Emerging Biomarkers for Immunotherapy: NSCLC as a model

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Disclosures

- **Institutional Research Grants: Amgen, AstraZeneca, Genentech, Merck**
- **Consultant/Advisory Board: AstraZeneca (institutional), Roche-Genentech (institutional), Guardant Health (institutional), Inivata, IO Biotech (institutional), Lilly, Merck, Novartis, Oncocyte (institutional)**

Immune Phenotype as potential Predictive Biomarkers for benefit from Checkpoint Immunotherapy

Tumor Neo-antigenicity

- Biomarkers indicative of hypermutation & neoantigens

Examples:

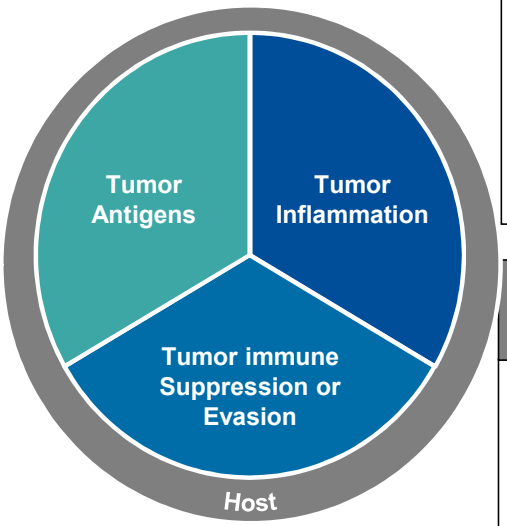
–TMB, MSI-high, neoantigen load

Tumor Microenvironment (Inflammation)

- Biomarkers (intra- or peri-tumoral) indicative of an inflamed phenotype

Examples:

–PD-L1, inflammatory signatures



Tumor Immune Suppression/Evasion

- Biomarkers that identify tumor immune system suppression or evasion beyond PD-1/CTLA-4

Examples:

–Tregs, MDSCs, IDO, LAG-3

–STK11 and KEAP1

–-ARID1A

Host Environment (e.g. Microbiome)

- Biomarkers that characterize the host environment, beyond tumor microenvironment

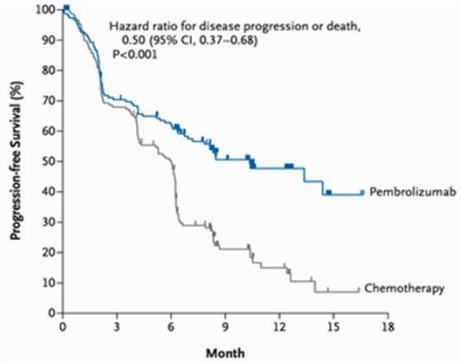
Examples:

–Microbiome, germline genetics

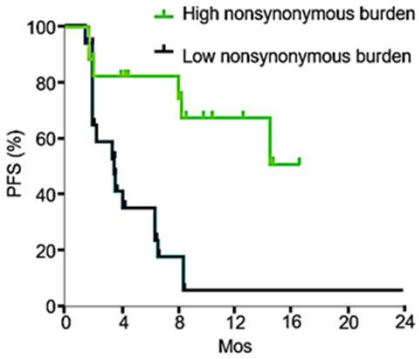
Adapted from Blank CU, et al. *Science* 2016;352:658–660

Selected Biomarkers associated with Checkpoint Immunotherapy efficacy in NSCLC

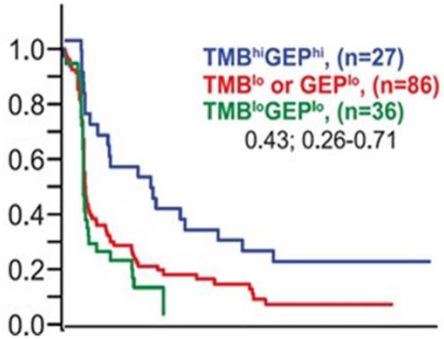
PD-L1 tumor IHC (22c3)[1]



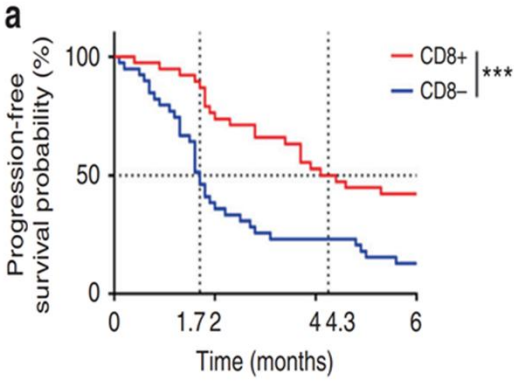
Tumor mutational burden (TMB) [2]



Gene expression signature (GEP)[3]



Tumor infiltrating lymphocytes (TILs)[14]



Hypothesis: Combination of biomarkers will provide largely non-redundant information and additional predictive value

1. Reck et al. 2016, NEJM; 2. Rizvi NA, et al. 2015, Science; 3. Cristescu et al. 2018, Science; 4. Fumet et al., 2018 BJC

Predictive Biomarkers for Checkpoint Immunotherapy (CPI)

Note: cannot be equated to a discrete variable like driver mutations (Present or Absent)

PD-L1 & TMB are dynamic & continuous variables across a context-specific range

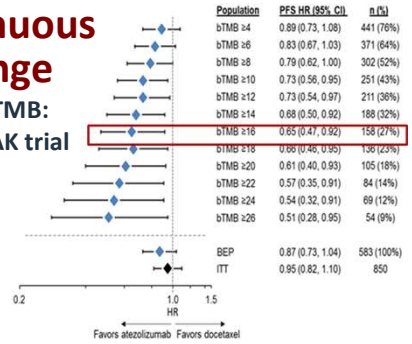
- **Which Biomarker(s)?**
  - PD-L1 IHC
  - TMB
  - PD-L1 IHC + TMB
  - PD-L1 + TMB + Other
  - Multitude of Others

- TMB Assessment
  - WES vs Neo-antigen Load vs NGS
    - Optimal Cutpoints for each
    - Tumor-type specific vs Agnostic
  - Tissue vs Blood

- **Which Analytic Algorithm for Analysis?**
  - Across a Continuous Range
  - As a Binary Variable

Continuous Range

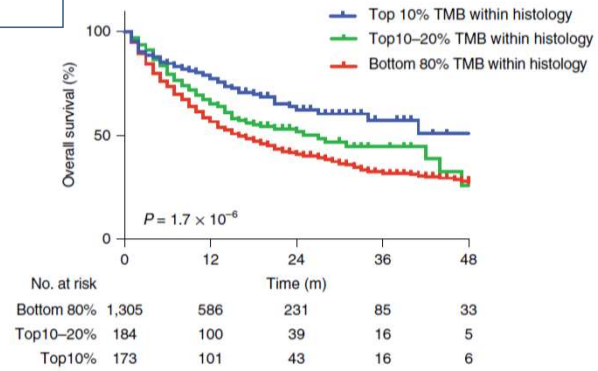
Blood TMB: PFS in OAK trial



Gandara et al: NatMed 2018

As a Binary Variable

TMB highest 10-20% across Tumor Types



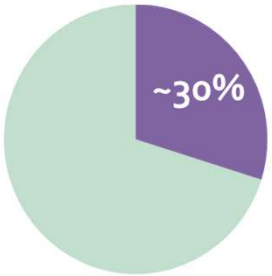
Samstein et al: NatGen 2018

- **What is the context?** (Biomarker for which type of CPI regimen)
  - NSCLC (Squamous or Non-Squamous) vs SCLC
  - CTLA-4 vs PD-1/PD-L1 vs PD-1/PD-L1 + CTLA-4
  - PD-1/PD-L1 + Platinum Chemotherapy

Chemotherapy likely "agnostic" to immuno-biomarker . "Dilutes out predictive value

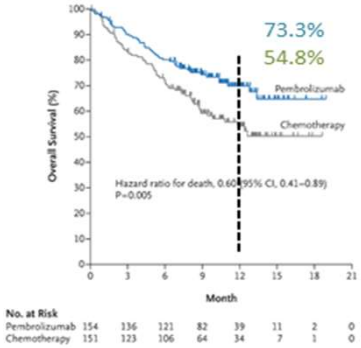
Gandara: Lung Cancer Summit. ESMO19

PD-L1 ≥50% distinguishes a Patient Subset with Substantial Benefit from CPI Monotherapy (KN024) as well as CPI + Chemotherapy (KN189)



PD-L1 ≥50%
EGFR/ALK WT

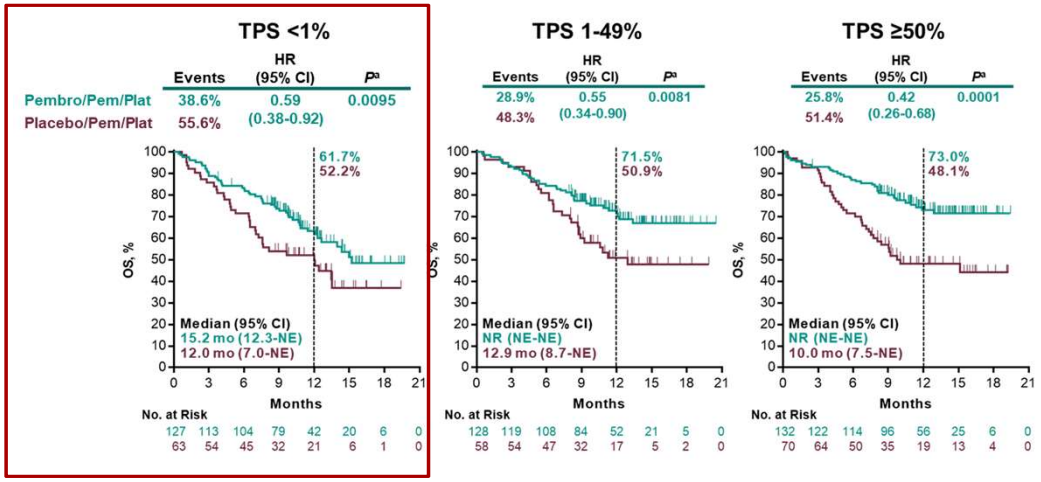
KeyNote 24: Pembro Monotherapy (OS by TPS ≥50%)



OS: HR 0.60 [95% CI 0.41-0.89]
p=0.005

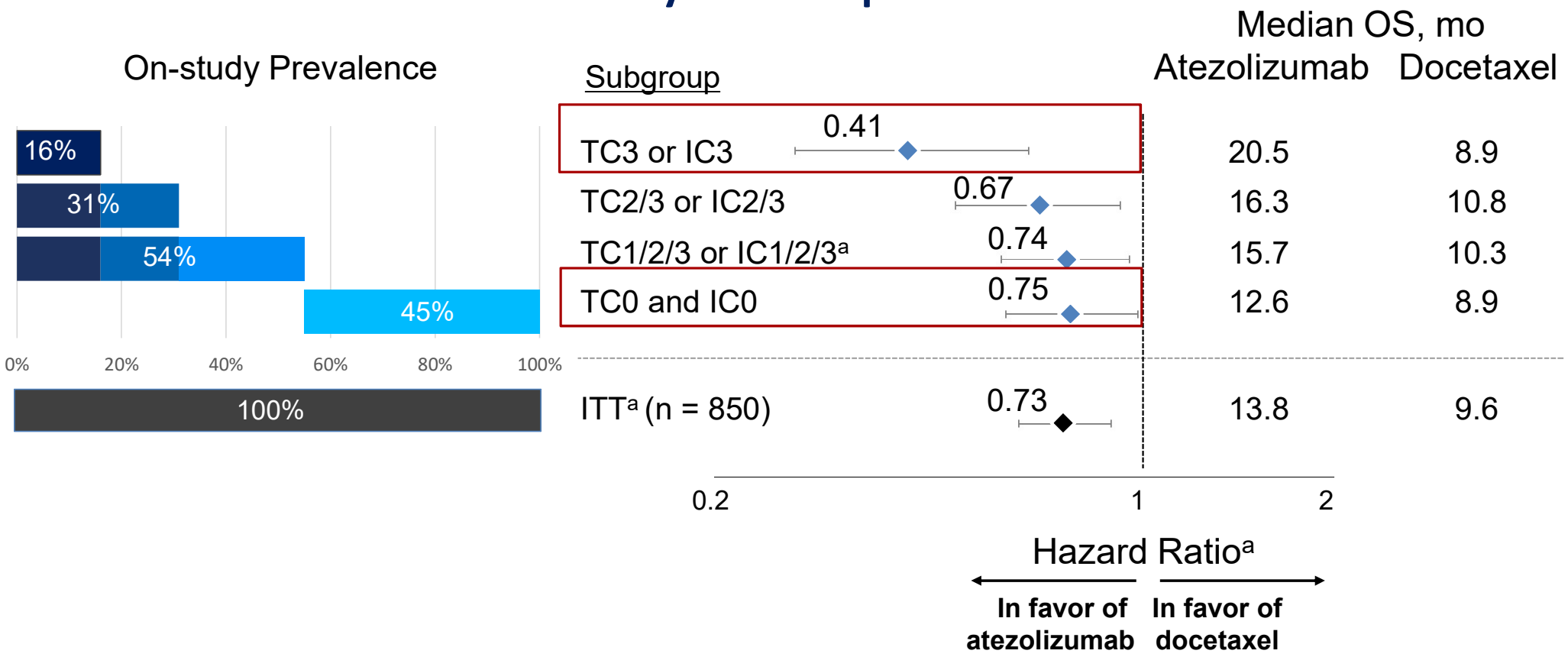
Reck et al. NEJM 2016; 275:1823-1833

KeyNote 189: Pembro-Chemo (OS by PD-L1 TPS)



