ESMO ADVANCED COURSE ON LUNG CANCER IN IMMUNOTHERAPY
Biomarkers

John Haanen MD PhD
Zürich, 3-4 July 2019

Netherlands Cancer Institute, Amsterdam
DISCLOSURE OF INTEREST

I have provided consultation, attended advisory boards, and/or provided lectures for: Amgen, AZ/Medimmune, Bayer, BMS, GSK, Ipsen, Merck Serono, MSD, Novartis, Pfizer, Roche/Genentech, Sanofi, Seattle Genetics for which NKI received honoraria.

I am on the SAB of AIMM, Celsius Therapeutics, Immunocore and Neon Therapeutics. Financial compensation goes to NKI.

Through my work NKI received grant support from Bayer, BMS, MSD, Novartis, Neon Therapeutics, Pfizer.
Tumor foreignness
Mutational load

Tumor sensitivity to immune effectors
MHC expression
IFN-γ sensitivity

Absence of inhibitory tumor metabolism
LDH, glucose utilization

Absence of soluble inhibitors
IL6->CRP/ESR

Absence of Checkpoints
PD-L1

General immune status
Lymphocyte count

Immune cell infiltration
Intratumoral T cells

Blank et al. Science 2016

The Cancer Immunogram
Describing the state of Cancer - Immune interaction
Solid predictive biomarkers of response or resistance to IO treatment are lacking

What do we have so far?

- PD-L1 IHC
- Tumor Mutational Burden (WES/WGS or targeted panel seq)
- TIL (IHC)
- Gene Expression Profiling
Solid predictive biomarkers of response or resistance to IO treatment are lacking

What do we have so far?

- PD-L1 IHC
- Tumor Mutational Burden (WES/WGS or targeted panel seq)
- TIL (IHC)
- Gene Expression Profiling
PD-L1 on human tumor cells mediates T cell inhibition

Pardoll DM, Nat Rev Cancer 2012
Categorical vs continuous biological variable as predictive biomarker of therapeutic benefit

Ross Camidge et al., Nat Rev Clin Oncol 2019
# Five-drug PD-L1 assay trial-validated combinations

<table>
<thead>
<tr>
<th>Drug</th>
<th>Company</th>
<th>PD-L1 Diagnostic Ab Clone</th>
<th>Staining Platform</th>
<th>Clinically Relevant Cutoffs&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nivolumab</td>
<td>Bristol-Myers Squibb</td>
<td>28-8 (Dako)</td>
<td>Dako Link 48</td>
<td>TC ≥ 1%, 5%, and 10%</td>
</tr>
<tr>
<td>Pembrolizumab</td>
<td>Merck/Merck Sharp and Dohme</td>
<td>22C3 (Dako)</td>
<td>Dako Link 48</td>
<td>TC ≥ 1% and 50%</td>
</tr>
<tr>
<td>Atezolizumab</td>
<td>Genentech/Roche</td>
<td>SP142 (Ventana)</td>
<td>Ventana BenchMark ULTRA</td>
<td>TC ≥ 1%, 10%, and 50%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IC ≥ 1%, 5%, and 10%</td>
</tr>
<tr>
<td>Durvalumab</td>
<td>AstraZeneca</td>
<td>SP263 (Ventana)</td>
<td>Ventana Benchmark</td>
<td>TC ≥ 25%</td>
</tr>
<tr>
<td>Avelumab</td>
<td>Pfizer/Merck Serono</td>
<td>73-10 (Dako)</td>
<td>Dako Link 48</td>
<td>TC ≥ 1%, 50%, and 80%</td>
</tr>
</tbody>
</table>

<sup>a</sup>Variable according to trials and line of therapy.

Modified with permission from Tsao et al. PD-L1, programmed death ligand 1; Ab, antibody; TC, tumor cells by percentage staining for PD-L1; IC, percentage of tumor area infiltrated by PD-L1-positive immune cells.
PD-L1 as a Biomarker: A summum in complexity

Expression of PD-L1 is heterogeneous¹

- Inter and intratumor heterogeneity
- Inducible and dynamic (IFN, post-treatment)
- Cell type (immune cell versus tumor versus both)
- Location (membrane versus cytoplasm)

Challenges Surrounding Biomarker

- Epitope stability
- Distribution (patchy versus diffuse)
- Different antibodies and platforms
- Different thresholds for expression
- Interobserver readability

Logistics: Tissue¹,⁸,⁹

- Interval between tissue and treatment (archived versus fresh)
- Primary versus metastatic disease
- Some circumstances not amenable to obtaining any tissue

Abs are not identical: >25% discordant¹,⁶,⁷

IFN = interferon; PD-L1 = programmed death ligand 1.

