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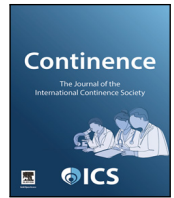
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Unlocking potential: Herpes Simplex Virus Type 1-based gene therapy in functional urology

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ABSTRACT

This literature review provides a comprehensive overview of the development and application of herpes simplex virus type 1 (HSV-1) vectors in gene therapy, with a specific focus on functional urology. Gene therapy is becoming more and more a clinical reality as more and more products getting market-approval. The text highlights the advantages of viral vectors, especially HSV-1, in delivering therapeutic genes due to their large cargo capacity, efficient growth in tissue culture, and tropism for neuronal cells. The neuronal system is crucial in functional urology because the intricate network of nerves or neuronal structures control and regulate the complex functions of the urinary system. In functional urology, HSV-1, vectors show promise in addressing conditions such as bladder pain syndrome, diabetic detrusor underactivity, and neurogenic detrusor overactivity. Studies demonstrate successful localized expression of therapeutic genes in animal models, indicating potential applications in treating bladder-related disorders.

This review underscores the promising frontier that HSV-1 vectors represent in functional urology, acknowledging the need for further research to optimize transduction efficiency, address potential immunogenic concerns, and ensure long-term effectiveness.

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1. Introduction

The concept of gene therapy has its origins in the 1960s, when early studies demonstrated the ability to introduce DNA sequences into mammalian cells for gene repair [1]. This groundbreaking approach not only offers the potential to treat disease symptoms but also targets the fundamental genetic issues embedded in our DNA. Despite being proposed over fifty years ago, the progress of gene therapy was initially hindered by technical challenges and ethical concerns [2]. However, recent breakthroughs in gene editing technologies, along with a deeper understanding of the human genome, have reignited interest and enthusiasm for gene therapy. Today, it has transformed from a theoretical concept into a practical and increasingly effective clinical reality.

A significant challenge in the development of gene therapy is devising a method to precisely deliver the therapeutic gene to the target tissue while ensuring the right level and duration of expression. Naked genetic material in the form of DNA and RNA is highly susceptible to degradation and can trigger unwanted immune responses when exposed to biological fluids [3]. This can lead to the loss of their therapeutic effectiveness. Hence, there is a demand for a system that can shield genes from degradation in the biological environment, traverse biological barriers, and facilitate precise targeting within cells. Because viruses can efficiently enter and replicate in human cells reflecting their unique biology and host cell preferences, it appeared that viruses could be engineered as effective gene delivery vehicles, called viral vectors [4].

To date, a plethora of viral vectors including adenovirus (AV), adeno-associated virus (AAV), lentivirus (LV) and herpes simplex virus type 1 (HSV-1) have proven their potential in safely and efficiently delivering gene therapies [2]. HSV-1 is a neurotropic virus and is particularly useful to deliver gene therapy locally in neurons that may modify or modulate their function. The neuronal system is crucial in functional urology because the intricate network of nerves or neuronal structures control and regulate the complex functions of the urinary system. In this literature review, we focus on the HSV-1 vector for gene therapies, its advantages and disadvantages and possible use in (functional) urology.

2. HSV biology

HSV-1 is an ubiquitous, double-stranded DNA human pathogen [5]. HSV-1 is primarily transmitted through close contact and primarily infects epithelial cells, which are found on the skin and mucous membranes, including the mouth, lips, eyes, and genitals. HSV-1 begins to replicate at the site of infection and then proceeds to travel by retrograde flow down an axon to the dorsal root ganglia (DRG) or trigeminal ganglia. It is in these ganglia, within the sensory neurons, that latency is established [6]. Here, it has the option to either reinstate a lytic phase or enter a dormant, non-infectious state. The lytic cycle is characterized by productive viral infection that can lead to sores. Due to several stimuli such as sunburn, stress or other infections, the virus can be reactivated from the latent state. Very rarely the viral particle is retrograde transported to the central nervous system and starts a latent or a lytic infection, which can evolve in encephalitis [7]. The life cycle is depicted in Fig. 1.

3. HSV molecular biology

The herpes simplex virus is among the largest DNA viruses. The virus consists of an envelope, a subenvelope structure referred to as the tegument, and a capsid, surrounding the internal DNA (figure 1) [8]. This linear double-stranded genome contains genes that can be classified as accessory and essential. Accessory genes can be individually deleted without substantially compromising virus replication. On the other hand, deletion of any essential gene completely blocks productive virus infection [5]. The manipulation of these viral genes has led to the creation of three types of HSV vectors: replication-defective recombinant vectors, replication-competent recombinant vectors and amplicon vectors.

3.1. Replication-defective recombinant vectors

The replication-defective recombinant vectors are viral vectors where ‘essential’ genes for in vitro viral replication are either mutated or deleted. As such, they are unable to replicate but retain many advantageous features of wild-type HSV-1, particularly the ability to express transgenes after having established latent infections in peripheral neurons. They are non-toxic to host cells [9]. This type of HSV-1 gene therapy was recently approved by FDA for the treatment of dystrophic epidermolysis bullosa by recurrent local cutaneous application and shows a very good safety profile in humans. [10]

3.2. Replication-competent recombinant vectors

Deletion of some non-essential viral genes results in viruses that retain the ability to replicate. Unlike replication-defective vectors, utilizing replication-competent vectors enables the initial delivery of genes to a small number of cells, with the potential for subsequent transfer to neighboring cells as the infection spreads. This approach can substantially enhance the effectiveness of gene delivery [11]. Attenuated HSV vectors have been tested mainly as oncolytic viruses, but also as live viral vaccines or as gene therapy vectors to deliver transgenes to the nervous system. [12]

3.3. Amplicon vectors

The genome of amplicon vectors does not carry any HSV-1 genes except one origin of DNA replication for production and one packaging signal allowing encapsidation [13]. The amplicon carries the gene(s) of interest. Amplicon vectors have the ability to accommodate very large sequences, compared to other viral vectors. The major limitation of amplicon-based gene delivery has been the generation of amplicon vector stocks that are free of the helper virus, which are needed to provide the functions necessary for packaging the amplicon into infectious viral particles. Amplicon stocks contaminated with helper virus are not desirable for gene therapy applications as it could lead to potential cytotoxicity and inflammatory responses [8].

