Immunotherapy for Non-Small Cell Lung Cancer: Integration of Predictive Biomarkers

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Immunotherapy Therapeutic Landscape in Advanced NSCLC: August 2021

Clinical Trial Results of 1st line Checkpoint Immunotherapy in Advanced NSCLC							
Study	**Drug (vs Chemo)**	**PDL1 Selection**	**Line of Tx**	**Control**	**Primary Endpoint**	**HR-Primary Endpoint**	**Result**
KN024	Pembro	≥50%	1st	Plat Chemo	PFS	0.50	Positive
CM026	Nivo	≥5%	1st	Plat Chemo	PFS	1.15	Negative
MYSTIC	Durva or Durva-Tremi	≥25%	1st	Plat Chemo	PFS & OS	NR	Negative
KN189 (Non-SQ)	Pembro-Chemo	≥1%	1st	Plat Chemo	PFS	0.52	Positive
KN042	Pembro	≥1%	1st	Plat Chemo	OS	0.81 for OS 0.69 for 50%	Positive
KN047 (SQ)	Pembro-Chemo	None	1st	Plat-Nab Paclitaxel	PFS & OS	0.64 for OS	Positive
Impower 150 (Non-SQ)	Atezo +Bev/ Pac/Carbo	None	1st	Bev/Pac Carbo	PFS OS	0.71	Positive
Impower 131 (SQ)	Atezo + Nab/Carbo	None	1st	Pac/ Carbo	PFS OS	0.71 (PFS)	Positive
CM227	Nivo or Nivo-Ipi	<1%/1% & TMB≥10	1st	Plat Chemo	PFS & OS	0.58 (in H-TMB)	Positive
IMpower 110	Atezo	≥1%	1st	Plat Chemo	OS in TC3/IC3	0.59	Positive
CM-9LA	Nivo-Ipi-Chemo	None	1st	Plat Chemo	OS	0.66	Positive

1st Line Trials

Test Regimen
CPI Monotherapy
CPI+Chemo
CPI+Chemo+Bev
CPI + CTLA4

Biomarker
None
PD-L1
TMB

Histology
All
Squamous
Non-Squamous

1 Endpoint
PFS
OS
Both

adapted from Gandara: ELCC 2021

Precision/Personalized Medicine in Non-Oncogene-driven NSCLC: Two Different & Shifting Viewpoints (Stereotyped)

Empiric Therapy (Non-Biomarker-Driven) "Lumper" Oncologist

Personalized Therapy (Biomarker-Driven) "Splitter" Oncologist

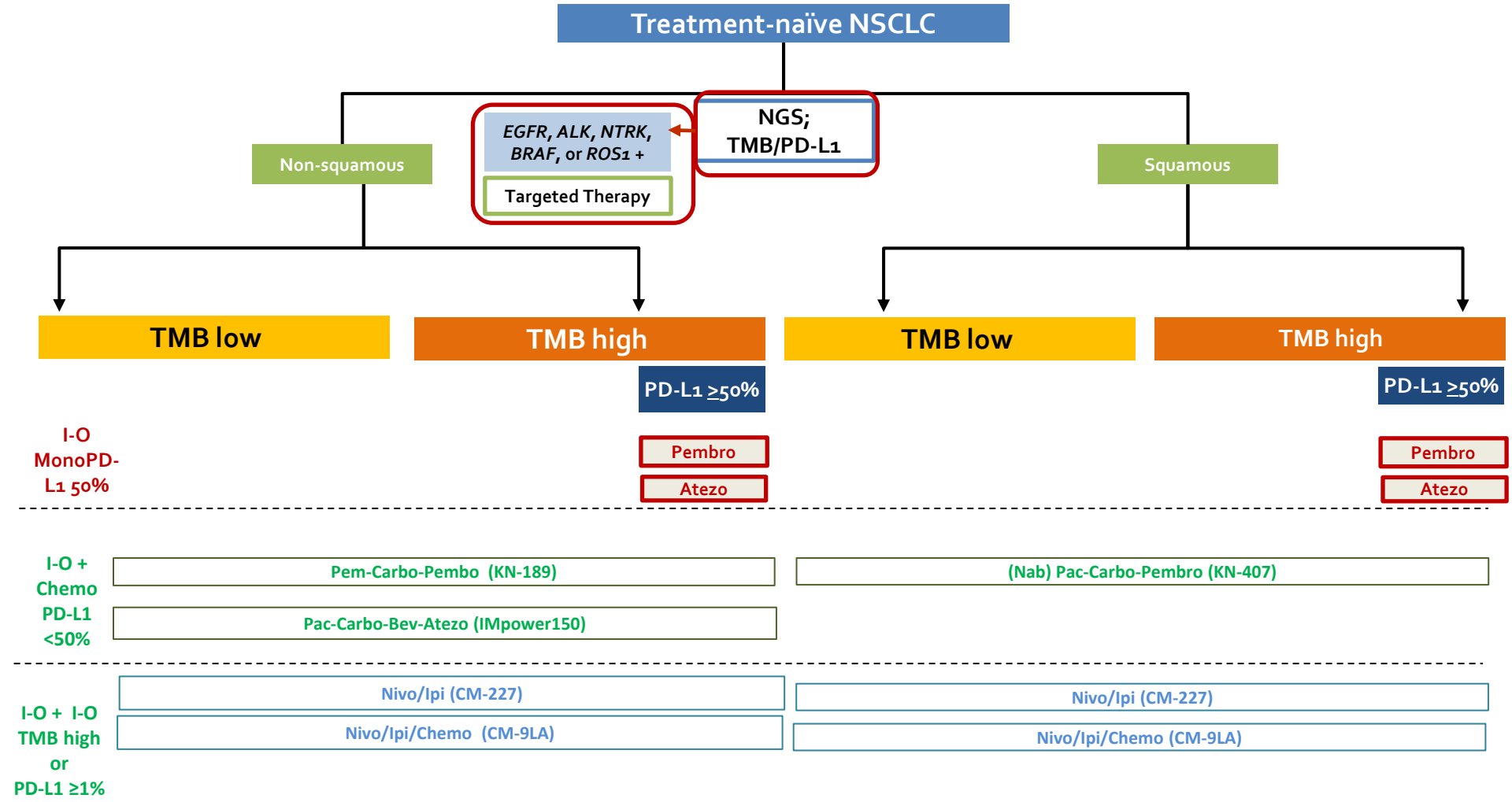
- One regimen for all (more or less)
- Primary goal is to initiate therapy rapidly
- Oncogene testing done "along the way"

- Attempts to individualize therapy for each patient
- Biomarker testing "early & often"
- Awaits initiation of 1st line therapy until oncogene testing returns
- Uses immunotherapy biomarkers (PD-L1 +/-TMB) to select 1st line IO therapy

from Gandara: Precision Medicine Symposium, 2021

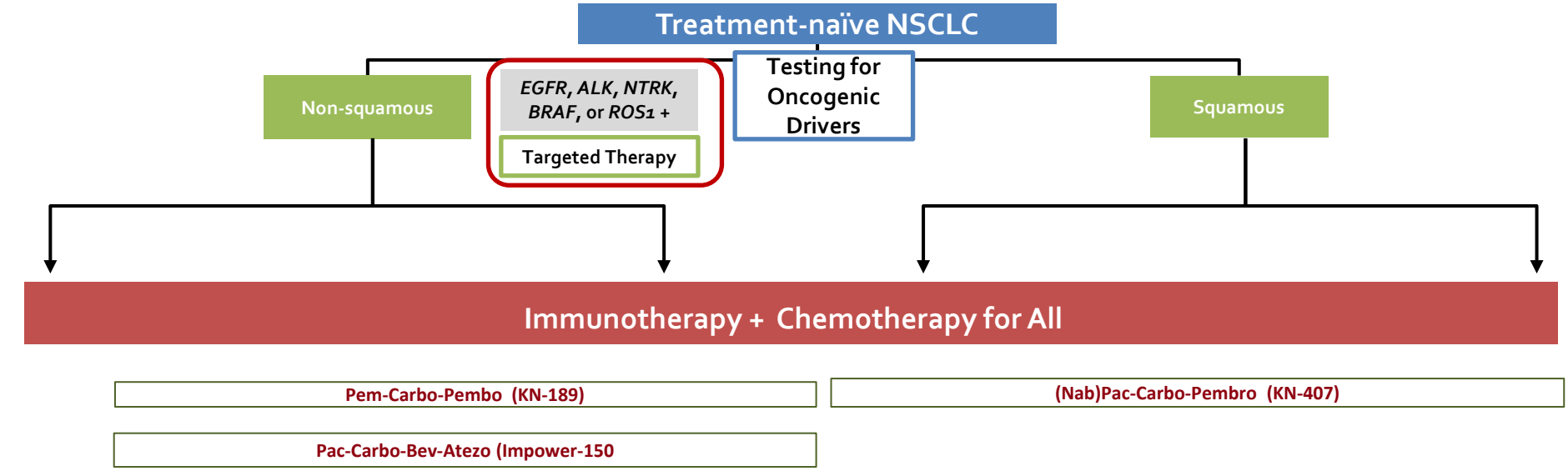
Stage IV NSCLC: Biomarker-driven Therapeutic Landscape

Algorithm of the "Splitter"



Gandara Prec Med 2021. (Adapted from S Peters: ILCC 2020)

Stage IV NSCLC: Biomarker-driven Therapeutic Landscape:
Algorithm of the "Lumper"



Gandara Prec Med 2021. (Adapted from S Peters: ILCC 2020)

Potential Predictive Biomarkers for benefit from Checkpoint Immunotherapy

Tumor Neo-antigenicity

- Biomarkers indicative of hypermutation & neoantigens

Examples:

–TMB, MSI-high, neoantigen load

- *Quantitation & Metrics of plasma ctDNA*

Tumor Microenvironment

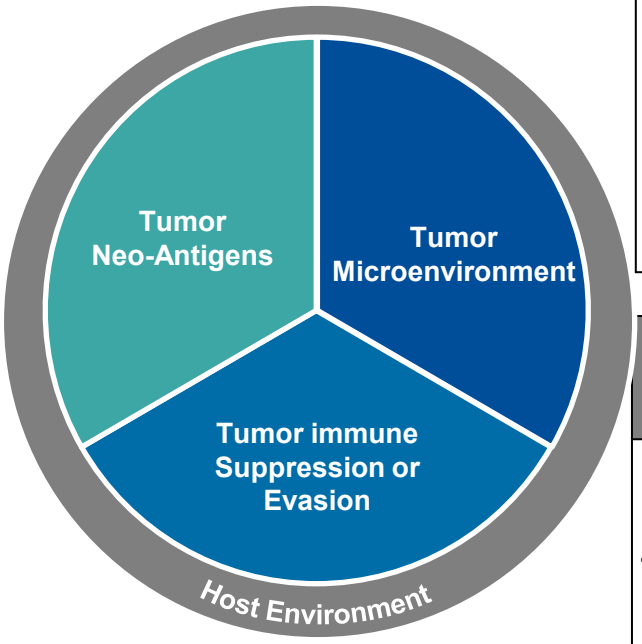
- Biomarkers (intra- or peri-tumoral) indicative of an inflamed phenotype

Examples:

–PD-L1, inflammatory signatures

- Biomarkers of immunoreactivity

Example: Gene Signature (Determa IO)



Tumor Immune Suppression/Evasion

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

Examples:

–Tregs, MDSCs, IDO, LAG-3

–STK11 and KEAP1

–-ARID1A

Host Environment)