Gandhi et al. NEJM 2016

OAK (Atezolizumab vs Docetaxel in 2nd line+ Advanced NSCLC: OS by PD-L1 Expression

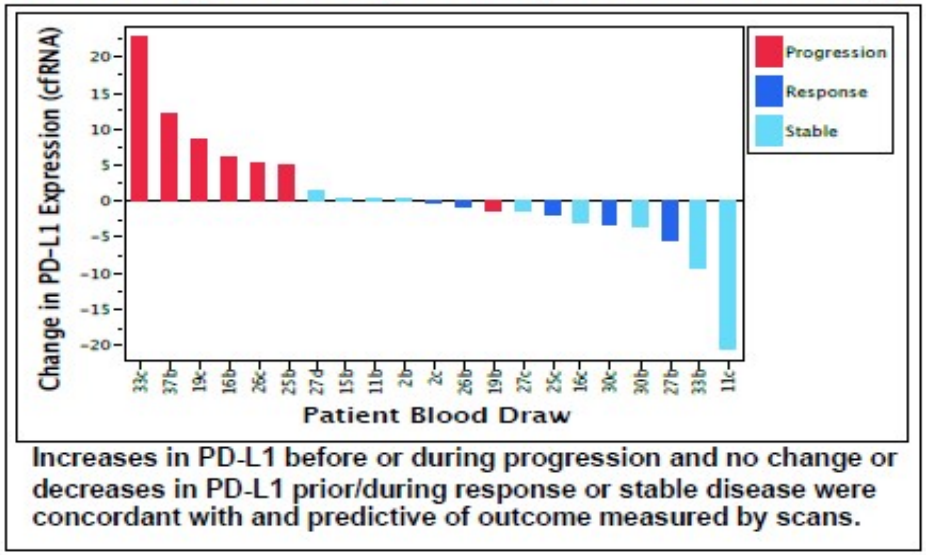


[a]Stratified HR for ITT and TC1/2/3 or IC1/2/3. Unstratified HR for other subgroups.
TC, tumor cells; IC, tumor-infiltrating immune cells; OS, overall survival.

Rittmeyer. Gandara et al. Lancet. 2017;389:255-265

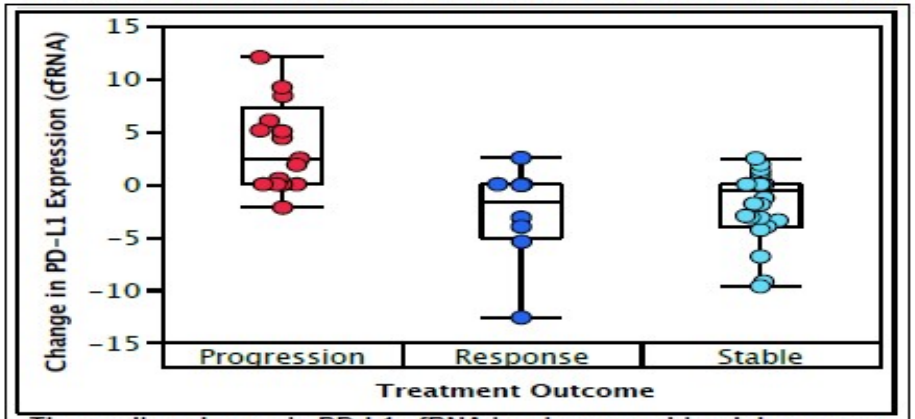
Changes in the Pd-L1 measured in cfRNA according to response to therapy in NSCLC

FIG 1. Changes in PD-L1 predict response to immunotherapy



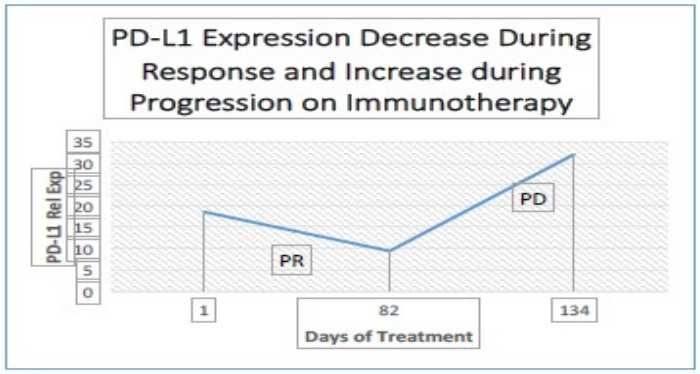
Increases in PD-L1 before or during progression and no change or decreases in PD-L1 prior/during response or stable disease were concordant with and predictive of outcome measured by scans.

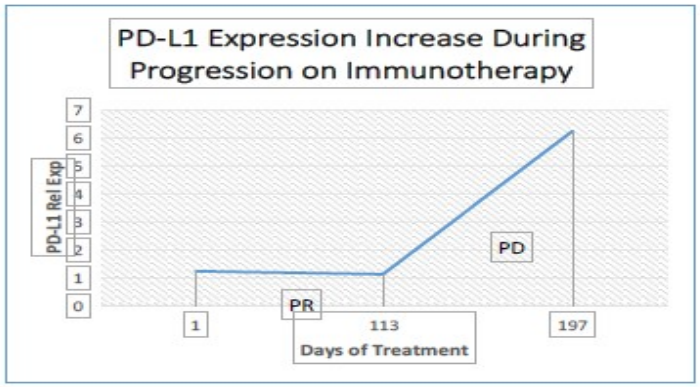
FIG 3. PD-L1 increases during emerging resistance to therapy



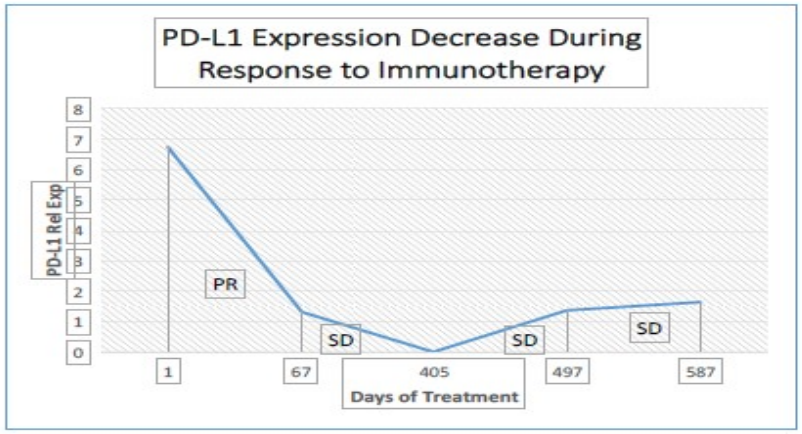
The median change in PD-L1 cfRNA levels across blood draws was statistically significantly higher in those leading up to PD compared with the groups with either SD or PR disease status, as measured by CT scans (p = 0.0001, Wilcoxon Rank Sums).

Raez LE, A, et al. J Clin Oncol 37, 2019 (suppl; abstr e14567)





Examples of the dynamics of PD-L1 expression during individual treatment of NSCLC patients with immunotherapy vs outcome*.



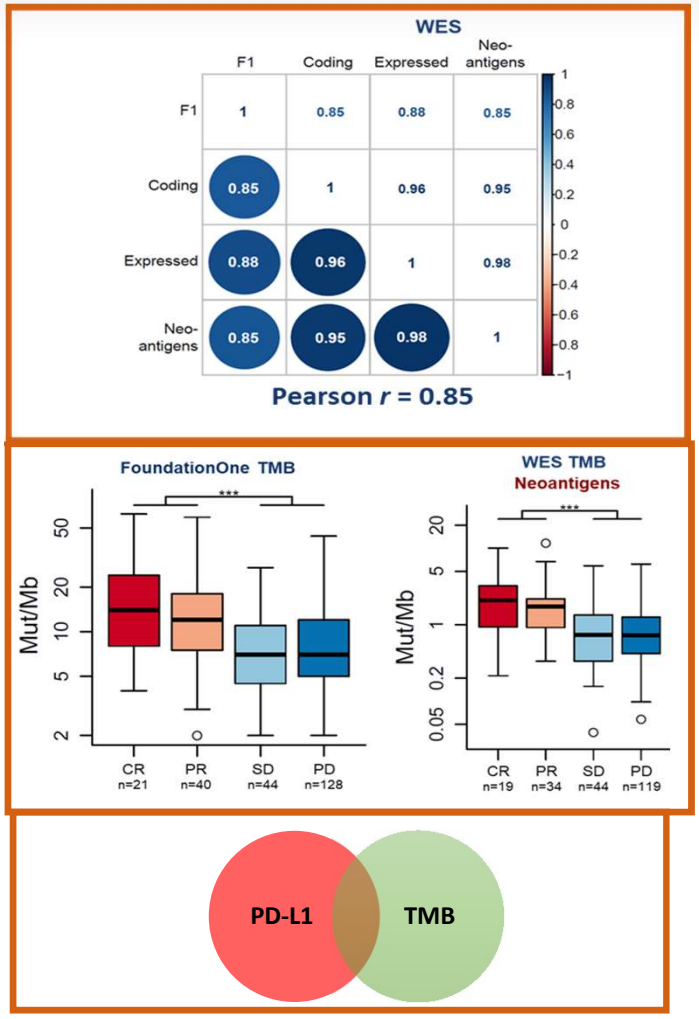
Raez LE, A, et al. J Clin Oncol 37, 2019 (suppl; abstr e14567)

Tumor Mutational Burden (TMB) as a Candidate Predictive Biomarker for Cancer Immunotherapy

- **Somatic mutations in cancers are multifactorial** (including DNA repair defects, carcinogens & enzymatic alterations in DNA polymerases)
- These mutations produce **neoantigens** that induce anti-tumor immune responses
- **TMB is an emerging predictive biomarker** for cancer checkpoint immunotherapy (CIT)
- TMB can be estimated using whole-exome sequencing **(WES)** or comprehensive genomic profiling by NGS **(e.g., FoundationOne & FACT in blood[bTMB]) . MSK-IMPACT. Guardant OMNI** in blood[1-8]
  - Studies show that TMB either by WES or CGP correlate with each other & with efficacy of CPI therapy in multiple cancer types[1-3]
- **Predicted neoantigen load (NAL),** a component of TMB most closely linked to immune response, correlates with F1 TMB & OMNI[4,5,7,8]
- **TMB identifies a distinct patient population** not currently captured by PD-L1 IHC or other immune biomarkers[5,6]

IHC, immunohistochemistry; PD-L1, programmed death-ligand 1; TMB, tumor mutational burden.

