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Neuromodulatory treatment for cavernous nerve injury
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·*Review*·

Emerging neuromodulatory molecules for the treatment of neurogenic erectile dysfunction caused by cavernous nerve injury

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Abstract

Advances in the neurobiology of growth factors, neural development, and prevention of cell death have resulted in a heightened clinical interest for the development of protective and regenerative neuromodulatory strategies for the cavernous nerves (CNs), as therapies for prostate cancer and other pelvic malignancies often result in neuronal damage and debilitating loss of sexual function. Nitric oxide released from the axonal end plates of these nerves within the corpora cavernosa causes relaxation of smooth muscle, initiating the haemodynamic changes of penile erection as well as contributing to maintained tumescence; the loss of CN function is primarily responsible for the development of erectile dysfunction (ED) after pelvic surgery and serves as the primary target for potential neuroprotective or regenerative strategies. Evidence from pre-clinical studies for select neuromodulatory approaches is reviewed, including neurotrophins, glial cell line-derived neurotrophic factors (GDNF), bone morphogenic proteins, immunophilin ligands, erythropoetin (EPO), and stem cells. *(Asian J Androl 2008 Jan; 10: 54–59)*

Keywords: erectile dysfunction; prostate cancer; radical prostatectomy; postoperative complications; neuroprotection; nerve regeneration; neurotrophins; brain-derived nerve growth factor; immunophilin ligands; stem cells

1 Introduction and background

The clinical potential of neuromodulatory therapy is based upon the recognition that although the peripheral nervous system demonstrates an intrinsic ability to regenerate after injury, this endogenous response is somewhat limited and does not usually allow for a full recovery of function [1]. Erectile dysfunction (ED) remains a common cause of significant post-operative morbidity for men undergoing radical therapies for prostate cancers or other pelvic malignancies, as the cavernous nerves (CNs) are inadvertently axotomized, lacerated, or stretched at time of surgery [2, 3]. Contemporary data indicates that the probability of ED following radical prostatectomy for clinically localized cancer of the prostate is 20–90% at 24 months [2]. Although refinements in anatomic surgical technique, as evidenced by an improved understanding of penile autonomic innervation and the implementation of such innovative technological advances as laparoscopic and robot-assisted surgery, has led to significant improvements in post-operative erectile function, most men demonstrate compromised erectile function (delayed, compromised or lack of post-surgical potency) as varying degrees of CN damage occur even with successful bilateral nerve-sparing procedures [4]. With CN compromise (ranging from neuropraxia to lethal axonal damage), well-defined pathobiological changes are observed in the penis, including apoptosis of smooth muscle and endothelium, reduction of nitric oxide synthase (NOS) nerve density, up-regulation of fibroproliferative cytokines such as transforming growth factor beta (TGF-β), and smooth muscle fibrosis or loss [1, 2]. Additionally, the chronic absence of erection secondary to CN neuropraxia results in failure to achieve

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cavernosal cycling between flaccid and erect state, with the potential for further structural damage to the cavernosal smooth muscle [5].

Preservation of the CNs during radical prostatectomy is a key variable for maintaining post-operative erectile function because downstream events, including smooth muscle apoptosis, cavernosal fibrosis, and venous leak are thought to result from CN injury; a clear clinical need for the development of therapeutic neuromodulatory interventions has been defined, as both sympathetic and parasympathetic pelvic innervation is at high risk of injury during surgery or radiation therapy for prostate, bladder, and colorectal malignancies [2–4]. For example, achieving cancer-control, continence and potency is limited to approximately 60% of men at 24 months after having open radical retropubic prostatectomy for clinically localized disease [6].

