Molecular subtyping and immunohistochemistry validation identifies muscle invasive bladder cancer subgroups with poorer overall survival

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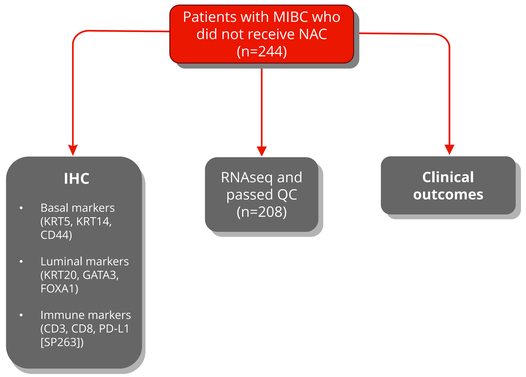
INTRODUCTION

- Muscle-invasive bladder cancers (MIBC) are molecularly heterogeneous and are associated with poorer clinical outcomes compared with non-muscle invasive disease
- Molecular characterization of tumor subtypes and immune status have demonstrated prognostic value and potential to guide precision intervention for different cancers
- A more comprehensive understanding of the association between tumor subtypes and immune cells is still needed
- In this study, an integrative multi-omics analysis was performed on MIBC tumor samples from whom the majority of patients did not receive treatment prior to cystectomy

METHODS

- Macro-dissected formalin-fixed paraffin embedded tissue slides were used to perform whole transcriptome RNA sequencing or immunohistochemistry (IHC) staining (**Figure 1**)
- Consensus single-sample classifier and TCGA classifier were applied to RNAseq data to determine molecular subtypes
- IHC scoring was assessed by two independent pathologists
- Tumor subtypes derived from either RNAseq or IHC were compared and correlated with disease-specific survival

FIGURE 1: Study design



IHC, immunohistochemistry; MIBC, muscle-invasive bladder cancers; NAC, neoadjuvant chemotherapy; QC, quality check.

RESULTS

- Among 244 patients with MIBC, 30.7% were T2, 47.6% were T3, and 21.7% were T4 (**Table 1**)

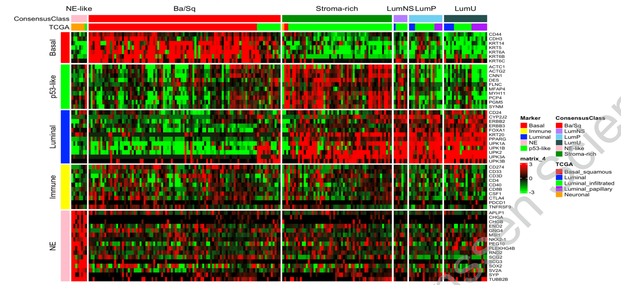
TABLE 1. Baseline characteristics and demographics

	Total N = 244
Age	
Median (range), y	70.7 (37, 91)
<65, n (%)	74 (30.3)
>=65, n (%)	170 (69.7)
Sex, n (%)	
Female	68 (27.9%)
Male	176 (72.1%)
pT stage, n (%)	
T2	75 (30.7)
T3	116 (47.6)
T4	53 (21.7)
Tumor grade (WHO 1973)	
G2	7 (2.9)
G3	237 (97.1)
Instillation history	
Yes, n (%)	11 (4.5)
No, n (%)	233 (95.5)
BCG	7
MMC	2
RCX	1
Unknown	1
FGFR3 driver status	
Any mutation/fusion, n (%)	21 (8.6)
S249C	10
Y373C	3
R248C	2
G370C	2
FGFR3-TACC3v1	3
FGFR3-TACC3v1 & R248C	1

BCG, Bacillus Calmette-Guérin; MMC, Mitomycin C; pT, primary tumor; RCX, radical cystectomy; WHO, World Health Organization.

- Consensus molecular classification identified mRNA subtypes and showed agreement with the TCGA molecular classification (**Figure 2**)
- Correlations with disease-specific survival revealed that luminal subtypes trended towards the best outcome, while stroma-rich subtypes trended towards poorer outcomes compared with other MIBC subtypes (**Figure 3**)

FIGURE 2: Molecular subtyping classification



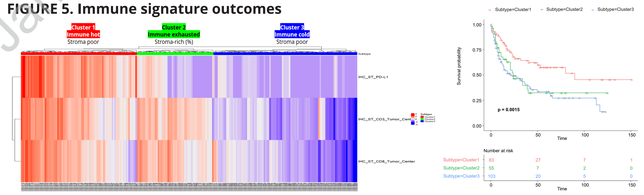
	Consensus Classifier (n=208)	Percent (%)
NE	8	3.8%
Basal	98	47.1%
Stroma	56	26.9%
LumP	17	8.2%
LumU	22	10.6%
LumNS	7	3.4%

FIGURE 3. Correlation between molecular subtypes and disease-specific survival

FIGURE 4. Canonical markers associate with molecularly-defined subtypes

- IHC markers associated with luminal-like and basal-like tumors recapitulated the molecularly defined luminal/basal subtype assignment (**Figure 4**)
- Stroma-rich subtypes were enriched with fibroblast signatures, implying high desmoplastic stromal cell infiltration and low immune cell infiltration

FIGURE 5. Immune signature outcomes



- IHC immune markers (PD-L1, CD3, and CD8) demonstrated 3 patient clusters that were differentially represented by unique consensus MIBC subtype spectrum **(Table 2)**
- The 3 IHC immune clusters were significantly associated with differential survival benefit **(Figure 5)**

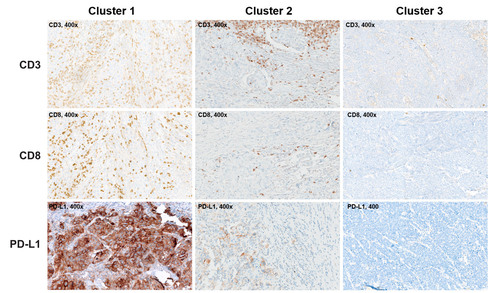
TABLE 2. Molecular subtype and immune signatures

	Ba/Sq	NE-like	Stroma rich	Luminal (combined)
Cluster 1 Immune-hot	34	6	25	20
Cluster 2 Immune-exhausted	21	2	17	6
Cluster 3 Immune cold	43	0	14	20

post-hoc Cluster 2 vs. Other in Stroma-rich ratep=0.03881

- Heterogeneity of IHC immune signatures were observed within mRNA subtypes (**Table 2**)
- Cluster 2 subjects were significantly enriched with higher stroma-rich subtypes than other clusters

FIGURE 6. IHC immune signatures



- IHC immune markers CD3, CD8, and PD-L1 identified 3 distinct immune signatures within MIBC (**Figure 6**)

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Urothelial Cancer

KEY TAKEAWAYS

- Integrating MIBC subtyping, IHC of immune markers, and patient outcomes data provided a biological framework from which results from this study underscore the existence of heterogeneity in immune phenotypes within MIBC subtypes
- Deeper understanding of the association between MIBC subtypes and their immunological states is crucial to guide treatment decisions, particularly for MIBC patients with worse prognostic outcomes

CONCLUSIONS

- Correlation analysis showed that luminal MIBC subtypes trended towards the best outcome, while stroma-rich MIBC subtypes trended towards poorer outcomes compared with other MIBC subtypes
- Integrating molecular subtyping and IHC immune markers demonstrated that immune signatures were significantly associated with survival benefit in patients with MIBC

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