LB-122 Significant Cytotoxic And Immunomodulatory Effects Of Continuous Low Dose Intravesical Gemcitabine In Rodent Bladder Tumor Models

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ABSTRACT

Traditional gemcitabine bladder instillations (40 mg/mL) exhibit limited efficacy possibly due to micturition and saturable nucleotide uptake limiting tumor exposure. The present study investigates gemcitabine's anti-tumor and immunomodulatory effects after continuous low dose intravesical administration to enhance bladder tumor exposure.

The direct effects of low dose gemcitabine at nominal bladder urine concentrations of 0, 20, 40, or 80 µg/mL were studied in athymic nude rats with human MIBC cells, T24-TurboFP635, injected into the bladder. After 3 days tumor growth, gemcitabine or vehicle was perfused into the bladder over 5 days, N=6 per group. Tumor bioluminescence was measured on day 5. A companion study in immunocompetent Wistar rats inoculated with syngeneic NBTII bladder tumors, provided tumors for immunocyte analysis. A significant reduction in bladder tumor bioluminescence of 47.6%, 70.5% and 91.2% vs. control was observed in the 20, 40, and 80 µg/mL groups, respectively. Flow cytometric analysis of residual tumors in immunocompetent rats demonstrated a significant decrease of 85.2% in the proportion of tumor T regulatory cells to T effector cells.

Gemcitabine immunogenicity was studied in immunocompetent rats with NBTII cells injected into the bladder and subcutaneous flank tissue; flank tumors were implanted either simultaneously with bladder tumors or 14 days after bladder tumor implantation. Control groups received only flank tumors, N=15, or bladder and flank tumors with vehicle perfusion, N=10. Treatment groups, N=10, received two 5-day bladder perfusions, yielding gemcitabine urine concentrations of 10, 20, and 40 µg/mL which were separated by 7 days. Flank tumor growth was similar with tumor doubling times of 4.5 days after subcutaneous injections of 1.0, 2.5, or 5.0 x 10^6 cells and were unchanged with concomitant bladder tumor implantation and vehicle perfusion. During the first gemcitabine perfusion cycle, flank tumor growth was fully arrested, with tumor sizes averaging only 50% of controls in rats with concomitant bladder tumors; this effect was maintained during the 7-day drug free period in the 40 µg/mL group. Initiation of the second perfusion resulted in rapid flank tumor ablation in all but one animal completing the study. A similar priming effect was observed in the 10 and 20 µg/mL groups. Delaying flank tumor implantation to 3 days before the second perfusion not only prevented tumor growth, but completely ablated all tumors.

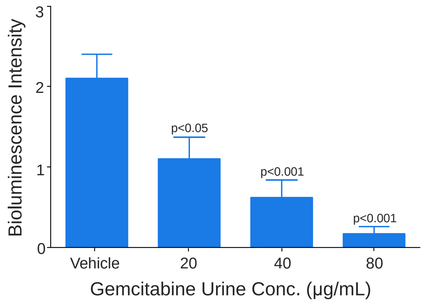
The results suggest continuous low dose intravesical gemcitabine inhibits bladder tumor growth, shifts the tumor microenvironment toward effector T cell dominance and induces a systemic immune response sufficient to suppress distant disease. Perhaps for the first time, the results also demonstrate a cytotoxic agent can induce a significant abscopal response when administered under proper conditions.

OBJECTIVES

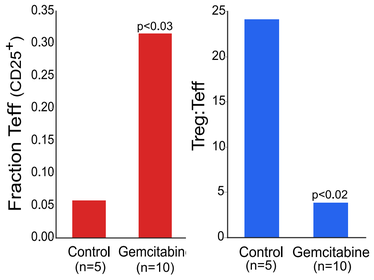
- Characterize the effects of continuous, low dose intravesical gemcitabine on bladder tumor growth
- Determine the impact of intravesical gemcitabine on the bladder tumor microenvironment
- Investigate the systemic immunological effects of local tumor treatment utilizing intravesical gemcitabine

RESULTS

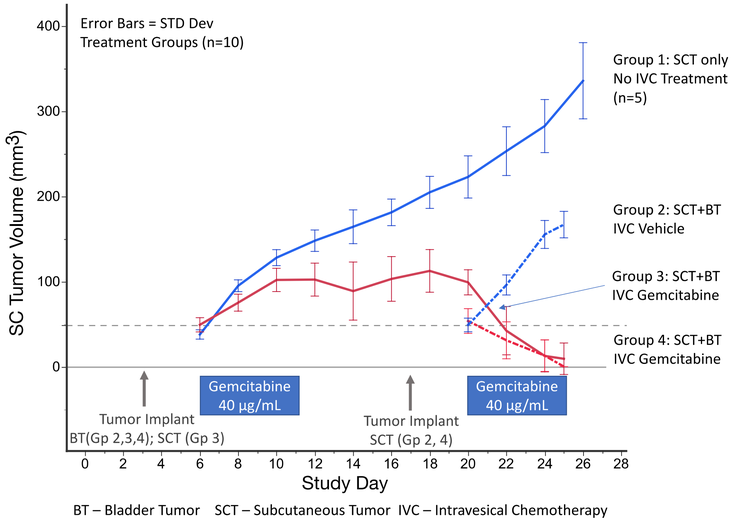
Gemcitabine Concentration Dependent Inhibition of Bladder Tumors



Enhanced T cell Activation, Reduced Treg Expression In Tumor Microenvironment



Systemic Tumor Ablation After Continuous Low Dose, Intravesical Gemcitabine Treatment of Bladder Tumors



BT – Bladder Tumor SCT – Subcutaneous Tumor IVC – Intravesical Chemotherapy

METHODS

- Indwelling bladder cannulas, permitting chronic perfusion, were surgically placed in rats 3 days prior to tumor implantations
- Gemcitabine (or vehicle) was perfused (0.3 mL/hr) over 1 or 2 - 5 day treatment cycles, yielding nominal urine concentrations of 0, 20, 40, or 80 µg/mL (T24 tumors), or 0, 10, 20 or 40 µg/mL (NBTII tumors)
- T24-TurboFP635 UC cells were injected into the bladder wall of athymic rats (Hsd:RH-Foxn1rnu, Harlan). Tumor bioluminescence was measured on day 5
- NBT-II UC cells were injected into the bladder wall and/or subcutaneous flank of Wistar immuno-competent rats (Vivo BioTech). Bladder tumors were dissected on day 5 for tumor immunocyte study
- Tumor immunocytes ($CD4^+$, $CD4^+CD25^+$, $CD4^+FOXP3^+$, $CD8^+$, $CD8^+CD25^+$, $CD8^+FOXP3^+$) were harvested by enzymatic and mechanical processing followed by magnetic bead enrichment and flow cytometry
- Subcutaneous tumor volumes were measured by caliper

CONCLUSIONS

- Continuous low dose, intravesical gemcitabine inhibits muscle invasive bladder tumors in a concentration dependent manner
- Gemcitabine increases activated T cells in the tumor microenvironment while reducing local Treg cells
- Local treatment of bladder tumors induced a systemic immune response sufficient to ablate tumors at distant sites
- Together, these results suggest continuous low dose intravesical gemcitabine may be useful in treating both organ confined and disease beyond the bladder