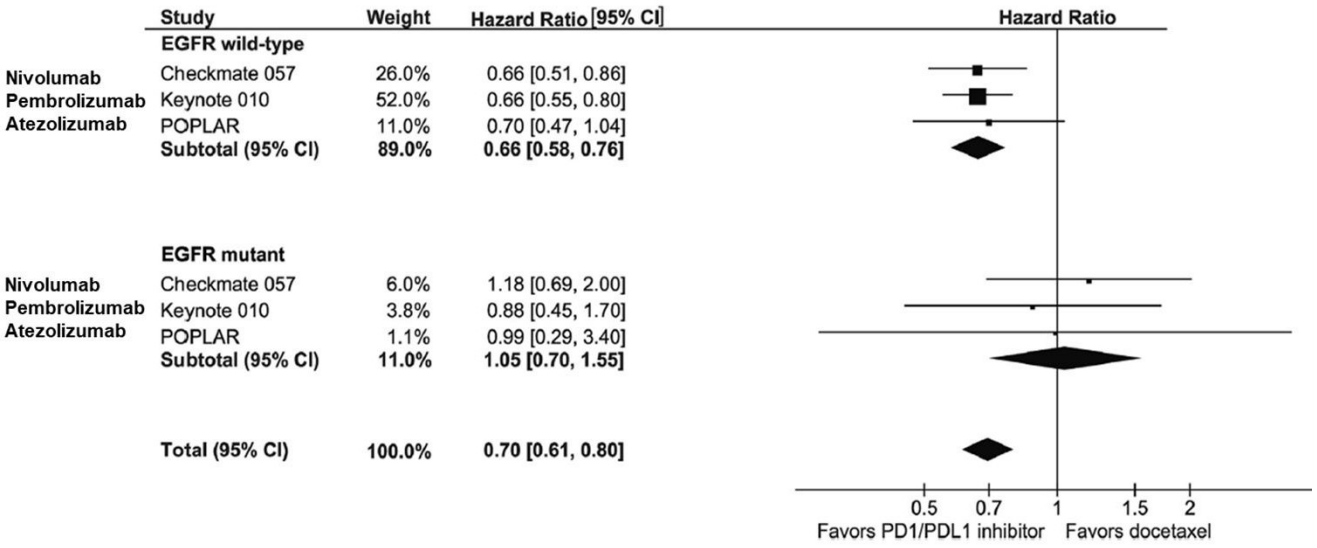
1. Yarchoan M, et al. *N Engl J Med.* 2017; 2. Chalmers ZR, et al. *Genome Med.* 2017; 3. Goodman AM, et al. *Mol Cancer Ther.* 2017; 4. Efremova M, et al. *Front Immunol.* 2017; 5. Topalian SL, et al. *Nat Rev Cancer.* 2016; 6. Kowanetz M, et al. WCLC 2017. 7. Mariathansan, et al. Nature 2018. 8. Rizvi et al: ESMO IO 2018.



From Gandara, LeGrand et al: ASCO 2018

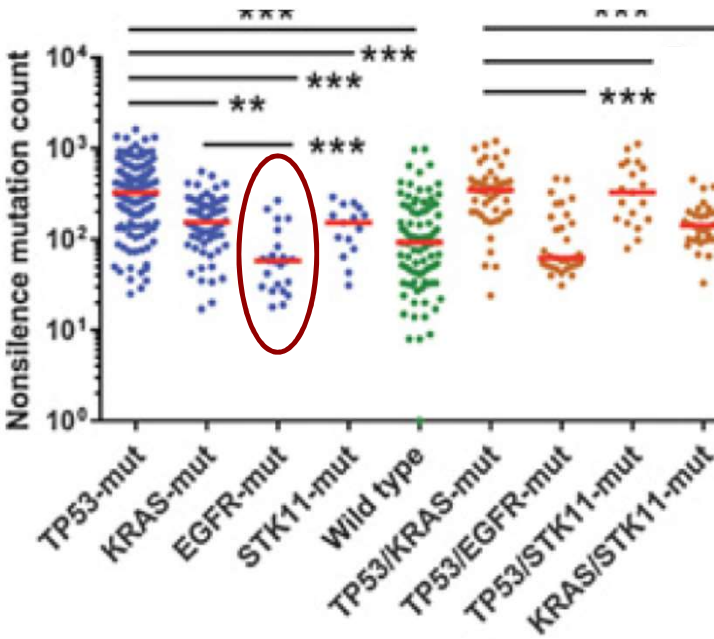
EGFR-mutated NSCLC: Efficacy of PD1/PD-L1 inhibitors is poor regardless of PD-L1 score and TMB is low

PD-(L)1 Inhibitors in EGFR-mutated NSCLC



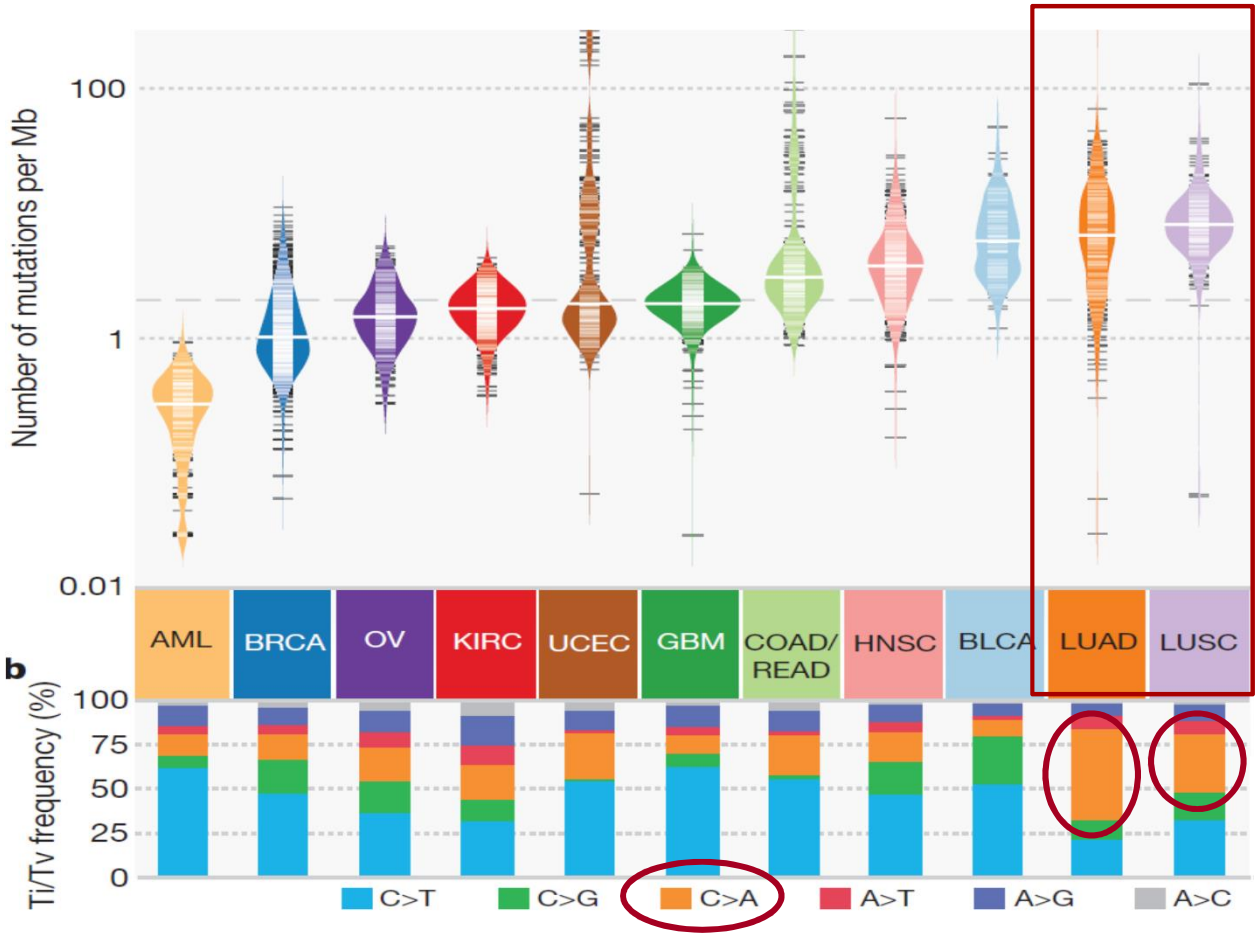
Lee CK, et al. J Thorac Oncol 2016

TMB is low in EGFR-mutated cancers



Dong, Wu et al CCR 2017

NSCLC is complex both genomically & immunologically, with Quantitative & Qualitative differences from other Cancer Types ("Mutational Load")



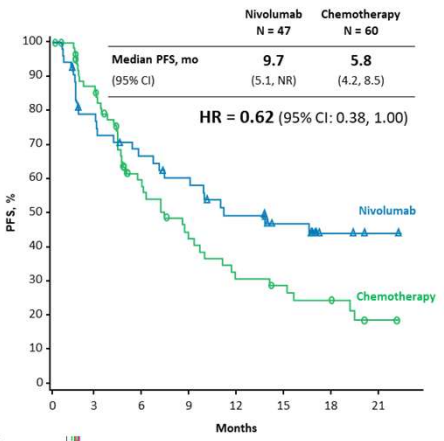
Adapted from The Cancer Genome Atlas Project: Kandoth et al *Nature* 2013.

C>A Transversions

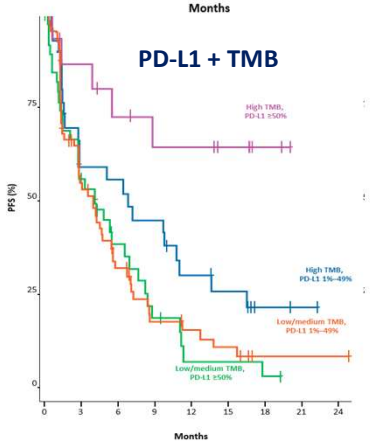
- **~Tobacco-related**
- **Most neo-antigenic**

High Tissue TMB is associated with increased efficacy of Checkpoint Inhibitor Monotherapy

WES: CM-026 NSCLC (Nivo -high TMB)

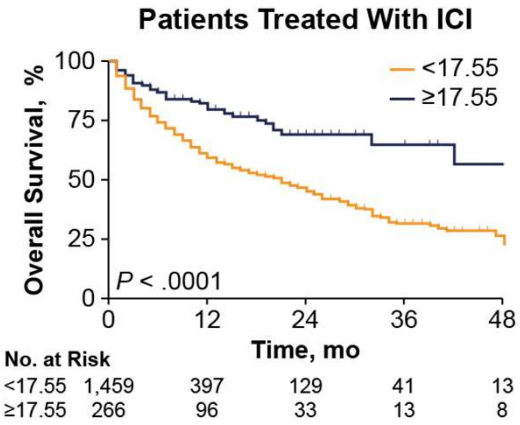


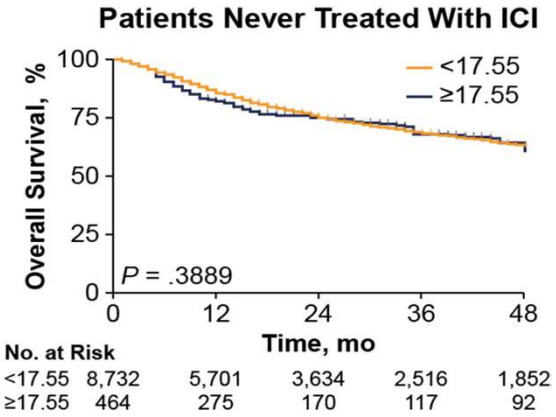
PD-L1 + TMB



Carbone et al: NEJM 2017

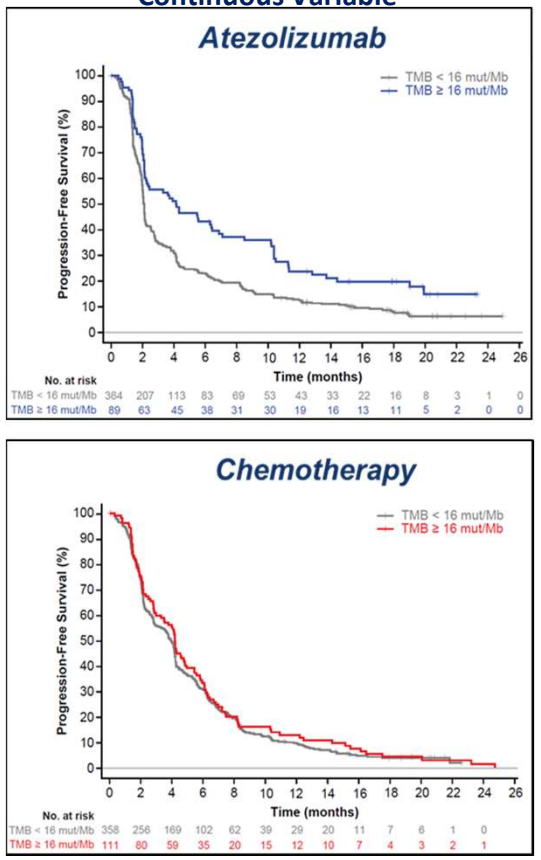
NGS -IMPACT: Multiple Tumor Types Binary Variable