Penile erection, controlled by adrenergic, cholinergic, and nonadrenergic noncholinergic (NANC) neuroeffectors of the CNs, is often compromised by these treatments and patient and partner quality-of-life is markedly reduced [7]. Accumulating evidence suggests that a return to potency following injury to the CNs is partially dependent upon axonal regeneration in the remaining neural tissues and several treatment strategies offering the potential to facilitate recovery are currently under investigation in animal models, including neurotrophins, growth factors, immunophilin ligands, and stem cells [8–10]. Collateral sprouting of axons occurs acutely following injury to adult peripheral neurons and growth cones target local environments supportive of regeneration [11]. However, the specific molecular mechanisms responsible for survival and the preservation of function for adult parasympathetic ganglion neurons, including the CNs, following injury remain incompletely understood.

Although phosphodiesterase type-5 (PDE-5) inhibitors have revolutionized the treatment of ED, compromised erectile function following radical prostatectomy remains a therapeutic challenge [12]. The restoration of erectile function is optimized only if it becomes possible to stimulate nerve growth to re-establish penile innervation and surviving or recoverable axons are protected in the post-traumatic period from further deterioration or death. As the molecular understanding of the neural response to injury and mechanisms of recovery expands, treatment strategies using signalling pathway modulators, neurotrophic factors, stem cells, or novel combinations of these molecules/agents, offer the potential to modulate the CN microenvironment and promote repair and survival [13].

2 Neurotrophins

Neurotrophin polypeptides regulate neuronal survival though a series of signaling pathways, including those mediated by G-proteins, ras, cdc-42/ras/rho, and PI-3 kinase cascades [14]. The ability of neurotrophic factors to enhance functional recovery after cavernosal nerve injury has been shown via direct injection of neurotrophic factors and gene therapy with adeno-associated virus-mediated neurotrophic factor production [15]. Brain-derived neurotrophic factor (BDNF), a member of the mammalian family of neurotrophins which also includes nerve growth factor (NGF), and neurotrophins 3, 4, and 5, has been the focus of intense investigation because of its central role in neuronal development, maturation, survival after injury, and demonstrable retrograde axonal transport to the cell body [16]. Retrograde transport of neurotrophic factors occurs as molecules are taken up by the neural synapses of the corpus cavernosum and travel to the major pelvic ganglion to exert their neuroprotective/regenerative effects [17].

2.1 BDNF

A growing body of literature suggests BDNF may represent a promising neuromodulatory therapeutic agent, enhancing neuronal survival, differentiation, and regeneration alone or synergistically with other molecules. Functional studies have determined that BDNF-secreting fibroblasts promote recovery of bladder and hindlimb function following spinal cord contusion, while Lue's group has demonstrated BDNF-enhanced recovery of erectile function, BDNF/vascular endothelial growth factor synergies, and regeneration of neuronal NOS (nNOS)-containing nerve fibres [18, 19]. BDNF has been shown to exert its effects on several classes of neurons, acting in an autocrine or paracrine fashion early after nerve injury when a rapid influx of growth factors occurs distally to the site of trauma. Recent identification of the JAK/STAT signaling pathway as the primary mechanism responsible for *in vitro* BDNF-mediated cavernous neurite outgrowth (Figures 1 and 2) and subsequent observations that CN axotomy up-regulates in *vivo* expression of penile BDNF and leads to endogenous activation of the JAK/STAT pathway illustrates both the gaps in contemporary knowledge and the potential for elucidating these important mechanisms [8, 14, 16]. Subsequently, the membrane receptors JAK1 and JAK2, and downstream molecules including STAT1, 2, 3 and 5 have become key components for further study of the CN response to injury.

