Disclosure Information

I hereby declare that I have had business or personal interests in the following industrial enterprises since 1 September 2016:

<table>
<thead>
<tr>
<th>Name of the enterprise / Nature of the interest</th>
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<tbody>
<tr>
<td>Nothing to declare</td>
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</tbody>
</table>
A Tissue Microarray Expression Analysis of Cell Signaling Pathways in Recurrent Non-Muscle Invasive Bladder Cancers

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Introduction

- NMIBC have a high risk of recurrence after resection and adjuvant therapy.
- The recurrent BC often show progression in stage and grade.
- Recurrent tumors may acquire molecular alterations as compared to primary tumors.

NMIBC- Non-Muscle Invasive bladder cancer; BC- Bladder cancer
p53/p21 pathway in bladder cancer progression

- Alterations in the cell cycle regulatory pathways occur in bladder cancers
- p53/p21 alterations have been shown to be associated with BC progression and poor prognosis

• PD-1 is expressed on lymphocytes
• PD-L1 is expressed on both tumor and immune cells
PD-L1 in Bladder cancer

• PD-L1 overexpression has been shown in advanced bladder tumors- a mechanism by which they develop tolerance to immune regulation.

• PD-L1 expression
  – is a poor prognostic factor
  – predictive of better response from both PD-1 and PD-L1 inhibitors

• However, there is scant data on its expression in superficial low grade bladder tumors and its recurrence.

Objectives

• To study the differences in expression of cell cycle pathway molecules including p53, p21/WAF1/Cip1 and Ki-67-index in primary and recurrent NMIBC.

• To study the expression of PDL-1 in these tumors to evaluate the benefit of anti-PD-1/PD-L1 directed therapy in recurrent tumors.
Material and methods

- Using FFPE tissue, TMA of 42 NMIBC (20 primary and 22 recurrent) was constructed.
- 2-3 cores of 3mm tissue from each tumor
Material and methods

Definitions

• **Recurrence** - bladder cancer after 3 months of initial treatment.
• **Progression** - advancement of stage/grade or both in the recurrent tumor.
Material and methods

- Immunohistochemistry was performed for:

<table>
<thead>
<tr>
<th>S.No</th>
<th>Antibody</th>
<th>Clone</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>p53</td>
<td>318-6-11</td>
<td>Dako</td>
</tr>
<tr>
<td>2</td>
<td>p21/WAF1/Cip1</td>
<td>SX118</td>
<td>Dako</td>
</tr>
<tr>
<td>3</td>
<td>Ki-67</td>
<td>MIB-1</td>
<td>Dako</td>
</tr>
<tr>
<td>4</td>
<td>PDL1</td>
<td>E1L3N</td>
<td>Cell Signaling Technology</td>
</tr>
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</table>
Immunohistochemical Evaluation

• p21-altered
  – nuclear expression in <10% cells

• p53 positive
  – nuclear expression in >10% cells

• p53 and p21 nuclear staining also expressed as semi-quantitative H-score
  – (range 0-300) based on the intensity (scale of 0-3) and percentage of 500 tumor cells analyzed

• PDL1 positive
  – membranous expression in >5% tumor cells
• Age at presentation of primary: 62.5± 11.8 years
• All males
• Primary tumors; LG NMIBC (n=20):
  – Lamina invasion 3/20 (15%)
  – No. of recurrences 1-8 (Median 2)
  – Progression 12/20 (70%) (Stage-1; Grade 5; Both 6)
• Time duration for
  – Recurrence (months): Mean 39 (Median 38; range 4-109)
  – Progression (months): Mean 41 (Median 33)

LG-Low grade
Results

• Recurrent tumors (n=22)
  – 11 high grade (50%)
    • Associated progression in stage- 6 [Muscle invasive-4; Lamina invasive-2]
  – 7 were of higher stage (35%)
    • Progression to MI-4; Lamina invasion-3
    • Associated transformation to high grade-6
p53 in Primary and Recurrent tumors

Case 4_p

H score: 30

Case 4_r (4yrs)

H score: 285
p21 in primary and recurrent tumors

Case 4_p

Case 4_r (4yrs)
Ki-67 (MIB-1) Proliferation Index in primary and recurrent tumors
### p53/p21 pathway in bladder cancer

<table>
<thead>
<tr>
<th>IHC Marker</th>
<th>Primary (n=20; LG)</th>
<th>Recurrent</th>
<th>Total (n=22)</th>
<th>LG (n=11)</th>
<th>HG (n=11)</th>
<th>Progression Stage (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>p53+ve</td>
<td>17 (85%)</td>
<td>19 (86%)</td>
<td>10 (91%)</td>
<td>9 (91%)</td>
<td>6 (86%)</td>
<td></td>
</tr>
<tr>
<td>p21-ve</td>
<td>5 (25%)</td>
<td>9 (41%)</td>
<td>3 (27%)</td>
<td>6 (55%)</td>
<td>3 (43%)</td>
<td></td>
</tr>
<tr>
<td>Ki-67 (≥10%)</td>
<td>3 (15%)</td>
<td>11 (50%)</td>
<td>5 (45%)</td>
<td>6 (55%)</td>
<td>3 (43%)</td>
<td></td>
</tr>
</tbody>
</table>
### Results: Paired samples T-test

<table>
<thead>
<tr>
<th>IHC</th>
<th>Primary/recurrent NMIBC</th>
<th>H score (Mean ±S.D)</th>
<th>Difference between means</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>p53</td>
<td>primary</td>
<td>31.4±23</td>
<td>48.1</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>recurrent</td>
<td>79.5±89</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p21</td>
<td>primary</td>
<td>94.3±68.6</td>
<td>3.12</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>recurrent</td>
<td>93±88.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ki-67</td>
<td>primary</td>
<td>9.1±17.1</td>
<td>1.0</td>
<td>0.84</td>
</tr>
<tr>
<td></td>
<td>recurrent</td>
<td>10.1±13.2</td>
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Significant increase in p53 expression in recurrent tumours ($p<0.05$)
PDL-1 expression in Primary and Recurrent tumors

Case 4_p

Case 4_r (4yrs)
PDL-1 expression in Primary and Recurrent tumors

Case 12_p

Case 12_r (4.25yrs)
• Alterations in p21 and p53 were seen in recurrent tumors
• Ki-67 index was higher in recurrent tumors.
• Loss of p21 was associated with p53 (p=0.03).
• PDL-1 expression was seen in one progressive HG MIBC (1/4;25%) (strong expression in 50% cells).
Conclusion

• Alteration in cell cycle regulators, cellular proliferation and immune tolerance is seen in recurrent NMIBCs as compared to primary tumors.

• Anti-PD-1/PD-L1 directed therapies may have a role in recurrent NMIBC progressing to high grade MIBC.
Acknowledgement

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Thank You