PD-L1 Immunohistochemistry: Results from the Blueprint 2 project

Table 1. Reliability (Intraclass Correlation Coefficient) of Scoring PD-L1 Expression on Tumor Cells among All Pathologists (Excluding the Trainer) for All Cases and NSCLC Biopsy Samples/Resected Cases

<table>
<thead>
<tr>
<th>Assay</th>
<th>Glass Slide Scoring</th>
<th>Digital Scoring</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All Cases</td>
<td>NSCLC Only</td>
</tr>
<tr>
<td>22C3</td>
<td>0.89</td>
<td>0.88</td>
</tr>
<tr>
<td>28-8</td>
<td>0.92</td>
<td>0.94</td>
</tr>
<tr>
<td>SP-142</td>
<td>0.88</td>
<td>0.86</td>
</tr>
<tr>
<td>SP-263</td>
<td>0.89</td>
<td>0.92</td>
</tr>
<tr>
<td>73-10</td>
<td>0.93</td>
<td>0.95</td>
</tr>
<tr>
<td>All assays</td>
<td>0.86</td>
<td>0.89</td>
</tr>
</tbody>
</table>

PD-L1, programmed death ligand 1.
Potential explanations why PD-L1 expression might not predict benefit from PD-1/PD-L1 inhibition

<table>
<thead>
<tr>
<th>Hypothesis</th>
<th>Evidence</th>
<th>Potential explanations</th>
</tr>
</thead>
</table>
| PD-L1 expression apparently not necessary | PD-L1 absent by IHC but clinical benefit seen from inhibition of PD-1 or PD-L1 | • Spatial and/or temporal variability in PD-L1 expression within tumour (sampling error)  
• Incomplete sensitivity of IHC in the detection of PD-L1, with variation between assays (false-negative result)  
• PD-L2, the alternative ligand for PD-1, could provide a bypass mechanism for immune suppression, leading to responses of PD-L1+ tumours to anti-PD-1 antibodies, although in theory, not to anti-PD-L1 antibodies |
| PD-L1 expression apparently not sufficient | PD-L1 present by IHC but no clinical benefit from inhibition of PD-1 or PD-L1 | • Elevation in PD-L1 expression for reasons other than in response to a primed immune attack (for example, intrinsic induction in some oncogene-addicted NSCLCs)  
• Engagement of other immune checkpoints in addition to the PD-1–PD-L1 axis and/or immune suppression or deficiencies with different causes  
• The measured extent of PD-L1 positivity (a continuous variable) might be insufficient for a response to PD-1 or PD-L1 inhibition, reflecting substantial heterogeneity in the underlying tumour biology (including neoantigen profiles and mechanisms of immune escape) |

- PD-L1 IHC is a validated and approved biomarker
- It is the only ‘drug specific’ biomarker
- Clinically it can be reliably delivered
- It does enrich for treatment benefit

- But it is not perfect
- Implementation can be complicated
Solid predictive biomarkers of response or resistance to IO treatment are lacking

What do we have so far?

- PD-L1 IHC
- Tumor Mutational Burden (WES/WGS or targeted panel seq)
- TIL (IHC)
- Gene Expression Profiling
Estimate of the neoantigen repertoire in human cancers

Comparison between mutational load and neoantigen load: Not a 1:1 relationship per se
Evolvement of TMB over time as a potential biomarker of response to immunotherapy
Connection between TMB, neoantigens and immunotherapy
Clinical evidence demonstrating TMB as a biomarker for response to immunotherapy