Samstein et al: NatGen 2019

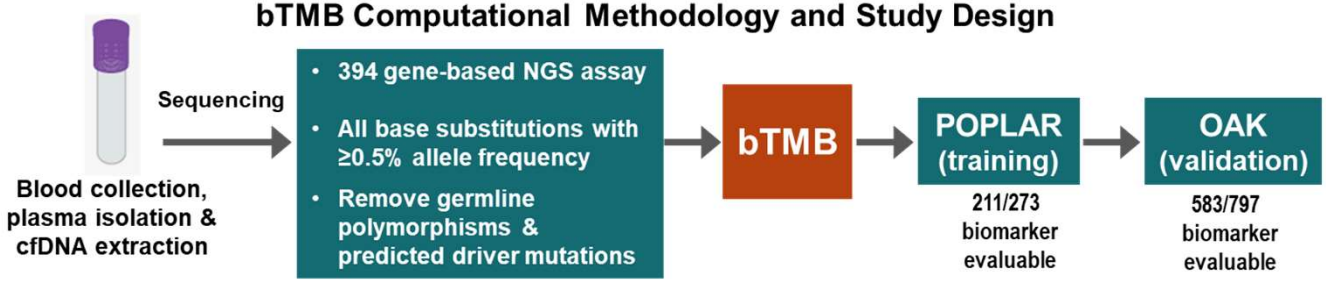
NGS Foundation-One: Multiple Tumor Types Continuous Variable

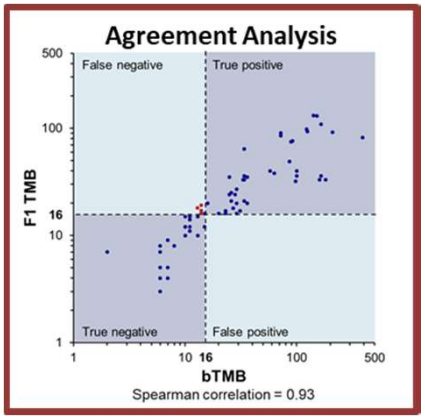


Gandara, Legrand et al: ASCO 2018

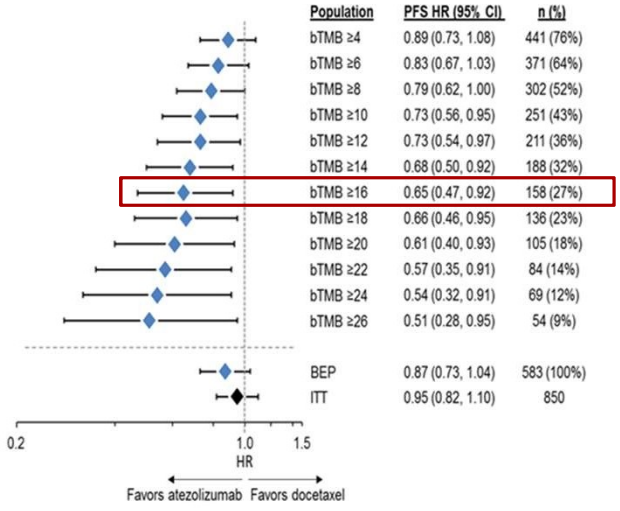
Analytical & Clinical Validation of Tumor Mutational Burden in Blood (bTMB) in association with Atezolizumab efficacy in advanced NSCLC (POPLAR & OAK Trials)

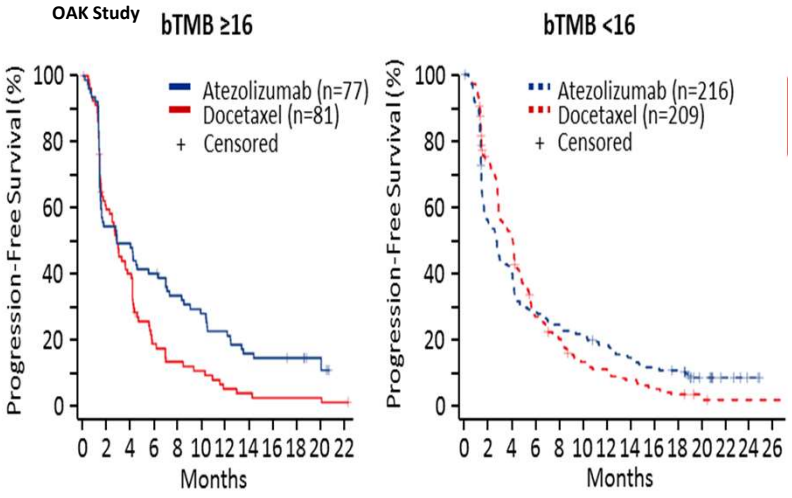
bTMB Computational Methodology and Study Design



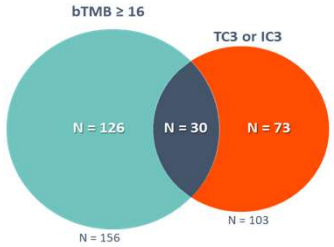


Progression-Free Survival – OAK



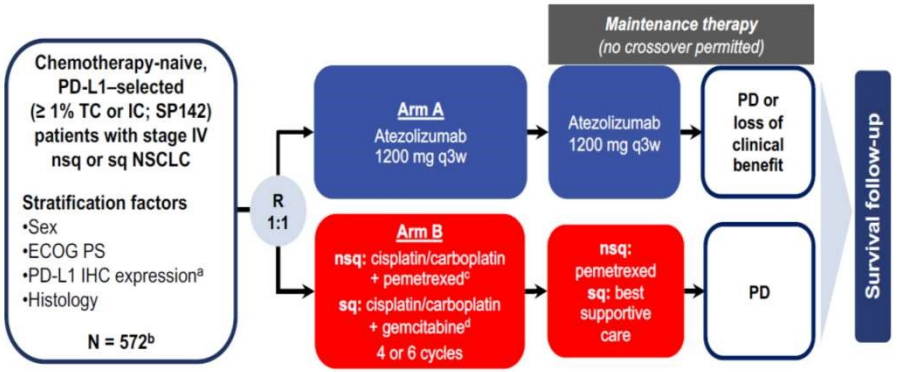


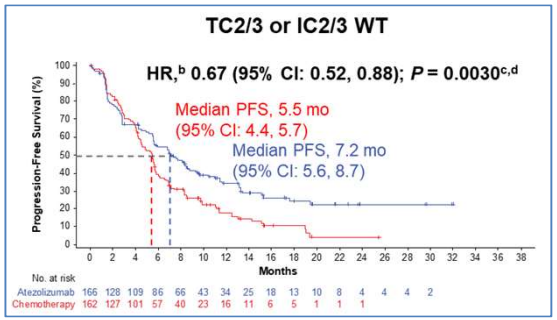
Gandara DR, et al. Nature Med 2018.

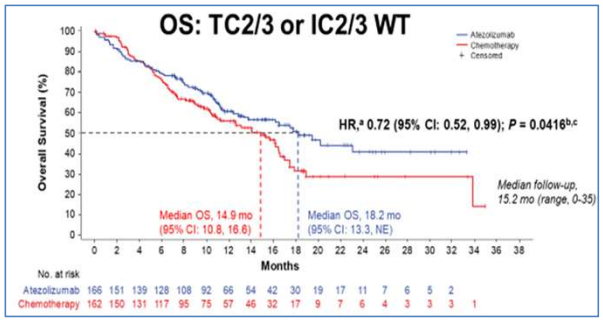


	PFS HR (95% CI)	OS HR (95% CI)
bTMB ≥16	0.64 (0.46, 0.91)	0.64 (0.44, 0.93)
TC3 or IC3	0.62 (0.41, 0.93)	0.44 (0.27, 0.71)
bTMB ≥16 and TC3 or IC3	0.38 (0.17, 0.85)	0.23 (0.09, 0.58)

Blood TMB in IMpower110 Trial of Atezolizumab vs Platinum-based Chemotherapy

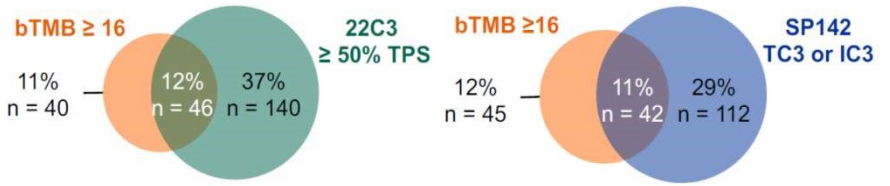






bTMB was assessed by Foundation assay*

- A bTMB score of 16 is equivalent to 16 mutations/1.1 Mb, or ≈14.5 mutations/Mb
- PD-L1 IHC (SP142 or 22C3) and bTMB identified distinct patient populations in IMpower110



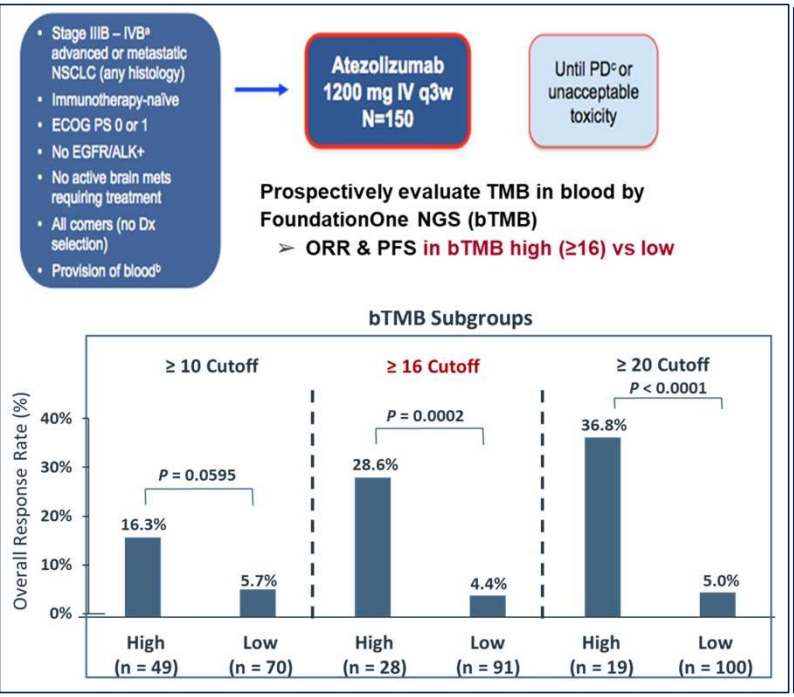
[a] By Foundation Medicine. bTMB and 22C3 overlap: BEP-WT population, n = 375; bTMB and SP142 overlap: BEP-WT population, n = 389. 1. Gandara DR, et al. *Nat Med*. 2018; 2. Socinski MA, et al. *ESMO*. 2019 [abstract 2239]; 3. Hellmann MD, et al. *N Engl J Med*. 2018; 4. Greillier L, et al. *Transl Lung Cancer Res*. 2018.

PFS in the bTMB BEP-WT in IMpower110

Subgroup	n (%)	PFS HR (95% CI)[a]	Median PFS, m Atezo	Chem
TC1/2/3 or IC1/2/3-WT	**554 (100)**	**0.77 (0.63, 0.93)**	**5.7**	**5.5**
bTMB BEP-WT	**389 (100)**	**0.88 (0.70, 1.11)**	**5.5**	**5.4**
bTMB ≥ 10	175 (45)	0.74 (0.53, 1.05)	5.5	4.3
bTMB ≥ 16	87 (22)	0.55 (0.33, 0.92)	6.8	4.4
bTMB ≥ 20	56 (14)	0.56 (0.30, 1.06)	6.8	5.2
bTMB < 10	214 (55)	1.03 (0.76, 1.39)	5.5	5.7
bTMB < 16	302 (78)	1.00 (0.78, 1.29)	4.5	5.5
bTMB < 20	333 (86)	0.95 (0.74, 1.21)	4.9	5.4

