THE SEARCH FOR BIOMARKERS IN BLADDER CANCER

CDDP and IO WORLD

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DISCLOSURE OF INTEREST

- **Advisory role:**
  - MSD, Pfizer, BMS, Astellas, Janssen, Clovis, Bayer, Roche

- **Speaker role:**
  - Pfizer, MSD, Astellas, Sanofi Aventis, Janssen, Bayer, BMS, Roche, AstraZeneca

- **Research funding:**
  - Takeda, Pfizer, MSD
CHEMOTHERAPY (CDDP)
Biomarkers
Molecular determinants of response to cisplatin-based neoadjuvant chemotherapy

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Predictive value</th>
<th>Biomarker status good outcome</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>p53</td>
<td>Controversial</td>
<td>Non overexpression</td>
<td>Related with higher RR to neoadjuvant MVAC</td>
<td>[11]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Related to better DFS and OS to RT-cisplatin</td>
<td>[12]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Not related with higher RR to neoadjuvant MVAC</td>
<td>[4]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Not related to better OS to RT-cisplatin</td>
<td>[10,13]</td>
</tr>
<tr>
<td>p21 and p53</td>
<td>Prognostic value</td>
<td>Non overexpression</td>
<td>Related to better DFS or OS to RT-cisplatin</td>
<td>[12]</td>
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<tr>
<td>pRB</td>
<td>No</td>
<td>Non overexpression</td>
<td>Not related to better DFS or OS to RT-cisplatin</td>
<td>[12]</td>
</tr>
<tr>
<td>Bcl2</td>
<td>Yes, not validated</td>
<td>Non overexpression</td>
<td>Related with better OS in cisplatin and radiotherapy-treated patients</td>
<td>[14]</td>
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<tr>
<td>Mdm-2</td>
<td>No</td>
<td>Non overexpression</td>
<td>Not related with pathologic downstaging after neoadjuvant MVAC</td>
<td>[15]</td>
</tr>
<tr>
<td>Ki67</td>
<td>No</td>
<td>Overexpression</td>
<td>Trend to better PFS and OS after neoadjuvant MVAC</td>
<td>[16]</td>
</tr>
<tr>
<td>XAF-1</td>
<td>Yes, not validated</td>
<td>High mRNA expression</td>
<td>Related with higher RR and PFS after neoadjuvant GC</td>
<td>[17]</td>
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<tr>
<td>VEGF</td>
<td>Prognostic value</td>
<td>Low expression</td>
<td>Related with better DFS after neoadjuvant MVAC</td>
<td>[18,19]</td>
</tr>
<tr>
<td>Her2</td>
<td>Yes, not validated</td>
<td>Non overexpression</td>
<td>Related with higher CR after RT-cisplatin</td>
<td>[20]</td>
</tr>
<tr>
<td>BCRA1</td>
<td>Yes, not validated</td>
<td>Low mRNA expression</td>
<td>Related with higher RR after neoadjuvant cisplatin regimen</td>
<td>[25]</td>
</tr>
<tr>
<td>ERCC-1</td>
<td>Yes, not validated (RT-Cisplatin)</td>
<td>Low expression</td>
<td>Related with higher RR after RT-cisplatin regimen</td>
<td>[29]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Not related with higher RR after neoadjuvant cisplatin regimen</td>
<td>[30,31]</td>
</tr>
<tr>
<td>14-gene expression</td>
<td>Yes, validated</td>
<td>Positive score</td>
<td>Related with higher RR after MVAC</td>
<td>[32,35]</td>
</tr>
<tr>
<td>20-gene expression</td>
<td>Prognostic value, validated</td>
<td>Cutoff high/low risk</td>
<td>Related with risk of nodal involvement in neoadjuvant-treated patients</td>
<td>[33]</td>
</tr>
</tbody>
</table>

Bellmunt J, Curr Opin Urol 2013, 23:466–471
• N=50
• DNA extracted from pre-NAC specimens.
• WES + correlation with pathologic response to NAC.
• Identification of genes selectively mutated in responders vs non-responders
• Nucleotide excision repair genes among others

Markers of pathologic response to neoadjuvant cisplatin-based chemotherapy

Data from 2 phase II trials NAC N=48, WES

**pCR**: 80% in ERCC2mut
31% in ERCC2wt
(p=0.01)

*Liu D. JAMA Oncol 2016*

ERCC2 mutations associated with pCR →
Bladder sparing approach for those with somatic ERCC2 mutations planned to be prospectively investigated
Markers of pathologic response to neoadjuvant cisplatin-based chemotherapy

