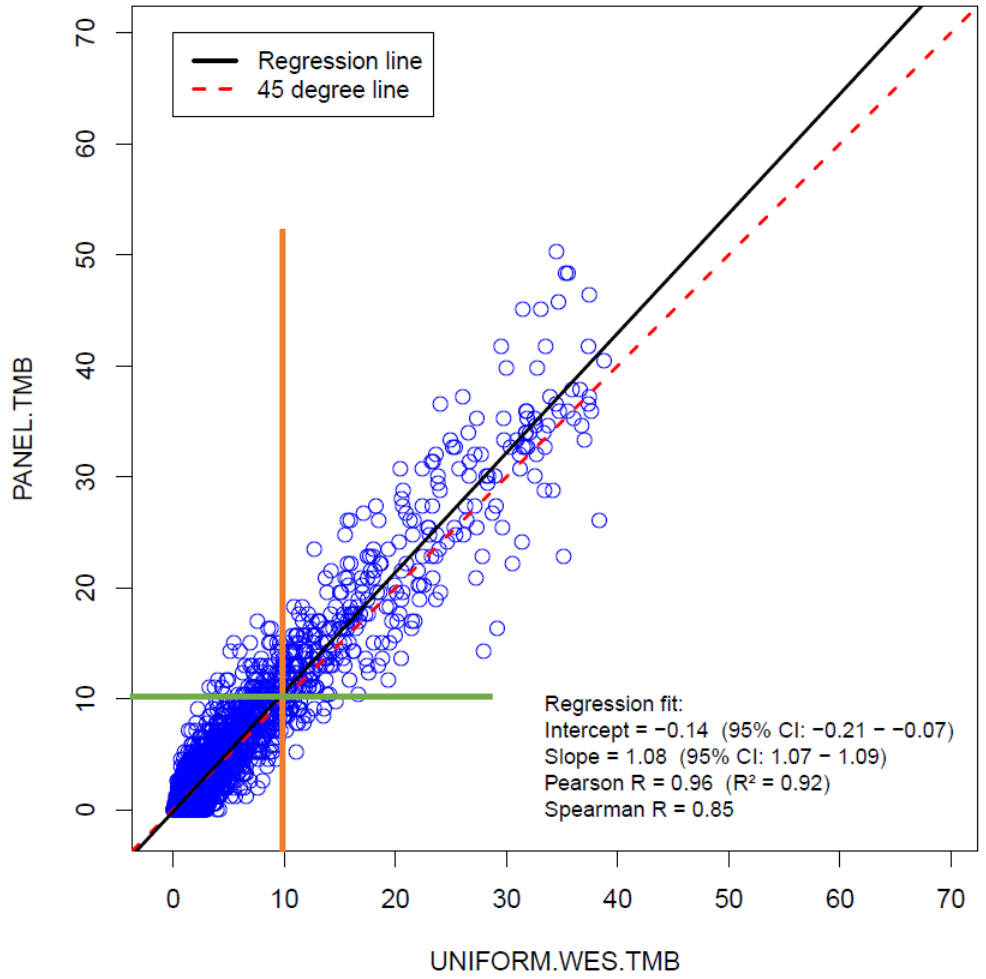
Panel 8



Impact of Panel-TMB Performance on cutoff

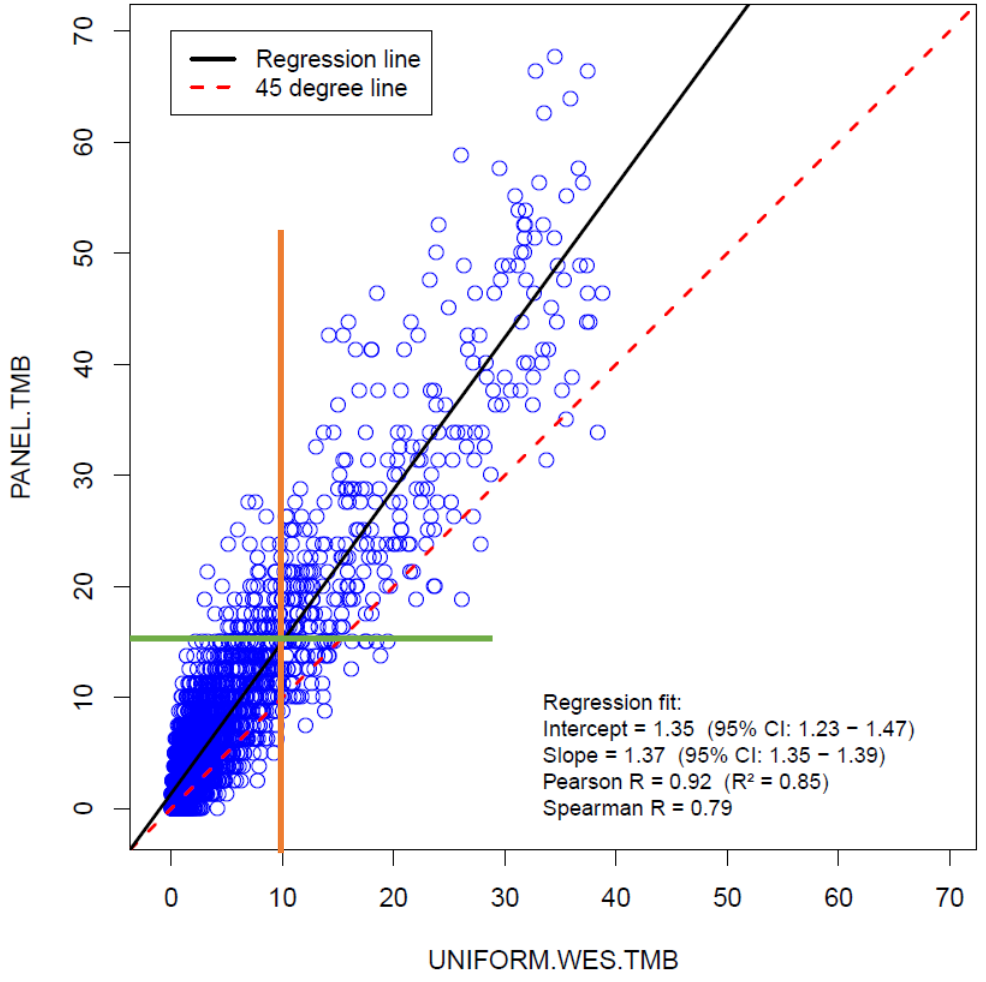
Panel 2

WES-TMB 10 = Panel-TMB 10



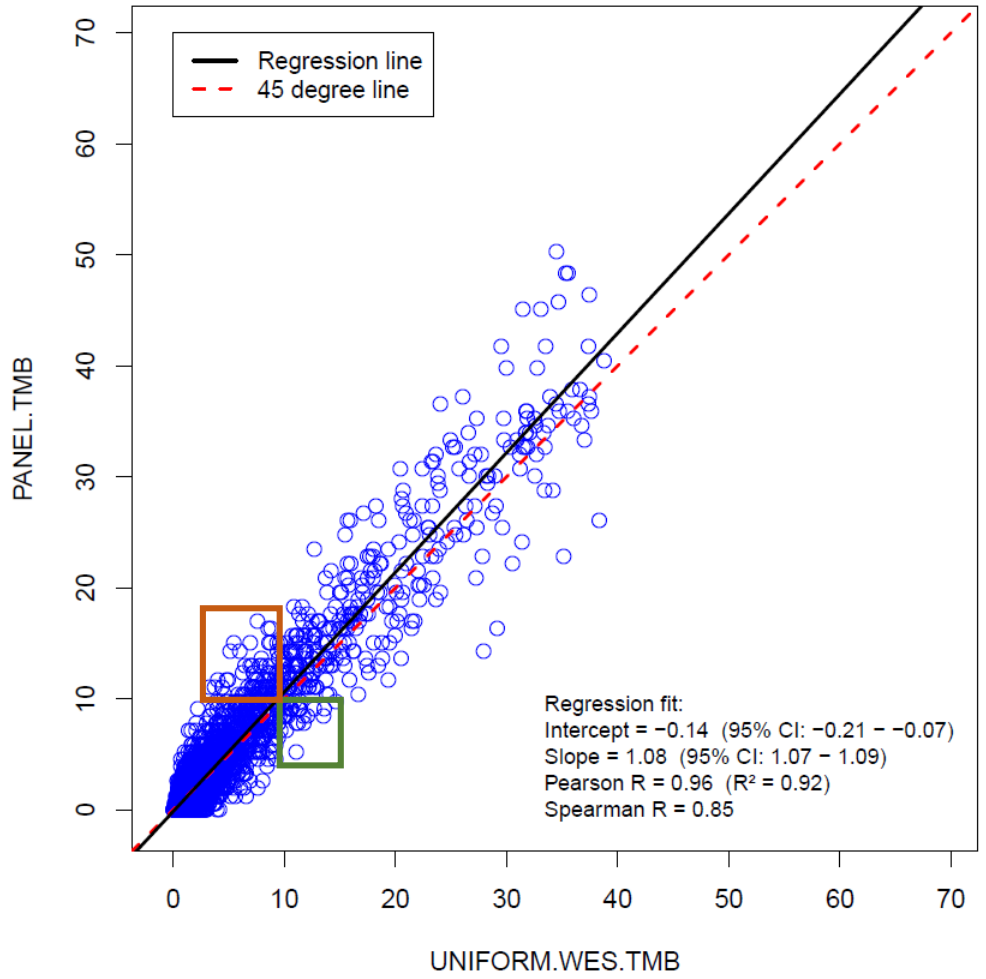
Panel 8

WES-TMB 10 = Panel-TMB 15

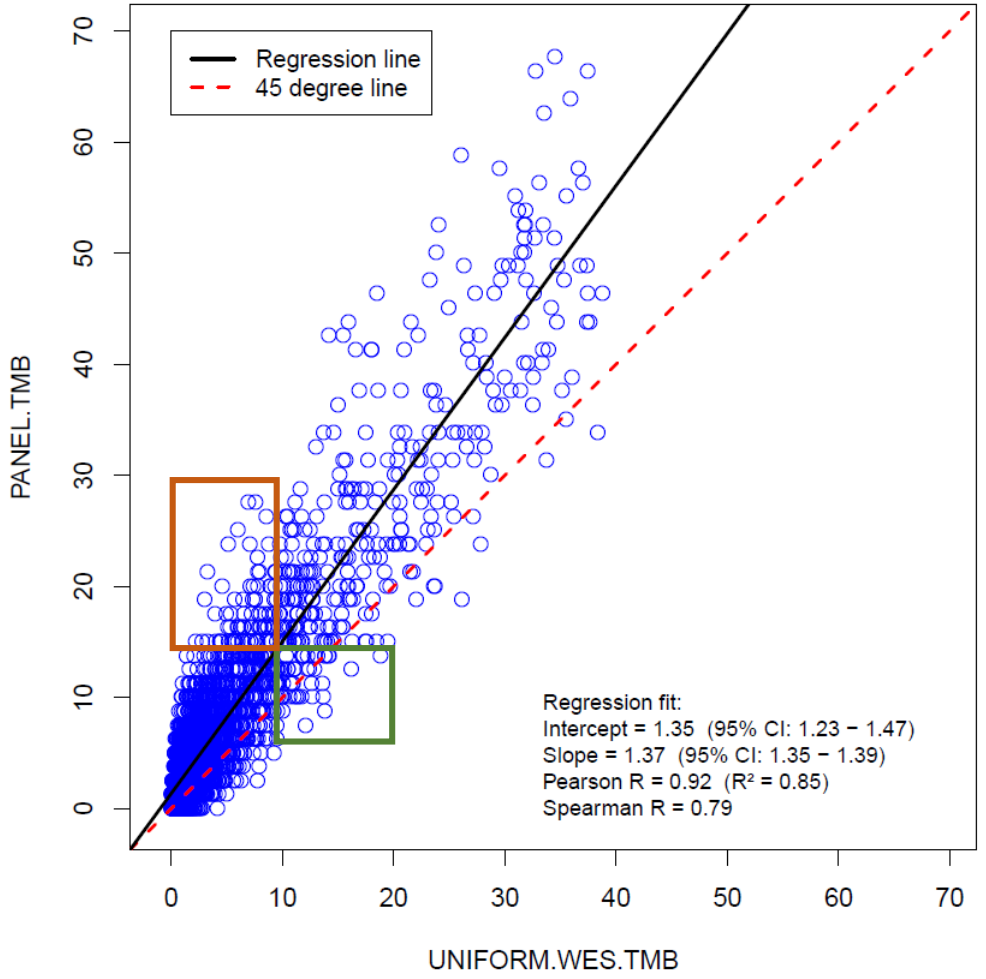


Impact of Panel-TMB Performance

Panel 2

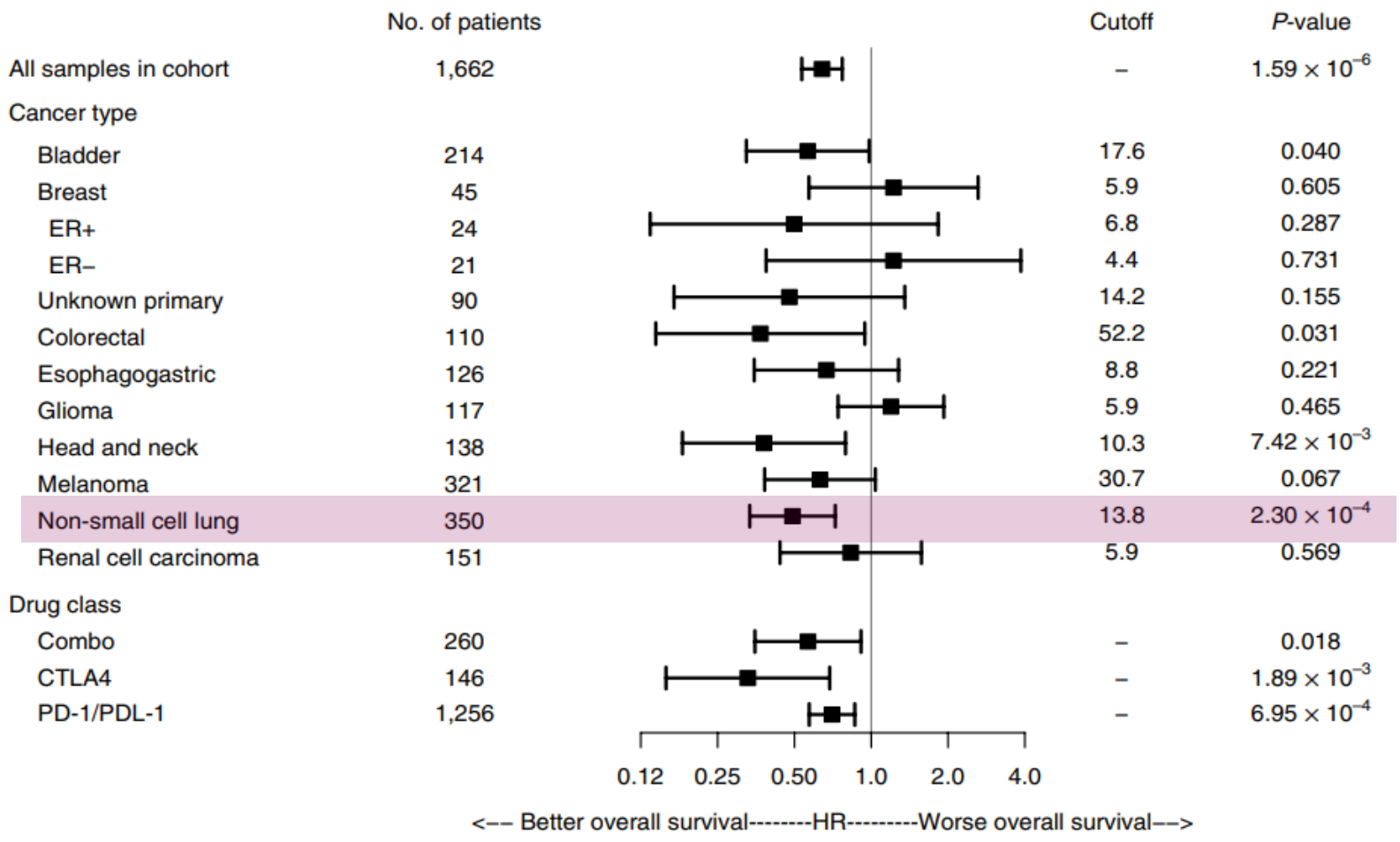


Panel 8



Cutoff matters too …

Using to 20% TMB as the cutoff for Checkpoint Inhibitor Predictor



Samstein et al. Nat Gen. 2019

Fig. 2 | Effect of nonsynonymous mutational load on overall survival after ICI treatment, by cancer subtype and drug class. Forest plot for all patients in the identified cohort or individual cancer subtypes. Indicated are the number of patients and HR comparing overall survival after ICI in patients in the highest twentieth-percentile TMB within each histology. Bars represent the 95% CI. The cutoff defining the top 20% of normalized mutational burden from MSK-IMPACT for each cancer type is shown, as well as the two-sided log-rank *P* value for the comparison of high and low mutational burden survival curves. ER, estrogen receptor. All cancer types in analysis are displayed.