- Biomarkers that characterize the host environment, beyond tumor microenvironment

Examples:

–Microbiome, germline genetics

Adapted from Blank CU, et al. *Science* 2016

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

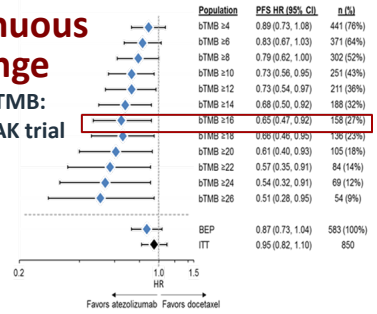
- **Which Biomarker(s)?**
  - PD-L1 IHC
  - TMB
  - PD-L1 IHC + TMB
  - PD-L1 + TMB + Other
  - Other (STK11, KEAP1)

- 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

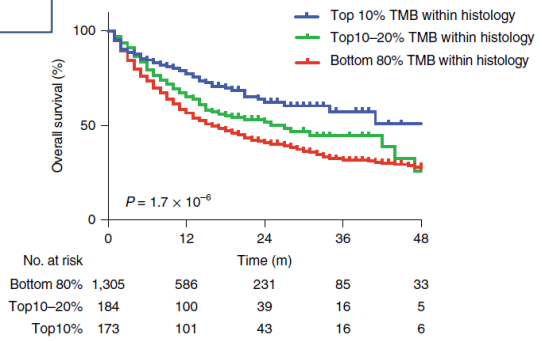
Blood TMB:
PFS in OAK trial



Gandara et al: NatMed 2018

As a Binary Variable

TMB highest 10-20% across Tumor Types



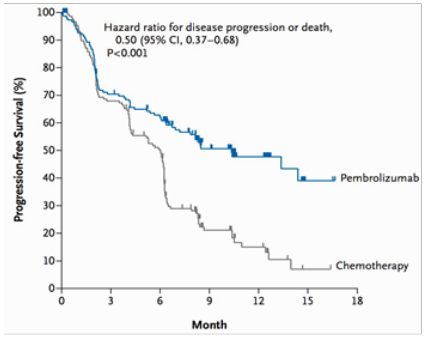
Samstein et al: NatGen 2018

- **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

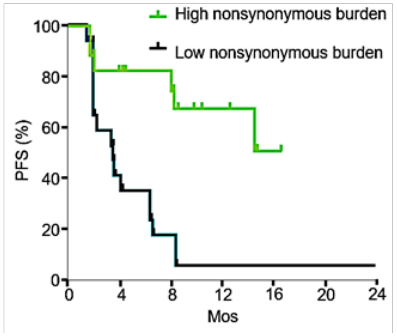
→ Chemotherapy likely "agnostic" to immuno-biomarker .
"Dilutes out predictive value

Selected Biomarkers associated with Checkpoint Immunotherapy efficacy in NSCLC

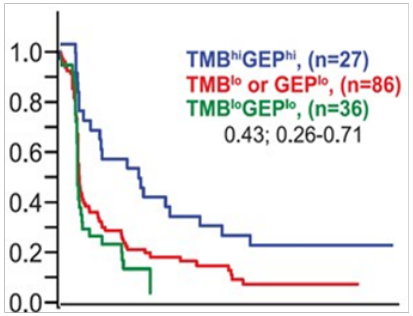
PD-L1 tumor IHC (22c3)[1]



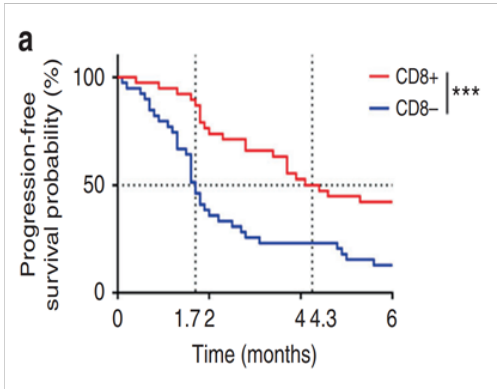
Tumor mutational burden (TMB) [2]



Gene expression signature (GEP)[3]



Tumor infiltrating lymphocytes (TILs)[14]

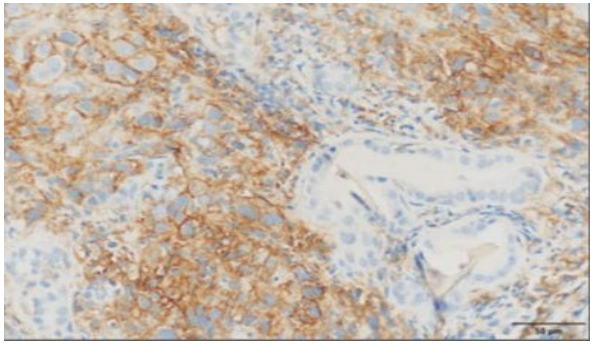


1. Reck et al. 2016, NEJM; 2. Rizvi NA, et al. 2015, Science; 3. Cristescu et al. 2018, Science; 4. Fumet et al., 2018 BJC

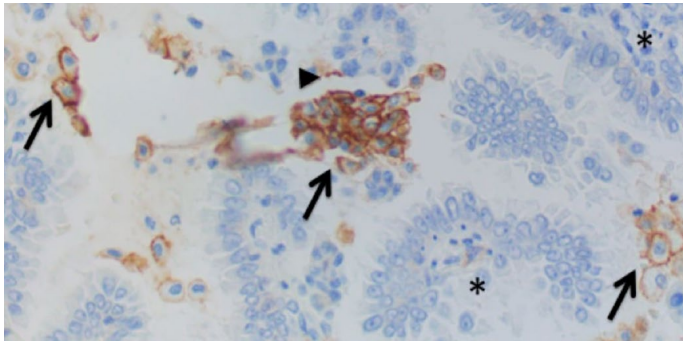
PD-L1 expression & response to PD-1/PD-L1 blockade across multiple assays, drugs & tumor types

	Nivolumab Solid Tumors (Topalian et al. NEJM 2012)	Nivolumab Melanoma (Weber ASCO 2013)	Nivolumab Melanoma (Grosso et al. ASCO 2013)	MPDL3280A Solid Tumors (Herbst et al. ASCO 2013)	MPDL3280A Melanoma (Hamid et al. ASCO 2013)	MPDL3280A NSCLC (Soria et al. ECC 2013)	Pembrolizumab Melanoma (Daud et al. AACR 2014)	Pembrolizumab NSCLC (Gandhi et al. AACR 2014)	MPDL3280A Bladder (Powles et al. ASCO 2014)	Pembrolizumab Head & Neck (Seiwert et al. ASCO 2014)	Pembrolizumab Melanoma (Ribas et al. ASCO 2014)
Patient number	42	44	34	94	30	53	113	129	65	55	411
Response Rates											
Unselected	21%	32%	29%	22%	23%	23%	40%	19%	26%	18%	40%
PD-L1 +	36%	67%	44%	39%	27%	46%	49%	37%	43%	46%	49%
PD-L1 -	0%	19%	17%	13%	20%	15%	13%	11%	11%	11%	13%

PD-L1 expression by TPS vs CPS



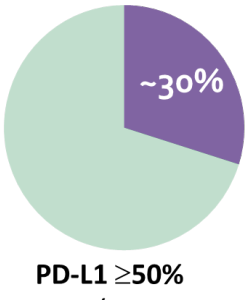
TPS >50% (TC)



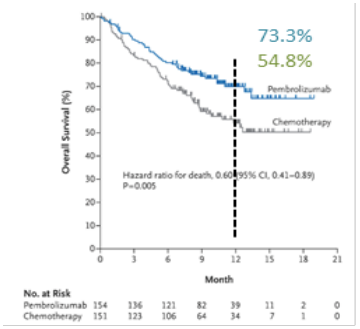
CPS 30% (TC=0, IC=30%)

Callahan: ASCO 2014; Marchi et al. J Clin Path 2020

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



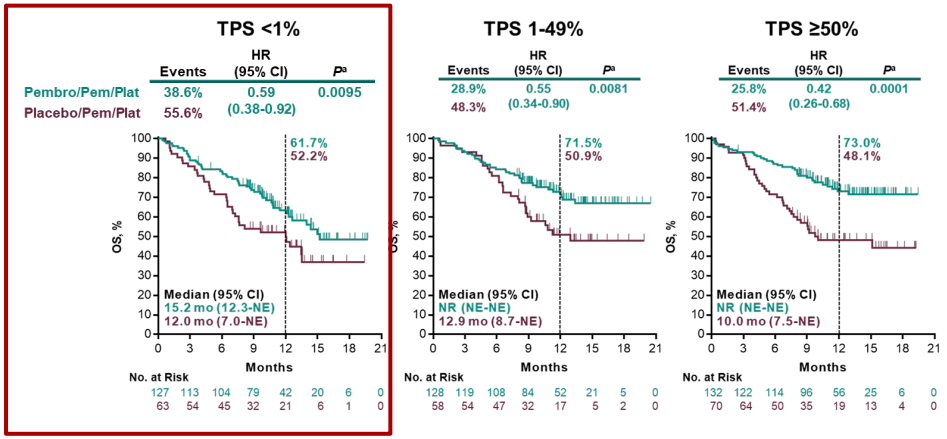
KeyNote 24: Pembro Monotherapy (OS by TPS ≥50%)



