REACTION BIOLOGY

Advanced Solutions for Oncology Drug Development

In Vivo Models of Bladder Cancer

At diagnosis, over 75% of bladder cancers (BC) are at the non-muscle-invasive stage (NMIBC). The standard of care for these patients is tumor excision coupled with BCG treatment via catheterization[1]. In the remaining 25–30% of patients, BC has already invaded deeper layers of the bladder wall (MIBC—muscle-invasive disease) or formed metastases. Despite advanced molecular research, surgical techniques improvements, and wide adoption of perioperative chemotherapy, the long-term survival rates of patients with bladder cancer have remained unchanged for decades. It is believed that individualized therapy and novel systemic as well as intravesical treatment modalities will lead to better oncological outcomes of patients. Therefore, the establishment of new *in vivo* models of bladder cancer for early drug development remains a primary need.

At Reaction Biology we have successfully established both NMIBC and MIBC models via orthotopic cancer cell instillation or bladder wall injection which are available for testing the *in vivo* efficacy of your compound.

The Need for Orthotopic Bladder Cancer Models

Over 70% of cases at diagnosis are at the NMIBC stages and are treated locally using intravesical instillation. Therefore, standard subcutaneous models are not applicable nor relevant for early discovery research in NMIBC. Furthermore, the microenvironment of BC at the bladder may be different from that at the subcutaneous space, thus requiring more physiologically relevant solutions.

Why work with Reaction Biology

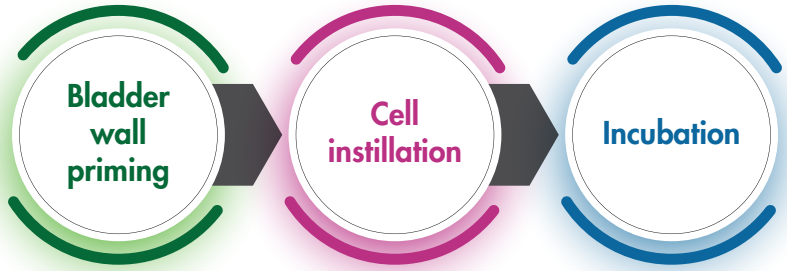
- Dedicated and highly experienced *in vivo* technical teams including a diverse group of PhDs
- Science-driven study setup with a focus on reproducible and meaningful results backed by our highly experienced QA personnel
- IND-ready study reports written by professional Medical Writers and custom-tailored for each project including meticulous documentation of drug efficacy testing

Orthotopic Models: Bladder Wall Instillation Model

To minimize surgical trauma associated with surgical inoculation, our orthotopic bladder cancer model is established by direct instillation of murine bladder cancer cells through the urethra into the bladder cavity of female C57BL/6 mice. To increase cancer cell attachment to the urothelial layer and engraftment, the bladder is treated with poly-L-lysine.

The murine bladder cancer cell lines MB49 and MB49_luc2F7 transduced with firefly luciferase and GFP were chosen for orthotopic model establishment via intravesical implantation in C57BL/6 mice.

Additionally, we are currently optimizing the development of a bladder cancer instillation models using the MBT-2 cell line in C3H/He mice.



Our available readouts for tumor progression include:

- Luminescent imaging
- Terminal tumor weight
- Clinical observations

MB49_luc Bladder Cancer Instillation Model

At Reaction Biology we have established orthotopic BC models by direct instillation through the urethra of MB49_luc murine bladder cancer cells into the bladder cavity of female C57BL/6 mice. Eleven MB49_luc clones with differing levels of luminescence *in vitro* and a range of doubling times (16.56-39.84 hrs) were chosen based on speed of growth and intensity of luminescence signal to provide a collection of *in vivo* models with specific characteristics for efficacy studies.

Our data provide a preclinical reference for cell line (Figure 1, 2, 3) selection for analysis of new drug candidates in C57BL/6 female mice bearing superficial bladder tumors.

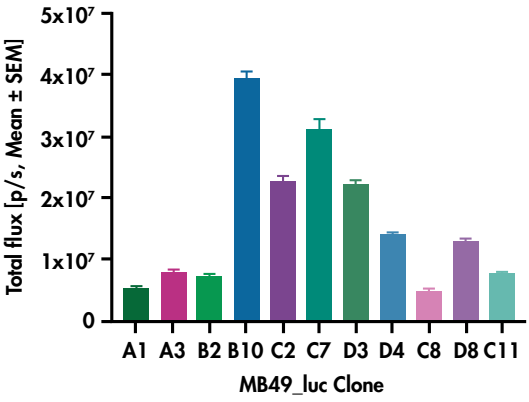


Figure 1. Generation and isolation of MB49_luc clones
The murine bladder cancer cell line MB49 was transduced with firefly luciferase and GFP. Multiple clones were isolated and analyzed *in vitro* for luminescent signal and growth. A limiting dilution assay was performed to select single cell MB49_luc clones with varying degrees of luminescent signal. 250,000 cells were plated per well, luciferin added, and signal detected within 10 minutes of addition.