Association between tTMB and Efficacy in NSCLC

cancers

MDPI

Article

In-house Implementation of Tumor Mutational Burden Testing to Predict Durable Clinical Benefit in Non-small Cell Lung Cancer and Melanoma Patients

Simon Heeke [1,2,3,4], Jonathan Benzaquen [1,2,5], Elodie Long-Mira [1,2,3,4], Benoit Audelan [1,6], Virginie Lespinet [1,3], Olivier Bordone [1,3], Salomé Lalvée [1,3], Katia Zahaf [1,3], Michel Poudenx [1,7], Olivier Humbert [1,8], Henri Montaudié [1,4,9], Pierre-Michel Dugourd [1,9], Madleen Chassang [1,10], Thierry Passeron [1,4,9,11], Hervé Delingette [1,4,6], Charles-Hugo Marquette [1,2,3,4,5], Véronique Hofman [1,2,3,4], Albrecht Stenzinger [12,13], Marius Ilié [1,2,3,4] and Paul Hofman [1,2,3,4,*]

Cancers (2019) 11:1271

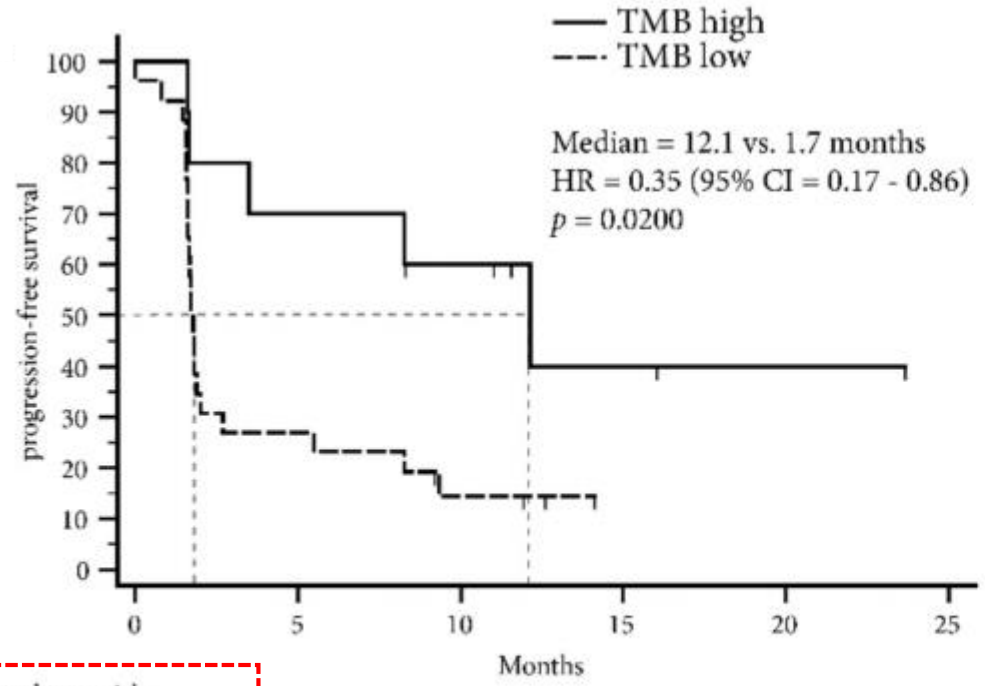
Tissue TMB

Oncomine TML panel
Cutoff: 9.4 mut/Mb

F1Cdx panel
Cutoff: 15 mut/mb

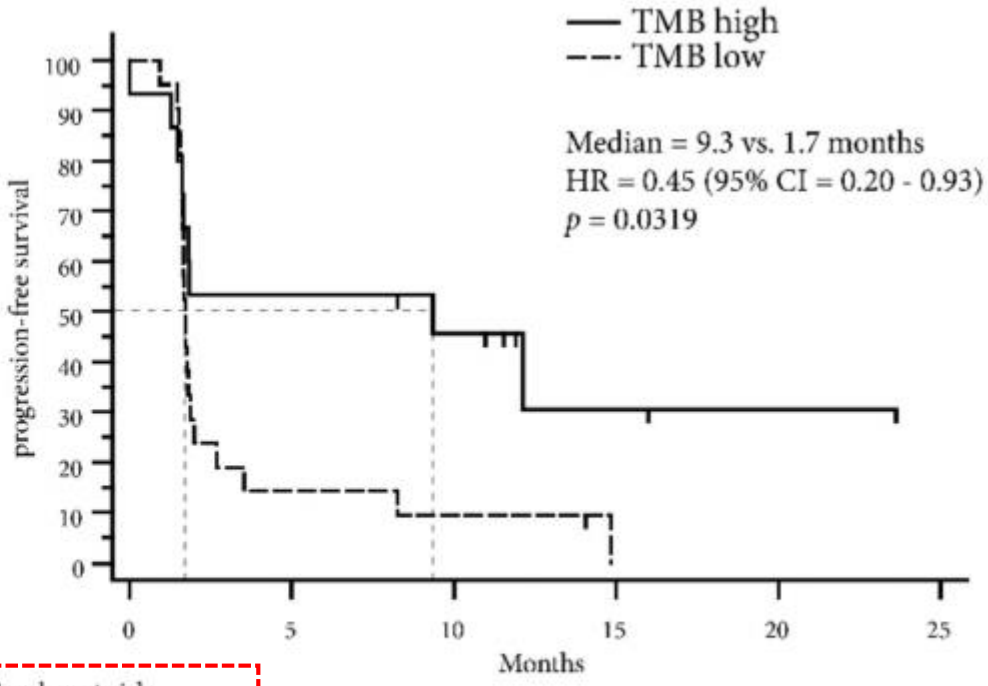
Progression free survival computed for NSCLC using panel testing

Oncomine TML panel
Cutoff: 9.4 mut/Mb



Oncomine TML panel

F1Cdx panel
Cutoff: 15 mut/mb



FoundationOne test (FO).

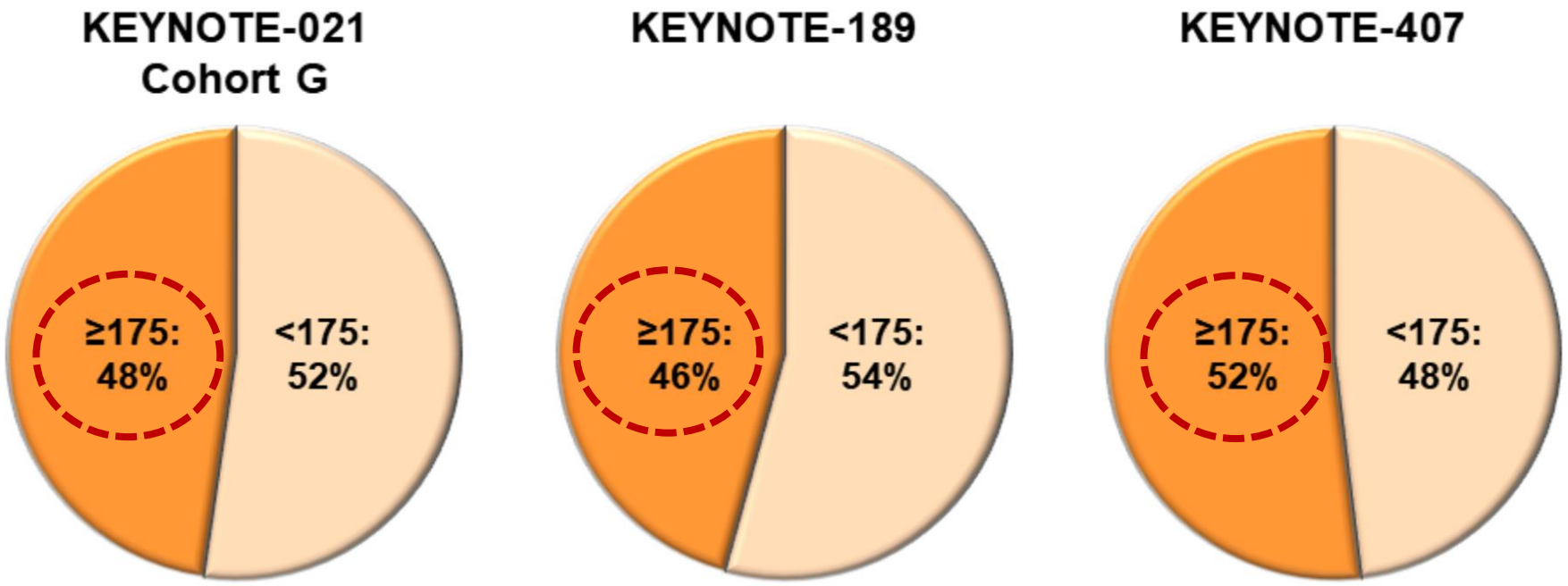
Note: Patients were treated with ICI monotherapy in either a first- or second line manner

Cancers (2019) 11:1271

Distribution of TMB-High Population in KN-021, 189 & 407

Paz-Ares KN021/189/407 TMB ESMO 2019

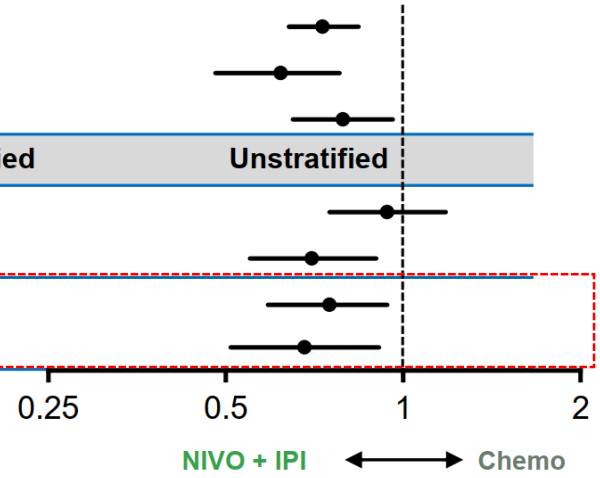
Prevalence of tTMB ≥175 and <175 mut/exome



Data cutoff dates: Dec 1, 2017 (KEYNOTE-021); Sep 21, 2018 (KEYNOTE-189); May 9, 2019 (KEYNOTE-407).

