Albrecht Stenzinger

UNIVERSITÄTS
KLINIKUM
HEIDELBERG

UK
HD

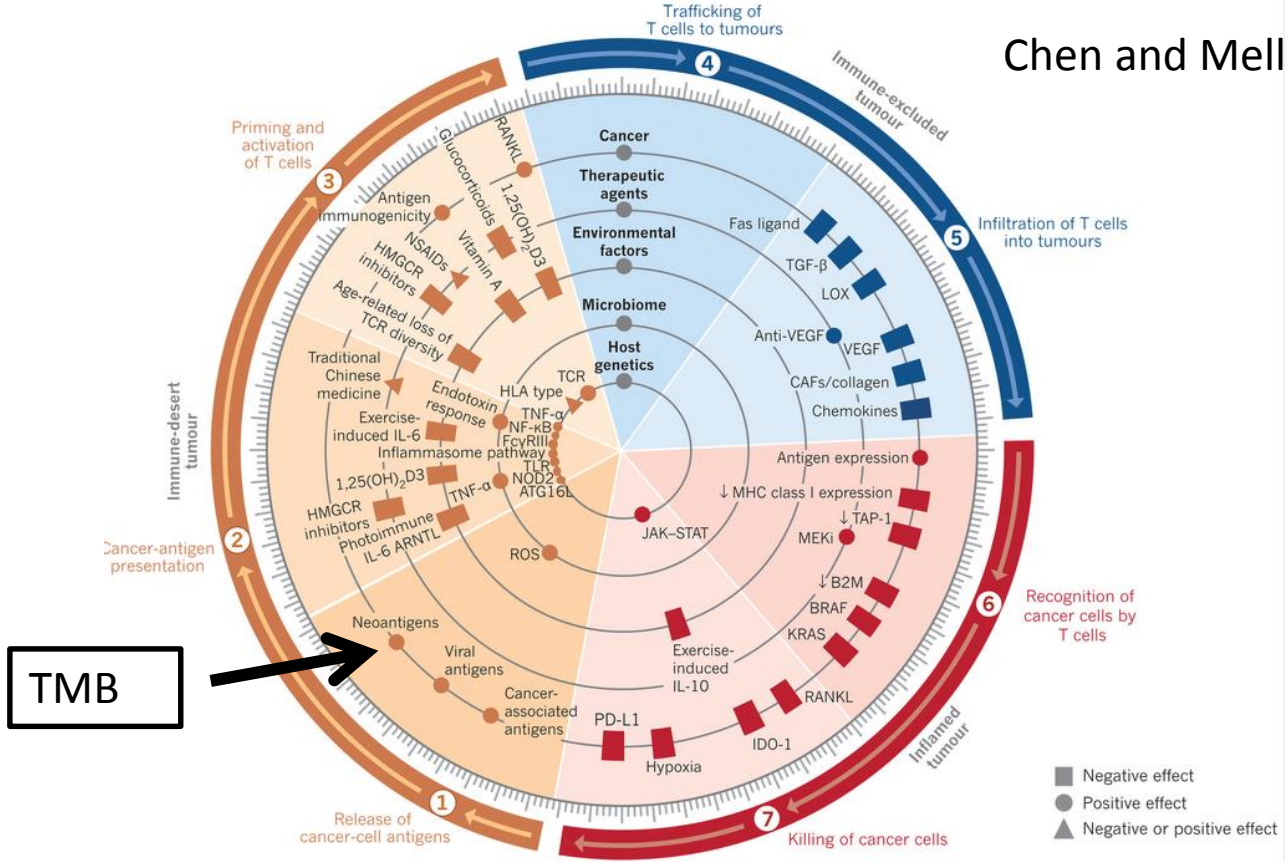
Disclosures

- Advisory Board/Speaker:

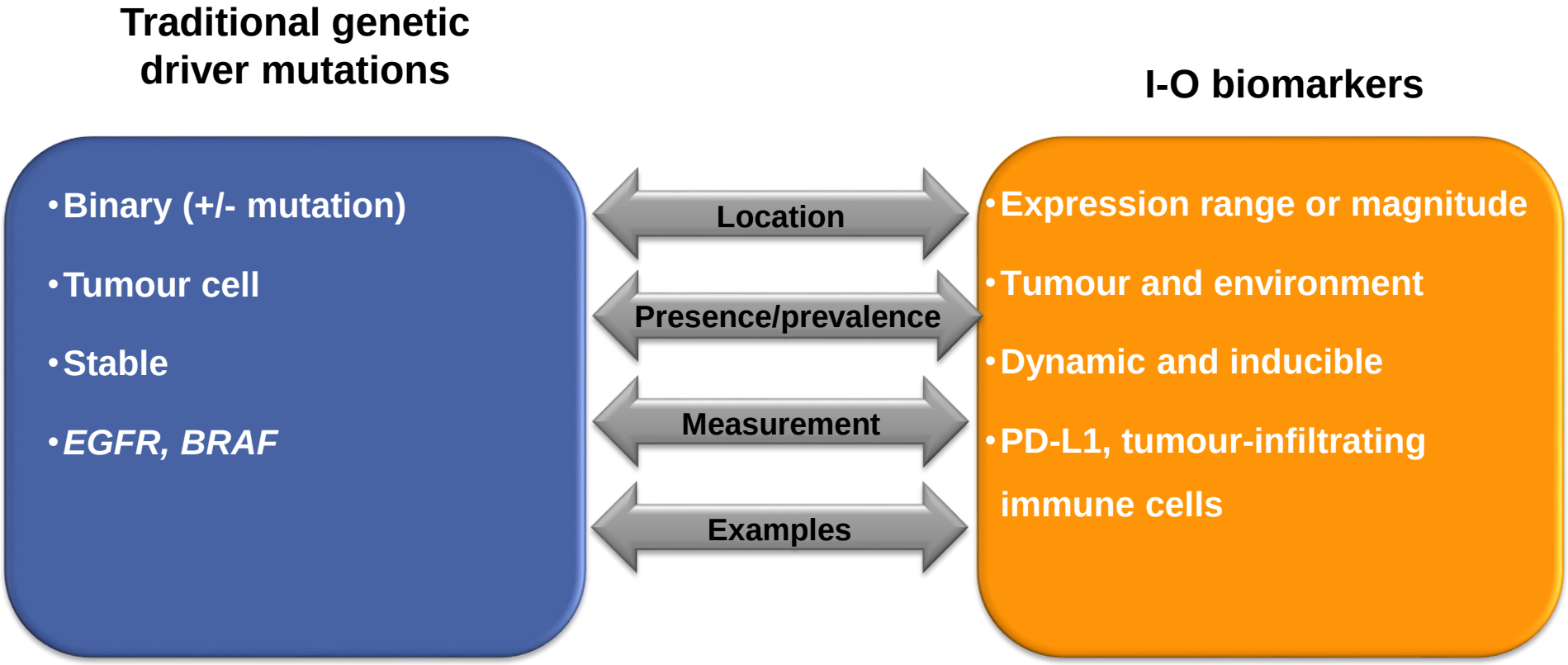
Astra Zeneca, Bayer, BMS, Illumina, MSD, Novartis, Pfizer, Roche, Seattle Genomics, Takeda, Thermo Fisher

The Cancer Immune Setpoint

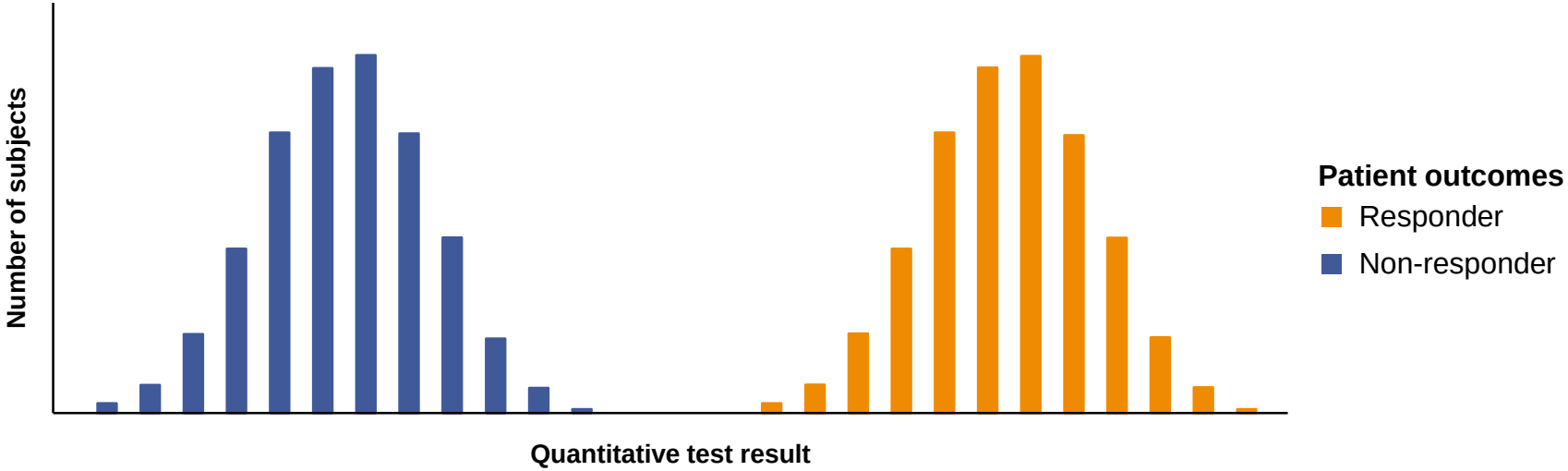
Chen and Mellman, 2017



The TMB concept from a diagnostic point of view



Interpretation of continuous biomarker data presents challenges for maximising patient benefit



The ideal predictive marker: bimodal population that can be interpreted categorically

Results can be presented with confidence as either predicted responders or predicted non-responders

Complex (Composite) Biomarkers

"measurement of complex, composite biomarkers may enable better predictions because multiple biomarkers each play a small role in the summative outcome of interest."