3 Glial cell-line derived neurotrophic factor (GDNF)

GDNFs include the molecules GDNF, neurturin (NTN), persephin, and artemin. This class of compounds represent a novel group of neuroprotective and neuroregenerative agents [20, 21]. Initial *in vitro* studies suggested NTN acts as a target-derived survival and/or

neuritogenic factor for penile erection-inducing postganglionic neurons via a neurotrophic signaling mechanism distinct from other parasympathetic neurons and mediated by the GDNF family receptors α1, α2 (predominant) and α4 [22]. Bella *et al.* [20] first demonstrated neurturin's ability to confer an *in vivo* advantage for the functional recovery of erectile function following CN injury, as neurturin applied directly to the area of CN injury facilitated the preservation of erectile function as compared to untreated control rats and those treated with extended release neurotrophin-4 [20]. Neurturin facilitated the preservation of erectile function, with a mean ICP increase of 55% (net increase of 62.0 ± 9.2 cm H_2O ($P < 0.05$ *vs*. control), and the extended 5-week course of treatment was well tolerated. Subsequently, Kato *et al.* [21] reported the use of a herpes simplex virus vector expressing GDNF as the delivery mechanism to the site of injury, with significant functional recovery observed as well. As penis-projecting pelvic neurons express nNOS and GFRα2, accumulating tissue culture, cell-line, *in vivo* signaling, and functional evidence suggests that neurturin and GDNF play a role in regeneration, as well as maintenance, of adult parasympathetic neurons.

4 GDF-5

Growth differentiation factor-5 (GDF-5), a member of the TGF-B superfamily, is a more recently isolated neurotrophic factor and is classified as a bone morphogenic protein [23]. GDF-5's molecular structure was first characterized in 2005 and effector pathways include intermediary mitogen-activated protein kinase (MAPK)-dependent pathways [24] that effector pathways include intermediary serine/threonine kinase receptors, namely, bone morphogenic protein (BMP) receptor Ib (BMPRIb), BMPR2 and activin receptor 2 (ACTR2), which modulate Smad and p38 MAPK-dependent pathways [24]. Fandel *et al.* [23] have shown dose-dependent functional improvements for recovery of erectile function following bilateral crush injury in the rat, with lower concentrations of this neurotrophic factor resulting in a doubling of mean peak intracavernous pressures (electrostimulation) compared to controls [23]. Follow-up studies have confirmed the neuromodulatory effects of GDF-5, as functional recovery is accompanied by nNOS neuronal preservation and decreased levels of apoptosis [25]. GDF-5 is an atypical neuromodulatory agent with some promise. Although neurobiological properties have not been fully determined (particularly in the peripheral nervous system), the bone regenerative properties have been well characterized in animal models and a human pilot clinical study is underway for this indication [26].

5 Immunophilin ligands

Immunophilin ligands represent an exciting new class of agents with well-characterized pre-clinical neuroprotective and neuroregenerative properties [1]. The neurotrophic characteristics of immunophilin ligands hold potential for the treatment of many urological and non-urological neurotraumatic or neurodegenerative conditions, including spinal cord injury, peripheral neuropathies, and ED following radical pelvic surgeries [13, 27]. Immunophilin ligands include cyclosporine and FK 506 (also known as tacrolimus), agents that bind to immunophilin receptors and cellular signaling proteins present in immune and neural tissue [6]. Using models of CN crush injury in the rat, tacrolimus was found to preserve function, reduce neural degeneration and stimulate axonal regrowth [9, 27, 28]. Valentine *et al.* [9] recently reported preserved CN architecture with prevention of CN axonal degeneration following 1-day and 7-day courses of FK 506 treatment, while Sezen *et al.* [27] clearly demonstrated functional recovery for FK 506 treated animals versus controls (treatment at time of crush

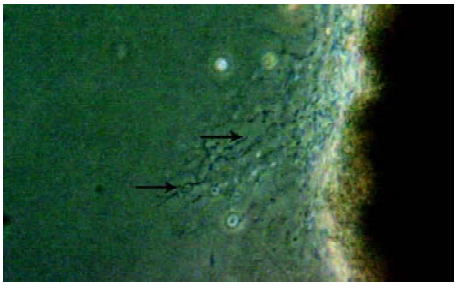

Figure 1. Endogenous neurite outgrowth (see arrows) from the nitric oxide synthase (NOS)-bearing dorsal-caudal region of the major pelvic ganglion (DCR-MPG) of the rat (tissue culture). Magnification, × 200.