After the viral vector is created, the next crucial step is to introduce the HSV-1 vector with the genes of interest into the target cells or tissues. This can be achieved through various delivery methods, such as direct injection, intravenous infusion, or local administration [2]. Once the HSV-1 vector enters the target cells, it follows the natural HSV-1 infection pathway. The vector infects the cells and delivers its cargo, including the therapeutic genes, into the host cell's nucleus. Inside this nucleus, the therapeutic genes carried by the HSV-1 vector are expressed allowing transcription and translation phases leading to the synthesis of functional proteins or molecules, which are responsible for mediating the intended therapeutic effect. [14] Fig. 2 depicts a schematic overview on the mechanism of viral vector gene delivery.

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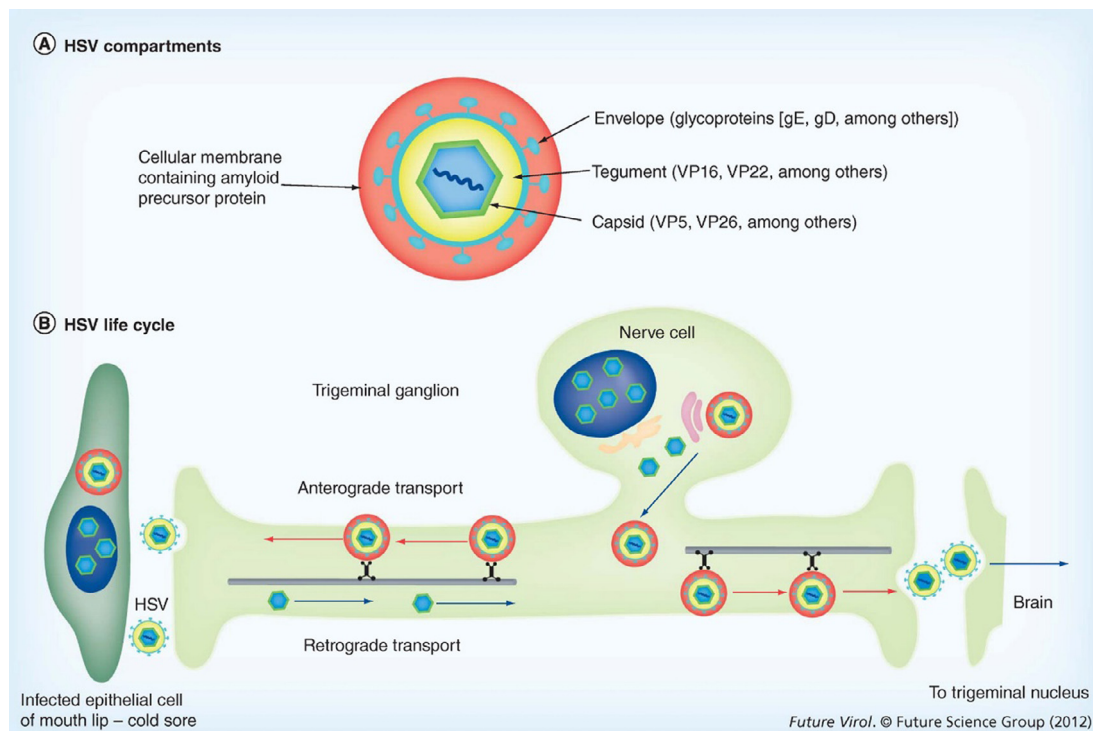


Fig. 1. HSV-1 structure (A) and life cycle (B). (A) The virus consists of an envelope, a subenvelope structure referred to as the tegument, and a capsid, surrounding the internal, double-stranded DNA. (B) HSV-1 is primarily transmitted through close contact and primarily infects epithelial cells, which are found on the skin and mucous membranes. HSV-1 begins to replicate at the site of infection and then proceeds to travel by retrograde flow down an axon to the dorsal root ganglia or trigeminal ganglia. It is in these ganglia, within the sensory neurons, that latency is established. Very rarely the viral particle is retrograde transported to the central nervous system.

Source: Adapted from Bearer EL. HSV, axonal transport and Alzheimer's disease: in vitro and in vivo evidence for causal relationships. *Future Virol.* 2012 Sep;7(9):885-899. doi: 10.2217/fvl.12.81. PMID: 23335944; PMCID: PMC3546524.

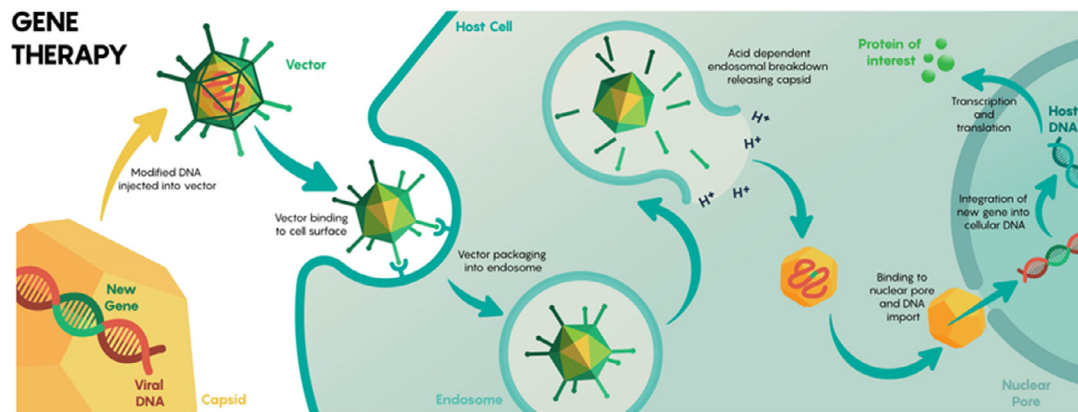


Fig. 2. Schematic overview of how viral vectors can be used to deliver genetic material to cells. After the viral vector is created, the HSV-1 vector is introduced into the target cell or tissue. Once the HSV-1 vector enters the target cell, it delivers its cargo, including the therapeutic genes, into the nucleus of the host cell. Inside the nucleus, the therapeutic genes are translated into functional proteins or molecules, which are responsible for mediating the intended therapeutic effect.

Source: Adapted from Ju, W. (2023, March 5). 3.6: Viral mediated delivery of genes to neurons. *Medicine LibreTexts*. [https://med.libretexts.org/Bookshelves/Pharmacology_and_Neuroscience/Neuroscience_\(Ju\)/03%3A_Fundamental_Neuroscience_Techniques/3.06%3A_Viral_Mediated_Delivery_of_Genes_to_Neurons](https://med.libretexts.org/Bookshelves/Pharmacology_and_Neuroscience/Neuroscience_(Ju)/03%3A_Fundamental_Neuroscience_Techniques/3.06%3A_Viral_Mediated_Delivery_of_Genes_to_Neurons).

