

DEVICE AND METHODS FOR ADMINISTERING A THERAPEUTIC COMPOSITION TO THE PHARYNGEAL MUSCLE

Cross-Reference to Related Applications

5 The present application claims priority from United States Provisional Patent Application No 63/510,750 filed on 28 June 2023, the content of which is incorporated herein by reference in its entirety. Furthermore, the present applicant has recently disclosed therapeutic agents and compositions for the treatment of oculopharyngeal muscular dystrophy (OPMD) throughout the following applications: US Provisional No. 62/812,187, filed 28
10 February 2019; US Provisional No. 62/747,089, filed 17 October 2018; International Patent Application PCT/AU2019/051134, filed 17 October 2019; and International Patent Application PCT/AU2020/050182, filed 28 February 2020, the complete contents of which are incorporated by reference herein in their entirety.

15 Technical Field

 The present disclosure relates to devices and methods for administration of a therapeutic composition to the pharyngeal muscle. In particular, the devices and methods of the disclosure may be used in treatment or prevention of dysphagia, including dysphagia associated with oculopharyngeal muscular dystrophy (OPMD) in individuals suffering
20 therefrom or predisposed thereto. Additionally, the present disclosure relates to devices and methods used to deliver modified adeno-associated virus (AAV) delivery vectors comprising 'silence and replace' DNA constructs, including compositions comprising same, for example.

Background

25 OPMD is a rare and slowly-progressing, late-onset muscular dystrophy that first manifests in midlife, advances with age, and is characterized by progressive ptosis, swallowing difficulties and proximal limb weakness. OPMD is caused by a specific mutation in the gene encoding PABPN1 on chromosome 14 (position q11.2-q13). As a result, a person with OPMD can find it increasingly difficult to swallow (dysphagia), putting them at risk of
30 harm or death from choking, inhaling food or drinks into their lungs (aspiration), aspiration pneumonia, weight loss and malnutrition.

 The mutation that causes OPMD is an abnormal expansion of a (GCN)_n trinucleotide repeat in the coding region of the poly(A) binding protein nuclear 1 (PABPN1) gene. The presence of the trinucleotide repeat leads to an expanded polyalanine tract at the N-terminus
35 of the PABPN1 protein: 10 alanines are present in the normal protein, however an expansion

of 11 to 18 alanines is present in the mutant form (expPABPN1). Intranuclear aggregates, designated as “intranuclear inclusions” comprise the primary histopathological hallmark of the disease. Misfolding of expanded PABPN1 protein results in the accumulation of insoluble polymeric fibrillar aggregates inside nuclei of affected cells, as PABPN1 is an aggregation
5 prone protein.

There are no medicinal products approved for the treatment of OPMD, and there are no medicinal products that have demonstrated significant alterations in the natural history of the disease. Palliative surgical interventions, such as cricopharyngeal myotomy, have been employed. Cricopharyngeal myotomy serves to release the tension on the cricopharyngeus
10 (CP) muscle, and this surgical procedure can transiently reduce the impact of dysphagia in some patients. Direct injection of botulinum toxin (Botox) into the CP muscle has also been used to affect temporary improvements in swallowing via chemical-induced relaxation of the targeted muscle. Although both techniques can provide transient reductions in the dysphagia experienced by some patients, the oropharyngeal symptoms inevitably return and disease
15 progression continues unabated. Repetitive dilatation of the upper esophageal sphincter has demonstrated some efficacy in OPMD patients with moderate dysphagia, however, additional clinical studies are required to corroborate results and characterize long-term outcomes.

By way of background, under normal conditions, a food bolus leaves the oral cavity and is able to traverse the length of the pharynx, en route to the esophagus, via the propulsive
20 activity of the coordinated constriction of the superior, middle, and inferior pharyngeal constrictor muscles. As the food bolus nears the opening of the upper esophagus, the subsequent relaxation of the cricopharyngeal muscle allows the bolus to enter the esophagus and travel to the stomach. In OPMD, the pharyngeal constrictor muscles are weakened and atrophic and, as a result, are unable to consistently exert the level of force required to support
25 the food bolus propulsion that defines the normal swallowing process. Restoration of muscle fiber size and muscle force generation capacity in the weakened and atrophic pharyngeal constrictor muscles would be expected to meaningfully enhance the ability of those muscle groups to support food bolus propulsion through the pharynx and towards the esophagus. The pharyngeal constrictor muscles (inferior and middle) are, then, more relevant therapeutic
30 targets, and an increase in muscle strength could enhance the function of these muscles, reducing dysphagia in OPMD patients.

The present applicant has recently disclosed therapeutic compositions for treatment of OPMD in WO2017/177277, WO2018/107228, WO2019/043630, WO2020/077412 and WO2020/172720. However, there remains a need to improve the method and means of
35 delivery of compositions to the pharyngeal muscles, such as those therapeutic compositions developed and disclosed by the applicant.

Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present disclosure as it existed before the priority date of each of the appended
5 claims.

