

## P75 NEUROTROPHIN RECEPTOR INHIBITION FOR TREATMENT OF RADIATION CYSTITIS

### Hypothesis / aims of study

Ionizing radiation used as a therapy for pelvic malignancies can induce cystitis leading to long-term bladder dysfunction. The urothelial cell layer, which is highly radiosensitive, has been shown to be a principal site of cellular damage in the bladder. This leads to inflammation and release of nerve growth factor (NGF) and brain derived neurotrophic factor (BDNF), the pro forms of which bind to p75 neurotrophin receptors (p75<sup>NTR</sup>) which are highly expressed on the urothelium and are initiators of the apoptotic cascade. We hypothesize that p75<sup>NTR</sup> blockade can prevent recurrent urothelial loss and treat radiation cystitis. Accordingly, the aims of this study were to determine the effects of the selective p75<sup>NTR</sup> blocker, LM11A-31, on urothelial cell survival and bladder function post irradiation.

### Study design, materials and methods

Adult female C57BL/6 mice were anesthetized (avertin, 5 mg/kg), a lower midline incision made and the urinary bladder externalized for selective irradiation (10 Gray; X-RAD 320 KV). LM11A-31 was administered by oral gavage (100 mg/kg in water) starting one day after irradiation and continued daily for 7 days when sacrificed. Bladder walls were histologically evaluated using Hematoxylin & Eosin staining while function was assessed using decerebrate cystometry.

The effects of LM11A-31 on cultured urothelial cells (UROtsa) were also assessed. Cells were plated at low density (250 cells / 15 mm well), irradiated at 5 Gy and treated with LM11A-31 (1  $\mu$ M) or vehicle (media) 1 hour post irradiation. Colonies were allowed to grow for 6 days after which they were stained with crystal violet and evaluated using automated colony counting.

Differences between data sets were tested with Student's t-test, data represented as mean  $\pm$  SD.

### Results

One week post irradiation, cystometry revealed decreased intercontractile intervals, reduced bladder compliances and the presence of non-voiding contractions compared to non-irradiated bladders (Figure 1). At 7 days, the urothelial layer looked intact, but the underlying tissue damage suggested urothelial disruption early after irradiation (Figure 2). LM11A-31 administration reduced bladder wall damage and functional changes.

LM11A-31 treatment also significantly increased UROtsa cell survival and colony growth following irradiation in comparison to untreated irradiated cells ( $70 \pm 9\%$  versus  $44 \pm 8\%$ , respectively,  $p < 0.05$ ).

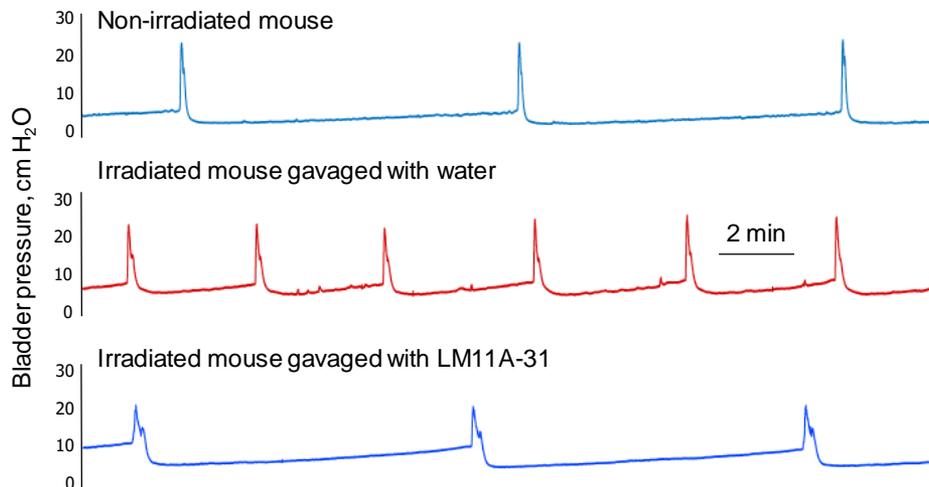
### Interpretation of results

LM11A-31 treatment eliminated the development of bladder edema and overactivity and increased the survival and growth of cultured urothelial cells following irradiation. These results suggest that p75<sup>NTR</sup> signaling pathways are involved in irradiation-induced bladder damage and that selective p75<sup>NTR</sup> inhibition may be beneficial in treating radiation cystitis.

### Concluding message

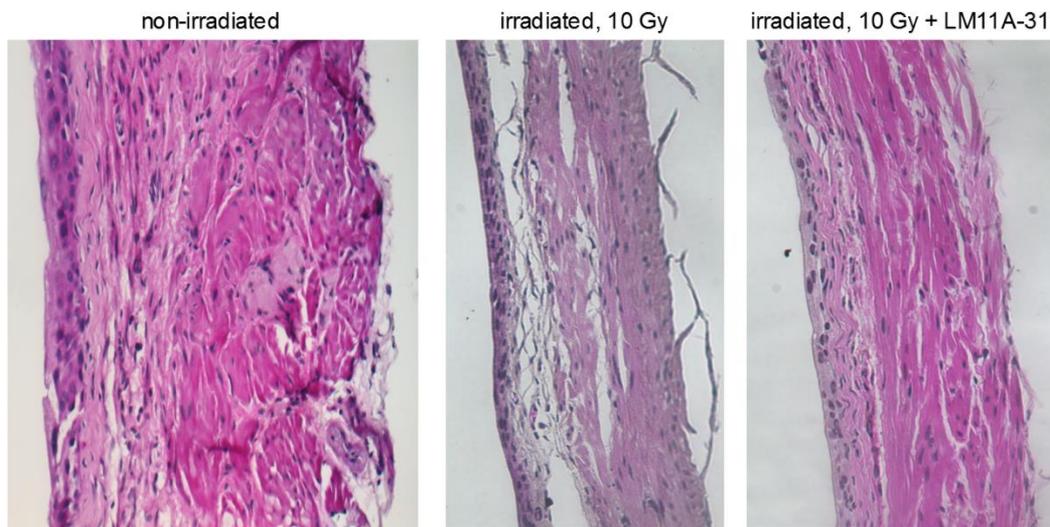
We have demonstrated that irradiation-induced urothelial cell death, decreased bladder compliance and development of overactivity is ameliorated by selective p75<sup>NTR</sup> inhibition post irradiation. Accordingly, p75<sup>NTR</sup> antagonists are potential therapeutic agents for both the prevention and treatment of radiation cystitis. They may also be beneficial in other conditions associated with compromised urothelial integrity including interstitial cystitis/bladder pain syndrome.

**Figure 1.** LM11A-31 treatment ameliorates bladder overactivity post irradiation (BP, baseline pressure; PT, pressure threshold; ICI, intercontractile interval; BC, bladder compliance.)



Mouse	BP, cmH <sub>2</sub> O	PT, cmH <sub>2</sub> O	ICI, sec	BC, $\mu$ /cmH <sub>2</sub> O
Non-irradiated	3 $\pm$ 1	12 $\pm$ 2	720 $\pm$ 180	25 $\pm$ 6
Irradiated, 10 Gy	4 $\pm$ 1	7 $\pm$ 1	251 $\pm$ 15	10 $\pm$ 2
Irradiated + LM11A-31	3 $\pm$ 1	6 $\pm$ 1	519 $\pm$ 49	29 $\pm$ 5

**Figure 2.** p75<sup>NTR</sup> inhibition improves bladder wall integrity post irradiation.



**Disclosures**

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