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(54) Title: PHARMACEUTICAL COMBINATION PRODUCT COMPRISING BOTULINUM TOXIN FOR THE TREATMENT OF ERECTILE DYSFUNCTION

(57) Abstract: The present invention is directed to a pharmaceutical combination product comprising botulinum toxin and at least one proerectile agent for simultaneous, sequential or separate use in the treatment of erectile dysfunction.

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**Pharmaceutical combination product comprising botulinum toxin for the treatment
of erectile dysfunction**

The present invention is directed to the treatment of erectile dysfunction.

5 Erectile dysfunction (ED) is defined as the inability to achieve or maintain a penile erection sufficient for satisfactory sexual performance and is a common sexual dysfunction in men. Indeed, epidemiological studies indicate that an estimated one-third of men will experience ED within their lifetime. ED incidence increases with age and may be of psychogenic, organic (e.g. vasculogenic, neurogenic, etc.) or mixed origin. Organic
10 etiologies associated with ED include cardiovascular diseases (CVD) and associated risk factors (e.g. smoking, obesity, dyslipidemia), diabetes mellitus, neurological diseases (e.g. spinal cord injury, multiple sclerosis, etc.), depression, pelvic surgery or radiotherapy for cancer, lower urinary tract symptoms (LUTS), side effects of medications, and sleep apnea, among others.

15 Erection is a complex neuro-vasculo-tissular process in which, in response to sexual stimulation, brain signals, transmitted via the spinal cord to the parasympathetic/sympathetic efferent neural pathways, induce neurotransmitter release by the parasympathetic terminations of the cavernous nerves while inhibiting neurotransmitter release by sympathetic terminations of the same nerves. This
20 combination of neurotransmitter release and inhibition ultimately leads to erection via penile arterial dilation, which is responsible for increased blood flow to the erectile tissue, and cavernosal smooth muscle fiber relaxation, ultimately causing corporal veno-occlusion and penile rigidity.

The main neurotransmitter responsible for penile erection is nitric oxide (NO). It is
25 released by both parasympathetic neural terminations and cavernosal endothelial cells lining the sinusoidal spaces in erectile tissue. NO stimulates intracellular guanylate cyclase activity in cavernosal smooth muscle fibers, converting GTP to cGMP. Increased cGMP initiates a cascade of events ultimately leading to increase in blood flow to the erectile tissue and cavernosal smooth muscle fiber relaxation, causing erection. Degradation of
30 cGMP to inactive GMP by phosphodiesterase 5 (PDE5) is a normal physiological process, in which smooth muscle relaxation is antagonized, ending erection. The sustained release of

norepinephrine by sympathetic neural terminations within the erectile tissue is responsible for the permanent contraction of cavernosal smooth muscle fibers, maintaining the penis in a flaccid state. Thus, release of norepinephrine must be inhibited during penile erection.

At the time of sexual stimulation, the two main pathophysiological mechanisms responsible for ED are: i) a decrease in NO release by neural terminations and/or endothelial cells and ii) an increase in contractile cavernosal smooth muscle tone or a lack of inhibition of this contractile tone, preventing penile blood flow increase and cavernosal smooth muscle relaxation.

Treatment of ED is largely empirical and performed in a step-wise manner, mainly comprising the use of proerectile agents. First-line pharmacological therapy consists of systemic delivery of proerectile phosphodiesterase type 5 (PDE5) inhibitors via oral administration. PDE5 inhibitors (PDE5i) are mild vasodilators that indirectly cause cavernosal smooth muscle relaxation by competitively inhibiting the PDE5 enzyme, thereby preventing intracellular cGMP degradation and thus potentiating cavernosal smooth muscle fiber relaxation. However, the mechanism of action, i.e. the facilitatory proerectile effect of PDE5i, requires sexual stimulation to create local arousal and initially triggers the release of NO in an effort to elicit cGMP production.

Although PDE5i have been beneficial in correcting ED in a wide range of patients with varying etiologies when taken on demand (e.g. any PDE5i) or once per day (e.g. tadalafil), they are ineffective in approximately one third of ED patients, regardless of ED etiology. For example, men whose cavernous nerves have been damaged during radical prostatectomy or men with diabetes mellitus are poor responders to PDE5i. Additionally, patients may not respond to PDE5i because their vascular condition is too severe, they take concomitant medications that interfere with the local or central mechanisms of penile erection, or they harbor severe psychological and/or interpersonal issues that overwhelm the facilitatory proerectile effect of PDE5i. Others will ultimately fail to respond to oral therapy as the causative disease progresses or evolves. The result is a large number of men who are unable, or who are no longer able, to benefit from oral PDE5i for ED.

Furthermore, in some cases, the use of PDE5i is associated with adverse events, including headache, dyspepsia, facial flushing, myalgia, nasal congestion and visual disturbances. Use of a PDE5i with nitrate-containing medications or nitrate donors, such as those used to

treat angina pectoris, or riociguat, a guanylate cyclase activator, for the treatment of pulmonary hypertension can result in dangerously low blood pressure. Accordingly, PDE5i are strictly contraindicated in combination with NO donors, nitrates or riociguat because of their synergistic hypotensive effects. Caution is also advised when PDE5i are administered
5 in combination with an alpha-blocker for the treatment of benign prostatic hyperplasia related LUTS, as co-administration may lead to symptomatic hypotension in susceptible individuals. Loss of vision in one eye because of non-arteritic anterior ischaemic optic neuropathy (NAION), regardless of whether this episode was in connection or not with previous PDE5i exposure, and known hereditary degenerative retinal disorders such
10 as retinitis pigmentosa are contraindications for the use of PDE5i. Concerns have also been raised regarding PDE5i use and sudden hearing loss.

Second-line pharmacological therapies include locally acting proerectile agents, such as vasoactive agents (also referred to as vasodilators), including prostaglandin E1 (PGE1, also known as alprostadil), papaverine, phentolamine, vasoactive intestinal polypeptide,
15 phenoxybenzamine, or mixtures thereof. These direct acting vasoactive agents represent an alternative treatment method, particularly in cases where PDE5i are not effective and/or are contraindicated. Like PDE5i, these drugs are symptomatic and usually do not have any disease modifying effect. They must also be administered on demand prior to each sexual intercourse attempt with local delivery. However, in contrast to PDE5i, these
20 vasoactive agents do not require sexual stimulation to be active. Indeed, these drugs are expected to exert an inducer effect, rather than the facilitator effect of PDE5i.

PGE1 is generally self-administered via intra-cavernosal injection. In addition, PGE1 is available as a cream for topical administration or as an intra-urethral suppository. However, these formulations have a lower efficacy than intra-cavernosal delivery. PGE1
25 itself can cause penile pain and/or burning in some patients. Severe hypotension has been reported after intra-urethral delivery of PGE1. These side effects are dose-related. Thus, it is of particular interest to reduce the dose of PGE1.

In case of lack of efficacy of these first and second-line pharmacological therapies, alternative methods of treating ED exist, including vacuum constriction devices and surgical
30 implantation of a penile prosthesis or implant. However, vacuum constriction devices are cumbersome and can sometimes cause pain; the penis can also be cold or numb. Vacuum constriction devices may also be difficult to use in obese men because of fatty tissue in the

lower abdomen. Penile prosthetic implants are the therapy of last resort because they are irreversible. After implantation, pharmacological therapies can no longer be used. Furthermore, penile implants are expensive, efficacy cannot be guaranteed, and implantation is associated with infection risks. The failure rate is estimated at 10% at 5
5 years due to infection and mechanical failure.

Recently, intra-cavernosal delivery of 50 units of onabotulinumtoxinA has been reported to have a positive effect on severe ED of vascular origin in patients who have failed to respond to PDE5i and intra-cavernosal injections (data not shown). Similarly, a study in aged rats has also suggested that onabotulinumtoxinA may improve erectile function (data not
10 shown). In these rats, intra-cavernosal delivery of onabotulinumtoxinA was shown to increase intra-cavernosal blood vessel diameter and sinusoidal space volume by inducing enhanced cavernous smooth muscle relaxation. Unfortunately, on the basis of the single study available in humans, the use of onabotulinumtoxinA is limited to ED having a single severe etiology of vascular origin and to cases where first and second-line
15 pharmacotherapies have failed. Similarly, the study performed in rats is limited only to ED associated with aging.

Given current drawbacks and limitations in the treatment of ED, both clinicians and patients would welcome new products and methods. In particular, there exists a need for new products and methods having new mechanisms of action. Indeed, the majority of
20 current products are proerectile agents, which facilitate or induce the relaxation of corporal smooth muscle fibers (e.g. PDE5i and PGE1, respectively). There also exists a need for new products and methods that can complement existing therapies, as well as for new products and methods having long-term effects, which do not need to be administered on demand prior to each sexual intercourse attempt. Finally, there is a need for new
25 products and methods that have fewer side effects, or that are able to reduce side effects or dosage of existing treatments. Preferably, new products and methods would be able to treat ED having different (or multiple) underlying etiologies. New products and methods expanding the population that is able to be treated by existing first and/or second line therapies as part of a combination therapy would be particularly advantageous.

30 A first object of the present invention is directed to a pharmaceutical combination product comprising botulinum toxin and at least one proerectile agent for simultaneous, sequential and/or separate use in the treatment of ED.

In a preferred embodiment of the first object of the present invention, the pharmaceutical combination product is a composition comprising botulinum toxin and at least one proerectile agent, according to the modalities described herein, as well as pharmaceutically acceptable vehicles. Pharmaceutically acceptable vehicles for botulinum toxin or proerectile agents are well-known in the art.

For the purpose of the present invention, the terms “combination product” and “composition” are intended to mean that the two active components are not mixed together but are administered simultaneously, sequentially or separately according to the modalities described herein.

Indeed, the inventors have surprisingly found that the botulinum toxin decreases permanent contractile tone, likely by preventing the sustained intra-cavernosal release of norepinephrine. Specifically, to maintain the penis in a flaccid state, norepinephrine is released by postganglionic sympathetic neurons in erectile tissue via synaptic vesicles from the presynaptic terminals, fusing with the membrane and releasing its contents into the synaptic cleft through a process called exocytosis. Following exocytosis, norepinephrine preferentially activates $\alpha 1a$, $\alpha 1b$ and $\alpha 1d$ adrenoceptors (with a predominant role likely for $\alpha 1a$ and $\alpha 1d$) on cavernosal smooth muscle cells, causing cavernosal smooth muscle contraction. In particular, the inventors have surprisingly found that botulinum toxin prevents muscle contraction mediated by permanent sympathetic nerve discharge via exocytotic blockade, thereby inducing a chronic or long-term modulatory relaxant effect on erectile tissue, which facilitates erectile response to sexual stimulation and proerectile drug efficacy.

As defined herein, the term “botulinum toxin” refers to a neurotoxin produced by *Clostridium botulinum*. The botulinum toxin may be a native botulinum toxin of serotype A, B, C (e.g. C₁), D, E, F, or G, and may be present in non-complex or complex form. The term “complex form” refers to the association of botulinum toxin with one or more additional proteins (e.g. non-toxin proteins, hemagglutinins, human serum albumin). When in complex form, these additional proteins may alter the properties of the botulinum toxin (e.g. improve its stability and/or activity). The term “botulinum toxin” as used herein further comprises any variant of said toxin (e.g. any fragment, derivative, modified, short-acting and/or recombinant toxin), said variant retaining the functional activity of the botulinum toxin to inhibit the release of norepinephrine and, optionally, other

neurotransmitters from sympathetic terminations in the corpora cavernosa and the penile vasculature by exocytosis. Said botulinum toxin variant may notably have had at least one of its amino acids deleted, modified, or replaced, as compared to a native botulinum toxin. For example, the botulinum toxin may be modified to improve or enhance desired toxin characteristics, or to reduce unwanted side effects thereof. Preferably, botulinum toxin is produced in *C. botulinum*. As an example, the botulinum toxin onabotulinumtoxinA of serotype A is produced in the *Clostridium botulinum* type A-Hall strain. However, botulinum toxin may also be produced in a different bacterial species, including, for example *Escherichia coli*.

10 The botulinum toxin of the invention comprises native botulinum toxins of serotype A (e.g. A1, A2, A3, A4), B, C (e.g. C₁), D, E, F, or G, as well as variants of native botulinum toxins of said serotypes, including any fragment, derivative, modified, short-acting and/or recombinant toxin of said toxins.

According to a preferred embodiment of the invention, the botulinum toxin is selected from among short-acting, recombinant or native botulinum toxins of any serotype. According to a preferred embodiment of the invention, the botulinum toxin is of serotype A, B, C, D, E, F, or G, preferably of serotype A, B, or F, more preferably of serotype A or B, even more preferably of serotype A. In a preferred embodiment, the botulinum toxin is onabotulinumtoxinA, abobotulinumtoxinA, or incobotulinumtoxinA, more preferably onabotulinumtoxinA or abobotulinumtoxinA. According to a preferred embodiment, the botulinum toxin is in complex form. According to a preferred embodiment, the botulinum toxin is a variant, preferably a recombinant toxin or a short-acting toxin.

As defined herein, the terms “chronic” or “long-term” refer to a length of time equivalent or superior to three months, preferably equivalent or superior to four months, more preferably equivalent or superior to five months, even more preferably equivalent or superior to six months. Current pharmacological therapies, including proerectile agents which are deliverable either orally or intra-cavernosally (e.g. PDE5i and PGE1), have short-term effects lasting no more than 36 hours, and generally no more than 4 to 6 hours. In contrast, botulinum toxin exerts a long-term effect, which potentializes the effects of the aforementioned proerectile agents.

According to a preferred embodiment, botulinum toxin is administered once per three-month period, preferably once per four-month period, more preferably once per five-month period. According to the most preferred embodiment, botulinum toxin is administered only once in a six-month period.

- 5 In the context of ED treatment, a subject is generally initially administered a first-line PDE5i treatment. If the subject does not respond or insufficiently responds to said PDE5i, they may then be administered a second-line treatment (e.g. local PGE1). The pharmaceutical combination product of the present invention is highly advantageous as it surprisingly enables subjects that insufficiently respond to a first and/or second-line
- 10 treatment to sufficiently respond. By “insufficiently responds” is meant herein that no clinically significant improvement in ED occurs in a subject in response to multiple treatment attempts. In particular, a subject that insufficiently responds will have an IIEF-EF domain score that remains equal or inferior to 25 even after treatment. (A IIEF-EF score of 26 to 30 is associated with normal erectile function; see section 7.1 of the Examples for
- 15 further details). A clinically significant improvement occurs when a subject’s IIEF-EF domain score increases by at least the Minimal Clinically Important Difference (MCID), which varies according to initial severity of ED (see section 7.1 of the Examples for further details of determining both ED severity and a clinically significant improvement). According to a particular aspect, an “insufficient response” corresponds to the inability to achieve or
- 20 maintain an erection adequate for intercourse. As used herein, the term “proerectile agent insufficient responder” refers to subjects that insufficiently respond to the administration of one or more proerectile agents (e.g. PDE5i, PGE1, etc.). By “sufficiently responds” is therefore meant herein a clinically significant improvement in a subject’s IIEF-EF domain score. According to a particular aspect, a “sufficient response” corresponds to
- 25 the ability to achieve or maintain an erection adequate for intercourse. Furthermore, for some subjects that insufficiently respond to PGE1, the pharmaceutical combination product of the present invention may comprise a PDE5i rather than PGE1. This is highly advantageous, in particular due to the greater ease of use of PDE5i (e.g. oral administration rather than intra-cavernosal injection).
- 30 A further object of the present invention is therefore directed to a pharmaceutical combination product comprising botulinum toxin and at least one proerectile agent for simultaneous, sequential and/or separate use in the treatment of ED in insufficient

responders. Preferably, said insufficient responders insufficiently respond to treatment with a proerectile agent (i.e. PDE5i or PGE1 alone).

