Disclosure Information

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

Name of the enterprise / Nature of the interest

<table>
<thead>
<tr>
<th>Enterprise</th>
<th>Interest</th>
</tr>
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<tbody>
<tr>
<td>Nothing to disclose – Authors declare no conflicts of interest related to the content of this work</td>
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</table>
DESCRIPTIVE STATISTICAL ANALYSIS OF MISMATCH REPAIR PROTEINS (MLH1, MSH2, MSH6 AND PMS2) IMMUNOHISTOCHEMICAL EXPRESSION IN PROSTATE CANCER: CORRELATION WITH GRADE GROUPS (ISUP/WHO 2016)

INTRODUCTION: MISMATCH REPAIR SYSTEM

- HUMAN MISMATCH REPAIR SYSTEM (MMR) GENES
  - MutS HOMOLOGUES (MSHs): $MSH2$, $MSH6$, $MSH3$
  - MutL HOMOLOGUES (MLHs): $MLH1$, $PMS2$, $PMS1$, $MLH3$

- HETERODIMERIC COMPLEXES
  - MutS$\alpha$ complex ($MSH2$-$MSH6$)
  - MutS$\beta$ complex ($MSH2$-$MSH3$)
  - MutL$\alpha$ complex ($MLH1$-$PMS2$)
  - MutL$\beta$ complex ($MLH1$-$PMS1$)
  - MutL$\gamma$ complex ($MLH1$-$MLH3$)
INTRODUCTION: MISMATCH REPAIR SYSTEM

Microsatellites (1-9 bp)

Minisatellites (10-100 bp)

Macrosatellites (>100 bp)

Short Tandem Repeats
...GCACACACACACACCT...
...CGTGTGTGTGTGGGA...

INTRODUCTION
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  - GCCACACACACACCT...
  - TGTGTGTGTGGAA...

- MSH1
- MSH2
- MSH6
- PMS2
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...GCCACAC...
...CGTGTG...

PMS2
MLH1
MSH6
MSH2
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Minisatellites (10-100 bp)

Macrosatellites (>100 bp)

Short Tandem Repeats

...GACACACACACAC... 
...CGTGTGTGTGGA...

PMS2

MLH1

MSH2

MSH6
INTRODUCTION: MICROSATELLITE INSTABILITY

Microsatellites (1-9 bp)

Minisatellites (10-100 bp)

Macrosatellites (>100 bp)

- **Sporadic cancer**: Acquired mutations, 
  *MLH1* promoter hypermethylation

- **Hereditary cancer (Lynch Sdr.)**: 
  Germline mutations
INTRODUCTION: MMR SYSTEM & PROSTATE CANCER

- In prostate cancer (PCa) mutations are reported in \textit{MSH2} and \textit{MSH6}. 
- MMR protein loss has been described in both primary and metastatic PCa, but few mutations have been defined (Pritchard CC et al)
- \textit{MSH6}, \textit{MLH1} and \textit{PMS2} become up-regulated during cancer development.

This immunohistochemical overexpression seems linked to tumor aggressiveness, early PSA recurrence and poor outcome, suggesting a prognostic relevance of MMR genes in cancers lacking ERG-fusion \textit{(p<0.0001)} (Wilczak et al)


AIM

To investigate alterations in the expression of mismatch repair proteins (MLH1, MSH2, MSH6 and PMS2) and their relationship with Grade Groups (GG) in prostate cancer
MATERIALS AND METHODS: IHC

• OUR SERIES
  ▪ Number of analysed cases: 126 patients
  ▪ Number of total analysed cores: 1472 cores
  ▪ Number of analysed cores per antibody: 368 cores
  ▪ Tumor histology and cases by GG:
    ▸ 126 Acinar ADC: GG1 (30), GG2 (47), GG3 (15), GG4 (17), GG5 (11), NV (6)
    ▸ 10 IDC
    ▸ 1 AIP

• ANTIBODIES USED (Ventana – Roche ©)
  ▪ Anti-MSH2 (G219-1129), Anti-MSH6 (44), Anti-MLH-1 (M1) and PMS2 (EPR3947)
### MATERIALS AND METHODS: IHC

**IMMUNOHISTOCHEMICAL ANALYSIS OF MMR PROTEIN EXPRESSION**

<table>
<thead>
<tr>
<th>MLH1</th>
<th>PMS2</th>
<th>MSH2</th>
<th>MSH6</th>
<th>SUGGESTED ALTERATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>NO ALTERATION</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>MLH1 (and PMS2)</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>PMS2</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>MSH2 (and MSH6)</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>MSH6</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>MSH2 (and MSH6), MLH1 (and PMS2)</td>
</tr>
</tbody>
</table>

