Intravesical Administration of VB-85247, a Novel STING Agonist, Induces Strong and Durable Antitumor Immune Response in a Non-Muscle Invasive Bladder Cancer Model

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BACKGROUND

Introduction: Bacillus Calmette-Guerin (BCG) unresponsive non-muscle invasive bladder cancer (NMIBC) patients are in great need of effective immunotherapies. STING (Stimulator of Interferon Genes) plays a central role in mounting innate and adaptive immune responses to tumor cells. Activation of the STING pathway leads to the induction of inflammatory cytokines (IFN-α/β, TNF-α, IL-6, and CXCL10), maturation and activation of dendritic cells (DC), and induction of anti-tumor T cells. Our group has developed a novel STING agonist, VB-85247. Here we use a murine NMIBC model to report the potent antitumor effect of VB-85247 compared to standard of care BCG and anti-PD1 checkpoint inhibitor therapy currently used in the management of NMIBC.

Methods: We developed a mouse model of Non-Muscle Invasive Bladder Cancer (NMIBC) utilizing orthotopically implanted MB49-Luc cells, permitting BLI (Bioluminescence) measurement of tumor growth by In Vivo Imaging Systems (IVIS).

Results: VB-85247 was found to bind to mouse STING and all major variants of human STING protein. VB-85247 induced high levels of IFN-β and other cytokines across cells from different species, including primary human bladder epithelial cells.

VB-85247 treatment by intravesical instillation in the mouse model of NMIBC resulted in dose dependent tumor regression starting after the first treatment, achieving up to a 100% complete response rate at the 40 µg dose level after 5 weekly treatments. The treatment was well tolerated, eliciting strong and durable anti-tumor immune responses without any mortality. All cured mice rejected a re-challenge with MB49-Luc cells with no further treatment, demonstrating long-lasting anti-tumor immunity. By contrast, BCG treatment in the same model was not efficacious. Combination with anti-PD1 treatment reduced the dose of VB-85247 needed to achieve 100% complete responses to 20 µg, whereas anti-PD1 treatment alone resulted in only 25% complete responses. In addition, a single dose of 40 µg VB-85247 by bladder instillation in the NMIBC model induced systemic immune responses including serum cytokines demonstrating Type I IFN responses plus DC mobilization and activation in the blood, draining lymph nodes, and spleen within 24 hours.

Conclusions: The STING agonist VB-85247 was well tolerated and displayed robust efficacy by bladder instillation in a mouse orthotopic tumor model of NMIBC, achieving up to 100% complete responses. All cured mice rejected fresh inoculations of tumor cells with no further treatment, demonstrating induction of immunologic memory. Treatment with BCG was not efficacious in the model. These results suggest the potential utility of the VB-85247 STING agonist in the treatment of BCG unresponsive NMIBC patients. Based on these data, VB-85247 is in clinical development.

SUMMARY

- VB-85247 induces an anti-tumor immune response with immunologic memory which protects from re-challenge with no further treatment.
- VB-85247 treatment achieved up to 100% complete responses in the MB49 model of BCG-unresponsive NMIBC.
- VB-85247 also provides combination benefit with anti-PD1 immunotherapy in the NMIBC model.
- Efficacious doses were well tolerated in the mouse model of NMIBC.
- VB-85247 treatment induced upregulation of immune cell stimulating cytokines and dendritic cell maturation *in vivo*.
- VB-85247 is in clinical development for BCG unresponsive NMIBC.