A further object of the present invention is therefore directed to a pharmaceutical composition comprising botulinum toxin and at least one proerectile agent as well as
5 pharmaceutically acceptable vehicles, the composition being useful for treatment of ED in insufficient responders. Preferably, said insufficient responders insufficiently respond to treatment with a proerectile agent (i.e. PDE5i or PGE1 alone). As indicated, pharmaceutically acceptable vehicles for botulinum toxin or proerectile agents are well-known in the art.

10 For the purpose of the present invention, the terms “combination product” and “composition” are intended to mean that the two active components are not mixed together but administered simultaneously, sequentially or separately according to the modalities described herein.

A further object of the present invention is a pharmaceutical agent/product for use in the
15 treatment of erectile dysfunction comprising botulinum toxin, which is used in combination with at least one proerectile agent. Preferably, the botulinum toxin is simultaneously, sequentially, or separately administered with the proerectile agent(s). The pharmaceutical agent/product for use in the treatment of erectile dysfunction comprising botulinum toxin, which is used in combination with at least one proerectile agent, encompasses all of the
20 modalities as described and/or defined herein.

A further object of the present invention is a pharmaceutical agent/product for use in the treatment of erectile dysfunction comprising at least one proerectile agent, which is used in combination with botulinum toxin. Preferably, said proerectile agent(s) are simultaneously, sequentially, or separately administered with botulinum toxin. The
25 pharmaceutical agent/product for use in the treatment of erectile dysfunction comprising at least one proerectile agent, which is used in combination with botulinum toxin, encompasses all of the modalities as described and/or defined herein.

In the context of the present invention, the proerectile agent is preferably administered sequentially to botulinum toxin. Sequential use as defined herein further refers to the
30 successive administration of each compound of the combination product wherein botulinum toxin is administered a single time for one or more sequential administrations of the

proerectile agent on demand, once per day, or every other day. According to a preferred embodiment, botulinum toxin is administered no more than once in a three-to-six-month period (e.g. administered once in three, four, five, or six months) while the proerectile agent is administered on demand (e.g. prior to sexual intercourse attempt) or daily during the same period. The person skilled in the art can easily determine the desired frequency of administration of the botulinum toxin in the context of ED. In particular, the person skilled in the art may further extend the interval of administration of the botulinum toxin by up to 12 months (i.e. administration of botulinum toxin once every 12 months), preferably by up to 6 to 8 months. The frequency of administration of the toxin may notably depend on the subject's response to treatment. In contrast, the proerectile agent is preferably administered on demand (e.g. prior to sexual intercourse attempt), daily, or every other day during the same period.

According to a preferred embodiment, the proerectile agent is administered at least one day to one week after administration of the botulinum toxin.

Indeed, the inventors have surprisingly shown that the use of a pharmaceutical combination product comprising botulinum toxin and at least one proerectile agent in the treatment of ED improves ED in an advantageous manner. Indeed, the use of said pharmaceutical combination product produces a surprising synergistic effect, superior to the expected effect of the combination of botulinum toxin and at least one proerectile agent (as illustrated, for example, in Table 2 and in Figures 5a-5c). This advantageous effect was highly unexpected as the prior art indicates that botulinum toxin exerts a pro-erectile (e.g. a vasodilator) effect, like current first and second-line treatment methods. Thus, no advantageous effect was expected when combining botulinum toxin with a proerectile agent. The inventors have found that the unexpected, advantageous effect of combining botulinum toxin and proerectile agents, such as PDE5i and PGE1, is due to a dual mechanism, which modulates the balance between contractile and relaxant factors governing flaccidity/rigidity within the penis. Indeed, botulinum toxin facilitates long-term cavernous smooth muscle relaxation through alteration of the balance within the penis between the permanent sympathetic contractile tone (applied to cavernosal smooth muscle fibers responsible for flaccidity) and the relaxed state of the cavernous smooth muscles (induced by the activation of the parasympathetic/NO-cGMP signaling pathway) in response to sexual stimulation. Thus, the combination of the inhibition of contractile mechanisms

(by botulinum toxin) and the activation of relaxing mechanisms (e.g. by first or second-line pharmacotherapies such as PDE5i or PGE1) promotes the local mechanisms of penile erection.

Advantageously, as the combined effect of botulinum toxin and proerectile agent improves treatment efficacy, when the dose of proerectile agent is maintained, an improved effect can be observed. Advantageously, proerectile agent dosage can be reduced without negatively influencing proerectile effects. Accordingly, side effects associated with proerectile agent administration are reduced. Advantageously, new population groups for which proerectile agents are not effective, or who suffer from side effects at the administered dose of proerectile agent, can now be treated, as lower doses of proerectile agents can be administered when in combination with botulinum toxin. In some cases, the frequency of use of proerectile agent treatment on demand may also advantageously be reduced. As a particular example, when the proerectile agent is administered daily (i.e. tadalafil), the frequency of use may advantageously be reduced e.g. every other day.

The term “erectile dysfunction” or “ED” as used herein refers to the inability to achieve or maintain a penile erection sufficient for sexual performance. The combination product and methods of the invention treat ED by eliciting, enhancing, and/or sustaining an erection. The combination product and methods of the invention may be used to treat ED of psychogenic, organic, or mixed etiologies, in which the activity of the sympathetic nervous system is abnormally increased. As an example, increased sympathetic contractile tone, due to an overactivity of the sympathetic nervous system, occurs in cardiovascular diseases including, hypertension, ischemic heart disease and chronic heart failure. Elevated sympathetic nervous system activity has also been reported in subjects with diabetes mellitus (in particular type II diabetes mellitus), obesity, metabolic syndrome, obstructive sleep apnea, depression and neurological disorders such as spinal cord injury. Furthermore, increased activity of the sympathetic nervous system occurs under stress, which is involved in ED of psychogenic origin. Lastly, increased sympathetic nervous system activity, via neurogenic (nor)adrenergic contractions, has also been reported to be enhanced after radical prostatectomy.

According to a preferred embodiment, the pharmaceutical composition is administered to subjects having ED of neurogenic, psychogenic, vasculogenic, cardio-metabolic, or endocrine (or hormonal) origin, or having ED of mixed origin (i.e. of more than one origin).

The different categories of ED origin are well-known in the art, and are discussed, for example, in Muneer et al., BMJ, (2014) 348:g129 and Davis-Joseph et al., Adult Urology (1995) 45:3, p. 498-502). According to a more preferred embodiment, the pharmaceutical composition is administered to subjects having ED of neurogenic origin, of psychogenic origin, of endocrine origin, or of mixed origin. According to a preferred embodiment, the pharmaceutical composition is administered to subjects having increased sympathetic nervous system activity (or sympathetic contractile tone), preferably to subjects suffering from cardiovascular disease, such as hypertension, ischemic heart disease, or chronic heart failure, or, from diabetes mellitus (in particular type II diabetes mellitus), obesity, metabolic syndrome, obstructive sleep apnea, depression, spinal cord injury, stress, after radical prostatectomy, or combinations thereof.

The terms “treat,” “treatment,” “treating,” and the like as used herein refer to obtaining a desired pharmacologic and/or physiological effect. These terms are not meant to be absolute, but rather indicate a clinical improvement in at least one aspect of ED. This may be an improvement in the occurrence of an erection in response to sexual stimulation or an enhancement of a characteristic of the erection itself (e.g. rigidity, duration, frequency, etc.). More particularly, this may be an increase in penile rigidity and/or duration of erection to provide an erection allowing satisfactory sexual activity, including vaginal intercourse. It is understood that said treatment method may not be successful in all subjects. Treatment may also correspond to a reduction in undesirable side effects following a reduction in dosage of at least one compound in the pharmaceutical combination product of the present invention. The term treatment may in some cases further comprise prophylaxis, wherein the pharmaceutical combination product is administered in subjects at risk of developing ED (e.g. in subjects having prostate cancer that will undergo radical prostatectomy).

According to a preferred embodiment, the pharmaceutical combination product and methods of the invention are used to treat ED in men having an overactive or elevated sympathetic nervous system activity or tone, or suspected of having an overactive or elevated sympathetic nervous system activity or tone (e.g. based on the presence of a causative etiology or risk factor or co-morbidity).

The term “proerectile agent” as used herein refers to drugs that target the corporal smooth muscle fiber relaxing mechanisms either at a systemic or local level (e.g. in the

penis). Said proerectile agents may function as “facilitator” or “inducer” drugs, promoting activity of the parasympathetic nervous system. The term “proerectile agent” as used herein is well-known in the art. Proerectile agents include phosphodiesterase inhibitor (e.g. PDE5i), direct vasodilator or vasoactive agents (e.g. papaverine, phentolamine, phenoxybenzamine, vasoactive intestinal polypeptide), adrenergic receptor antagonist, as well as pharmaceutically acceptable salts, derivatives, precursors, pharmaceutically active sequences or regions, and mimetics, thereof.

The proerectile agent of the present invention may be a PDE5i, as well as pharmaceutically acceptable salts, derivatives, precursors, pharmaceutically active sequences or regions, and mimetics, thereof. The proerectile agent of the invention may further comprise combinations of two or more proerectile agents.

According to a preferred embodiment, the proerectile agent of the invention comprises at least one proerectile agent. According to another preferred embodiment, the proerectile agent of the invention comprises a combination of two, three or four proerectile agents. Said proerectile agents include those listed above.

According to a preferred embodiment, the at least one proerectile agent of the invention is a PDE5i or PGE1. According to a preferred embodiment, the at least one proerectile agent comprises a PDE5i and PGE1.

The term “PDE5 inhibitor” or “PDE5i” refers to a chemical compound that competitively inhibits the PDE5 enzyme, permitting accumulation of cGMP.

According to a first embodiment, the at least one proerectile agent of the invention comprises a PDE5i, even more preferably a cyclic guanosine 3', 5'-monophosphate PDE5i. According to a preferred embodiment, the at least one proerectile agent consists of a PDE5i, even more preferably a cyclic guanosine 3', 5'-monophosphate PDE5i.

PDE5 inhibitors are well-known in the art and may be selected from known compounds, such as avanafil, lodenafil, mirodenafil, sildenafil, tadalafil, vardenafil, udenafil, and the like, or identified using established criteria of selectivity and potency using well-known techniques. The PDE5i of the present invention may be any pharmaceutically acceptable salt, solvate or polymorph of the PDE5i, such as sildenafil citrate.

According to a preferred embodiment, the PDE5i of the present invention is selected from avanafil, lodenafil, mirodenafil, sildenafil, tadalafil, vardenafil, udenafil, or a pharmaceutically acceptable salt thereof. Preferably, the PDE5i is sildenafil, tadalafil, vardenafil or a pharmaceutically acceptable salt thereof. More preferably, the PDE5 inhibitor is sildenafil citrate or tadalafil, even more preferably sildenafil citrate.

Preferably, when said PDE5i comprises or consists of avanafil, lodenafil, mirodenafil, sildenafil, tadalafil, vardenafil, udenafil, or a pharmaceutically acceptable salt thereof, said PDE5i is administered on demand (e.g. prior to sexual activity). It is understood in the context of the invention that said PDE5i may be administered more than once per day, depending on the dosage.

According to a first embodiment, when the PDE5i is sildenafil, preferably in the form of sildenafil citrate, 25, 50, 75, or 100 mg is administered per dose, more preferably 50 or 100 mg, even more preferably 100 mg. According to a second embodiment, when the PDE5i is tadalafil, 10 to 20 mg is administered per dose, preferably 10 or 20 mg, even more preferably 20 mg. According to a further embodiment, when the PDE5i is avanafil, 50 to 200 mg, preferably 50, 100 or 200 mg, more preferably 200 mg is administered per dose. According to a further embodiment, when the PDE5i is vardenafil, 2.5 to 20 mg, more preferably 2.5, 5, 10, or 20 mg is administered per dose. According to a further embodiment, when the PDE5i is udenafil, 100 to 200 mg, even more preferably 100 or 200 mg is administered per dose. According to a further embodiment, when the PDE5i is mirodenafil, 50 to 100 mg, even more preferably 50 or 100 mg, is administered per dose. According to a further embodiment, when the PDE5i is lodenafil, 80 mg is preferably administered per dose. According to this embodiment, the PDE5i (sildenafil citrate, tadalafil, etc.) is preferably administered on demand prior to sexual intercourse attempt.

According to an alternative embodiment, when the PDE5i is tadalafil, 2.5 to 5 mg is administered per dose, preferably 2.5 or 5 mg, even more preferably 5 mg per dose. According to this embodiment, tadalafil is preferably administered once per day or every other day.

Preferably, the proerectile agent comprising or consisting of PDE5i is administered separately or sequentially to botulinum toxin. More preferably, the proerectile agent comprising or consisting of PDE5i is administered at least one day to one week after

administration of botulinum toxin. According to a second embodiment, the proerectile agent comprises PGE1. According to a preferred embodiment, the proerectile agent comprising PGE1 further comprises at least one other vasoactive agent. Preferably, said vasoactive agent is selected from papaverine, phentolamine, phenoxybenzamine, or vasoactive intestinal polypeptide, or a combination of two of more thereof. According to a preferred embodiment, the proerectile agent comprising PGE1 further comprises papaverine or phentolamine, or a combination thereof. According to a preferred embodiment, the proerectile agent comprises PGE1 in combination with papaverine and/or phentolamine.

Alternatively, the proerectile agent consists of PGE1. According to a preferred embodiment, the proerectile agent consists of PGE1 in combination with papaverine, phentolamine, phenoxybenzamine, or vasoactive intestinal polypeptide, or a combination of two of more thereof. According to a preferred embodiment, the proerectile agent consists of PGE1, and papaverine or phentolamine, or a combination thereof. According to a preferred embodiment, the proerectile agent consists of PGE1 in combination with papaverine and/or phentolamine.

Preferably, the proerectile agent comprising or consisting of PGE1 is administered separately or sequentially to botulinum toxin. More preferably, the proerectile agent comprising or consisting of PGE1 is administered at least one day to week after administration of botulinum toxin. Preferably, when said proerectile agent comprises or consists of PGE1, said PGE1 is administered on demand (e.g. prior to sexual activity).

In the context of the invention, when the proerectile agent comprising or consisting of PGE1 is administered intra-cavernosally, 1 to 60 micrograms (mcg), preferably 2.5 to 40 mcg, 10 to 50 mcg, 15 to 45 mcg, more preferably 10 to 20 mcg or 15 to 25 mcg of PGE1 is administered per dose. When the proerectile agent comprising or consisting of PGE1 is administered intra-urethrally, 125 to 1000 mcg, preferably 250 to 1000 mcg, 500 to 1000 mcg, 750 to 1000 mcg, more preferably 1000 mcg is administered per dose. When the proerectile agent comprising or consisting of PGE1 is administered topically, 50 to 300 µg, preferably 100 to 300 µg, 200 to 300 µg, more preferably 300 µg, is administered per dose.