Summary

In a first aspect of the present disclosure there is provided an injection needle comprising:
10 a needle cannula comprising:
a proximal portion having a proximal end,
a distal portion having a distal end,
a middle portion disposed between the proximal portion and distal portion, and
a lumen extending through the proximal portion, the middle portion and the
15 distal portion between the proximal end of the proximal portion and the distal end of the distal portion, the lumen opening at an outlet at or adjacent the distal end of the distal portion;
wherein the proximal portion and the distal portion are each substantially straight and
wherein the middle portion is curved such that the distal portion extends at an angle of
between 75° and 105° relative to the proximal portion; and
20 wherein the length of the proximal portion is shorter than the length of the distal portion.

The distal portion may extend at an angle of between about 85° and 95° relative to the proximal portion. For example, the distal portion may extend at an angle of about 90° relative to the proximal portion.

25 A radius of curvature of the middle portion may be between about 2.5mm and 3.1mm. For example, the radius of curvature of the middle portion may be about 2.8mm, for example.

The proximal portion may have a length that is less than 90%, less than 80%, less than 70%, or less than 60%, or less than 50% of a length of the distal portion, for example.

30 A length of the distal portion may be greater than the sum of the length of the proximal portion and a length of the middle portion (e.g. an arc length of the middle portion).

A total length of the needle cannula may be between about 10mm and about 27mm. In some examples, the needle cannula may have a total length of about 16mm to about 22mm. For example, the needle cannula may have a total length of about 18mm to about 20mm e.g.,
35 about 19mm. For example, the length of the distal portion may be between about 7mm and about 15mm, the length (e.g. arc length) of the middle portion may be between about 3mm

and about 6mm, and the length of the proximal portion may be between about 2mm and about 6mm.

In one example:

the distal portion extends at an angle of between about 85° and about 95° (e.g., about
5 90°) relative to the proximal portion; and
a radius of curvature of the middle portion is between about 2.5mm and 3.1mm.

In one example:

the proximal portion has a length that is less than 70% of a length of the distal
portion;
10 the length of the distal portion is greater than the sum of the length of the proximal
portion and the length of the middle portion (e.g. an arc length of the middle portion); and
the total length of the needle cannula is between about 10mm and 27mm.

In one example:

the distal portion extends at an angle of between about 85° and 95° relative to the
15 proximal portion;
the length of the distal portion is between about 7mm and 15mm; and
the length of the proximal portion is between about 2mm and 6mm.

The needle cannula may be between about a 26-28 Gauge e.g., a 27 Gauge needle.
An outer diameter of the needle cannula may be between about 0.3mm and 0.5mm, for
20 example. An inner diameter of the needle cannula may be between about 0.1mm and 0.3mm,
for example. A thickness of a wall of the needle cannula may be between about 0.03mm and
0.17mm, for example.

The needle cannula may comprise a dead volume of between about 0.5μL/25.4mm
and 1.5μL/25.4mm, for example.

25 The needle cannula may comprise a bevel formed at a distal end of the needle. The
bevel may include a bevelled edge that faces away from an inner bend side of the needle
cannula, e.g., from a center point of curvature of the middle portion of the needle cannula.
The distal tip may be bevelled at an angle of between about 5° to about 15°. A bevel length
may be between about 0.5mm to about 3.5mm. The outlet of the needle cannula may be
30 located at the bevelled edge.

The injection needle may further comprise a needle hub connected to the needle
cannula. The needle cannula may project distally from the needle hub. The proximal end of
the proximal portion of the needle cannula may be defined at a boundary where the needle
cannula extends from the needle hub. In some embodiments the needle cannula may further
35 comprise a support portion, which extends proximally from the proximal end of the proximal

portion of the needle cannula, and which is disposed inside the needle hub. The lumen may further extend through the support portion.

The needle cannula may have an inlet which is at or adjacent to the proximal end of the proximal portion, or at a proximal end of the support portion when present. The lumen
5 may extend between the inlet and the outlet.

The needle hub may have a distal portion adjacent the proximal end of the proximal portion of the needle cannula. The distal portion of the needle hub may be swaged.

The injection needle may be used specifically as a pharyngeal muscle injection
10 needle, for example. The injection needle may be used to directly inject a therapeutic into a pharyngeal muscle. Direct injection may occur, for example, during an open surgical procedure. That is, the direct injection into the pharyngeal muscle may occur following an incision in a subject's neck to obtain direct access to the pharyngeal muscle. The injection
15 needle may be used for direct injection specifically into a pharyngeal constrictor muscle and/or any one of the following specific muscles: an inferior pharyngeal constrictor muscle, a middle pharyngeal constrictor muscle, a superior pharyngeal constrictor muscle, a
palatopharyngeus muscle, a salpingopharyngeus muscle, a stylopharyngeus muscle, or any combination thereof.