4. Advantages and drawbacks

Depending on the specific therapeutic goals and safety considerations, both viral and non-viral vectors have their unique advantages,

and the choice between them depends on the specific requirements of the gene therapy approach. Compared to their non-viral alternatives, viral vectors are known for their high efficiency in delivering genetic material to target cells. As mentioned before, naked genetic material is highly susceptible to degradation. Viral vectors however can integrate into the host genome, allowing longer-lasting gene expression. Moreover, viral vectors are excellent at infecting a wide range of cell types, making them suitable for a variety of applications. They offer greater flexibility in terms of cargo capacity and can be engineered for specific purposes [15].

On the other hand, non-viral vectors are generally safer and less immunogenic than viral vectors. Additionally, non-viral vectors are often easier and more cost-effective to manufacture and have fewer

Table 1
Advantages and disadvantages of viral vectors versus non-viral vectors.

Advantages	Disadvantages
Highly efficient in delivering genetic material	Possibility to trigger immune responses and inflammatory reactions
Can be used in a wide range of different cells	Complex and costly production methods
Flexible cargo capacity	Possibility to cause mutagenesis (integrative vector only)
Variety of viral vectors with different specific characteristics, which can be exploited for different targets	Questions about persistence in dividing cells
Longer persistence in cells	

regulatory hurdles to overcome. Table 1 comprises some benefits and drawbacks of viral vectors.

Four viral vectors are most commonly used in preclinical and clinical studies: adenovirus, adeno-associated virus, lentivirus and the herpes simplex virus type 1. The main advantage of HSV-1-based vectors is the large cargo capacity, because the DNA coding for non-essential genes can be substituted with transgene material.

Apart from the large cargo, there are other features of HSV biology which also make it attractive as a vector. The virus grows well in tissue culture and high-titer stocks (large enough to consider manufacturing) can be obtained. Genetic manipulation of the viral genome is straightforward [16]. Furthermore, its tropism for neuronal cells has been exploited to obtain efficient gene delivery to neurons distal from the site of inoculation, making it ideal for treating neurogenic diseases [16]. Especially intriguing in this treatment paradigm is its ability to “peacefully” coexist within the neuron in a latent state and possibly remain there for life, as it has the ability to evade the host immune system [17]. In addition, the virus DNA will not integrate into cellular DNA, but remains episomal as a closed circular molecule, thus reducing the risk of insertional mutagenesis [18]. Finally, there are antivirals available for HSV in the event of unintended exposure.

There has traditionally been a concern with safety for the HSV-1, as the virus makes products that are highly toxic. However, Fink et al. demonstrated the safety of defective HSV-1 in phase 1 clinical trials testing of NP2, a replication-defective vector expressing preproenkephalin in sensory neurons to treat pain in patients with cancer: no serious adverse events or modifications in immune status occurred, even in individuals who were seropositive to HSV-1 at treatment initiation [19]. The harmlessness of HSV-1 vectors was confirmed by preclinical studies that demonstrated that non-replicative vector inoculation does not induce reactivation of resident latent viral genomes. In addition, as of December 2019, the US clinical trials portal has listed up to 38 clinical trials assessing the safety and effectiveness of oncolytic HSV-1 against several types of cancers [20].

Persistence is another issue for viral vectors. The question remains whether engineered vectors can establish a quasi-latent state in cells and whether gene expression can remain lifelong. For example HSV-1 replication-defective vectors, as they are not incorporated in the host cell genome, are lost during cell division. Avoiding an immune response from viral vectors also commits to the efficacy of the treatment. Both innate and adaptive immune responses against viral vectors can occur in human patients. This can limit the repeated use of the same viral vector due to neutralizing antibodies, and transduction efficiency due to immune response to the introduced viral structures [2]. This is particularly true for AAV [21]. In the case of HSV-1 vectors, the interaction between HSV-1 and the host’s immune system is notably harmonious. HSV-1 has developed multiple strategies to evade host innate responses and facilitate a latent-state [22]. Recent estimates by the WHO show that more than 3,5 billion people worldwide are infected with HSV-1, most of them being asymptomatic [23]. This suggests that the immune

Table 2
Advantages and disadvantages of HSV-1 vectors versus other viral vectors.

Advantages	Disadvantages
Large cargo capacity (up to 150 kb)	Possible toxicity
Tropism for a wide variety of cells, especially the nervous system	Lack of persistence in dividing cells
Easy genetic manipulation	
Ability for a latent state and theoretically durable gene-expression	
Antivirals available	
Harmonious relationship between vector and host’s immune system	
Easy to obtain high-titer stocks	
No mutagenesis as vector is non-integrative	

response against HSV-1 is sufficiently robust to restrain the reactivated virus’s spread. Advantages and disadvantages of HSV-1 vectors are depicted in Table 2.

5. Current HSV-1 viral vector use in humans

5.1. Replication-competent recombinant vectors

Almost all of the trials with HSV-1 replication-competent vectors currently focus on cancer applications [2]. The principal mechanism of action of these products is based on: (1) the engineered viral vectors have intrinsic oncolytic properties and/or (2) the carried gene leads to expression of tumor suppressors or immunomodulators that enhance the anti-tumor efficacy [24]. Currently more than 50 different types of oncolytic HSV-1 have been constructed and studied in animal models of cancer. Nine of them are currently being tested in phase 1 or 2 clinical trials.

Talimogene Laherparepvec (T-VEC, IMLYGIC) has become the first oncolytic vector to gain approval for clinical use by the EMA and FDA [25]. It was approved for the treatment of local recurrent unresectable melanoma after initial surgery via direct intralesional administration. The viral vector contained the granulocyte-macrophage colony-stimulating factor (GM-CSF) gene. The expression of GM-CSF attracts dendritic cells that lead to an improved immune response against the tumor, apart from tumor lysis [26]. Currently, it is being tested for a multitude of other cancers, including ovarian cancer, pancreatic cancer and soft tissue sarcoma.