Integration of several individual parameters/biomarkers

- TMB
- Gene expression or methylation profiles
- HRD signature: e.g. LOH, LST, TAI

Califf et al, 2018

TMB testing in Heidelberg routine DX

male, 28 yrs, pre-treated, metastasized choriocarcinoma

Mutationslast per MB:

21.61

Mikrosatelliten-Instabilität

Untersuchte MSI Positionen	*Instabile Positionen*	*% instabile Positionen*	*MSI-Status*
99	23	**23.23**	Mikrosatelliten-instabil (MSI high)

Gene mit möglicher Kopienzahl-Veränderung

Gen	*RefSeq-ID*	*CNV-Typ*	*Cytoband*
		Keine CNVs identifiziert.	

Gen	*Mutation*	*Allel-frequenz*	*Beurteilung der Variante*	*COSMIC-ID*
RAC1	p.Gln61Arg	**11.9 %**	**Vermutlich aktivierende Mutation**	COSM1131540
BRCA2	p.Gly2528*	**5.2 %**	**Deletäre Mutation**	
EED	p.Thr50fs*5	**21.7 %**	**Deletäre Mutation**	
ERBB2	p.Gln513*	**7.2 %**	**Deletäre Mutation**	
LAMP1	p.Phe300fs*7	**5.8 %**	**Deletäre Mutation**	
MSH3	p.Lys383fs*32	**10.2 %**	**Deletäre Mutation**	COSM1438888
NBN	p.Arg466fs	**7.9 %**	**Deletäre Mutation**	COSM1458550
NF1	p.Tyr628fs*3	**9.8 %**	**Deletäre Mutation**	
SPEN	p.Arg724*	**20.3 %**	**Deletäre Mutation**	COSM3802617
IL7R	p.Leu259*	**8.1 %**	**Vermutlich Deletär**	
NFE2L2	p.Arg34Gly	**29.8 %**	**Vermutlich Deletär**	COSM132847
NSD1	p.spl?	**6.9 %**	**Vermutlich Deletär**	

Potential ICI Biomarkers beyond PD-L1

Sensitivity

Overall mutational load and neoantigen burden
Rizvi et al. Science 2015

Neoantigen intratumoral heterogeneity
McGranahan et al. Science 2016

Immunogenic insertion/deletion mutations
Turajlic et al. Lancet Oncol 2017

***PDL1* amplification and/or overexpression**
Ansell et al. N Engl J Med 2015

Structural rearrangements of *PDL1/2*
Steidl et al. Nature 2011

Disruption of *PDL1* 3' untranslated region
Kataoka et al. Nature 2016

Loss-of-function *PBRM1* mutations
Miao et al. Science 2018

T-cell-inflamed gene expression profile
Cristescu et al. Science 2018

Resistance

Inactivating *JAK* family member and *B2M* mutations
Zaretsky et al. N Engl J Med 2016

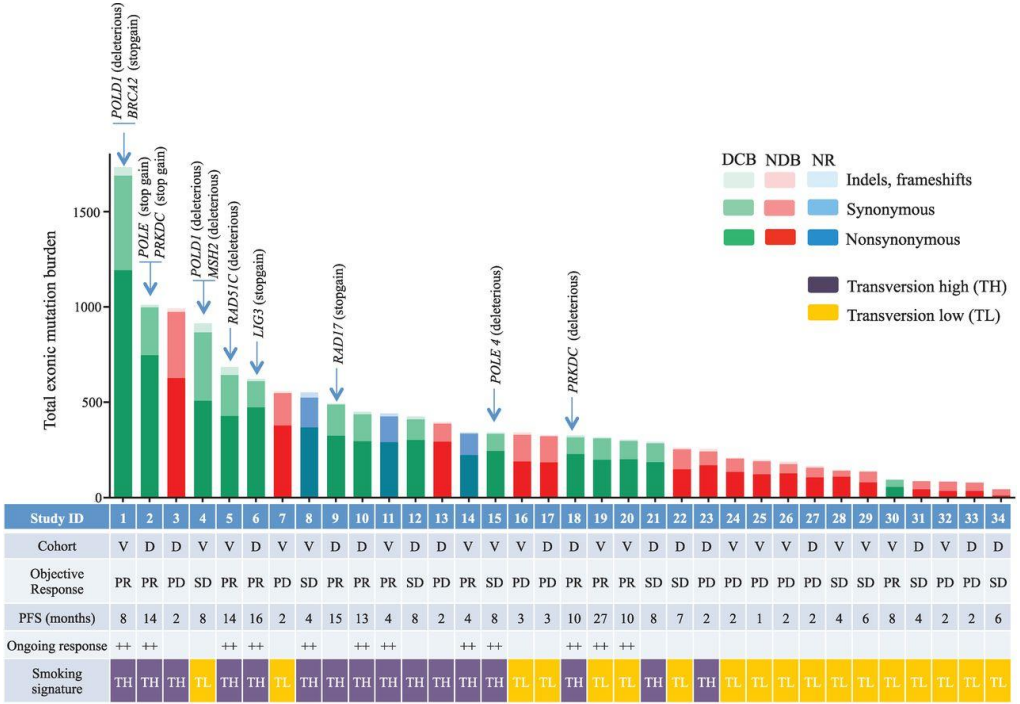
***MDM2/4* amplification**
Kato et al. Clin Cancer Res 2017

***PTEN* loss**
Peng et al. Cancer Discov 2016

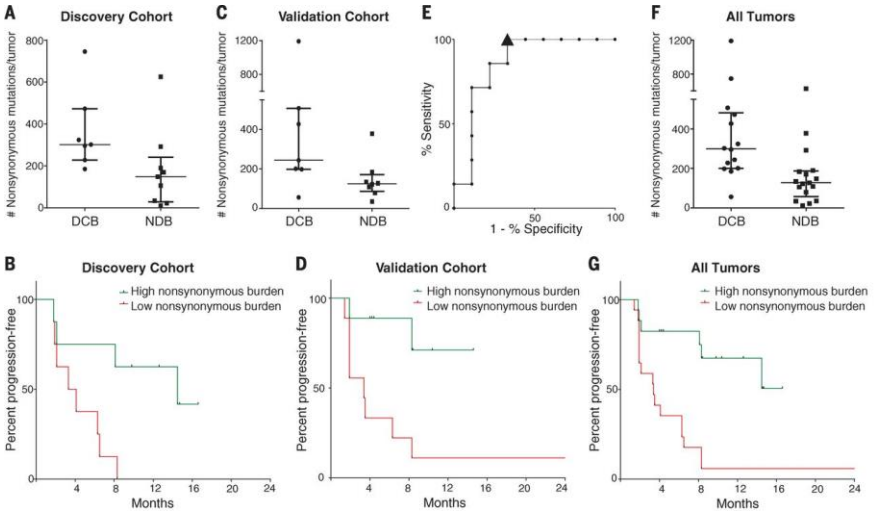
Inactivating *STK11* mutations
Skoulidis et al. Cancer Discov 2018

PD-1 blockade in NSCLC and TMB

„More mutations predict better efficacy"



