Molecular taxonomy of bladder cancer
Current status

31st European Congress of Pathology, Nice

Yves.Allory@curie.fr
Disclosures

• Board Advisor : AstraZeneca
• Invited speaker : AstraZeneca, MSD, BMS
Current status…at ECP 2019?

20 abstracts including “Molecular subtype” or “Molecular classification” words

Bladder cancer, heterogeneous disease

- **Tis** Carcinoma in situ
- **Ta**
  - Low grade
- **Ta**
  - High grade
- **T1**
- **≥ T2**

Muscle Invasive

- **METASTASIS**

© Renaud Chabrier 2009 - 06 60 74 01 43 - renaud.chabrier@fastx.org - www.renaudchabrier.com
Six reference and independent classifications with successive revisions for muscle invasive bladder cancer (MIBC) within the last 10 years. Based on gene expression (transcriptomics):

- Lindgren et al. (Lund.v1) 2010
- Volkmer et al. (Baylor.v1)
- Sjödahl et al. (Lund.v2) 2012
- Choi et al. (MDA) 2014
- Damrauer et al. (UNC)
- Rebouissou et al. (CIT-Curie)
- TCGA. v1
- Sjödhal et al. (Lund.v3) 2017
- Mo et al. (Baylor.v2) 2018
- Robertson et al. (TCGA.v2)
- Marzouka et al. (Lund.v4)
- Tan et al. (Singapore) 2019
The distinct classifications support the existence of MIBC intrinsic molecular subtypes....

BUT

- Subtype numbers highly variable according to the classifications: 2 to 10
- Namings very heterogeneous (link with histology / biology / classification methods)
Subtypes might be useful to predict treatment response

Choi et al, 2014

Classification MDA

Neoadjuvant chemotherapy

Anti PD-L1 immunotherapy
(atezolizumab in metastatic setting)

Rosenberg et al, 2016

Classification TCGA.v1

% patients

Mariathasan et al, 2018

Classification Lund.v2

% patients

Subtypes might be useful to predict treatment response

Choi et al, 2014

Classification MDA

Neoadjuvant chemotherapy

Anti PD-L1 immunotherapy
(atezolizumab in metastatic setting)

Rosenberg et al, 2016

Classification TCGA.v1

% patients

Mariathasan et al, 2018

Classification Lund.v2
To get consensus reconciling the distinct MIBC molecular classifications?

10 countries, 20 institutions, 4 meetings (2015-2018)
A six-class consensus classification

Subtypes « Basal »

Subtypes « infiltrated »

Subtypes « Neuroendocrine-like »

Initial classifications

Consensus classes identified

Subtypes « Luminal » or « differenciated »

A. Kamoun et al, Eur Urol, in press
<table>
<thead>
<tr>
<th>Differentiation</th>
<th>Oncogenesis</th>
<th>Mutations</th>
<th>Stroma</th>
<th>Immunity</th>
<th>Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urothelial / Luminal</td>
<td>FGFR3+ PPARG+ CDKN2A-</td>
<td>FGFR3 (40%) KDM6A (38%) STAG2 (22%)</td>
<td>Fibroblasts</td>
<td></td>
<td>Papillary (59%)</td>
</tr>
<tr>
<td>Stroma-rich</td>
<td>PPARG+ E2F3+, ERBB2+ Genomic instability, cell cycle.</td>
<td>ELF3 (35%) TP53 (76%) ERCC2 (22%) TMB+, APOBEC+</td>
<td>Smooth muscle Fibroblasts Myofibroblasts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuroendocrine-like (NE-like)</td>
<td>EGFR</td>
<td>TP53 (61%) RB1 (25%)</td>
<td>Fibroblasts Myofibroblasts</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Tumor microenvironment

MCPcounter

-log10(t-test $P$)

Estimate

(Colors represent different cell types and scores)

Higher score

Lower score

[Diagram showing various cell types and their scores]
Differential outcomes in retrospective studies

**Patients overall survival (n = 873)**

- LumP: 241
- LumNS: 53
- LumU: 110
- Stroma-rich: 137
- Ba/Sq: 314
- NE-like: 18

\[ P(\text{logrank}) = 2.5 \times 10^{-5} \]

**Patients treated with Atezolizumab (IMvigor210)**

**Overall survival (n=348)**

- LumP: 81
- LumNS: 9
- LumU: 65
- Stroma-rich: 78
- Ba/Sq: 109
- NE-like: 6

\[ P(\text{logrank}) = 0.19 \]

**Patients treated with Neoadjuvant Chemotherapy**

**Overall survival (n=367)**

\[ P(\text{logrank}) = 0.37 \]
Potential therapeutic targets?