Teserpaturev (Delytact) is a oncolytic replication-competent HSV-1 derived vector used for the treatment of malignant glioma. Delytact is expected to have a tumor lysis as well as immunogenic activity [27]. Based on preliminary results, a conditional and time-limited approval in Japan has been issued as of 2021. This means the company has to verify the safety and efficacy of the drug in the next seven years, after which the drug can be fully approved in the market. No FDA or EMA approval has been granted so far.

5.2. Replication-defective recombinant vectors

5.2.1. Monogenic diseases

In May 2023, the FDA approved Beremagene Geperpavec (B-VEC, Vyjuvek), a HSV-1 vector-based gene therapy, for the treatment of wounds in patients suffering from dystrophic epidermolysis bullosa with mutations in the COL7A1 gene. These patients experience painful and persistent skin wounds and rarely survive beyond 30 years of age. The skin wounds arise from loss of dermal and epidermal cohesion because of genetic mutations in the COL7A1 gene. Vyjuvek replaces the collagen gene with two normal copies of the gene in keratocytes and fibroblasts. This leads to better wound healing [10]. It is a topical treatment and repeat dosing is necessary. Currently, there are two other HSV-1 based replacement trials running in clinical trials: one targeting cystic fibrosis and one for the improvement of aging skin. [27]

5.2.2. Pain

HSV-1 based vectors can be effectively used to deliver pain-modulating transgenes to sensory spinal neurons [28]. In 2011, Fink et al. conducted a phase-1 trial in which they injected replication-defective HSV-based vectors carrying the proenkephalin (PENK) gene intradermally in 10 patients with intractable pain resistant to high doses of morphine due to terminal cancer [19]. The human PENK gene encodes preproenkephalin, a precursor protein that is processed to produce endogenous opioid peptides. The study revealed that the treatment exhibited a high level of safety and tolerability. It is crucial to note that, given the advanced stage of the cancer in the patients studied, the maximum follow-up period extended to just 4 months, with only 4 out of the initial 10 patients reaching this milestone. Unfortunately, 4 patients died because of their condition during the course of the study. Subjects receiving a low dose of NP2 reported no meaningful effect, while subjects in the middle and high dose cohorts reported clinically meaningful pain relief for four weeks following injection of NP2 [29]. Subsequently, a phase-2 trial was conducted. This trial however failed to show substantial improvement in pain score compared to the placebo group. On the other hand, the treatment exhibited a high degree of safety, as evidenced by the similar incidence of serious adverse events in both treatment groups and the absence of any serious adverse events attributed to the treatment.

6. HSV1 gene vectors in functional urology

Capitalizing on the clinical success of HSV-1-based therapies in diverse medical domains, researchers are now turning their attention to its use in functional urology.

We aim to provide a concise overview of the (ongoing) pre-clinical research and their results on HSV-1 viral vectors in functional urology and potential applications in this emerging therapeutic landscape.

6.1. Bladder pain syndrome

Interstitial cystitis (IC)/ bladder pain syndrome (BPS) have been a major challenge to understand and treat. In the early 2000, Yoshimura et al. hypothesized that targeted and localized expression of enkephalin in bladder afferent nerves by PENK gene transfer using HSV vectors can treat bladder pain [30]. In 2013, Yokoyama et al. demonstrated high levels of enkephalin gene expression and significantly lower measures of pain in treated compared to untreated animals when exposed to stimuli intended to induce bladder irritation [31]. However, no current clinical trials are underway to look at its potential.

Another research focus pertains to the hypersensitivity of bladder sensory afferents contributing to IC and BPS [32]. The transient receptor potential vanilloid-1 (TRPV1) receptors, largely located in unmyelinated C-fibers, are associated with afferent sensitization in these disorders. Majima et al. studied how gene transfer of a negative regulator of the TRPV1 (i.e. PP1 α) modulates afferent activation in the spinal cord in rats. The study had two interesting results; firstly, the gene therapy did indeed reduce the detrusor overactivity in rats significantly. Secondly, they showed that injection of the HSV-vector into the bladder wall resulted in the presence of the vector not only in the bladder but also in the cellular body of neurons within the L6 and S1 dorsal root ganglia, but not in L4, which is the level that does not innervate the bladder. The authors conclude that these results demonstrate that the HSV vectors can deliver and express therapeutic genes in the bladder and its primary sensory neurons, but not in afferent neurons projecting to other organs after the bladder inoculation. Therefore, they suggest that HSV vector-based gene therapy could be organ-specific in nature thereby avoiding systemic adverse effects. [32]

6.2. Diabetic detrusor underactivity

The pathogenesis of diabetes-induced bladder dysfunction is likely to be multifactorial. Alterations in detrusor muscle components, urothelial dysfunction and neuronal impairment are thought to be crucial factors in diabetic cystopathy leading to diabetic detrusor underactivity [33]. The nerve growth factor (NGF) has been considered as a very powerful and selective growth factor for sympathetic and sensory neurons [34]. The decreased synthesis of NGF in the bladder or the defective transport of NGF to the lumbosacral dorsal root ganglia is associated with diabetic neuropathy and bladder dysfunction [35]. Sasaki et al. developed a replication-defective HSV-1 derived vector to deliver and express the NGF-gene in cells [36]. Intravesical injection lead to increased access of NGF in dorsal root ganglia. Diabetic HSV-NGF-injected rats also showed significantly reduced bladder capacity and post-void residual volume indicating that voiding function was improved after HSV vector-mediated NGF gene delivery. [36]

6.3. Neurogenic bladder dysfunction

Neurogenic bladder dysfunction refers to [urinary bladder](#) problems due to disease or injury of the [central nervous system](#) or peripheral nerves involved in the control of [urination](#) [37]. It consists of neurogenic detrusor overactivity (NDO) with or without detrusor sphincter dyssynergia (DSD) or a hypocontractile detrusor. NDO is caused by lesions in the brain or spinal cord associated with a congenital or acquired condition (e.g., myelomeningocele, stroke, spinal cord injury, multiple sclerosis, Parkinson's disease, dementia) [38]. DSD is the urodynamic description of bladder outlet obstruction from detrusor muscle contraction with concomitant involuntary urethral sphincter activation. It is associated with suprasacral lesions of the spinal cord, such as spinal cord injury, multiple sclerosis, and spina bifida [39]. Currently, HSV-1 gene therapy is used in three research areas aimed at treating neurogenic bladder dysfunction: intracellular synthesis of botulinum toxin in dorsal root ganglia to address neurogenic detrusor overactivity, suppression of TRPV1 channels in C-fiber afferents for the same purpose, and silencing A δ -fibers to target detrusor sphincter dyssynergia.