<table>
<thead>
<tr>
<th>Immunotherapy agent and tumour type</th>
<th>Study/trial*</th>
<th>TMB assay used</th>
<th>Type of benefit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nivolumab</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSCLC (1 L)</td>
<td>CheckMate 026[^2]</td>
<td>WES</td>
<td>ORR, PFS</td>
</tr>
<tr>
<td>NSCLC</td>
<td>Flatiron Health[^17]</td>
<td>Foundation CGP panel</td>
<td>OS</td>
</tr>
<tr>
<td>Melanoma (1 L or 2 L)</td>
<td>CheckMate 038[^22]</td>
<td>WES</td>
<td>ORR, OS, PFS</td>
</tr>
<tr>
<td>Melanoma</td>
<td>CheckMate 064[^23]</td>
<td>WES</td>
<td>ORR, OS</td>
</tr>
<tr>
<td>Bladder</td>
<td>CheckMate 275[^83]</td>
<td>WES</td>
<td>ORR, OS, PFS</td>
</tr>
<tr>
<td>GBM</td>
<td>Bouffet et al, 2016[^118]</td>
<td>WES</td>
<td>DRR</td>
</tr>
<tr>
<td>Ipilimumab</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melanoma</td>
<td>Van Allen et al, 2015[^119]</td>
<td>WES</td>
<td>CBR</td>
</tr>
<tr>
<td></td>
<td>Snyder et al, 2014[^56]</td>
<td>WES</td>
<td>CBR, OS</td>
</tr>
<tr>
<td>Nivolumab and ipilimumab in combination</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSCLC (1 L)</td>
<td>CheckMate 012[^35]</td>
<td>WES</td>
<td>ORR, DCB, PFS</td>
</tr>
<tr>
<td>NSCLC (1 L)</td>
<td>CheckMate 2271[^8]</td>
<td>FoundationOne CDx</td>
<td>ORR, PFS</td>
</tr>
<tr>
<td>NSCLC (1 L)</td>
<td>CheckMate 568[^9]</td>
<td>FoundationOne CDx</td>
<td>ORR</td>
</tr>
<tr>
<td>SCLC (2 L)</td>
<td>CheckMate 032[^84]</td>
<td>WES</td>
<td>ORR, OS, PFS</td>
</tr>
<tr>
<td>Pembrolizumab</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSCLC (1 L)</td>
<td>KEYNOTE-001[^36]</td>
<td>WES</td>
<td>ORR, DCB, PFS</td>
</tr>
<tr>
<td>CRC</td>
<td>Le et al, 2015[^64]</td>
<td>WES</td>
<td>ORR, PFS</td>
</tr>
<tr>
<td>Multiple solid tumours</td>
<td>KEYNOTE-012/KEYNOTE-028[^120]</td>
<td>WES</td>
<td>ORR</td>
</tr>
</tbody>
</table>

[^118]: Büttner et al., ESMO Open 2019
TMB has predictive predictive value for IO activity across cancer types

Requirements for a response to anti-PD1: MSI-high tumors

A Biochemical Response

- Mismatch repair–proficient colorectal cancer
- Mismatch repair–deficient colorectal cancer
- Mismatch repair–deficient noncolorectal cancer

Change in Tumor Marker Level (%)

Days

Mismatch Repair–Deficient Noncolorectal Cancer
(N = 7)

<table>
<thead>
<tr>
<th>Type</th>
<th>Mismatch Repair–Deficient Noncolorectal Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 (14)*</td>
</tr>
<tr>
<td></td>
<td>4 (57)†</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2 (29)</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>71 (29–96)</td>
</tr>
<tr>
<td></td>
<td>Not reached</td>
</tr>
<tr>
<td></td>
<td>12 (10–13)</td>
</tr>
</tbody>
</table>
TMB correlates to tumor type specific response rate

