Biomarkers for Immunotherapy: Any Hope of Progress?

Prof. Dr. med. Viktor Grünwald

Medizinische Hochschule Hannover

Klinik für Hämatologie, Hämostaseologie, Onkologie und Stammzelltransplantation
The role of surrogates and how important is response in mRCC
Most trial-level validation studies of surrogate endpoints in oncology find low correlations with survival. All validation studies use only a subset of available trials. The evidence supporting \textit{the use of surrogate end points in oncology is limited}. 
Disease control is valuable for HD-IL2 treated patients

Table 3 Landmark analysis of 1-, 2- and 3-year survival based on initial response to one course of high-dose IL-2 therapy

<table>
<thead>
<tr>
<th>Percent survival (%)</th>
<th>Progressive disease (PD) (%)</th>
<th>Stable disease (SD) (%)</th>
<th>Partial response or complete response (PR/CR) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>One year</td>
<td>36.2</td>
<td>92.5</td>
<td>92.0</td>
</tr>
<tr>
<td>Two year</td>
<td>9.9</td>
<td>70.5</td>
<td>87.5</td>
</tr>
<tr>
<td>Three year</td>
<td>3.6</td>
<td>47.5</td>
<td>78.3</td>
</tr>
</tbody>
</table>

Responders perform better than other patients (sunitinib; RCC)

N=1059

Non-responders include patients with SD as best response

http://doi.org/10.1016/j.ejca.2013.08.021
Lead-time bias

Tumor shrinkage is prognostic (6-mo. landmark)

<table>
<thead>
<tr>
<th>Overall cohort</th>
<th>Overall survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$p$ value</td>
</tr>
<tr>
<td>Overall cohort</td>
<td></td>
</tr>
<tr>
<td>$-100%$ to $-60%$</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>$-60%$ to $-30%$</td>
<td>0.005</td>
</tr>
<tr>
<td>$-30%$ to $&lt;0%$</td>
<td>Reference</td>
</tr>
<tr>
<td>0% to $&lt;+20%$</td>
<td>0.002</td>
</tr>
<tr>
<td>$\geq +20%$</td>
<td>0.011</td>
</tr>
<tr>
<td>No post-baseline imaging</td>
<td>0.5</td>
</tr>
</tbody>
</table>

PBI: post baseline imaging

Tumor shrinkage is independent from type of therapy

TKIs (n=1773)

Temsriolimus (n=416)

Interferon (n=560)

<table>
<thead>
<tr>
<th>Agent</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sunitinib</td>
<td>1059</td>
</tr>
<tr>
<td>Axitinib</td>
<td>359</td>
</tr>
<tr>
<td>Sorafenib</td>
<td>355</td>
</tr>
<tr>
<td>Temsirolimus ±IFN</td>
<td>416</td>
</tr>
<tr>
<td>IFN</td>
<td>560</td>
</tr>
</tbody>
</table>

Grünwald V et al. ESMO 2013, Amsterdam, #2702
Early tumor shrinkage (eTS) is prognostic in mRCC

**Table 2. Optimal thresholds of eTS.**

<table>
<thead>
<tr>
<th></th>
<th>Optimal Threshold (Sensitivity / Specificity)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OS</td>
</tr>
<tr>
<td>Overall population (N=4334)</td>
<td>8% (0.603 / 0.632)</td>
</tr>
<tr>
<td>Type of therapy</td>
<td></td>
</tr>
<tr>
<td>VEGF-targeted agenta (n=2905)</td>
<td>10% (0.653 / 0.659)</td>
</tr>
<tr>
<td>Temsirolimusb (n=943)</td>
<td>12% (0.616 / 0.621)</td>
</tr>
<tr>
<td>IFN-α (n=486)</td>
<td>1% (0.712 / 0.508)</td>
</tr>
<tr>
<td>Line of therapy</td>
<td></td>
</tr>
<tr>
<td>1st-line (n=2763)</td>
<td>8% (0.596 / 0.621)</td>
</tr>
<tr>
<td>2nd-line (n=1571)</td>
<td>5% (0.673 / 0.704)</td>
</tr>
</tbody>
</table>

*a Includes sunitinib, axitinib, sorafenib, and bevacizumab + temsirolimus. 
*b Includes single-agent temsirolimus and temsirolimus + IFN-α.

eTS=early tumor shrinkage; IFN=interferon; OS=overall survival; PFS=progression-free survival; VEGF=vascular endothelial growth factor.
Role of response in mRCC

- Tumor shrinkage is an important treatment effect
- Optimal threshold for prognostication varies between agents used
- Threshold of tumor shrinkage seems different for immune therapies vs. TKI
- Response rate is an adequate surrogate of anti-tumor efficacy
Can we predict response?
TCC: immune staining is associated with better OS

Rosenberg et al. Lancet 2016
Amount of tumor shrinkage matters in IC2/3+ TCC

Response to atezolizumab shown in IC2/3+ patients according to RECIST defined responses

Rosenberg et al. Lancet 2016
Genetic instability – possible confounder of response?