- **Loss of nuclear expression** in tumor cells has to be complete
- **MSH2 and MLH1** are the essential / dominant components
MATERIALS AND METHODS: IHC

• ASSESSED FEATURES
  ▪ Internal control: lymphocytes, stromal and endothelial cells
  ▪ Nuclear staining intensity (% cells): “constitutive” expression
    o 0: negative, 1: weak, 2: moderate, 3: strong
  ▪ Cytoplasmic staining intensity: “aberrant” expression

• QUANTIFICATION BY HISTOSCORE
  ▪ Scores: 0-300
  ▪ Nuclear Histoscore groups: 0 (0-10), 1 (>10-100), 2 (>100-300)

RESULTS: MMR PROTEIN EXPRESSION

<table>
<thead>
<tr>
<th>Gene</th>
<th>POSITIVE</th>
<th>NEGATIVE</th>
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<tbody>
<tr>
<td>MSH2</td>
<td>87.8</td>
<td>12.2</td>
</tr>
<tr>
<td>MSH6</td>
<td>39.8</td>
<td>60.2</td>
</tr>
<tr>
<td>MLH1</td>
<td>88.8</td>
<td>11.2</td>
</tr>
<tr>
<td>PMS2</td>
<td>96.6</td>
<td>3.4</td>
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</table>
RESULTS: MSH2 EXPRESSION AND GG

<table>
<thead>
<tr>
<th>GG</th>
<th>MSH2 POSITIVE</th>
<th>MSH2 NEGATIVE</th>
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</thead>
<tbody>
<tr>
<td>GG1</td>
<td>88,5</td>
<td>11,5</td>
</tr>
<tr>
<td>GG2</td>
<td>87,2</td>
<td>12,8</td>
</tr>
<tr>
<td>GG3</td>
<td>86,7</td>
<td>13,3</td>
</tr>
<tr>
<td>GG4</td>
<td>88,2</td>
<td>11,8</td>
</tr>
<tr>
<td>GG5</td>
<td>90</td>
<td>10</td>
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P = 0.999
RESULTS: MLH1 EXPRESSION AND GG

<table>
<thead>
<tr>
<th></th>
<th>MLH1 POSITIVE</th>
<th>MLH1 NEGATIVE</th>
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<tbody>
<tr>
<td>GG1</td>
<td>81.5</td>
<td>18.5</td>
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<tr>
<td>GG2</td>
<td>91.3</td>
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<tr>
<td>GG3</td>
<td>86.7</td>
<td>13.3</td>
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<tr>
<td>GG4</td>
<td>94.1</td>
<td>5.9</td>
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<tr>
<td>GG5</td>
<td>90.9</td>
<td>9.1</td>
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$P = 0.714$
RESULTS: PMS2 EXPRESSION AND GG

<table>
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<th>PMS2 NEGATIVE</th>
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<tbody>
<tr>
<td>GG1</td>
<td>100</td>
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<tr>
<td>GG2</td>
<td>95.7</td>
<td>4.3</td>
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<tr>
<td>GG3</td>
<td>100</td>
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<tr>
<td>GG4</td>
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<tr>
<td>GG5</td>
<td>90.9</td>
<td>9.1</td>
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</table>

$P = 0.510$
RESULTS: MSH6 EXPRESSION AND GG

**MSH6 POSITIVE**  **MSH6 NEGATIVE**

<table>
<thead>
<tr>
<th>GG</th>
<th>MSH6 POSITIVE</th>
<th>MSH6 NEGATIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG1</td>
<td>26.1</td>
<td>73.9</td>
</tr>
<tr>
<td>GG2</td>
<td>41.9</td>
<td>58.1</td>
</tr>
<tr>
<td>GG3</td>
<td>26.7</td>
<td>73.3</td>
</tr>
<tr>
<td>GG4</td>
<td>35.3</td>
<td>64.7</td>
</tr>
<tr>
<td>GG5</td>
<td>90</td>
<td>10</td>
</tr>
</tbody>
</table>

\*P = 0.007
CONCLUSIONS

• Few studies have defined MMR gene alterations in prostate cancer outside Lynch syndrome

• MHS2, MLH1 and PMS2 expression did not show significant association with prostate cancer Grade Groups

• MSH6 expression showed a statistical correlation with the more aggressive tumors (ISUP/WHO GG5)

• In prostate cancer, expression of MSH6 could be a surrogate marker of genomic damage and tumor aggressiveness
FUTURE DIRECTIONS

• The molecular mechanisms controlling the variable expression of MSH6 among the different grade groups in prostate cancer deserve further study.

• In addition, cases with loss of the dominant partner are more eligible for being molecularly analyzed to confirm the microsatellite instability and to correlate with immunohistochemical data.
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Dank je wel! Thank you!