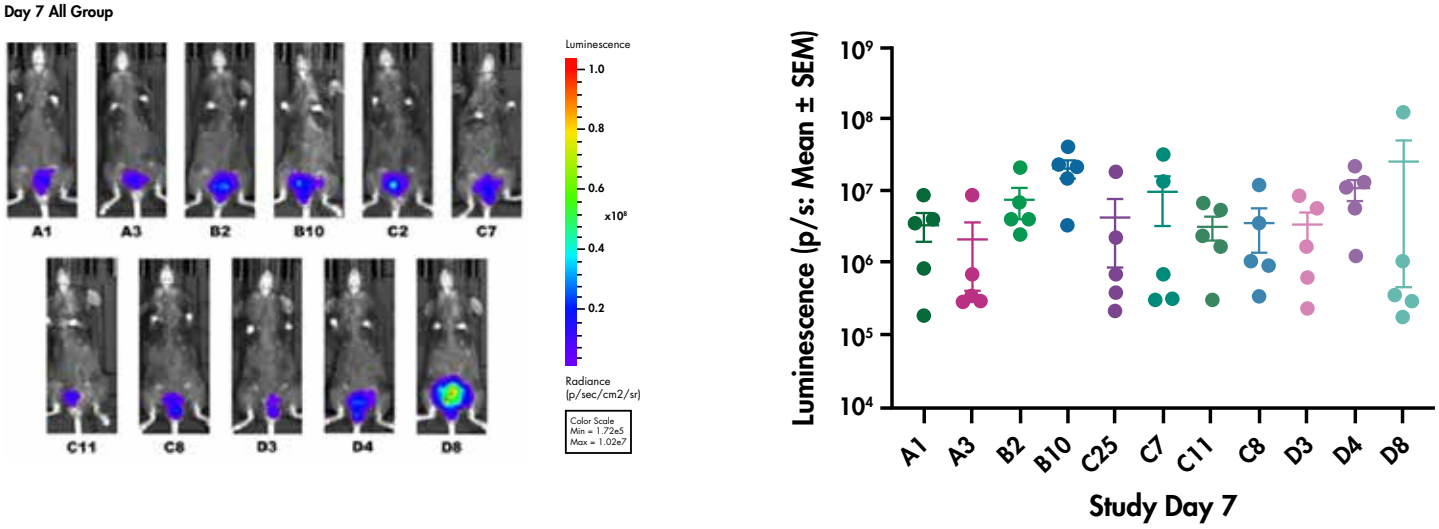


Figure 2. *In Vivo* MB49_luc Clone Analysis
Representative *in vivo* luminescent signal seven days post inoculation (left). Average luminescence for each clone (5 animals per group) seven days post inoculation (right).

