Advancing Insights Into Disease Biology of Non-Muscle Invasive Bladder Cancer (NMIBC) Through Comprehensive Multi-Omics Analysis

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Key Takeaway

Findings provide insights into NMIBC disease biology and highlight the importance of integrating genomic and transcriptomic profiling to better understand molecular markers associated with risk stratification

Conclusions

- *FGFR3* mutations were found to be more common in low- and intermediate-risk NMIBC, consistent with known disease biology
- High-risk NMIBC showed enrichment of cell-cycle pathway activity, consistent with the role of cell-cycle dysregulation in tumor aggressiveness
- BCG Response Subtype 3 was validated as a poor-prognosis subtype in BCG-treated patients, reinforcing the robustness of BRS classification for predicting BCG response
- High-risk NMIBC is marked by cell-cycle dysregulation, while BRS3 predicts poor BCG response, emphasizing the importance of integrated multi-omics for risk stratification

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Poster

https://www.congresshub.com/Oncology/AACR2026/Gem-iDRS/Low

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Background

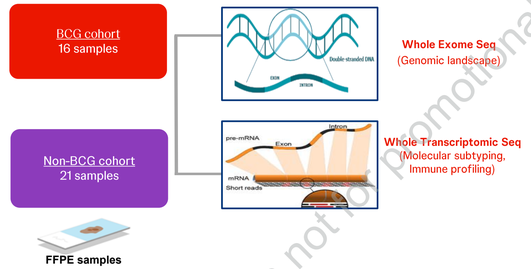
- Non-muscle–invasive bladder cancer (NMIBC) accounts for approximately 75% of all bladder cancer cases and has a high rate of recurrence and potential progression to muscle-invasive bladder cancer (MIBC)[1-3]
- Treatment strategies for NMIBC include transurethral resection of bladder tumor (TURBT) followed by Bacillus Calmette-Guérin (BCG) immunotherapy or chemotherapy.[1,3] Although NMIBC management has progressed, understanding its molecular basis and post-treatment changes remains critical for better prognosis, patient stratification, and informed treatment strategies[2]
- Transcriptomics-based molecular subtyping has shown that BCG Response Subtypes (BRS) provide clinically meaningful prognostic stratification in NMIBC patients receiving BCG therapy
- Here, we conducted whole exome and transcriptomic sequencing to characterize the molecular profiling of NMIBC tumor samples

Objectives

- To investigate genomics and transcriptomics architecture among low/intermediate-risk from high-risk NMIBC
- To validate molecular subtypes BRS associated with recurrence risk among the patients with NMIBC who received BCG treatment

Methods

Figure 1: Study design



BCG, Bacillus Calmette–Guérin; FFPE, formalin-fixed paraffin-embedded; Seq, sequencing.

Patient cohort

- Pre-treatment tumor specimens were obtained from 37 patients with NMIBC who underwent either TURBT followed by BCG immunotherapy (BCG cohort) or TURBT alone (Non-BCG cohort)

Genomic and transcriptomic sequencing

- Comprehensive molecular profiling was conducted using whole-exome sequencing (WES) and transcriptomic sequencing (RNA-seq) to characterize the genomic and transcriptomic landscape of NMIBC

BCG response subtype classification

- Transcriptomic data were analyzed using a BCG response subtype predictor to classify tumors into three BCG Response Subtypes (BRS1–3). This classification was used to investigate molecular differences associated with BCG response subtype

Pathway and gene set analysis

- Transcriptomic pathway activities were evaluated using single-sample Gene Set Enrichment Analysis (ssGSEA) based on Kyoto Encyclopedia of Genes and Genomes (KEGG) gene signatures to compare pathway-level transcriptional differences across NMIBC risk groups

Survival analysis

- Progression-free survival (PFS) was assessed to evaluate clinical associations of BRS. PFS was compared across BRS groups to determine the relevance of transcriptomic subtype in the context of BCG treatment

Results

- Among 37 patients with NMIBC, 16 with intermediate/high risk were treated with BCG post-TURBT (**Table 1**)
  - These patients had other aggressive features (eg, T1, high tumor grade, multiple and larger tumors) compared with the 21 patients who received TURBT alone
- Concomitant CIS was low (10.8%), and time to recurrence was fast (median 6.5 months) in this study