In a preferred embodiment, the at least one proerectile agent of the invention comprises or consists of combination of sildenafil and PGE1, preferably 100 mg sildenafil in the form

of sildenafil citrate and 40 mcg PGE1, wherein said PGE1 is administered intra-cavernosally.

Proerectile agents are preferably administered orally, intra-nasally, intra-cavernosally, topically (e.g. by applying a cream to the glans penis), subcutaneously or intra-urethrally.

5 According to a first embodiment, the at least one proerectile agent is administered orally, intra-nasally or subcutaneously, preferably orally or subcutaneously, even more preferably orally, preferably when said proerectile agent comprises or consists of a PDE5i. According to a second embodiment, the at least one proerectile agent is administered topically to the glans penis, intra-urethrally, or intra-cavernosally, preferably when said proerectile agent
10 comprises or consists of PGE1 or a combination comprising PGE1 and at least one other agent, preferably at least one other proerectile (e.g. vasoactive) agent. According to preferred embodiment, said proerectile agent may comprise PGE1, administered intra-cavernosally, topically, or intra-urethrally, and at least one PDE5i, administered orally, intra-nasally or subcutaneously.

15 According to a preferred embodiment, the therapeutic dose of proerectile agent is reduced when administered sequentially to the botulinum toxin. Preferably, the therapeutic dose of proerectile agent is reduced by at least 25%, 50%, 66%, or 75%. More preferably, the therapeutic dose of proerectile agent is reduced by at least 50%, 55%, 60%, 65%, 70% or 75%. Advantageously, the reduction of the therapeutic dose of proerectile agent reduces
20 the risk of side effects associated with said proerectile agent.

According to particular example, the therapeutic dose of PGE1 is reduced by at least 50% (e.g. a reduction in the dose of PGE1 from 40 mcg to 20 mcg, or a reduction in the dose of tadalafil from 20 to 10 mg) when administered sequentially to the botulinum toxin, more preferably by at least 66% (e.g. a reduction in the dose of PGE1 from 60 mcg to 20 mcg).

25 According to a preferential mode, a therapeutic dose of botulinum toxin is administered by injection. Preferably, a therapeutic dose of botulinum toxin is administered by parenteral injection. According to a preferred embodiment, botulinum toxin is injected intra-cavernosally.

30 The term "oral administration" as used herein comprises orogastric administration, as well as oromucosal administration, including buccal, sublingual, or sublabial administration. According to a preferred embodiment, when the proerectile agent is PDE5i, said PDE5i is

administered orally via the orogastric or oromucosal route, even more preferably via the orogastric, sublingual, or buccal route.

As used herein, the term “intra-cavernosal injection” or “intra-cavernosally” refers to an injection into the corpora cavernosa of the penis. The corpora cavernosa comprises two
5 bodies, or cavernous bodies, of erectile tissue in which arterial blood accumulates during erection. The corpora are separated in the center by a septum. The septum is incomplete distally, perforated on its dorsal margin by vertically orientated openings in the pectiniform septum that provides communication between the two corpora. Injection into the corpora cavernosa is preferably bilateral, the term “bilateral” referring to the
10 administration of at least one injection in each cavernous body. Preferably, at least one injection into each cavernous body is performed. More preferably, at least two injections into each cavernous body are performed, even more preferably at least three injections into each cavernous body are performed. When two or more injections are performed in a cavernous body, different injection sites are preferably used for each injection. As an
15 example, injection sites may be chosen from proximal, midshaft, and distal sites. The term “proximal” as used herein refers to a site in the cavernous body that located near the penile crus (e.g. closer to the penile crus than the glans of the penis). The term “distal” as used herein refers to a site in the cavernous body that is in proximity to the glans of the penis (e.g. closer to the glans than the penile crus). The term “midshaft” as used herein
20 refers to a site that is located approximately equidistant between the proximal and distal sites (e.g. approximately equidistant between the penile crus and the glans of the penis). The use of at least two separate injection sites within a cavernous body, is also referred to herein as a “multisite injection.” Preferably, when performing a multisite injection, injection sites are spaced apart from each other by at least one centimeter, or as
25 appropriate depending on the size of the penis, as would be easily determined by the skilled person. As a non-limiting example, when two or more injections are performed in a cavernous body, at least one injection may be performed at the proximal site of the cavernous body (near the penile crus) and at least one injection may be performed at the midshaft site of the cavernous body.

30 According to a first embodiment of the invention, a therapeutic dose of botulinum toxin is bilaterally injected into the corpora cavernosa, with at least one injection in each cavernous body. According to a second embodiment, a therapeutic dose of botulinum toxin

is bilaterally injected into the corpora cavernosa, with at least two injections into each cavernous body. Preferably, the at least two injections are both performed at the proximal site, at the midshaft site, or at the distal site. A proximal site, a midshaft site, and a distal site are comprised in each cavernous body. Preferably, when the at least two injections
5 are performed at the same site, they are separated by at least 1 cm. More preferably, the at least two injections are performed at two different sites (e.g. at the proximal and midshaft sites, at the proximal and distal sites, or at the midshaft and distal sites). Even more preferably, the at least two injections are performed at the proximal and distal sites. According to a third embodiment, a therapeutic dose of botulinum toxin is injected by
10 multisite injection to the corpora cavernosum, with two or three injections in each cavernous body. Preferably, the three injections are performed at the proximal, midshaft, and/or distal sites, preferably at the proximal, midshaft, and distal sites of the cavernous body.

Preferably, when performing bilateral injection, the therapeutic dose is divided
15 approximately equally between the two cavernous bodies. As an example, if a therapeutic dose of 100 units of botulinum toxin (such as onabotulinumtoxinA or incobotulinumtoxinA) is administered, when performing a bilateral injection, 50 units are administered to each cavernous body. When only one injection is performed in each cavernous body, each injection comprises 50 units. If more than one injection is performed in each cavernous
20 body, the therapeutic dose administered per injection is adapted appropriately, as can be easily determined by the skilled person. Preferably, if more than one injection is performed in each cavernous body, the therapeutic dose administered per injection is divided approximately equally between the injections. As an example, if 50 units of botulinum toxin (such as onabotulinumtoxinA or incobotulinumtoxinA) are injected in a
25 cavernous body at two separate sites (e.g. at the proximal and midshaft sites), each injection may preferably comprise approximately 25 units. According to a preferred embodiment of the invention, 50 units of botulinum toxin (such as onabotulinumtoxinA or incobotulinumtoxinA) are administered, preferably with a single injection of 25 units of botulinum toxin in each cavernous body. According to a more preferred embodiment, 100,
30 150, or 200 units of botulinum toxin (such as onabotulinumtoxinA or incobotulinumtoxinA) are administered, more preferably with a single injection of 50, 75, or 100 units, respectively, in each cavernous body. According to an alternative preferred embodiment,

250 units of toxin of botulinum toxin (such as abobotulinumtoxinA) are administered, more preferably with a single injection of 125 units, respectively, in each cavernous body.

The term “injection” as defined herein comprises administration by direct injection by syringe, pen, auto-injector, high pressure injection device, or by injecting an implantable
5 drug or device providing sustained release. According to a preferred embodiment, direct injection is by syringe, pen, auto-injector or high-pressure injection device. More preferably, injection is by syringe, pen, or auto-injector. Even more preferably, the needle of said syringe, pen, or auto-injector has a length of 13 mm and a 30-gauge diameter.

Injection of a product into the corpora cavernosa is a well-established procedure that
10 corresponds to a minor routine intervention with a low degree of complexity which does not belong to the core of medical activities. Indeed, the procedure involves no significant health risks when carried out with the required care and skill and can be performed by subjects themselves. Self-administered intra-cavernosal injection is already performed for PGE1, such as Caverject® and Edex® or Viridal®.

15 To retain the composition within the corpora cavernosa following injection, thereby avoiding systemic spreading, the penis is preferably constricted at or near the penile crus. Constriction is preferably performed for approximately 15-30 minutes. Penile constriction may be performed using a rubber band, cuff, ring, loop, or any other appropriate constricting device, as is known by the skilled person. Preferably, said constricting device
20 is adjustable, preferably an adjustable penile loop ring. According to a preferred embodiment, penile constriction is performed prior to injection of botulinum toxin. According to an alternative preferred embodiment, penile constriction is performed after injection of botulinum toxin.

According to a preferred embodiment, penile constriction located at the penile crus is
25 initiated prior to injection of botulinum toxin and continues for up to 30 minutes following injection of botulinum toxin. According to a preferred embodiment, penile constriction lasts for up to 30 minutes following injection (e.g. 5, 10, 15, 20, 25, or 30 minutes). According to a preferred embodiment, penile constriction lasts for 30 minutes following injection.

30 In a preferred embodiment, the pharmaceutical combination product comprises 10 to 600 units of botulinum toxin per dose, preferably 20 to 300 units, 30 to 250 units, 40 to 225

units, 50 to 200, more preferably to 50 to 100 units or 100 to 200 units, even more preferably 50 units per dose. In a preferred embodiment, the pharmaceutical combination product comprises 50 to 200 units of botulinum toxin, preferably 50, 100, 150, or 200 units of botulinum toxin, when said botulinum toxin is onabotulinumtoxinA, incobotulinumtoxinA, or abobotulinumtoxinA. In a preferred embodiment, the pharmaceutical combination product comprises 100 to 200 units of botulinum toxin when said botulinum toxin is incobotulinumtoxinA. According to a most preferred embodiment, the pharmaceutical combination product comprises 50, 100, or 150 units of onabotulinumtoxinA or incobotulinumtoxinA per dose, even more preferably 100 units per dose. According to a preferred embodiment, the pharmaceutical combination product comprises 150 to 600 units, more preferably 200 to 500 units of abobotulinumtoxinA, per dose. According to a particularly preferred embodiment, the pharmaceutical combination product comprises 250 units of abobotulinumtoxinA per dose.

In a preferred embodiment, the pharmaceutical combination product for administration comprises an aqueous solution of at least the botulinum toxin. Preferably, the total volume of said aqueous solution to be administered is from about 0.2 to about 4 milliliters (ml), preferably, from about 0.3 to about 3 ml, more preferably from about 0.4 to about 2 ml. Preferably the volume to be administered per injection is from about 0.5 to about 1 ml.

The term “dose” is defined herein amount of the pharmaceutical combination that is administered at a given time. Said dose is administered to each cavernous body, and may be further divided into two or more separate injections in each cavernous body. Botulinum toxin dose may be increased, decreased, or maintained over time according to the presence and/or the level of therapeutic effect. In particular, the dose, or frequency of administration, may be increased if therapeutic effect is diminished, for example due to disease progression. A person skilled in the art can easily determine the appropriate dose for administration and adapt it according to the needs of a particular subject. The dose may be expressed herein in terms of weight, volume, or in units of botulinum toxin. In a particular example, a dose of 100 units of onabotulinumtoxinA may be administered by intra-cavernosal injections of 50 units in the right corpus cavernosum and 50 units in the left corpus cavernosum.

The term “therapeutically effective dose” or “therapeutic dose” means that the amount administered is sufficient to provide a clinical improvement in ED. Said clinical improvement may concern the achievement, the enhancement, and/or the sustainment of an erection, more specifically an increase in penile rigidity and/or duration of erection to provide with an erection allowing satisfactory sexual activity, including vaginal intercourse. Said clinical improvement may also be a reduction in side effects associated with the use of higher doses of a therapy in which botulinum toxin is not present. A therapeutically effective dose may be administered in one or more independent administrations, particularly when administration of the pharmaceutical combination product is sequential.

5 A “therapeutically effective dose” of the pharmaceutical combination product will vary between subjects, depending on a variety of factors including underlying etiology, disease progression, age, existing health conditions, route of administration, and the like.

Advantageously, the pharmaceutical combination product of the present invention comprising botulinum toxin for intra-cavernosal administration is a lyophilized or aqueous solution that is commercially available. As a non-limiting example, commercialized botulinum toxins include onabotulinumtoxinA (e.g. Botox®), abobotulinumtoxinA (e.g. Dysport®), incobotulinumtoxinA (e.g. Xeomin®) and rimabotulinumtoxinB (e.g. Myobloc®). If the pharmaceutical combination product comprising botulinum toxin used in the present invention is not commercially available, the galenic formulation of said botulinum toxin can easily be selected by the skilled person based on existing formulations, for example such as those described in Picket and Perrow (2010) Journal of Drugs in Dermatology, 9(9):1085-1091, and according to their general knowledge. Preferably, botulinum toxin of the combination product comprises onabotulinumtoxinA, abobotulinumtoxinA, incobotulinumtoxinA or rimabotulinumtoxinB. Preferably, botulinum toxin of the combination product consists of onabotulinumtoxinA, abobotulinumtoxinA, incobotulinumtoxinA or rimabotulinumtoxinB.

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Preferably, said at least one proerectile drug is administered at least one day to one week after administration of botulinum toxin. If said proerectile drug is taken once per day, preferably administration is interrupted before administration of botulinum toxin, preferably by at least one day to one week, or at the time of administration of botulinum toxin, and also following administration of botulinum toxin for at least one day to one week.

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According to a particular embodiment, the pharmaceutical combination product for use according to the invention comprises 50 U or 100 U of onabotulinumtoxinA or incobotulinumtoxinA, and 100 mg of sildenafil for use in the treatment of erectile dysfunction.

- 5 According to a particular embodiment, the pharmaceutical combination product for use according to the invention comprises 100 U of onabotulinumtoxinA or incobotulinumtoxinA, and 200 mg of avanafil or 10 mg of tadalafil for use in the treatment of erectile dysfunction.

- 10 According to a particular embodiment, the pharmaceutical combination product for use according to the invention comprises 250 U of abobotulinumtoxinA, and 100 mg of sildenafil for use in the treatment of erectile dysfunction.

- 15 According to a particular embodiment, the pharmaceutical combination product for use according to the invention comprises 100 U of onabotulinumtoxinA or incobotulinumtoxinA, and 5 mg of tadalafil administered once per day for use in the treatment of erectile dysfunction.

According to a particular embodiment, the pharmaceutical combination product for use according to the invention comprises 150 U of onabotulinumtoxinA or incobotulinumtoxinA, 100 mg sildenafil, and 20 µg PGE1 for use in the treatment of erectile dysfunction.

- 20 According to a particular embodiment, the pharmaceutical combination product for use according to the invention comprises 50 U or 100 U of onabotulinumtoxinA or incobotulinumtoxinA, and 15 to 25 mcg of PGE1, preferably 20 mcg of PGE1.

- 25 The pharmaceutical combination product of the invention, comprising botulinum toxin and at least one proerectile agent is particularly advantageous for use in the treatment of ED in subjects that insufficiently respond to one or more proerectile agents taken alone. Indeed, the inventors have surprisingly found that subjects that insufficiently respond to one or more proerectile agents (i.e. PGE1, PDE5i) responded to the pharmaceutical combination product of the invention, even in cases where the dose of proerectile agent (e.g. tadalafil, PGE1) was significantly reduced with respect to the dose of proerectile agent administered alone.

A further object of the present invention is a method for preventing or treating ED in a subject in need thereof comprising administering botulinum toxin and simultaneously, sequentially, or separately, administering at least one proerectile agent.