OS for NIVO + IPI vs Chemo by Tumor PD-L1 Expression, TMB Status, and Combined Subgroups in All Randomized Patients

		Median OS, months NIVO + IPI n = 583	Chemo n = 583	HR	HR (95% CI)
Randomized groups				**Stratified**	**Stratified**
PD-L1	All randomized (N = 1166)	17.1	13.9	0.73	
	PD-L1 < 1% (n = 373)	17.2	12.2	0.62	
	PD-L1 ≥ 1% (n = 793)	17.1	14.9	0.79[a]	
Additional exploratory subgroups analyses[b,c]				**Unstratified**	**Unstratified**
PD-L1	1–49% (n = 396)	15.1	15.1	0.94	
	≥ 50% (n = 397)	21.2	14.0	0.70	
TMB[d] (mut/Mb)	679 (58%) low, < 10 (n = 380) 56%	16.2	12.6	0.75	
	high, ≥ 10 (n = 299) 44%	23.0	16.4	0.68	



- No consistent correlation was observed between survival outcomes with NIVO + IPI vs chemo and PD-L1 or TMB alone or in combination[1]

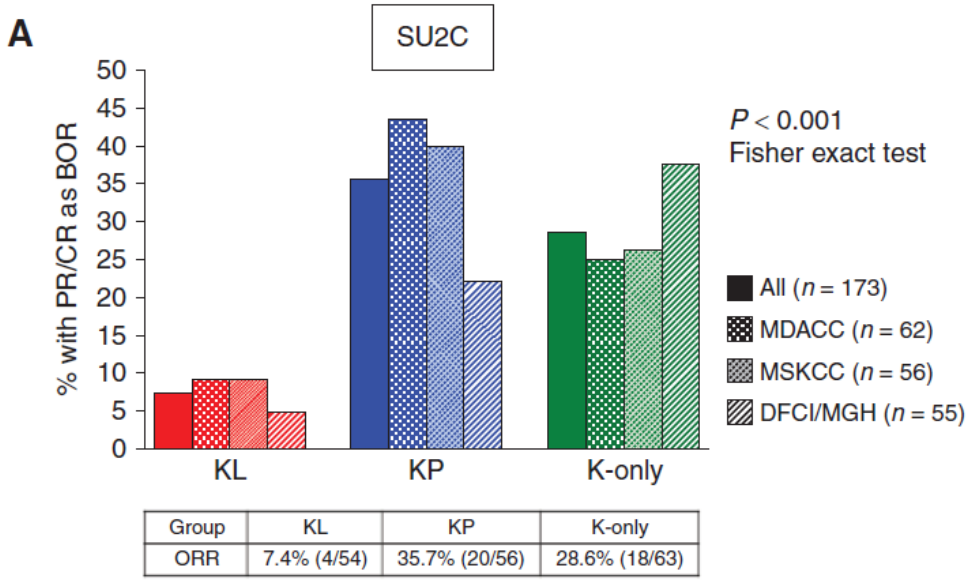
[a]Stratified HR (97.72% CI); [b]Patients were not stratified by TMB or PD-L1 ≥ or < 50% – subgroup analyses therefore may be impacted by imbalances and should be interpreted with caution; [c]Not controlled by randomization; [d]Unstratified HR for NIVO + IPI vs chemo in TMB-evaluable (n = 679) and non-evaluable (n = 487) patients was 0.74 (95% CI, 0.61–0.88) and 0.74 (95% CI, 0.60–0.92), respectively.

[1]Hellmann MD, et al. N Engl J Med 2019. doi: www.nejm.org/doi/full/10.1056/NEJMoa1910231. 2019 Sept 28 [Epub ahead of print].

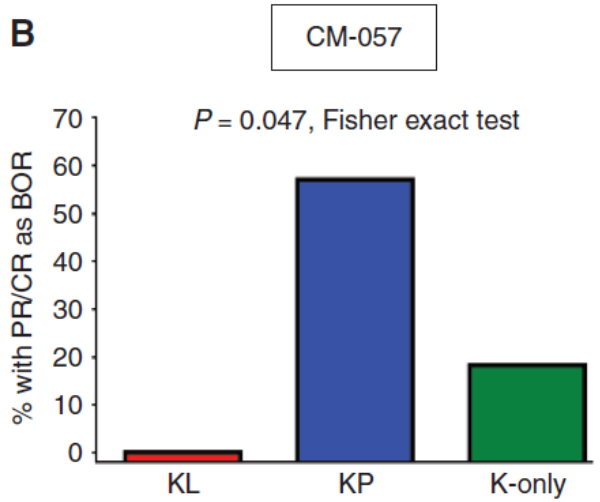
Additional consideration …

STK11 deficiency confers resistance to checkpoint blockade

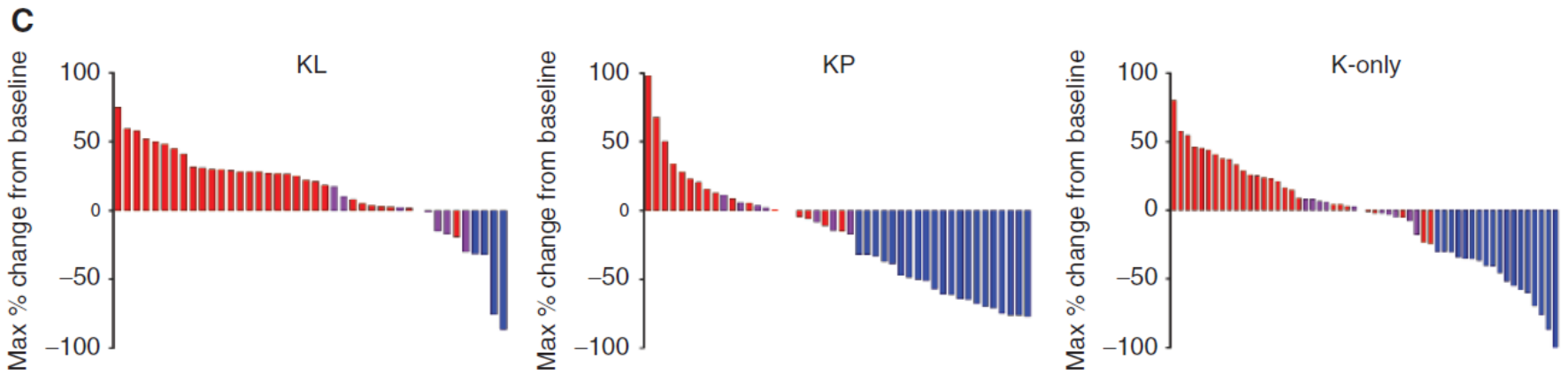
- STK11 aka LKB1 gene
- STK11/LKB1 co-mutations are associated with inferior ORR with checkpoint blockade in KRAS-mutant NSCLC



Group	KL	KP	K-only
ORR	7.4% (4/54)	35.7% (20/56)	28.6% (18/63)



Group	KL	KP	K-only
ORR	0% (0/6)	57.1% (4/7)	18.2% (2/11)

