EG 427

PINPOINT GENE THERAPY

Gene therapy of bladder dysfunctions by a replication-incompetent recombinant HSV-1 vector expressing the light chain of botulinum neurotoxin F in C-type afferent bladder neurons.

A novel HSV-based gene therapy for chronic bladder disease.

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BACKGROUND

EG110A is a non-replicative recombinant herpes simplex virus type 1 (HSV-1)-derived vector that expresses the light chain of the botulinum neurotoxin F (BoNT/F-LC) driven by the human calcitonin gene-related protein (hCGRP) promoter, designed to achieve long-term sensory neuron-selective transgenic expression in bladder afferents. The mechanism of action of EG110A is based on the natural ability of HSV-1 to infect peripheral sensory neurons, and to express the BoNT/F-LC inside the targeted neurons, leading to the cleavage of the VAMP2 protein that is essential for neurotransmission (Figure 1). The hCGRP promoter intends to reinforce the selectivity of the vectors for C-fibre bladder afferents, whereas the introduction of the transgene in the LAT region of the vector genome promotes long-term expression. The aim for EG110A is to provide a long-term efficacy, possibly lasting several years, for the treatment of neurogenic detrusor overactivity (NDO) and other lower urinary tract dysfunctions such as OAB (overactive bladder) or interstitial cystitis/bladder pain syndrome (IC/BPS), while preserving the ability of the bladder to contract and not cause urinary retention.

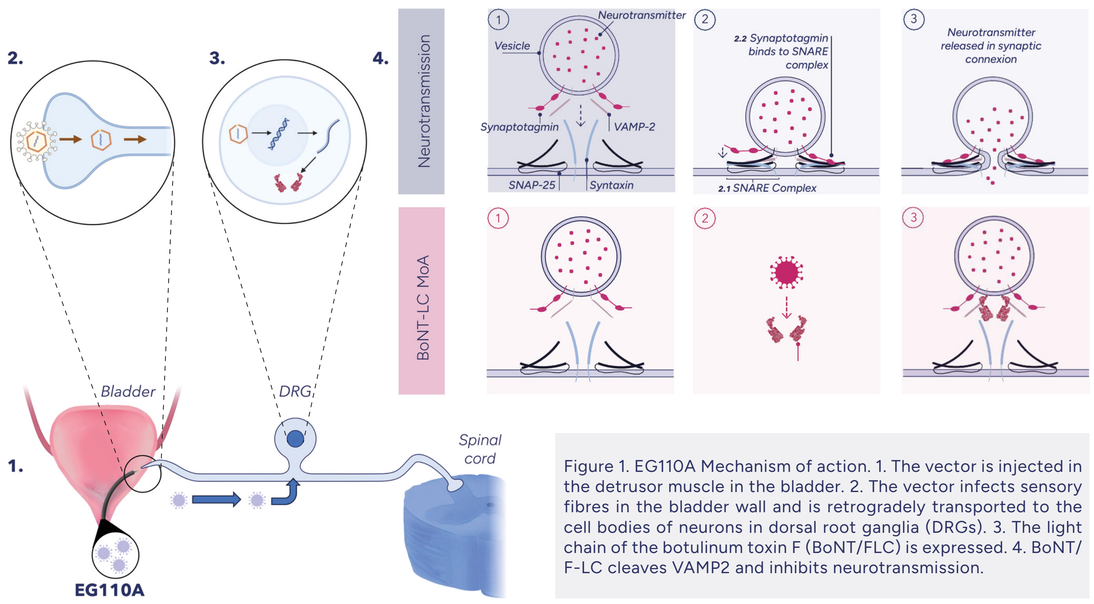


Figure 1. EG110A Mechanism of action. 1. The vector is injected in the detrusor muscle in the bladder. 2. The vector infects sensory fibres in the bladder wall and is retrogradely transported to the cell bodies of neurons in dorsal root ganglia (DRGs). 3. The light chain of the botulinum toxin F (BoNT/FLC) is expressed. 4. BoNT/F-LC cleaves VAMP2 and inhibits neurotransmission.