6.3.1. Intracellular synthesis of botulinum toxin

Botulinum toxin is a potent neurotoxin produced by the bacteria *Clostridium botulinum*. Botox consists of a light chain (BoNT LC), which is the biological active molecule, and a heavy chain (BoNT HC) that is involved in binding and delivery of the neurotoxin to cells [40]. The botulinum toxin acts by inhibiting calcium-mediated release of acetylcholine vesicles at the pre-synaptic neuromuscular junction in peripheral nerve endings, resulting in temporary flaccid muscle paralysis. To date, seven distinct forms of botulinum toxin exist, including serotypes A, B, C, D, E, F, and G. Only serotypes A (Dysport[®], Xeomin[®] and Botox[®]) and B (Myobloc[®]) are currently commercially available for therapeutic use.

Neurogenic bladders in spinal cord injury patients often cause insufficient bladder emptying, high detrusor pressures and can lead to kidney failure [41]. A possible treatment for NDO is intravesical botox injections in combination with intermittent catheterization. Even though intravesical botox injections have proven effective in addressing bladder overactivity, they come with significant drawbacks. One notable limitation is the necessity for repeated injections due to the protein's limited half-life, approximately every 6–9 months.

A French group currently focuses on long-term intracellular synthesis of the BoNT LC in dorsal root ganglia using herpes viral vectors [42]. The rationale for expressing the botulinum toxin light chain with these type of vectors is that they are fully safe as the de novo synthesized LC cannot spread to other cells in the absence of the heavy chain [43]. Moreover, the tropism for peripheral neurons, and particularly for sensory neurons, make HSV-1-derived vectors particularly well suited

for delivering the transgenic LC in the appropriate cellular targets. The intention is to deliver the transgene only in bladder sensory neurons, sparing the motor output (i.e. the efferents), so that micturition can be triggered on demand by an electronic implantable device, similarly to the Brindley procedure which was developed 40 years ago. The Brindley procedure consists of a stimulator for sacral anterior-root stimulation and a rhizotomy of the dorsal sacral roots to abolish neurogenic detrusor overactivity. The sacral dorsal rhizotomy is a neurosurgical procedure that aims to selectively sever the posterior sacral roots from S2 to S5 that convey the afferent sensory fibers from the bladder and the pelvi-perineal organs. This procedure efficiently abolishes the signals that trigger NDO but it is also responsible for an abolition of reflex penile erection and ejaculation, the remaining bowel reflex functions and the sensation of the skin in the sacral area. Due to these major side effects, it is poorly accepted by patients. If this gene therapy succeeds, the gene therapy would represent an excellent alternative for the bladder selective dorsal rhizotomy and avoid the invasiveness (and side-effects) of the procedure.

In 2019, BoNT LC was successfully induced in cultures of embryonic rat dorsal root ganglia neurons using an amplicon HSV-1 based vector [42]. In 2022 Joussain et al. identified a sensory neuron-specific promoter displaying selective expression in sensory neurons only, which is important to spare the motor efferents as much as possible [44]. This resulted in the construction of EG110 A, a non-replicative recombinant HSV-1 derived vector that expresses BoNT LC. Recently, a study where they injected EG110 A in adult female rats was conducted. The authors found a stable expression of BoNT LC in the dorsal root ganglia. More importantly, EG110 A counteracted bladder irritation (induced by capsaicin) as evidenced by a significant decrease in the frequency of micturition and increase in bladder capacity. Thirdly, EG110 A did not alter the normal bladder function, meaning the bladder efferents stay intact. Lastly, administration of the viral vector seemed safe. Although still early in the research phase, these results are promising for delivering a long-lasting efficient therapy without the adverse effects on voiding.

6.3.2. Suppression of TRPV1 channels in C-fiber afferents

In NDO C-fiber afferents play an important role [45]. Its mechanism is (partially) related to the presence of TRPV1 channels. Patients with NDO exhibit increased expression of TRPV1 channels in urothelial cells and nerve fibers, compared to healthy subjects [46]. In humans with NDO, intravesical administration of C-fiber neurotoxins reduces the density of TRPV1 immunoreactive nerve fibers. Randomized controlled trials with SCI patients revealed that these neurotoxins effectively improved both urodynamic and clinical parameters [47]. Therefore, TRPV1 channels in the C-fiber afferents are a potential target to tackle NDO. It has also been reported that HSV-1 vector-mediated gene delivery of inactivated TRPV1, in which the segment in C terminus of TRPV1 receptor is deleted to suppress TRPV1 activation, or protein phosphate 1 α (PP1 α), which negatively modulates TRPV1 activation, had a therapeutic effect on bladder overactivity in rats with acute chemical irritation [32]. Shimizu et al. investigated the effect of HSV-1 replication-defective vector-mediated gene delivery of a non-functional TRPV1 channels or PP1 α to C-fiber afferents in SCI mice. Detrusor overactivity was significantly reduced, while bladder function remained intact. The authors conclude that gene therapy with inactivated TRPV1 or PP1 α could become a novel treatment for NDO.

6.3.3. Silencing $\alpha\delta$ -fibers

To address the problem of DSD, the Pittsburgh group is currently directing their efforts toward $\alpha\delta$ -fibers, positing that these fibers play a key role in the excitation of the external urethral sphincter following SCI. Their approach involves utilizing the 'designer receptor exclusively activated by designer drugs' (DREADD) principle, a concept which is part of the so-called 'chemogenetics'. Essentially, this entails designing receptors to remain unresponsive to natural ligands while being activated by pharmacologically inert, drug-like small molecules [48]. By

introducing these receptors into target cells, such as neurons, they can be remotely manipulated using specific designed drugs [48]. Similar techniques in this field include thermogenetics and optogenetics, which respectively utilize temperature or light instead of molecules to control cells.

The Pittsburgh group engineered a replication-defective HSV-1 vector carrying a modified glycine receptor (the designer receptor) with high sensitivity to ivermectin (the designer drug). In 2017, Shimizu et al. successfully developed an HSV-1 vector tailored to target $\alpha\delta$ -bladder afferent neurons in mice [49]. Subsequently, in 2020, Saito et al. administered this viral vector containing the modified glycine receptor sensitive to ivermectin into mouse bladders [50]. They observed that ivermectin-induced silencing of these $\alpha\delta$ -fiber afferents led to improved voiding efficiency in SCI-mice. However, significant further research is necessary in this area before it can be translated into clinical therapeutics.