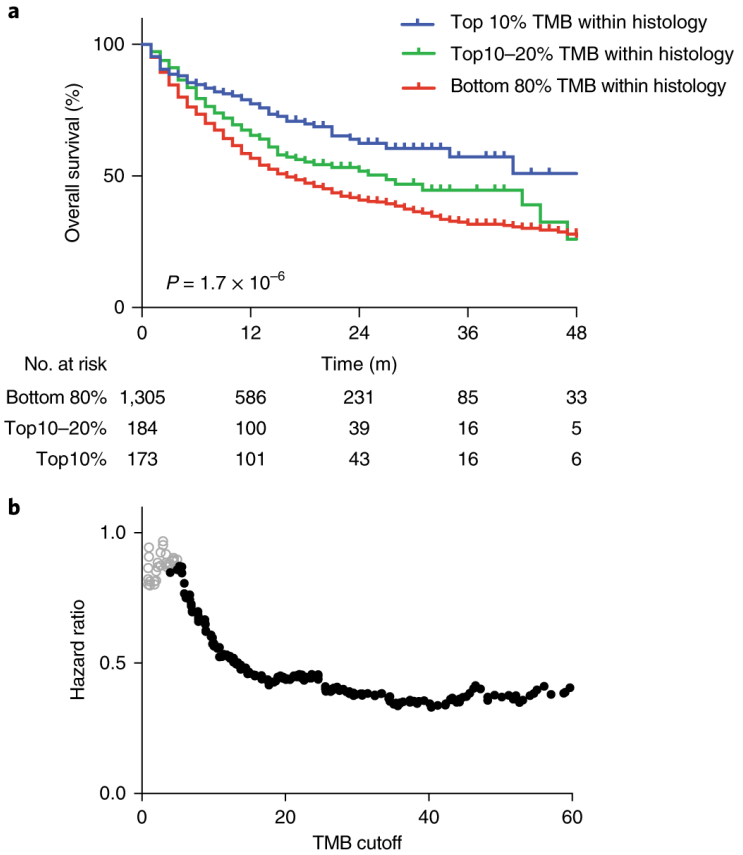
Study ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
Cohort	V	D	D	V	V	D	V	V	D	D	V	D	D	V	V	V	D	D	V	V	D	D	D	V	V	V	D	V	V	V	D	V	D	D
Objective Response	PR	PR	PD	SD	PR	PR	PD	SD	PR	PR	PR	SD	PD	PR	SD	PD	PD	PR	PR	PR	SD	SD	PD	PD	PD	PD	PD	SD	SD	PR	SD	PD	PD	SD
PFS (months)	8	14	2	8	14	16	2	4	15	13	4	8	2	4	8	3	3	10	27	10	8	7	2	2	1	2	2	4	6	8	4	2	2	6
Ongoing response	++	++			++	++		++		++	++			++	++			++	++	++														
Smoking signature	TH	TH	TH	TL	TH	TH	TL	TH	TH	TH	TH	TH	TH	TH	TH	TL	TL	TH	TL	TL	TH	TL	TH	TL	TL	TL	TL	TL	TL	TL	TL	TL	TL	TL