Accordingly, the method of the present invention encompasses the use of the
5 pharmaceutical combination product for treating ED based on the modalities described herein.

The term “subject” or “patient” is defined herein as an individual having ED, wherein said ED may be due, at least in part, to increased sympathetic contractile tone. According to a preferred embodiment, said subject having ED is diagnosed with a disease or a condition or
10 a personal situation (psychogenic ED) associated with increased sympathetic tone, such as those described previously. Preferably, said subject is an insufficient responder. More preferably, said insufficient responder insufficiently responds to treatment with a proerectile agent (i.e. PDE5i or PGE1 alone).

According to a preferred embodiment, botulinum toxin of said method is administered by
15 injection. More preferably, botulinum toxin of said method is administered to the corpora cavernosa. According to an even more preferred embodiment, at least one, preferably two, even more preferably three, injections are administered to each cavernous body.

According to preferred embodiment, botulinum toxin of said method is selected from among: short-acting, recombinant or native botulinum toxins of any serotype, more
20 preferably type A or type B botulinum toxin, even more preferably type A.

According to preferred embodiment, botulinum toxin of said method is administered no more than once in a 3-month to twelve-month period, preferably no more than once in a four, five, or six-month period.

According to a first preferred embodiment, the at least one proerectile agent of said
25 method is administered on demand (e.g. prior to sexual stimulation). According to a second preferred embodiment, proerectile agent of said method is administered once per day or every other day.

Preferably, the at least one proerectile agent of the said method is a PDE5i and/or PGE1.

Preferably, the PDE5i of the method is selected from avanafil, lodenafil, mirodenafil, sildenafil, tadalafil, vardenafil, udenafil, or a pharmaceutically acceptable salt thereof, even more preferably sildenafil citrate.

Preferably, when the at least one proerectile agent of the method is a PDE5i, it is
5 administered orally or subcutaneously.

Preferably, when the at least one proerectile agent of the method is PGE1, it is administered locally, even more preferably topically, intra-urethrally or intra-cavernosally. Preferably, when PGE1 is administered topically, it is administered to the tip of the penis.

According to a preferred embodiment, both botulinum toxin and PGE1 of the method are
10 administered intra-cavernosally.

According to a preferred embodiment, penile constriction, preferably using a penile loop ring, is performed prior to or after injection of botulinum toxin. According to a more preferred embodiment, penile constriction is performed prior to injection of botulinum toxin. Penile constriction preferably lasts for up to 30 minutes (e.g. for 5, 10, 15, 20, 25,
15 or 30 minutes), preferably for 30 minutes, following injection.

Preferably, said at least one proerectile drug, comprising at least PDE5i or PGE1 is administered at least one day to one week after administration of botulinum toxin.

A further object of the present invention is a method of administering botulinum toxin and at least one proerectile agent to a subject, said method comprising administering
20 botulinum toxin followed by administration of said at least one proerectile agent at least one day to one week after administration of botulinum toxin.

A further object of the present invention is a method for the treatment of erectile dysfunction, said method comprising the steps of:

- a. selecting a subject that is an insufficient responder,
- 25 b. administering botulinum toxin intra-cavernosally as described herein, and
- c. administering at least one proerectile agent as described herein, preferably via oral or intra-cavernosal administration.

According to a particular embodiment, a method for the treatment of erectile dysfunction
30 is provided herein, said method comprising the steps of:

- a. selecting a subject that is a proerectile agent insufficient responder,
- b. administering botulinum toxin serotype A by intra-cavernosal injection at two different sites, and
- c. orally or intra-cavernosally administering at least one proerectile agent after step b).

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Step c) of the above methods may be performed once or repeated several times. Thus, according to a preferred embodiment, step c) is repeated, preferably according to the modalities described herein.

According to a particular embodiment, the methods described herein further comprise recording information related to the effectiveness of the treatment. "Effectiveness of the treatment" may comprise any beneficial effect observed, such as an improvement in one or more of the following parameters: ability to get a penis hard enough for penetration, ability to maintain erection for the completion of sexual intercourse, obtention of a satisfactory sexual experience, hardness of erection, etc. As a non-limiting example, the subject may rate one or more of the above-mentioned parameters on a predetermined scale, and/or may provide a yes/no response to one or more questions. The subject may provide such information for recording. Preferably, the subject rates the hardness of erection according to the Erection Hardness Score (EHS) and/or provides answers to Sexual Encounter Profile (SEP) questions. The Erection Hardness Score (EHS) is a single-item Likert scale according to which hardness of an erection is rated on a scale from zero to four, as follows: 0 - Penis does not enlarge; 1 - Penis is larger, but not hard; 2 - Penis is hard, but not hard enough for penetration; 3 - Penis is hard enough for penetration, but not completely hard; 4 - Penis is completely hard and fully rigid.

The SEP is a log diary that is completed after each sexual attempt, comprising questions to which a "yes" or "no" response is provided, including:

Question 1: "Were you able to achieve at least some erection (some enlargement of the penis)?"

Question 2: "Were you able insert your penis into your partner's vagina?"

Question 3: "Did your erection last long enough for you to have successful intercourse?"

Question 4: "Were you satisfied with the hardness of your erection?"

Question 5: "Were you satisfied overall with this sexual experience?"

Preferably, the subject provides information concerning each sexual attempt. Preferably, the subject himself records information relating to the effectiveness of the treatment. Preferably, said subject records information relating to the effectiveness of the treatment on a first computer device. Preferably, information relating to the effectiveness of the treatment recorded on a first computer device is accessed on a second computer device.

As a non-limiting example, said first and/or second computer device may comprise a housing, a processor, a memory (e.g. one or more of a Flash memory, SRAM, ROM, DRAM, RAM, EPROM, dynamic storage), an input device, an indicating device (e.g. display), and/or an output device. The housing may be of any shape and of any size and may be configured for containing the processor and memory and may further be configured for containing or otherwise being associated with an input and a display device and/or a suitable power supply. The input device may be any suitable device capable of inputting data into the indicator device. For instance, the data input device may be a keyboard, a touch pad, such as on a display screen, or a receiver capable of receiving a wire or wireless transmission.

Said computer device may be portable. Said at least first device is configured to receive user (i.e. subject) information. Preferably, information relating to the effectiveness of the treatment is recorded into a software program that is configured to receive the information. Preferably, the method described herein further comprises storing the information regarding effectiveness of the treatment in a database. As a non-limiting example, said computer device may refer to a computer.

According to a further aspect of the invention, a computer system programmed to perform the steps of a computer-implemented method is provided herein, said method comprising:

- a. receiving information related to the effectiveness of a treatment method comprising the administration of an effective amount of botulinum toxin simultaneously, sequentially or separately to the administration of an effective amount of at least one proerectile agent in a subject;
- b. storing the information in a database and, optionally, transmitting the information to a medical practitioner.

The present invention has for further object a kit comprising at least the botulinum toxin of the present invention and preferably, a means for administration of said product.

Preferably, the kit comprises:

- at least the botulinum toxin of the invention, and
- optionally, a notice of use.

Preferably, the at least one proerectile agent is further comprised in the kit.

- 5 The present invention has for a further and more preferred object a kit comprising the botulinum toxin of the present invention, at least one proerectile agent and, preferably, a means for administration of said products.

Optionally, a means is provided for dispensing the botulinum toxin. The term “means” is defined herein as any device for local application, preferably within the corpora cavernosa, more preferably within each of the two cavernous bodies. Preferably, said “means” is a device for injection of botulinum toxin into the corpora cavernosa, such as a syringe and a needle, a pen injection device, or an auto-injector, said means preferably further comprising a constricting device, such as a rubber band, cuff, ring, loop, or any other appropriate constricting device known to the person skilled in the art.

15

FIGURE LEGENDS

Figure 1: Erectile function in spontaneously hypertensive rats (SHR) as compared to WKY rats.

- 20 Erectile responses elicited by electrical stimulation (ES) of the cavernous nerve (CN), at 6 V, 1 ms for 45 s, at increasing stimulation frequencies in anaesthetized SHRs, as compared to WKY rats. Erectile responses were expressed as $\Delta\text{ICP}/\text{MAP}$ (A) $\text{AUC}_{45}/\text{MAP}$ (B) or $\text{AUC}_{\text{tot}}/\text{MAP}$ (C). ICP is intra-cavernous pressure, MAP is basal mean arterial pressure during the plateau phase of the erectile response, AUC is area under the curve. ICP and MAP are expressed in mm Hg. ΔICP represents the difference between ICP in the flaccid state (i.e. before stimulation) and ICP during the plateau phase of the erectile response. AUC_{45} represents the AUC measured during the first 45 seconds after beginning electrical stimulation, while AUC_{tot} represents the AUC measured during the entire response.

- 30 **Figure 2: Baseline MAP values under anesthesia prior to the evaluation of erectile function.** Data are mean $\pm$ SEM. ** $p < 0.01$, Student's t-test.

Figure 3: Effect of intra-cavernosal injection of botulinum toxin on erectile function.

Erectile responses elicited by ES CN (6 V, 1 ms for 45 s) at increasing stimulation frequencies in anaesthetized SHRs that received either an intra-cavernosal (IC) injection of vehicle (saline) 7 days before erectile function evaluation followed by an intravenous (*iv*) injection of saline (Group I_vehicle, n=8) or acute sildenafil 0.3 mg/kg 4 minutes prior to
 5 erectile function evaluation (Group II_*iv* sild 0.3mg/kg, n=8) or an IC injection of 10 U onabotulinumtoxinA (BTX-A) 7 days before erectile function evaluation followed by an *iv* injection of saline 4 minutes prior to erectile function evaluation (Group III_BTX-A 10 U, n=8). Erectile responses were expressed as Δ ICP/MAP (A) AUC_{45}/MAP (B) or AUC_{tot}/MAP (C). Data represent mean values $\pm$ SEM. ns, non-significant; * $p < 0.05$; Two-way ANOVA.

10 **Figure 4: Effect of intra-cavernosal injection of botulinum toxin on erectile function when combined with the oral PDE5i sildenafil.**

Erectile responses elicited by ES CN (6 V, 1 ms for 45 s) at increasing stimulation frequencies (0, 2, 3, 4, 5, 7.5 and 10 Hz) in anaesthetized SHRs that received either an IC
 15 injection of vehicle (saline), 7 days before erectile function evaluation followed by an *iv* injection of saline (Group I vehicle, n=8) or acute sildenafil 0.3 mg/kg 4 minutes prior to erectile function evaluation (Group II *iv* sild 0.3 mg/kg, n=8), or an IC injection of 10 U BTX-A, 7 days before erectile function evaluation followed by an *iv* injection of either saline (Group III BTX-A 10 U, n=8), or acute sildenafil 0.3 mg/kg (Group IV BTX-A 10U+ *iv*
 20 sild 0.3mg/kg, n=7) 4 minutes prior to erectile function evaluation. Erectile responses were expressed as Δ ICP/MAP (A) AUC_{45}/MAP (B) or AUC_{tot}/MAP (C). Data represent mean values $\pm$ SEM. ns, non-significant; *** $p < 0.001$; ** $p < 0.01$, * $p < 0.05$, Two-way ANOVA.

25 **Figure 5: Synergistic effect of intra-cavernosal injection of botulinum toxin on erectile function when combined with the oral PDE5i sildenafil.**

The mean percent increase in erectile response elicited by ES CN (6 V, 1 ms for 45 s) at 10 Hz in anaesthetized SHRs that received an IC injection of vehicle (saline) 7 days before
 erectile function evaluation followed by an acute *iv* injection of sildenafil 0.3 mg/kg 4 minutes prior to erectile function evaluation (*iv* sild 0.3 mg/kg, n=8, corresponding to
 30 Group II), or an IC injection of 10 U BTX-A, 7 days before erectile function evaluation followed by an *iv* injection of either saline (BTX-A 10 U, n=8, corresponding to Group III), or acute sildenafil 0.3 mg/kg (BTX-A 10 U + *iv* sild 0.3 mg/kg, n=7, corresponding to Group IV) 4 minutes prior to erectile function evaluation are shown, with each group normalized against the control group in which the erectile response elicited by ES CN (6 V, 1 ms for 45

s) at 10 Hz was determined in anaesthetized SHRs that received an IC injection of saline 7 days before erectile function evaluation followed by an *iv* injection of saline (n=8, corresponding to Group I) (% increase vs control). The theoretical expected effect of the administration of botulinum toxin followed by sildenafil in view of results obtained with botulinum toxin alone (solid dark grey bars) and sildenafil alone (solid light grey bars) is shown (hatched bar), as is the actual effect observed with the administration of botulinum toxin followed by sildenafil (solid black bar), illustrating a surprising synergistic effect. The mean percent increase in erectile response was expressed as the mean percent increase in $\Delta\text{ICP}/\text{MAP}$ (A) $\text{AUC}_{45}/\text{MAP}$ (B) or $\text{AUC}_{\text{tot}}/\text{MAP}$ (C) in each treatment group (Groups II, III, IV) normalized against Group I.

Examples

The following examples are included to demonstrate preferred embodiments of the invention. All subject-matter set forth or shown in the following examples and accompanying drawings is to be interpreted as illustrative and not in a limiting sense. The following examples include any alternatives, equivalents, and modifications that may be determined by a person skilled in the art.

Example 1: Methodology

This preclinical study was designed to further illustrate that the combination of botulinum toxin and a proerectile agent is an effective and novel therapy for the treatment of ED, particularly in non-responders to the proerectile agent alone.

In this study, the effect of botulinum toxin was investigated in a well-established and clinically predictive model of ED associated to hypertension (HTN), the spontaneously hypertensive rat (SHR). Indeed, Behr-Roussel et al. first characterized ED and its pathophysiology in the SHR and clearly showed that SHRs display impaired erectile responses compared to their age-matched counterparts Wistar Kyoto (WKY) rats (Behr-Roussel et al. (2003) *Am J Physiol Regul Integr Comp Physiol* 284: R682-R688). Since then, this model has been widely used and several papers have shown that SHR with established HTN had ED compared with normotensive (WKY) rats (e.g. Fibbi et al., (2008) *J Androl* 29: 70-84). Indeed, as illustrated in Figure 1, SHR rats show a significant decrease in erectile response elicited by electrical stimulation of the cavernous nerve (ES CN), as compared to WKY rats.

Adult male SHR (from Janvier Labs, France) of 11 weeks of age were housed for at least 7 days prior to the beginning of the experiments with free access to standard chow and water and maintained on an inversed 12 h dark/light cycle (10:00 a.m./10:00 p.m.). All procedures were performed in accordance with legislation currently in force in France, and
 5 by the Animal Care Ethical Committee of the University of Versailles Saint-Quentin, France. This study included 32 rats randomized into 4 groups, as shown in Table 1 (below).