The injection needle may be used to treat or prevent dysphagia. In some examples,
20 the dysphagia is associated with oculopharyngeal muscular dystrophy (OPMD) e.g., the dysphagia may be secondary to a genetically confirmed diagnosis of OPMD.

In one aspect, there is provided a pharyngeal muscle injection needle, the pharyngeal muscle injection needle comprising:

a proximal portion having a proximal end,
a distal portion having a distal end,
25 a middle portion disposed between the proximal portion and distal portion, and
a lumen extending through the proximal portion, the middle portion and the distal portion between the proximal end of the proximal portion and the distal end of the distal portion, the lumen opening at an outlet at or adjacent to the distal end of the distal portion;
wherein the proximal portion and the distal portion are each substantially straight and
30 wherein the middle portion is curved such that the distal portion extends at an angle relative to the proximal portion.

The pharyngeal muscle injection needle may include any one or more of the features of the injection needle described above with respect to the first aspect of the present disclosure. For example, the middle portion may be curved such that the distal portion extends
35 at an angle of between 75° and 105° (such as at an angle of between about 85° and about 95°,

or at an angle of about 90°) relative to the proximal portion. As another example, the length of the proximal portion may be shorter than the length of the distal portion.

The pharyngeal muscle injection needle may be for the treatment or prevention of dysphagia. In one example, the dysphagia may be associated with oculopharyngeal muscular dystrophy (OPMD) e.g., the dysphagia may be secondary to a genetically confirmed diagnosis of OPMD.

In a further aspect, there is provided a method of forming an injection needle, such as a pharyngeal muscle injection needle, wherein the method comprises:

providing a needle cannula comprising a proximal end and a distal end, and a lumen extending through the needle cannula between the proximal end and the distal end, the lumen opening at an outlet at or adjacent to the distal end of the distal portion; and

bending the needle cannula such that the needle cannula comprises a substantially straight proximal portion having the proximal end, a substantially straight distal portion having the distal end, and a middle portion disposed between the proximal portion and the distal portion, the middle portion being curved such that the distal portion extends at an angle relative to the proximal portion.

The method may be such that the injection needle includes any one or more of the features of the injection needle described above with respect to the first aspect of the present disclosure. For example, the middle portion may be curved such that the distal portion extends at an angle of between 75° and 105° (such as at an angle of between about 85° and about 95°, or at an angle of about 90°) relative to the proximal portion. As another example, the length of the proximal portion may be shorter than the length of the distal portion.

In one aspect of the present disclosure there is provided a method of administering a therapeutic composition to a pharyngeal muscle of a subject, comprising directly injecting the therapeutic composition into the pharyngeal muscle of the subject using a bent or curved injection needle. In some examples, the bent or curved injection needle comprises:

a proximal portion having a proximal end,
a distal portion having a distal end,
a middle portion disposed between the proximal portion and distal portion, and
a lumen extending through the proximal portion, the middle portion and the distal portion between the proximal end of the proximal portion and the distal end of the distal portion, the lumen opening at an outlet at or adjacent to the distal end of the distal portion;
wherein the proximal portion and the distal portion are each substantially straight and wherein the middle portion is curved such that the distal portion extends at an angle relative to the proximal portion.

The bent or curved injection needle may include any one or more of the features of the injection needle described above with respect to the first aspect of the present disclosure.

For example, the middle portion may be curved such that the distal portion extends at an angle of between 75° and 105° (such as at an angle of between about 85° and about 95°, or at an angle of about 90°) relative to the proximal portion. As another example, the length of the proximal portion may be shorter than the length of the distal portion.

- 5 In one preferred example, the method comprises directly injecting the therapeutic composition into the pharyngeal muscle of the subject using the injection needle as described herein according to the first aspect.

10 The pharyngeal muscle may include any one of the following specific muscles: an inferior pharyngeal constrictor muscle, a middle pharyngeal constrictor muscle, a superior pharyngeal constrictor muscle, a palatopharyngeus muscle, a salpingopharyngeus muscle, a stylopharyngeus muscle, or any combination thereof. In one particular example, the method comprises administering the therapeutic composition into the middle pharyngeal constrictor muscle and the inferior pharyngeal constrictor muscle.

15 The therapeutic composition may be administered by direct injection into the pharyngeal muscle (i.e., intramuscular injection) following an incision in a subject's neck which is sufficient to obtain direct access to at least part of the subject's pharyngeal muscle. Accordingly, the method may comprise making one or more incisions (to the skin) in the subject's neck to provide direct access to the subject's pharyngeal muscle and then administering the therapeutic composition by direct intramuscular injection into the
20 pharyngeal muscle. For example, an incision may be made to the right side of the pharyngeal area at the right side of a human or animal body, and the area sufficiently dissected to access at least the right side of one or more of the pharyngeal muscles. The needle cannula may then be inserted directly into the right side of one or more of the pharyngeal muscles. Alternatively or additionally, an incision may be made to the left side of the pharyngeal area
25 at the left side of a human or animal body, and the area sufficiently dissected to access the left side of one or more of the pharyngeal muscles. The needle cannula may then be inserted directly into the left side of one or more of the pharyngeal muscles.