Table 1: Baseline clinical profile by treatment received

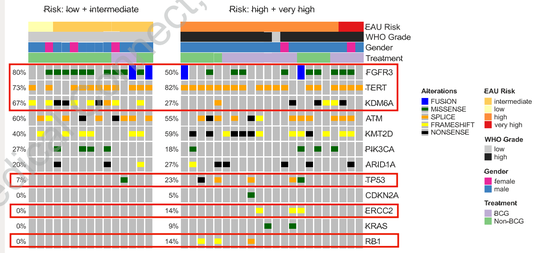
	Total (N=37)	TURBT+BCG (n=16)	TURBT Alone (n=21)
Age	72.0 (48–87)	73.0 (56–87)	69.0 (48–87)
Sex, Male	32 (86.8)	13 (81.2)	19 (90.5)
Primary tumor	32 (86.5)	13 (81.2)	19 (90.5)
Pathological T stage (TNM classification)			
Ta	29 (78.4)	9 (56.2)	20 (95.2)
T1	8 (21.6)	7 (43.8)	1 (4.8)
Concomitant CIS	4 (10.8)	3 (18.8)	1 (4.8)
NMIBC tumor grade			
Low grade	16 (43.2)	4 (25.0)	12 (57.1)
High grade	21 (56.8)	12 (75.0)	9 (42.9)
Multiple lesions	30 (78.9)	15 (93.8)	15 (68.2)
Tumor size			
<3cm	30 (78.9)	10 (62.5)	20 (90.9)
≥3cm	8 (21.1)	6 (37.5)	2 (9.1)
EAU risk group[a]			
Low	3 (8.1)	0	3 (14.3)
Intermediate	12 (32.4)	3 (18.8)	9 (42.9)
High	19 (51.4)	10 (62.5)	9 (42.9)
Very high	3 (8.1)	3 (18.8)	0
EORTC progression risk			
Low	7 (18.9)	0	7 (33.3)
Intermediate	18 (48.6)	7 (43.8)	11 (52.4)
High	12 (32.4)	9 (56.2)	3 (14.3)
EORTC recurrence risk			
Low	4 (10.8)	0	4 (19.0)
Intermediate	33 (89.2)	16 (100)	17 (81.0)
Intravesical chemotherapy	1 (2.6)	0	1 (4.5)
Time from baseline to recurrence	6.5 (2.6–56.6)	6.3 (3.4–15.4)	9.4 (2.6–56.6)

Data presented as n (%) or median (range). [a]Sylvester RJ, et al. *Eur Urol* 2021;79(4):480-488.
BCG, Bacillus Calmette–Guérin; CIS, Carcinoma in situ; EAU, European Association of Urology; EORTC, European Organisation for Research and Treatment of Cancer; NMIBC, non–muscle–invasive bladder cancer; TNM, tumour, node, metastasis.

Baseline genetic alteration profile by EAU risk group

- Alteration frequencies were consistent with published literature, with highest prevalence in *TERT*, *FGFR3,* and *ATM* (**Figure 2**).
- *FGFR3* and *KDM6A* mutations were more frequent in low/intermediate-risk groups compared with high/very high-risk groups
- *KMT2D*, *TP53*, *ERCC2*, and *RB1* mutations were more common in high-/very high-risk groups
- *FGFR3* alterations were mutually exclusive with majority of *TP53*, *RB1*, and *ERCC2* mutations

Figure 2: Genetic alterations at baseline by EAU NMIBC risk group

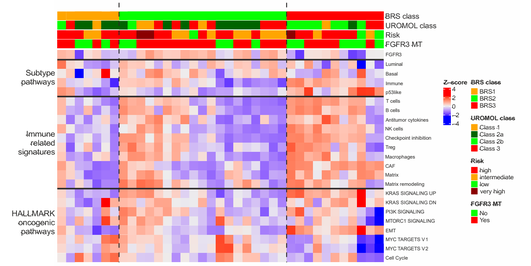


ARID1A, AT-rich Interaction Domain 1A; ATM, Ataxia telangiectasia mutated; BCG, Bacillus Calmette–Guérin; *CDKN2A*, Cyclin-dependent kinase inhibitor 2A; EAU, European Association of Urology; *ERCC2*, Excision repair cross-complementation group 2; *FGFR3*, Fibroblast growth factor receptor 3; *KDM6A*, Lysine-specific demethylase 6A; *KMT2D*, Lysine methyltransferase 2D; *KRAS*, Kirsten rat sarcoma viral oncogene homolog; NMIBC, Non–muscle-invasive bladder cancer; *PIK3CA*, Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; *RB1*, Retinoblastoma 1; *TERT*, Telomerase reverse transcriptase; *TP53*, Tumor Protein p53; *TSC1*, Tuberous sclerosis complex 1; WHO, World Health Organization.