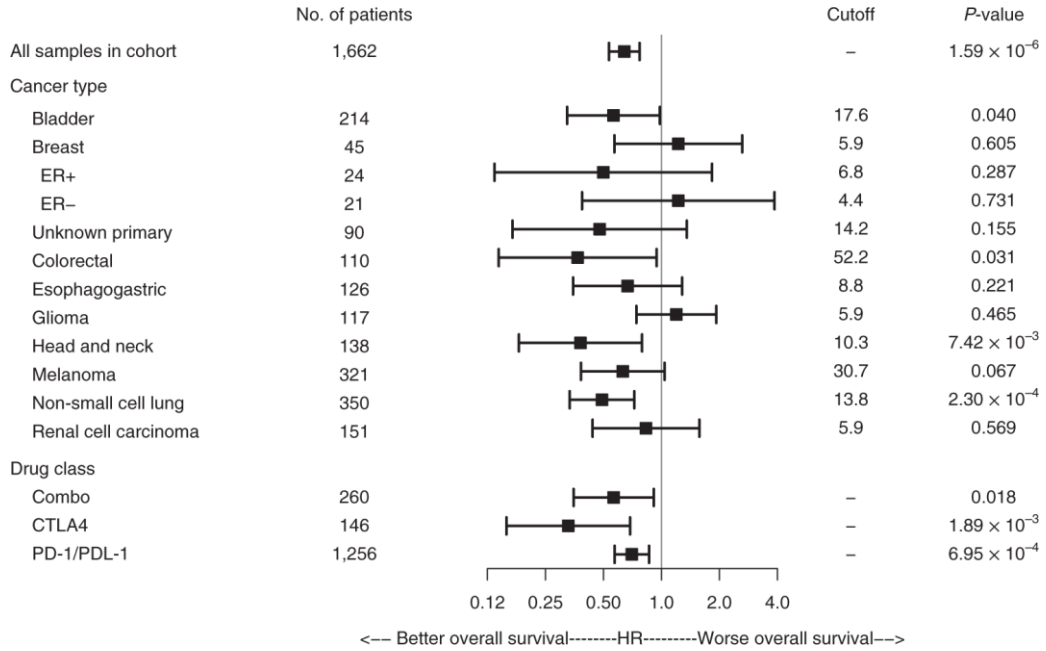


Cutpoint: 200 missense mutations

Rizvi et al., 2015

MSKCC- single center experience – 1,662 pts

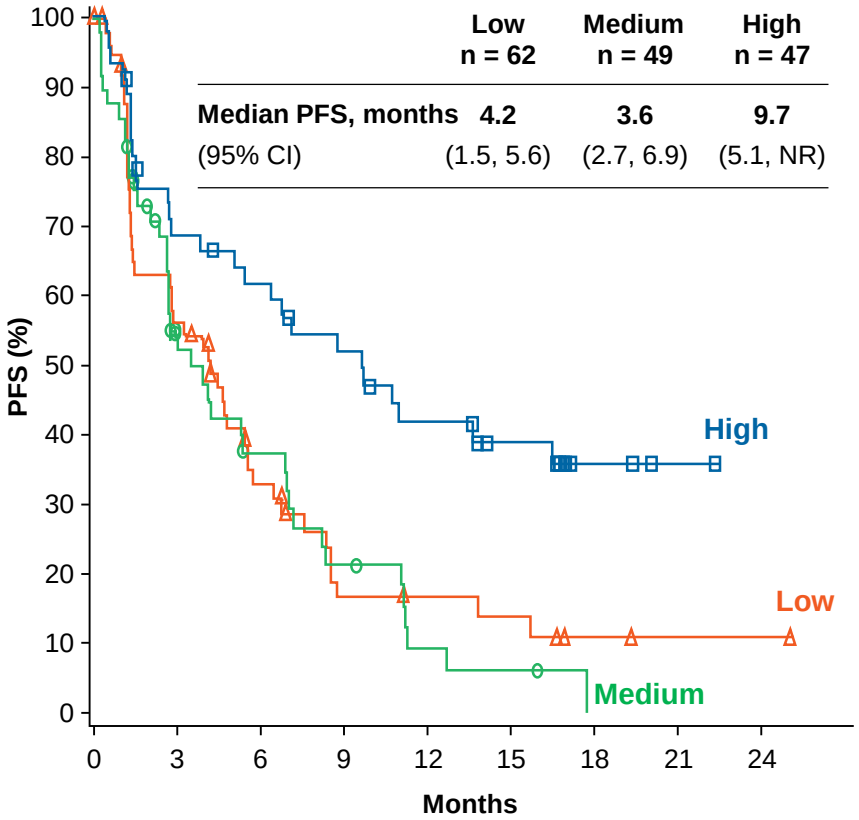




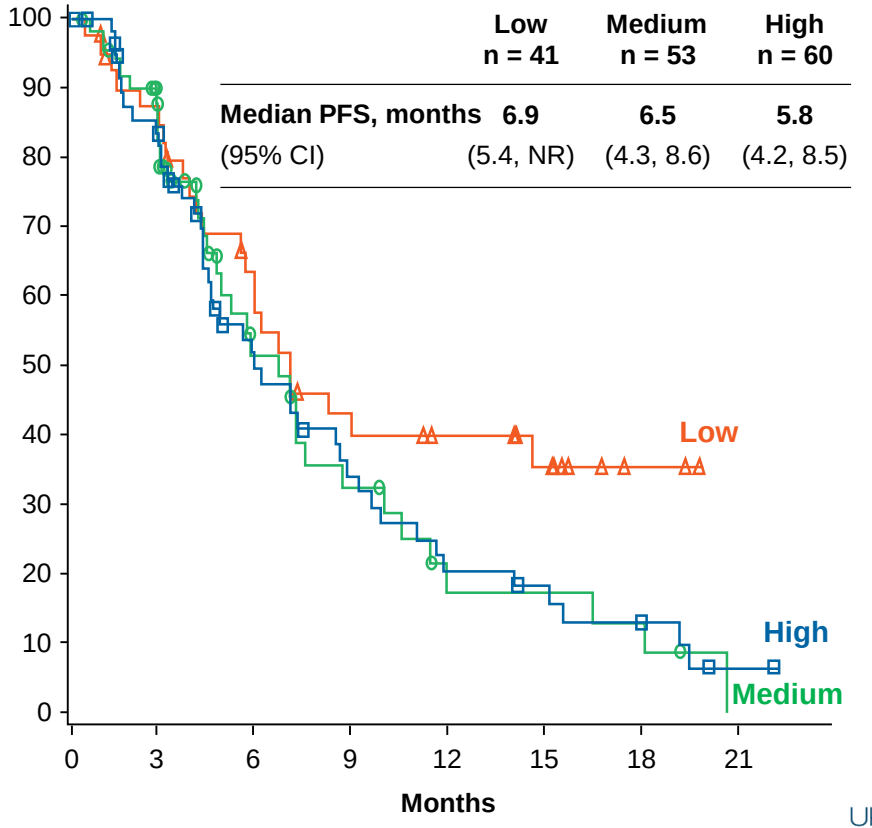
Samstein et al, 2019

CM 26

Nivolumab Arm



Chemotherapy Arm



Peters et al., 2017

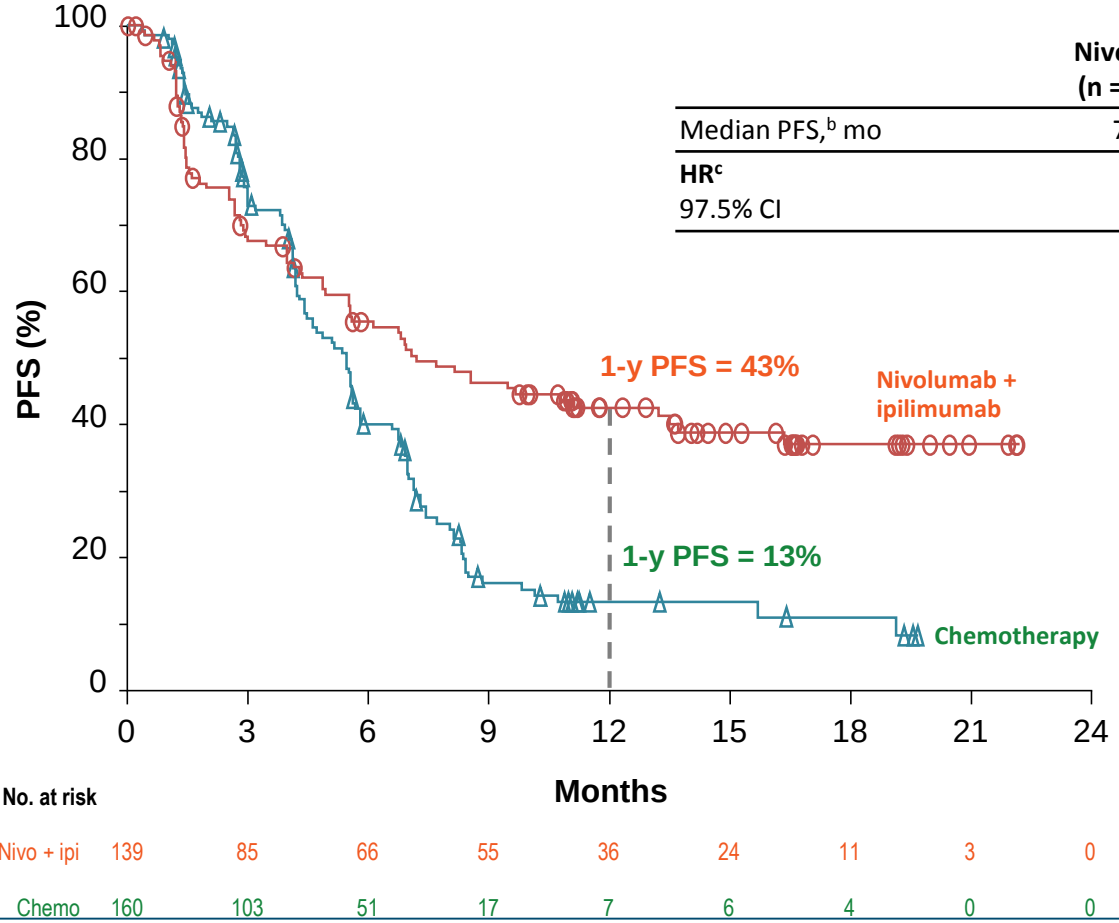
(Initial) definition TMB: Total number of non-synonymous mutations

~22.000 human genes, (30) 50-60 Mbp region (depending on enrichment kits used)
Parallel germline sequencing: filtering of private and common polymorphisms.

Pros:
- Comprehensive
- Used in clinical trials

Cons:
- only limited use for FFPE material
- hard to implement in routine MDx
- additional germline sequencing mandatory
- expensive
- long turnaround time

CM227



	Nivo + ipi (n = 139)	Chemo (n = 160)
Median PFS,[b] mo	7.2	5.4
HR[c]	**0.58**	
97.5% CI	0.41, 0.81	
	***P* = 0.0002**	

The NEW ENGLAND JOURNAL of MEDICINE

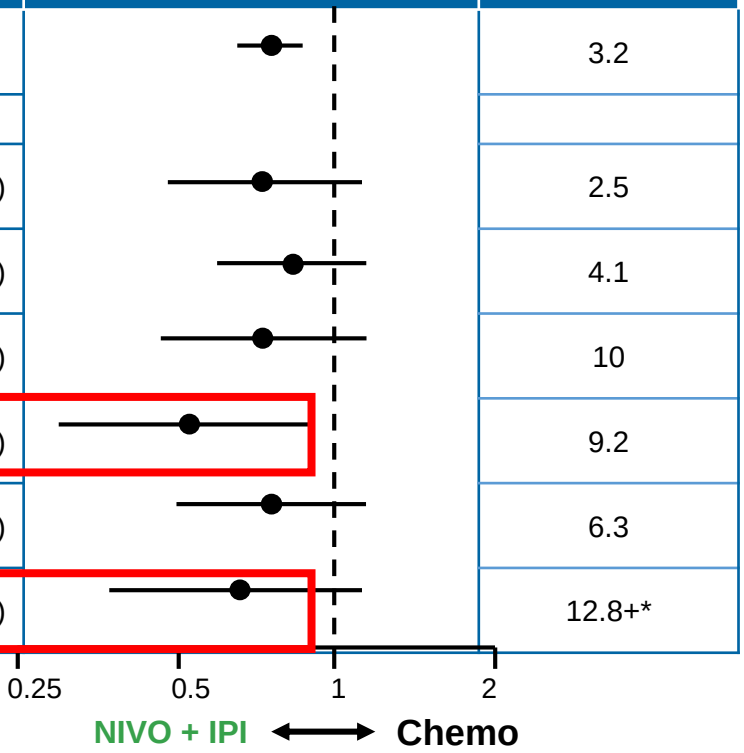
ORIGINAL ARTICLE

Nivolumab plus Ipilimumab in Advanced Non–Small-Cell Lung Cancer

M.D. Hellmann, L. Paz-Ares, R.B. Caro, B. Zurawski, S.-W. Kim, E.C. Costa, K. Park, A. Alexandru, L. Lupinacci, E. de la Mora Jimenez, H. Sakai, I. Albert, A. Vergnenegre, S. Peters, K. Syrigos, F. Barlesi, M. Reck, H. Borghaei, J.R. Brahmer, K.J. O'Byrne, W.J. Geese, P. Bhagavatheeswaran, S.K. Rabindran, R.S. Kasinathan, F.E. Nathan, and S.S. Ramalingam, for the CheckMate 227 Investigators*

Risk of Death According to Tumor PD-L1 Expression Level and Tumor Mutational Burden

	Median OS, months			
	NIVO + IPI n=583	Chemo n=583	Unstratified HR (95% CI)	Difference in OS
Overall (n=1166)	17.1	13.9	0.73[a] (0.64-0.84)	3.2
Interplay subgroups				
<1% PD-L1;<10 mut/Mb TMB (n=111)	15.5	13.0	0.69 (0.46-1.05)	2.5
≥1% PD-L1;<10 mut/Mb TMB (n=269)	16.2	12.1	0.78 (0.57-1.06)	4.1
≥50% PD-L1;<10 mut/Mb TMB (n=125)	18.1	8.1	0.67 (0.44-1.03)	10
<1% PD-L1;≥10 mut/Mb (n=86)	20.4	11.2	0.51 (0.30-0.87)	9.2
≥1% PD-L1;≥10 mut/Mb TMB (n=213)	24.4	18.1	0.77 (0.52-1.15)	6.3
≥50% PD-L1;≥10 mut/Mb TMB (n=111)	NR	17.2	0.63 (0.37-1.07)	12.8+*



[a]Stratified HR (unstratified HR, 0.74: 95% CI, 0.64-0.85)
Minimum of 29-month follow up.
*Estimated based on minimum follow-up since mOS not reached.