RESULTS

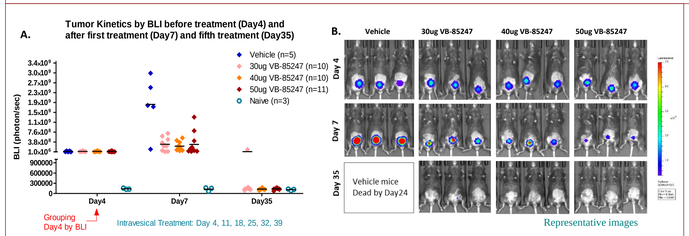

Figure 1. VB-85247 monotherapy provides robust efficacy in the mouse model of BCG-unresponsive NMIBC. Orthotopic bladder tumors were established in C57BL/6 mice by instilling MB49-Luc cells in the bladder on Day 0. The growth of bladder-implanted tumor cells was monitored by bioluminescence (BLI) signal using In vivo Imaging System (IVIS). Animals were assigned to treatment groups using a randomization based on BLI signal on Day 4. VB-85247 was administered via intravesical instillation as 6 weekly doses. A. Mean BLI signal from intravesical implanted MB49Luc cells on Day 4, 7 and 35. B. Bioluminescent images of representative mice from each experimental group on Day 4, 7, and 35.

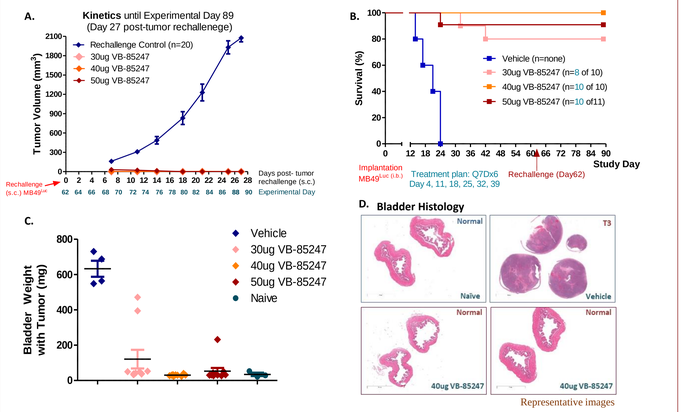

Figure 2. VB-85247 induces immunological memory. To determine whether immunologic memory was induced in all tumor-free mice from Figure 1, mice were re-implanted on the flank with the MB49-luc tumor cells on Day 62 post-primary tumor implantation. Naïve, age-matched, mice were used as controls. A. Tumor growth kinetics of subcutaneously implanted tumor at the indicated time points. B. Kaplan-Maier survival curves for the Vehicle control and VB-85247 treated groups. Survival is noted in parenthesis; *** P<0.0001 (Mantel-Cox Test). C. Bladder weight with the tumor as outlined in B, or treated groups at study termination on Day 89. D. Representative images of H&E-stained bladder sections from naïve, control vehicle group and 40µg VB-85247group with pathological evaluation.

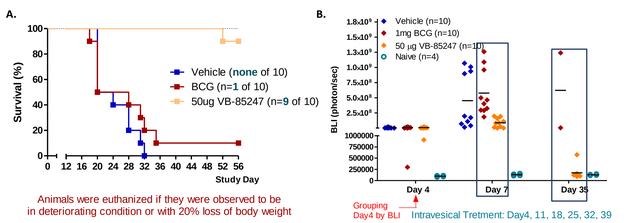

Figure 3. VB-85247 is markedly superior to BCG treatment in the mouse model of NMIBC. Bladder tumors and animal assignment to treatment groups as described in Figure 1. A. Kaplan-Maier survival curves for the Vehicle control and VB-85447 treatment groups. Survival is noted in parenthesis; *** P<0.0001 (Mantel-Cox Test). B. Tumor growth kinetics of MB49Luc cells based on BLI signal on Day 4, 7, and 35. VB-85247 treatment inhibited tumor growth within 3 days of first treatment.

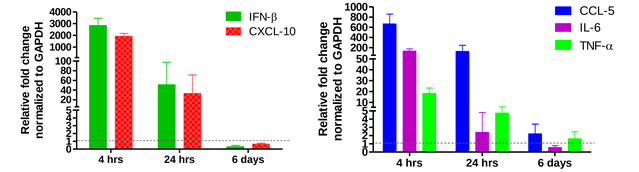

Figure 4. VB-85247 induces expression of immune cell stimulating cytokines and chemokines in the bladder. Tumor bearing mice received a single dose of either saline or 40 µg VB-85247 via intravesical instillation. Figures show relative fold change in gene expression between vehicle and treated mice as determined by quantitative RT-PCR analysis of bladder tissue.

