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An Immune-Competent, Durable MB49-Luc Orthotopic Bladder Cancer Model Demonstrates Bladder-Specific Tumor Immunity for Translational Immunotherapy Research

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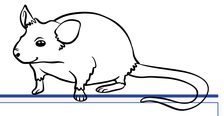


ABSTRACT

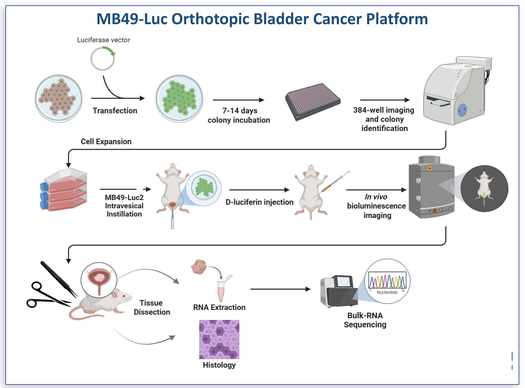
Background and Objective:
Bladder cancer remains a significant health concern in the United States, with approximately 85,000 new cases and 17,000 deaths annually. Nearly 70% of cases present as non–muscle-invasive bladder cancer (NMIBC), commonly treated with transurethral resection followed by intravesical immunotherapy. However, limited and inconsistent responses to current therapies underscore ongoing clinical challenges. To enable effective therapeutic development, a reliable, immune-competent, and longitudinally trackable preclinical model capable of representing the unique immune and physiological features of bladder cancer is essential. Despite decades of effort to refine syngeneic orthotopic bladder cancer models in mice, low tumor take rates and limited in-life study duration remain major challenges. Here, we present a syngeneic orthotopic bladder cancer model with extended in-life monitoring capacity that recapitulates bladder tumor immunity and enables robust immunotherapy evaluation.

Method and Result:
A luciferase-expressing MB49 cell line was generated via lentiviral transduction. Female C57BL/6 mice were implanted intravesically to establish an orthotopic tumor model. In-life bioluminescence imaging (BLI) using IVIS revealed stable and quantifiable bladder-localized tumor signals for more than 21 days, with a 100% tumor take rate. Tumor presence in the bladder lumen was confirmed by H&E histopathology.

Tumor-bearing bladders collected on Days 10 and 20 underwent next-generation sequencing (NGS) and subsequent enrichment analysis. Differential gene expression revealed biomarker signatures reflective of early- and late-stage tumor burden, along with dynamic immune-profiling changes. Pathway enrichment analysis linked these gene-level differences to functional networks governing immune regulation, tumor progression, and bladder cancer physiology.

Conclusion:
We established an optimized MB49-Luc orthotopic bladder cancer model that preserves immune relevance, provides a prolonged imaging window, and demonstrates distinct molecular transitions during tumor progression. This long-duration, immune-competent platform enables rigorous evaluation of intravesical and immuno-oncology therapeutics and offers meaningful translational insights to support next-generation NMIBC immunotherapy development.

Conceptual Diagram



Prolonged MB49 Orthotopic Tumor Growth Kinetics

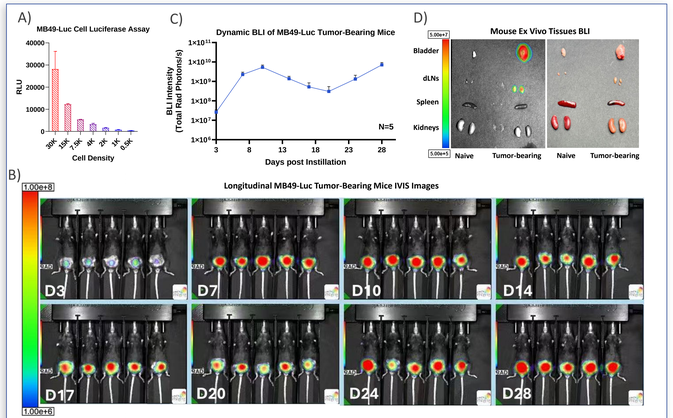


Figure 1. Establishment and longitudinal characterization of an orthotopic MB49-Luc bladder tumor model in mouse.
In vitro validation of MB49-Luc cells demonstrated a cell density–dependent increase in luciferase activity, confirming stable reporter expression **(A)**. Representative serial in vivo BLI images collected from Day 3 to Day 28 illustrated sustained bladder-associated tumor signal throughout the in-life phase of the study **(B)**. The quantitation of longitudinal BLI showed persistent signal in the bladder region following orthotopic instillation, supporting successful tumor engraftment, and enabling noninvasive monitoring over time **(C)**. Ex vivo tissues BLI further demonstrated predominant signal localization in the bladder of tumor-bearing mice, with additional detectable signal in the draining iliac lymph nodes (dLNs) **(D)**. Notably, longitudinal BLI level revealed that signal intensity peaked around Day 10–11, declined and partially diminished thereafter, and became increasing again around Day 20. This temporal pattern indicated that tumor progression in this model may involve distinct biological phases. Novel mechanisms may contribute to these shifts, requiring further research.