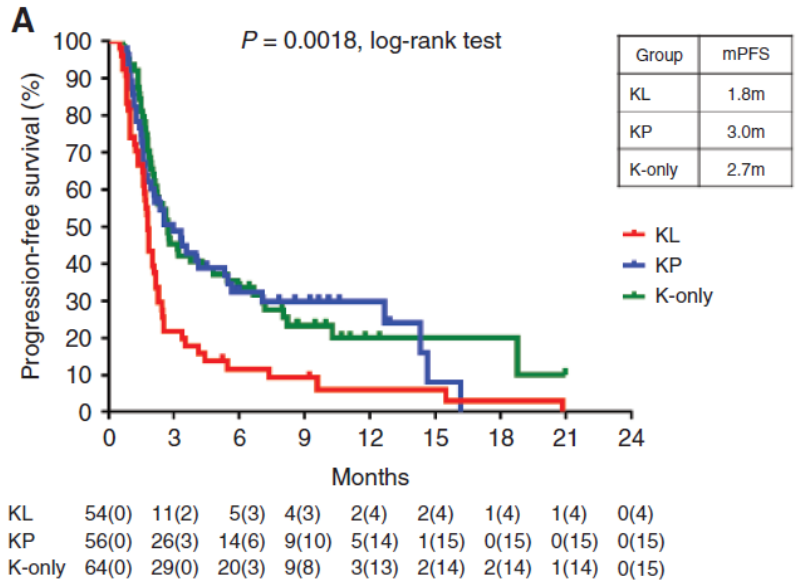


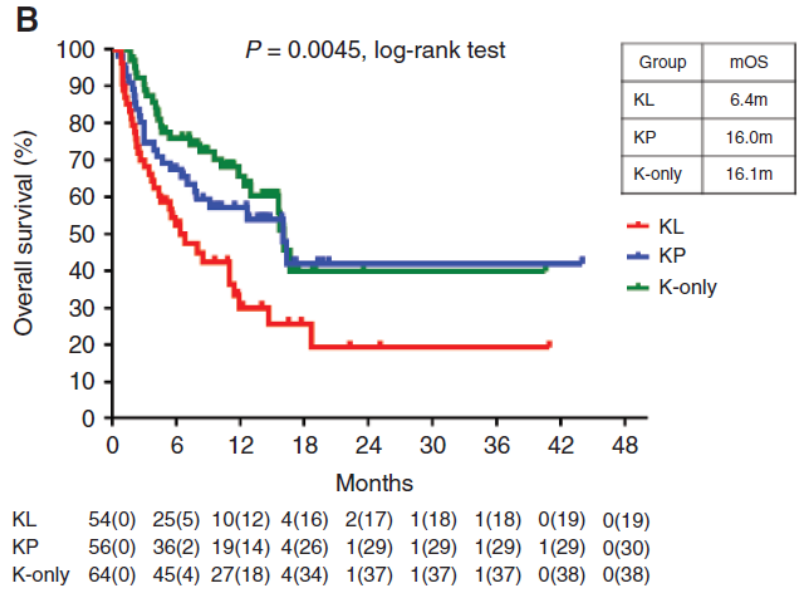
KL	$KRAS^{mut}STK11^{mut}TP53^{wt}$
KP	$KRAS^{mut}STK11^{wt}TP53^{mut}$
K-only	$KRAS^{mut}STK11^{wt}TP53^{wt}$

Skoulidis et al. Cancer Discov 2018

STK11 deficiency confers resistance to checkpoint blockade

- STK11/LKB1 genetic alterations are associated with shorter PFS and OS with checkpoint blockade among KRAS-mutant NSCLC



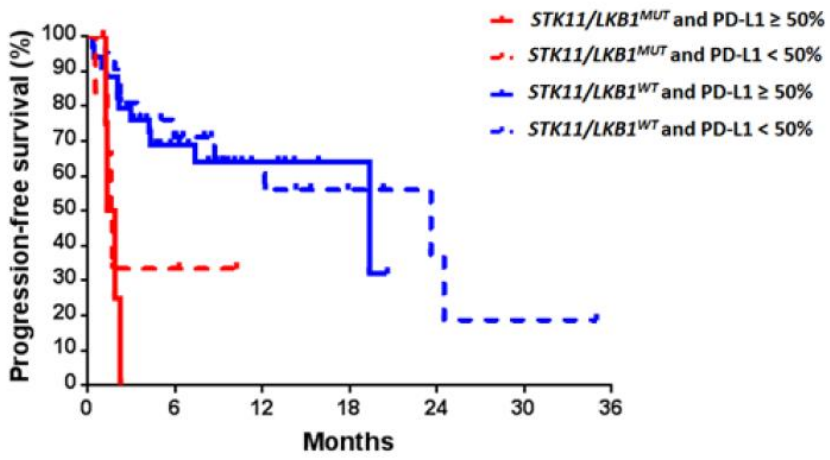


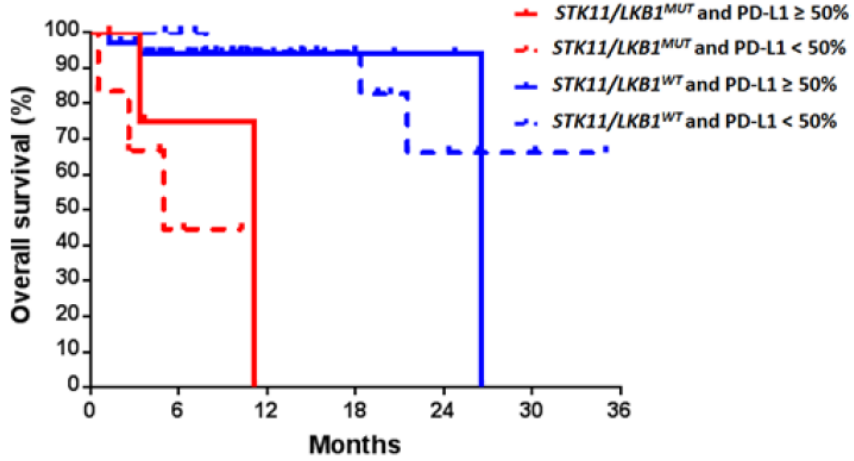
KL	$KRAS^{mut}STK11^{mut}TP53^{wt}$
KP	$KRAS^{mut}STK11^{wt}TP53^{mut}$
K-only	$KRAS^{mut}STK11^{wt}TP53^{wt}$

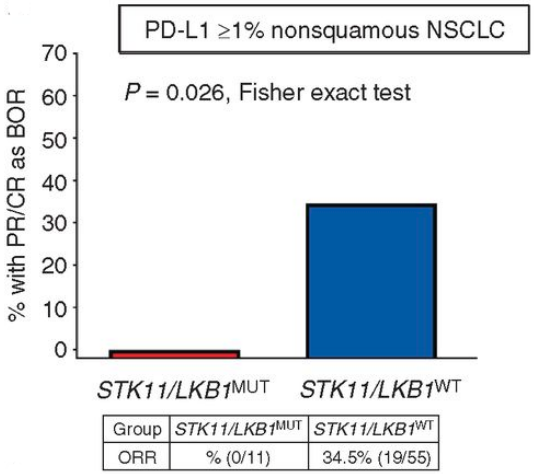
Skoulidis et al. Cancer Discov 2018

STK11 deficiency confers resistance to checkpoint blockade

- STK11/LKB1 genetic alterations affect response and outcome independently of PD-L1 status (all patients PD-L1+ve by 22C3 pharmDx assay)







Group	*STK11/LKB1*^{MUT}	*STK11/LKB1*^{WT}
ORR	% (0/11)	34.5% (19/55)

A. Progression-free survival

	loglik	Chisq	df	p-value
Null model	-107.38			
PD-L1	-107.36	0.0356	1	0.850374
STK11	-102.08	10.5654	1	0.001152
PD-L1 STK11 interaction	-102.08	0.4896	1	0.484087

PD-L1 ≥ 50% group:
HR 0.14 (95% CI, 0.04 - 0.5), P= 0.0005, log-rank test

PD-L1 <50% group:
HR 0.27 (95% CI, 0.08 - 0.94), P=0.0278, log-rank test

B. Overall survival

	loglik	Chisq	df	p-value
Null model	-36.765			
PD-L1	-36.765	0.0515	1	0.8204261
STK11	-30.502	12.5266	1	0.0004012
PD-L1 STK11 interaction	-30.358	0.2883	1	0.5913080

PD-L1 ≥ 50% group:
HR 0.11 (95% CI, 0.015 - 0.78), P= 0.0075, log-rank test

PD-L1 <50% group:
HR 0.05 (95% CI, 0.004 - 0.49), P=0.0278, log-rank test

Skoulidis et al. Cancer Discov 2018

One more thing …

Hyper-progressive disease upon checkpoint inhibitor treatment

Hyperprogressive disease (HPD) is a new pattern of progression in cancer patients treated by anti-PD-1/PD-L1

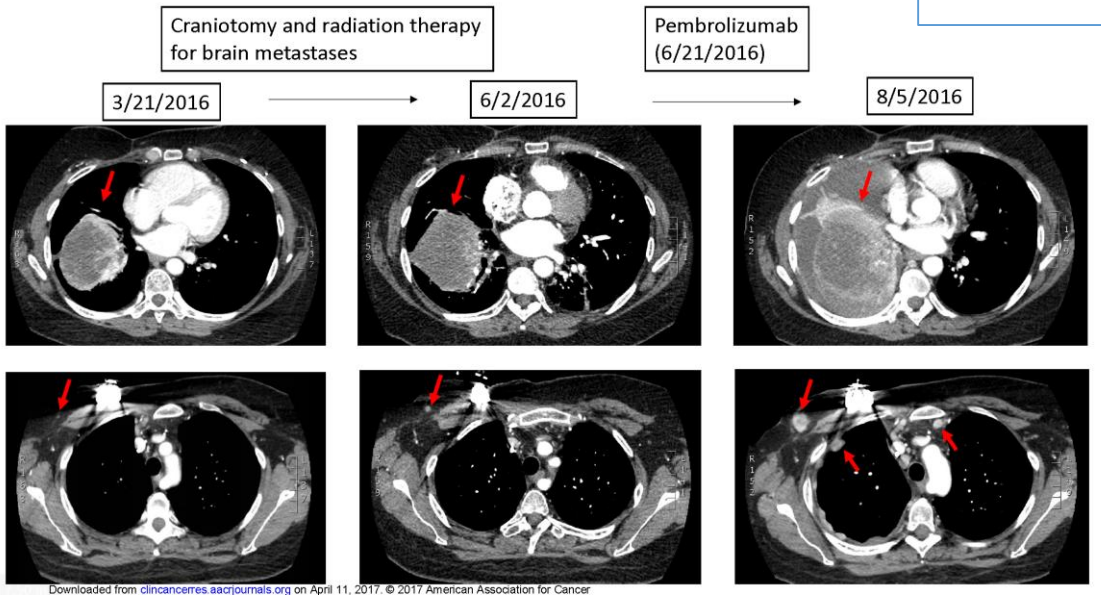
Authors.