Trials stage 4 NSCLC – tissue TMB (WCLCL 2019)

- **Keynote 21**: **Pembro+pem-carbo** vs permetrexed-carboplatinum alone
  - 70 pts where tissue available (total study population 145)
  - WES, cutpoint: 175 muts
  - No association TMB and PD-L1 ➔ fine
  - OR, PFS, OS: no effect
- **Keynote 189**: **Pembro+pem-carbo** vs permetrexed-carboplatinum alone
  - 293 pts where tissue available (total study population: 616)
  - WES; cutpoint: 175 muts
  - OR, PFS, OS: no effect
- **S1400I phase III**: nivo + ipi vs nivo alone
  - 231 where tissue available for TMB, 149 for TMB and PD-L1
  - Panel, 10 muts/Mb + various PD-L1 cutpoints
  - OS: no effect; trend for combined view on PD-L1 and TMB

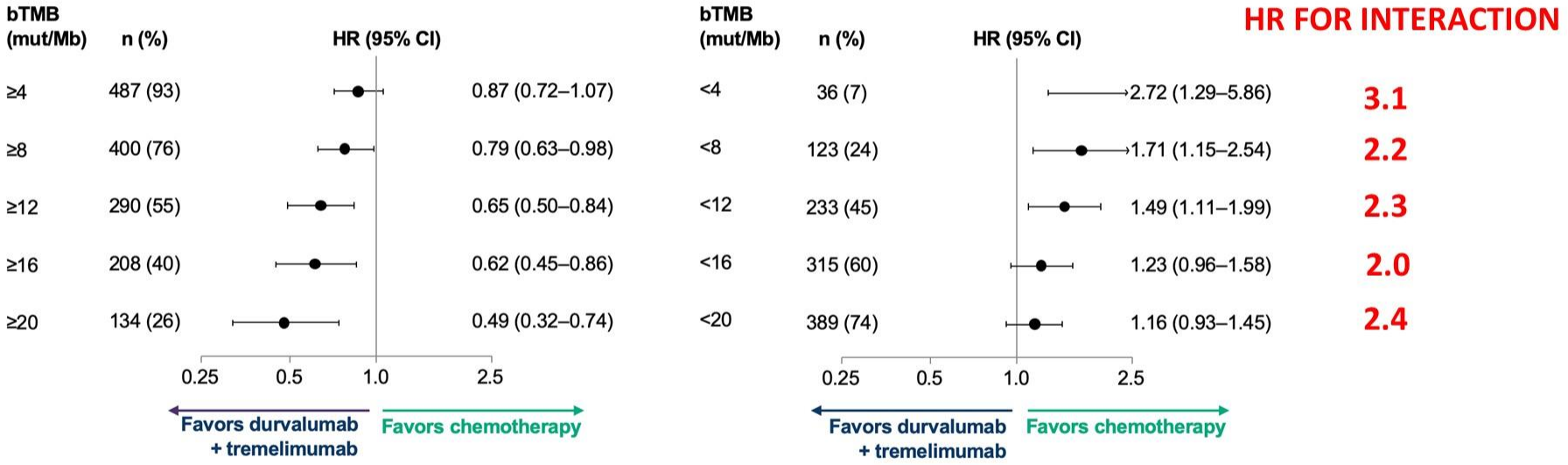
Trials stage 4 NSCLC – tissue TMB (ESMO 2019)

- **Keynote 21 (nonsq), 189 (nonsq), 407 (sq)**: pembro+platinum vs. chemo alone
  - 48%, 48%, 56% of total study population, respectively
  - WES, cutpoint 175 muts
  - OS: no effect
- **Keynote 10**: **Pembro mono** vs chemo
  - 253 pts where tissue available (24% of total study population)
  - PD-L1: >/=1%
  - WES, cutpoint: 175 muts
  - median OS: 14.1 vs 7.6 mos
- **Keynote 42**: **Pembro mono** vs chemo
  - 793 pts where tissue available (62% of total study population)
  - PD-L1: >/=1 1%
  - WES, cutpoint: 175 muts
  - median OS: 21.9 vs 11.6 mos

Results cont'd

Figure 3. Overall Survival Across bTMB Cut-offs (cont'd)

B. Durvalumab + tremelimumab vs chemotherapy



bTMB is predictive of durva treme at all cut-off levels, however magnitude of interaction is always the same

bTMB evaluable population.

Neptune phase III

- **21 August 2019 07:00 BST**

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.

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.

Reasons for mixed results in NSCLC trials so far

- No prospective analysis; **retrospective subsets** investigated
- **Different therapy regimen**: PD1/PD-L1 mono, combo w/ CTLA4, combo w/ chemo
- **Different IO drugs**
- **Differently stratified pts**: PD-L1 status included/not included
- **Different cut-points** (e.g. 200 vs 175 muts in WES)
- **Different assays**: WES vs (different) panels
- **Different input source**: tissue vs blood (**where** and **when**?)
- **Crucial assay parameters** often **not well defined**, e.g. coverage
- Are we missing **biology**? (think MSI-H)
- Integration with abundance/activity of **effector compartment** needed?

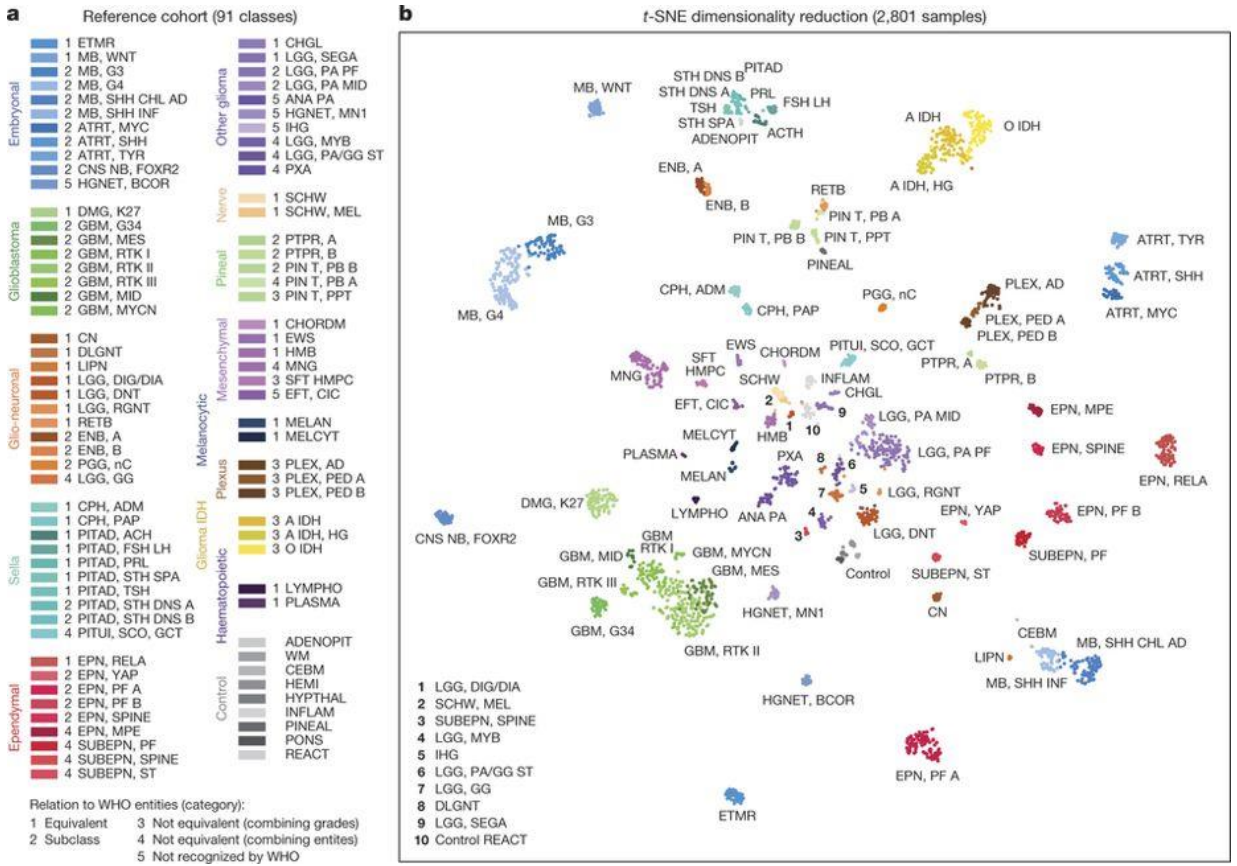
Harmonization is key

Adjei Alex, Mayo, Rochester @WCLC:

„I think therein lies the problem – We are not even talking about the same thing.