30 Injection of the therapeutic composition described herein may be made directly into one or more locations of a pharyngeal muscle, into one or more pharyngeal muscles, into one or more sides (e.g. right side or left side) of a pharyngeal muscle, or any combination thereof. Injection at multiple locations may assist with improved delivery of the therapeutic composition. For example, in the case of delivery of a viral vector-based therapeutic composition, injection at multiple locations may improve delivery to the pharyngeal muscle tissue. In one example, the therapeutic composition may be injected into the middle
35 pharyngeal constrictor muscle and the inferior pharyngeal constrictor muscle.

 The present disclosure also provides a method of administering a therapeutic composition to a pharyngeal muscle of a subject, comprising directly injecting the therapeutic

composition into the pharyngeal muscle of the subject using a bent or curved injection needle. In some preferred examples, the injection needle is an injection needle as described herein with reference to the first aspect.

5 The therapeutic composition may be administered to one or more pharyngeal muscles selected from: an inferior pharyngeal constrictor muscle, a middle pharyngeal constrictor muscle, a superior pharyngeal constrictor muscle, a palatopharyngeus muscle, a salpingopharyngeus muscle, a stylopharyngeus muscle, and any combination thereof. For example, the method may comprise administering the therapeutic composition into the middle pharyngeal constrictor muscle and the inferior pharyngeal constrictor muscle.

10 The therapeutic composition may be administered to the pharyngeal muscle by direct injection following an incision in a subject's neck which is sufficient to provide direct access to at least part of the subject's pharyngeal muscle. In accordance with this example, the method may comprise making one or more incisions in the subject's neck to provide direct access to at least part of the subject's pharyngeal muscle and then administering the
15 therapeutic composition by direct intramuscular injection to the pharyngeal muscle.

In some examples, the therapeutic composition is for treatment or prevention of dysphagia e.g., such as dysphagia associated with oculopharyngeal muscular dystrophy (OPMD). Accordingly, the therapeutic composition may be effective for treatment or prevention of dysphagia associated with OPMD. The therapeutic composition may also be
20 effective to treat or prevent one or more other symptoms of OPMD.

A therapeutic composition which is effective for treatment or prevention of dysphagia associated with OPMD for use in the method of the disclosure may comprise a viral delivery vector comprising a 'silence and replace' construct comprising:

- 25 (i) a DNA-directed RNAi (ddRNAi) construct comprising a nucleic acid comprising a sequence which encodes a short hairpin micro-RNA (shmiR); and
- (ii) a PABPN1 construct comprising a nucleic acid comprising a sequence encoding a functional PABPN1 protein having a mRNA transcript which is not targeted by the shmiR(s) encoded by the ddRNAi construct.

In some examples, the viral delivery vector is an adeno-associated virus (AAV)
30 comprising a viral capsid protein from AAV9 comprising a modified subunit 1 (VP1) sequence wherein the amino acids at positions 26, 40, 43, and 44 are modified relative to a corresponding wildtype AAV9 VP1 sequence set forth in SEQ ID NO: 17. For example, the AAV may comprise a viral capsid protein from AAV9 comprising a VP1 sequence with a modified phospholipase 2 (PLA2) domain comprising the sequence set forth in SEQ ID NO:
35 27. For example, the AAV may comprise a viral capsid protein from AAV9 comprising mutations A67E, Q81R, K84D and A85S with respect to the full length wild-type AAV

serotype 9 capsid sequence set forth in SEQ ID NO: 19, optionally wherein the viral capsid protein comprises the amino acid sequence set forth in SEQ ID NO: 28.

In some examples, the viral delivery vector is an adeno-associated virus (AAV) comprising a viral capsid protein from AAV9 comprising a modified subunit 1 (VP1) sequence wherein the amino acids at positions 1, 26, 40, 43, and 44 are modified relative to a corresponding wildtype AAV9 VP1 sequence set forth in SEQ ID NO: 17. For example, the AAV may comprise a viral capsid protein from AAV9 comprising a VP1 sequence with a modified phospholipase 2 (PLA2) domain comprising the sequence set forth in SEQ ID NO: 18. For example, the AAV may comprise a viral capsid protein from AAV9 comprising mutations A42S, A67E, Q81R, K84D and A85S with respect to the full length wild-type AAV serotype 9 capsid sequence set forth in SEQ ID NO: 19, optionally wherein the viral capsid protein comprises the amino acid sequence set forth in SEQ ID NO: 20

In some examples, the viral delivery vector is an adeno-associated virus (AAV) comprising a viral capsid protein from AAV8 comprising a modified subunit 1 (VP1) sequence wherein the amino acids at positions 1, 26, 40, 43, 44 and 64 are modified relative to a corresponding wildtype AAV8 VP1 sequence set forth in SEQ ID NO: 21. For example, the AAV may comprise a viral capsid protein from AAV8 comprising a VP1 sequence with a modified PLA2 domain comprising the sequence set forth in SEQ ID NO: 22. For example, the AAV may comprise a viral capsid protein from AAV8 comprising mutations A42S, A67E, Q81R, K84D, A85S and Q105K with respect to the full length wild-type AAV serotype 8 capsid sequence set forth in SEQ ID NO: 23, optionally wherein the viral capsid protein comprises the amino acid sequence set forth in SEQ ID NO: 24.