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

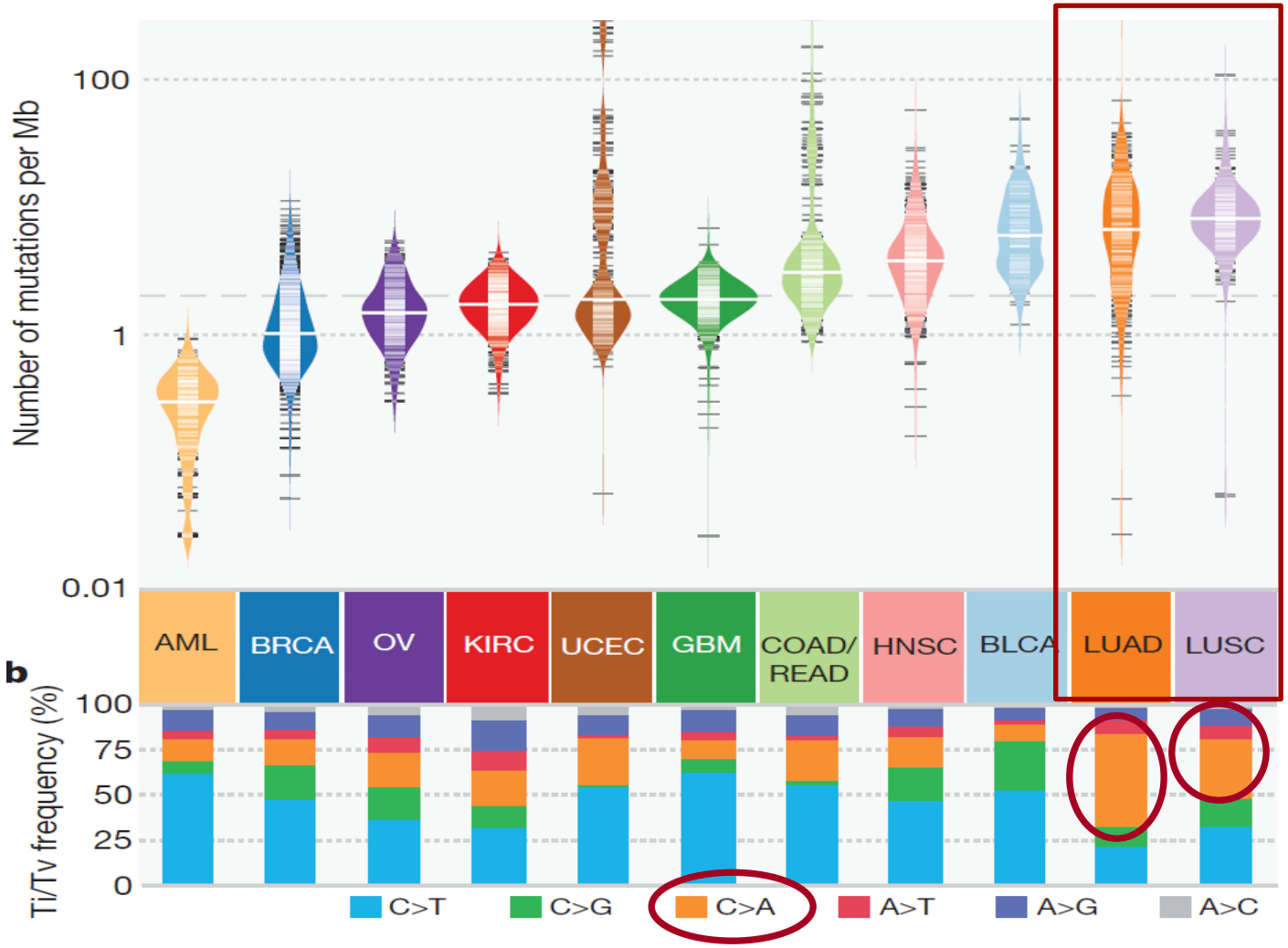
Reck et al. NEJM 2016; 275:1823-1833

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



Gandhi et al. NEJM 2016

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



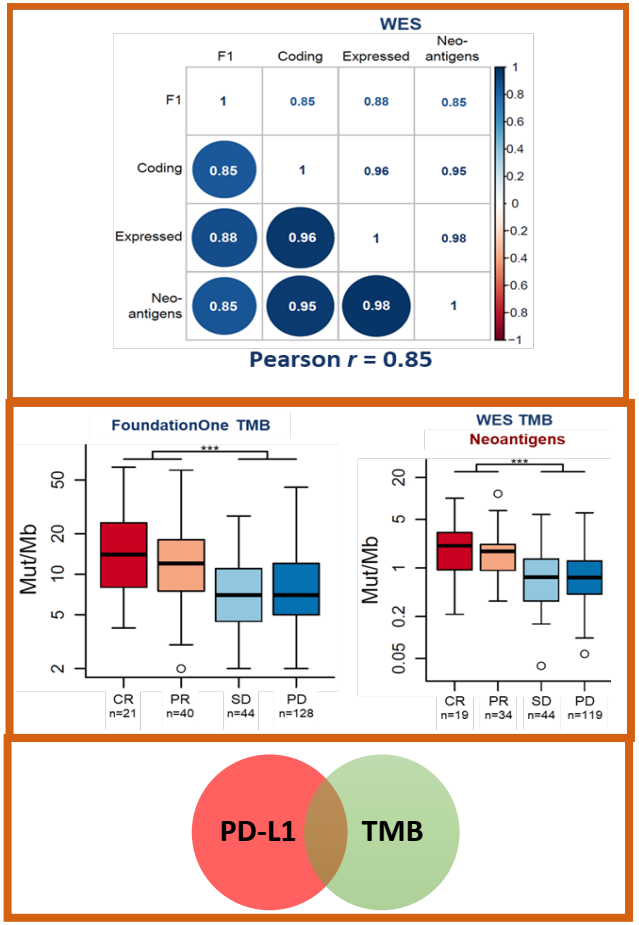
C>A Transversions

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

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

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]



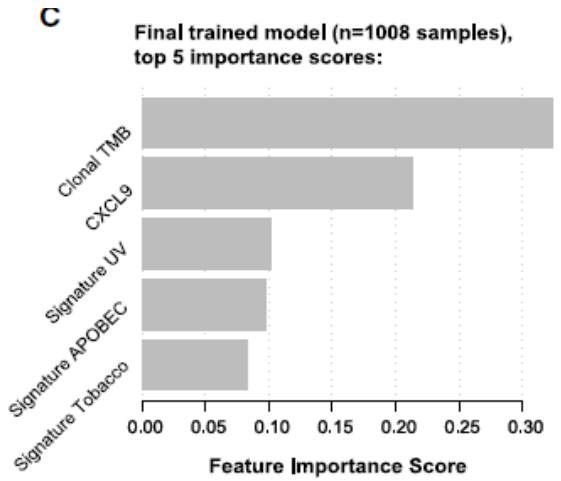
From Gandara, LeGrand et al: ASCO 2018

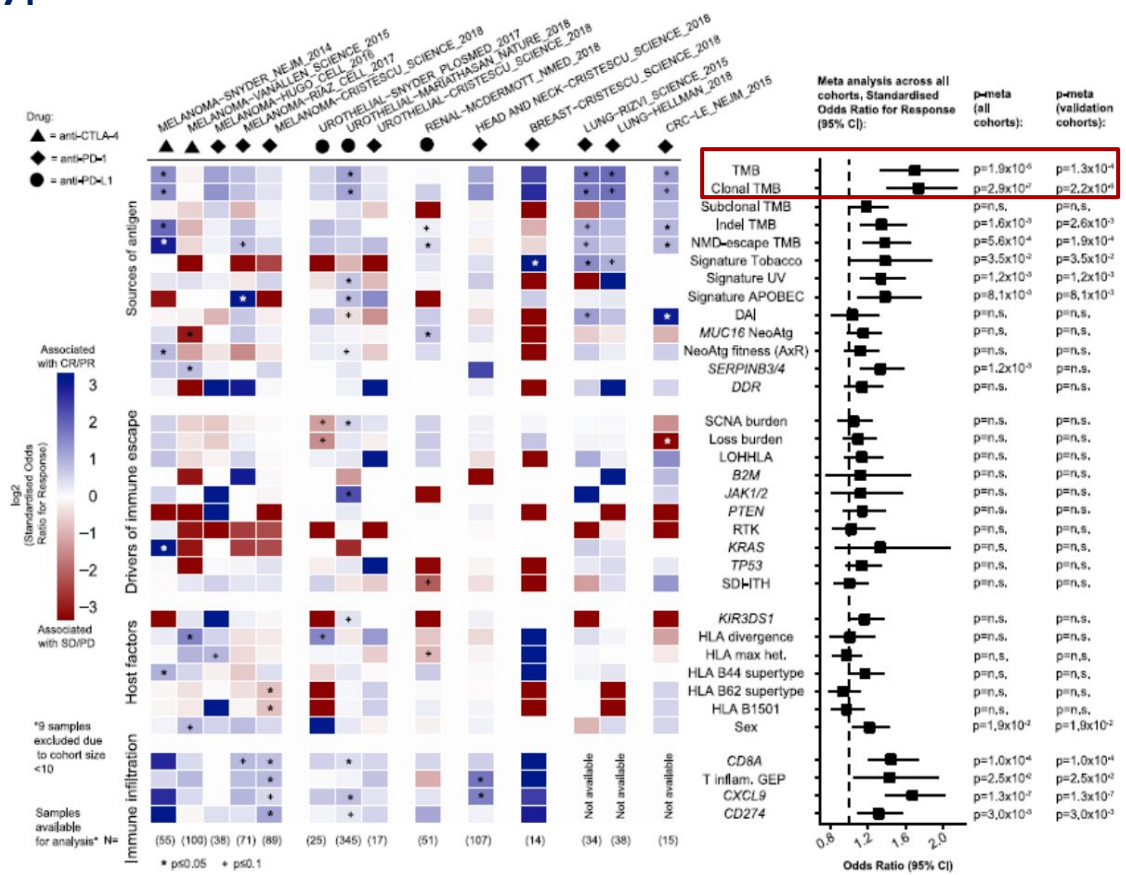
IHC, immunohistochemistry; PD-L1, programmed death-ligand 1; TMB, tumor mutational burden.

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.

Pan-cancer Outcomes in >1,000 patients treated with Checkpoint Immunotherapy (CPI) by WES & Transcriptome analysis

- **Clonal TMB** is strongest predictor of CPI response. OR for CR/PR: 1.74 [1.41–2.15]
- **Total TMB:** OR for CR/PR: 1.70 [1.33–2.17]
- **Sub-clonal TMB** not associated with CPI response: OR for CR/PR 1.18 [0.99–1.41]
- A **Multivariable Predictor** adding CXCL9/CXCL13 expression, 9q34 loss & CCND1 amplification improves TMB as a predictor of CPI response

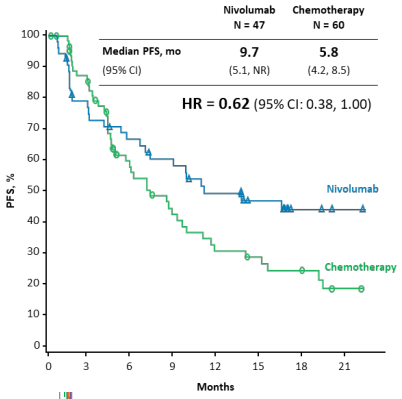


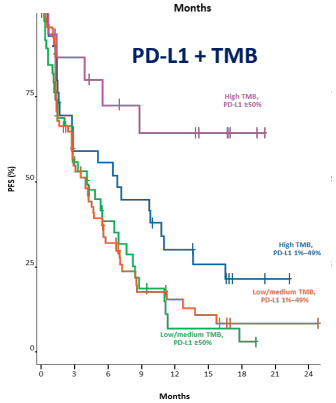


Lichtfield, Swanton et al. Cell, 2021

Regardless of Methodology, High Tissue TMB is associated with increased efficacy of Checkpoint Inhibitor Monotherapy in Advanced NSCLC

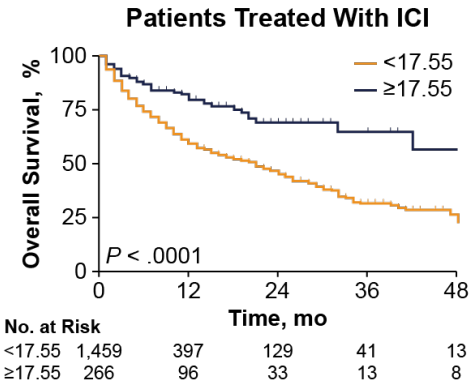
WES: CM-026 NSCLC (Nivo -high TMB)