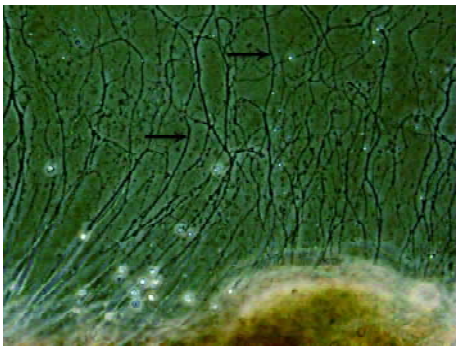

Figure 2. Brain-derived neurotrophic factor (BDNF) enhanced neurite outgrowth (concentration 50 ng/mL) from the dorsal-caudal region of the major pelvic ganglion (DCR-MPG) of the rat (tissue culture) occurs primarily via the JAK/STAT pathway. Magnification, × 150.

and on successive days). Concerns remain about the potential applicability of these therapies in patients treated for malignancies due to FK 506's immunosuppressant qualities. However, dose levels of FK 506 used in humans with rheumatoid arthritis (typically 2–3 mg/day) do not induce immunosuppression (versus daily 5-mg doses utilized following transplantation procedures), therefore supporting further research efforts focused on this promising pathway [13, 29].

5.1 GPI 1046 and FK 1706 non-immunosuppressant immunophilin ligands

Non-immunosuppressant forms of immunophilin ligands, such as FK 1706 and GPI 1046, represent a new class of candidate neurogenerative and neuroprotective compounds which may ultimately may be preferred to the immunosuppressive immunophilins (FK 506, cyclosporine A, and rapamycin). Although the mechanism by which FK 1706 promotes preservation and functional recovery of neurons is incompletely understood, it is likely independent of FKBP-12 binding, subsequent calcineurin inhibition, and the disruption of the cytokine synthesis cascade [30, 31]. Current evidence suggests the neuromodulatory actions occur via an anti-apoptotic effect, protecting neurons by the upregulation of glutathione (antioxidant) and production of neurotrophic factors. Immunophilins target only injured nerves and molecular signaling for FK 1706 and GPI 1046 likely involves immunophilin type-specific binding proteins expressed by damaged neurons, as reported for FK 506 [30–32]. For example, the neurotrophic effects specific to FK 1706 appear to be putatively mediated via FK 506 binding protein (FKBP) subtype-52 and activation of the Ras/Raf/MAPK signaling pathway, resulting in NGF-mediated neurite outgrowth [33]. Neither calcineurin inhibition nor binding to FKBP-12 are necessary for the neurotrophic activity of immunophilin ligands, suggesting that the neuroregenerative and immunosuppressive properties can be separated.

In a bilateral CN crush model, FK 1706 has been shown to enhance the recovery of erectile function in a concentration dependent manner, with higher dose treatment group showing a statistically significant elevation of Intracavernous pressure (ICP) compared to vehicle-only treated control animals (73.9 *vs.* 34.4 mean cm H_2O) and low/medium FK 1706 groups (improvement of more than 60%) [34]. Groups were treated with subcutaneous injection of vehicle (control) alone (1.0 mL/kg), or low (0.1 mg/kg), medium (0.32 mg/kg) or high dose (1.0 mg/kg) FK 1706 5 days per week for 8 weeks. It is uncertain whether FK 1706 has more potential *in vivo* than FK 506, but FK 1706 has proven more effective at provoking NGF-induced neurite outgrowth *in vitro* [33]. Oral and intraperitoneal administration of GPI 1046 results in similar erectile function recovery to that of FK 506 in both unilateral and bilateral CN-injured animals following short-term 1- and 7-day administration. Prevention of axonal degeneration is observed in 83% of unmyelinated axons [9]. Animals exposed to longer than 5 days duration of FK 506 treatment have been reported to lose their ability to gain weight and some expired secondary to chronic, high-dose FK 506 administration [13, 35]; this morbidity has not been seen to date with either the Guildford (now MGI) Pharmaceuticals compound GPI 1046 or Astellas Pharmaceuticals' FK 1706. An initial clinical trial with this class of agents using GPI 1485 (phase II multicenter, randomized double blind placebo-controlled three armed study) in 197 men undergoing bilateral CN sparing radical prostatectomy for prostate cancer did not reveal significant differences between treatment groups [36]. However, further study of the potential of non-immunosuppressant immunophilin ligands seems warranted as other neuromodulatory compounds from this group may demonstrate meaningful efficacy for promoting the recovery of potency after radical prostatectomy. From a safety standpoint, GPI 1046 has not shown mitogenic effects on human prostate cancer cells *in vitro*, making it less likely that these molecules will negatively impact cancer progression or recurrence biology [13, 37].