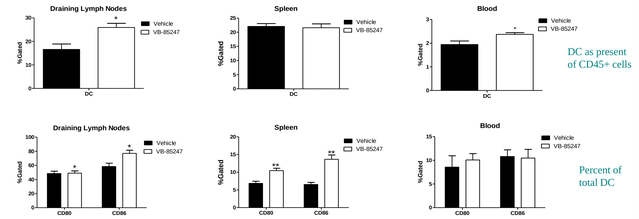

Figure 5. Single treatment of VB-85247 in MB49-luc NMIBC model resulted in dendritic cell maturation and migration to draining lymph node and spleen. Tumor bearing mice received a single dose of 40ug VB-85247 via intravesical instillation. Flow cytometry was used to detect DCs and costimulatory CD80 CD86 molecules from draining lymph nodes, spleen, and blood at 24 hours. A. Percent gated DCs. Statistical significance was calculated using unpaired t-test. Error bars are ± SEM. B. Percent gated CD80⁺ and CD86⁺ cells. Statistical significance was calculated using unpaired t-test. Error bars are ± SEM. *P<0.05, **P<0.01.

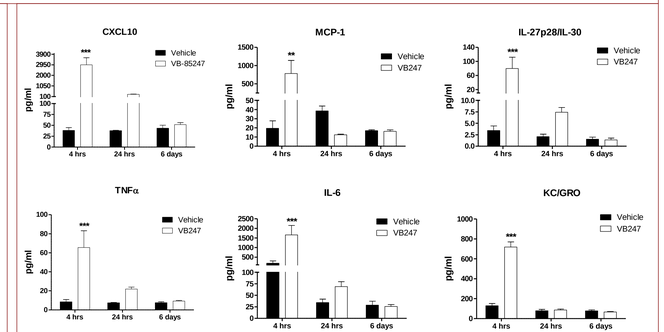

Figure 6. Local administration of single dose VB-85247 elicited pro-inflammatory cytokines and chemokines *in vivo*. Multiplex analysis of serum cytokines and chemokines at 4 hours, 24 hours and 6 days after intravesical delivery of single dose of 40 µg VB-85247 in mice with bladder MB49-Luc tumors. Results are shown as mean ± SEM. **P<0.01, ***P<0.001.

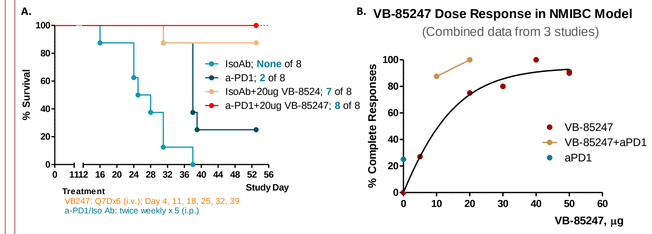

Figure 7. VB-85247 provides an additive effect to anti-PD1 immunotherapy in the mouse model of NMIBC. Bladder tumors were established and animals assigned to experimental groups as described in Figure 1. Mice were treated on Days 4, 11, 18, 25, 32, and 39 with 20µg VB-85247 via intravesical delivery in combination with anti-PD1 (10 mg/kg) or equal amount of isotype control twice weekly for total of 5 weeks. Control groups were treated with isotype antibody or anti-PD1 (10 mg/kg) alone. A. Kaplan-Maier survival curves. Survival is noted in parenthesis; *** P<0.0001 (Mantel-Cox Test). B. Percent complete response to VB-85247, anti-PD1 and combination therapy. Cumulative data from three different studies.

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