In some examples, the PABPN1 construct within the 'silence and replace' construct comprises a nucleic acid molecule encoding functional PABPN1 protein which is codon optimised such that its corresponding mRNA sequence is not recognised by the shmiR(s) encoded and expressed from the ddRNAi construct. For example, the codon-optimised nucleic acid may comprise the sequence set forth in SEQ ID NO: 15. The codon-optimised nucleic acid preferably encodes the amino acid sequence set forth in SEQ ID NO: 16.

In some examples, the ddRNAi construct comprises a DNA sequence encoding a shmiR comprising an effector sequence which is substantially complementary to the sequence set forth in SEQ ID NO:1 and/or an effector sequence which is substantially complementary to the sequence set forth in SEQ ID NO:2. In one example, the ddRNAi construct comprises a DNA sequence encoding a shmiR comprising an effector sequence which is substantially complementary to the sequence set forth in SEQ ID NO:1 and a DNA sequence encoding a shmiR comprising an effector sequence which is substantially complementary to the sequence set forth in SEQ ID NO:2. For example, the ddRNAi construct may comprise a DNA sequence encoding a shmiR comprising an effector sequence set forth in SEQ ID NO: 4 and

an effector complement sequence set forth in SEQ ID NO: 3 (shmiR13); and a DNA sequence encoding a shmiR comprising an effector sequence set forth in SEQ ID NO: 6 and an effector complement sequence set forth in SEQ ID NO: 5 (shmiR17). For example, the ddRNAi construct may comprise a nucleic acid comprising or consisting of a DNA sequence set forth in SEQ ID NO: 12 (shmiR13) and a nucleic acid comprising or consisting of a DNA sequence set forth in SEQ ID NO: 13 (shmiR17).

In one particular example, the therapeutic composition which is effective for treatment or prevention of dysphagia associated with OPMD and which is administered in the method of the disclosure is designated BB-301, and comprises an AAV9 vector comprising:

- 10 (a) a viral capsid protein from AAV9 comprising mutations A67E, Q81R, K84D and A85S determine relative to the full length wild-type AAV9 capsid sequence set forth in SEQ ID NO: 19, optionally wherein the viral capsid protein comprises the amino acid sequence set forth in SEQ ID NO: 28; and
 - (b) a 'silence and replace' construct comprising:
 - 15 (i) a muscle-specific promoter (optionally wherein the promoter is a Spc512 muscle-specific promoter);
 - (ii) a DNA-directed RNAi (ddRNAi) construct comprising: a nucleic acid comprising or consisting of a sequence which encodes shmiR13 as described herein (optionally the DNA sequence set forth in SEQ ID NO: 12); and a nucleic acid
 - 20 comprising or consisting of a sequence which encodes shmiR17 as described herein (optionally the DNA sequence set forth in SEQ ID NO: 13); and
 - (iii) a PABPN1 construct comprising a nucleic acid sequence encoding a functional PABPN1 protein comprising the amino acid sequence set forth in SEQ ID NO: 16, wherein the nucleic acid sequence encoding the functional PABPN1 protein is
 - 25 a DNA sequence which is codon-optimised such that its corresponding mRNA sequence is not targeted by the shmiR(s) encoded by the ddRNAi construct (optionally wherein the codon-optimised nucleic acid comprises the sequence set forth in SEQ ID NO: 15);
- wherein the muscle-specific promoter is operably-linked to the ddRNAi construct and the PABPN1 construct.

In each of the foregoing examples, the method may comprise administering the therapeutic composition for treatment or prevention of dysphagia associated with OPMD (such as the example described herein and designated BB-301) in a volume ranging from about 25µl to about 75µl per injection site. The therapeutic composition may be administered at a concentration between about 1.0+E12 vg/mL and about 1.0+E15 vg/mL. For example, the concentration of the therapeutic composition for treatment of dysphagia associated with

OPMD which is administered to the subject may be between about 5.0×10^{12} vg/mL and about 1.0×10^{14} vg/mL, such as between about 5.0×10^{12} vg/mL and 5.0×10^{13} vg/mL.