6 Erythropoetin (EPO)

EPO receptor expression has been localized to human penile tissues and in the periprostatic neurovascular bundles responsible for erectile function [38]. Allaf *et al*. [39] have also investigated the effects of recombinant human EPO (rhEPO), a cytokine-hormone, on erectile function recovery in a rat model of CN injury, demonstrating statistically significant normalization of intracavernous pressures compared to controls (treatment group-rhEPO 5 000 U/kg daily for 14 days versus one day prior plus one hour prior to injury administration) for treated cohorts [39]. Electron microscopy confirmed significant improvement in axonal regeneration for rhEPO treated groups 14 days after injury. This agent has also shown CNs efficacy via cytoprotection, neurogenesis, and decreased subventricular zone morphologic changes following ischemic brain injury in a rat model of stroke [40]. As well, EPO increased the percentage of newly generated neurons. Given these observations, further molecular and functional studies seem warranted following CN trauma.

7 Stem cells

Using stem-cell based therapy as a treatment for ED is an attractive concept and warrants mention; the reader is also referred to Kendirci's [41] excellent review of

this topic in this issue. In time, stem cells may become key neuromodulatory agents following CN injury given that the time of injury is known prior to surgery, penile anatomy (external) allows for intracavernous introduction, and retrograde transport of potential therapeutic agents to the site of injury from the corpora is widely described. Several animals studies confirm proof-of-concept and encourage further research into this exciting potential neuromodulatory approach. Embryonic stem cells (ESC) that have differentiated along the neuronal cell line have been injected into the corpus cavernosum, influencing cavernosal nerve regeneration and functional erectile status after bilateral crush injury in the rat [40]. In this study, the maximal increase in intracavernous pressure following CN electrostimulation at three months was markedly enhanced for ESC treated groups, and examination of penile nerves demonstrated a greater degree of nerve regeneration by immunohistochemical NOS-containing nerve and neurofilament staining [40]. Kendirci *et al.* [41] have demonstrated that injection of nonhematopoetic bone marrow stem cells that are selected according to p75 NGF receptor status confer a treatment effect in the bilateral CN crush rat model as measured by intracavernous pressure response to electrostimulation. The same group has also demonstrated similar neuromodulatory potential using mesechymal stem cells in aged rats [42]. Finally, Lue's group at the University of California San Francisco have demonstrated that adult adipose tissue-derived stem cells (ADSCs) increase *in vitro* neurite growth from the major pelvic ganglion (from which the CNs originate) of the rat [43]. The most intriguing aspect of the latter investigation is that ADSCs were not induced towards a particular lineage (ie. endothelial or neural) prior to use.