METHODS

EG110A was injected in the detrusor muscle of adult female rats (2E+08 PFU in 40µL per rat) after open laparotomy exposing the bladder. Control animals received 40µL of vehicle buffer. One week after injection five rats injected with EG110A were euthanized and L6/S1 dorsal root ganglia (DRGs) were collected for the measurement of the transgene expression by digital droplet PCR (ddPCR). At five weeks after injection, rats (n=14 rats per group) were anesthetized and a catheter connected to a pressure transducer was inserted in the bladder through the dome. The bladder was continuously perfused with saline during a stabilization period of 90 min followed by perfusion with 30 µM of capsaicin for 60 min. Intravesical pressure was recorded via cystometry throughout the experiment and parameters of reflex-evoked bladder contractions were measured. Data were computed over 15 min intervals during the capsaicin irritation period and normalized for each rat by the value of the stabilization period. Three control rats and two EG110A-injected rats could not be analyzed due to leakage of the bladder during cystometry experiment. Following cystometry measurements, L6/S1 DRGs were collected for RNA expression analysis. Expression of BoNT/F-LC was measured by ddPCR on cDNAs following reverse transcription. Expression of BoNT/F-LC was also investigated in L6/S1 DRG neurons by RNAscope and immunostaining for neuronal markers.

RESULTS

1. Construction of EG110A

Two essential genes, ICP4 and ICP27, were deleted from the HSV-1 genome to make the vector non replicative (Figure 2). The sequence coding for the BoNT/F-LC, placed under the control of the human CGRP promoter, was inserted in the LAT region of the virus genome, between long-term expression (LTE) and downstream DNA insulator (Ins) motifs.

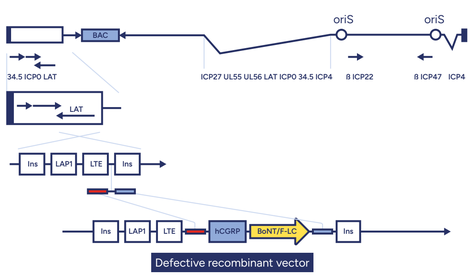


Figure 2. EG110A genome map.

2. EG110A cleaves VAMP2 and inhibits CGRP release

Primary rat sensory neurons were infected with EG110A at increasing multiplicity of infection (MOI). After 24 hours, dose-dependent VAMP2 cleavage was observed by western blot (Figure 3A). Dose-dependent inhibition of the release of neuropeptide CGRP in medium was measured with ELISA (Figure 3B).

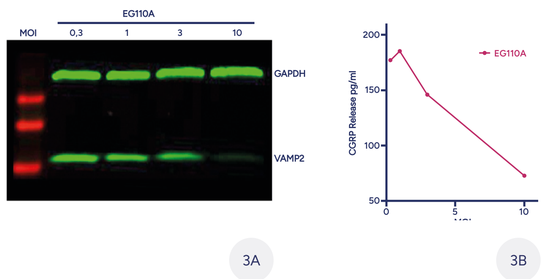


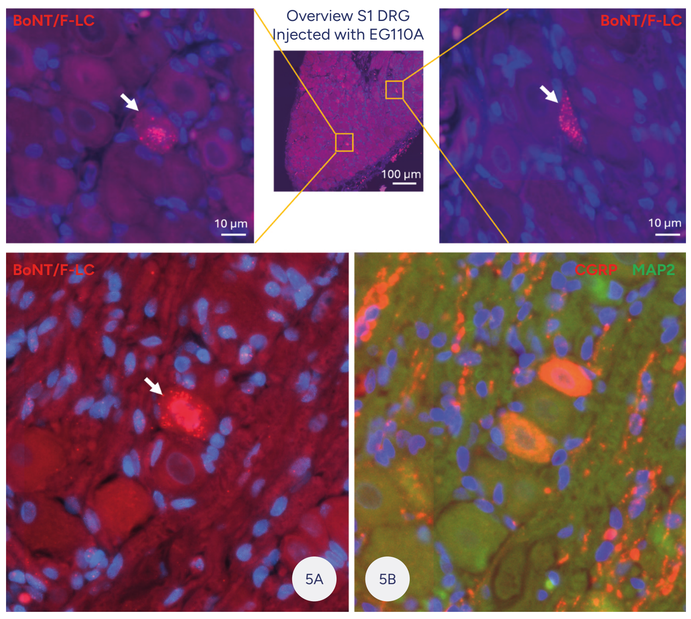
Figure 3. EG110A mediates VAMP2 cleavage thereby inhibiting CGRP release in primary rat sensory neurons.