\[ R = 0.74 \ (P < .001) \]
<table>
<thead>
<tr>
<th>Status</th>
<th>Test name</th>
<th>Number of genes</th>
<th>Coverage (Mb)*</th>
<th>Gene variants</th>
<th>Sample type</th>
</tr>
</thead>
<tbody>
<tr>
<td>FDA-approved or authorised diagnostic assays†</td>
<td>MSK-IMPACT[56 68]</td>
<td>468</td>
<td>1.5</td>
<td>SNVs, indels, rearrangements/ fusions, CNAs, parallel analysis of genomic signatures (eg, TMB and dMMR/MSI)</td>
<td>FFPE</td>
</tr>
<tr>
<td></td>
<td>Foundation Medicine FoundationOne CDx[34 49]</td>
<td>324</td>
<td>0.8</td>
<td>SNVs, indels, CNAs, select rearrangements, parallel analysis of genomic signatures (eg, TMB and dMMR/MSI)</td>
<td>FFPE</td>
</tr>
<tr>
<td>Commercial assays for research use only</td>
<td>Caris Molecular Intelligence[32]</td>
<td>592</td>
<td>1.4</td>
<td>Somatic missense mutations</td>
<td>FFPE</td>
</tr>
<tr>
<td></td>
<td>Illumina TruSight 500 gene panel[33]</td>
<td>500</td>
<td>2.0</td>
<td>SNVs and indels</td>
<td>FFPE</td>
</tr>
<tr>
<td></td>
<td>Thermo Fisher Scientific Oncomine Tumor Mutation Load Assay[37]</td>
<td>409</td>
<td>1.7</td>
<td>SNVs</td>
<td>FFPE</td>
</tr>
<tr>
<td></td>
<td>NEO New Oncology NEOplus v2 RUO[34]</td>
<td>&gt;340</td>
<td>1.1</td>
<td>SNVs, indels, fusions, CNAs, parallel analysis of TMB, MSI, and driver mutations</td>
<td>FFPE</td>
</tr>
<tr>
<td></td>
<td>Foundation Medicine FoundationOne[60]</td>
<td>315</td>
<td>1.1</td>
<td>SNVs, indels, CNAs, select gene rearrangements, genomic signatures for MSI and TMB</td>
<td>FFPE</td>
</tr>
<tr>
<td></td>
<td>Foundation Medicine bTMB assay[132]</td>
<td>394</td>
<td>1.1</td>
<td>SNVs</td>
<td>Blood</td>
</tr>
<tr>
<td></td>
<td>TruSight Tumor 170[135]</td>
<td>170</td>
<td>0.5</td>
<td>Fusions, splice variants, SNVs, indels, amplifications</td>
<td>FFPE</td>
</tr>
<tr>
<td></td>
<td>QIAGEN GeneRead DNaseq Comprehensive Cancer Panel[67]</td>
<td>160</td>
<td>0.7</td>
<td>SNVs, CNAs, indels, and fusions</td>
<td>FFPE</td>
</tr>
<tr>
<td></td>
<td>NEO New Oncology NEOplus[105 136]</td>
<td>94</td>
<td></td>
<td>SNVs, indels, CNAs, rearrangements, and fusions</td>
<td>FFPE</td>
</tr>
</tbody>
</table>
TMB is not correlated to PD-L1 Expression
Both biomarkers might be additive
First-Line Nivolumab Plus Ipilimumab in Advanced Non–Small-Cell Lung Cancer (CheckMate 568): Outcomes by Programmed Death Ligand 1 and Tumor Mutational Burden as Biomarkers

Neal Ready, MD, PhD; Matthew D. Hellmann, MD; Mark M. Awad, MD, PhD; Gregory A. Otterson, MD; Martin Gutierrez, MD; Justin F. Gainor, MD; Hossein Borghaei, DO; Jacques Jolivet, MD; Leora Horn, MD; Mihaela Mates, MD; Julie Brahmer, MD; Ian Rabinowitz, MD; Pavan S. Reddy, MD; Jason Chesney, MD, PhD; James Orcutt, MD; David R. Spigel, MD; Martin Reck, PhD; Kenneth John O’Byrne, MD; Luis Paz-Ares, MD, PhD; Wenhua Hu, PhD; Kim Zerba, PhD; Xuemei Li, MD; Brian Lestini, MD, PhD; William J. Geese, PhD; Joseph D. Szuslakowski, PhD; George Green, PhD; Han Chang, PhD; and Suresh S. Ramalingam, MD

Stage IV NSCLC
Non-Sq or Sq histology
No prior systemic therapy for stage IV disease
EGFR/ALK wild type
ECOG PS 0 or 1

Nivo 3 mg/kg Q2 wks + Ipi 1 mg/kg Q6 wks
N = Approximately 300
Enrollment stops when at least 120 PD-L1 positive and 100 negative subjects are treated

Treat until disease progression or unacceptable toxicity, or maximum 2 years
Progression free survival according to PD-L1 expression or TMB

Ready et al. J Clin Oncol 2019
### ORR following ipilimumab + nivolumab according to PD-L1 or TMB