Stéphane Champiat[1,2], Laurent Dercle[3], Samy Ammari[4], Christophe Massard[1], Antoine Hollebecque[1], Sophie Postel-Vinay[1,2], Nathalie Chaput[5,6,7,8], Alexander Eggermont[9], Aurélien Marabelle[1,10], Jean-Charles Soria[1,2,*], Charles Ferté[1,11,12,*]

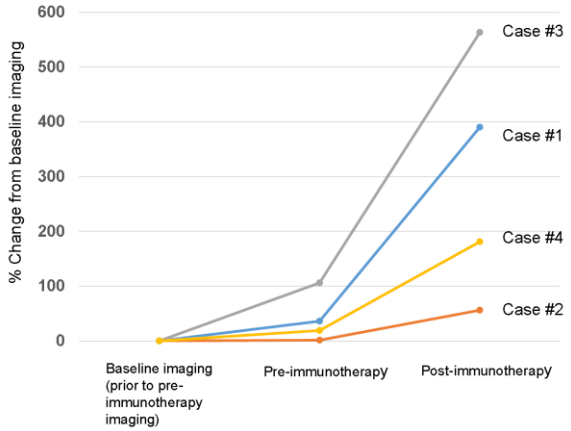
Hyper-progressors after Immunotherapy:

Analysis of Genomic Alterations Associated with Accelerated Growth Rate

Shumei Kato* [1], Aaron Goodman* [1], Vighnesh Walavalkar [2], Donald A. Barkauskas [3], Andrew Sharabi [1,4], Razelle Kurzrock [1]



Downloaded from clincancerres.aacrjournals.org on April 11, 2017. © 2017 American Association for Cancer



TMB in the NCCN Guideline for NSCLC (Sep. 2019)

National Comprehensive Cancer Network®

NCCN Guidelines Version 7.2019
Non-Small Cell Lung Cancer

NCCN Guidelines Index
Table of Contents
Discussion

EMERGING BIOMARKERS TO IDENTIFY NOVEL THERAPIES FOR PATIENTS WITH METASTATIC NSCLC

Genetic Alteration (ie, Driver event)	Available Targeted Agents with Activity Against Driver Event in Lung Cancer
High-level *MET* amplification or *MET* exon 14 skipping mutation	Crizotinib[1-5]
RET rearrangements	Cabozantinib[6,7] Vandetanib[8]
ERBB2 (HER2) mutations	Ado-trastuzumab emtansine[9]
Tumor mutational burden (TMB)*	Nivolumab + ipilimumab[10] Nivolumab[11]

*TMB is an evolving biomarker that may be helpful in selecting patients for immunotherapy. There is no consensus on how to measure TMB.

[4]Paik PK, Drilon A, Fan PD, et al. Response to MET inhibitors in patients with stage IV lung adenocarcinomas harboring MET mutations causing exon 14 skipping. Cancer Discov 2015;5:842-849.
[5]Awad MM, Oxnard GR, Jackman DM, et al. MET exon 14 mutations in non-small-cell lung cancer are associated with advanced age and stage-dependent MET genomic amplification and cMET overexpresion. J Clin Oncol 2016;34:721-730.
[6]Drilon A, Wang L, Hasanovic A, et al. Response to cabozantinib in patients with RET fusion-positive lung adenocarcinomas. Cancer Discov 2013; 3:630-635.
[7]Drilon A, Rekhtman N, Arcila M, et al. Cabozantinib in patients with advanced RET-rearranged non-small-cell lung cancer: an open-label, single-centre, phase 2, single-arm trial. Lancet Oncol 2016;17:1653-1660.
[8]Lee SH, Lee JK, Ahn MJ, et al. Vandetanib in pretreated patients with advanced non-small cell lung cancer-harboring RET rearrangement: a phase II clinical trial. Ann Oncol 2017;28:292-297.
[9]Li BT, Shen R, Buonocore D, et al. Ado-trastuzumab emtansine in patients with HER2 mutant lung cancers: Results from a phase II basket trial. J Clin Oncol 2018;36:2532-2537.
[10]Hellmann MD, Ciuleanu TE, Pluzanski A et al. Nivolumab plus ipilimumab in lung cancer with a high tumor mutational burden. N Engl J Med 2018; 378:2093-2104.
[11]Carbone DP, Reck M, Paz-Ares L et al. First-line nivolumab in stage IV or recurrent non-small-cell lung cancer. N Engl J Med 2017;376:2415–2426.

Note: All recommendations are category 2A unless otherwise indicated.
Clinical Trials: NCCN believes that the best management of any patient with cancer is in a clinical trial. Participation in clinical trials is especially encouraged.

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NSCL-H

Diagnostic tools for immunotherapy

- PD-L1 staining
  - IHC-based
  - Companion diagnostics for some indications, complimentary for others
- Microsatellite instability (MSI) or mismatch repair deficiency (MMRd)
  - PCR- or sequencing-based microsatellite assay
  - IHC or sequencing of MMR genes (MLH1, MSH6, MSH6 & PMS2)
- Tumor mutational burden (TMB)
  - A measure of the total number of somatic mutations per million bases of coding sequence in a tumor genome
  - WES or panel sequencing
- Tumor microenvironment assessment
  - Identification of T-cell inflamed or “hot” tumors
  - mRNA- or protein-based assays

Biomarkers for immune checkpoint inhibitors

	Factor	Association with favourable clinical outcome	Validated in phase III clinical trial?	Predictive versus prognostic[a]	Cancer type	Tissue type for biomarker assessment[b]	Possible assay type for biomarker assessment
*	Tumour mutation burden	Positive	Yes	Predictive	Multiple cancer types	Blood or tumour tissue	NGS WES or targeted gene panel sequencing
*	PDL1 expression	Positive	Yes	Predictive	Multiple cancer types	Tumour tissue	Immunohistochemistry
*	Copy number variation	Negative	TBD	Prognostic, predictive or both	Multiple cancer types	Tumour tissue	NGS WES or targeted gene panel sequencing
*	HLA class I diversity	Positive	TBD	Predictive	Melanoma and NSCLC	Blood	NGS WES or PCR-based typing
	LOH at HLA class I alleles	Negative	TBD	Predictive	Melanoma	Tumour tissue	TBD
	T cell repertoire clonality change	Positive	TBD	Predictive	Melanoma	Tumour tissue or blood	TBD
*	T cell-inflamed microenvironment	Positive	TBD	Prognostic, predictive or both	Multiple cancer types	Tumour tissue	NGS RNA-seq or immunostaining
	SERPINB3 or *SERPINB4* mutations	Positive	TBD	Predictive	Melanoma	Tumour tissue	NGS WES
	Gut microbial diversity	Positive	TBD	Predictive	Melanoma	Oral or gut	PCR or NGS
	Specific gut microbial species	Positive or negative	TBD	Predictive	Melanoma	Oral or gut	PCR or NGS
	TGFβ expression	Negative	TBD	Predictive	Colon cancer and urothelial cancer	Tumour tissue	NGS RNA-seq or expression panel
	Mutations in the β-catenin pathway	Negative	TBD	Predictive	Melanoma	Tumour tissue or blood	NGS WES, targeted gene panel sequencing or RNA-seq
	JAK2 mutations (rare)[c]	Negative	TBD	Predictive	Melanoma	Tumour tissue or blood	NGS WES or targeted gene panel sequencing
	B2M mutations (rare)[c]	Negative	TBD	Predictive	Melanoma	Tumour tissue or blood	NGS WES or targeted gene panel sequencing
*	*STK11* mutations (common)	Negative	TBD	Predictive	NSCLC	Tumour tissue or blood	NGS WES or targeted gene panel sequencing

HLA, human leukocyte antigen; LOH, loss of heterozygosity; NSCLC, non-small-cell lung cancer; NGS, next-generation sequencing; PDL1, programmed cell death 1 ligand 1; RNA-seq, RNA sequencing; TBD, to be determined; TGFβ, transforming growth factor-β; WES, whole-exome sequencing. [a]Predictive refers to a given biomarker that has an effect dependent on the immune checkpoint inhibitor therapy, and prognostic refers to a biomarker that has a specific effect independent of the therapy. [b]Blood detection of mutations refers to cell-free DNA analysis. [c]*JAK2* and *B2M* mutations are controversial. Responses have been seen in patients with these mutations. Intratumoural heterogeneity likely needs to be assessed along with these mutations.