- **Luminal Papillary (24%)**
  - FGFR3 inhibitors

- **Luminal Non-Specified (8%)**
  - Chimiotherapy
  - Immunotherapy

- **Luminal Unstable (15%)**
  - ERBBs inhibitors
  - Radiotherapy
  - Chimiotherapy
  - Immunotherapy

- **Basal (35%)**
  - EGFR inhibitors
  - Chimiotherapy
  - Immunotherapy

- **Neuroendocrine-like (3%)**
  - Radiotherapy
  - Immunotherapy

- **Stroma-rich (15%)**
  - TGFß inhibitors
  - Immunotherapy?
Single sample classifier from RNA expression


Future reduced RNA classifier? (cf. NanoString approach for TCGA classification)
Some hot questions for molecular taxonomy in bladder cancer

1) Is there a clinical utility (prognosis, treatment response prediction)?
2) Can immunohistochemistry be used instead of RNA profile?
3) How much does intra-tumoral heterogeneity add complexity to molecular classification?
4) Does MIBC molecular classification apply to NMIBC?
5) Does molecular classification apply to histological variants?
Is there a clinical utility?

Promising... but retrospective studies


These results need to be confirmed in prospective studies before recommending the use of molecular classification in clinical setting.

Can immunohistochemistry be used?

(1) the simple approach: luminal versus basal

Need for validation and consensus
- For molecular classification: compare with RNA based subtype
- For treatment response: prospective studies...

Need to define the threshold
% and/or intensity and/or location?
Can immunohistochemistry be used? (2) the systematic approach: from RNA to IHC

Lund classification

**Tumour cell phenotype definitions**

- **Uro**
  - FGFR3 +
  - CCND1 +
  - RB1 +
  - p16 -

- **GU**
  - FGFR3 -
  - CCND1 -
  - RB1 -
  - p16 +

- **Basal/SCC-like**
  - KRT5 +
  - KRT14 +
  - FOXA1 -
  - GATA3 -

- **Mes-like**
  - VIM +
  - ZEB2 +
  - CDH1 -
  - EPCAM -

- **Sc/NE-like**
  - TUBB2B +
  - EPCAM +
  - CDH1 -
  - GATA3 -

**Critics**
- Lack of feasibility
- Utility?

---

*Sjödahl, J Pathol 2017; Sjödahl, Methods Mol Biol 2018; Mazourka, Sci Rep 2018; Bernardo, J Pathol 2019*
Can immunohistochemistry be used?  
Provisional consideration

Immunohistochemical panel for subtyping? Yes, if
- the subgroup identified by the panel is well defined [sensibility, specificity related to RNA subtyping gold standard]
- it is standardized
- utility is demonstrated (prognosis or treatment response prediction)

### Table of Immunohistochemical Panel for Subtyping

<table>
<thead>
<tr>
<th>IHC</th>
<th>CK5/6</th>
<th>GATA3</th>
<th>FGFR3</th>
<th>p16</th>
<th>RB</th>
<th>TUBB2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Papillary</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>-</td>
<td>+/++</td>
<td></td>
</tr>
<tr>
<td>basal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NOS</td>
<td>-/+</td>
<td>++</td>
<td>++</td>
<td>-/+</td>
<td>+/++</td>
<td></td>
</tr>
<tr>
<td>Unstable</td>
<td>-</td>
<td>+++</td>
<td>-/+</td>
<td>+++</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Stroma rich</td>
<td>- or +</td>
<td>+ or -</td>
<td>-/+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal/squamous</td>
<td>+++</td>
<td>-/+</td>
<td>-/+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NE-like</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td>+++</td>
</tr>
</tbody>
</table>
For a fraction of admixed cases, intra-tumoral heterogeneity adds complexity to molecular classification.
Does MIBC molecular classification apply to NMIBC?

- Only the Lund classification was developed both for NMIBC and MIBC
- Several teams proposed to extend the luminal versus basal MIBC molecular classification to NMIBC

Subtyping NMIBC using MIBC classification system might provide prognosis information but it does not imply that it identifies intrinsic NMIBC entities that match with MIBC counterparts...
Does molecular classification apply to histological variants?

• Frequent and/or highly distinct variant contributed to the molecular consensus classification:
  o Squamous divergence → Basal / squamous subtype
  o Neuroendocrine carcinoma → Neuroendocrine like

• Micropapillary → luminal or stroma-rich (with luminal differentiation) subtypes

• Sarcomatoid → mainly Basal / squamous subtype

• Others (to be done) : they might fit with known molecular subtypes or form rare and distinct molecular subtypes

Conclusions depend also on subtype definitions : RNA versus IHC panel....
Take home messages

• For MIBC, there is now an international molecular consensus classification
• IHC panel might be used to identify the subtypes (or at least some of them) but needs standardization and validation by comparison with RNA subtypes
• A lot of work for pathologists: clinical utility, histological variants, NMIBC classification...?