<table>
<thead>
<tr>
<th>Variable</th>
<th>All Treated (N = 288)</th>
<th>&lt; 1% PD-L1 Expression (n = 114)</th>
<th>≥ 1% PD-L1 Expression (n = 138)</th>
<th>≥ 50% PD-L1 Expression (n = 68)</th>
<th>PD-L1 Expression Not Quantifiable (n = 36)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>86</td>
<td>17</td>
<td>57</td>
<td>34</td>
<td>12</td>
</tr>
<tr>
<td>Percentage of patients (95% CI)</td>
<td>29.9 (24.6 to 35.5)</td>
<td>14.9 (8.9 to 22.8)</td>
<td>41.3 (33.0 to 50.0)</td>
<td>50.0 (37.6 to 62.4)</td>
<td>33.3 (18.6 to 51.0)</td>
</tr>
<tr>
<td>Best overall response, No. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete response</td>
<td>7 (2.4)</td>
<td>3 (2.6)</td>
<td>4 (2.9)</td>
<td>3 (4.4)</td>
<td>0</td>
</tr>
<tr>
<td>Partial response</td>
<td>79 (27.4)</td>
<td>14 (12.3)</td>
<td>53 (38.4)</td>
<td>31 (45.6)</td>
<td>12 (33.3)</td>
</tr>
<tr>
<td>Stable disease</td>
<td>104 (36.1)</td>
<td>53 (46.5)</td>
<td>40 (29.0)</td>
<td>17 (25.0)</td>
<td>11 (30.6)</td>
</tr>
<tr>
<td>Progressive disease</td>
<td>76 (26.4)</td>
<td>36 (31.6)</td>
<td>31 (22.5)</td>
<td>13 (19.1)</td>
<td>9 (25.0)</td>
</tr>
<tr>
<td>Could not be determined</td>
<td>22 (7.6)</td>
<td>8 (7.0)</td>
<td>10 (7.2)</td>
<td>4 (5.9)</td>
<td>4 (11.1)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variable</th>
<th>&lt; 5 (n = 23)</th>
<th>≥ 5 to &lt; 10 (n = 27)</th>
<th>≥ 10 (n = 48)</th>
<th>≥ 10 to &lt; 15 (n = 20)</th>
<th>≥ 15 (n = 28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>2</td>
<td>4</td>
<td>21</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>Percentage of patients (95% CI)</td>
<td>8.7 (1.1 to 28.0)</td>
<td>14.8 (4.2 to 33.7)</td>
<td>43.8 (29.5 to 58.8)</td>
<td>50.0 (27.2 to 72.8)</td>
<td>39.3 (21.5 to 59.4)</td>
</tr>
<tr>
<td>Best overall response, No. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete response</td>
<td>0</td>
<td>1 (3.7)</td>
<td>4 (8.3)</td>
<td>2 (10.0)</td>
<td>2 (7.1)</td>
</tr>
<tr>
<td>Partial response</td>
<td>2 (8.7)</td>
<td>3 (11.1)</td>
<td>17 (35.4)</td>
<td>8 (40.0)</td>
<td>9 (32.1)</td>
</tr>
<tr>
<td>Stable disease</td>
<td>11 (47.8)</td>
<td>12 (44.4)</td>
<td>14 (29.2)</td>
<td>5 (25.0)</td>
<td>9 (32.1)</td>
</tr>
<tr>
<td>Progressive disease</td>
<td>6 (26.1)</td>
<td>7 (25.9)</td>
<td>13 (27.1)</td>
<td>5 (25.0)</td>
<td>8 (28.6)</td>
</tr>
<tr>
<td>Could not be determined</td>
<td>4 (17.4)</td>
<td>4 (14.8)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Receiver operating characteristic curves (ROC) of objective response rate by tumor programmed death ligand 1 (PD-L1) expression and tumor mutational burden (TMB)

No data were provided on using the combination of both biomarkers
Solid predictive biomarkers of response or resistance to IO treatment are lacking

What do we have so far?

• PD-L1 IHC
• Tumor Mutational Burden (WES/WGS or targeted panel seq)
• TIL (IHC)
• Gene Expression Profiling
Tumor Infiltrating Lymphocytes (TIL)

Diffuse infiltration with CD8+ TILs

Absence of TILs

Keck et al., Clin Canc Res 2014
Role for T cells in cancer

Intratumoral T Cells, Recurrence, and Survival in Epithelial Ovarian Cancer

Lin Zhang, M.D., Jose R. Conejo-Garcia, M.D., Ph.D., Dionysios Katsanos, M.D., Ph.D., Phyllis A. Gimotty, Ph.D., Marco Massobrio, M.D., Giorgia Regnani, M.D., Antonis Makrigiannakis, M.D., Ph.D., Heidi Gray, M.D., Katja Schienger, M.D., Ph.D., Michael N. Lieberman, Ph.D., Stephen C. Rubin, M.D., and George Coukos, M.D., Ph.D.