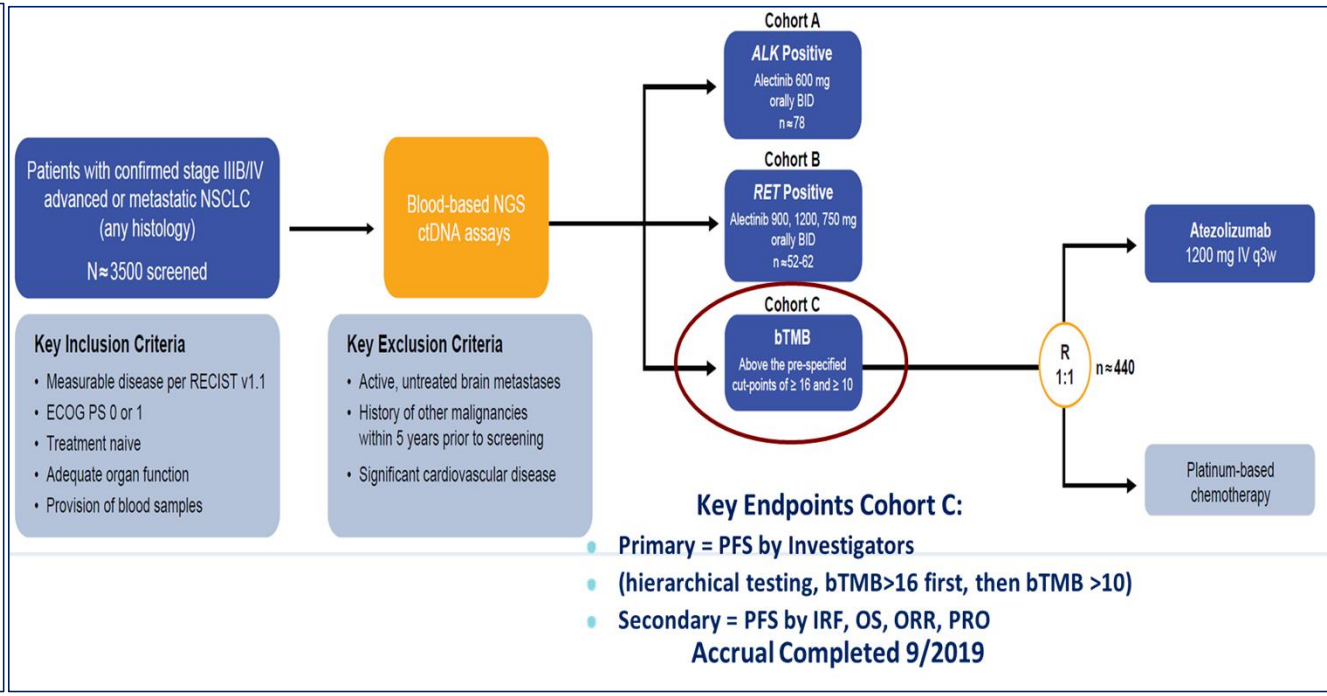
Herbst et al: ESMO IO19; Herbst et al: NEJM 2020

B-F1RST
Proof of Principle trial for bTMB Selection of Atezolizumab Immunotherapy



Kim ES, et al. ESMO 2018. Abstract LBA55.

BFAST
(Blood First Assay Screening Trial): Phase II/III Trial in Advanced Treatment-naïve Advanced NSCLC



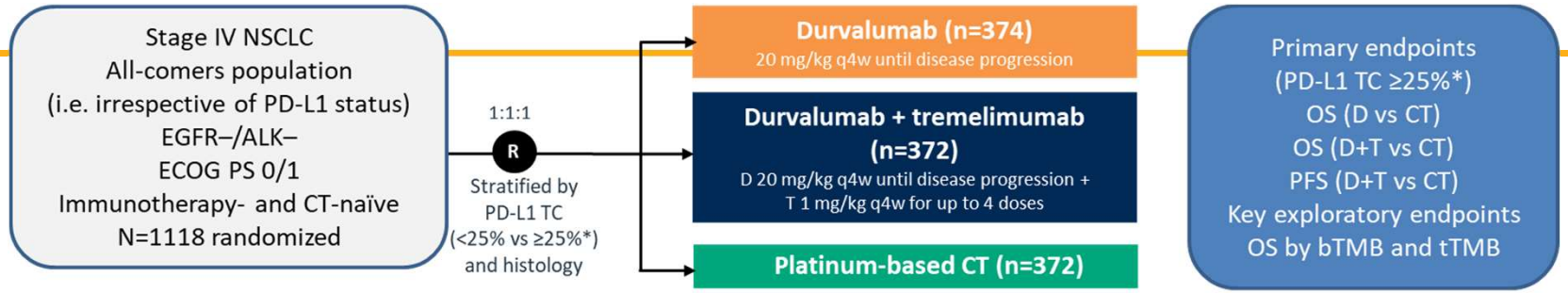
FDA Approval of FoundationOne NGS CDx Assay

CAMBRIDGE, Mass. – Wednesday, August 26 – Foundation Medicine, Inc. today announced that the U.S. Food and Drug Administration (FDA) approved FoundationOne®Liquid CDx, the Company's comprehensive pan-tumor liquid biopsy test. FoundationOne Liquid CDx will be commercially available on Friday, August 28 and is covered across all solid tumors for eligible Medicare and Medicare Advantage beneficiaries in accordance with the Centers for Medicare and Medicaid Services National Coverage Decision Memo criteria.

FoundationOne Liquid CDx is part of Foundation Medicine's proven portfolio of FDA-approved tests and acts as:

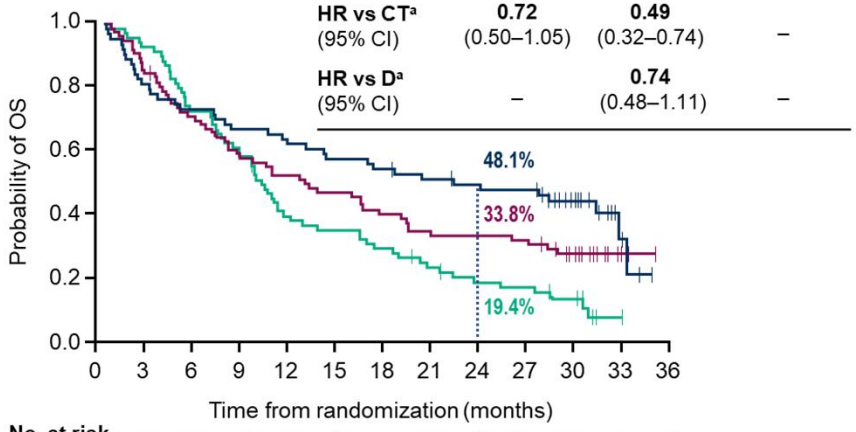
- a companion diagnostic to identify patients who may benefit from treatment with specific FDA-approved targeted therapies, including an indication for Rubraca (rucaparib), a poly (ADP-ribose) polymerase (PARP) inhibitor for treatment in patients with BRCA 1/2-mutant metastatic castration-resistant prostate cancer, and three first-line tyrosine kinase inhibitors (TKIs) for the treatment of non-small cell lung cancer;
- an FDA-approved test to enable accelerated companion diagnostic development for biopharma companies developing precision therapeutics; and
- a comprehensive genomic profiling test that reports genomic alteration results, including genomic signatures such as blood tumor mutational burden and high microsatellite instability, as well as single gene alterations, including all NTRK fusions, for patients with any solid tumor as an aid in patient care.

MYSTIC: 1L durvalumab ± tremelimumab vs chemotherapy in adv NSCLC – bTMB by GH OMNI



bTMB ≥20 mut/Mb

	D (n=77)	D+T (n=64)	CT (n=70)
mOS, months (95% CI)	**12.6** (7.8–18.6)	**21.9** (11.4–32.8)	**10.0** (8.1–11.7)
HR vs CT[a] (95% CI)	**0.72** (0.50–1.05)	**0.49** (0.32–0.74)	–
HR vs D[a] (95% CI)	–	**0.74** (0.48–1.11)	–

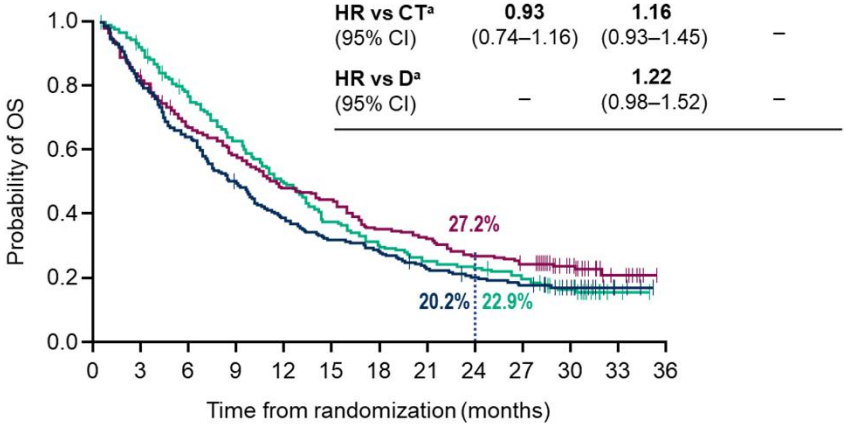


No. at risk

D	77	64	53	44	39	35	30	25	25	23	10	1	0
D+T	64	50	47	43	40	37	35	32	29	29	14	2	0
CT	70	65	51	41	27	25	21	16	12	11	6	0	0

bTMB <20 mut/Mb

	D (n=209)	D+T (n=204)	CT (n=185)
mOS, months (95% CI)	**11.0** (8.9–14.9)	**8.5** (6.7–9.8)	**11.6** (9.6–13.1)
HR vs CT[a] (95% CI)	**0.93** (0.74–1.16)	**1.16** (0.93–1.45)	–
HR vs D[a] (95% CI)	–	**1.22** (0.98–1.52)	–



D	209	167	134	114	98	86	72	63	55	49	21	8	0
D+T	204	161	129	98	75	65	55	45	39	35	18	4	0
CT	185	162	135	110	89	68	53	45	41	34	17	1	0

Rizvi NA, et al. ASCO 2019. ESMO 2019

Predictive Biomarkers for Checkpoint Immunotherapy (CPI)

Note: cannot be equated to a discrete variable like driver mutations (Present or Absent)

PD-L1 & TMB are dynamic & continuous variables across a context-specific range

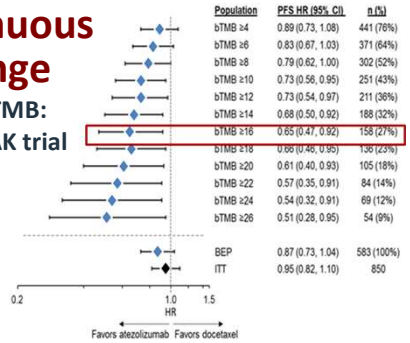
- **Which Biomarker(s)?**
  - PD-L1 IHC
  - TMB
  - PD-L1 IHC + TMB
  - PD-L1 + TMB + Other
  - Other (STK11, KEAP1, ARID1A)

- **Which Analytic Algorithm for Analysis?**
  - Across a Continuous Range
  - As a Binary Variable

- TMB Assessment
  - WES vs Neo-antigen Load vs NGS
    - Optimal Cutpoints for each
    - Tumor-type specific vs Agnostic
  - Tissue vs Blood

Continuous Range

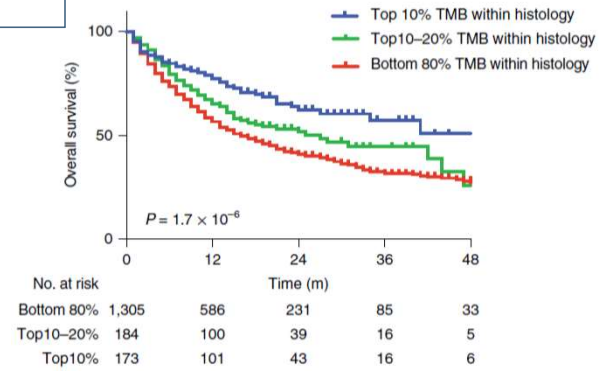
Blood TMB:
PFS in OAK trial



Gandara et al: NatMed 2018

As a Binary Variable

TMB highest 10-20% across Tumor Types



Samstein et al: NatGen 2018

What is the context? (Biomarker for which type of CPI regimen)

- NSCLC (Squamous or Non-Squamous) vs SCLC
- CTLA-4 vs PD-1/PD-L1 vs PD-1/PD-L1 + CTLA-4
- PD-1/PD-L1 + Platinum Chemotherapy

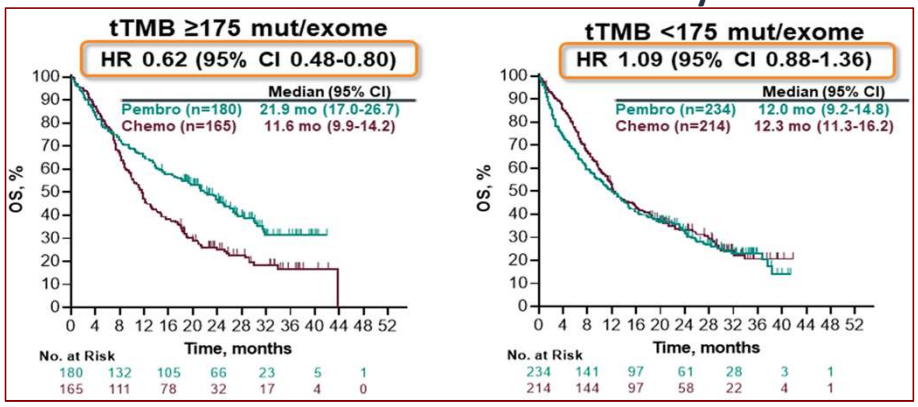
Chemotherapy likely "agnostic" to immuno-biomarker .
"Dilutes out predictive value