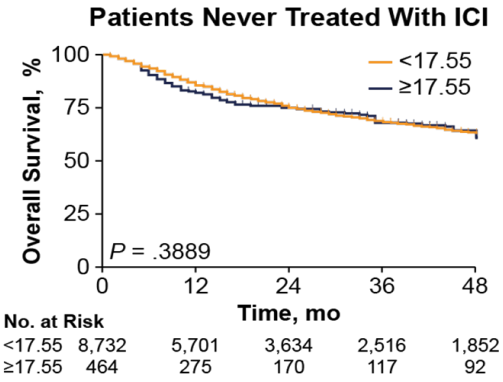




Carbone et al: NEJM 2017

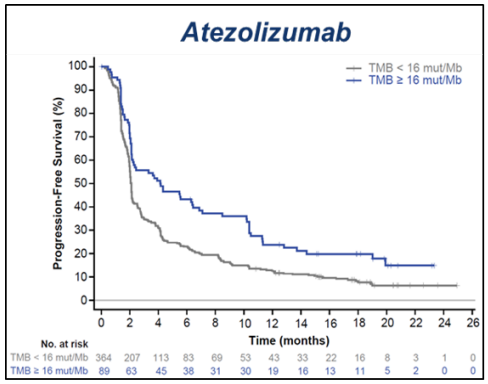
NGS -IMPACT: Multiple Tumor Types

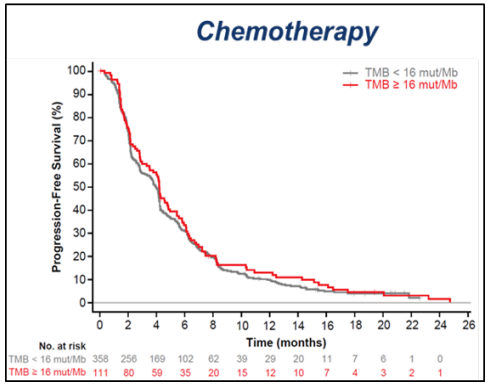




Samstein et al: NatGen 2019

NGS Foundation-One: Multiple Tumor Types





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

Blood collection, plasma isolation & cfDNA extraction

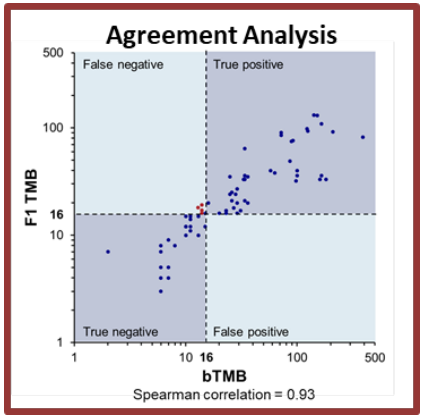
Sequencing

- 394 gene-based NGS assay
- All base substitutions with ≥0.5% allele frequency
- Remove germline polymorphisms & predicted driver mutations

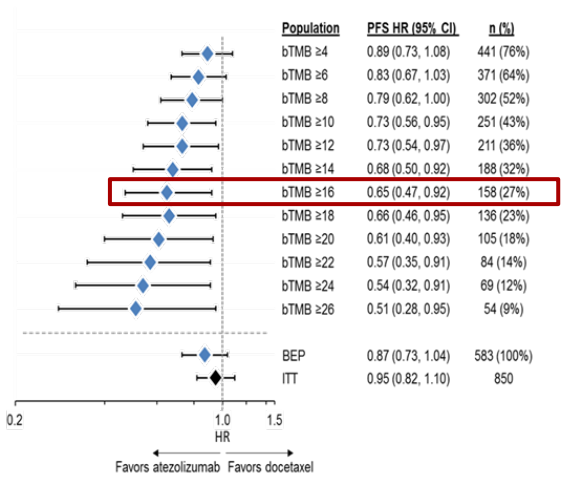
bTMB

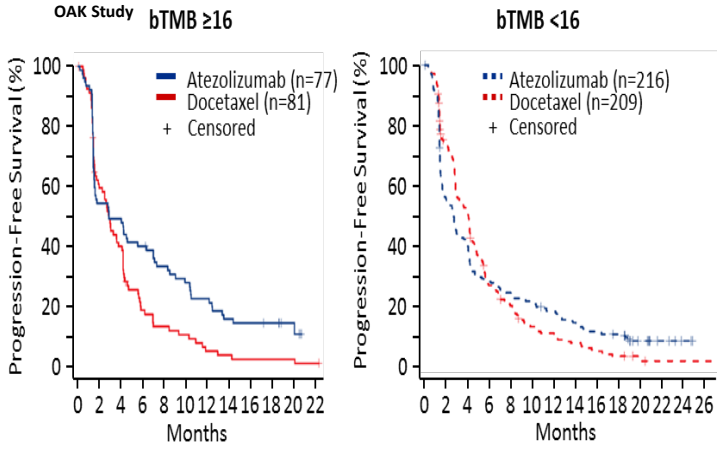
POPLAR (training)
211/273 biomarker evaluable

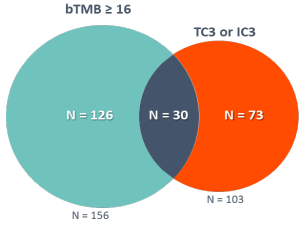
OAK (validation)
583/797 biomarker evaluable



Progression-Free Survival – OAK



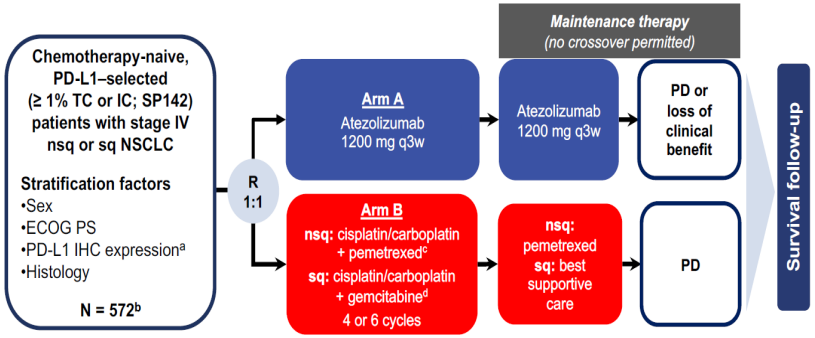


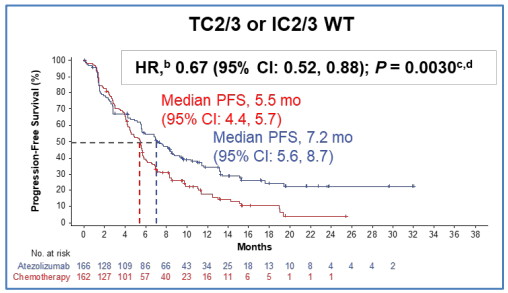


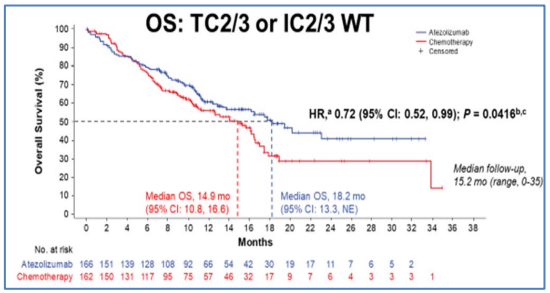
	PFS HR (95% CI)	OS HR (95% CI)
bTMB ≥16	0.64 (0.46, 0.91)	0.64 (0.44, 0.93)
TC3 or IC3	0.62 (0.41, 0.93)	0.44 (0.27, 0.71)
bTMB ≥16 and TC3 or IC3	0.38 (0.17, 0.85)	0.23 (0.09, 0.58)

Gandara DR, et al. Nature Med 2018.

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

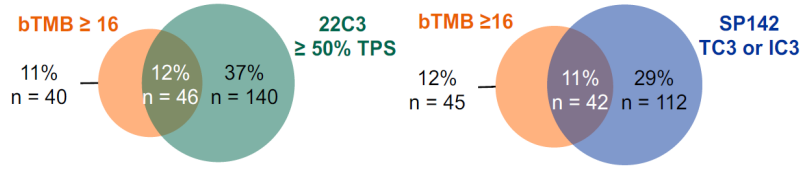






bTMB was assessed by Foundation assay*

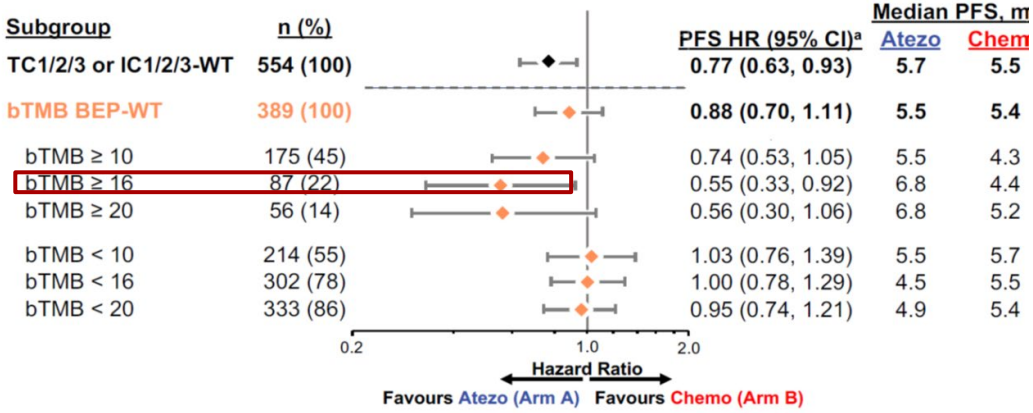
- A bTMB score of 16 is equivalent to 16 mutations/1.1 Mb, or ≈14.5 mutations/Mb
- PD-L1 IHC (SP142 or 22C3) and bTMB identified distinct patient populations in IMpower110



[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.

Herbst et al. IMpower110 Biomarkers https://bit.ly/33XGN7P

PFS in the bTMB BEP-WT in IMpower110



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

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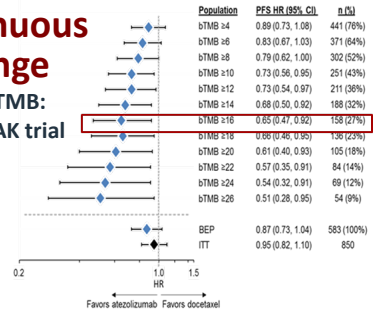
Continuous Range

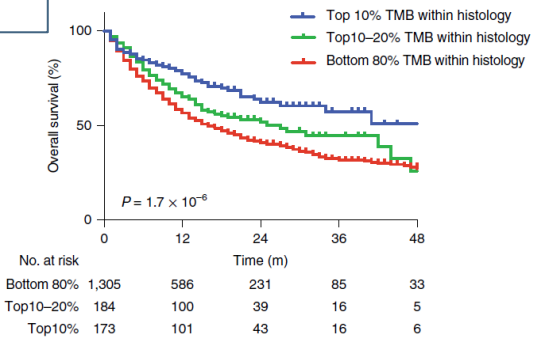
Blood TMB: PFS in OAK trial



Gandara et al: NatMed 2018

As a Binary Variable

TMB highest 10-20% across Tumor Types



Samstein et al: NatGen 2018

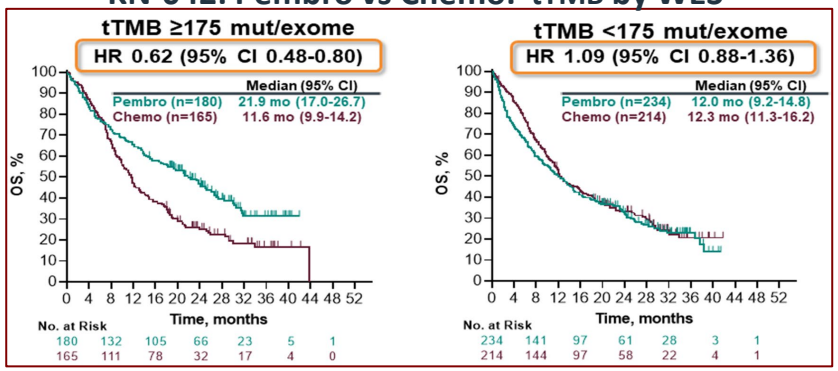
- **What is the context?** (Biomarker for which type of CPI regimen)
  - NSCLC (Squamous or Non-Squamous) vs SCLC
  - CTLA-4 vs PD-1/PD-L1 vs PD-1/PD-L1 + CTLA-4
  - PD-1/PD-L1 + Platinum Chemotherapy