Two-Phase Dynamics: Late-Stage TME Remodeling

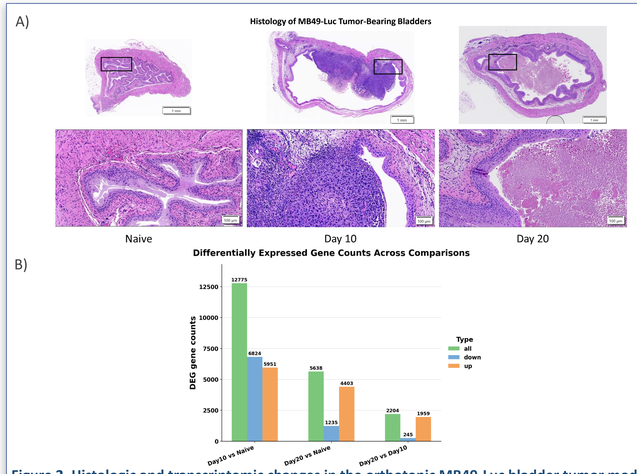


Figure 2. Histologic and transcriptomic changes in the orthotopic MB49-Luc bladder tumor model.
Representative H&E staining showed preserved normal bladder architecture in naïve tissue. Early tumor formation was confirmed at Day 10 with immune cells infiltration observed in tumor associated tissues. Advanced tumor-associated changes with intraluminal necrotic features was observed at Day 20, relevant with later-stage bladder pathology **(A)**. The two-phase tumor progression was further characterized by RNA-seq. Differential expression analysis revealed extensive gene expression changes in Day 10 and Day 20 bladder tissues relative to naïve controls, indicating substantial transcriptional remodeling. In addition, the Day 20 vs. Day 10 comparison identified a smaller yet distinct set of differentially expressed genes **(B)**. These results suggest that the durable MB49-Luc model exhibits a biphasic pattern of orthotopic tumor growth. Enrichment analyses were subsequently performed to elucidate pathway-level alterations occurring during the early and late tumor stages.

Differential Pathway Enrichment in Immunology-Related Signatures

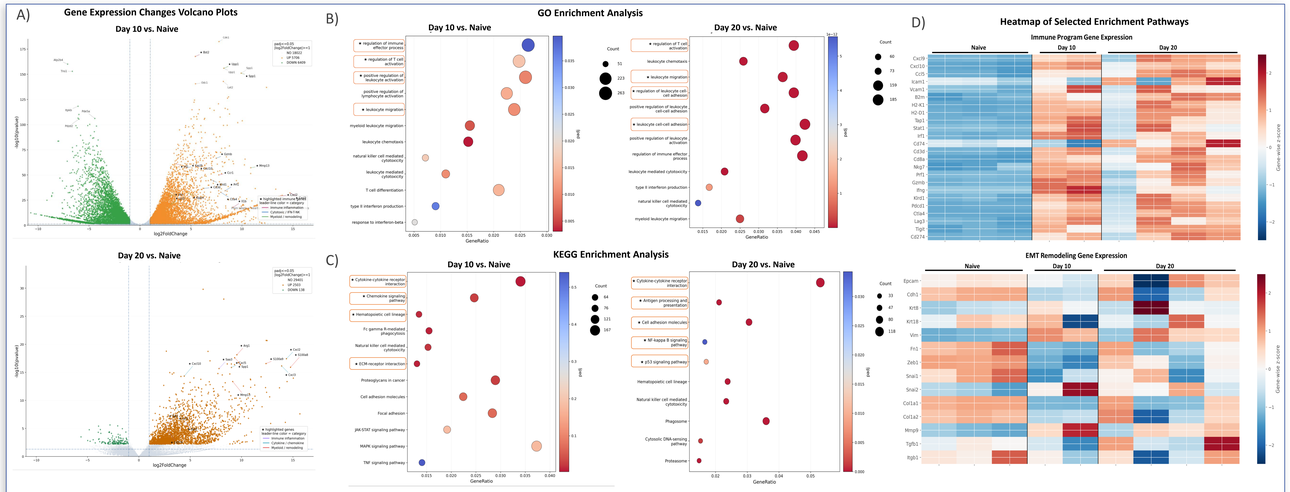


Figure 3. Pathway enrichment and gene-level analyses across Day 10 and Day 20 tumor-bearing bladders.
Volcano plots of Day 10 vs. Naïve and Day 20 vs. Naïve comparisons highlighted differentially expressed genes **(A).** Enrichment analysis demonstrated that the differentially expressed genes are preferentially associated with specific pathways. GO analysis further revealed that these altered pathways are largely immune-related at both Day 10 and Day 20, with T cell-associated pathways exhibiting the most significant modulation **(B)**. KEGG enrichment analysis further identified pathway-level changes, with cytokine signaling, oncogenic pathways, and EMT remodeling enriched at Day 10. At Day 20, enrichment extended to include pathways associated with immune responses and cell survival **(C).** Immune program and EMT remodeling heatmaps further supported these stage-associated changes at the individual gene expression level **(D).** These results suggest a shift from an early immune-active state at Day 10 toward a later state at Day 20 that retains immune-associated features while showing broader adhesion, antigen-processing, and remodeling-related changes.