8 Conclusion

A paradigm shift in the management of prostate cancer occurred with the introduction of CN-sparing radical prostatectomy by Walsh and Donker, and the widespread availability of effective, safe, and well-tolerated oral therapies for ED. Although cancer-control is the most important outcome measure for any treatment of malignancy, a growing emphasis on health-related quality of life has thrust sexual function into the forefront of post-operative clinical concerns. Increasing attention has been given to strategies enhancing CN recovery in the face of treatments for prostate cancer and possibly other nontraumatic neurogenic ED disease states such as diabetes mellitus. Unfortunately, clinical management of CN injury remains 'reactive', as there are currently no treatments that have been shown to confer therapeutic benefits if given at or around the time of injury. The identification of novel molecules that promote CN regeneration or offer neuroprotection, combined with new insights for the mechanism(s) of CN recovery, may translate into novel treatments for neuropathic ED via neuromodulatory interventions.

Disclosures

Dr Author J. Bella: Eli Lilly Inc., Pfizer Inc., American Medical Systems: Consultant/Advisor and Meeting Participant/Lecturer; Bayer, Boehringer-Ingelheim: Meeting Participant/Lecturer. Dr Bella is a 2007 Canadian Urological Association Research Scholar.

Dr Illias Cagiannos and Dr Guiting Lin: None declared.

Dr Tom F. Lue: Consultant/Advisor, Investigator, or Scientific Study/Trial: Biopharm GmbH, GSK/Schering-Plough, Eli Lilly Inc., Pfizer Inc., Sanofi Aventis.

References

1 Burnett AL, Lue TF. Neuromodulatory therapy to improve erectile function recovery outcomes after pelvic surgery. J Urol 2006; 176: 882–7.
2 Mulhall JP, Morgentaler A. Penile rehabilitation should become the norm for radical prostatectomy patients. J Sex Med 2007; 4: 538–43.
3 Wang R. Penile rehabilitation after radical prostatectomy: where do we stand and where are we going? J Sex Med 2007; 4: 1085–97.
4 Mulhall J, Land S, Parker M, Waters WB, Flanigan RC. The use of an erectogenic pharmacotherapy regimen following radical prostatectomy improves recovery of spontaneous erectile function. J Sex Med 2005; 2: 532–40.
5 Mooreland RB. Is there a role of hypoxemia in penile fibrosis: a viewpoint presented to the Society for the Study of Impotence. Int J Impot Res 1998; 10: 113–20.
6 Bianco FJ Jr, Scardino PT, Eastham JA. Radical prostatectomy: long-term cancer control and recovery of sexual and urinary function ("trifecta"). Urology 2005; 66 (5 Suppl): 83–94.
7 Miles C, Candy B, Jones L, Williams R, Tookman A, King M. Interventions for sexual dysfunction following treatments for cancer. Cochrane Database Syst Rev 2007: 4; CD005540.
8 Bella AJ, Lin G, Tantiwongse K, Garcia M, Lin CS, Brant W, *et al.* Brain-derived neurotrophic factor (BDNF) acts primarily via the JAK/STAT pathway to promote neurite growth in the major pelvic ganglion of the rat: Part 1. J Sex Med 2006: 3: 815–20.
9 Valentine H, Chen Y, Guo Y, McCormick J, Wu Y, Sezen SF, *et al.* Neuroimmunophilin ligands protect cavernous nerves after crush injury in the rat: new experimental paradigms. Eur Urol 2007; 51: 1724–31.
10 Bochinski D, Lin GT, Nunes L, Carrion R, Rahman N, Lin CS, *et al.* The effect of neural embryonic stem cell therapy in a rat model of cavernosal nerve injury. BJU Int 2004, 94: 904–9.
11 Palma CA, Keast JR. Structural effects and potential changes in growth factor signaling in penis-projecting autonomic neurons after axotomy. BMC Neurosci 2006; 7: 41.
12 Bella AJ, DeYoung L, al-Numi M, Brock GB. Daily administration of phosphodiesterase type 5 inhibitors for urological and nonurological indications. Eur Urol 2007; 52: 990–1005.
13 Mulhall J. Neuroimmunophilin ligands protect cavernous nerves after crush injury in the rat: new experimental paradigms. Eur Urol 2007; 51: 1488–9.
14 Lin G, Bella AJ, Lue T, Lin CS. Brain-derived neurotrophic factor (BDNF) acts primarily via the JAK/STAT pathway to promote neurite growth in the major pelvic ganglion of the rat: Part 2. J Sex Med 2006; 3: 821–9.