Table 1: Treatment groups

Group #	Treatment	Rat strain	Intra-cavernosal (IC) injection one week prior to ES CN	Intravenous (iv) injections 4 mins prior to ES CN	N
I	Vehicle	SHR	Saline	Saline	8
II	Sildenafil (sild) 0.3 mg/kg		Saline	Sildenafil 0.3 mg/kg	8
III	BTX-A*		BTX-A* 10 U	Saline	8
IV	BTX-A + sildenafil (sild) 0.3 mg/kg*		BTX-A* 10 U	Sildenafil 0.3 mg/kg	8

10 *BTX-A= onabotulinumtoxinA, N is the number of animals per group

After the 1-week acclimation period, each rat received one intra-cavernous (IC) injection of onabotulinumtoxinA (BTX-A, Botox®) at 10 U (groups III and IV) or normal saline (groups I and II). To perform the IC injection, both corpora cavernosa were visualized by gently
 15 pulling the glans to expose the penis and the tip of a 30-gauge needle was inserted into one corpus cavernosum. Correct implantation of the needle in the corpus cavernosum was confirmed before injection under microscope. Then, each rat received 10 U of BTX-A in 50 µl (50µl of a solution containing 200U of BTX-A per ml) or normal saline using a microperfusor at a rate of 15 µl/min.

20 One week post IC injection, erectile responses were elicited by ES CN, and measured by monitoring intra-cavernous pressure (ICP) in anesthetized rats according to a previously described and well-standardized experimental procedure (Giuliano et al., (1993) J Urol 150: 519-524, and Giuliano et al., (1995) J Auton Nerv Syst 55: 36-44).

Rats were anesthetized using a mixture of xylazine (10 mg/kg) and ketamine (90 mg/kg) and their temperature maintained at 37°C using a homeothermic blanket. Rats were tracheotomized to prevent aspiration of saliva, and when required to perform artificial ventilation. The carotid artery was catheterized with polyethylene tubing filled with
 5 heparinized saline (50 U/ml) to record blood pressure (BP) via a pressure transducer (Elcomatic 750, Glasgow, UK). Simultaneous computerized measure of BP and ICP were performed. The cavernous nerve (CN) was exposed at the lateral aspect of the prostate, with the aid of a dissecting microscope and mounted on a bipolar platinum electrode connected to an electrical stimulator (AMS 2100, Phymep, France).

10 At **T=0**, the continuous recording of BP and ICP was started. If diastolic BP was inferior to 40 mmHg during the first 5 minutes of recording, rats were not further evaluated.

At **T=5 min**, 2 stimulations of the CN at 6V, 10Hz, 1 ms for 45 s were performed to elicit an increase of ICP to verify correct catheter implantation.

At **T+16 min**, sildenafil (0.3 mg/kg) or vehicle (saline) was intravenously injected. This
 15 dose of sildenafil has previously been demonstrated to ameliorate erectile function in anesthetized rats.

At **T+20 min**, and at **3 minute intervals** thereafter, the CN was stimulated at 6V, 1 ms for 45 s by different stimulation frequencies (0, 2, 3, 4, 5, 7.5 and 10 Hz) in a randomized manner in order to assess the erectile response. These different electrical stimulations
 20 were repeated twice in view of establishing a frequency-response curve for each animal.

At **T+60 min**, the recording ended.

Following the *in vivo* erectile function evaluation, rats were euthanized.

Example 2: Experimental endpoints and statistical analyses

25 Erectile responses elicited by each ES CN were quantified for each rat and averaged for each experimental group by calculating:

- $\Delta\text{ICP (mmHg)} / \text{MAP (mmHg)} \times 100$ with ΔICP being the difference between ICP in the flaccid state, i.e. before ES CN and ICP during the plateau phase of the erectile response. MAP is the mean arterial pressure during the plateau phase.
- 30 • $\text{AUC}_{45} / \text{MAP}$ with AUC_{45} being the area under the ICP-time curve during the 45 s electrical stimulation period. AUC_{45} was determined using the ICP level in the flaccid state prior to the onset of ES CN

- AUC_{tot} / MAP with AUC_{tot} being the area under the curve during the entire erectile response, measured from the beginning of the electrical stimulation until the end of the erectile response and determined using the ICP level in the flaccid state prior to the onset of ES CN.

5

ICP increase and AUC were normalized with MAP to account for the influence of the systemic blood pressure on the amplitude of ICP increase during the plateau phase of the erectile response (Giuliano et al., (1993) J Urol 150: 519-524).

All results are presented as mean $\pm$ SEM. Grubbs test was used for the detection and exclusion of outliers. This statistical method allowed determining when a value is unlikely to have come from the same Gaussian population as the other values in the group.

Comparisons of mean MAP were performed using a Student's t-test and comparisons of frequency-response curves were performed with a two-way ANOVA statistical analysis test. Statistical analyses were performed with GraphPad Prism® 6.05 software. P values < 0.05 are considered significant.

Example 3: Summary of Events

No unexpected events occurred during the transportation/acclimatization period. The occurrence of experimental hazards following surgical procedure/anesthesia during the *in vivo* evaluation of the erectile function (1 rat from group 4) was in accordance with standard practice in the laboratory and not specific to any experimental group. Consequently, 8 rats of groups I, II and IV and 7 rats of group III were included in the final analysis.

Of note, body weights were recorded before starting the treatment period and daily following post intra-cavernous (IC) injection. All rats were also weighed just before the *in vivo* evaluation of erectile function.

Overall, the rats that received an IC injection of BTX-A at 10 U showed a 19% loss in body weight 7 days post-injection as compared to rats that received a saline injection.

Example 4: Baseline MAP under anesthesia

At the time of *in vivo* experiments and after anesthesia, basal mean arterial pressure (MAP) was measured for each rat before electrical stimulation of the cavernous nerve began. The baseline MAP under anesthesia was significantly lower in rats that received IC injection of BTX-A at 10 U compared to vehicle-injected rats (Figure 2).

Example 5: Effect of administration of botulinum toxin or a proerectile drug alone on erectile response *in vivo*.

The SHRs that received a single IC injection of BTX-A at 10 U showed a significant increase in the erectile responses elicited by ES CN at increasing frequencies, as compared to SHRs that received an IC injection of saline in terms of $\Delta\text{ICP}/\text{MAP}$ (Figure 3A) and $\text{AUC}_{45}/\text{MAP}$ (Figure 3B).

Likewise, acute *iv* sildenafil at 0.3 mg/kg administered 4 minutes prior to erectile function evaluation significantly improved the erectile responses in terms of $\Delta\text{ICP}/\text{MAP}$ (Figure 3A) and $\text{AUC}_{45}/\text{MAP}$ (Figure 3B) in SHRs when compared to responses recorded in SHRs that received an IV injection of saline. Rats from both groups received an IC injection of saline 7 days before the erectile function evaluation.

In both cases, i.e., following BTX-A or sildenafil alone, this improvement was not statistically significant as compared to vehicle-treated SHRs for $\text{AUC}_{\text{tot}}/\text{MAP}$.

Example 6: Effect of the combination treatment of botulinum toxin with a proerectile drug on erectile response *in vivo*.

Surprisingly, IC injection of BTX-A at 10 U administered 7 days before erectile function evaluation, significantly potentiated erectile responses of SHRs treated with acute *iv* sildenafil 4 minutes prior to the erectile function evaluation.

Indeed, SHRs who received the combination therapy i.e., IC injection of BTX-A at 10 U, 7 days before the erectile function evaluation combined with acute *iv* sildenafil at 0.3 mg/kg administered 4 minutes prior to erectile function evaluation, showed an increase of 19% ($p<0.05$) in $\Delta\text{ICP}/\text{MAP}$ (Figure 4A), 17% ($p<0.05$) in $\text{AUC}_{45}/\text{MAP}$ (Figure 4B) and 33% ($p<0.001$) in $\text{AUC}_{\text{tot}}/\text{MAP}$ (Figure 4C) at 10Hz, as compared to SHRs treated with sildenafil alone and that received an IC injection of saline.

Surprisingly, SHRs that received the combination therapy also presented a significant increase in erectile responses in terms of $\Delta\text{ICP}/\text{MAP}$ (Figure 4A) and $\text{AUC}_{\text{tot}}/\text{MAP}$ (Figure 4C) compared to SHRs that received BTX-A alone. This increase was not statistically significant in terms of $\text{AUC}_{45}/\text{MAP}$ (Figure 4B).

These data are further summarized in Table 2 (below).

Table 2: Summary of treatment effect on erectile response

Comparison of groups	Quantification of erectile responses elicited by each ES CN		
	$\Delta\text{ICP}/\text{MAP}$ (%)	$\text{AUC}_{45}/\text{MAP}$ (s^{-1})	$\text{AUC}_{\text{tot}}/\text{MAP}$ (s^{-1})
II vs I	+13%, *	+15%, *	+13%, ns (p=0.2)
III vs I	+12%, *	+15%, *	+9%, ns (p=0.051)
IV vs I	+35%, ***	+35%, ***	+50%, ***
III vs II	+0%, ns	+0%, ns	+0%, ns
IV vs II	+19%, *	+17%, *	+33%, ***
IV vs III	+21%, *	+17%, ns (p=0.08)	+37%, ***

Erectile responses elicited by ES CN at 10 Hz were quantified as detailed in Example 2, and the difference in response between treatment groups was determined (expressed as % difference in response). ns, non-significant; ***p<0.001; **p<0.01, *p<0.05 by two-way ANOVA.

- 5 As indicated above and as illustrated in Figures 3 and 4, either a single IC injection of BTX-A or an acute *iv* injection of sildenafil slightly but significantly improved the erectile responses elicited by ES CN in SHR. However, the combination effect of BTX-A and sildenafil surprisingly shows an even greater effect.

Indeed, these results clearly show that IC injection of botulinum toxin (e.g. BTX-A)
 10 significantly potentiates the erectile response in SHRs treated with the oral PDE5i, sildenafil.

As is further illustrated in Figure 5, a synergistic effect on erectile response was clearly observed when BTX-A and the proerectile agent (in this case sildenafil) were administered sequentially. Indeed, the extent of the effect of the pharmaceutical combination product
 15 comprising BTX-A and the proerectile agent is surprisingly greater than the expected additive effect of BTX-A and sildenafil for each parameter (i.e. $\Delta\text{ICP}/\text{MAP}$ or $\text{AUC}_{45}/\text{MAP}$), and is even greater for the $\text{AUC}_{\text{tot}}/\text{MAP}$ (see Fig. 5c). As $\text{AUC}_{\text{tot}}/\text{MAP}$ depicts the full erectile response (i.e. both the amplitude of the erectile response (ICP/MAP) and the maintenance of the erectile response beyond ES CN including the detumescence phase), this parameter
 20 particularly illustrates the striking synergistic response observed by the sequential combination of BTX-A and a proerectile agent.

Example 7: Clinical evaluation of the effect of the combination of botulinum toxin and one or more proerectile drugs

13 male patients between the ages of 24 and 75 with ED with insufficient response to proerectile drugs were treated with a combination of intra-cavernosal injections of botulinum toxin and one or more proerectile drugs. An informed consent was obtained from all patients prior to treatment. In each case, treatment comprised the administration of
5 botulinum toxin.

7.1 Determination of the International Index of Erectile Function - Erectile Function (IIEF-EF) domain score

To assess the efficacy of the combination of botulinum toxin with one or more proerectile drugs (e.g. botulinum toxin A with a PDE5i administered either on demand or daily (i.e. once per day) or with PGE1 (IC injections)), we have used the Erectile Function domain score of the International Index of Erectile Function (IIEF-EF) comprising 6 questions (maximum score: 30) (Rosen et al., Int J Impot Res. 2002;14(4):226-44) The IIEF-EF domain score has been used to assess the Minimal Clinically Important Difference (MCID) according to ED severity. Baseline ED severity is as follows: No ED (normal erectile function): IIEF-EF
10 score of 26-30; Mild ED: IIEF-EF score of 22-25; moderate ED: IIEF-EF score of 11-21; severe ED: IIEF-EF score of 6-10. MCIDs are 2, 5, and 7 for patients with mild, moderate, and severe baseline ED, respectively. (Rosen et al Eur. Urol. 2011;60(5):1010-6). Thus, as an example, a patient with moderate ED (i.e. IIEF-EF score of 11-21) must show at least a 5-point improvement in the IIEF-EF score to be considered as having a clinical improvement
15 and therefore sufficiently respond to treatment, while a patient with severe ED (i.e. IIEF-EF score of 6-10) must show at least a 7-point improvement in the IIEF-EF score to be considered as having a clinical improvement and therefore sufficiently respond to treatment.
20

This method allowed us to evaluate the effect of the pharmaceutical combination product on ED in terms of clinically relevant improvement.
25

7.2 Clinical evaluation of the effect of the administration of the combination of botulinum toxin followed by a PDE5i

Patient 1 (MO... E), was a 60-year-old male, with ED for 3.5 years, and treated with 5 mg of tadalafil daily. Patient 1 had an asymptomatic Steinert myopathy and was diagnosed with
30 sleep apnea as a co-morbid condition for ED (thus ED of mainly organic etiology). He did not have any cardiovascular risk factors for ED. Over time he became an insufficient

responder to 5 mg of tadalafil daily, having an IIEF-EF domain score of 17 with this treatment. He received an intra-cavernosal injection of 50 U of onabotulinumtoxinA in the right corpus cavernosum and 50 U of onabotulinumtoxinA in the left corpus cavernosum. Prior to the injections, a loop ring was placed around the penile crus and left for 30 minutes following the injections. The patient continued to take 5 mg tadalafil and reported an immediate and significant improvement. Four weeks after IC injection of the botulinum toxin, the IIEF-ED domain score increased to 28 with the patient continuing to take 5 mg of tadalafil daily.

The pharmaceutical combination product comprising botulinum toxin and 5 mg tadalafil daily surprisingly enabled the patient having moderate ED of mainly organic etiology to sufficiently respond.

Patient 2 (BA... G) was a 59-year-old male with type 2 diabetes, having had a stroke responsible for hemiplegia 5 months prior to his first visit for ED. He was treated for arterial hypertension by diuretics and by a selective serotonin reuptake inhibitor. He was an insufficient responder to 100 mg sildenafil on demand (IIEF-EF domain score of 15, corresponding to moderate ED). He received an intra-cavernosal injection of 50 U of onabotulinumtoxinA in the right corpus cavernosum and 50 U of onabotulinumtoxinA in the left corpus cavernosum. Prior to the injections, a loop ring was placed around the penile crus and left for 30 minutes following the injections. Ten weeks after the onabotulinumtoxinA intra-cavernosal injections, the IIEF-EF domain score had increased to 28 with 5 mg tadalafil administered daily.

The pharmaceutical combination product comprising botulinum toxin and 5 mg tadalafil daily surprisingly enabled the patient having moderate ED of mainly organic etiology (diabetes and a severe cardio-vascular condition) to sufficiently respond.

Patient 3 (DI...D) was a 28-year-old male, initially complaining of primary ED. The only medical event was a 60 kg weight loss due to morbid obesity. Patient 3 self-administered 5 µg of PGE1 for 2.5 years on demand via intra-cavernosal injection. The efficacy of different PDE5 inhibitors was insufficient. With 100 mg sildenafil on demand, for example, the patient's IIEF-EF domain score was 12 (moderate ED). Patient 3 received an intra-cavernosal injection of 50 U of onabotulinumtoxinA in the right corpus cavernosum and 50 U of onabotulinumtoxinA in the left corpus cavernosum. Prior to the injections, a loop ring

was placed around the penile crus and left for 30 minutes following the injections. One month later the IIEF-EF domain score increased to 27 with 100 mg sildenafil on demand.

The pharmaceutical combination product comprising botulinum toxin and 100 mg sildenafil surprisingly enabled the patient with moderate ED insufficiently responding to PGE1 to sufficiently respond.

