Biomarker studies in S1314: The CoXEN Trial

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Translational Medicine
SWOG GU Committee
Disclosures

- Grant support: Astra-Zeneca, Janssen, Rainier Pharmaceuticals
- Honoraria: Janssen, Rainier, H3 Biomedicine

NON FDA Approved use of drugs or products referenced in this presentation – None.

David J. McConkey, PhD
Background

- Cisplatin-based combination neoadjuvant chemotherapy is the standard of care in eligible patient with muscle-invasive, localized disease
  - Both Gemcitabine + Cisplatin (GC) and dose-dense MVAC (dd-MVAC: Methotrexate, Vinblastine, Doxorubicin + Cisplatin) are acceptable regimens
- The use of this therapy, despite category 1 support, remains suboptimal
- There are no predictive biomarkers in use for cytotoxic chemotherapy in this setting
Downstaging as a surrogate for survival

- Radical cystectomy – removes primary tumor and lymph nodes (extent: S1011)
- Downstaging to <pT2 (i.e., no muscle-invasive disease) is associated with excellent outcomes
- In this setting, chemosensitivity of the primary tumor is considered a surrogate for the sensitivity of sub-clinical metastatic disease
- *However, the correlation is not perfect (i.e., disconnect with ctDNA, Dyrsjkot, JCO 2019)*
Neoadjuvant chemotherapy in bladder cancer

SWOG 8710:
• Rate of pT0 was 38% with chemotherapy and 15% without
• 8 year survival
  • pT0 ~75%
  • > pT0 ~30%

NCI WORKSHOP

NOVEL NEOADJUVANT THERAPY FOR BLADDER CANCER

AGENDA

MONDAY, SEPTEMBER 19TH FROM 8:00 AM – 6:45 PM ET
TUESDAY, SEPTEMBER 20TH FROM 7:30 AM – 1:15 PM ET

GAITHERSBURG MARRIOTT WASHINGTONIAN CENTER
9751 WASHINGTONIAN BLVD
GAITHERSBURG, MD

ROOM: SALONS EFG (FOR MAIN SESSION)

Session 1: Candidate biomarkers (McConkey and Theodorescu)

S1314 TM was the product of the meeting.
The CoXEN algorithm

Downstaging vs. COXEN Score

Downstaging defined as ≤pT1 or ≤T1 after two courses of MVAC

Proportion Surviving

Ref: Clin Can Res 2005;11(7): 2625
Tx: Neoadjuvant MVAC (N=45) + surgery or XRT
Outcome: Downstaging, Overall survival
Trial schema

Stage cT2-T4aN0M0
Urothelial carcinoma
Planned cystectomy

Randomize

Mandatory tissue collection

Arm 1:
Gemcitabine + Cisplatin (GC)
• Growth factor support (as deemed appropriate)

Cystectomy with lymph node dissection – Tissue collection

Arm 2:
Dose-dense methotrexate, vinblastine, doxorubicin & cisplatin (ddMVAC)
• Mandatory growth factor support
Integrated translational medicine

- CoXEN (Theodorescu, Flaig): *primary objective*
- miRNA-based molecular subtypes (Dinney, Choi, McConkey)
- Molecular subtypes (Lerner, Choi, others)
- DDR mutations (Rosenberg, Iyer, Plimack)
- SNPs associated with drug metabolism (O’Donnell)
BISQFP funding

- RNA isolation and Affymetrix gene expression profiling ( Flaig, Theodorescu )
- RNA and DNA isolation and Nanostring miRNA expression profiling ( Dinney, Choi and McConkey )
- Blood germline and tumor MSK IMPACT panel exome sequencing ( Rosenberg )
Tissue collection and processing

- Collected 20 unstained slides per patient
- 10x went to ALMAC for RNA isolation and Affymetrix gene expression profiling (HU133 chips)
- 5x went to MDACC for RNA and DNA extraction and miRNA profiling (NanoString)
- 5x remain in the SWOG tissue bank (Nationwide)
Total Randomized: N=237