15 Hsieh PS, Bochinski DJ, Lin GT, Nunes L, Lin CS, Lue TF. The effect of vascular endothelial growth factor and brain-derived neurotrophic factor on cavernosal nerve regeneration in a nerve-crush rat model. BJU Int 2003; 92: 470–5.
16 Bella AJ, Lin G, Garcia MM, Tantiwonge T, Lin CS, Brant WO, *et al*. Upregulation of penile brain-derived neurotrophic factor (BDNF) and activation of the JAK/STAT signaling pathway in the major pelvic ganglion of the rat following cavernous nerve transection. Eur Urol 2007; 52: 574–81.
17 Hiltunen JO, Laurikainen A, Klinge E, Saarma M. Neurotrophin-3 is a target derived neurotrophic factor for penile erection-inducing neurons. Neuroscience 2005; 133: 51–8.
18 Chen KC, Minor TX, Rahman NU, Ho HC, Nunes L, Lue TF. The additive erectile recovery effect of brain-derived neurotrophic factor combined with vascular endothelial growth factor in a rat model or neurogenic impotence. BJU Int 2005; 95: 1077–80.
19 Mitsui T, Fischer I, Shumsky JS, Murray M. Transplants of fibroblasts expressing BDNF and NT-3 promote recovery of bladder and hindlimb function following spinal contusion injury in rats. Exp Neurol 2005; 194: 410–31.
20 Bella AJ, Fandel TM, Tantiwongse K, Brant WO, Klein R, Garcia CA, *et al*. Neurturin enhances the recovery of erectile function following bilateral cavernous nerve crush injury in the rat. J Brachial Plex Peripher Nerve Inj 2007: 2; 5.
21 Kato R, Wolfe D, Coyle CH, Huang S, Wechuck JB, Goins WF, *et al*. Herpes simplex virus vector-mediated delivery of glial cell line-derived neurotrophic factor rescues erectile dysfunction following cavernous nerve injury. Gene Ther 2007: 14; 1344–52.
22 Laurikainen A, Hiltunen JO, Thomas-Crusells J, Vanhatalo S, Arumae U, Airaksinen MS, *et al*. Neurturin is a neurotrophic factor for penile parasympathetic neurons in adult rat. J Neurobiol 2000: 43; 198–205.
23 Fandel T, Bella AJ, Tantiwongse K, Garcia M, Nunes L, Thuroff JW, *et al*. The effect of intracavernous growth differentiation factor-5 in a rat model of cavernous nerve injury. BJU Int 2006; 98: 632–36.
24 Sullivan AM, O'Keeffe GW. The role of growth/differentiation factor 5 (GDF5) in the induction and survival of midbrain dopaminergic neurons: relevance to Parkinson's disease treatment. J Anat 2005; 207: 219–26.
25 Fandel TM, Bella AJ, Lin G, Tantiwongse K, Banie L, Thurhoff JW, *et al*. Intracavernous growth differentiation factor-5 therapy enhances the recovery of erectile function in a rat model of cavernous nerve injury. J Urol 2007; 177: 225 (Abstract 670).
26 Magit DP, Maak T, Trioano N, Raphael B, Hamouria Q, Polzhofer G, *et al*. Healos/recombinant human growth and differentiation factor-5 induces posterolateral lumbar fusion in a New Zealand white rabbit model. Spine 2006;31:2180–8.
27 Sezen SF, Hoke A, Burnett AL, Snyder SH. Immunophilin ligand FK 506 is neuroprotective for penile innervation. Nat Med 2001; 710: 1073–4.
28 Lagoda G, Jin L, Lehrfeld TJ, Liu T, Burnett AL. FK506 and sildenafil promote erectile function recovery after cavernous nerve injury through antioxidative mechanisms. J Sex Med 2007; 4: 908–16.