Objective Measurement and Clinical Significance of TILs in Non-Small Cell Lung Cancer

Kurt A. Schalper, Jason Brown, Daniel Carvajal-Hausdorf, Joseph McLaughlin, Vamsidhar Velcheti, Konstantinos N. Syrigos, Roy S. Herbst, David L. Rimm

Type, Density, and Location of Immune Cells Within Human Colorectal Tumors Predict Clinical Outcome

Jérôme Galon, et al.
Science 313, 1960 (2006);
DOI: 10.1126/science.1129139

Immunotype and Immunohistologic Characteristics of Tumor-Infiltrating Immune Cells Are Associated with Clinical Outcome in Metastatic Melanoma

The association of immune cell infiltrates with prognosis in cancer

Patients with a pre-existing immune response derive the most benefit from checkpoint inhibitors

Teff/IFNy: CD8A, GZMA, GZMB, CXCL9, EOMES, IFNg, CXCL10, T-bet
A single dose of neoadjuvant PD-1 blockade predicts clinical outcomes in resectable melanoma

Alexander C. Huang,1,2,3,4,16*, Robert J. Orlowski,1,13,16, Xiaowei Xu,4,5, Rosemarie Mick,2,4,6, Sangeeth M. George,1,2, Patrick K. Yan,1,2,7, Sasikanth Manne,2,7, Adam A. Kraya,1,4, Bradley Wubbenhorst,1,4, Liza Dorfman,1,4, Kurt D'Andrea,1,4, Brandon M. Wenz,1,4, Shujing Liu,4,5, Lakshmi Chilukuri,2,7, Andrew Kozlov,4,8, Mary Carberry,1,4, Lydia Giles,1,4, Melanie W. Kier,1, Felix Quagliarello,2,13, Suzanne McGettigan,1,4, Kristin Kreider,1,4, Lakshmanan Annamalai,9, Qing Zhao,1, Robin Mogg,1,4, Wei Xu,1,4, Wendy M. Blumenschein,9, Jennifer H. Yearley,9, Gerald P. Linette,1,2,3,4, Ravi K. Amaravadi,1,4, Lynn M. Schuchter,1,4, Ramin S. Herati,1,2, Bertram Bensch,2,15, Katherine L. Nathanson,1,2,3,4, Michael D. Farwell,4,8,17, Giorgos C. Karakousis,4,10,17, E. John Wherry,1,2,3,4,7,17*, and Tara C. Mitchell,1,4,17*
Early pathological response to neoadjuvant anti-PD-1 or anti-CTLA-4 + anti-PD-1 is correlated with improved survival

Huang et al., Nat Med 2019, Blank et al., Nat Med 2018; Amaria et al., Nat Med 2018
Early TIL infiltration following anti-PD-1 correlated with improved survival

Huang et al., Nat Med 2019
IFN-γ-related mRNA profile predicts clinical response to PD-1 blockade


The Journal of Clinical Investigation
Melanoma discovery set

19 patients

10-gene "Preliminary IFN-γ" signature developed to correlate with clinical response

IFN-γ score

Nonresponder Responder

Best overall response, RECIST v1.1

Melanoma discovery set

19 Patients

Melanoma validation set

62 Patients independent data set

Correlation matrix of top significant genes in the discovery set evaluated in the validation set

“Preliminary expanded immune" (28-gene) signature: coherent set correlated with the 10-gene "preliminary IFN-γ" signature genes (bolded text)
Tumor mutational burden does not correlate with T cell gene signature in any cancers among TCGA

Presented by Tom Gajewski at ASCO 2019
Integrated analysis of immune gene signature + TMB enriches for responders

Pan-tumor

SCCHN

Melanoma

Cristescu et al. Science 2018
Ines Pires da Silva et al: samples analyzed

- Samples n=92
  - Cutaneous n=77
    - Acral n=11
    - Mucosal n=4

Treatment

- Inhibitor: PD1 n=64
  - Response
    - Good=33
    - Poor=31
- Inhibitor: IPI+PD1 n=28
  - Response
    - Good=21
    - Poor=7

Sample collection before treatment

- WGS n=92
- RNAseq n=53
- IHC PD-L1/TILs n=41

SNV: single nucleotide variant
SV: structural variant
CNV: copy number variation

Presented by Tom Gajewski at ASCO 2019
Combined immune gene signature and TMB was best predictive biomarker

TMB and IFNg.6 score predictive model

AUC=0.83

Plan to study outliers and to identify mechanisms of resistance

Presented by Tom Gajewski at ASCO 2019
Changes in TIL score upon anti-CTLA4 or anti-PD-1 treatment in metastatic melanoma
The Cancer Immunogram