Eligible: N=228

Ineligible: N=9

Received < 3 cycles chemo: N=23

Did not receive cystectomy within 100 days: N=38

Evaluable: N=167
Pathologic response by treatment arm in evaluable subjects

<table>
<thead>
<tr>
<th></th>
<th>GC (N=82)</th>
<th>ddMVAC (N=85)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chemotherapy Response</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR (pT0)</td>
<td>28 (35%)</td>
<td>27 (32%)</td>
</tr>
<tr>
<td>PR (downstaged to ≤T1)</td>
<td>12 (15%)</td>
<td>20 (24%)</td>
</tr>
<tr>
<td><strong>CR + PR</strong></td>
<td>40 (50%)</td>
<td>47 (56%)</td>
</tr>
<tr>
<td><strong>Non-responders</strong></td>
<td>42 (50%)</td>
<td>38 (44%)</td>
</tr>
</tbody>
</table>
Correlation of repeat samples between Batch 1 and 2

- $r=0.96$ 
- $r=0.97$ 
- $r=0.94$ 
- $r=0.97$ 
- $r=0.98$ 
- $r=0.98$ 
- $r=0.97$
Cluster 1
Cluster 2
Cluster 3

Red: Batch 2
Green: Batch 1
S1314: Primary Analysis

<table>
<thead>
<tr>
<th>Coxen Score</th>
<th>Outcome</th>
<th>Arm</th>
<th>Number</th>
<th>Odds Ratio**</th>
<th>95% CI**</th>
<th>P-value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC*</td>
<td>pT0</td>
<td>GC</td>
<td>82</td>
<td>2.63</td>
<td>(0.82, 8.36)</td>
<td>0.10</td>
</tr>
<tr>
<td>GC*</td>
<td>≤pT1</td>
<td>GC</td>
<td>82</td>
<td>1.75</td>
<td>(0.60, 5.34)</td>
<td>0.30</td>
</tr>
<tr>
<td>ddMVAC*</td>
<td>pT0</td>
<td>ddMVAC</td>
<td>85</td>
<td>1.12</td>
<td>(0.42, 2.95)</td>
<td>0.82</td>
</tr>
<tr>
<td>ddMVAC*</td>
<td>≤pT1</td>
<td>ddMVAC</td>
<td>85</td>
<td>0.92</td>
<td>(0.37, 2.27)</td>
<td>0.86</td>
</tr>
<tr>
<td>GC*</td>
<td>≤pT1</td>
<td>Both</td>
<td>167</td>
<td>2.33</td>
<td>(1.11, 4.89)</td>
<td>0.02</td>
</tr>
<tr>
<td>ddMVAC*</td>
<td>≤pT1</td>
<td>Both</td>
<td>167</td>
<td>0.90</td>
<td>(0.46, 1.75)</td>
<td>0.76</td>
</tr>
</tbody>
</table>

Moderate Spearman correlation between GC and MVAC Coxen scores: 0.39

* favorable based on prespecified algorithm and dichotomous cut point
** adjusted for two stratification factors – clinical stage at baseline (T2 vs T3, T4a), PS (0 vs 1)
TCGA final analyses: k = 5
18 MBC cohorts
10 profiling techniques
1750 mRNA profiles

Subtype predictions from 6 classification systems in each cohort

Aggregated data (matrix D)
1750 samples x 29 molecular subtypes

Network construction and identification of consensus subtypes

Assignment of each of the 29 input subtypes to a consensus subtype

Single sample classifier construction for subtype prediction

Consensus subtype predictions for 1750 MBC samples

Molecular, pathological and clinical characterization of consensus subtypes

6 classification systems x 29 molecular subtypes

<table>
<thead>
<tr>
<th>Classification</th>
<th>Number of Subtypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baylor (Volkmer et al, 2012)</td>
<td>N=2</td>
</tr>
<tr>
<td>Chapel Hill (Demuynck et al, 2014)</td>
<td>N=2</td>
</tr>
<tr>
<td>GIT-Cure (Reboulissou et al, 2014)</td>
<td>N=7</td>
</tr>
<tr>
<td>MDA (Choi et al, 2014)</td>
<td>N=3</td>
</tr>
<tr>
<td>Lund (Spångel et al, 2017)</td>
<td>N=10</td>
</tr>
<tr>
<td>TCGA (Robertson et al, 2017)</td>
<td>N=5</td>
</tr>
</tbody>
</table>