Patient 4 (RO... J) was a 75-year-old male with no cardiovascular risk factors or disease. As ED co-morbidity, this patient had sleep apnea treated by the use of continuous positive airway pressure. He complained of decreased libido and of mainly organic ED. Patient 4 was initially treated with androgen replacement therapy and 5 mg tadalafil daily, and later with 20 mg tadalafil on demand. The drug caused side effects (headache), and the patient was an insufficient responder with an inability to penetrate. He was then treated with 200 mg of avanafil on demand, with improved tolerability, but again with poor efficacy (IIEF-EF domain score was 5 with 200 mg avanafil on demand, corresponding to severe ED). He received an intra-cavernosal injection of 50 U of onabotulinumtoxinA in the right corpus cavernosum and 50 U of onabotulinumtoxinA in the left corpus cavernosum. Prior to the injections, a loop ring was placed around the penile crus and left for 30 minutes following the injections. Two months later with 200 mg avanafil on demand, he was able to perform sexual intercourse with vaginal penetration twice and the IIEF-EF domain score increased to 17 (moderate ED).

The pharmaceutical combination product comprising botulinum toxin and a PDE5i surprisingly allows patients having non-vascular ED to sufficiently respond.

Patient 5 (NI... M) was a 32-year old male with an L1 AIS A C spinal cord lesion due to an injury sustained in a road accident two years earlier. He complained of neurogenic ED. Neurogenic detrusor activity was managed with intradetrusor onabotulinumtoxin A injections (200 U) and self-intermittent catheterizations. He was an insufficient responder to 20 mg tadalafil on demand (IIEF-EF domain score of 17 corresponding to moderate ED). He did not have any other risk factors or identified causes of ED, including vascular etiology, apart from the spinal cord lesion. He received an intra-cavernosal injection of 50 U of onabotulinumtoxin A in the right corpus cavernosum and 50 U of onabotulinumtoxin A in the left corpus cavernosum. Prior to the injections, a loop ring was placed around the

penile crus and left for 30 minutes following the injections. Thirteen weeks later, with only 10 mg tadalafil on demand, the IIEF-EF domain score increased to 27.

The pharmaceutical combination product comprising botulinum toxin and tadalafil on demand surprisingly enabled the patient to reduce the required dose of PDE5i (in this case tadalafil) by 50% (i.e. dose reduced from 20 mg to 10 mg) in addition to sufficiently responding to treatment. These results clearly indicate that the pharmaceutical combination product comprising botulinum toxin in combination with at least one proerectile agent, in this case the PDE5i tadalafil, has a synergistic effect.

7.3 Clinical evaluation of the effect of the administration of the combination of botulinum toxin followed by PGE1

Patient 6 (PO... D) was a 70-year-old male with ED for 9 years. As ED co-morbidities, Patient 6 was treated for arterial hypertension and later used nightly continuous positive airway pressure for sleep apnea (thus this patient had vascular ED). Over time he became an insufficient responder to 100 mg sildenafil on demand and 7 year earlier was switched to on demand intra-cavernosal injections of 60 µg PGE1 taken in combination with 100 mg sildenafil to obtain rigid erection for vaginal penetration. With this on demand treatment IIEF-EF domain score was 22 corresponding to mild ED. He received an intra-cavernosal injection of 50 U of onabotulinumtoxinA in the right corpus cavernosum and 50 U of onabotulinumtoxinA in the left corpus cavernosum. Prior to the injections, a loop ring was placed around the penile crus and left for 30 minutes following the injections. One month later he was able to experience fully rigid erection with only 20 µg of PGE1, and no longer had any need for additional sildenafil. The IIEF-EF domain score increased to 30.

Patient 7 (PA...A) was a 75-year-old male with ED for 10 years and the following risk factors: dyslipidemia and arterial hypertension. The patient was initially treated with PDE5i and then by intra-cavernosal injections of PGE1 at increasing doses from 20 µg up to 60 µg. He then underwent a radical prostatectomy for a pT3a N0 R0 prostate cancer. Four months after the surgical procedure he received intra-cavernosal delivery of 25 U of onabotulinumtoxinA in each corpus cavernosum (50 U total). Prior to the injections, a loop ring was placed around the penile crus and left for 30 minutes following the injections. One month after injection, the patient reported improved penile erection following intra-cavernosal delivery of only 20 µg of PGE1.

Patient 8 (WA... T) was a 58-year-old male with no cardiovascular risk factors or conditions, with psychogenic ED related to a recent widowhood. He was able to obtain a rigid erection for vaginal intercourse with intra-cavernosal administration of 60 µg of PGE1. With this high dose he had an IIEF-EF domain score of 19 (moderate ED). He received an intra-cavernosal injection of 50 U of onabotulinumtoxinA in the right corpus cavernosum and 50 U of onabotulinumtoxinA in the left corpus cavernosum. Prior to the injections, a loop ring was placed around the penile crus and left for 30 minutes following the injections. Five weeks after intra-cavernosal injection of onabotulinumtoxinA, he was able to decrease the posology of PGE1 to 20 µg. The IIEF-EF domain score increased up to 29.

The pharmaceutical combination product comprising botulinum toxin and 20 µg PGE1 on demand surprisingly allowed insufficiently responding patients to respond and furthermore surprisingly enabled patients to reduce the required dose of PGE1 (e.g. dose reduced from 40-60 µg to 20 µg). These results further indicate that the administration of botulinum toxin in combination with a proerectile agent, in this case PGE1, has a synergistic effect.

7.4 Clinical evaluation of the effect of the administration of the combination of botulinum toxin followed by two proerectile agents

Patient 9 (LO... N) was a 48-year-old male who experienced a myocardial infarction eight years earlier, in addition to paraplegia T3 AIS A, also 8 years earlier. Patient 9 had severe ED from both vascular and neurogenic origin and was an insufficient responder to a combination of 20 µg PGE1 administered by intra-cavernosal injection and 100 mg sildenafil on demand (IIEF-EF domain score of 6). He received an intra-cavernosal injection of 75 U of onabotulinumtoxinA in the right corpus cavernosum and 75 U of onabotulinumtoxinA in the left corpus cavernosum. Prior to the injections, a loop ring was placed around the penile crus and left for 30 minutes following the injections. Six weeks later the IIEF-EF domain score was significantly improved with an increase to 12 with the combination of 20 µg PGE1 administered by intra-cavernosal injection and 100 mg sildenafil on demand. This improvement was confirmed by the patient's partner.

The pharmaceutical combination product comprising botulinum toxin, in this case 150 U, and the proerectile agents sildenafil and PGE1 surprisingly improved ED (improvement from severe to moderate ED).

7.5 Clinical evaluation of the effect of the administration of the combination of botulinum toxin followed by a proerectile agent, wherein said botulinum toxin is abobotulinumtoxinA

Patient 10 (MA... B) was a 44-year old male who had had a stroke 18 months earlier with
5 sequelar hemiparesia. He reported ED after the stroke, thus ED was of vascular origin. He
was an insufficient responder to 100 mg sildenafil on demand, despite multiple attempts
(IIEF-EF domain score was 18 with 100 mg sildenafil on demand, corresponding to moderate
ED). He received an intra-cavernosal injection of 125 U of abobotulinumtoxinA in the right
corpus cavernosum and 125 U of abobotulinumtoxinA in the left corpus cavernosum. Prior
10 to the injections, a loop ring was placed around the penile crus and left for 30 minutes
following the injections. Six days later, he reported significant improvement in the effect
of 100 mg sildenafil on demand, and his IIEF-EF domain score increased to 21 (mild ED).

Patient 11 (PR... B) was a 37-year old male with a spinabifida T10 AIS A. He initially used
intra-cavernosal injections of 20 µg PGE1 on demand, and later switched to 100 mg
15 sildenafil on demand. The IIEF-EF domain score was 14 (moderate ED). He then received
an intra-cavernosal injection of 125 U of abobotulinumtoxinA in the right corpus
cavernosum and 125 U of abobotulinumtoxinA in the left corpus cavernosum. Prior to the
injections, a loop ring was placed around the penile crus and left for 30 minutes following
the injections. Four weeks later the IIEF-EF domain score increased to 21 (mild ED) with
20 100 mg sildenafil on demand.

These results demonstrate that the surprising effect of the pharmaceutical combination
product comprising botulinum toxin and at least one proerectile agent is not limited to
onabotulinumtoxinA, but that the pharmaceutical combination product comprising other
botulinum toxins (i.e. abobotulinumtoxinA) also has the same surprising effect, allowing
25 patients to sufficiently respond.

7.6 Clinical evaluation of the duration of the effect of the administration of the combination of botulinum toxin followed by a proerectile agent

Patient 12 (DA... Y) was a 43-year-old male diagnosed with Type 2 diabetes 8 years earlier,
who became an L3 ASIA A paraplegic after a traumatic spinal cord injury, with ED of both
30 diabetic and neurogenic origins. He was an insufficient responder to 100 mg sildenafil on
demand. He was then switched to self-administered intra-cavernosal injections of 20 µg

- PGE1 approximately 3 times per week for three months, to which he responded. He then received an intra-cavernosal injection of 25 U of onabotulinumtoxinA in the right corpus cavernosum and 25 U of onabotulinumtoxinA in the left corpus cavernosum. Prior to the injections, a loop ring was placed around the penile crus and left for 30 minutes following the injections. He was then able to respond to 100 mg sildenafil on demand. The efficacy of 100 mg sildenafil on demand decreased over time with the patient complaining of an insufficient response 11 months following onabotulinumtoxinA injection. The IIEF-EF domain score was 17 (moderate ED) with 100 mg sildenafil on demand prior to a second intra-cavernosal injection of 100 U onabotulinumtoxinA (50 U of onabotulinumtoxinA in the right corpus cavernosum and 50 U of onabotulinumtoxinA in the left corpus cavernosum). Prior to the injections, a loop ring was placed around the penile crus and left for 30 minutes following the injections. Six weeks following this second injection, the IIEF-EF domain score increased to 21 (moderate ED). The patient reported increased efficacy when compared to the injection of 50 U of onabotulinumtoxinA one year earlier.
- 15 Patient 13 (CH... T) was a 27-year-old male with tetraplegia due to spinal cord injury C6 AIS A occurring seven years earlier and complaining of neurogenic ED. He was an insufficient responder to 100 mg sildenafil on demand. He had no other risk factors or identified causes of ED, including vascular ED, apart from the spinal cord lesion. He received an intra-cavernosal injection of 50 U of onabotulinumtoxinA in the right corpus cavernosum and 50 U of onabotulinumtoxinA in the left corpus cavernosum. Prior to the injections, a loop ring was placed around the penile crus and left for 30 minutes following the injections. Prior to the intra-cavernosal treatment with onabotulinumtoxinA, with 100 mg sildenafil on demand, the IIEF-EF domain score was 16 (moderate ED). One month later he reported a significant improvement of the efficacy of 100 mg sildenafil on demand. Five months later, the IIEF-EF domain score had increased to 29 with 100 mg sildenafil on demand.

These results demonstrate that the pharmaceutical combination product comprising botulinum toxin and at least one proerectile agent surprisingly has long-term effects (e.g. for at least five to eleven months), during which patients sufficiently responded to treatment.

- 30 None of these patients have reported any undesired side effects following intra-cavernosal delivery of onabotulinumtoxinA or abobotulinumtoxinA comprised in the pharmaceutical combination product.

Claims

1. Pharmaceutical combination product comprising botulinum toxin and at least one proerectile agent for simultaneous, sequential or separate use in the treatment of erectile dysfunction.
- 5 2. Pharmaceutical combination product according to claim 1, wherein the botulinum toxin is selected from among: short-acting, recombinant or native botulinum toxins of any serotype.
- 10 3. Pharmaceutical combination product according to claim 1 or 2 wherein the botulinum toxin is administered by injection.
4. Pharmaceutical combination product according to claim 3, wherein the botulinum toxin is injected in the corpora cavernosa.
- 15 5. Pharmaceutical combination product according to claim 4, wherein at least one injection, preferably at least two injections, is/are performed in each cavernous body.
6. Pharmaceutical combination product according to any of the preceding claims, wherein the botulinum toxin is type A or B.
- 20 7. Pharmaceutical combination product according to claim 6, wherein the type A botulinum toxin is selected from onabotulinumtoxinA, abobotulinumtoxinA, and incobotulinumtoxinA.
- 25 8. Pharmaceutical combination product according to any of the preceding claims, wherein the botulinum toxin is administered no more than once in a 3-to-12 month period.
9. Pharmaceutical combination product according to any of the preceding claims, wherein said at least one proerectile agent is a phosphodiesterase type five (PDE5) inhibitor or prostaglandin E1 (PGE1).
- 30

10. Pharmaceutical combination product according to claim 9, wherein the PDE5 inhibitor is selected from avanafil, lodenafil, mirodenafil, sildenafil, tadalafil, vardenafil, udenafil, or a pharmaceutically acceptable salt thereof.
- 5 11. Pharmaceutical combination product according to claim 10, wherein the PDE5 inhibitor is sildenafil citrate.
12. Pharmaceutical combination product according to claim 10 or 11, wherein said PDE5 inhibitor is administered on demand, or, when said PDE5 inhibitor is tadalafil, once per day
10 or every other day.
13. Pharmaceutical combination product according to any of the claims 9 to 12, wherein the PDE5 inhibitor is administered orally.
- 15 14. Pharmaceutical combination product according to claim 9, wherein PGE1 is administered locally, preferably wherein PGE1 is administered topically, intra-urethrally or intra-cavernosally.
15. Pharmaceutical combination product according to any of claims 1 to 9 and 12,
20 wherein both botulinum toxin and PGE1 are administered intra-cavernosally.
16. Pharmaceutical combination product according to any of the preceding claims, for use in the treatment of insufficient responders.
- 25 17. A method for the treatment of erectile dysfunction, the method comprising administering an effective amount of botulinum toxin simultaneously, sequentially or separately to the administration of an effective amount of at least one proerectile agent.
18. The method of claim 17, for the treatment of erectile dysfunction in proerectile
30 agent insufficient responders.
19. A method for the treatment of erectile dysfunction, said method comprising the steps of:

- a. selecting a subject that is a proerectile agent insufficient responder;
- b. administering botulinum toxin serotype A by intra-cavernosal injection at two different sites, and
- c. orally or intra-cavernosally administering at least one proerectile agent after
5 step b).

20. The method of any one of claims 17 to 19, further comprising a subject recording information related to the effectiveness of the treatment, preferably on a first computer device.

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21. The method of claim 20, wherein the information related to the effectiveness of the treatment is recorded on a first computer device and is accessed on a second computer device.

15 22. The method of claim 20 or 21, wherein the information related to the effectiveness of the treatment is recorded into a software program that is configured to receive the information.

20 23. The method of any one of claims 20 to 22, further comprising storing the information related to the effectiveness of the treatment in a database.

24. A computer system programmed to perform steps of a computer-implemented method, said method comprising:

- a. receiving information related to effectiveness of a treatment method comprising
25 the administration of an effective amount of botulinum toxin simultaneously, sequentially or separately to the administration of an effective amount of at least one proerectile agent in a subject;
- b. storing the information in a database and transmitting the information to a medical practitioner.