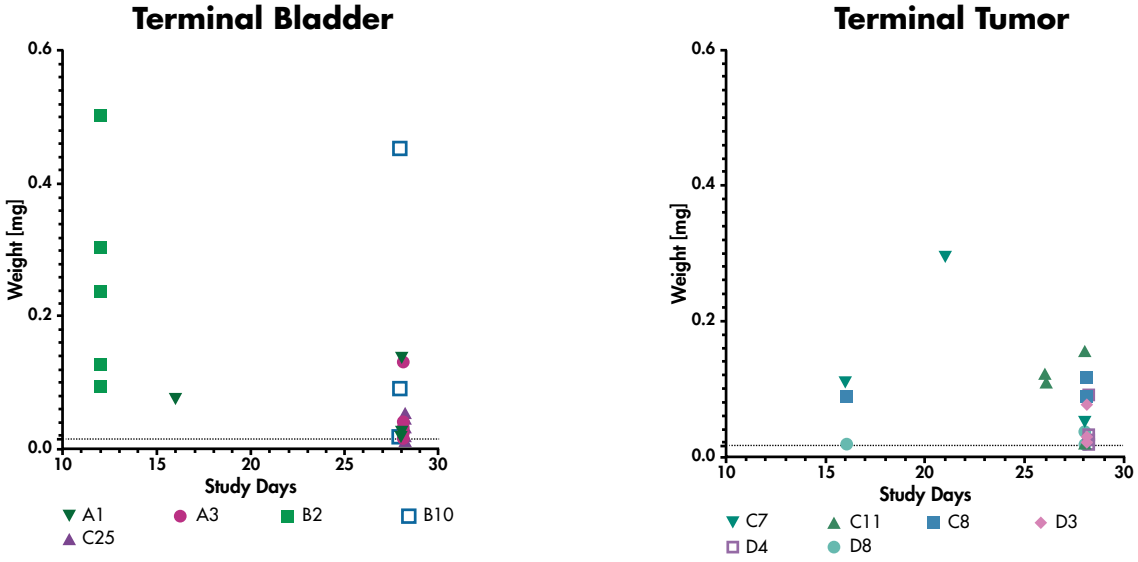


Figure 3. Terminal Tumor Weigh Comparison Across Clones.
Bladders and tumors were excised at termination from all animals still alive and weights recorded (dotted line represents average of bladder weights from sham inoculated mice).

TILs infiltration and T cell proliferation was assessed across different clones to provide a means for selecting hotter or colder tumor models for downstream analysis (Figure 4).

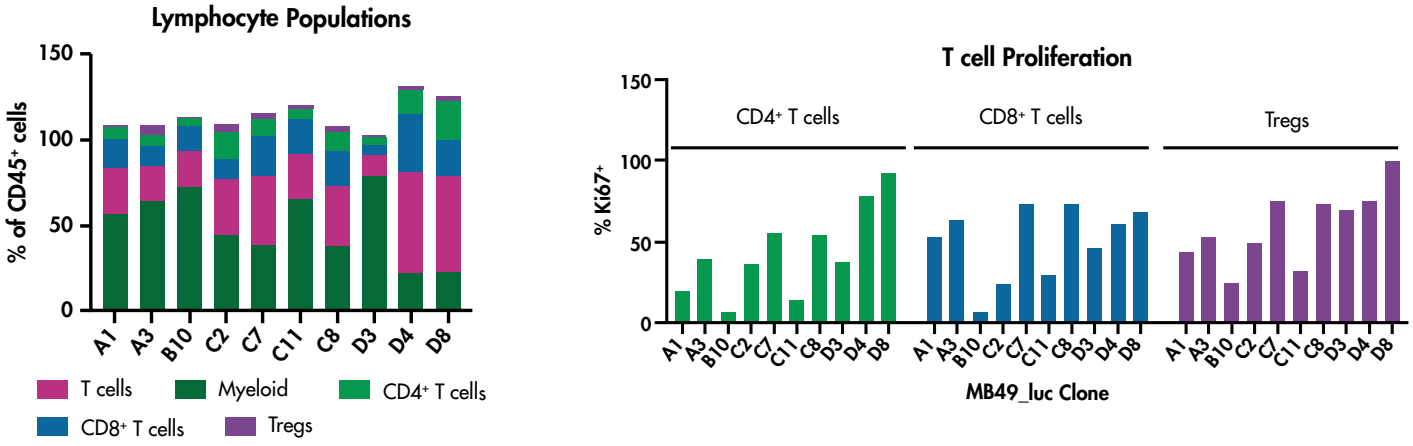


Figure 4. Tumor Infiltrating Lymphocytes 28 Days Post Inoculation.
Excised tumors were dissociated and analyzed via flow cytometry to determine if clones varied in tumor infiltrating lymphocyte populations (TILs). Flow cytometric analysis of Ki67 staining was used to determine proliferation of CD4+ T cells, CD8+ T cells and Tregs present in tumors. Panel: LDA, CD45, CD3, CD11b, CD4, CD8, FoxP3, Ki67

Our MB49_luc model characterization data provide a preclinical reference for cell line selection for analysis of new drug candidates in C57BL/6 female mice bearing superficial bladder tumors.

Our established MB49_luc orthotopic *in vivo* models represent an invaluable tool for the assessment of novel therapies targeting NMIBC in a physiologically relevant settings.

MBT-2 Bladder Cancer Instillation Model

In addition to our established MB49_luc model, our team is also optimizing the establishment of MBT-2 via intravesical implantation in C3H/He mice.

Weekly intravesical treatment regimen is also available in this model and tumor progression is assessed via body weight measurement and clinical observations.