Nb of subtypes in each classification

\[ D_{m,s} = \begin{cases} 1 & \text{if sample s belongs to molecular subtype } m \\ 0 & \text{otherwise} \end{cases} \]

Cohen's Kappa metrics + MCL clustering x 500

< 6-class solution >

1084 core consensus samples defined by hypergeometric tests

Pearson-based nearest centroid classifier construction

Leading cancer research

SWOG
<table>
<thead>
<tr>
<th>Class Name</th>
<th>Luminal Papillary (LumP)</th>
<th>Luminal Non-Specified (LumNS)</th>
<th>Luminal Unstable (LumU)</th>
<th>Stroma-rich</th>
<th>Basal/Squamous (BaSq)</th>
<th>Neuroendocrine-like (NE-like)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of MIBC</td>
<td>24%</td>
<td>8%</td>
<td>15%</td>
<td>15%</td>
<td>32%</td>
<td>3%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Differentiation</th>
<th>Urothelial / Luminal</th>
<th>Basal</th>
<th>Neuroendocrine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oncogenic mechanisms</td>
<td>FGFR3 +, PPARG +, CDKN2A -</td>
<td>PPARG +, E2F3 +, ERBB2 +, Genomic instability, Cell cycle +</td>
<td>EGFR +, TP53 - Rb1 +, Cell cycle +</td>
</tr>
<tr>
<td>Mutations</td>
<td>FGFR3 (40%), KDM6A (38%)</td>
<td>ELF3 (35%), TP53 (76%), ERCC2 (22%), TMB +, APOBEC +</td>
<td>TP53 (61%), Rb1 (25%), TP53 (94%), Rb1 (39%)*</td>
</tr>
<tr>
<td>Stromal infiltrate</td>
<td>Fibroblasts</td>
<td>Smooth muscle, Fibroblasts, Myofibroblasts</td>
<td>Fibroblasts, Myofibroblasts</td>
</tr>
<tr>
<td>Immune infiltrate</td>
<td>Q cells</td>
<td>CD8 T cells, NK cells</td>
<td>Neoendocrine differentiation (72%)</td>
</tr>
<tr>
<td>Histology</td>
<td>Papillary morphology (59%), Micropapillary variant (36%)</td>
<td>Squamous differentiation (42%), Neuroendocrine differentiation (72%)</td>
<td></td>
</tr>
<tr>
<td>Clinical</td>
<td>T2 stage +</td>
<td>Older patients + (80+)</td>
<td>Women + T3/T4 stage +</td>
</tr>
<tr>
<td>Median overall survival (years)</td>
<td>4</td>
<td>1.8</td>
<td>2.9</td>
</tr>
</tbody>
</table>

* 94% of these tumors present either Rb1 mutation or deletion
### Relationship between subtype membership and NAC response

<table>
<thead>
<tr>
<th>_subtype membership</th>
<th>Basal</th>
<th>p53-like</th>
<th>Luminal</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of pts</td>
<td>2.50</td>
<td>-1.67</td>
<td>-0.83</td>
</tr>
<tr>
<td>% of pts</td>
<td>0.00</td>
<td>0.83</td>
<td>1.67</td>
</tr>
<tr>
<td>% of pts</td>
<td>2.50</td>
<td>-1.67</td>
<td>-0.83</td>
</tr>
</tbody>
</table>