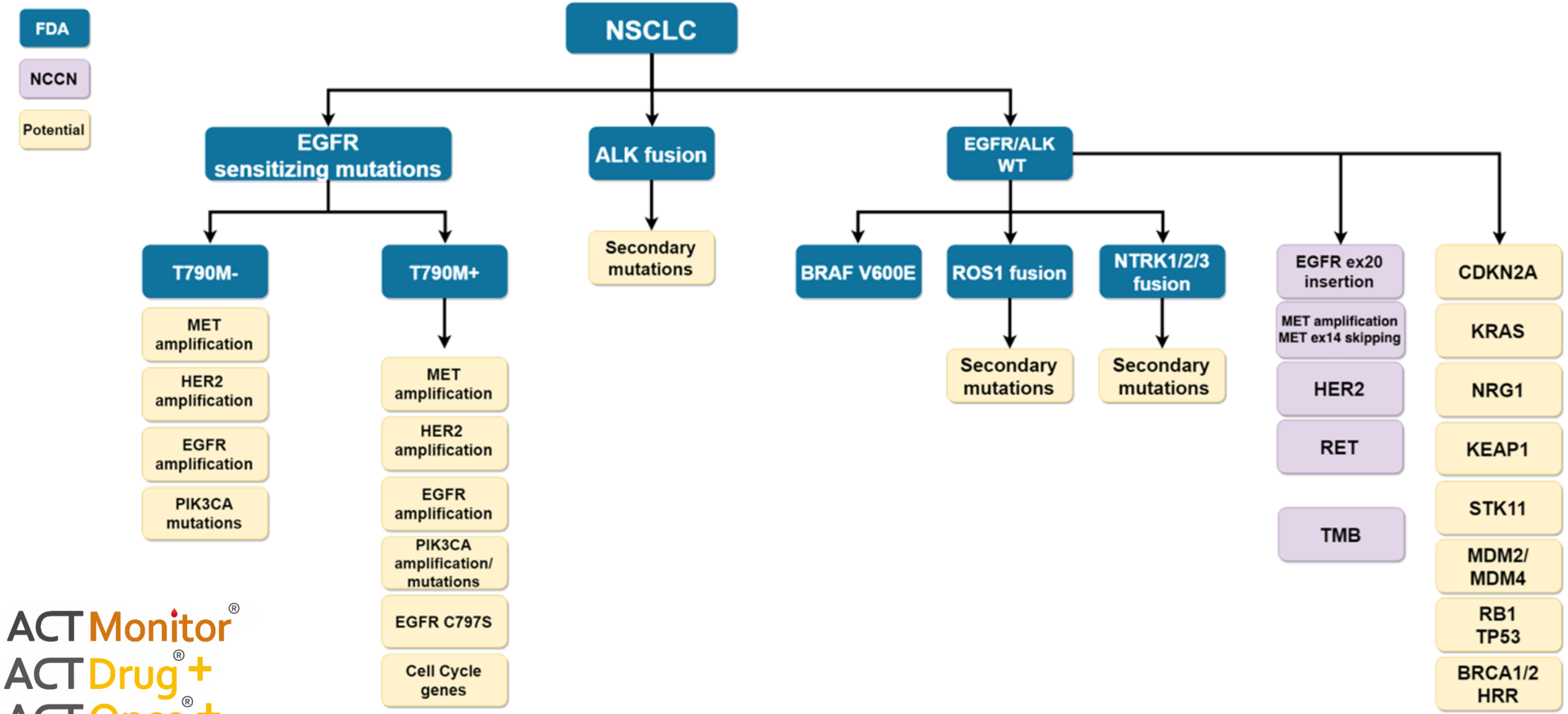
* Evidence in NSCLC

Havel et al. Nat Rev Cancer 2019

To TMB or Not to TMB?

- WES-TMB
  - Most studies show positive correlation to treatment response
  - Cutoff should be 175 mutations per exome or higher
- tTMB
  - Mixed positive and negative data
  - Positive data typically associated with higher cutoff
  - Cutoff depends on the panel and algorithm
  - TMB harmonization program should help to align individual panel-TMB
- bTMB
  - Not much data available on bTMB
  - Failure of NEPTUNE left the utility of bTMB in doubt
- Other factors
  - Mutations in resistance pathway (e.g. STK11) should be taken into consideration

The Potential Molecular Testing Guidelines for NSCLC



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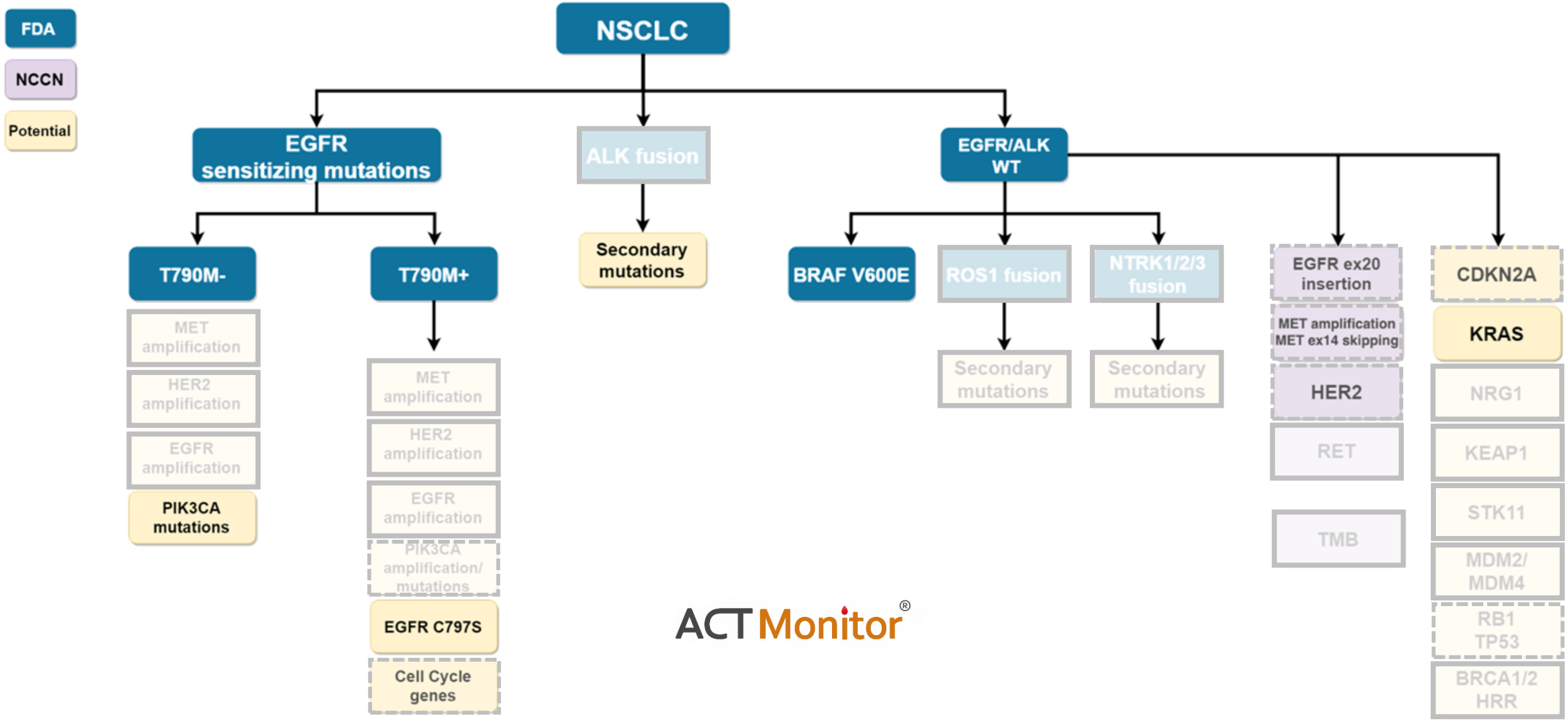
The Potential Molecular Testing Guidelines for NSCLC