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

TMB in CPI Monotherapy vs CPI + Chemo (or Ipi) Trials in NSCLC

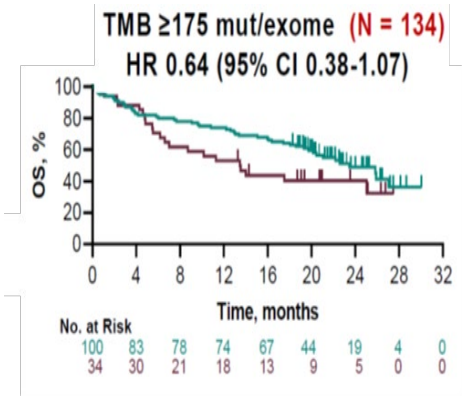
Phase III Trials	Mono- or Combination	TMB	PFS	OS
KN-010	Pembro Mono	**WES-tissue**	√	√
KN-042	Pembro Mono	WES-tissue	√	√
KN-189	Pembro + Chemo	**WES-tissue**	No	No
KN-407	Pembro + Chemo	WES-tissue	No	No
CM-227	Nivo + Ipi	Fone-tissue	√	No
S1400i (LungMAP)	Nivo + Ipi	Fone-tissue	No	√
MYSTIC	Durva + Treme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

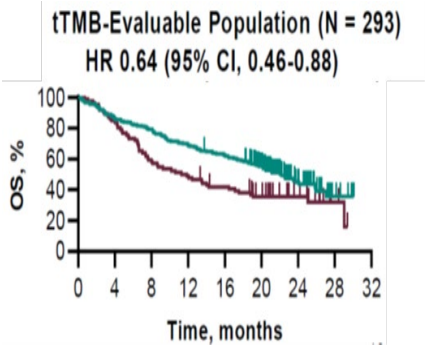
KN-042: Pembro vs Chemo: tTMB by WES



Herbst: ESMO 2019

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



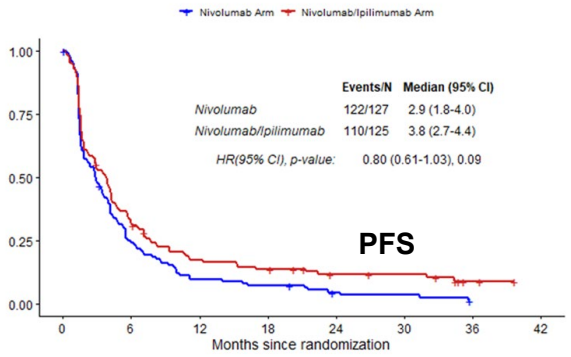


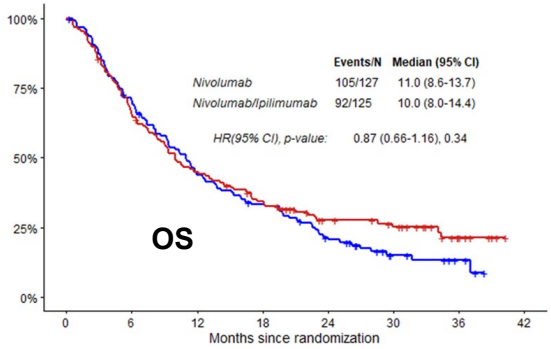
Garissino: ESMO 2019

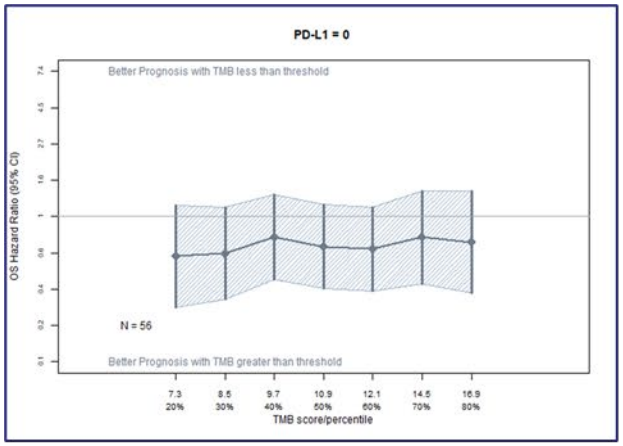
from Gandara: Master Lecture Series 2020

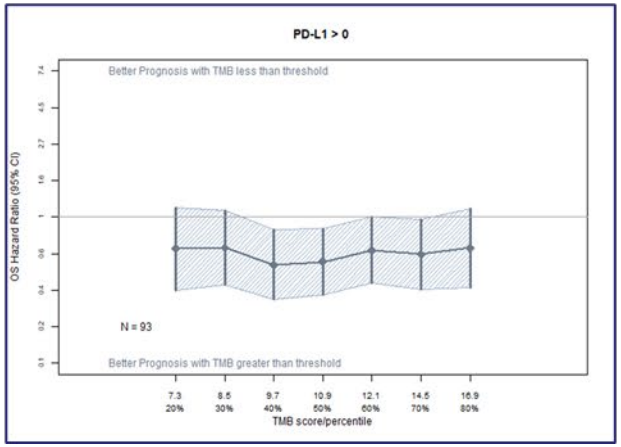
Associations of Tumor Mutational Burden & Combination Index of TMB + PD-L1 in Lung MAP S1400i (Nivo +/-Ipi in 2nd line Squamous)

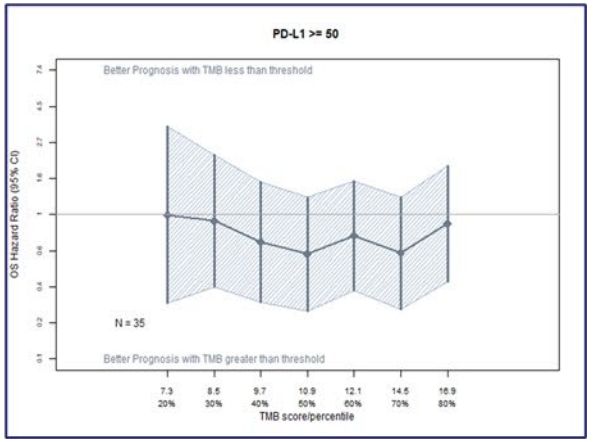
S1400I: Phase III study: Nivolumab + Ipilumumab vs Nivolumab