29 Fleischmann R, Iqbal I, Stern RL. Tacrolimus in rheumatoid arthritis. Expert Opinion Pharmacother 2006; 7: 91–9.
30 Tanaka K, Asanuma M, Ogawa N. Molecular basis of anti-apoptotic effect of immunophilin ligands on hydrogen peroxide-induced apoptosis in human glioma cells. Neurochem Res 2004; 29:1529–36.
31 Hayashi N, Minor TX, Carrion R, Price RD, Nunes L, Lue TF. Erectile recovery effect of FK1706 following a bilateral cavernous nerve crush injury in a rat model. J Urol 2006; 176:824–9.
32 Khan Z, Ferrari F, Kasper M, Tonge DA, Steiner JP, Hamilton GS, *et al*. The non-immunosuppressive immunophilin ligand GPI-1046 potently stimulated regenerating axon growth from adult mouse dorsal root ganglia cultured in Matrigel. Neuroscience 2002;114:601–9.
33 Price RD, Yamaji T, Yamamoto H, Higashi Y, Hanaoka K, Yamazaki S, Ishiye M, *et al*. FK1706, a novel non-immunosuppressive immunophilin: neurotrophic activity and mechanism of action. Eur J Pharmacol 2005; 509: 11–9.
34 Bella AJ, Hayashi N, Carrion R, Price R, Lue TF. FK1706 enhances functional recovery following bilateral cavernous nerve crush injury in a rat model. J Sex Med 2007; 4: 341–47.
35 Burnett AL, Becker RE. Immunophilin ligands promote penile neurogenesis and erection recovery after cavernous nerve injury. J Urol 2004; 171: 495–500.
36 Burnett AL, McCullough AR, Smith JA Jr, Montie JE, Walsh PC, Steiner JP. Neuromodulation to preserve erectile function after radical prostatectomy: results from the GPI 1485 neuroimmunophilin ligand clinical trial. J Urol 2007; 177: 383–4 (Abstract 1162).
37 Burnett AL, Kramer MF, Dalrymple S, Isaacs JT. Nonimmunosuppressant immunophilin ligand GPI-1046 does not promote in vitro growth of prostate cancer cells. Urology 2005; 65: 1003–7.
38 Liu T, Allaf ME, Lagoda G, Burnett AL. Erythropoetin receptor expression in the human urogenital tract: immunolocalization in the prostate, neurovascular bundle, and penis. BJU Int 2007; 100: 1103–6.
39 Allaf ME, Hoke A, Burnett AL. Erythropoetin promotes the recovery of erectile function following cavernous nerve injury. J Urol 2005; 174: 2060–4.
40 Bochinski D, Lin GT, Nunes L, Carrion R, Rahman N, Lin CS, *et al*. The effect of neural embryonic stem cell therapy in a rat model of cavernosal nerve injury. BJU Int 2005; 94: 904–9.
41 Kendirci M, Spees JL, Trost L, Whitney MJ, Prockop DJ, Hellstrom WJ. Adult bone marrow stem cells isolated by the p75 nerve growth factor receptor into the corpora cavernosa promoted recovery of erectile function in cavernous nerve injury. J Sex Med 2006; 3 (Suppl 5): 384 (Abstract OR-008).
42 Bivalacqua TJ, Deng W, Kendirci M, Usta MF, Robinson C, Taylor BK, *et al*. Mesenchymal stem cells alone or *ex vivo* gene modified with endothelial nitric oxide synthase reverse age-associated erectile dysfunction. Am J Physiol Heart Circ Physiol 2007; 292: H1278–90.
43 Bella AJ, Garcia MM, Lin G, Fandel TM, Brant WO, Lue TF. Adult adipose tissue derived stem cells enhance neurite outgrowth from the major pelvic ganglion of the rat. CUAJ 2007; 1: 200 (Abstract 2.01).