Gandara: Lung Cancer Summit. ESMO19

Summary of TMB in CPI Monotherapy vs CPI + Chemo (or Ipi) Trials: New Data from WCLC & ESMO 2019

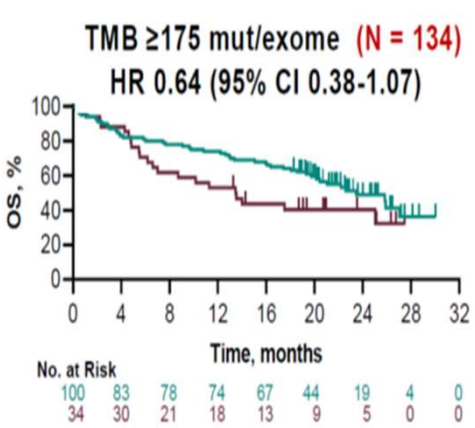
Phase III Trials	Mono- or Combination	TMB	PFS	OS
KN-010	Pembro Mono	WES-tissue	√	√
KN-042	Pembro Mono	WES-tissue	√	√
KN-189	Pembro + Chemo	WES-tissue	No	No
KN-407	Pembro + Chemo	WES-tissue	No	No
CM-227	Nivo + Ipi	Fone-tissue	√	No
S1400i (LungMAP)	Nivo + Ipi	Fone-tissue	No	√
MYSTIC	Durva + Treme	OMNI-blood	√	√

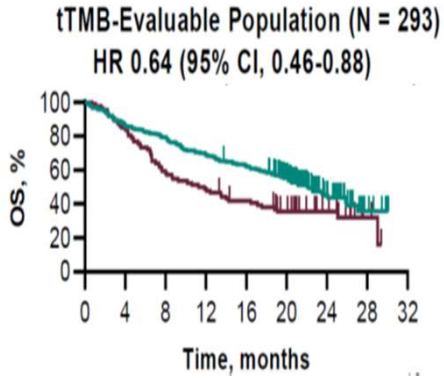
KN-042: Pembro vs Chemo: tTMB by WES



Herbst: ESMO 2019

KN-189: Pembro+Chemo vs Chemo (Non-Squamous): tTMB by WES





Garissino: ESMO 2019

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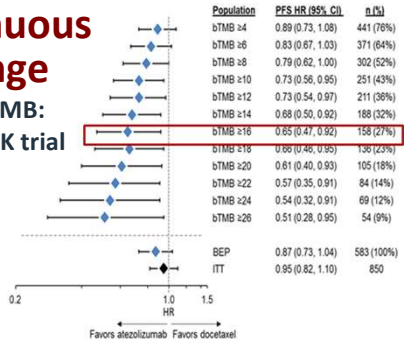

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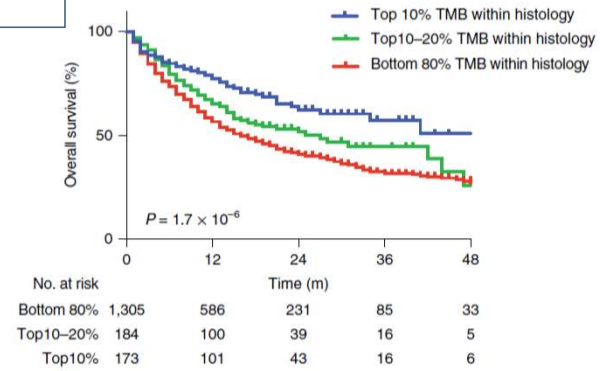

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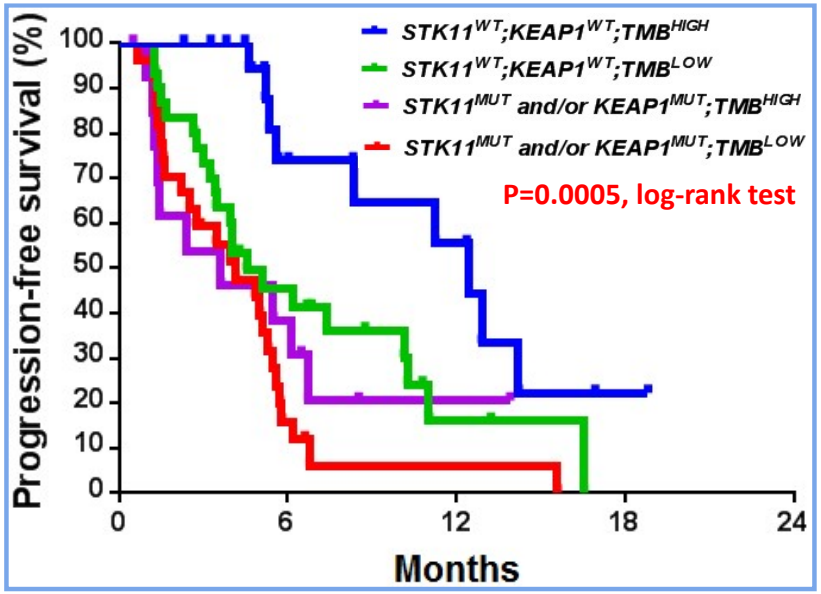
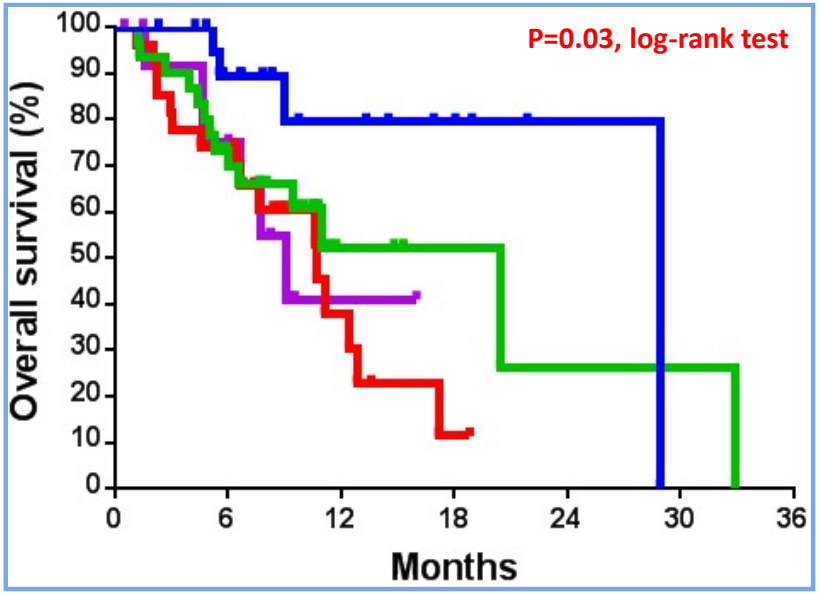
Gandara: Lung Cancer Summit. ESMO19

Integration of *STK11* and *KEAP1* genomic alterations with TMB and other biomarkers: Moving towards a composite panel?

Group	PFS
$STK11^{WT}$;$KEAP1^{WT}$;TMB^{HIGH}	12.4m
$STK11^{WT}$;$KEAP1^{WT}$;TMB^{LOW}	4.5m
$STK11^{MUT}$ and/or $KEAP1^{MUT}$;TMB^{HIGH}	4.1m
$STK11^{MUT}$ and/or $KEAP1^{MUT}$;TMB^{LOW}	3.6m



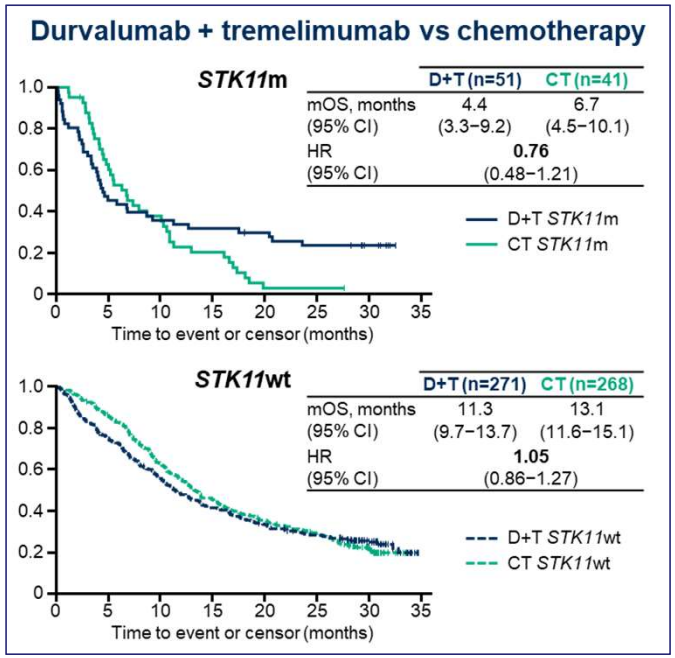
Group	OS
$STK11^{WT}$;$KEAP1^{WT}$;TMB^{HIGH}	28.9m
$STK11^{WT}$;$KEAP1^{WT}$;TMB^{LOW}	20.4m
$STK11^{MUT}$ and/or $KEAP1^{MUT}$;TMB^{HIGH}	10.7m
$STK11^{MUT}$ and/or $KEAP1^{MUT}$;TMB^{LOW}	9.1m



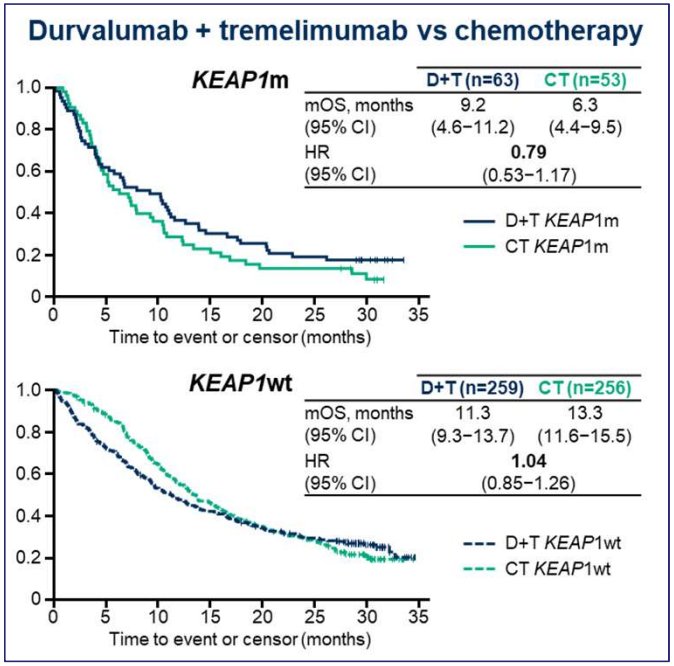
Skoulidis: ASCO 2019.

Analysis of MYSTIC trial by STK11-KEAP1 & ARID1A mutational status (ctDNA by Guardant360)

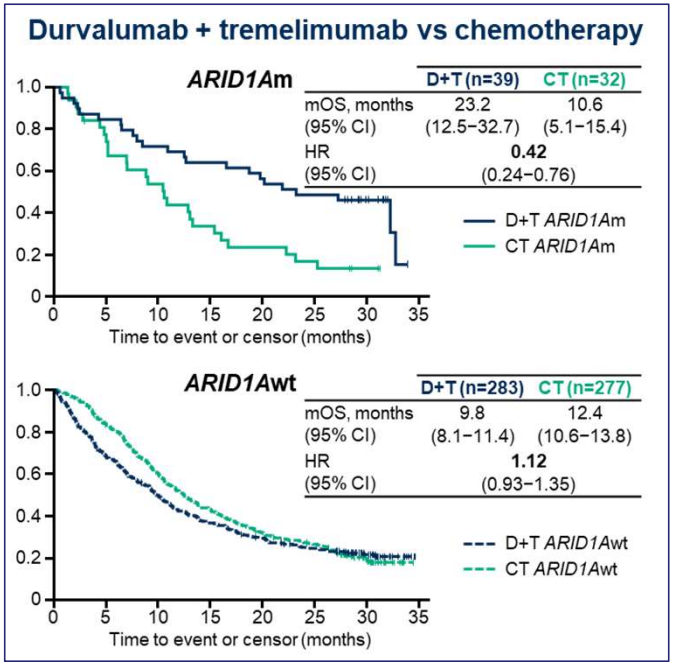
STK11 by ctDNA



KEAP1 by ctDNA



ARID1A by ctDNA



- **STK11 & KEAP1 may be primarily prognostic & not predictive of IO efficacy**
- **ARID1A may be predictive for efficacy of Durva + Treme combination**