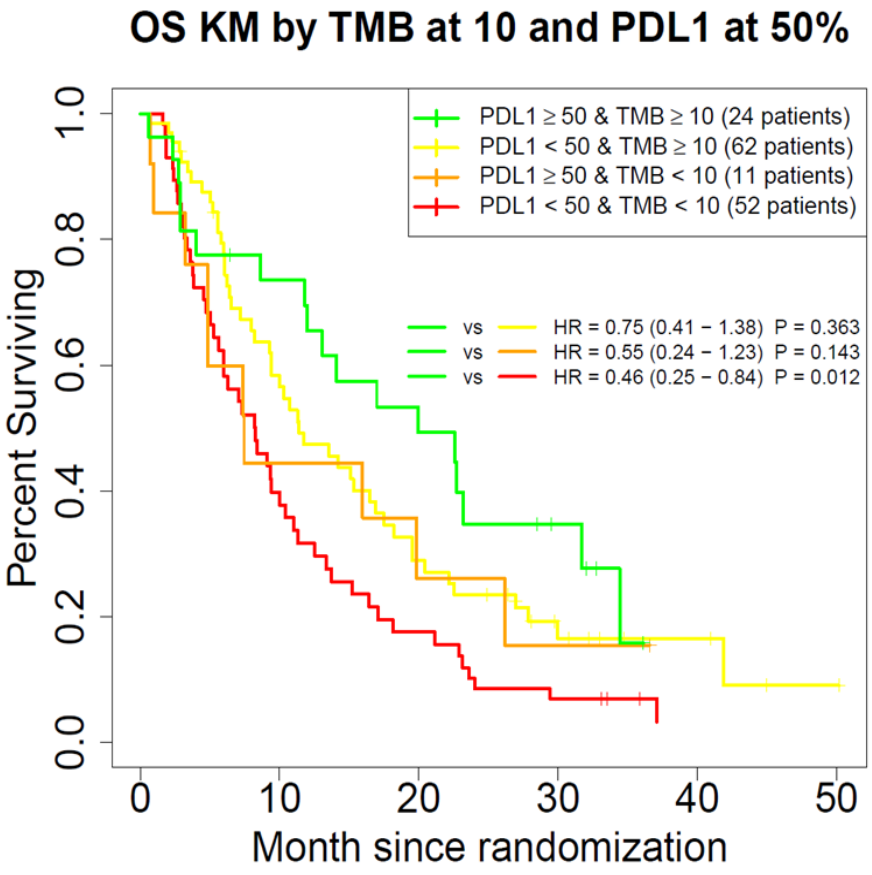


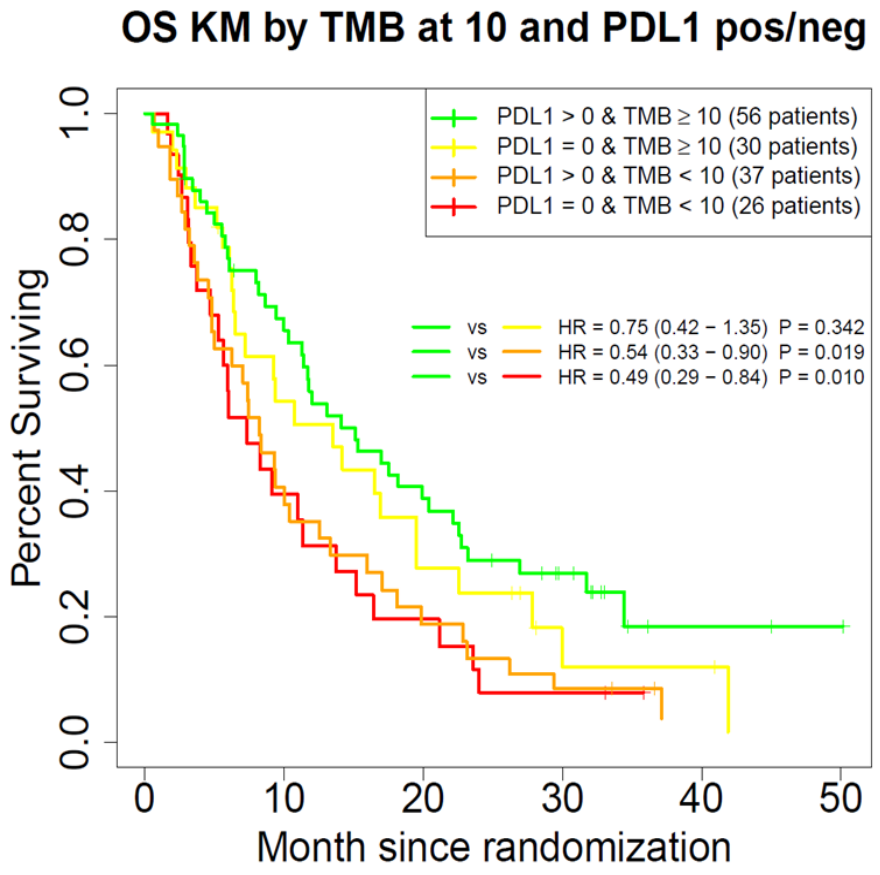




HRs ≤ 1.0 for OS by TMB in all PD-L1 subgroups

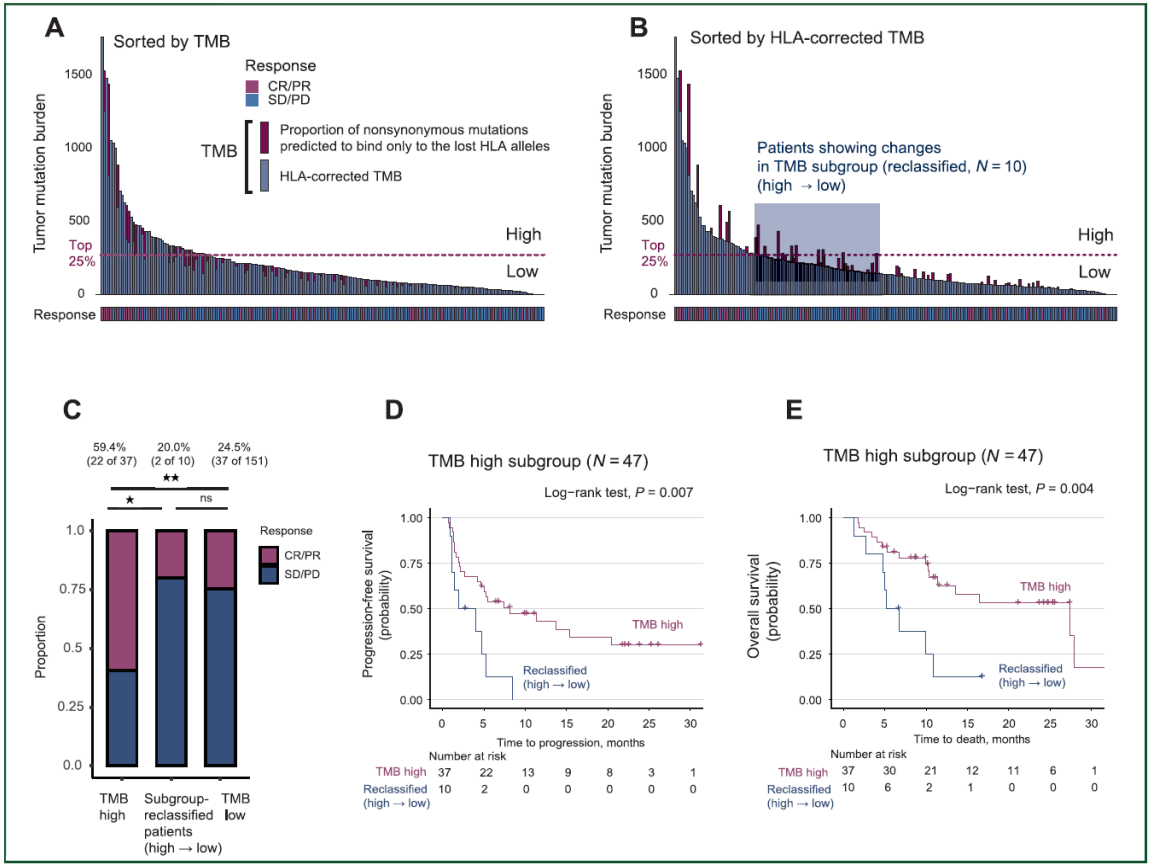
Combination Index of TMB + PD-L1





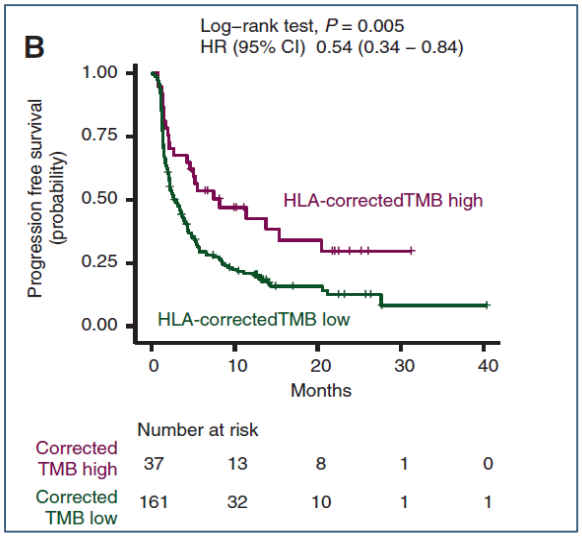
Patient OS was best in patients with Combination Index of TMB-high + PD-L1-high

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



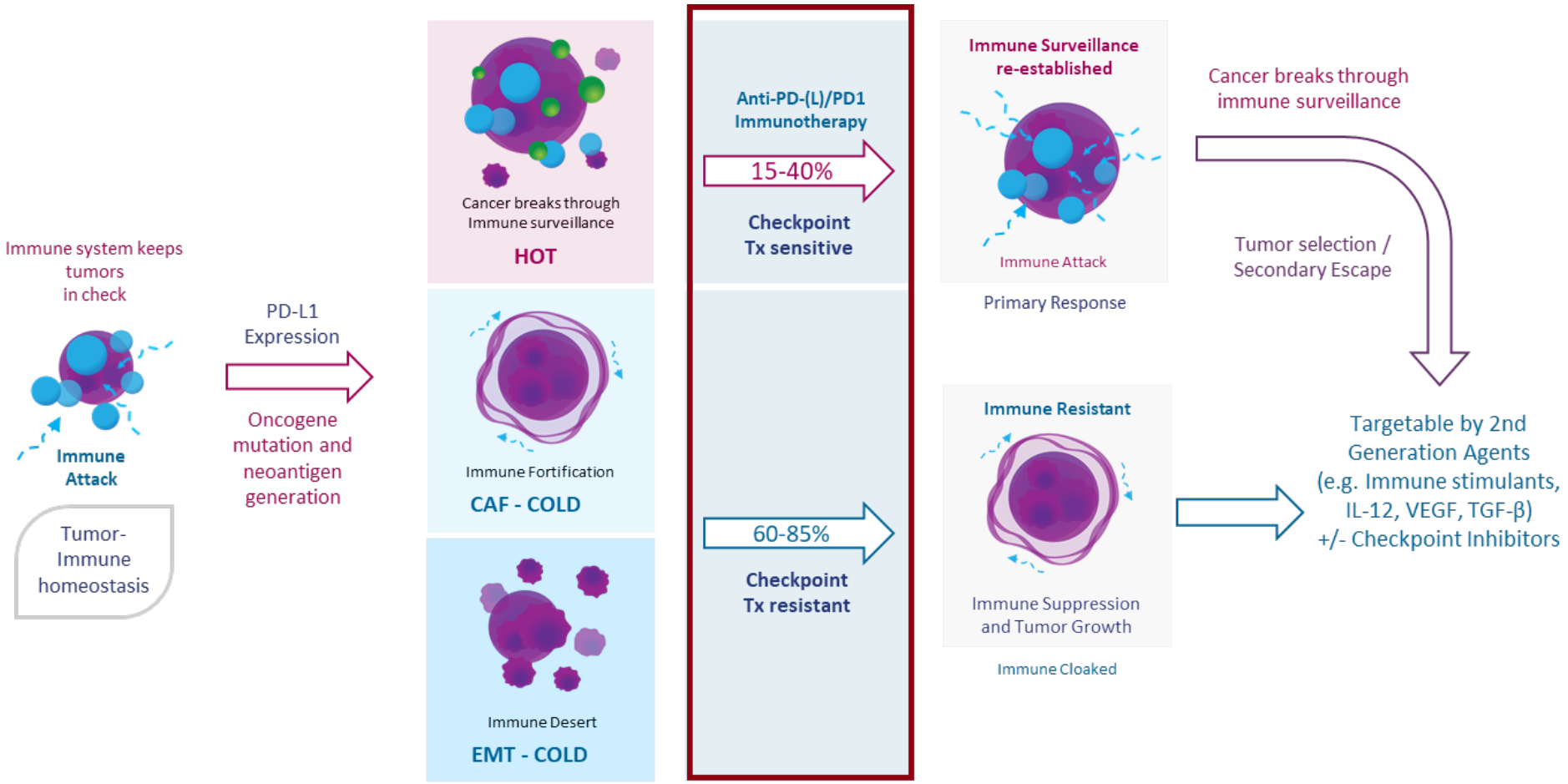
Shin et al: Ann Oncol 2020

- 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



Determa-IO: A Predictive 27 Gene Signature for Checkpoint Immunotherapy Efficacy

American Association for Cancer Research®
FINDING CURES TOGETHER®

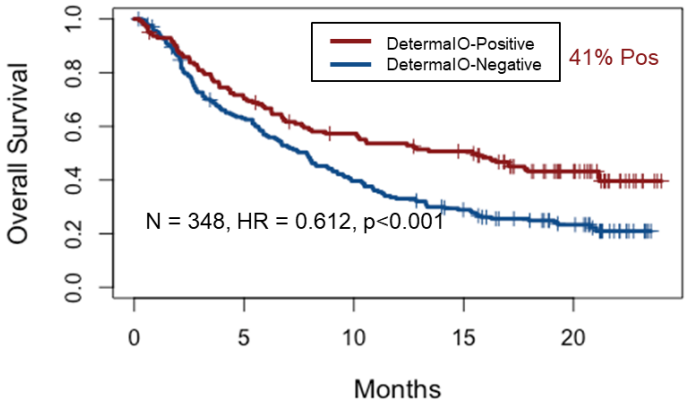


Applying Determa-IO to Bladder cancer IMVigor210 – Results: Primary endpoint (OS)

American Association for Cancer Research®
FINDING CURES TOGETHER®

DetermaIO

27 Gene Predictor

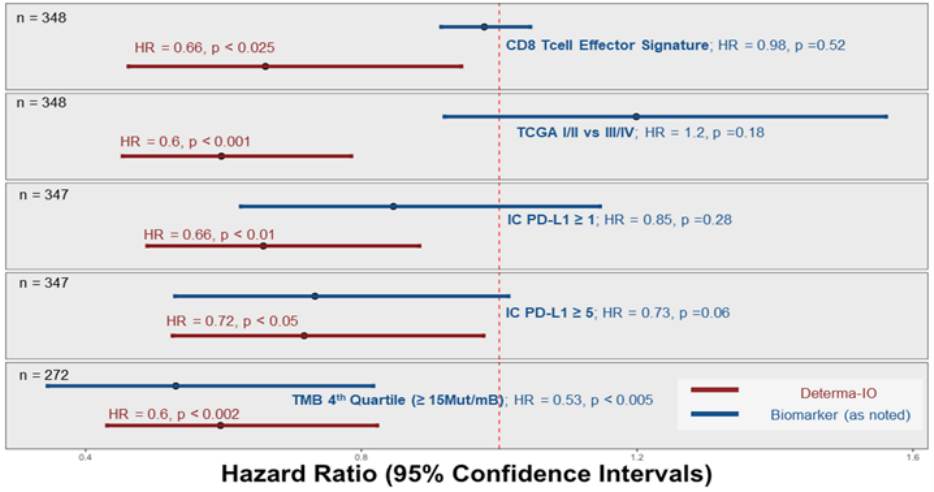


	Median OS (mos)	2 Year OS
DetermaIO-Positive	15.4	39.6%
DetermaIO-Negative	7.9	20.9%