RCC: less variability in the amount of somatic mutations
MMR deficiency - a putative surrogate for response to pembrolizumab

A  Progression-free Survival in Cohorts with Colorectal Cancer

B  Overall Survival in Cohorts with Colorectal Cancer

Genetic instability as predictor for ipilimumab

Melanoma B Survival in Discovery Set

- >100 mutations (N=17)
- ≤100 mutations (N=8)

P=0.04 by log-rank test

Snyder et al. NEJM 2014
Neoantigens are predictive for ipilimumab

Snyder et al. NEJM 2014
What is the concept behind neoantigens/mutations?

What is its predictive nature for immune therapies?
Neoantigens in cancer immunotherapy

Ton N. Schumacher and Robert D. Schreiber

The clinical relevance of T cells in the control of a diverse set of human cancers is now beyond doubt. However, the nature of the antigens that allow the immune system to distinguish cancer cells from noncancer cells has long remained obscure. Recent technological innovations have made it possible to dissect the immune response to patient-specific neoantigens that arise as a consequence of tumor-specific mutations, and emerging data suggest that recognition of such neoantigens is a major factor in the activity of clinical immunotherapies. These observations indicate that neoantigen load may form a biomarker in cancer immunotherapy and provide an incentive for the development of novel therapeutic approaches that selectively enhance T cell reactivity against this class of antigens.
Identification of immunogenic antigens

No predictive mutational signature for nivolumab response (RCC)

N=9 (ORR: 3/9)

- 1 outlier revealed up-regulation of immune related genes (PD-L1, PD-L2, PD-1, CTLA4, PRF-1, CD8A, GZMA, BTLA)
- Immune related genes may relate to response
Tumor heterogeneity during carcinogenesis

A. Linear evolution

B. Clonal separation (allopatric speciation)

C. Clonal competition (antagonist evolution)

D. Clonal cooperation (symbiotic evolution)

Antagonism

Cooperation

McGranahan & Swanton Cancer Cell 2015
Penetrance of antigens is essential for efficacy of immune therapies

- antigen penetrance varies
- immune response detects only cells with (neo)antigen
- subclonal neoantigens may hamper response
- DTIC induces mutations
- But, they are mainly subclonal (melanoma)
- This may be deliterious for response

McGranahan et al. Science 2016
Chemotherapy induced cell death induces antigen release

CAVE: not all antigens are immunogenic

PD-L1 expression
PD-L1 expression – a negative prognostic marker

Meta-analysis: PD-L1 is prognostic

Metaanalysis: N=1323, ccRCC/nccRCC, stages I-IV

*PD-L1-expression is higher in pts. with ccRCC*
PD-L1 Expression: a negative prognostic marker for TKI treatment


<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Median OS, months (95% CI)</th>
<th>HR</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pazopanib low</td>
<td>35.6 (27.2, 40.8)</td>
<td>1.52</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Pazopanib high</td>
<td>15.1 (9.4, 45.1)</td>
<td>1.55</td>
<td>0.0005</td>
</tr>
<tr>
<td>Sunitinib low</td>
<td>27.8 (23.7, 32.9)</td>
<td>1.43</td>
<td>0.028</td>
</tr>
<tr>
<td>Sunitinib high</td>
<td>15.3 (11.2, 30.5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P = 0.0302

HR: Hazard Ratio

Organe >2 vs. ≤2
KPS 70-80 vs. 90-100
PD-L1 HS>55 vs. HS≤55
<table>
<thead>
<tr>
<th>Agent</th>
<th>Nivolumab</th>
<th>Pembrolizumab</th>
<th>Atezolizumab</th>
<th>Durvalumab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mechanism</td>
<td>PD-1</td>
<td>PD-1</td>
<td>PD-L1</td>
<td>PD-L1</td>
</tr>
<tr>
<td>Test (Ab clone)</td>
<td>28-8 (DAKO)</td>
<td>22C3 (DAKO)</td>
<td>SP142 (Ventana)</td>
<td>SP263 (Ventana)</td>
</tr>
<tr>
<td>Staining</td>
<td>Tumor</td>
<td>Tumor/Stroma</td>
<td>Infiltrating immune cells and/or tumor</td>
<td>Tumor</td>
</tr>
<tr>
<td>Tissue</td>
<td>archiviert</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cut-offs</td>
<td>≥1%, or ≥5%, or ≥10%</td>
<td>≥50%</td>
<td>intensity: 3: TC ≥50%/IC ≥10% 2-3: ≥5% +: ≥1% -: &lt;1%</td>
<td>≥25%</td>
</tr>
</tbody>
</table>

Ab, antibody; IHC, immunohistochemistry; PD-L1, programmed death ligand-1.
mAbs detect different domains of PD-L1

2A3, 5A4, 7G11, 015

9A11, E1L3N, SP142

Figure 1.
Domain specificity of PD-L1 mAbs. 9A11, E1L3N, and SP142 recognize an epitope in the cytoplasmic domain of PD-L1, whereas most other mAbs used for therapeutics, flow cytometry, and IHC recognize an epitope in the extracellular domain of PD-L1, including 2A3, 5A4, 7G11, and 015.