7. Conclusion

The utilization of HSV-1 derived vectors in functional urology presents a promising frontier for innovative therapeutic strategies. The unique neurotropic and neuroinvasive properties of these vectors have shown considerable potential in addressing urological disorders, particularly those involving the intricate neural circuitry of the genitourinary system. While progress has been made in preclinical studies, the translation of herpes simplex viral vectors to clinical applications necessitates further investigation, with a focus on optimizing transduction efficiency, addressing potential immunogenic concerns and delivering long-term effect. As research advances, the prospect of leveraging these vectors for targeted and effective gene therapy in urological conditions holds considerable promise, providing new avenues for precision medicine in the realm of urological disorders.

Declaration of competing interest

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References

- [1] T. Wirth, N. Parker, S. Ylä-Herttuala, History of gene therapy, *Gene* 525 (2) (2013) 162–169, <http://dx.doi.org/10.1016/j.gene.2013.03.137>, Epub 2013 Apr 23. PMID: 23618815.
- [2] Z. Zhao, A.C. Anselmo, S. Mitragotri, Viral vector-based gene therapies in the clinic, *Bioeng. Transl. Med.* 7 (1) (2021) e10258, <http://dx.doi.org/10.1002/btm2.10258>, PMID: 35079633; PMCID: PMC8780015.
- [3] T. Liu, Y. Liang, L. Huang, Development and delivery systems of mRNA vaccines, *Front Bioeng. Biotechnol.* 9 (2021) 718753, <http://dx.doi.org/10.3389/fbioe.2021.718753>; *Front Bioeng. Biotechnol.* 9 (2021) 766764 (Erratum). PMID: 34386486; PMCID: PMC8354200.
- [4] Y. Shen, L. Post, Viral vectors and their applications, in: B.N. Fields, et al. (Eds.), *Fields Virology*, Wolters Kluwer Health/Lippincott Williams & Wilkins, Philadelphia, 2007, pp. 539–564.
- [5] J.C. Glorioso, Herpes simplex viral vectors: late bloomers with big potential, *Hum. Gene Ther.* 25 (2) (2014) 83–91, <http://dx.doi.org/10.1089/hum.2014.2501>, PMID: 24502405; PMCID: PMC3922308.
- [6] D. Saleh, S.N.S. Yarrarapu, S. Sharma, Herpes simplex type 1. 2023 jul 24, in: *StatPearls [Internet]*, StatPearls Publishing, Treasure Island (FL), 2023, PMID: 29489260.
- [7] O.O. Koyuncu, I.B. Hogue, L.W. Enquist, Virus infections in the nervous system, *Cell Host Microbe* 13 (4) (2013) 379–393, <http://dx.doi.org/10.1016/j.chom.2013.03.010>, PMID: 23601101; PMCID: PMC3647473.
- [8] S. De Silva, W.J. Bowers, Herpes virus amplicon vectors, *Viruses* 1 (3) (2009) 594–629, <http://dx.doi.org/10.3390/v1030594>, PMID: 19956558; PMCID: PMC2783652.
- [9] R. Manservigi, R. Argnani, P. Marconi, HSV recombinant vectors for gene therapy, *Open Virol. J.* 4 (2010) 123–156, <http://dx.doi.org/10.2174/1874357901004030123>, PMID: 20835362; PMCID: PMC2936037.
- [10] S.V. Guide, M.E. Gonzalez, I.S. Bağcı, B. Agostini, H. Chen, G. Feeney, M. Steimer, B. Kapadia, K. Sridhar, L. Quesada Sanchez, F. Gonzalez, M. Van Ligt, T.J. Parry, S. Chitra, L.A. Kammerman, S. Krishnan, M.P. Marinkovich, Trial of beremagene geperpavec (B-VEC) for dystrophic epidermolysis bullosa, *N. Engl. J. Med.* 387 (24) (2022) 2211–2219, <http://dx.doi.org/10.1056/NEJMoa2206663>, PMID: 36516090.