Comparison to Clinical Trial Endpoint:
DetermaIO met Primary Endpoint of IMVigor210 Trial (ORR > 10%):

32% ORR (24 - 41 % CI); Δ10% p < 0.001

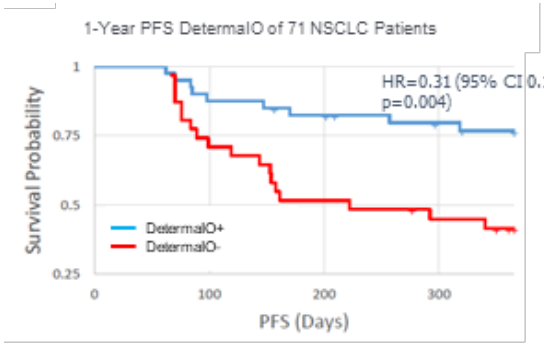
Bivariate Analysis with Various Biomarkers

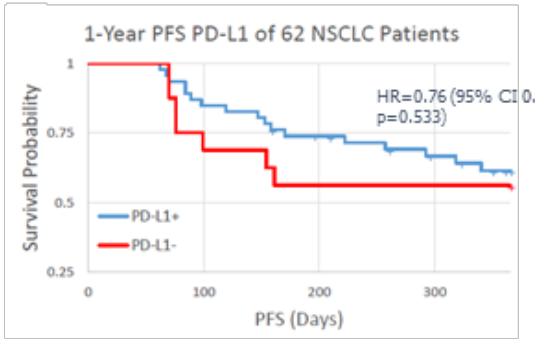


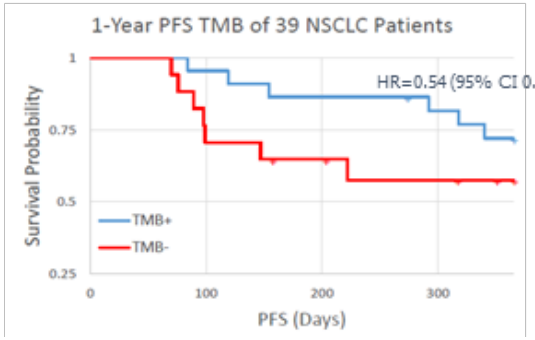
Pilot Study in Advanced NSCLC treated with Checkpoint Immunotherapy: Progression free survival comparing DetermaIO to PD-L1 & TMB analysis

NSCLC (N=71)

Marker	Cases	Neg.	Pos.	Percent Positive	
DetermaIO (-/+)	71	32	39	55%	
PD-L1	66	19	47	71%	1% threshold
TMB	41*	17	24	59%	>10 mutations/MgB







DetermaIO was predictive of Checkpoint Inhibitor treatment outcome (PFS), independent of PD-L1 or TMB scores, demonstrating superiority to both biomarkers

Ranganath et al. SITC 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

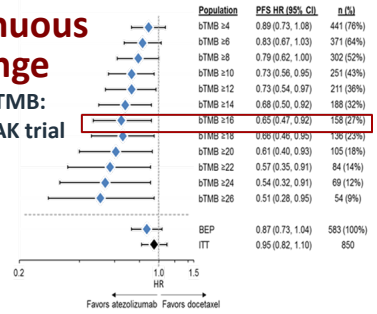
- **Which Biomarker(s)?**
  - PD-L1 IHC
  - TMB
  - PD-L1 IHC + TMB
  - PD-L1 + TMB + Other
  - Other (STK11, KEAP1)

- 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

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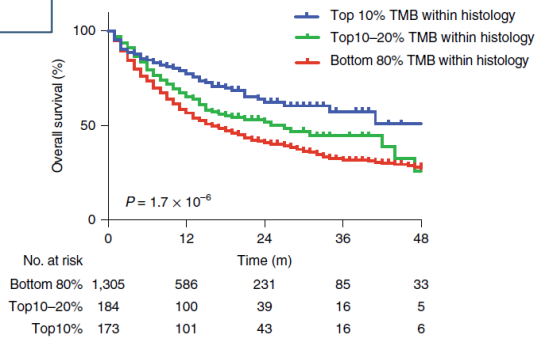

Samstein et al: NatGen 2018

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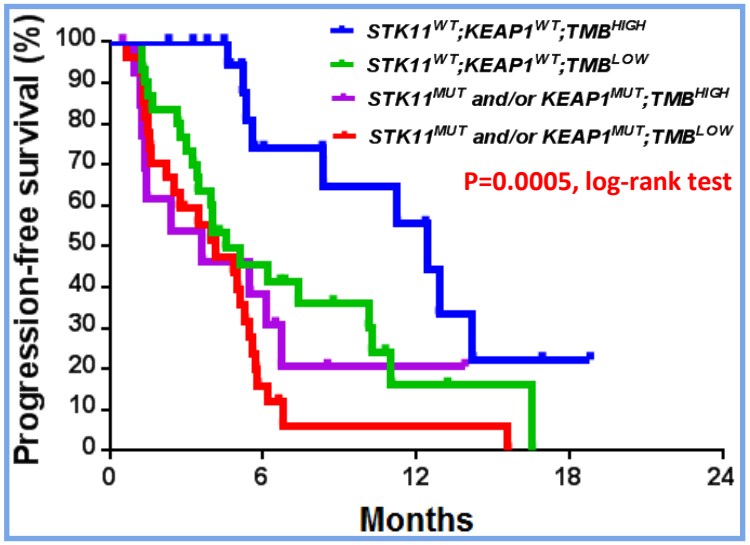
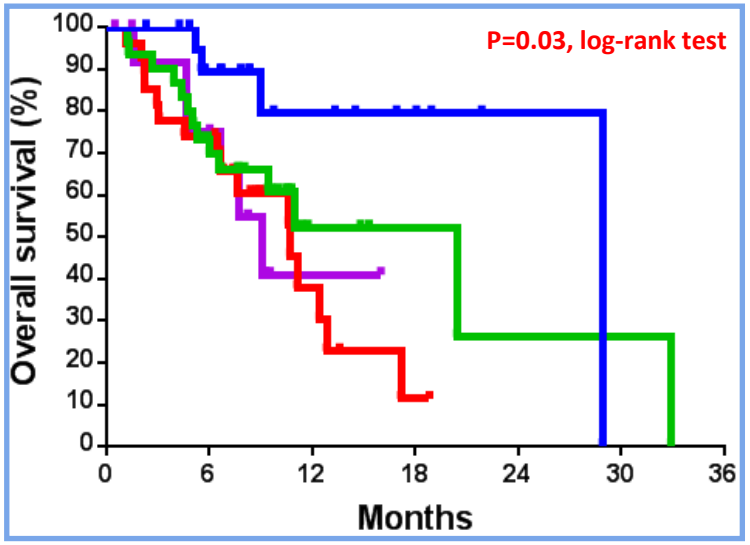
Integration of *STK11* and *KEAP1* genomic alterations with TMB & other biomarkers: Moving towards a composite panel?

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 et al: Cancer Disc 2018; ASCO 2019.

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

PD-L1+ or PD-L1–

- Advanced or metastatic NSCLC
- Patients with EGFR and ALK WT NSCLC
- No prior chemotherapy for recurrent/metastatic NSCLC
- WHO/ECOG PS 0 or 1
- N = 1092

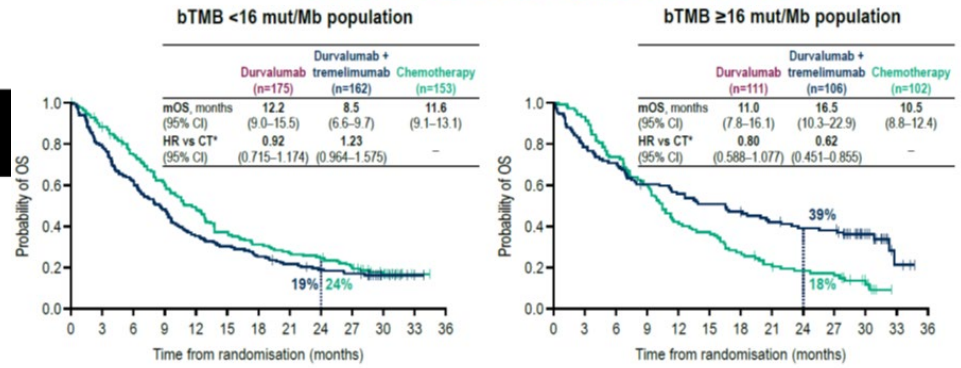
1:1:1

- **Durvalumab + Tremelimumab** (n = 364)
- **Durvalumab monotherapy** (n = 364)
- **SOC (platinum-based doublet chemotherapy)** (n = 364)
  Carboplatin + paclitaxel
  *Carboplatin/cisplatin + gemcitabine
  †Carboplatin/cisplatin + pemetrexed

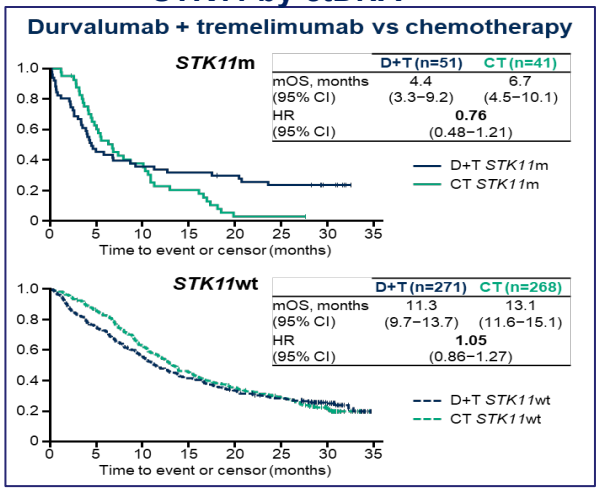
Stratification factors:
1. PD-L1 status
2. Histology: squamous/nonsquamous

Co-primary endpoints: PFS and OS

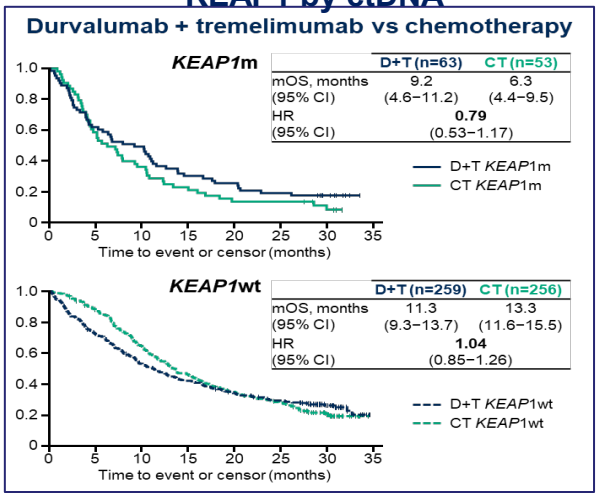
OS: bTMB SUBGROUPS (EXPLORATORY ANALYSIS)