Baseline transcriptomic profile by BRS

- Baseline transcriptomic profiles showed clear separation by BCG response subtype (BRS)[4] (**Figure 3**), that is consistent with previous reported classification
  - BRS1: Enriched for cell-cycle, autophagy, protein secretion, and antigen-presentation pathways
  - BRS2: Characterized by luminal-papillary, *FGFR3*, and *MYC* target signatures
  - BRS3: Enriched for *EMT*-basal, immunosuppressive, IL-6/JAK/STAT, angiogenesis, and *MAPK* signaling pathways

Figure 3: Baseline transcriptomic profiles by BRS

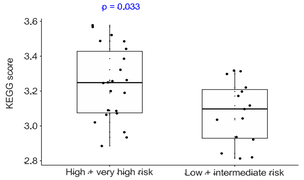


BRS, BCG response subtypes; CAF, Cancer-associated fibroblasts; *FGFR3*, Fibroblast growth factor receptor 3; *FGFR3 MT*, *FGFR3* mutation status; *KRAS*, Kirsten rat sarcoma viral oncogene homolog; MTORC1, Mechanistic target of rapamycin complex 1; *MYC*, MYC proto-oncogene, BHLH Transcription Factor; NK cells, natural killer cells; *p53*, Tumor protein p53; *PI3K*, Phosphatidylinositol 3-kinase; UROMOL, Urothelial Carcinoma Molecular Classification.

Cell cycle pathways are upregulated in high-risk NMIBC

- High-risk tumors show enrichment of *TP53* and *RB1* mutations, supporting dysregulated cell cycle control (**Figure 4**)[5]

Figure 4: Cell cycle pathway in KEGG score by risk group

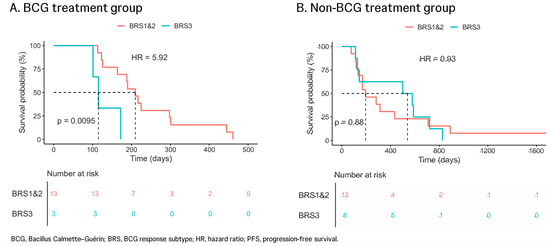


KEGG, Kyoto Encyclopedia of Genes and Genomes.

BRS3 has poor prognosis in the BCG treatment group

- Patients with BRS3 tumors had significantly shorter PFS compared with BRS1/2 (HR=5.92; p=0.0095) following BCG treatment (**Figure 5A**). This trend was not observed with Non-BCG treatment (**Figure 5B**)

Figure 5: PFS stratified by BCG response subtypes



BCG, Bacillus Calmette–Guérin; BRS, BCG response subtype; HR, hazard ratio; PFS, progression-free survival.

BRS1 and BRS3 are enriched in high-risk NMIBC

- High-risk and very high-risk NMIBC tumors are enriched in BRS1 and BRS3 relative to low/intermediate risk (**Table 2**), however the difference is not statistically significant
  - BRS1 is associated with cell-cycle–related biology, as supported by transcriptomic data
  - BRS3 is associated with immune-enriched immunosuppressive features, supported by transcriptomic and multiplex immunofluorescence data

Table 2: Association between BRS classification and NMIBC clinical risk groups[a]

BRS class	High/very-high risk	Low/intermediate risk	Total (100%)
BRS1	5 (71.4%)	2 (28.6%)	7
BRS2	10 (52.6%)	9 (47.4%)	19
BRS3	7 (63.6%)	4(36.4%)	11

Data presented as n (%).
[a]Chi square test p = 0.65. BRS, BCG response Subtype; NMIBC, non–muscle-invasive bladder cancer.

References

1. Lopez-Beltran A, et al. *BMJ* 2024;384:e076743. **2.** Holzbeierlein J, et al. *Nat Rev Urol.* 2025;22(2):75–91. **3.** Holzbeierlein J, et al. *J Urol. 2024.* 211(4):533–538. **4.** De Jong FC, et al. *Sci. Transl. Med.* 2023 15(697),eabn4118. **5.** Robertson AG, et al. *Cell* 2018;174(4):1033.

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