Rizvi et al. IASLC WCLC 2019

Patients with f*ARID1A* mutations were more frequently Squamous histology and had a significantly higher number of mutations (N=33,086 NSCLC patients; N=3,115 with ARID1A mutations)

NSCLC histology	Number of Patients	% of patients with f*ARID1A*	p-value
Adenocarcinoma	26,935	3.8	<0.0001
Squamous	4,197	5.1	

f*ARID1A* mutations were significantly more frequent in squamous NSCLC

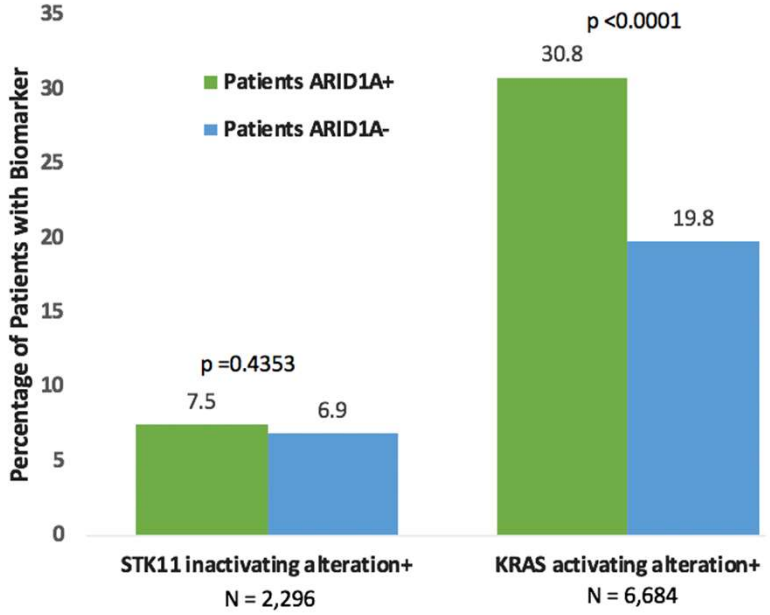
Sample f*ARID1A* status	Median Number of Alterations*	p-value
f*ARID1A*+	6	<0.0001
f*ARID1A*-	3	

Previous work suggests that *ARID1A* is associated with a higher mutational burden – we found that samples with f*ARID1A* had a significantly higher number of alterations per sample

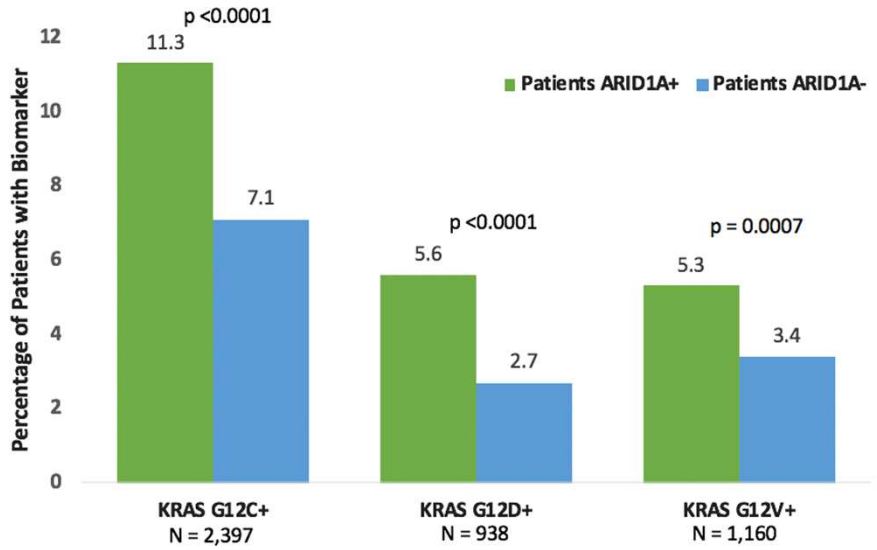
***All reported alterations detected in each sample, including synonymous variants and variants of uncertain significance, were included when calculating the median number of alterations per sample**

References: Rizvi N, et al. World Conference on Lung Cancer 2019; Okamura R, et al. *J Immunother Cancer*, 2020

Activating *KRAS* mutations were significantly more frequent in patients with f*ARID1A* mutations



KRAS mutations associated with smoking (G12C/V) and non- smoking (G12D) were significantly more frequent in patients with f*ARID1A* mutations



References: Herbst R, et al. ESMO Immuno-Oncology Congress, 2019; Aggarwal C, et al. *Clin Canc Res*, 2020; Skoulidis F, et al. *Cancer Discovery,* 2018; Skoulidis F, et al. World Conference on Lung Cancer, 2018

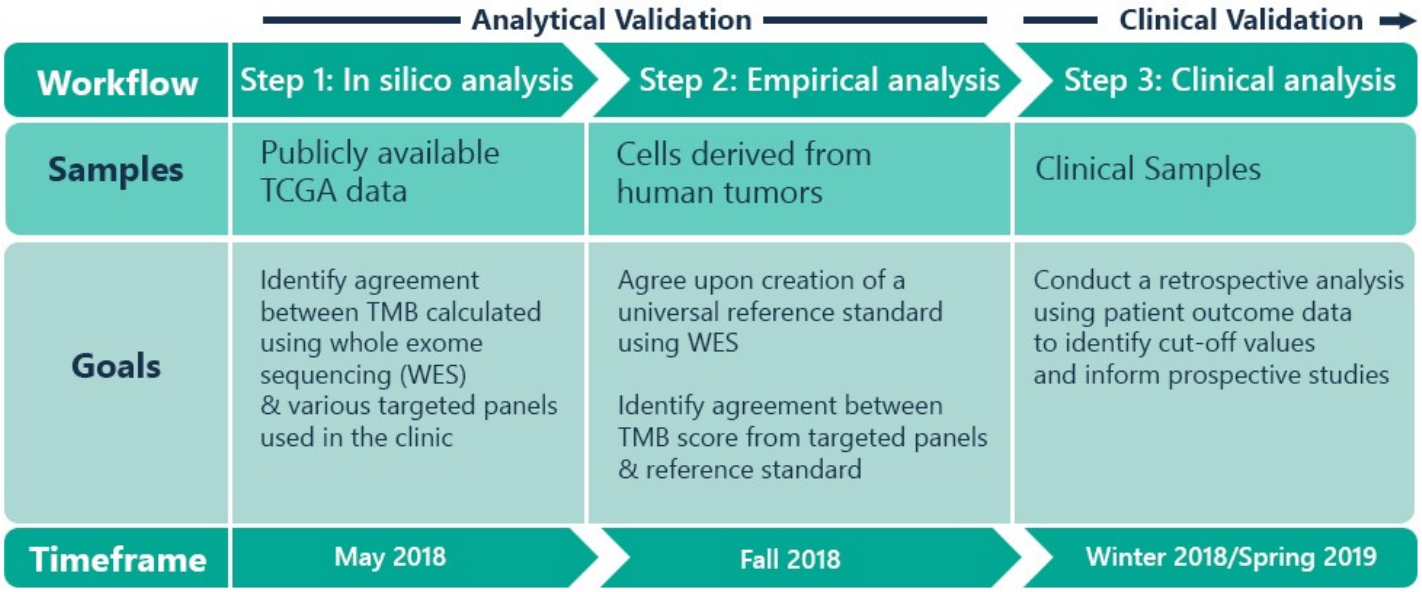
Friends of Cancer Research: TMB Harmonization Effort

Friends of Cancer Research has convened a multi-stakeholder working group to align on and publish universal best practices for defining TMB, analytic validation, and alignment against reference standards.

Participants:

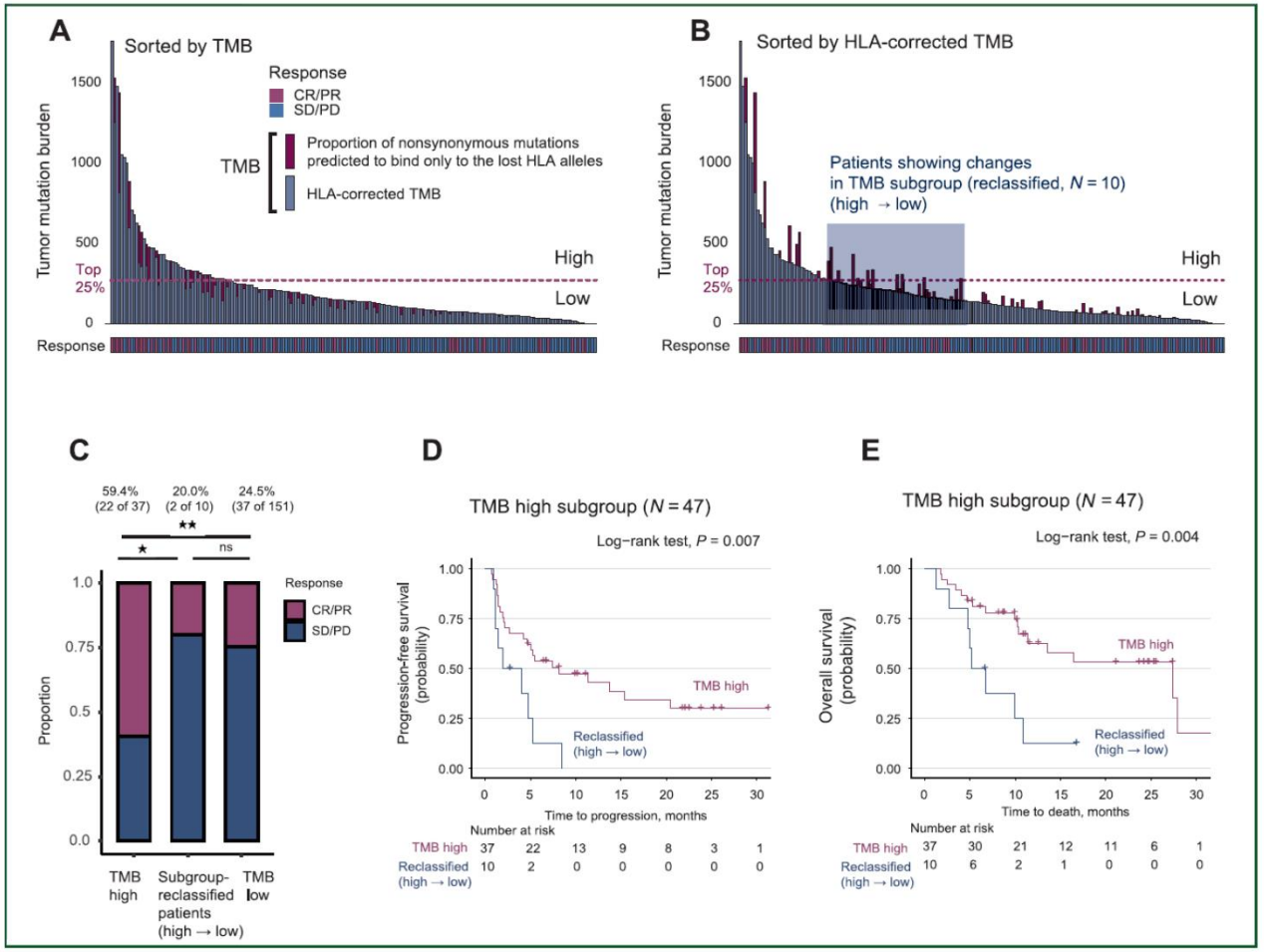
- Seven test developers
- Six pharma companies
- FDA
- NCI
- Academia

TMB Workflow



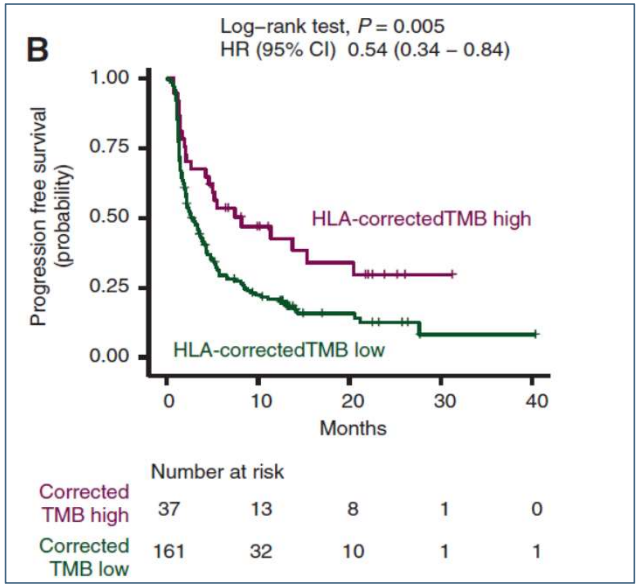
https://www.focr.org/tmb

HLA-corrected TMB:
Impact of HLA-correction on TMB classification (High vs Low)