**TURBT Cystectomy**

- **Basal**
- **p53-like**
- **Luminal**

**Basal p53-like Luminal**

**TURBT**

- **Basal**
- **p53-like**
- **Luminal**

**Cystectomy**

- **Basal**
- **p53-like**
- **Luminal**

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*Leading cancer research. Together.*

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*SWOG*
Molecular subtypes: prognostic for survival

Siefker-Radtke, Eur Urol 2016
Basal tumors and NAC benefit

Seiler et al, Eur Urol 2017
MD Anderson subtypes in S1314

oneNN subtype assignments
Relationship with downstaging

- **Pooled data**
  - Basal
  - p53-like
  - Luminal

- **By treatment arm**
  - Basal
  - p53-like
  - Luminal

- **Legend**
  - pT0
  - <pT2
  - GC
  - MVAC

- **Molecular subtype**
  - Basal
  - p53-like
  - Luminal
MVAC-sensitive basal tumors were infiltrated with lymphocytes
Future plans

- Train a CoXEN classifier on cisplatin alone, and reapply to the S1314 dataset
- Apply the other molecular subtyping algorithms to the Affy dataset and correlate with downstaging
- Use the leftover RNA at MDACC to perform RNAseq (Theodorescu)
- Correlate molecular subtype membership with survival (18-24 months from now)
- ctDNA?
Somatic ERCC2 Mutations Correlate with Cisplatin Sensitivity in Muscle-Invasive Urothelial Carcinoma


available at www.europeanurology.com
journal homepage: www.europeanurology.com

Platinum Priority – Bladder Cancer

Defects in DNA Repair Genes Predict Response to Neoadjuvant Cisplatin-based Chemotherapy in Muscle-invasive Bladder Cancer


* Fox Chase Cancer Center, Philadelphia, PA, USA; ** Foundation Medicine Inc., Cambridge, MA, USA; *** Thomas Jefferson University Hospital, Philadelphia, PA, USA; **** MD Anderson Cancer Center, Houston, TX, USA
Figure. Overall Survival With and Without Somatic ERCC2 Mutations

A. Overall survival with and without somatic ERCC2 mutations in the current Fox Chase Cancer Center (FCCC) validation cohort. Kaplan-Meier analysis of overall survival by the presence or absence of a somatic ERCC2 mutation. There is a statistically significant difference in survival (log rank test; $P = 0.03$).

B. Overall survival with and without somatic ERCC2 mutations in a previously reported cohort (Dana Farber Cancer Institute and Memorial Sloan Kettering Cancer Center [DFCI/MSKCC] combined) discovery cohort. Kaplan-Meier analysis of overall survival by the presence or absence of a somatic ERCC2 mutation. There is a statistically significant difference in survival, log rank test ($P = 0.049$).

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Levi A. Garraway, MD, PhD
Joaquim Bellmunt, MD, PhD
Ellezer Van Allen, MD
Jonathan E. Rosenberg, MD
Ongoing studies

- Amendment to allow panel DNA exome sequencing (MSK IMPACT and Caris) was approved
- BISQFP funding is in place for MSK IMPACT
- DNA from MDACC will be sent to MSK
- Germline DNA will be isolated at MSKCC and shared with Peter O’Donnell
- Correlate ctDNA and CTCs with path responses and outcomes (Goldkorn, R01)
For the future

- Public Affy and Illumina RNAseq datasets
- Residual ALMAC RNA
- 5x unstained slides
- Urine
- Post-treatment tumors
The SWOG S1314 team

Tom Flaig (Colorado)
Cathy Tangen (FHCRC)
Melissa Plets (FHCRC)
Dan Theodorescu (Cedars-Sinai)
Dan Gustafson (Colorado State)
Scott Lucia (Colorado)
Seth Lerner (Baylor)
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Matt Milowski (UNC)
Gary MacVicar (Illinois CancerCare)
Bruno Bastos (Baptist Health)
Ian Thompson (San Antonio)