Purpose:
To explore the feasibility of a urine based epigenetic marker assay to monitor response to neoadjuvant therapy for muscle invasive bladder cancer.

Methods:
Inclusion: All patients undergoing neoadjuvant therapy, chemotherapy or immunotherapy of any type (NAT), for MIBC

**Bladder CARE™ test** (Pangea Laboratory, LLC)

- **DNA methylation** levels of 3 bladder-cancer specific biomarkers (TRNA-Cys, SIM2, and NKX1-1) and two internal control loci using *methylation-sensitive restriction enzymes coupled with qPCR*.

- Results reported as **Bladder CARE Index (BCI)**:
  - “Positive”, “High-risk”, or “Negative”,
  - Proportional to the number of cancer cells identified in the urine sample

MIBC Patient Monitoring During NAC

Baseline (pre-NAT) = Initial BCI

After each NAT cycle

Post-NAT to RC = Final BCI
## Results:

10 patients have undergone cystectomy after NAT

<table>
<thead>
<tr>
<th>ID</th>
<th>cTN</th>
<th>Histology</th>
<th>NAT</th>
<th>pT</th>
<th>Initial Bladder CARE</th>
<th>Final Bladder CARE</th>
<th>%Δ BCI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>T4N0</td>
<td>UC</td>
<td>GemCis</td>
<td>TisN1</td>
<td>High risk</td>
<td>Negative</td>
<td>-84.8</td>
</tr>
<tr>
<td>3</td>
<td>T2N0</td>
<td>UC glandular</td>
<td>GemCis</td>
<td>TisN0</td>
<td>Positive</td>
<td>High risk</td>
<td>-54</td>
</tr>
<tr>
<td>5</td>
<td>T3N0</td>
<td>UC</td>
<td>GemCis</td>
<td>T0N0</td>
<td>Positive</td>
<td>High risk</td>
<td>-95</td>
</tr>
<tr>
<td>7</td>
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<td>UC</td>
<td>GemCis</td>
<td>T0N0</td>
<td>Negative</td>
<td>Negative</td>
<td>45.5</td>
</tr>
<tr>
<td>8</td>
<td>T4aN0</td>
<td>UC</td>
<td>GemCis</td>
<td>T0N0</td>
<td>Positive</td>
<td>Negative</td>
<td>-99.8</td>
</tr>
<tr>
<td>11</td>
<td>T2N0</td>
<td>UC</td>
<td>GemCis</td>
<td>T2bN0</td>
<td>Positive</td>
<td>Positive</td>
<td>-59.9</td>
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<tr>
<td>12</td>
<td>T3N0</td>
<td>Small cell</td>
<td>Carbo Etop</td>
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<td>Positive</td>
<td>Positive</td>
<td>148</td>
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<td>Pembro EphB4</td>
<td>TaN0</td>
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<td>Positive</td>
<td>64.4</td>
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<tr>
<td>15</td>
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<td>Pembro EphB4</td>
<td>T2bN0</td>
<td>Positive</td>
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<td>-94.7</td>
</tr>
</tbody>
</table>

- 7/10 (70%) patients showed decrease in BCI.
  - 6/7 (86%) had at least partial response to neoadjuvant therapy.
  - 5/6 (83%) responders had pure urothelial histology and 5/6 (83%) received GemCis

- 1 patient had ypT0 disease and negative BCI at baseline and throughout NAT (#7)
  - All had residual disease
  - 2/2 variant histology (#3 and #12)
  - 1 had prostatic urethral disease (#11)
  - 2 received alternative NAT (#12 and #14)

## Conclusion:

The Bladder CARE™ test may have utility in monitoring response to NAT

Further studies are underway