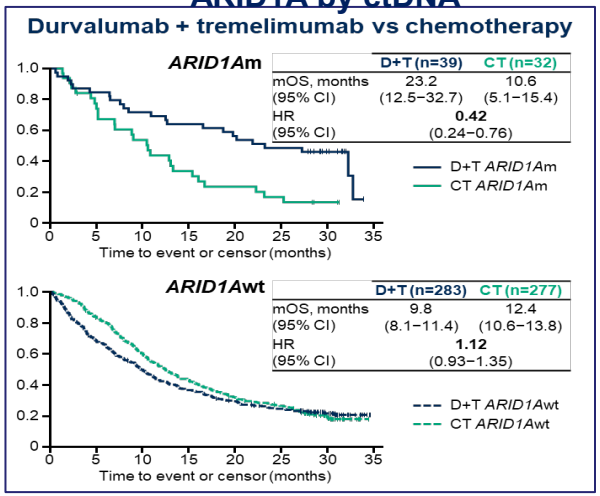
STK11 by ctDNA



KEAP1 by ctDNA



ARID1A by ctDNA

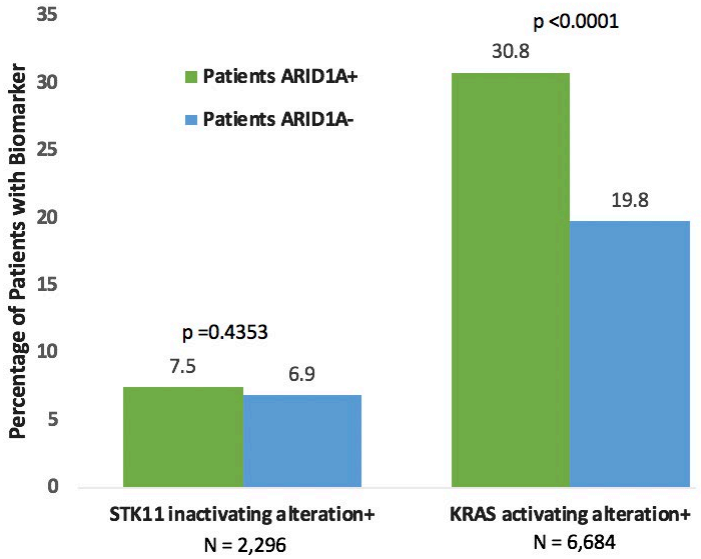


- **STK11 & KEAP1 may be primarily prognostic & not predictive of IO efficacy**
- **ARID1A may be predictive for efficacy of Durva + Treme IO combination**

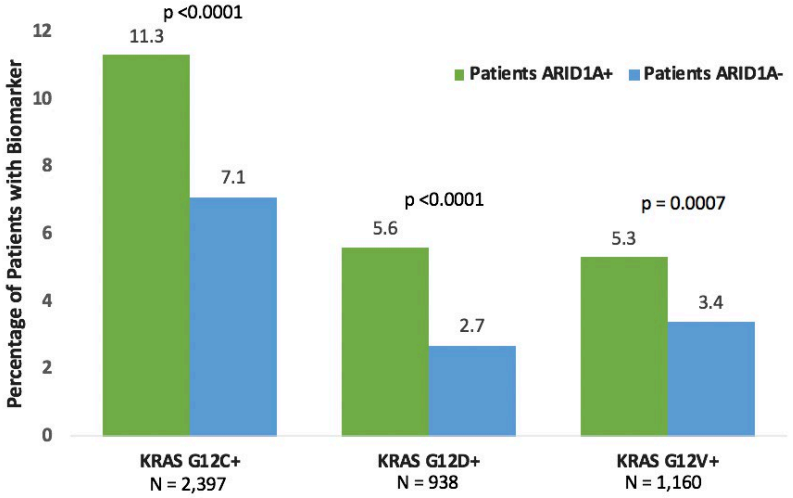
Rizvi et al. JAMA Onc 2020

Analysis of ARID1A Mutations in NSCLC by plasma ctDNA (N=33,086 NSCLC patients; N=3,115 with ARID1A mutations)

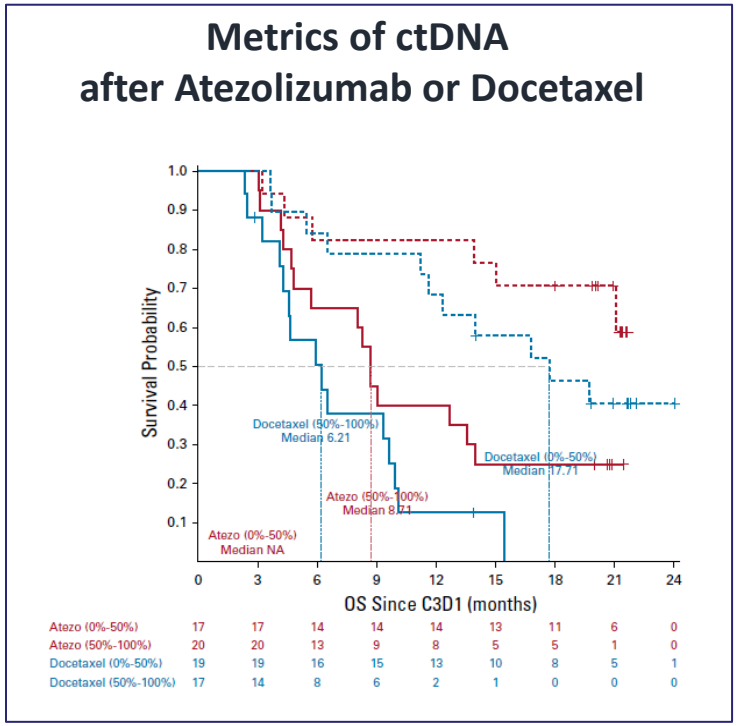
Activating *KRAS* mutations were significantly more frequent in patients with f*ARID1A* mutations



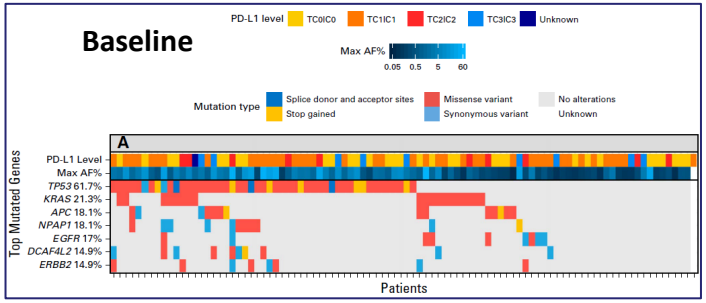
KRAS mutations associated with smoking (G12C/V) and non- smoking (G12D) were significantly more frequent in patients with f*ARID1A* mutations

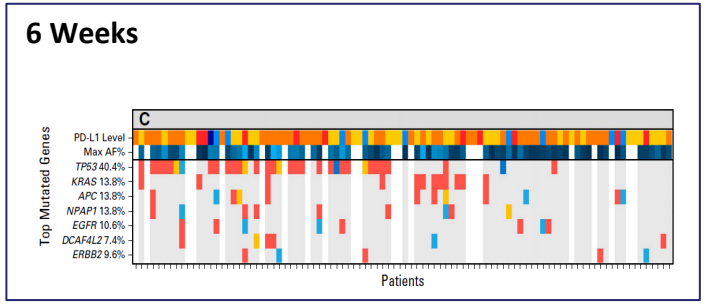


Plasma ctDNA in Advanced Stage NSCLC response monitoring: Checkpoint Immunotherapy & Chemotherapy



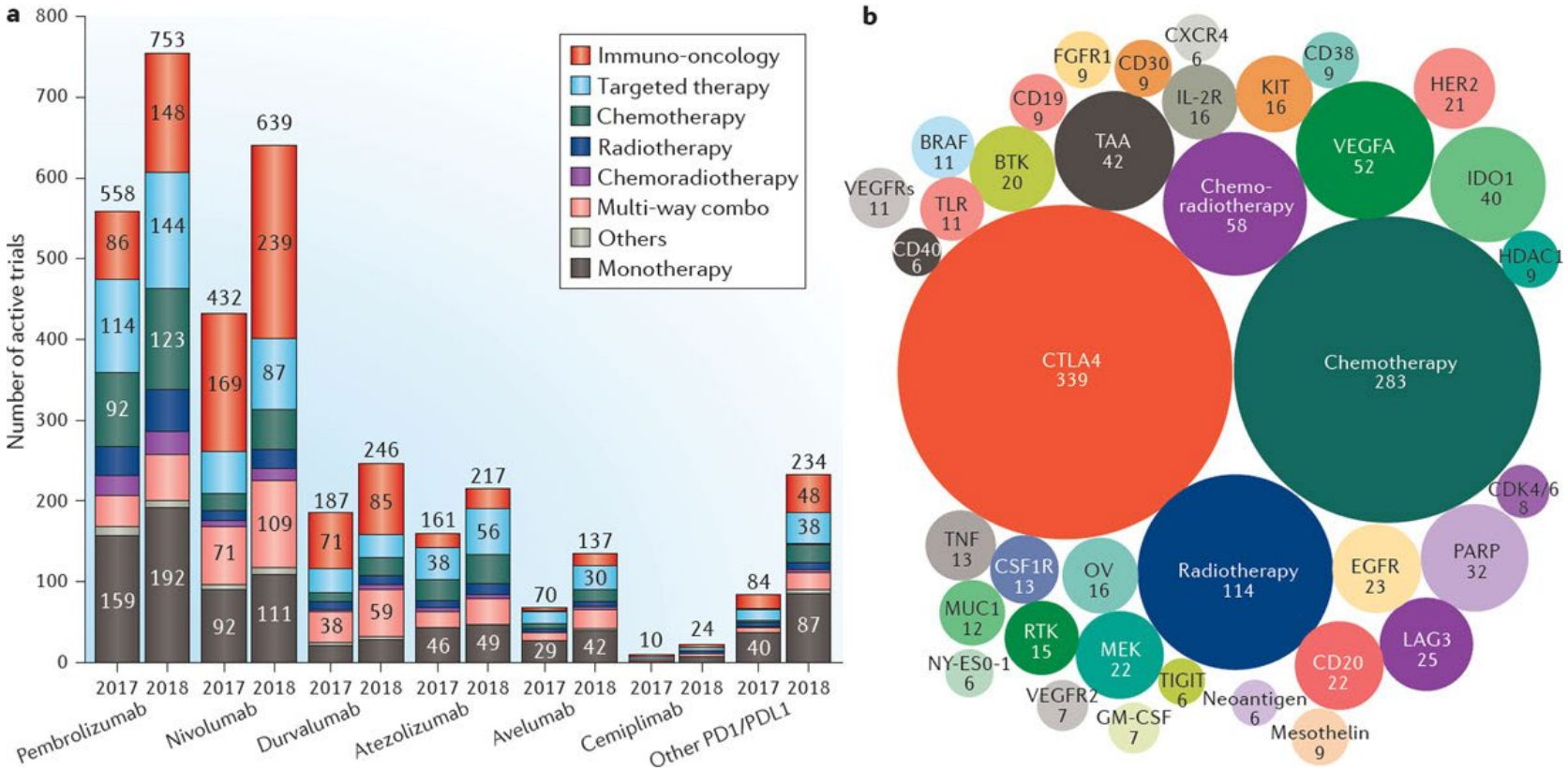
Reduction in mutation VAFs over time





Zou, Peters, Shames, Gandara, Patil et al. JCO Precis Oncol 2021

Ongoing Unmet Need for Predictive Biomarkers of Checkpoint Immunotherapy Efficacy



Over 2,250 clinical trials ongoing as of January 2019 (~4,000 trials as of 1-2021) requiring >500,000 patients
~750 trials in NSCLC

Tang: Nat RD 2018