3. Long-term transgene expression in dorsal root ganglia neurons

EG110A was injected in the bladder detrusor in rats. One week and 5 weeks after administration, DRGs (L6/S1, where cell bodies of bladder sensory neurons are located) were isolated. BoNT/F-LC transgene expression was measured by reverse transcription ddPCR on extracted RNAs. Figure 4A shows presence of the transcript and stable levels of expression in DRGs at 1- and 5-weeks following administration. Hence, EG110A injected in the bladder is able to transduce bladder sensory neurons and achieve long term expression of BoNT/F-LC in those cells. Similar experiments conducted with a reporter vector expressing firefly luciferase under the same hCGRP promoter showed transgene expression in DRGs for at least 12 weeks (Figure 4B).

Figure 4. A. BoNT/F-LC is expressed in DRGs following intradetrusor injection of EG110A. DRG L6/S1 were collected and pooled at 1 and 5 weeks after injection. ddPCR was performed to quantify absolute number of copies of BoNT/F-LC (mean +/- SD). B. Injection of a reporter vector EG111A (expressing firefly luciferase under hCGRP promoter) in the detrusor leads to long term (at least 12 weeks) luciferase expression in DRGs as measured by ddPCR (mean).

4. BoNT/F-LC is expressed in type-C neurons



Five weeks following administration of EG110A in bladder detrusor muscle, DRGs L6/S1 were collected. BoNT/F-LC transcripts were detected by RNAscope. Consecutive sections were stained for neuronal markers MAP2 (pan neuronal marker) and CGRP (C-type sensory neuron). Positive neurons for BoNT/F-LC were identified by RNAscope (Figure 5A). BoNT/F-LC positive cells were also positive for CGRP (Figure 5B) showing expression of the transgene in the targeted C-type neuronal population.

Figure 5. BoNT/F-LC is expressed in C-type DRG neurons following intradetrusor injection of EG110A. DRG L6/S1 were collected 5 weeks after injection. Sections were stained by RNAscope for the BoNT/F-LC (A) and consecutive sections were stained with MAP2 and CGRP antibodies (B). Arrow indicates BoNT/F-LC positive neurons.

5. Efficacy in capsaicin model

EG110A efficacy was tested in the acute intravesical capsaicin model in rat. Capsaicin stimulates specifically the C-type sensory neurons through its receptor TRPV1. Capsaicin perfusion in the bladder induces an increase of the frequency of bladder voiding contractions mimicking the pathophysiology of NDO.

EG110A was injected in the bladder detrusor in rats (2E+08PFU, 10 points of injection) (Figure 6A). After 5 weeks, under anesthesia, saline was perfused first in the bladder and intrabladder pressure was recorded (stabilization period). Capsaicin was then perfused for 60 min. Capsaicin instillation increased the frequency of bladder contractions in vehicle treated animals (Figure 6B). In EG110A treated rats, this increased frequency was prevented. Quantification of the intercontraction interval (time between two contractions) showed a significant increase in EG110A treated animals (Figure 6C). We conclude that EG110A inhibits capsaicin-stimulated C-type neurons in this acute animal model of NDO.