Patients with pCR had more genomic alterations than those with RD (p=0.024)

N=34 (NAC)

<table>
<thead>
<tr>
<th>Gene</th>
<th>P value</th>
<th>Residual Disease</th>
<th>Complete Response (pT0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATM</td>
<td>0.001542</td>
<td>22</td>
<td>1</td>
</tr>
<tr>
<td>RB1</td>
<td>0.001542</td>
<td>No Variants</td>
<td>1</td>
</tr>
<tr>
<td>FANCC</td>
<td>0.050980</td>
<td>0</td>
<td>13</td>
</tr>
</tbody>
</table>

ATM/RB1/FANCC alterations also predictive of better OS


DNA repair gene variants associated with pCR
Is the ERCC2 genomic test ready for prime time?

- ERCC2 is linked to pCR in only around 40% of patients across studies
- No “hot spot” mutation. Not all the mut are drivers
- pCR is seen in ERCC2 negative pts
- Tumor heterogeneity is an issue in all tumors
- We can only enrich the positive prediction
RNA extracted from pre-NAC specimens. N=27.
cDNA microarray obtaining via RT-PCR consisting of 27,648 genes.
Identification of genes expressed differently between responders and non-responder tumors.
We developed a gene expression model to predict the pathological node status from primary tumor tissue in 3 independent cohorts of patients who were clinically node negative.

- Cutoff system identified patients with high RR (1.74) and low RR (0.70) of N+ disease.
- Multivariate analysis: GEM predictor independent of age, sex, pathological T stage, and lymphovascular invasion.
- Potential to select high-risk patients for NAC while sparing the rest from patients toxic effects and delay to cystectomy.
SWOG TRIAL: COXEN-directed neoadjuvant chemotherapy
Prospective validation of the COXEN biomarker to predict pT0/pT1

Muscle-Invasive Bladder Cancer SWOG 8710 criteria - T2-T4a N0M0, cisplatin eligible

Cystectomy to assess pT0 or pT1 pathology

Correlate with COXEN prediction pT0/pT1

COXEN Model Predicting response to chemotherapy
Cisplatin, Gemcitabine, Methotrexate, Doxorubicin, Vinblastine

Gene Expression Model

NCI-60 Cell Line Panel (IC50)

Human Bladder Cancer Cell Lines

Bladder Cancer patient samples

MVAC (N=16)

P = 0.0469

GC (N=14)

P = 0.0303
Whole transcriptome profiling
Pre-NAC TURb samples
N= 343 MIBC
Classified according to four published molecular subtyping methods.
Outcome after NAC varies by molecular subtype.

Luminal tumors had the best prognosis, irrespective of the treatment strategy, implying that these patients may not need to receive NAC.

Luminal immune-infiltrated tumors did significantly worse than those with luminal non-infiltrated tumors. Patients with luminal-infiltrated tumors appear to have poor prognosis with and without NAC.

Patients with basal tumors appear to derive the most benefit from NAC.

Patients with claudin-low tumors had the worst prognosis irrespective of treatment strategy, suggesting also that these patients derived little or no benefit from NAC.

IMMUNOTHERAPY

Biomarkers
## Summary of FDA-Approved and Investigational PD-L1 Assays in Urothelial Carcinoma*

<table>
<thead>
<tr>
<th>Ab clone/ epitope</th>
<th>Pembrolizumab$^1$</th>
<th>Atezolizumab$^{2,3}$</th>
<th>Nivolumab$^4$</th>
<th>Durvalumab$^5$</th>
<th>Avelumab$^6$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell type scored</td>
<td>TCs and ICs</td>
<td>ICs</td>
<td>TCs</td>
<td>TCs or ICs</td>
<td>TCs</td>
</tr>
<tr>
<td>Scoring method</td>
<td>CPS: % of PD-L1 positive TCs and ICs relative to the total number of tumor cells</td>
<td>% of PD-L1 expressing ICs</td>
<td>% of PD-L1 expressing TCs</td>
<td>% of PD-L1 expressing TCs or ICs</td>
<td>% of PD-L1 expressing TCs</td>
</tr>
<tr>
<td>FDA status for urothelial carcinoma</td>
<td>NA</td>
<td>Complementary</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>PD-L1 thresholds under evaluation</td>
<td>≥1%, ≥10%</td>
<td>IC2/3 (≥5%), IC1 (≥1% but &lt;5%), IC0 (&lt;1%)</td>
<td>≥1%, ≥5%</td>
<td>≥25%</td>
<td>≥5%</td>
</tr>
</tbody>
</table>

* No head-to-head studies have been conducted and direct comparisons cannot be made between these studies.