The therapeutic composition (e.g., as BB-301) may be administered (by injection) to multiple sites within the pharyngeal muscle group. For example, the method may comprise
5 injecting the therapeutic composition at about 2 to about 30 or more sites within a pharyngeal muscle. In some example, the method may comprise injecting the therapeutic composition at about 2 to about 30 sites (e.g., about 4 to about 8 sites) at one or more locations within a pharyngeal muscle. For example, the method may comprise injecting the therapeutic
10 composition at about 4 to about 8 sites within one or more, or each of, the following sections of the pharyngeal muscle: a section which is left, lateral to the pharyngeal raphe of the middle pharyngeal constrictor muscle; a section which is right, lateral to the pharyngeal raphe of the middle pharyngeal constrictor muscle; a section which is left, lateral to the pharyngeal raphe of the inferior pharyngeal constrictor muscle; and a section which is right, lateral to the pharyngeal raphe of the inferior pharyngeal constrictor muscle. For example, the method may
15 comprise: injecting the therapeutic composition at about 4 sites within a section which is left, lateral to the pharyngeal raphe of the middle pharyngeal constrictor muscle; injecting the therapeutic composition at about 4 sites within a section which is right, lateral to the pharyngeal raphe of the middle pharyngeal constrictor muscle; injecting the therapeutic composition at about 8 sites within a section which is left, lateral to the pharyngeal raphe of
20 the inferior pharyngeal constrictor muscle; and injecting the therapeutic composition at about 8 sites within a section which is right, lateral to the pharyngeal raphe of the inferior pharyngeal constrictor muscle.

In each of the foregoing examples describing administering multiple injections of the therapeutic composition (e.g., BB-301) to the pharyngeal muscle, including methods
25 describing injections at multiple locations or sites within a pharyngeal muscle, each injection volume may be from about 12.5 μ L to about 25 μ L to about 75 μ L, preferably about 50 μ L to about 75 μ L. In one example, each injection volume may be about 50 μ L. In one example, each injection volume may be about 75 μ L. The therapeutic composition may be administered to one or more injection sites as a single injection per injection site, or as a plurality of injections per
30 injection site. The volume of therapeutic composition administered per injection site may therefore be a single injection volume, or the cumulative volume of a plurality of injections administered at the injection site. For example, administering a volume of about 75 μ L per injection site may comprise administering three injections having an injection volume of about 25 μ L per injection site.

35 In each of the foregoing examples describing intramuscular injection of the therapeutic composition (e.g., BB-301) to the pharyngeal muscle, including multiple injections of the therapeutic composition at one or more locations within the pharyngeal muscle, the total dose

of the therapeutic composition which is administered to the subject in a single treatment may be about 1.0×10^{13} vg/subject to about 5.5×10^{13} vg/subject. For example, the total dose of the therapeutic composition which is administered to the subject in a single treatment may be about 1.2×10^{13} vg/subject ('low dose'). For example, the total dose of the therapeutic composition which is administered to the subject in a single treatment may be about 3.6×10^{13} vg/subject ('medium dose'). For example, the total dose of the therapeutic composition which is administered to the subject in a single treatment may be about 5.4×10^{13} vg/subject ('high dose').

In each of the forgoing examples, the therapeutic composition described herein (e.g., BB-301) may be provided in a pre-filled syringe.

The present disclosure also provides a pre-filled syringe containing a therapeutic composition (e.g., BB-301) which is effective for treatment or prevention of dysphagia associated with OPMD. As described herein, the therapeutic composition (e.g., BB-301) may comprise an AAV9 vector comprising:

- (a) a viral capsid protein from AAV9 comprising mutations A67E, Q81R, K84D and A85S determine relative to the full length wild-type AAV9 capsid sequence set forth in SEQ ID NO: 19, optionally wherein the viral capsid protein comprises the amino acid sequence set forth in SEQ ID NO: 28; and
- (b) a 'silence and replace' construct comprising:
 - (i) a muscle-specific promoter (optionally wherein the promoter is a Spc512 muscle-specific promoter);
 - (ii) a DNA-directed RNA interference (ddRNAi) construct comprising: a nucleic acid comprising or consisting of a sequence which encodes shmiR13 as described herein (optionally the DNA sequence set forth in SEQ ID NO: 12); and a nucleic acid comprising or consisting of a sequence which encodes shmiR17 as described herein (optionally the DNA sequence set forth in SEQ ID NO: 13); and
 - (iii) a PABPN1 construct comprising a nucleic acid sequence encoding a functional PABPN1 protein comprising the amino acid sequence set forth in SEQ ID NO: 16, wherein the nucleic acid sequence encoding the functional PABPN1 protein is a DNA sequence which is codon-optimised such that its corresponding mRNA sequence is not targeted by the shmiR(s) encoded by the ddRNAi construct (optionally wherein the

codon-optimised nucleic acid comprises the sequence set forth in SEQ ID NO: 15);

wherein the muscle-specific promoter is operably-linked to the ddRNAi construct and the PABPN1 construct.