- **Tumor foreignness**
  - Mutational load

- **Tumor sensitivity to immune effectors**
  - MHC expression
  - IFN- sensitivity

- **Absence of inhibitory tumor metabolism**
  - LDH, glucose utilization

- **Absence of soluble inhibitors**
  - IL6->CRP/ESR

- **General immune status**
  - Lymphocyte count

- **Immune cell infiltration**
  - Intratumoral T cells

- **Absence of Checkpoints**
  - PD-L1

**Multiparameter biomarkers are key**

Blank et al. Science 2016
An evolving immunogram: increasing complexity requiring big data

Tumor foreignness
Mutational load
Neoantigen load

Tumor sensitivity to immune effectors
MHC expression
IFN- sensitivity
Heterozygocity at all MHC loci

Absence of inhibitory tumor metabolism
LDH, glucose utilization
IDO, TDO, tumor acidity
Argenase, glutamase
CD39 → CD73 → adenosine

Absence of soluble inhibitors
IL-6 → CRP/ESR
IL-8, IL-1β

Absence of checkpoints
PD-L1
PD-L2

General immune status
Lymphocyte count
LAG3, TIGIT, CD27
Ki67 PD1+ CD8; NLR

Microbiome
Diversity of microbiome

Immune cell infiltration
Intratumoral T cells
T cell clonality
B7-H3/CD276 macrophages
CCL5 attracting T cells
B-cells, CXCL13 Tertiary Lymphoid Structures
CCR5 expressing BATF3 DC
PD-1, LAG3, TIM3, TIGIT, CTLA-4, CD39
GEP: IFN-γ, TIL, TIS
β-catenin, p53, Myc, PTEN, LKB1

Adapted from Blank Science 2016
Gut microbiome modulates response to anti–PD-1 immunotherapy in melanoma patients.
Manipulation of the Gut Microbiome to Enhance Responses to Cancer Immunotherapy

<table>
<thead>
<tr>
<th>Trial Number</th>
<th>Patient Population</th>
<th>Intervention</th>
<th>Outcome(s)</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT02843425</td>
<td>all cancer patients treated at MDACC</td>
<td>addition of ½ cup beans per day to regular diet in a crossover design</td>
<td>primary: change in fecal microbiome profile from baseline (via 16S profiling)</td>
<td>open and recruiting (MDACC)</td>
</tr>
<tr>
<td>NCT02079682</td>
<td>stages II and III breast cancer patients treated at MDACC ages 18+</td>
<td>randomized intensive lifestyle change (diet, exercise, psychosocial)</td>
<td>primary: disease-free survival (DFS) secondary: change in fecal and oral microbiome (via 16S profiling)</td>
<td>open and recruiting (MDACC)</td>
</tr>
<tr>
<td>NCT01895530</td>
<td>CRC patients ages 18+ undergoing elective CRC resection</td>
<td>randomized probiotic (S. Boulardii) administration</td>
<td>primary: cytokine expression in colonic mucosa (via qPCR) secondary: post-operative complications</td>
<td>completed (Consoli et al., 2016)</td>
</tr>
<tr>
<td>NCT03072641</td>
<td>CRC patients ages 18+</td>
<td>randomized probiotic (Probiot Clinica B. lactis BI-04, L. acidophilus NCFM + Inulin) administration</td>
<td>primary: change in fecal and tumor microbiota from baseline secondary: changes in epigenetic patterns of tumor tissue from baseline</td>
<td>completed (Hibberd et al., 2017)</td>
</tr>
<tr>
<td>NCT03356511</td>
<td>post-menopausal breast cancer patients stages I-II</td>
<td>single-arm probiotic (Primal Defense Ultra multi-strain probiotic formula) administration</td>
<td>primary: change in mean number of CD8+ cells from baseline</td>
<td>open and recruiting (Mayo Clinic)</td>
</tr>
<tr>
<td>NCT029928523</td>
<td>acute myeloid leukemia patients ages 18-65 treated with intensive chemo and antibiotics</td>
<td>single-arm autologous FMT (frozen inoculum)</td>
<td>primary: diversity of the gut microbiome, multi-drug-resistant bacteria eradication secondary: signature of dysbiosis of gut microbiome</td>
<td>ongoing, closed to recruiting (France)</td>
</tr>
<tr>
<td>NCT03350422</td>
<td>metastatic melanoma patients ages 18+ who previously failed standard therapies</td>
<td>single-arm FMT (colonoscopy or gastroscopy) from patient donors who responded to immunotherapy</td>
<td>primary: safety (AEs associated with FMT), engraftment of FMT secondary: changes in immune cell populations and activity, objective response rate</td>
<td>open and recruiting (Israel)</td>
</tr>
</tbody>
</table>
Hierarchical loss of effector functions and increased inhibitory receptor expression in exhausted T cells