Papillary RCC are „cold“ tumors

ccRCC

Papillary RCC

No. at Risk
Both Negative   93  46  35  20
Either Positive 41  24  13  9  1
Both Positive   8   2

No. at Risk
Negative  173  101  37  6
Positive  11   4   1

No. at Risk
Negative  61   34  11  3
Positive  123  71  27  4
Regions within 1 tumor are heterogenous

McLaughlin et al. Jama Oncol 2015
PD-L1: disconcordant in 21%

Table 3. PD-L1 expression in primary tumors versus corresponding metastases

<table>
<thead>
<tr>
<th>Metastases</th>
<th>PD-L1</th>
<th>PD-L1$^+$</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary tumors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD-L1$^-$</td>
<td>33</td>
<td>3</td>
<td>36</td>
</tr>
<tr>
<td>PD-L1$^+$</td>
<td>8</td>
<td>9</td>
<td>17</td>
</tr>
<tr>
<td>Total</td>
<td>41</td>
<td>12</td>
<td>53</td>
</tr>
</tbody>
</table>

N=54
Intraindividual variability: PD-L1

automated quantitative analysis of PD-L1. N=34 (promiry & mets.; RCC)

Metastases have higher PD-L1 scores

Poor correlation of PD-L1 expression between primary & mets. (R=0.24)

Expression levels in primaries may not be used to predict PD-1i outcome in metastatic disease

No correlation between PD-L1 status and response to treatment

- 90% (NIVO) and 94% (EVE) with quantifiable PD-L1
- Presumably mainly primaries included
- Median time from diagnosis to randomization: 32 mo.

Motzer et al. NEJM 2015
PD-L1 expression is not predictive for ORR in mRCC

First Author, PY, Clinical Trial name

Amin, 2014, CA209-016 (P arm)
Amin, 2014, CA209-016 (S arm)
Choueiri, 2014, CA209-009
Motzer, 2015, CA209-010

Summary OR for objective clinical response:
1.70 (95%CI: 0.32, 9.02)
I²=33%

Fig. 4. Forest plot of ORs of clinical response in PD-L1+ (cut-off > 5%) vs. PD-L1- treated renal cell cancer patients.
PD-L1 is dynamic – response to treatment

Lieu et al. ESMO 2014

Arm A 1L RCC Patient

<table>
<thead>
<tr>
<th>Primary or Metastatic Tissue</th>
<th>Visit</th>
<th>IC %</th>
<th>IC Score</th>
<th>TC %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metastatic</td>
<td>Pretreatment</td>
<td>&lt;1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Metastatic</td>
<td>C3</td>
<td>10</td>
<td>3</td>
<td>40</td>
</tr>
</tbody>
</table>

Arm A Melanoma Patient

<table>
<thead>
<tr>
<th>Primary or Metastatic Tissue</th>
<th>Visit</th>
<th>IC %</th>
<th>IC Score</th>
<th>TC %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metastatic</td>
<td>Pretreatment</td>
<td>&lt;1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Metastatic</td>
<td>C3</td>
<td>8</td>
<td>2</td>
<td>50</td>
</tr>
</tbody>
</table>

a Dr. Johanna Bendell, Sarah Cannon Research Institute.
b Dr. John Powderly, Carolina BioOncology.
VEGF inhibition triggers PD-L1 expression
The microbiom may interact with immune response

- **Bacteroides thetaiotaomicron** or **B. fragilis** modulate CTLA-4 response
- **Microbiom** affects Th1 immune response
Composition of immune environment has prognostic potential

Tumor metabolism alters immune cell activity

Different approaches in non-immunogenic tumors needed

**hot tumor**

- Tumor cell with PD-L1 expression
- CD8 T cell with granzyme B expression
- CD4 T cell

**Immunogenic tumor microenvironment**

- Immune checkpoint therapy and durable clinical benefit

**cold tumor**

- Nonimmunogenic tumor microenvironment
- Combination therapies with agents that create immunogenic tumor microenvironment and immune checkpoint therapy
- CD8 T cell with CD45RO expression
- CD8 T cell with PD-L1 expression
- CD4 T cell

- Durable clinical benefit

---

PD-1 inhibition may have direct anti-tumor efficacy

Kleffel et al., 2015, Cell 162, 1242–1256
Conclusion

• Surrogates are important tools in oncology
• Tumor shrinkage is an important event of cancer treatment, irrespective of agent used
• Immune markers can predict response
• PD-L1 is not an optimal marker
• Composition of the immune environment is key
• Mutational load is not the solution for predictivity
• Identification of immunogeneic mutations would be of great help
• A panel of markers for immune therapies is needed
Is there any progress on markers for immunotherapies?

Yes

Is there any hope on a future marker panel?

Yes, but this is a long journey