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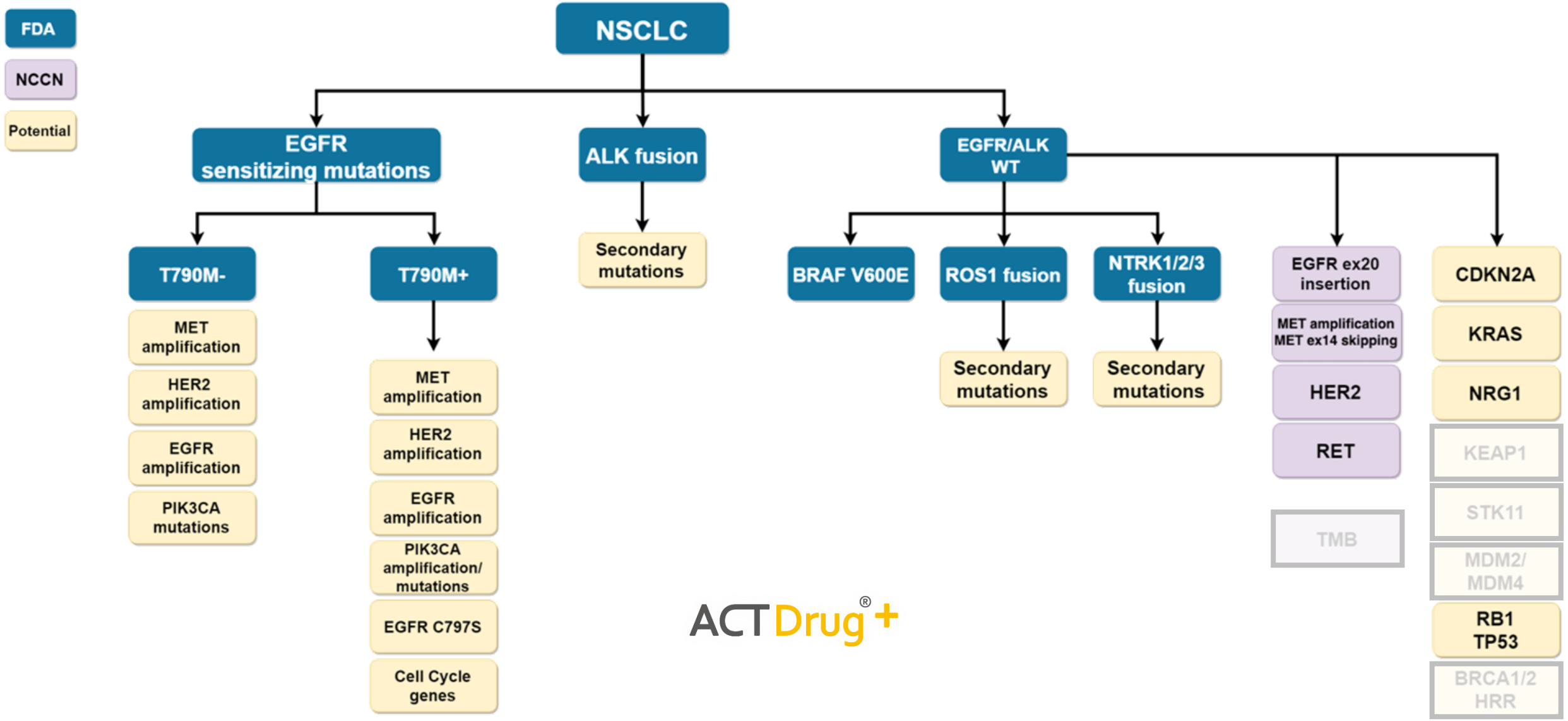

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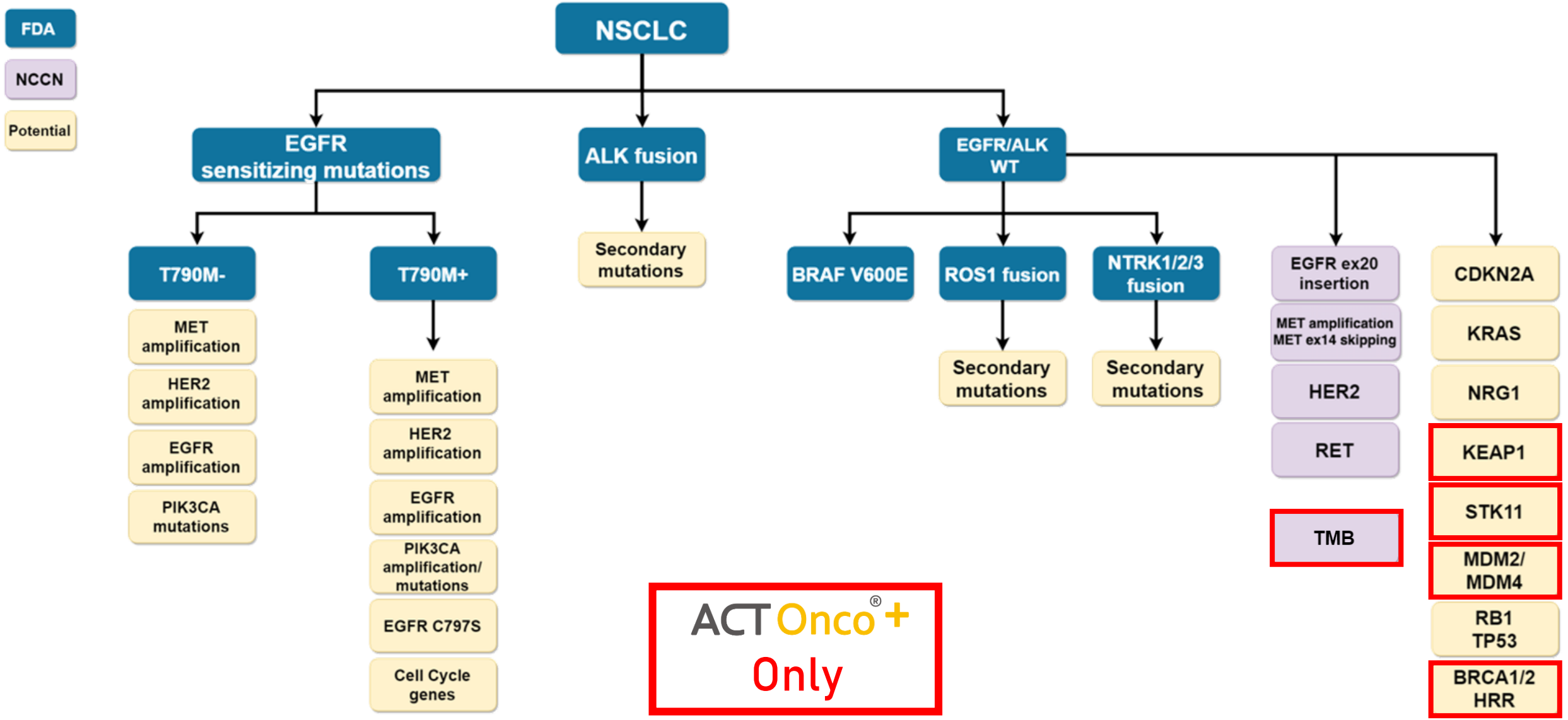

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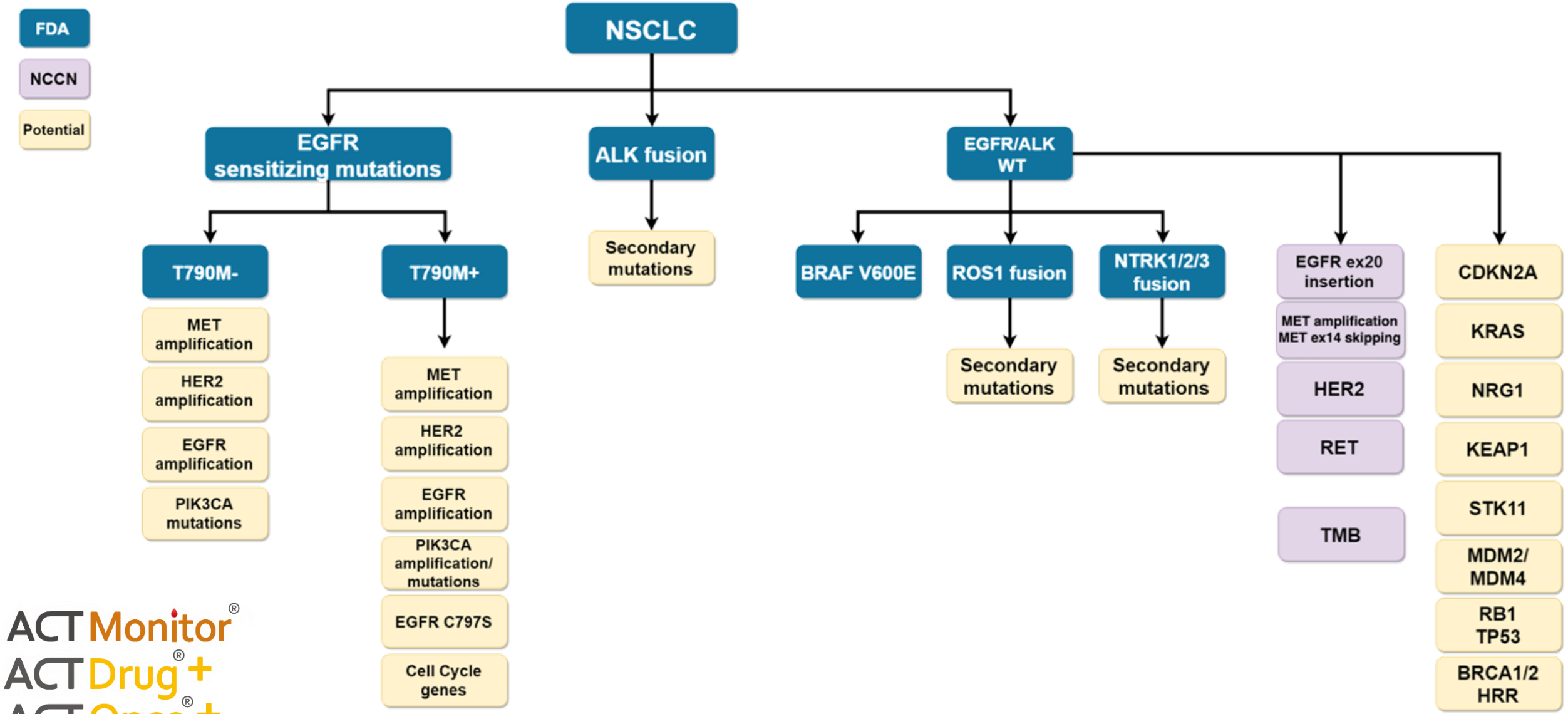

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