Testing the progressive exhaustion model in human NSCLC

Wherry et al, 2011, Nat Immunol
Hierarchical loss of effector functions and increased inhibitory receptor expression in exhausted T cells

Would the presence of cells with low expression of dysfunction-associated molecules be the best biomarker?

Testing the progressive exhaustion model in human NSCLC

Wherry et al, 2011, Nat Immunol
Adapted from Ghoneim et al, Trends in Mol Med, 2016
Standardized identification of T cells with different PD-1 expression levels in NSCLC

Thommen et al. Nat Med, 2018
PD-1\textsuperscript{T} TILs show loss of effector function

Sorting of TIL subsets from NSCLC specimens

Functional analyses  RNA sequencing

Impaired secretion of classical effector cytokines by PD-1\textsuperscript{T} TILs

Thommen et al. Nat Med, 2018
A novel function of PD-1\textsuperscript{T} CD8 T cells in the TME

Expression of CXCL13 mRNA in PD-1\textsuperscript{T} TILs

PD-1\textsuperscript{T} TILs produce and secrete CXCL13 protein

Thommen et al. Nat Med, 2018
PD-1^T TILs have an increased capacity for tumor recognition

PD-1^T TILs upregulate genes involved in proliferation and T cell activation

Antigen-driven expansion of PD-1^T TILs?

PD-1^T TILs are highly tumor reactive

Sorting of TIL subsets

Co-Culture with autologous digest

Expansion in high-dose IL2

PD-1^T TILs have an increased capacity for tumor recognition

Antigen-driven expansion of PD-1^T TILs?

PD-1^T TILs are highly tumor reactive

Thommen et al. Nat Med, 2018
Chronic antigen exposure +?

Biomarker for response to anti-PD-1 therapy?

Chronic viral infection

- Responding to anti-PD-1
- NOT responding to anti-PD-1
  - Partially exhausted
  - Terminally exhausted

Exhaustion

Cancer

- No tumor reactivity
- Tumor reactivity
  - PD-1^−
  - PD-1^+
  - PD-1^−

Dysfunction

Biomarker for response to anti-PD-1 therapy?

Tumor reactivity

Chronic antigen exposure
Algorithm-based digital image analysis for quantification of PD-1 expression levels

- **Development PD-1\(^T\) detection algorithm**
  - Flow cytometry
  - Immunohistochemistry
  - Definition PD-1 subsets
  - Setup Algorithm
  - [%] PD-1\(^T\) TILs by flow = [%] PD-1\(^T\) TILs by IHC

- **PD-1\(^T\) TIL quantification using HALO™**
  - Conventional IHC

- **Algorithm-based detection**
  - Test cohort initial staining

- **Thommen et al. Nat Med, 2018**
Presence of PD-1$^+$ TILs correlates with response and survival upon PD-1 blockade

- 21 patients treated with anti-PD-1 therapy
- Stage IV NSCLC
- No previous IT treatment
- Pathologists were blinded to treatment outcome

Correlation with clinical outcome

- PD-1$^+$ TILs correlate with response and survival upon PD-1 blockade
- Median OS
  - PD-1$^+$ >1%: Not reached
  - PD-1$^+$ <1%: 193 days
- HR=0.16, **p<0.005

Thommen et al. Nat Med, 2018
Cancer Immunogram: a multiparameter framework to check cancer-immune interactions

Working towards a complex multifaceted biomarker to predict response to IO
Unlike predictive biomarkers for targeted therapy, biomarkers for response to IT will be complex:

• Not one single marker predicts response/resistance
• Often no good cut off (PD-L1, TMB, TIL, GEP) as these are continuous biomarkers
• Combination of markers is being examined and appears better than single biomarkers
• New potential biomarkers are emerging:
  • Microbiome
  • PD-1
  • TLS, CXCL13
  • Myeloid markers
  • Metabolic biomarkers
  • TCR clonality
• We are in need of liquid biomarkers!
• We should start looking at early time points following IT (change timing of liquid and tumor biopsies)