Muscle Invasive Bladder Cancer (MIBC) Models

The therapeutic landscape for patients with advanced disease is constantly expanding with immune-checkpoint inhibitors and a pan-FGFR inhibitor approved by the FDA in recent years. In this rapidly evolving landscape, robust preclinical models can contribute to make better informed decisions before moving a new agent to the clinic.

At Reaction Biology we have established a MIBC model from the MB49_luc2F7 cancer cell line, which are injected directly into the bladder wall in C57Bl/6 mice (Figure 5).

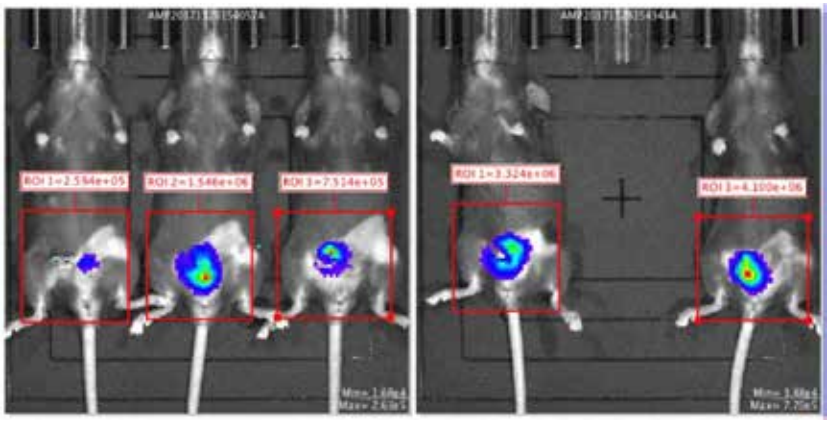


Figure 5. *In Vivo* MB49_luc2F7 Model Establishment.
Region of interest (ROI) visualized via bioluminescent imaging.

We have further compared tumor size at multiple time points (Days 5, 8, 12, 15, 19, 23) for the MB49_luc2F7 model established via either direct injection into the bladder wall or instillation, by measuring the intensity of the bioluminescent signal (Figure 6). This allows a direct comparison of the impact of the route of administration on tumor progression in this model

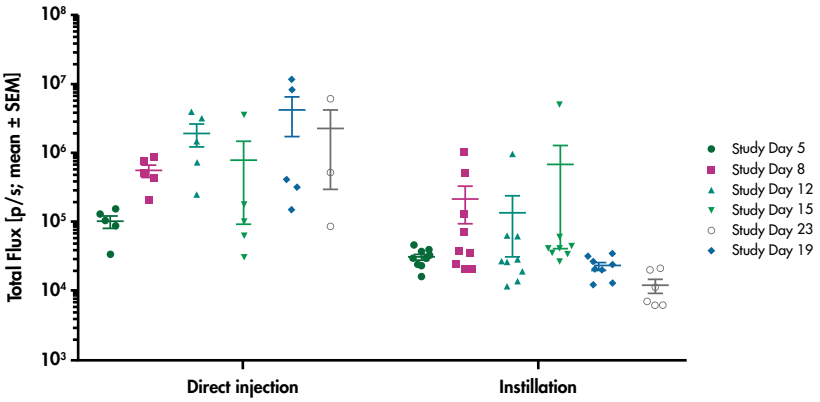


Figure 6. Tumor size comparison
across two different routes of establishment for MB49_luc2F7 model

Summary

The treatment landscape for BC is rapidly evolving, driven by novel discoveries and the virtually unchanged long-term survival rates of patients with bladder cancer over the last few decades.

Preclinical models of BC are required for early-stage drug development of novel therapeutics to improve patient outcome.

At Reaction Biology we have successfully established both NMIBC and MIBC models via orthotopic cancer cell instillation or bladder wall injection which are available for testing the *in vivo* efficacy of your compound.

Our experienced, PhD level scientists can offer tailored consultations to ensure you receive reproducible and meaningful results in IND-ready study reports.

Our *in vivo* solutions for BC drug development include models established via orthotopic bladder instillation for NIBC and direct bladder wall injection to mimic the more aggressive phenotypes of MIBC.

All our established models can be treated via weekly intravesical compound delivery to mimic the clinical administration route.

Connect with our team to find out how we can help you accelerate your BC development programs.

References

[1]Al Hussein Al Awamlh B, Chang SS. Novel Therapies for High-Risk Non-Muscle Invasive Bladder Cancer. Curr Oncol Rep. 2023

Get started today

Let's partner and develop the next generation of BC therapies!

REACTION BIOLOGY

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