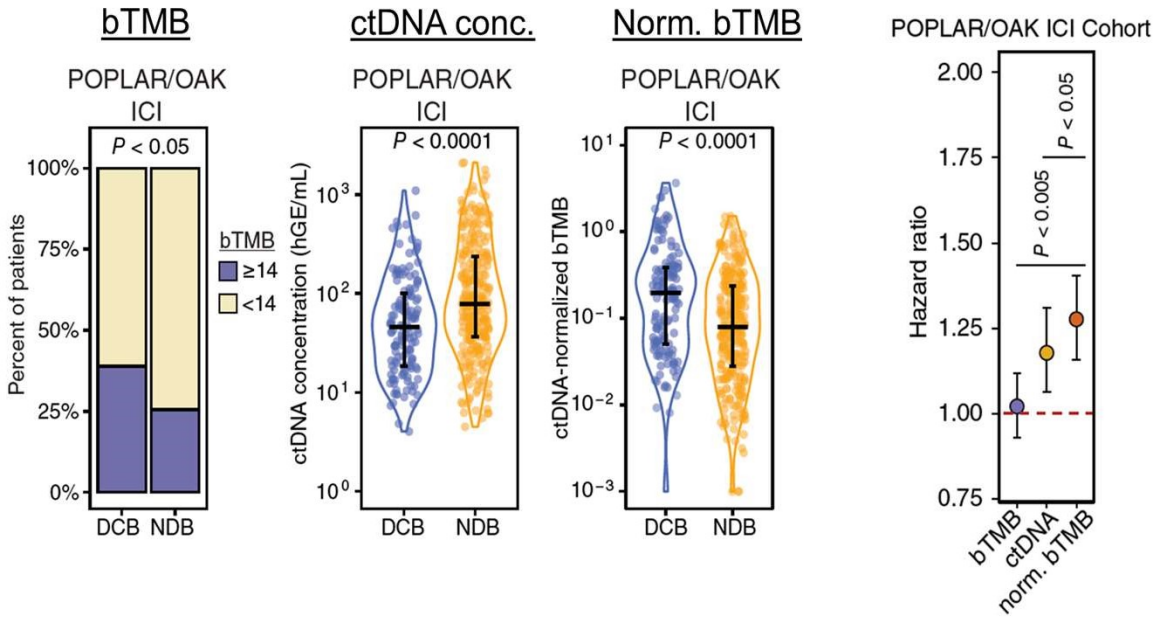
Shin et al: Ann Oncol 2020

- HLA-LOH is present in ~30% of NSCLC cases
- HLA-LOH is associated with increased somatic nonsynonomous mutations
- But HLA-LOH is not associated with increased efficacy of CPIs
- HLA-corrected TMB reclassifies the TMB score by removal of HLA-LOH effects

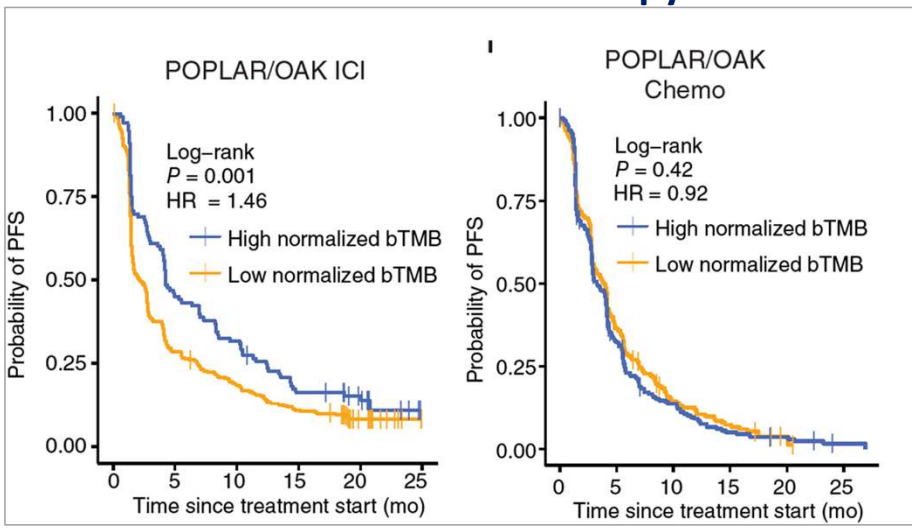


Liquid Biopsy and Checkpoint Immunotherapy (ICI)

Pre-treatment ctDNA features predict ICI outcomes



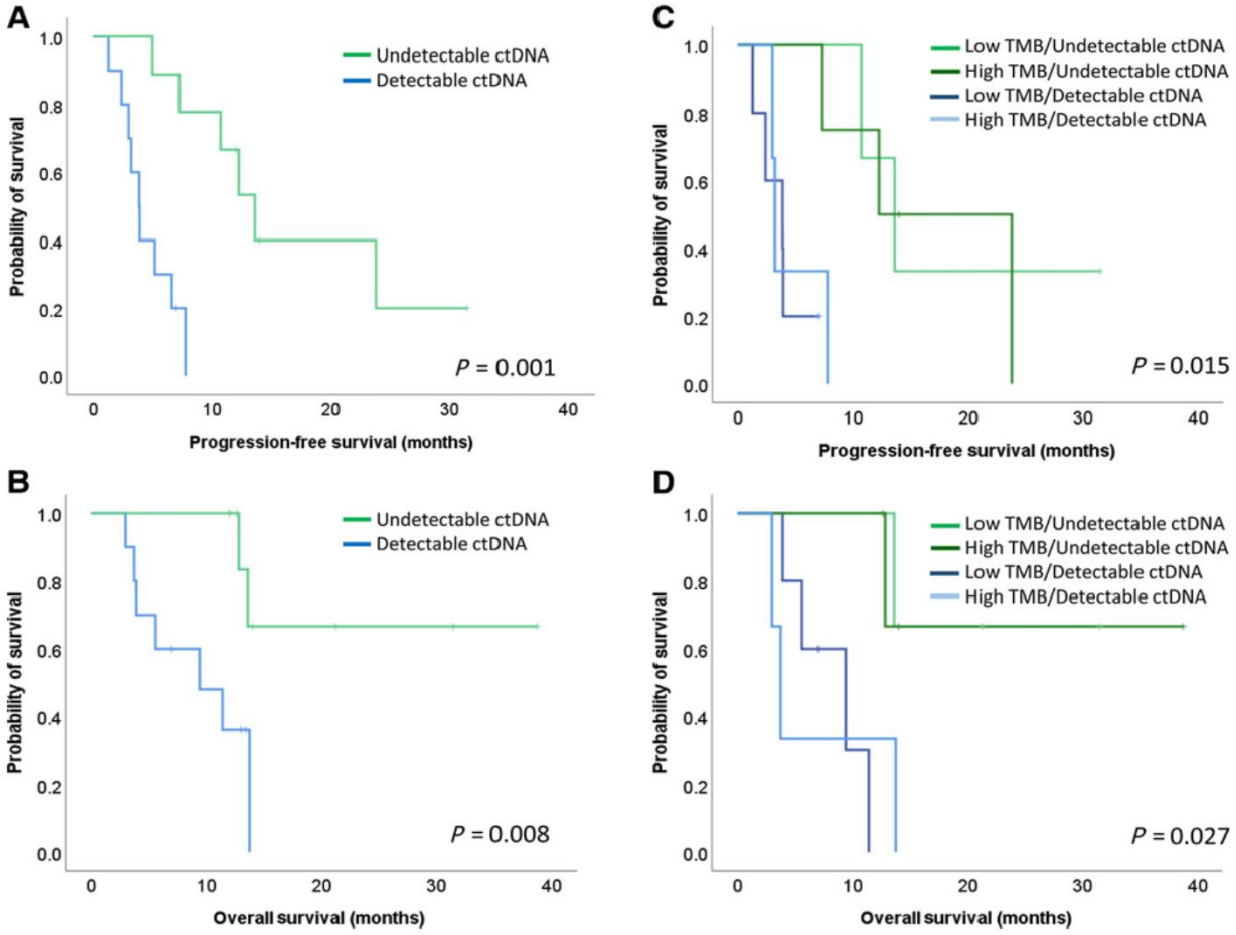
Normalized bTMB predictive for ICI but not chemotherapy



Data from Gandara *et al., Nat. Med.* (2018)

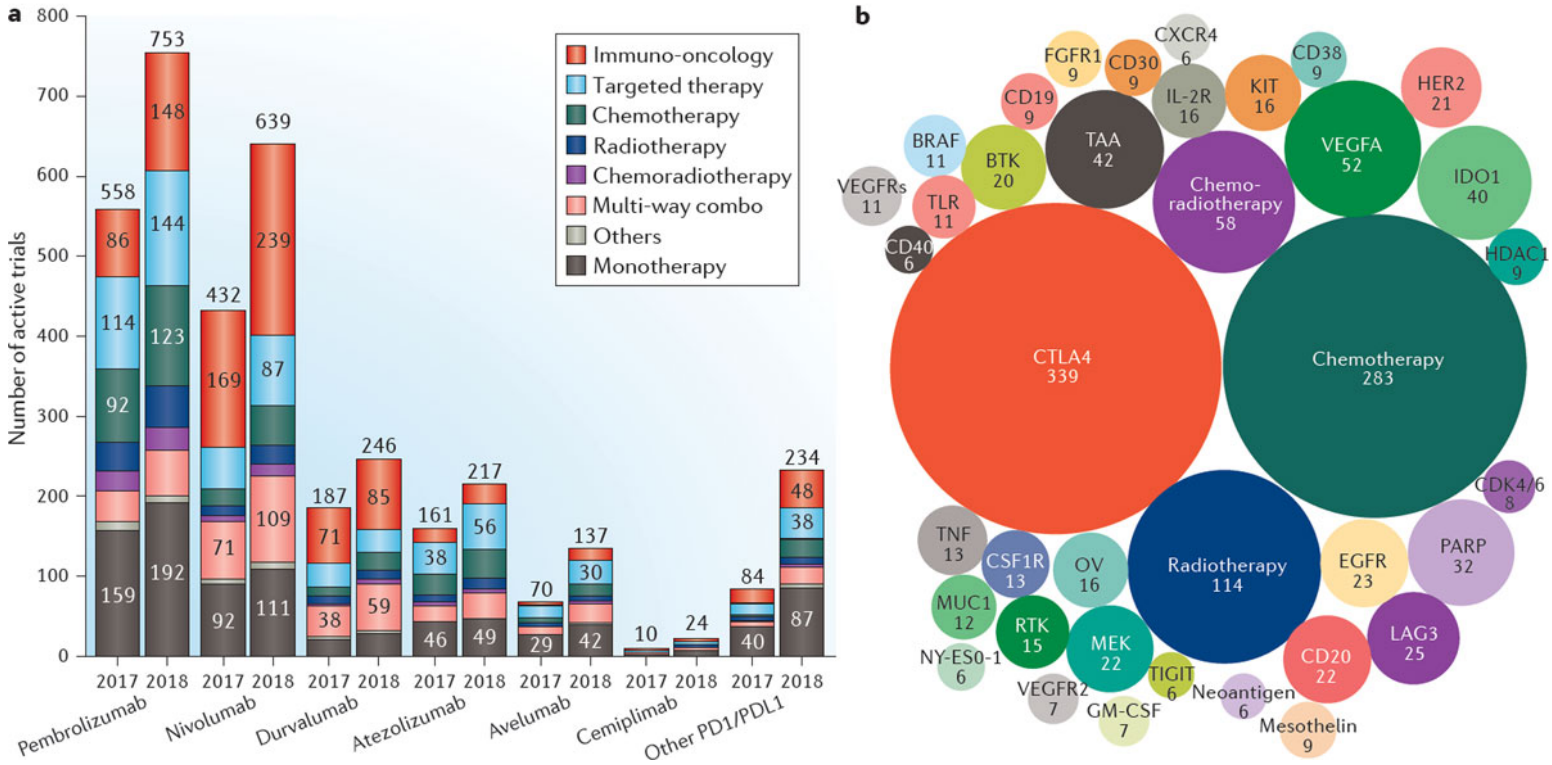
Diehn: IASLC LB Workshop; Nabet & Esfahani *et al., Cell* (2020)

Dynamics of ctDNA following Checkpoint Immunotherapy



Anagnostou et al: Cancer Res 2019

Unmet Need for Predictive Biomarkers in Clinical Trials of Checkpoint Immunotherapy



Over 2,250 clinical trials ongoing as of January 2019 (~3,500 trials as of 8-2020) requiring 380,900 patients
~750 trials in NSCLC

Tang: Nat RD 2018