PD-L1 Expression as a Predictor of Checkpoint Blockade Sensitivity in UC


6/9 studies reported positive association with PD-L1 staining
Opposite results in the cis-ineligible 1st line single arm trials

Balar AV, et al. J Clin Oncol 36, 2018 (suppl; abstr 4523)

In KN052 – Cisplatin ineligible front line pembrolizumab, low PDL1 (CPS <10) patients were 74% of the study population and had worse median OS.

In contrast, in IMvigor210 – Cisplatin ineligible front line atezolizumab - low PDL1 (IC0/1) patients were 70% of the study population and had similar to slightly better median OS.
FDA limits the use of Tecentriq and Keytruda for some urothelial cancer patients

FDA has limited the use of Tecentriq and Keytruda for patients with locally advanced or metastatic urothelial cancer who are not eligible for cisplatin-containing therapy.

The Agency took this action on June 19, 2018, due to decreased survival associated with the use of Keytruda (pembrolizumab) or Tecentriq (atezolizumab) as single therapy (monotherapy) compared to platinum-based chemotherapy in clinical trials to treat patients with metastatic urothelial cancer who have not received prior therapy and who have low expression of the protein programmed death ligand 1 (PD-L1).

The labels of both drugs have been revised to reflect the limitation in the indication. The indications read as follows:

KEYTRUDA is indicated for the treatment of patients with locally advanced or metastatic urothelial carcinoma who are not eligible for cisplatin-containing therapy and whose tumors express PD-L1 (Combined Positive Score ≥ 10), or in patients who are not eligible for any platinum-containing chemotherapy regardless of PD-L1 status.

TECENTRIQ is indicated for the treatment of patients with locally advanced or metastatic urothelial carcinoma who:

- Are not eligible for cisplatin-containing therapy, and whose tumors express PD-L1 (PD-L1 stained tumor-infiltrating immune cells [IC] covering ≥5% of the tumor area), as determined by an FDA-approved test, or
- Are not eligible for any platinum-containing therapy regardless of PD-L1 status.
Novel Biomarkers: Beyond PD1

Early data suggests the following may enrich for response to PD1 pathway inhibition:

- Higher mutational load
- TCGA Molecular Subtypes (Luminal II vs basal III)
- CD8 infiltration
- Immune related gene expression signatures (Nanostring)
- Peripheral expansion of certain TCR clones
But in TCGA:
- Luminal II have high immune markers expression
- Basal subtypes have the strongest immune expression phenotype (T eff cell markers)
Biomarkers beyond PD-L1: Mutation load is associated with OS and RR with ICI

Atezolizumab 1st line Unfit Phase II Trial

Nivolumab 2nd line Phase II Trial

Atezolizumab Phase III Trial

In the TMB-high subgroup, mOS was numerically longer with atezolizumab

Galsky et al. LBA 31. ESMO 2016
Powles, et al. GU ASCO 2018
Biomarkers beyond PD-L1: Alterations in DDR genes as marker of benefit with ICI

Nivolumab or atezolizumab retrospective analysis

N=60, WES

ORR: DDR+ vs wt: 67.9% vs 18.8% (p <0.001)

Atezolizumab Phase III Trial

Fig 1. (A) Progression-free survival by DNA damage response and repair (DDR) alteration status. (B) Overall survival by DDR alteration status. + DDRref, (-) DDRref. DDRref, non-deleterious DDR alterations; DDRref, deleterious DDR gene alterations; HR, not reached.

DDR Mutations and Efficacy

- However, tumors with DDR mutations did not enrich for increased efficacy in the atezolizumab arm
- Multiple factors contribute to TMB — e.g., DDR, APOBECs, proliferation


Powles, et al. GU ASCO 2018
Biomarkers beyond PD-L1: Alterations in DDR genes as marker of benefit with ICI

Deleterious DNA Damage Response (DDR) genomic alterations and TMB (all pts at first-stage, n=43)

PURE-01 Pembrolizumab Neoadjuvant Phase II Trial

DDR/RB1-GA, PD-L1 CPS and pathologic response

DDR and/or RB1 genomic alterations: 25/43=58.1%
DDR and/or RB1 genomic alterations AND PD-L1 CPS≥20%: 10/43=23.3%

<table>
<thead>
<tr>
<th>Pathologic Complete</th>
<th>All treated patients N=43</th>
<th>PD-L1 CPS ≥20% N=22</th>
<th>DDR and/or RB1 GA N=25</th>
<th>PD-L1 CPS ≥20% AND DDR/RB1-GA N=10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Response, n (%), 95% CI</td>
<td>17 (39.5)</td>
<td>11 (50.0)</td>
<td>15 (60.0)</td>
<td>9 (90)</td>
</tr>
<tr>
<td></td>
<td>26.3–54.4</td>
<td>30.7–69.3</td>
<td>40.7–76.6</td>
<td></td>
</tr>
</tbody>
</table>

Necchi et al. ASCO 2018
TGF-β: A potential new resistance mechanism and therapeutic target?