5 The present disclosure also provides an injection device comprising: a pre-filled syringe containing a therapeutic composition (e.g., BB-301) which is effective for treatment or prevention of dysphagia associated with OPMD as described herein; and an injection needle of the present disclosure, which is coupled to the pre-filled syringe. For example, the injection device may comprise the pre-filled syringe as described above, coupled to a curved or bent injection needle including any one or more of the features of the injection needle described above with respect to the first aspect of the present disclosure (e.g., Needle A described herein). For example, the pre-filled syringe may be coupled to an injection needle comprising a needle cannula comprising: a proximal portion having a proximal end; a distal portion having a distal end, a middle portion disposed between the proximal portion and distal portion, and a lumen extending through the proximal portion, the middle portion and the distal portion between the proximal end of the proximal portion and the distal end of the distal portion, the lumen opening at an outlet at or adjacent to the distal end of the distal portion. The proximal portion and the distal portion may be substantially straight and the middle portion may be curved such that the distal portion extends at an angle relative to the proximal portion. The distal portion may extend at an angle of between 75° and 105° (such as at an angle of between about 85° and about 95°, or at an angle of about 90°) relative to the proximal portion. Alternatively, or in addition, the length of the proximal portion may be shorter than the length of the distal portion. The needle cannula may be between about 10mm and about 27mm e.g., have a total length of about 16mm to about 22mm e.g., have a total length of about 18mm to about 20mm e.g., about 19mm. The needle cannula may be between about a 26-28 Gauge e.g., a 27 Gauge needle. The needle cannula may have a bevelled needle tip. In one preferred example, the injection needle comprises a needle cannula comprising: a proximal portion having a proximal end; a distal portion having a distal end, a middle portion disposed between the proximal portion and distal portion, and a lumen extending through the proximal portion, the middle portion and the distal portion between the proximal end of the proximal portion and the distal end of the distal portion, the lumen opening at an outlet at or adjacent to the distal end of the distal portion, and wherein proximal portion and the distal portion are substantially straight and the middle portion is curved such that the distal portion extends at an angle of between about 85° and about 95°, (optionally at an angle of about 90°) relative to the proximal portion. The length of the proximal portion may be shorter than the length of the distal portion, the needle cannula may have a total length of about 18mm to about 20mm e.g., about 19mm, the needle cannula may be between about a 26-28 Gauge e.g., a 27 Gauge needle, and/or the needle cannula may have a bevelled needle tip.

The injection device may be capable of delivering the therapeutic composition (e.g., BB-301) in multiple injections. The injection device may be configured to deliver the therapeutic composition (e.g., BB-301 as described herein) from the pre-filled syringe in a series of one or more injections. Each injection may have a pre-determined injection volume.

5 For example, each injection volume may be from about 12.5 μ l to about 75 μ l, preferably from about 50 μ l to about 75 μ l. For example, each injection volume may be 50 μ l. For example, each injection volume may be 75 μ l.

The injection device may further comprise an injection volume setting mechanism operable to set and/or adjust the injection volume that is dispensed with any of the one or

10 more injections.

The injection volume setting mechanism may be operable to set the injection volume to between about 12.5 μ L and about 75 μ L, or between about 50 μ L and about 75 μ L. For example, the injection volume setting mechanism may be operable to set the injection volume to 50 μ l. For example, the injection volume setting mechanism may be operable to set the

15 injection volume to 75 μ l. The injection setting mechanism may be operable to select from a discrete set of injection volumes. The discrete set of injection volumes may comprise one or more or each of: about 12.5 μ L; about 25 μ L; about 50 μ L; and about 75 μ L. The injection device may be configured to provide audible and/or tactile feedback to a user to indicate completion of an injection.

Accordingly, it is contemplated that the present disclosure may provide an injection device comprising a pre-filled syringe coupled to a curved or bent injection needle as described herein, wherein the pre-filled syringe is filled with a therapeutic composition (e.g., BB-301 as described herein) for treatment or prevention of dysphagia associated with OPMD and which is capable of delivering the therapeutic composition (e.g., BB-301 as described

20 herein) in multiple injections, each injection having a pre-determined injection volume. Each injection volume may be from about 12.5 μ l to about 75 μ l, from about 25 μ l to about 75 μ l, preferably from about 50 μ l to about 75 μ l. For example, each pre-determined injection volume may be 50 μ l. For example, each pre-determined injection volume may be 75 μ l.

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The present disclosure also provide a kit comprising a set of two or more injection devices as described herein, the pre-filled syringes of the injection devices collectively containing a single therapeutic dose of the therapeutic composition (e.g., BB-301 as described herein) for treatment of a single subject. The single therapeutic dose may comprise: about 1.2e13 vg/subject; about 3.6e13 vg/subject; or about 5.4e13 vg/subject.