- [11] R. Argnani, M. Lufino, M. Manservigi, R. Manservigi, Replication-competent herpes simplex vectors: design and applications, *Gene Ther.* 12 Suppl 1 (2005) S170–7, <http://dx.doi.org/10.1038/sj.gt.3302622>, PMID: 16231051.
- [12] P. Marconi, R. Argnani, A.L. Epstein, et al., HSV as a vector in vaccine development and gene therapy, in: *Madame Curie Bioscience Database* [Internet], Landes Bioscience, Austin (TX), 2000–2013, Available from: <https://www.ncbi.nlm.nih.gov/books/NBK7024/>.
- [13] A.L. Epstein, HSV-1-derived amplicon vectors: recent technological improvements and remaining difficulties—a review, *Mem. Inst. Oswaldo Cruz* 104 (3) (2009) 399–410, <http://dx.doi.org/10.1590/s0074-02762009000300002>, PMID: 19547864.
- [14] B.H. Yip, Recent advances in CRISPR/Cas9 delivery strategies, *Biomolecules* 10 (6) (2020) 839, <http://dx.doi.org/10.3390/biom10060839>, PMID: 32486234; PMCID: PMC7356196.
- [15] M.H. Butt, M. Zaman, A. Ahmad, R. Khan, T.H. Mallhi, M.M. Hasan, Y.H. Khan, S. Hafeez, E.E.S. Massoud, M.H. Rahman, S. Cavalu, Appraisal for the potential of viral and nonviral vectors in gene therapy: A review, *Genes (Basel)* 13 (8) (2022) 1370, <http://dx.doi.org/10.3390/genes13081370>, PMID: 36011281; PMCID: PMC9407213.
- [16] R. Lachmann, Herpes simplex virus-based vectors, *Int. J. Exp. Pathol.* 85 (4) (2004) 177–190, <http://dx.doi.org/10.1111/j.0959-9673.2004.00383.x>, PMID: 15312123; PMCID: PMC2517519.
- [17] A.L. Verzosa, L.A. McGeever, S.J. Bhark, T. Delgado, N. Salazar, E.L. Sanchez, Herpes simplex virus 1 infection of neuronal and non-neuronal cells elicits specific innate immune responses and immune evasion mechanisms, *Front. Immunol.* 12 (2021) 644664, <http://dx.doi.org/10.3389/fimmu.2021.644664>, PMID: 34135889; PMCID: PMC8201405.
- [18] S.A. Jackson, N.A. DeLuca, Relationship of herpes simplex virus genome configuration to productive and persistent infections, *Proc. Natl. Acad. Sci. USA* 100 (13) (2003) 7871–7876, <http://dx.doi.org/10.1073/pnas.1230643100>, Epub 2003 Jun 9. PMID: 12796511; PMCID: PMC164680.
- [19] D.J. Fink, J. Wechuck, M. Mata, J.C. Glorioso, J. Goss, D. Krisky, D. Wolfe, Gene therapy for pain: results of a phase I clinical trial, *Ann. Neurol.* 70 (2) (2011) 207–212, <http://dx.doi.org/10.1002/ana.22446>, Epub 2011 Jul 27. PMID: 21796661; PMCID: PMC3152623.
- [20] A.L. Epstein, HSV-1's contribution as a vector for gene therapy, *Nat. Biotechnol.* 40 (9) (2022) 1316, <http://dx.doi.org/10.1038/s41587-022-01449-1>, PMID: 36085499.
- [21] V. Louis Jeune, J.A. Joergensen, R.J. Hajjar, T. Weber, Pre-existing anti-adenovirus-associated virus antibodies as a challenge in AAV gene therapy, *Hum. Gene Ther. Methods* 24 (2) (2013) 59–67, <http://dx.doi.org/10.1089/hgtb.2012.243>, Epub 2013 Apr 3. PMID: 23442094; PMCID: PMC3732124.
- [22] I. Amin, S. Younas, S. Afzal, M. Shahid, M. Idrees, Herpes simplex virus type 1 and host antiviral immune responses: An update, *Viral. Immunol.* 32 (10) (2019) 424–429, <http://dx.doi.org/10.1089/vim.2019.0097>, Epub 2019 Oct 10. PMID: 31599707.
- [23] K.J. Looker, A.S. Magaret, M.T. May, K.M. Turner, P. Vickerman, S.L. Gottlieb, L.M. Newman, Global and regional estimates of prevalent and incident herpes simplex virus type 1 infections in 2012, *PLoS One* 10 (10) (2015) e0140765, <http://dx.doi.org/10.1371/journal.pone.0140765>, PMID: 26510007; PMCID: PMC4624804.
- [24] R. Goswami, G. Subramanian, L. Silayeva, I. Newkirk, D. Doctor, K. Chawla, S. Chattopadhyay, D. Chandra, N. Chilukuri, V. Betapudi, Gene therapy leaves a vicious cycle, *Front. Oncol.* 9 (2019) 297, <http://dx.doi.org/10.3389/fonc.2019.00297>, PMID: 31069169; PMCID: PMC6491712.
- [25] J. Pol, G. Kroemer, L. Galluzzi, First oncolytic virus approved for melanoma immunotherapy, *Oncoimmunology* 5 (1) (2015) e1115641, <http://dx.doi.org/10.1080/2162402X.2015.1115641>, PMID: 26942095; PMCID: PMC4760283.
- [26] S.S. Raman, J.R. Hecht, E. Chan, Talimogene laherparepvec: review of its mechanism of action and clinical efficacy and safety, *Immunotherapy* 11 (8) (2019) 705–723, <http://dx.doi.org/10.2217/imt-2019-0033>, Epub 2019 May 2. PMID: 31045464.
- [27] A. Mullard, FDA approves first topical gene therapy, *Nat. Rev. Drug Discov.* 22 (7) (2023) 527, <http://dx.doi.org/10.1038/d41573-023-00095-9>, PMID: 37286779.
- [28] J.C. Glorioso, D.J. Fink, Herpes vector-mediated gene transfer in the treatment of chronic pain, *Mol. Ther.* 17 (1) (2009) 13–18, <http://dx.doi.org/10.1038/mt.2008.213>, Epub 2008 Oct 7. PMID: 18841093; PMCID: PMC2613169.
- [29] D.J. Fink, D. Wolfe, Gene therapy for pain: A perspective, *Pain Manag.* 1 (5) (2011) 379–381, <http://dx.doi.org/10.2217/pmt.11.49>, PMID: 22461859; PMCID: PMC3313471.
- [30] N. Yoshimura, M.E. Franks, K. Sasaki, W.F. Goins, J. Goss, T. Yokoyama, M.O. Fraser, S. Seki, J. Fink, J. Glorioso, W.C. de Groat, M.B. Chancellor, Gene therapy of bladder pain with herpes simplex virus (HSV) vectors expressing preproenkephalin (PPE), *Urology* 57 (6 Suppl 1) (2001) 116, [http://dx.doi.org/10.1016/s0090-4295\(01\)01060-3](http://dx.doi.org/10.1016/s0090-4295(01)01060-3), PMID: 11378092.
- [31] H. Yokoyama, T. Oguchi, W.F. Goins, J.R. Goss, O. Nishizawa, W.C. de Groat, D. Wolfe, D.M. Krisky, J.C. Glorioso, N. Yoshimura, Effects of herpes simplex virus vector-mediated enkephalin gene therapy on bladder overactivity and nociception, *Hum. Gene Ther.* 24 (2) (2013) 170–180, <http://dx.doi.org/10.1089/hum.2011.180>, Epub 2013 Feb 14. PMID: 23316929; PMCID: PMC3581021.