Normally when we are talking about a biomarker, such as *EGFR* mutation, we are talking about the same thing. If you tell me high TMB, I don‘t know what it is because everyone‘s definition is different.“

Harmonization is key



One Assay covering 850,000 data points (CpG sites) **used** around the world **in all labs**

Capper et al., 2019

Across solid tumors (preliminary data @ESMO)

Association of tumour mutational burden with outcomes in patients with select advanced solid tumours treated with pembrolizumab in KEYNOTE-158

phase II basket study (NCT02628067)
progression on or intolerance to 1line of standard therapy
ECOG PS 0-1
TMB, panel 10 muts/Mb
1032 patients: 120 TMB-high (15.9%) – 15/120: MSI-High
Low correlation between TMB and PD-L1 expression
TMB-high associated with higher ORR, tail of PFS curve favored TMB-high

Main parameters influencing panel-based TMB measurement

- Tumor heterogeneity — biology

- Tumor purity
- DNA quality, FFPE artefacts
- DNA input amounts — pre-analytics

- Target region size
- Gene content — panel design

- Complexity of libary prep
- Coverage — lab

- Substraction of germline events
- Cutpoint allelic frequency
- Mutation types — bioinformatics

Exome (coding region – approx. 1-2% of genome) – 1 Mb = 1 million nucleotides

➔ Variability regarding definition

Definition:

➔**Somatic**

➔**coding region**

? missense mutations

? nonsense mutations

? frameshift mutations

? Indels

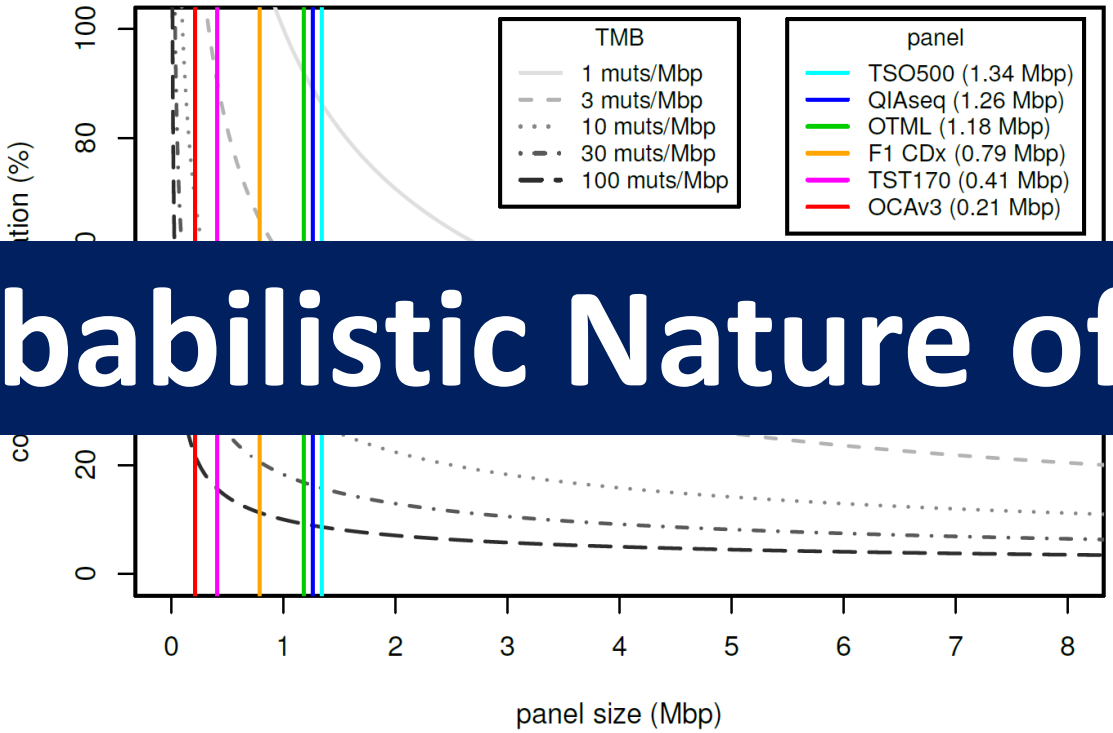
TMB:

➔**is a continous variable**

➔**does not follow a linear distribution**

Allgäuer et al., in press; Budczies et al., in press; Buchhalter et al., 2018, Endris et al., 2018 , Chan et al., 2018

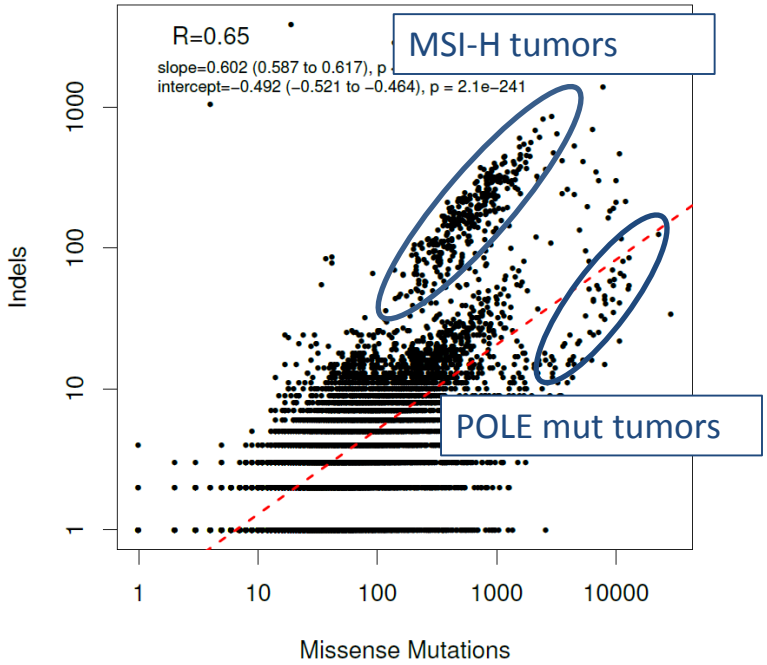
CV decreases proportionally to square root of panel size



