Disclosure Information

I hereby declare that I have had business or personal interests in the following industrial enterprises since 1 September 2018:

<table>
<thead>
<tr>
<th>Name of the enterprise / Nature of the interest</th>
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**The authors declare no conflict of interest**
Immunohistochemical evaluation of CXCR4 chemokine receptor expression in metastatic and non-metastatic well-differentiated Pancreatic Neuroendocrine Tumors

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INTRODUCTION: The CXCR4 Molecular Pathways

C-X-C chemokine receptor type 4

- Belongs to the family of G-protein coupled chemokine receptors;
- Induces downstream signaling by several different pathways in response to its ligand - stromal cell derived factor 1α (SDF-1);
- Overexpressed in over 20 types of cancer;
- Promotes tumor growth, progression and spreading;
- Regulates migration and homing of cancer cells to specific metastatic sites;
- An attractive target for therapy;
- Data on CXCR4 expression in neuroendocrine tumors are limited.
To assess the expression of CXCR4 chemokine receptor in a series of patients with pancreatic neuroendocrine tumors (PanNETs)

52 primary PanNETs:
- 25 - metastatic PanNETs (mts in liver, lung, bone, peritoneum, brain)
- 27 – non-metastatic PanNETs
- 20 - liver metastases

The results have been correlated with various clinicopathological parameters and prognosis
RESULTS: PanNETs. WHO 2017/2019

Pathology & Immunohistochemistry of PanNETs

<table>
<thead>
<tr>
<th>NET Grade</th>
<th>Count</th>
<th>Percentage</th>
<th>Non-metastatic</th>
<th>Metastatic</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12</td>
<td>23%</td>
<td>8 (66.7%)</td>
<td>4 (33.3%)</td>
</tr>
<tr>
<td>2</td>
<td>28</td>
<td>54%</td>
<td>16 (55.1%)</td>
<td>12 (42.9%)</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>23%</td>
<td>3 (25.0%)</td>
<td>9 (75.0%)</td>
</tr>
</tbody>
</table>

Ki-67:
- 1%
- 8%
- 30%
RESULTS: CXCR4

The Patterns of CXCR4 immunostaining in PanNETs: Rabbit monoclonal anti-CXCR4 antibody [clone UMB2]

CXCR4 – Low protein expression (IRS - 0-3)

CXCR4 – High protein expression (IRS- 4-9)

Immunoreactive score: [percentage of positive cells] × [intensity of staining] = IRS (0 - 9)

Primary PanNETs - 35 (67.3%)

- 13 metastatic & 22 non-metastatic

Primary PanNETs - 17 (32.7%)

- 12 metastatic & 5 non-metastatic

CXCR4 - 0% (0)

CXCR4 - < 10% (1)

CXCR4 - 10 - 50% (2)

CXCR4 - > 50% (3)
RESULTS: CXCR4

Expression of CXCR4 in WHO Grade 1 / Grade 2 / Grade 3 primary PanNETs

<table>
<thead>
<tr>
<th>Parameters</th>
<th>G1-NETs</th>
<th>G2-NETs</th>
<th>G3-NETs</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>12</td>
<td>28</td>
<td>12</td>
</tr>
<tr>
<td>Non-metastatic/metastatic</td>
<td>8/4</td>
<td>16/12</td>
<td>3/9</td>
</tr>
<tr>
<td>Ki-67 index</td>
<td>&lt; 3%</td>
<td>3-20%</td>
<td>23 - 45%</td>
</tr>
<tr>
<td>PR nuclear</td>
<td>10</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>SSTR-2A membrane</td>
<td>9</td>
<td>20</td>
<td>8</td>
</tr>
<tr>
<td>ATRX/DAXX nuclear</td>
<td>10</td>
<td>19</td>
<td>3</td>
</tr>
</tbody>
</table>

CXCR4 High

G1 16.7% (2/12) CXCR4 2+ (IRS-4) / Ki-67 - 2%

G2 28.6% (8/28) CXCR4 3+ (IRS-6) / Ki-67 – 16%

G3 58.4% (7/12) CXCR4 3+ (IRS-9) / Ki-67 – 35%
RESULTS: CXCR4

Overexpression of CXCR4 in Non-metastatic & Metastatic PanNETs

PanNETs G1 – 16.7% (2/12)
- Non-metastatic – 12.5% (1/8)
- Metastatic – 25.0% (1/4)

PanNETs G2 – 28.6% (8/28)
- Non-metastatic – 18.8% (3/16)
- Metastatic – 41.7% (5/12)

PanNETs G3 – 58.4% (7/12)
- Non-metastatic – 33.3% (1/3)
- Metastatic – 66.7 (6/9)
RESULTS: CXCR4

CXCR4 overexpression & other prognostic markers in PanNETs

- CXCR4$^{\text{high}} \rightarrow$ ATRX$^{\text{low}} \rightarrow$ SSTR2$^{\text{low}}$
- PanNETs

- PR$^{\text{low}}$
- PR$^{\text{high}}$
- p = 0.012

<table>
<thead>
<tr>
<th>Metastases −</th>
<th>Metastases +</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
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<tr>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>8</td>
<td>9</td>
</tr>
</tbody>
</table>

PR
- CXCR4$^{\text{high}} –$ PanNETs $\rightarrow$
- ATRX$^{\text{low}} \rightarrow$ SSTR2$^{\text{low}} \rightarrow$ PR$^{\text{low}}$

ATRX
- ATRX$^{\text{low}}$

SSTR2
- SSTR2$^{\text{high}}$

CXCR4
- CXCR4$^{\text{high}}$

PR
- PR$^{\text{low}}$
- PR$^{\text{high}}$

31.0% (16/52)

11.6% (6/52)
RESULTS: CXCR4

Correlation of CXCR4 high expression in PanNETs to clinicopathological parameters and survival

**No Correlation to**

- Age, gender, tumor size, angioinvasion, lymph node metastasis, pTNM stage
- Overall and disease free survival, % (Kaplan-Meier) according to CXCR4 expression in 52 cases of PanNETs

**Correlation to**

- Positively: higher WHO Grade ($p=0.001$), Ki-67 index ($p=0.003$), distant metastasis ($p=0.047$), and loss of ATRX nuclear expression ($p=0.033$)
- Negatively: nuclear PR ($p=0.047$) and membranous SSTR 2A staining patterns ($p=0.05$)
CONCLUSION

CXCR4 CHEMOKINE RECEPTOR STATUS IN PanNETs

- The overexpression of CXCR4 in primary tumors is associated with tumor progression and mainly observed in highly proliferative and metastatic PanNETs.

- CXCR4 may represent a potential tissue-based biomarker of an aggressive phenotype, which likely plays a significant role in driving metastatic disease among PanNETs.

- CXCR4 also may serve as a promising target for NET diagnostics and therapy.
Pathology is Nice

Thank you for your attention!