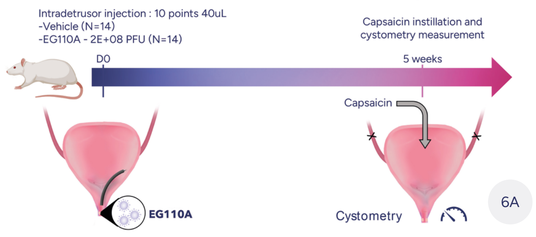
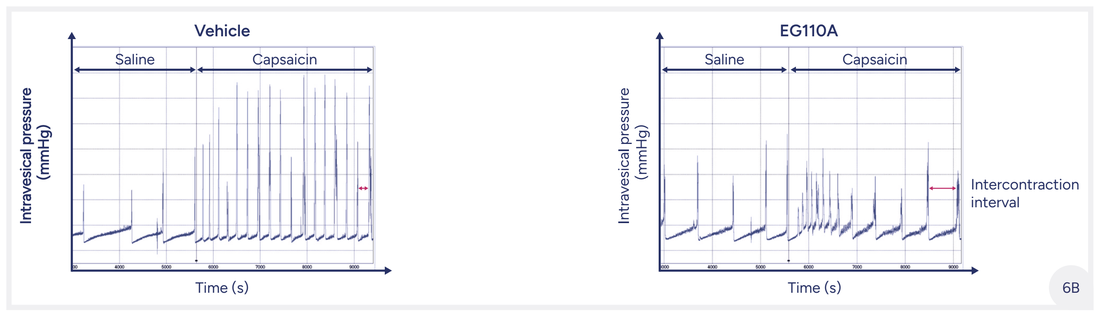
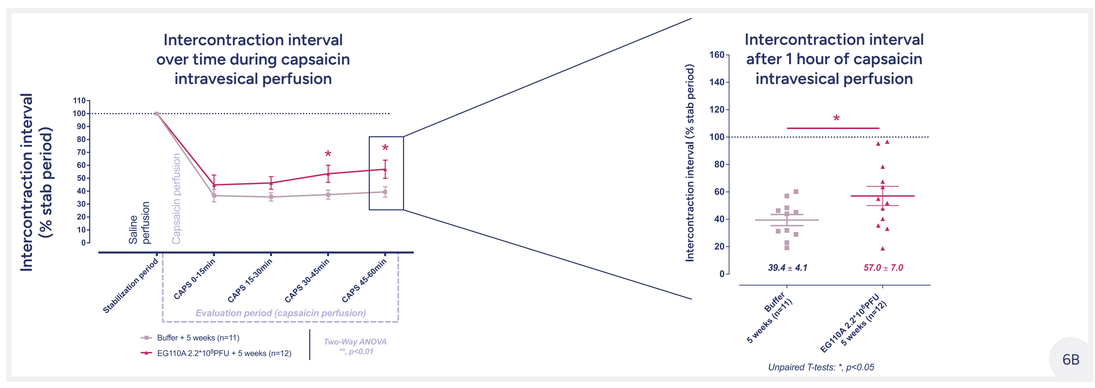


Figure 6. Efficacy of EG110A in the rat capsaicin model. A. Experimental design. B. Cystometric measurement in a vehicle and EG110A-treated animals, during saline and capsaicin perfusion. C. Quantification of the intercontraction interval between two voiding contractions. Means +/- SEM.





6. No effect of EG110A on normal bladder function

In the above capsaicin experiment, effect of EG110A on normal bladder function was also investigated by cystometry before capsaicin instillation (saline perfusion – stabilization period). No effect on any bladder functional parameter was found showing the selective effect of EG110A on C-type sensory neurons which are normally silent in normal bladder (Figure 7A). No change in post-void residual volume was observed showing that EG110A does not cause urine retention (Figure 7B).

Stabilization period comparison between buffer and EG110A vector groups (before capsaicin perfusion)

PARAMETERS	Buffer (n=11)	EG110A vector 2.2x10⁸ PFU/40µL (n=12)	Unpaired T-test
Maximal Amplitude (mmHg)	No significant difference between vehicle- and vector-treated group		ns
AUC of the micturition contraction (mmHg x s)			ns
Duration of the micturition contraction (s)			ns
Δ Pressure Threshold (mmHg)			ns
Baseline intravesical pressure (mmHg)			ns
Intercontraction interval (s)			ns
Voided volume (µl)			ns
Infused volume (µl)			ns
Voiding efficiency (%)			ns
Compliance (ml/mmHg)			ns

7A

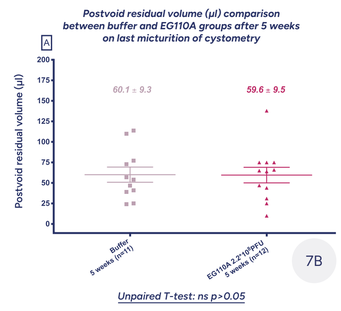


Figure 7. A. Bladder urodynamic parameters during stabilisation period in vehicle- and EG110A-treated rats. B. Post-void residual volume measured after cystometric measurements.

CONCLUSIONS

HSV-1-based non replicative vectors are being developed as an efficient tool to achieve long term expression of a transgene in sensory neurons. EG110A is a promising approach to treat C-fibre related NDO and other lower urinary tract disorders. EG110A has the potential to provide long term efficacy without adverse effects on voiding. Finally, these results show for the first time that intraneuronal expression of BoNT/F-LC can have a significant therapeutic potential. Noteworthy, beyond bladder dysfunction, the mode of action of EG110A makes this vector potentially suitable to treat any pathology driven by type C sensory neurons.