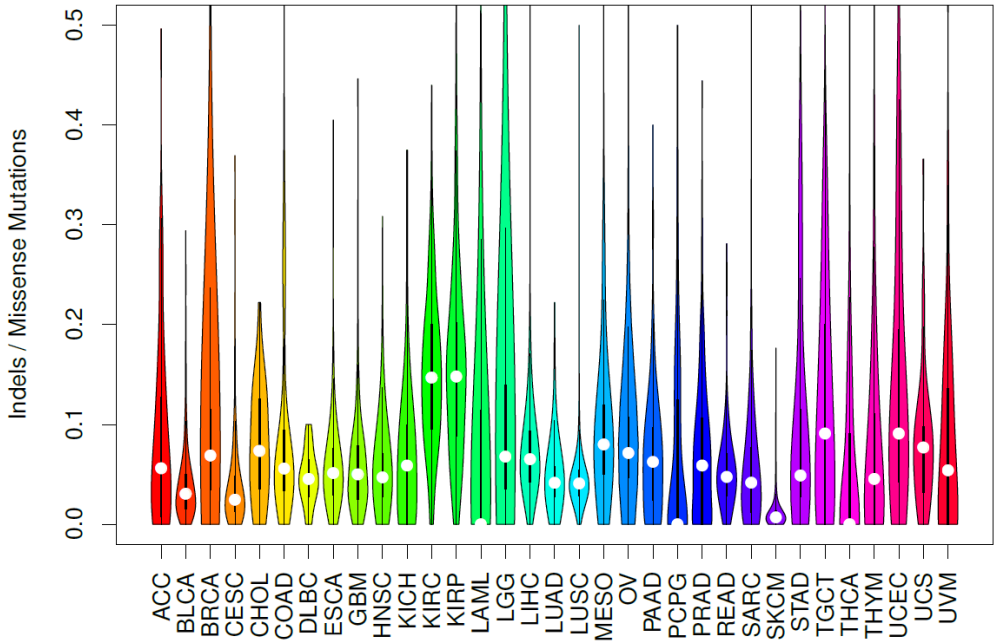
Probabilistic Nature of TMB

Budczies et al., 2019

Indels vs. missense mutations

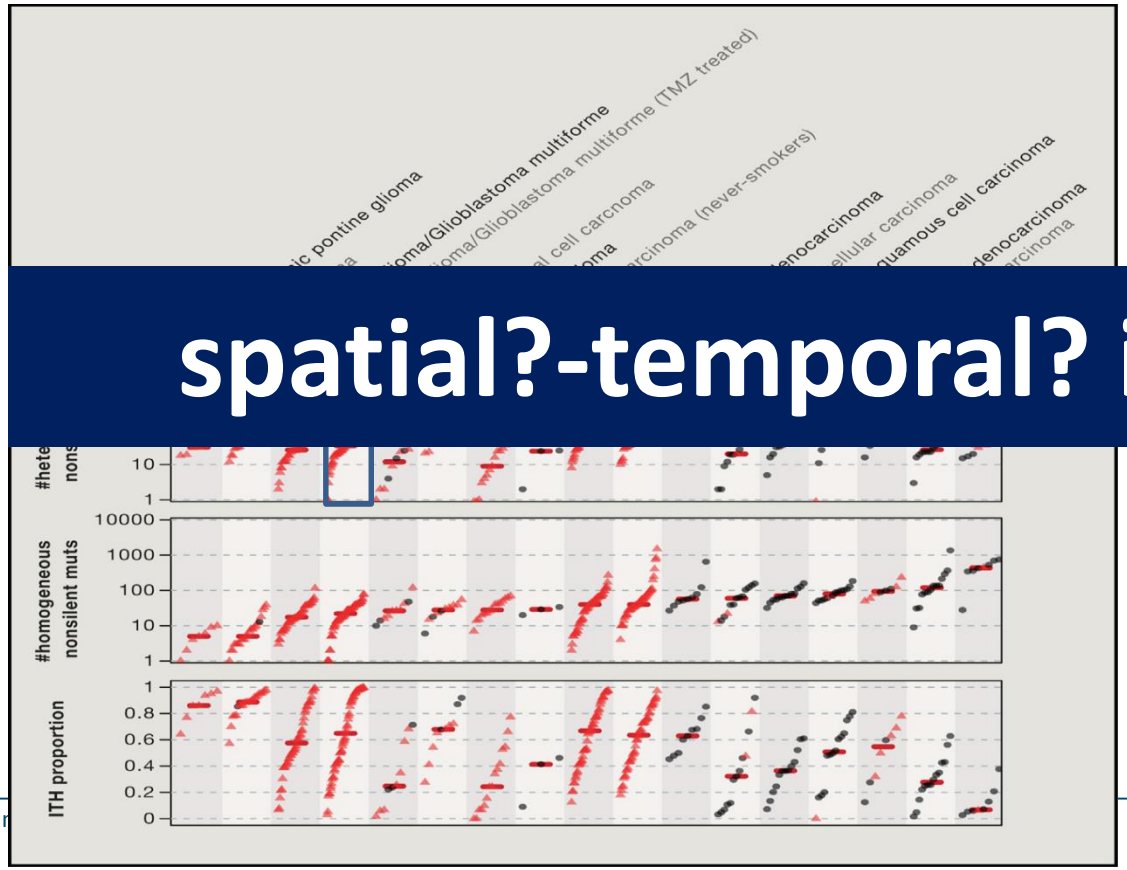


approx. 10%



Budczies et al., 2019

Clonality and subclonality in TMB



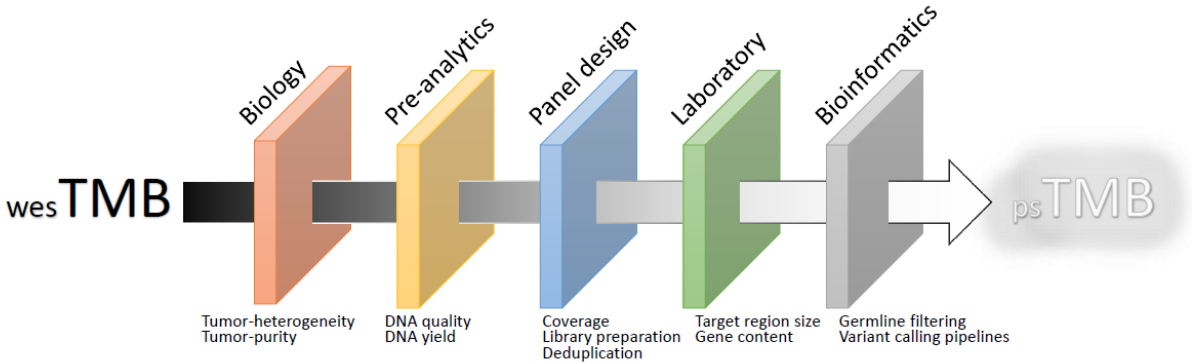
spatial?-temporal? influence

Genomic diversity comprising clonal and

entity specific manner and is also influenced by therapy.

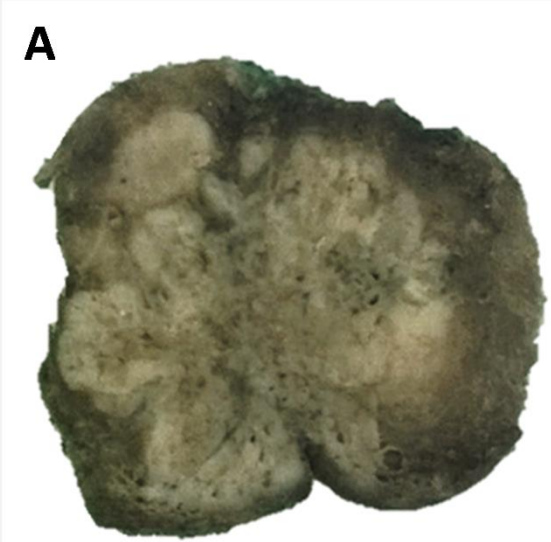
Granahan and Swanton, 2017

A first glimpse: Recommendations

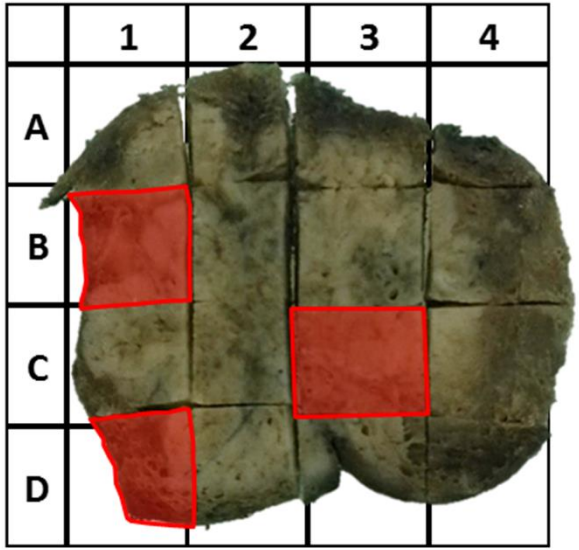


CONFIDENTIAL
Do not post

Spatial TMB heterogeneity: Multi-regional analysis



Central tumor section



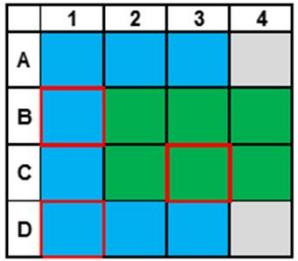
Segmentation

Tumor cell content [%]

	1	2	3	4
A	13	5	19	0
B	22	24	24	28
C	7	22	25	7
D	22	26	43	0

DNA content [ng/µl]

