Albrecht Stenzinger
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

TMB
The TMB concept from a diagnostic point of view

Traditional genetic driver mutations

- Binary (+/- mutation)
- Tumour cell
- Stable
- EGFR, BRAF

I-O biomarkers

- Expression range or magnitude
- Tumour and environment
- Dynamic and inducible
- PD-L1, tumour-infiltrating immune cells
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
“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
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

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

Disruption of PDL1 3’ untranslated region

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

MDM2/4 amplification

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”

Cutpoint: 200 missense mutations
MSKCC- single center experience – 1,662 pts

No. of patients

Cancer type

All samples in cohort
1,662

Cutoff
P-value

Bladder
214
17.6
0.040

Breast
45
5.9
0.605

ER+
24
6.8
0.287

ER-
21
4.4
0.731

Unknown primary
90
14.2
0.155

Colorectal
110
52.2
0.031

Esophagogastric
126
8.8
0.221

Glioma
117
5.9
0.465

Head and neck
138
10.3
7.42 × 10⁻³

Melanoma
321
30.7
0.067

Non-small cell lung
350
13.8
2.30 × 10⁻⁴

Renal cell carcinoma
151
5.9
0.569

Drug class

Combo
260
–
0.018

CTLA4
146
–
1.89 × 10⁻³

PD-1/PDL-1
1,256
–
6.95 × 10⁻⁴

<--- Better overall survival-----HR------Worse overall survival--->

Samstein et al, 2019
Nivolumab Arm

<table>
<thead>
<tr>
<th></th>
<th>Low</th>
<th>Medium</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>62</td>
<td>49</td>
<td>47</td>
</tr>
<tr>
<td>Median PFS, months</td>
<td>4.2 (1.5, 5.6)</td>
<td>3.6 (2.7, 6.9)</td>
<td>9.7 (5.1, NR)</td>
</tr>
</tbody>
</table>

Chemotherapy Arm

<table>
<thead>
<tr>
<th></th>
<th>Low</th>
<th>Medium</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>41</td>
<td>53</td>
<td>60</td>
</tr>
<tr>
<td>Median PFS, months</td>
<td>6.9 (5.4, NR)</td>
<td>6.5 (4.3, 8.6)</td>
<td>5.8 (4.2, 8.5)</td>
</tr>
</tbody>
</table>
(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

Heidelberg University Hospital, Institute of Pathology | October 2019 | Albrecht Stenzinger

The graph illustrates the progression-free survival (PFS) of patients treated with Nivolumab + Iplimumab (Nivo + ipi) compared to chemotherapy. The median PFS for patients in the Nivo + ipi group was 7.2 months, while it was 5.4 months for the chemotherapy group. The hazard ratio (HR) for the Nivo + ipi group was 0.58, with a 97.5% confidence interval (CI) of 0.41, 0.81. The one-year PFS rate for the Nivo + ipi group was 43%, and for the chemotherapy group, it was 13%. The statistical significance is indicated by a P-value of 0.0002.
Nivolumab plus Ipilimumab in Advanced Non–Small-Cell Lung Cancer

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

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

<sup>a</sup>Stratified HR (unstratified HR, 0.74: 95% CI, 0.64-0.85)

Minimum of 29-month follow up.

<sup>*</sup>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):** pembrolizumab+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 + tremelimunab vs chemotherapy

<table>
<thead>
<tr>
<th>bTMB (mut/Mb)</th>
<th>n (%)</th>
<th>HR (95% CI)</th>
<th>bTMB (mut/Mb)</th>
<th>n (%)</th>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥4</td>
<td>467 (93)</td>
<td>0.87 (0.72–1.07)</td>
<td>&lt;4</td>
<td>36 (7)</td>
<td>2.72 (1.29–5.86)</td>
</tr>
<tr>
<td>≥8</td>
<td>400 (76)</td>
<td>0.79 (0.63–0.98)</td>
<td>&lt;8</td>
<td>123 (24)</td>
<td>1.71 (1.15–2.54)</td>
</tr>
<tr>
<td>≥12</td>
<td>290 (55)</td>
<td>0.65 (0.50–0.84)</td>
<td>&lt;12</td>
<td>233 (45)</td>
<td>1.49 (1.11–1.99)</td>
</tr>
<tr>
<td>≥16</td>
<td>208 (40)</td>
<td>0.62 (0.45–0.86)</td>
<td>&lt;16</td>
<td>315 (60)</td>
<td>1.23 (0.96–1.58)</td>
</tr>
<tr>
<td>≥20</td>
<td>134 (26)</td>
<td>0.49 (0.32–0.74)</td>
<td>&lt;20</td>
<td>389 (74)</td>
<td>1.16 (0.93–1.45)</td>
</tr>
</tbody>
</table>

HR FOR INTERACTION

3.1  2.2  2.3  2.0  2.4

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
- Tumor purity
- DNA quality, FFPE artefacts
- DNA input amounts
- Target region size
- Gene content
- Complexity of library prep
- Coverage
- Subtraction of germline events
- Cutpoint allelic frequency
- Mutation types
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 continuous 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

Budczies et al., 2019

approx. 10%
Clonality and subclonality in TMB

Genomic diversity comprising clonal and subclonal events affects TMB assessment in an entity-specific manner and is also influenced by therapy.

Granahan and Swanton, 2017
A first glimpse: Recommendations

# 1 Suitble sample
- Valid psTMB score

# 2 Insufficient amount of sample material
- Invalid psTMB score (mutations missed)

# 3 Insufficient tumor purity
- Invalid psTMB score (mutations missed)

# 4 No deduplication implemented
- Invalid psTMB score [artificial mutations]
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

**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
A composite biomarker approach

Effector cell compartment (TME)
- PD-L1 (directly or indirectly)
- GEP Signatures

Tumor compartment
- Positive genetic predictors: TMB; MSI-H (dMMR) and others
- Negative genetic predictors: SKT11/KB1, HLA loss and others

?Microbiome?
Acknowledgments

Peter Schirmacher
Jan Budczies
Daniel Kazdal
Michael Allgäuer
Volker Endris

Mark Kriegsmann, Jonas Leichenring, Anna-Lena Volckmar, Alexander Harms, Martina Kirchner, Katharina Kriegsmann, Olaf Neumann, Regine Brandt, Suranand B. Talla, Eugen Rempel, Carolin Plöger, Moritz von Winterfeld, Roland Penzel

Thoraxklinik at University Hospital Heidelberg:
Petros Christopoulos, Michael Thomas, Hauke Winter, Helge Bischoff, Michael Meister, Thomas Muley, Felix Herth

Friends of Cancer Research, Washington, DC:
Diana M. Merino, Mark Stewart, Jeff Allen

QuIP and all academic partners in this study

Friends of Cancer Research, Washington, DC:
Diana M. Merino, Mark Stewart, Jeff Allen

National Center for Tumor Diseases, Heidelberg:
Stefan Fröhling

Lausanne University:
Solange Peters

Francis Crick Institute, London:
Charles Swanton