- [32] T. Majima, K. Mori, K. Kadekawa, S. Takai, Y. Funahashi, B. Reinhart, W.F. Goins, M. Gotoh, J.C. Glorioso, N. Yoshimura, The effect of herpes simplex virus vector-mediated gene therapy of protein phosphatase 1α on bladder overactivity and nociception, *NeuroUrol. Urodyn.* 38 (2) (2019) 582–590, <http://dx.doi.org/10.1002/nau.23882>, Epub 2018 Nov 29. PMID: 30499116.
- [33] N. Yoshimura, M.B. Chancellor, K.E. Andersson, G.J. Christ, Recent advances in understanding the biology of diabetes-associated bladder complications and novel therapy, *BJU Int.* 95 (6) (2005) 733–738, <http://dx.doi.org/10.1111/j.1464-410X.2005.05392.x>, PMID: 15794773.
- [34] L. Aloe, M.L. Rocco, P. Bianchi, L. Manni, Nerve growth factor: from the early discoveries to the potential clinical use, *J. Transl. Med.* 10 (2012) 239, <http://dx.doi.org/10.1186/1479-5876-10-239>, PMID: 23190582; PMCID: PMC3543237.
- [35] J.S. Brown, H. Wessells, M.B. Chancellor, S.S. Howards, W.E. Stamm, A.E. Stapleton, W.D. Steers, S.K. Van Den Eeden, McVary K. T., Urologic complications of diabetes, *Diabetes Care* 28 (2005) 177–185, <http://dx.doi.org/10.2337/diacare.28.1.177>.
- [36] K. Sasaki, M.B. Chancellor, W.F. Goins, M.W. Phelan, J.C. Glorioso, W.C. de Groat, et al., Gene therapy using replication defective herpes simplex virus vectors expressing nerve growth factor in a rat model of diabetic cystopathy, *Diabetes* 53 (2004) 2723–2730.
- [37] A.M. Suskind, Neurogenic bladder, in: J.W. McAninch, T.F. Lue (Eds.), *Smith & Tanagho's General Urology*, McGraw Hill, 2020, p. 19e, <https://accessmedicine.mhmedical.com/content.aspx?bookid=2840§ionid=241662745>. (Accessed 25 April 2024).
- [38] F. Haab, Chapter 1: The conditions of neurogenic detrusor overactivity and overactive bladder, *NeuroUrol. Urodyn.* 33 Suppl 3 (2014) S2–5, <http://dx.doi.org/10.1002/nau.22636>, PMID: 25042138.
- [39] J.T. Stoffel, Detrusor sphincter dyssynergia: a review of physiology, diagnosis, and treatment strategies, *Transl. Androl. Urol.* 5 (1) (2016) 127–135, <http://dx.doi.org/10.3978/j.issn.2223-4683.2016.01.08>, PMID: 26904418; PMCID: PMC4739973.
- [40] D. Dressler, F. Adib Saberi, Botulinum toxin: mechanisms of action, *Eur. Neurol.* 53 (1) (2005) 3–9, <http://dx.doi.org/10.1159/000083259>, Epub 2005 Jan 12. PMID: 15650306.
- [41] W.A. Taweel, R. Seyam, Neurogenic bladder in spinal cord injury patients, *Res. Rep. Urol.* 7 (2015) 85–99, <http://dx.doi.org/10.2147/RRU.S29644>, PMID: 26090342; PMCID: PMC4467746.
- [42] C. Joussain, O. Le Coz, A. Pichugin, P. Marconi, F. Lim, M. Sicurella, A. Salonia, F. Montorsi, F. Wandosell, K. Foster, F. Giuliano, A.L. Epstein, A. Aranda Muñoz, Botulinum neurotoxin light chains expressed by defective herpes simplex virus type-1 vectors cleave SNARE proteins and inhibit CGRP release in rat sensory neurons, *Toxins (Basel)* 11 (2) (2019) 123, <http://dx.doi.org/10.3390/toxins11020123>, PMID: 30791373; PMCID: PMC6409900.
- [43] Q. Teng, D. Tanase, J.K. Liu, M.E. Garrity-Moses, K.B. Baker, N.M. Boulis, Adenoviral clostridial light chain gene-based synaptic inhibition through neuronal synaptobrevin elimination, *Gene Ther.* 12 (2) (2005) 108–119, <http://dx.doi.org/10.1038/sj.gt.3302400>; *Gene Ther.* 12 (2) (2005) 194, Tanase, DK [corrected to Tanase, DJ] (Erratum). PMID: 15496959.
- [44] C. Joussain, O. Le Coz, A. Pichugin, P. Marconi, F. Lim, M. Sicurella, K. Foster, F. Giuliano, A.L. Epstein, A. Aranda Muñoz, Development and assessment of herpes simplex virus type 1 (HSV-1) amplicon vectors with sensory neuron-selective promoters, *Int. J. Mol. Sci.* 23 (15) (2022) 8474, <http://dx.doi.org/10.3390/ijms23158474>, PMID: 35955608; PMCID: PMC9369297.
- [45] N. Wada, S. Karnup, K. Kadekawa, N. Shimizu, J. Kwon, T. Shimizu, D. Gotoh, H. Kakizaki, W.C. de Groat, N. Yoshimura, Current knowledge and novel frontiers in lower urinary tract dysfunction after spinal cord injury: Basic research perspectives, *Urol. Sci.* 33 (3) (2022) 101–113, <http://dx.doi.org/10.4103/urol.uros.31.22>, Epub 2022 Aug 25. PMID: 36177249; PMCID: PMC9518811.
- [46] A. Apostolidis, C.M. Brady, Y. Yiangou, J. Davis, C.J. Fowler, P. Anand, Capsaicin receptor TRPV1 in urothelium of neurogenic human bladders and effect of intravesical resiniferatoxin, *Urology* 65 (2) (2005) 400–405, <http://dx.doi.org/10.1016/j.urology.2004.10.007>, PMID: 15708075.
- [47] C.J. Fowler, D. Griffiths, W.C. de Groat, The neural control of micturition, *Nat. Rev. Neurosci.* 9 (6) (2008) 453–466, <http://dx.doi.org/10.1038/nrn2401>, PMID: 18490916; PMCID: PMC2897743.
- [48] E.J. Campbell, N.J. Marchant, The use of chemogenetics in behavioural neuroscience: receptor variants, targeting approaches and caveats, *Br. J. Pharmacol.* 175 (7) (2018) 994–1003, <http://dx.doi.org/10.1111/bph.14146>, Epub 2018 Feb 26. PMID: 29338070; PMCID: PMC5843707.
- [49] N. Shimizu, M.F. Doyal, W.F. Goins, K. Kadekawa, N. Wada, A.J. Kanai, W.C. de Groat, A. Hirayama, H. Uemura, J.C. Glorioso, N. Yoshimura, Morphological changes in different populations of bladder afferent neurons detected by herpes simplex virus (HSV) vectors with cell-type-specific promoters in mice with spinal cord injury, *Neuroscience* 364 (2017) 190–201, <http://dx.doi.org/10.1016/j.neuroscience.2017.09.024>, Epub 2017 Sep 20; *Neuroscience* (2018) (Erratum). PMID: 28942324; PMCID: PMC5768486.
- [50] T. Saito, et al., Ivermectin-induced activation of mutant glycine receptors delivered to Aα-fiber bladder afferents by herpes simplex virus vectors driven by a subpopulation-specific neurofilament promoter improves detrusor-sphincter dyssynergia and inefficient voiding in mice with Spinal Cord Injury, 1970, ICS. Available at: <https://www.ics.org/2019/abstract/272>. (Accessed 25 April 2024).