Elevated Immune Activation in the Late-Stage Bladder TME

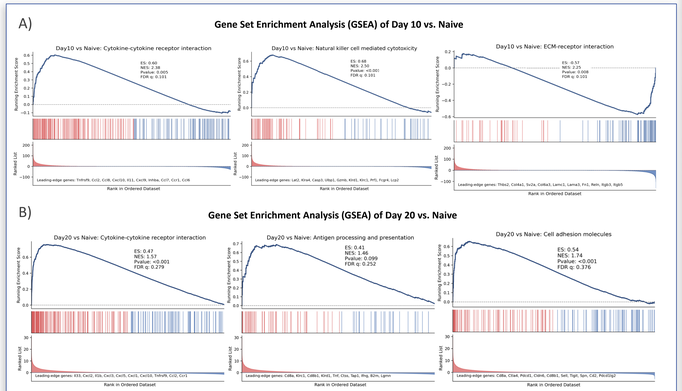


Figure 4. Representative GSEA plots validate pathway-level changes in the orthotopic MB49-Luc bladder tumor model.
In addition to the GO and KEGG analysis in Figure 3, GSEA of Day 10 vs. Naïve groups, cytokine-cytokine receptor interaction and natural killer cell-mediated cytotoxicity pathways were up-regulated, reflecting early activation of inflammatory and cytotoxic immune responses. Conversely, ECM-receptor interaction pathways showed inverse enrichment, potentially contributed to tissue remodeling toward a less fibrotic environment that likely permits early-stage immune cell infiltration **(A)**. In Day 20 vs. Naïve groups, GSEA further demonstrated up-regulation of pathways related to cytokine–cytokine receptor interactions, antigen processing and presentation, and cell adhesion molecules. These findings indicate enhanced immune recognition, immune stress within the tumor microenvironment, and processes associated with immune cell recruitment, tumor progression, and metastatic potential during the late stage **(B).** Leading-edge genes contributing to these enrichments included chemokines, cytotoxic mediators, antigen-presentation genes, and adhesion-related molecules, further supporting a shift from an early immune-active phase toward a later state with persistent immune signaling and tumor microenvironment.

Discussion

We established a stable orthotopic MB49-Luc bladder tumor model with bladder-localized tumor growth, longitudinal trackability, and intraluminal appearance, supporting its relevance to an NMIBC-like setting. In this model, longitudinal BLI showed a non-linear course, with signal peaking around Day10-11, declining during the intermediate phase, and rising again later, suggesting that tumor evolution may proceed through distinct stages rather than a simple linear increase.

Transcriptomic analyses further supported the biphasic pattern. Day 10 tumors exhibited an early immune-active state marked by leukocyte recruitment, cytokine/interferon signaling, and T-cell/NK-associated cytotoxic programs. By Day 20, immune-associated features remained evident but appeared more heterogeneous and were accompanied by stronger antigen-processing, leukocyte-interaction, checkpoint-related, and EMT remodeling-associated signals.

The observed biphasic tumor growth has clinical relevance, since NMIBC progression and treatment outcomes are governed not only by immune activation, but also by mechanisms of immune evasion, alterations in antigen presentation, checkpoint signaling, and ECM remodeling within the tumor microenvironment. Although NMIBC is managed with risk-adapted intravesical therapy, recurrence and progression remain major challenges, underscoring the need for novel therapeutic approaches. This model provides a preclinical platform that closely mimics the complex clinical context of NMIBC.

These findings suggest that the orthotopic MB49-Luc model captures dynamic immune and tissue-state evolution over time and may be useful for future mechanistic and therapeutic studies in an immune-competent bladder tumor setting.

Key Findings

- Stable orthotopic MB49-Luc bladder tumor model established and longitudinally tracked by IVIS.
- Ex vivo imaging and histology confirmed bladder-localized intraluminal tumor growth in an NMIBC setting.
- BLI revealed a non-linear pattern, with peak signal around Day 10-11 followed by decline and later re-emergence after Day 20.
- Day 10 was characterized by early immune activation, including leukocyte recruitment, cytokine/interferon signaling, and T-cell/NK cytotoxic programs.
- Day 20 retained immune-associated features but showed stronger antigen-processing, leukocyte-interaction, and tumor microenvironment immune stress.
- EMT remodeling alternation suggested changes in cell plasticity, increased potential of tumor progression and metastasis overtime.
- Findings provide a validated model with preliminary mechanistic insights, which is able to support future studies of tumor evolution and immune therapeutic response.

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