30

Fig. 1a

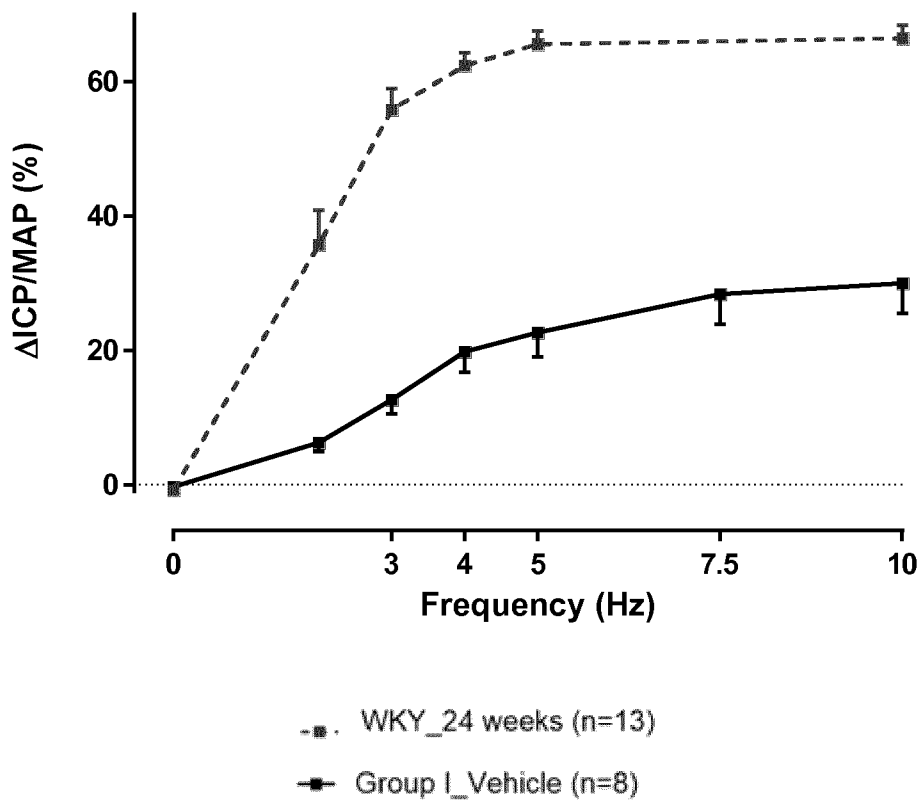


Fig. 1b

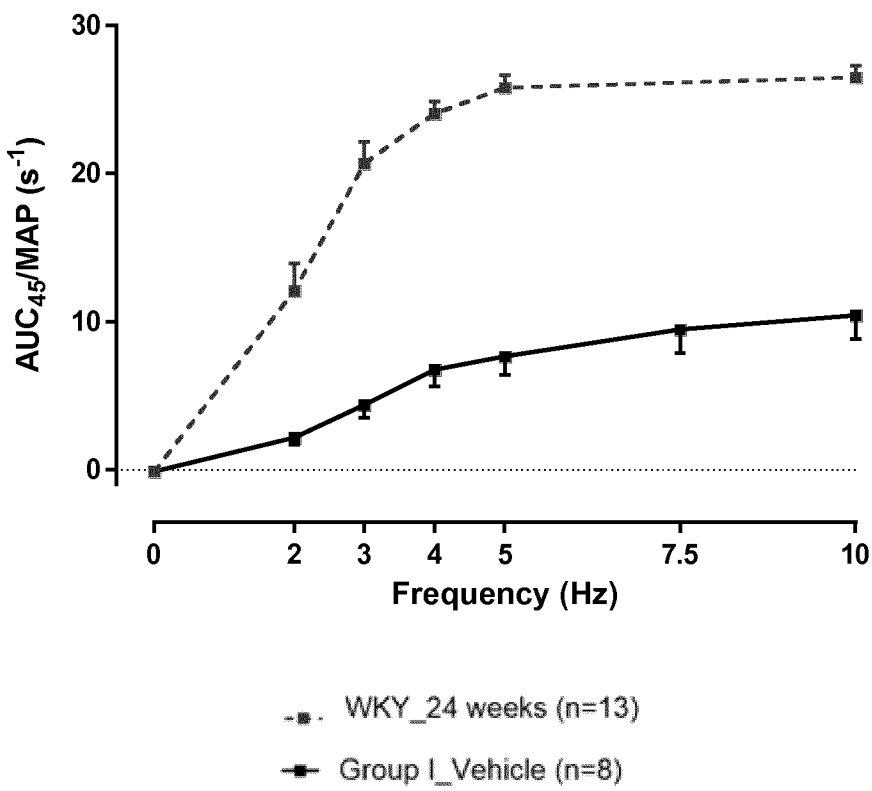


Fig. 1c

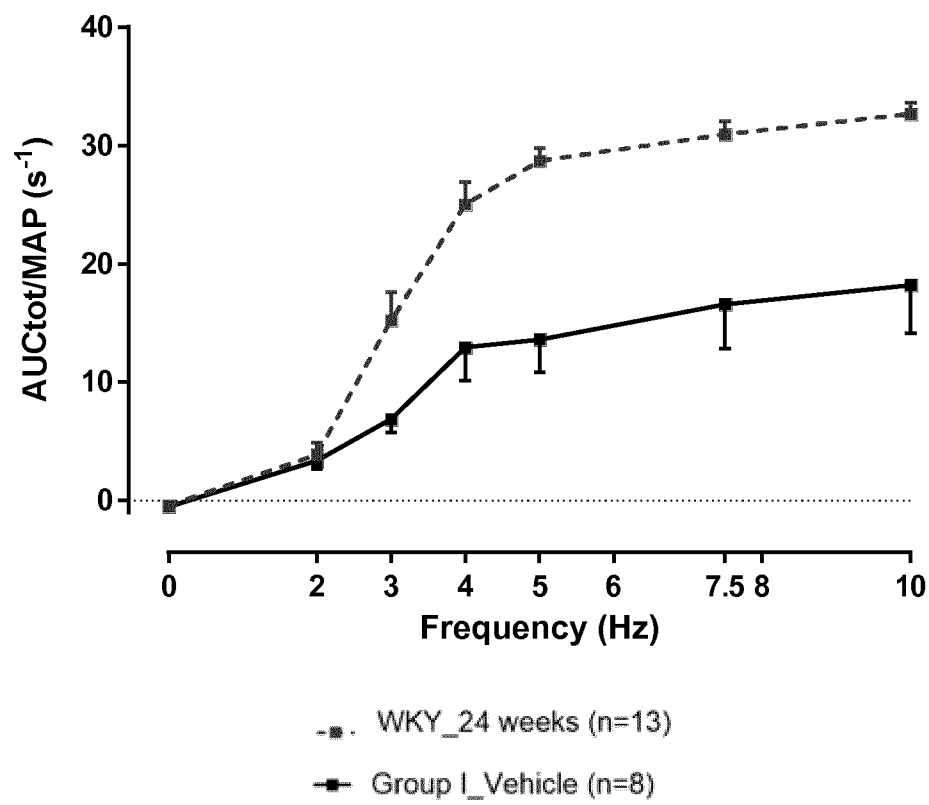


Fig. 2

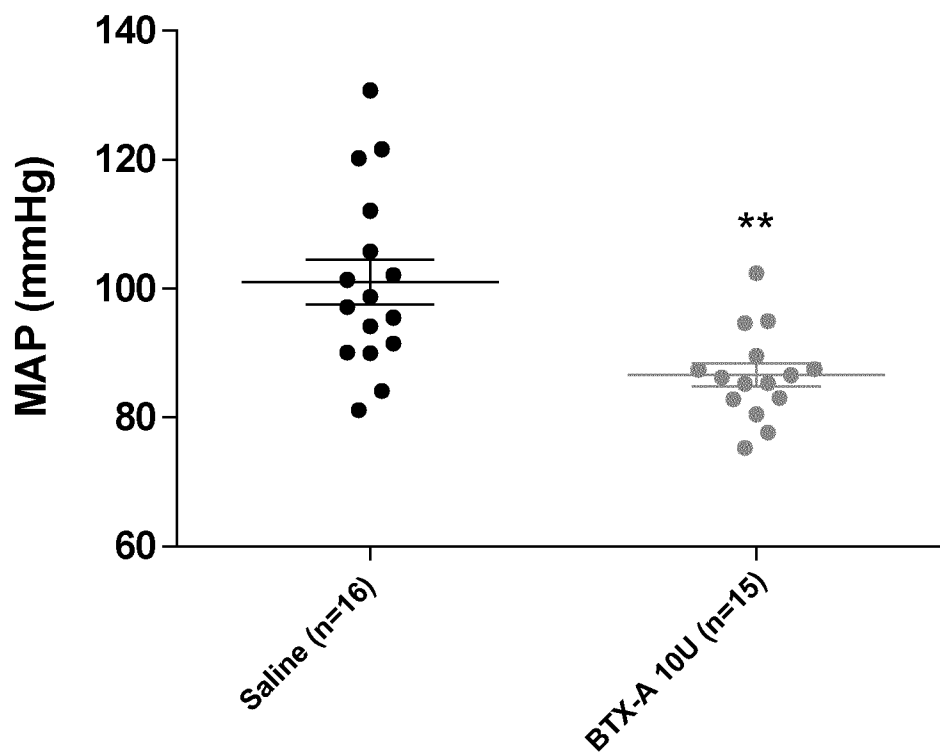


Fig. 3a

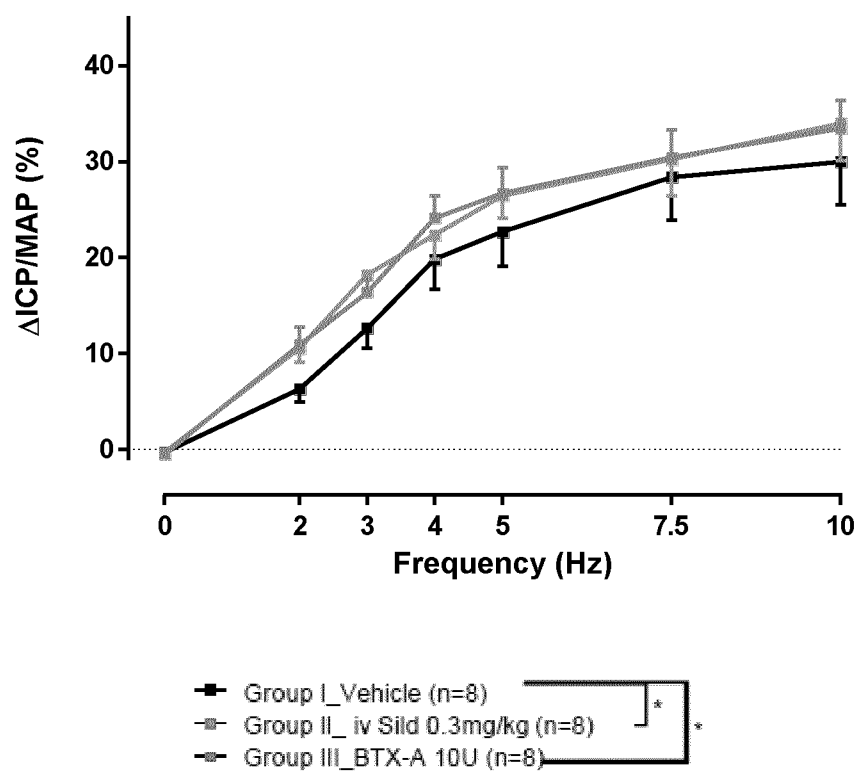


Fig. 3b

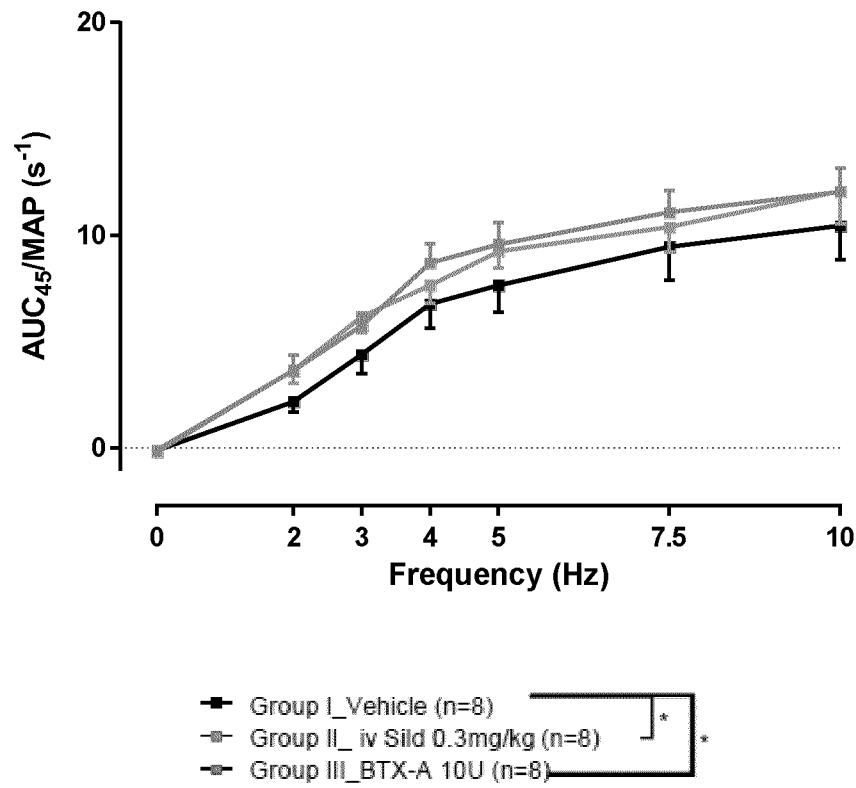


Fig. 3c

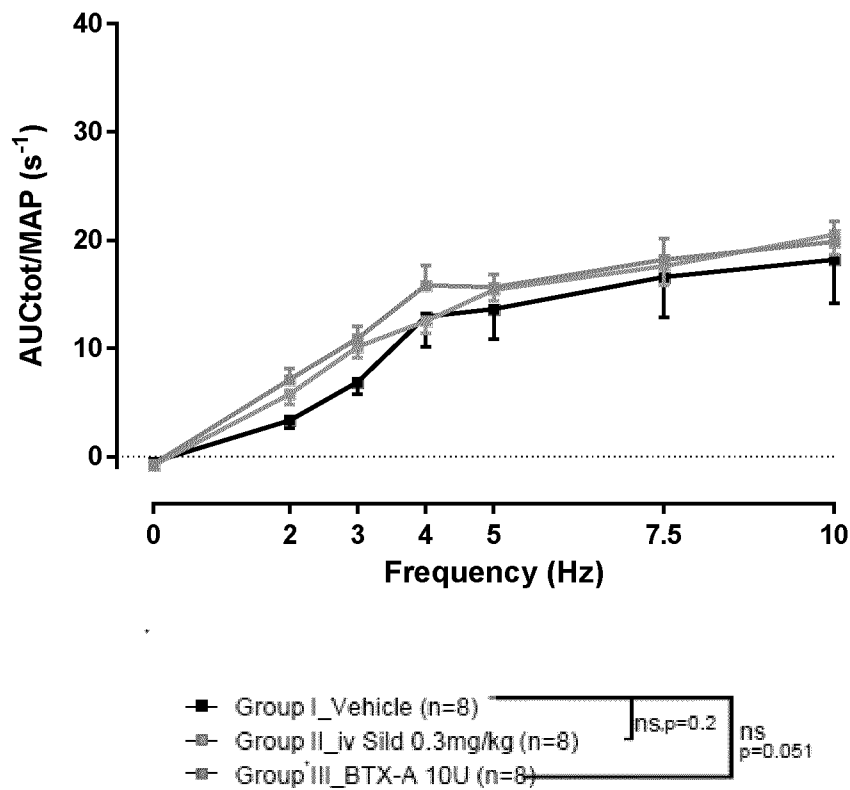


Fig. 4a

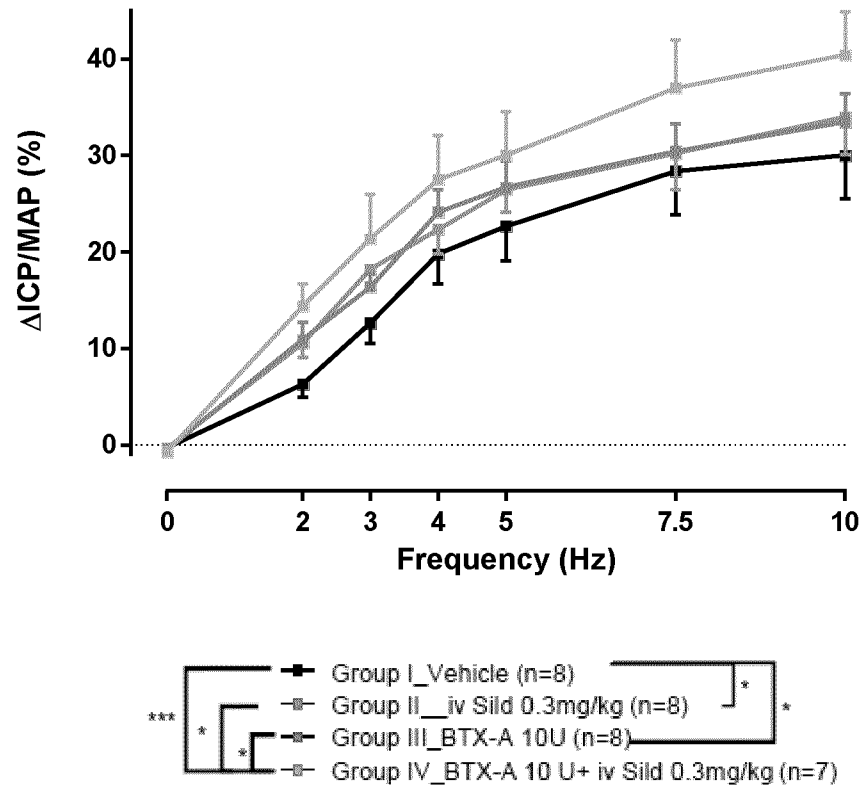


Fig. 4b

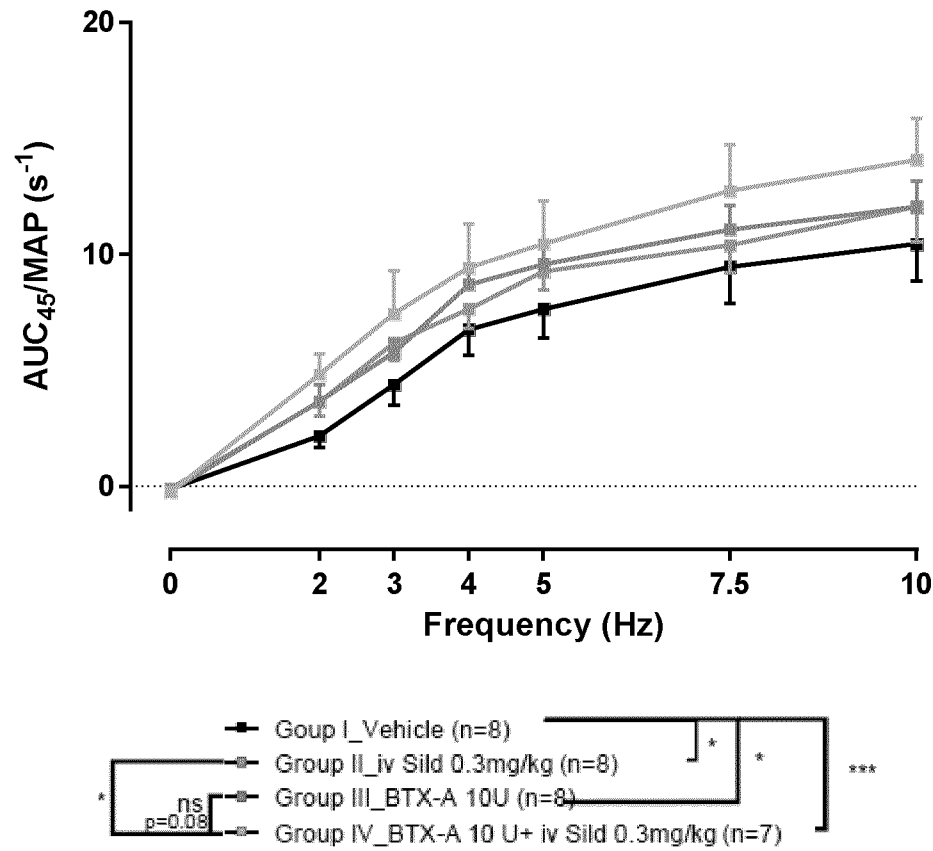


Fig. 4c

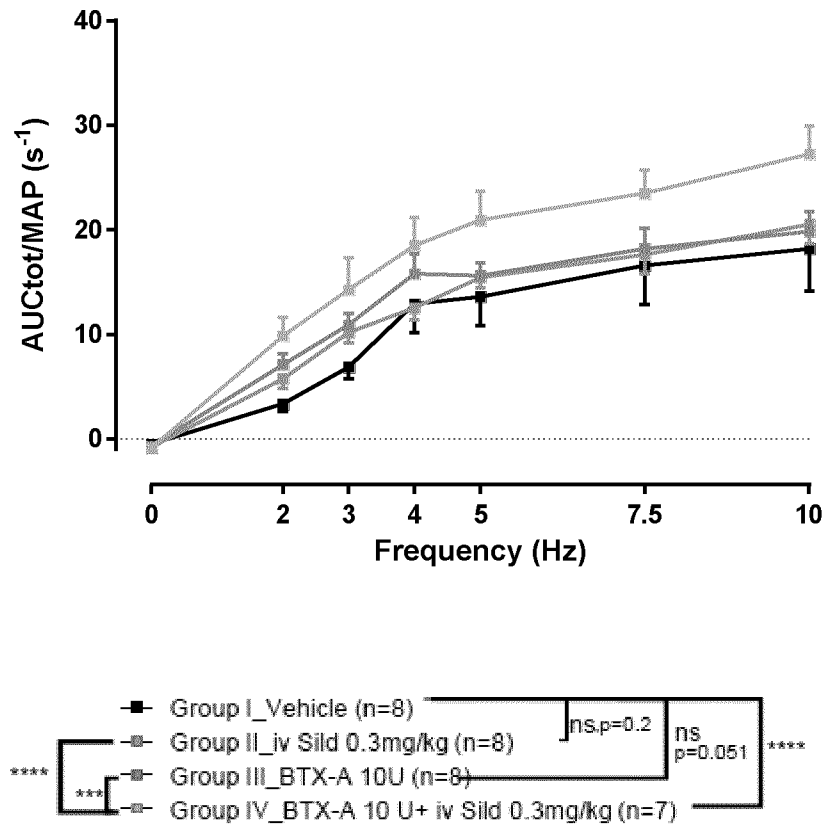


Fig. 5a

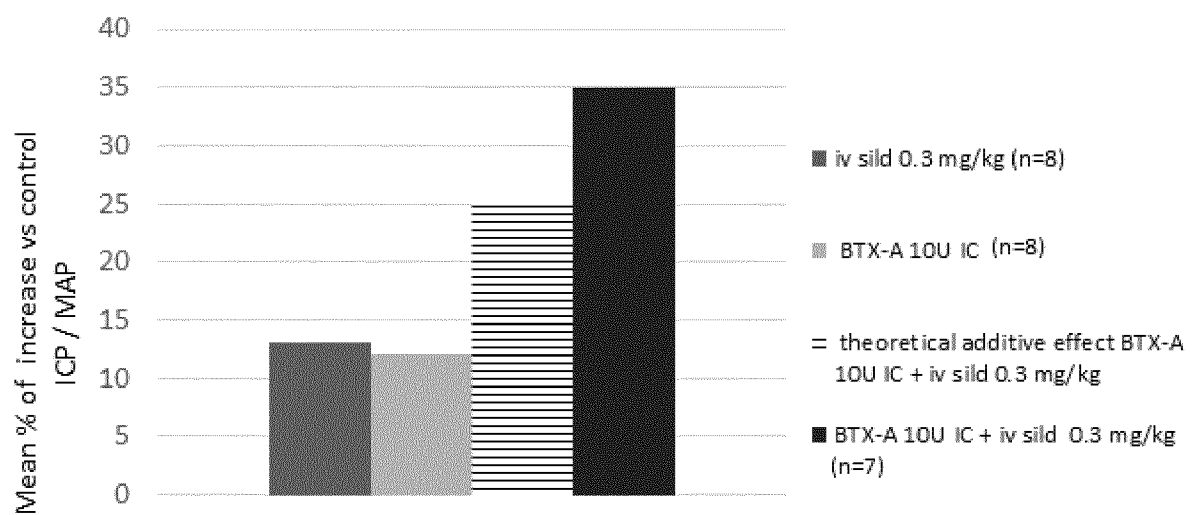


Fig. 5b

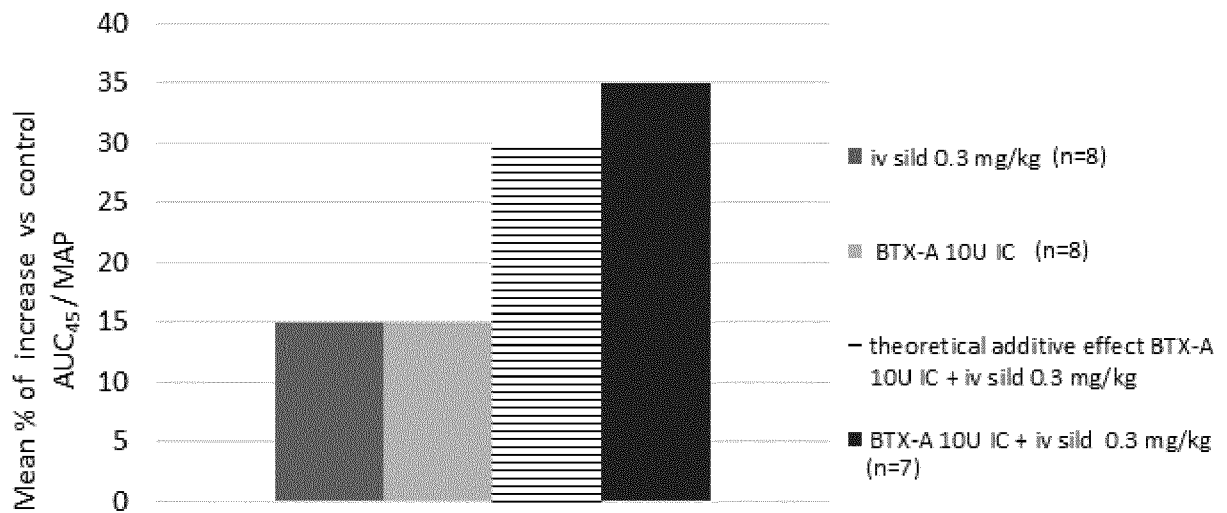
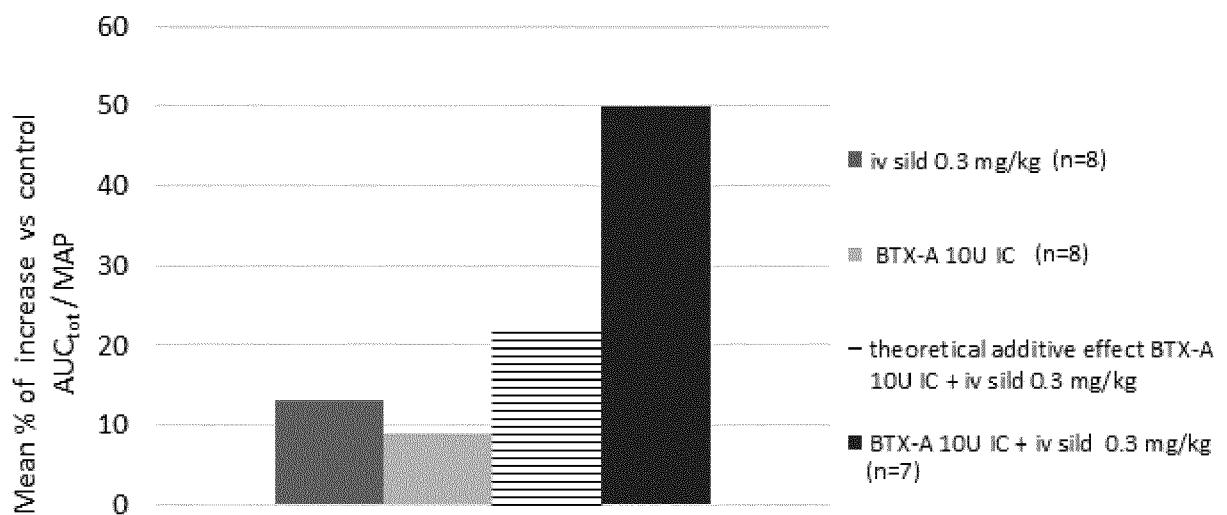


Fig. 5c



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2018/064656

A. CLASSIFICATION OF SUBJECT MATTER		
INV.	A61K38/48 A61K31/53	A61K45/06 A61K31/5575
	A61K31/4985 G06F17/00	A61K31/506 G07F17/00
		A61K31/519
ADD.		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols) A61K A61P G07F		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal, BIOSIS, EMBASE, WPI Data		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
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X	Anonymous: "Intracavernosal Injection of Botulinum Toxin Type A in the Treatment of Vascular Erectile Dysfunction - Full Text View - ClinicalTrials.gov", 16 January 2017 (2017-01-16), XP055427096, Retrieved from the Internet: URL:https://clinicaltrials.gov/ct2/show/study/NCT02584686 [retrieved on 2017-11-21] page 4	1-9, 14-19
Y		10,12, 13,20-24
Y	----- WO 02/11729 A1 (UNIV BOSTON [US]; GOLDSTEIN IRWIN [US]; TRAISH ABDUL [US]; KIM NOEL [U] 14 February 2002 (2002-02-14) claims 1, 5 page 2, lines 16-18 ----- -/-	1-10, 12-24
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 29 August 2018		Date of mailing of the international search report 06/09/2018
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Collins, Sally

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2018/064656

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
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A	US 2011/052636 A1 (GAXIOLA GUSTAVO M [MX] ET AL) 3 March 2011 (2011-03-03) claims 1, 5 -----	1-14

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Information on patent family members

International application No

PCT/EP2018/064656

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