	1	2	3	4
A	18.3	10.9	21.2	4.9
B	26.8	30.2	68.3	12.1
C	26.1	27.5	36.9	15.9
D	12.4	19.1	20.7	5.3



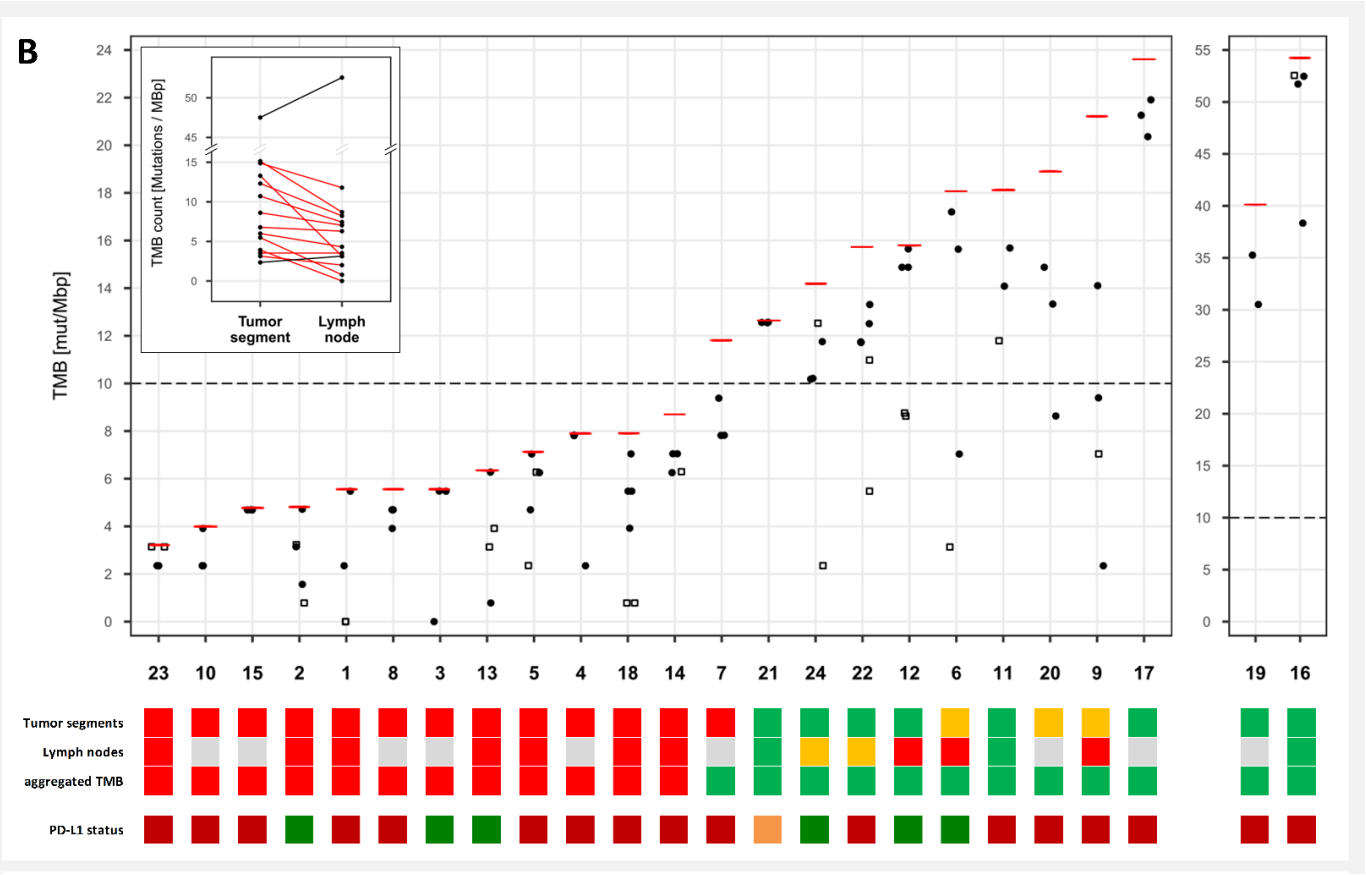
Histological growth pattern

24 patients:

69 tumor segments and 23 locoregional lymph node metastases → 2-6 samples per tumor/patient

Kazdal et al., 2019

Spatial TMB heterogeneity: Multi-regional analysis



Overall TMB range:
0 - 52.55 mut/Mbp

29% with ITH of ≥5 mut/Mbp;
max. diff. 14.13

*varying tumor cell content (4/7)
*distinct mutational profiles (3/7)

13 cases with region specific mutations -> simulation of pooled sample as aggregated TMB

17% patients with divergent TMB status

TMB in lymph nodes lower (p=0.02)

Independent of PD-L1 expression

Kazdal et al., 2019

TMB harmonization initiatives in Germany and USA

Patient Advocacy Organization, Washington, DC

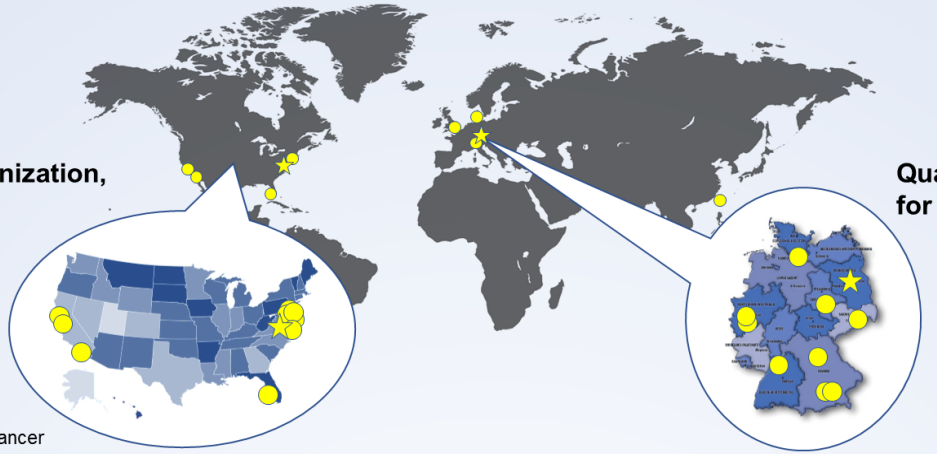
Partners:

Diagnostic partners
- ACT Genomics
- AstraZeneca
- Caris Life Sciences
- Foundation Medicine
- Guardant Health
- Illumina
- Memorial Sloan Kettering Cancer Center
- NeoGenomics
- OmniSeq
- Personal Genome Diagnostics
- QIAGEN
- Thermo Fisher Scientific

Academic partners
- Columbia University

Pharmaceutical partners
- Bristol-Myers Squibb
- EMD Serono
- Genentech
- Merck
- Pfizer

Other
- National Cancer Institute
- US FDA



***Friends* and QuIP TMB Standardization and Harmonization Initiative Objectives**

- Identify TMB assessment parameters and cutoff values used in published and ongoing clinical trials in a range of different tumor types
- 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

Quality Assessment Service for Pathology, Berlin, Germany

Partners:

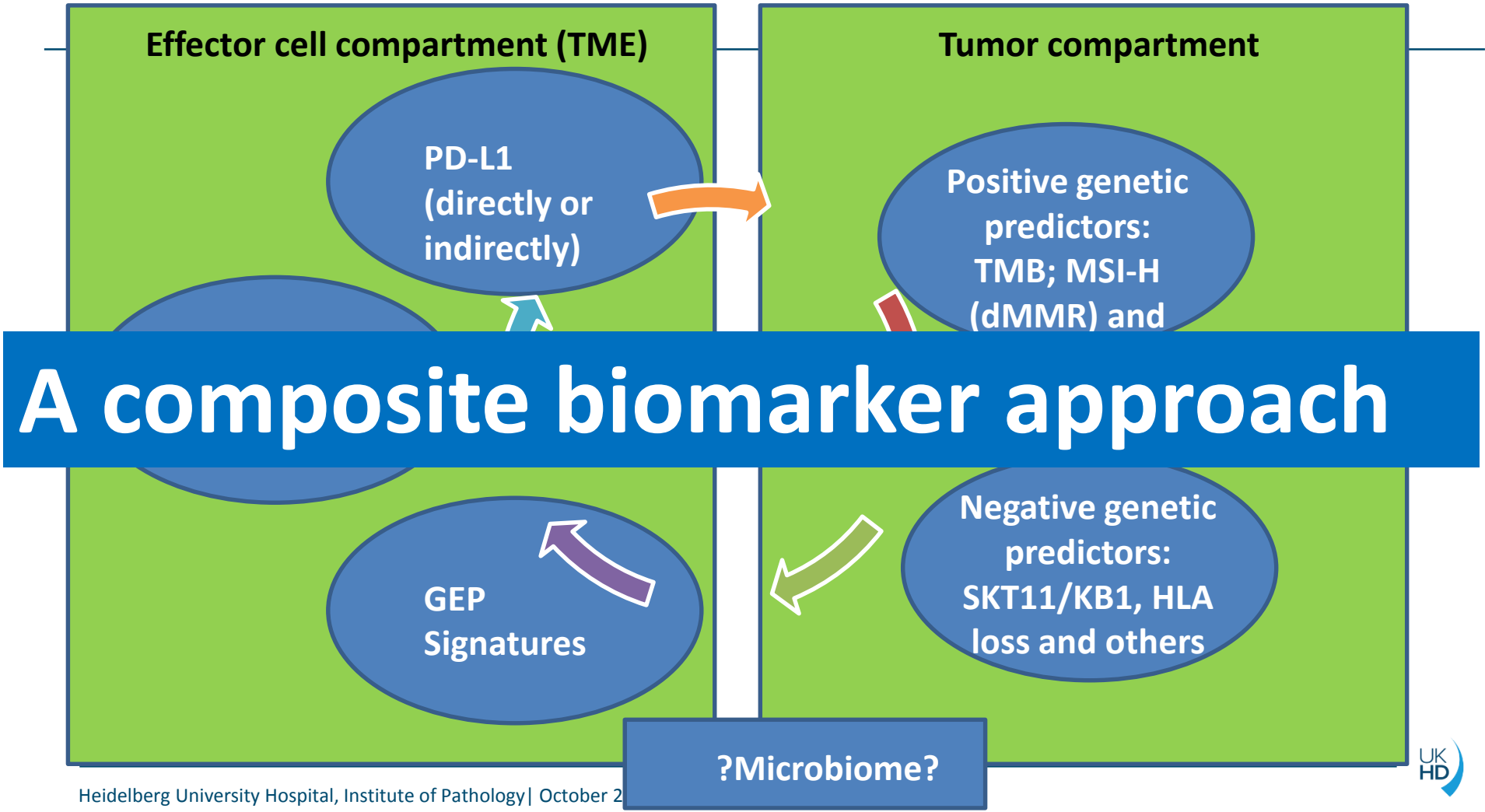
Diagnostic partners
- Foundation Medicine
- Illumina
- NEO NewOncology
- QIAGEN
- Thermo Fisher Scientific

Academic partners
- 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 partners
- Bristol-Myers Squibb
- Merck
- Roche

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

Stenzinger, Allen et al., 2019


Effector cell compartment (TME)
Tumor compartment
PD-L1 (directly or indirectly)
Positive genetic predictors: TMB; MSI-H (dMMR) and
A composite biomarker approach
GEP Signatures
Negative genetic predictors: SKT11/KB1, HLA loss and others
?Microbiome?

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