TGFβ attenuates tumour response to PD–L1 blockade by contributing to exclusion of T cells

Sanjeev Mariathasan*, Shannon J. Turley*, Dorothee Nickles*, Alessandra Castigioni, Kobe Yuen1, Yukui Wang1, Edward E. Kadel III1, Hartmut Koeppe1, Jillian L. Astarita1, Rafael Cubas1, Suchit Jhunjhunwala1, Romain Banchereau1, Yagui Yang1, Yinghui Guan1, Cécile Chalouni1, James Ziai1, Yasin Şenbabaoğlu1, Stephen Santoro1, Daniel Sheinson1, Jeffrey Hung1, Jennifer M. Gilman1, Andrew A. Pierce1, Kathryn Mesh1, Steve Lianoglou1, Johannes Riegler1, Richard A. D. Carano1, Pontus Eriksson1, Mattias Höglund1, Loa Somarriba1, Daniel L. Halligan1, Michiel S. van der Heijden4, Yohann Loriot1, Jonathan E. Rosenberg5, Lawrence Fong7, Ira Mellman1, Daniel S. Chen1, Marjorie Green7, Christina Derleth1, Gregg D. Fine1, Priti S. Hegde1, Richard Bourgon1 & Thomas Powles8

TGFβ drives immune evasion in genetically reconstituted colon cancer metastasis

Daniele V. F. Tauriello1,2, Sergio Palomo-Ponce1,2, Diana Stork1, Antonio Berenguer-Llengo1, Jordi Badia-Ramentol1, Mar Iglesias3,4,5, Marta Sevillano1,2, Sales Ibiza1, Adrià Cañellas1, Xavier Hernando-Momblona1,2, Daniel Byrom1, Joan A. Matarin1, Alexandre Calon1,2, Elisa I. Rivas1,2, Angel R. Nebreda1,4, Antoni Riera1,2, Camille Stephan-Otto Attolini2 & Eduard Batlle1,2,6
The impact of checkpoint inhibition on patient outcome in mUC is dictated by three core biological pathways:

(i) Pre-existing T-cell immunity
(ii) TMB, which is positively associated with outcome,
(iii) Absence of TGFβ expression, which is associated with lack of response and reduced survival

The enrichment of the fibroblast TGFβ -response signature in non-responding immune-excluded tumours, combined with preclinical models showing that co-inhibition of TGFβ and PD-L1 converted tumours from an excluded to an inflamed phenotype, support a model in which TGFβ signalling may counteract anti-tumour immunity by restricting the movement of T-cells in the TME.
Future Directions: Microbiota as Biomarkers

Primary, Adaptive, and Acquired Resistance to Cancer Immunotherapy
Padmanee Sharma,1* Siwen Hu-Lieskovsk,2 Jennifer A. Wago,3 and Antoni Ribas4,5
Cell 168, February 9, 2017 © 2017 Elsevier Inc.

Molecular analysis
Mutational load
Driver mutations
Gene expression

Immune analysis
CD8
PD-L1
Clonality

Baseline Early-on treatment Progression

Tumor
PBMC
Microbiome (fecal)
Microbiome (oral)

Cell. 2017 Feb 9;168(4):707-723.
Analysis of 113 fecal samples of patients with MM treated with anti PD-1:

- The gut microbiota of responders had a greater diversity
- Responders had increased abundance of fecal Clostridiales (specifically Ruminococcaceae family)
- No association between oral microbiome and response to therapy
Take Home Messages

- Several predictive biomarkers to chemotherapy mainly in the neoadjuvant setting to predict response rate

- Most validated: ERCC2 mutations, DDR alterations, gene expression profiles and molecular subtypes

- Even a more preliminary situation regarding immunotherapy biomarkers

- Controversial role of PD-L1 expression

- Promising: TMB, DDR alterations, molecular subtypes, TGF-β, microbiota,…

Currently, no biomarkers have been translated into daily clinical practice in bladder cancer
Acknowledgement: Joaquim Bellmunt

Thank You

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