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In some examples, the kit may comprise four of the injection devices. For example, a kit comprising four of the injection devices may be configured for treating a subject according to the method described herein in which: one of the injection devices is used to inject the therapeutic composition at multiple sites (e.g., 4 or more injection sites) within a section

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which is left, lateral to the pharyngeal raphe of the middle pharyngeal constrictor muscle; one of the injection devices is used to inject the therapeutic composition at multiple sites (e.g., 4 or more injection sites) within a section which is right, lateral to the pharyngeal raphe of the middle pharyngeal constrictor muscle; one of the injection devices is used to inject the therapeutic composition at multiple sites (e.g., 8 or more injection sites) within a section which is left, lateral to the pharyngeal raphe of the inferior pharyngeal constrictor muscle; and one of the injection devices is used to inject the therapeutic composition at multiple sites (e.g., 8 or more injection sites) within a section which is right, lateral to the pharyngeal raphe of the inferior pharyngeal constrictor muscle. Each of the injection devices within the kit may contain at least 200 μ L of the therapeutic composition (e.g., BB-301). For example, each of the injection devices may contain between about 200 μ L and about 2000 μ L, or between about 400 μ L and about 1000 μ L, such as about 200 μ L, about 300 μ L, about 400 μ L, about 500 μ L, about 600 μ L, about 800 μ L, about 1000 μ L, about 1500 μ L or about 2000 μ L of the therapeutic composition (e.g., BB-301).

In some examples, the kit comprises two injection devices containing between about 200 μ L and about 1000 μ L of the therapeutic composition; and two injection devices containing between about 400 μ L and about 2000 μ L of the therapeutic composition. In some examples, the kit comprises two injection devices containing between about 200 μ L and about 500 μ L of the therapeutic composition; and two injection devices containing between about 400 μ L and about 1000 μ L of the therapeutic composition.

One or more of the injection devices in the kit may be configured to dispense the therapeutic composition (e.g., BB-301) in a series of injections, each having an injection volume of at least 25 μ L. One or more of the injection devices may be configured to dispense the therapeutic composition (e.g., BB-301) in a series of injections, each having an injection volume of about 50 μ L. One or more of the injection devices may be configured to dispense the therapeutic composition (e.g., BB-301) in a series of injections, each having an injection volume of about 75 μ L.

In some examples, the method comprises administering the therapeutic composition to one or more locations of a pharyngeal muscle, to one or more pharyngeal muscles, to one or more sides of a pharyngeal muscle, or any combination thereof.

In any of the above aspects, the therapeutic composition may be administered by direct injection into the pharyngeal muscle following an incision in a subject's neck to obtain direct access to the pharyngeal muscle.

Key to the Sequence Listing

- SEQ ID NO: 1: RNA sequence for region within mRNA transcript corresponding to PABPN1 protein designated PABPN1 mRNA Region 13.
- 5 SEQ ID NO: 2: RNA sequence for region within mRNA transcript corresponding to PABPN1 protein designated PABPN1 mRNA Region 17.
- SEQ ID NO: 3: RNA effector complement sequence for shmiR designated shmiR13.
- SEQ ID NO: 4: RNA effector sequence for shmiR designated shmiR13.
- SEQ ID NO: 5: RNA effector complement sequence for shmiR designated shmiR17.
- SEQ ID NO: 6: RNA effector sequence for shmiR designated shmiR17.
- 10 SEQ ID NO: 7: RNA stem loop sequence for shmiRs
- SEQ ID NO: 8: 5' flanking sequence of the pri-miRNA backbone.
- SEQ ID NO: 9: 3' flanking sequence of the pri-miRNA backbone
- SEQ ID NO: 10: RNA sequence for shmiR designated shmiR13.
- SEQ ID NO: 11: RNA sequence for shmiR designated shmiR17.
- 15 SEQ ID NO: 12: DNA sequence coding for shmiR designated shmiR13.
- SEQ ID NO: 13: DNA sequence coding for shmiR designated shmiR17.
- SEQ ID NO: 14: DNA sequence for double construct version 2 coding for coPABPN1 and shmiRs designated shmiR17 and shmiR13 under control of Spc512.
- SEQ ID NO: 15: DNA sequence for Human codon-optimized PABPN1 cDNA sequence.
- 20 SEQ ID NO: 16: Amino acid sequence for human wildtype PABPN1 protein.
- SEQ ID NO: 17: Wildtype VP1 subsequence for AAV serotype 9, comprising the PLA2 domain and flanking sequence.
- SEQ ID NO: 18: Modified VP1 subsequence variant 1 for AAV serotype 9, comprising the PLA2 domain and flanking sequence.
- 25 SEQ ID NO: 19: Full-length wild-type AAV serotype 9 capsid.
- SEQ ID NO: 20: Full-length modified AAV serotype 9 capsid variant 1.
- SEQ ID NO: 21: Wildtype VP1 subsequence for AAV serotype 8, comprising the PLA2 domain and flanking sequence.
- SEQ ID NO: 22: Modified VP1 subsequence for AAV serotype 8, comprising the PLA2 domain and flanking sequence.
- 30 SEQ ID NO: 23: Full-length wild-type AAV serotype 8 capsid.

- SEQ ID NO: 24: Full-length modified AAV serotype 8 capsid.
- SEQ ID NO: 25: AAV2 5' ITR Sequence variant 1.
- SEQ ID NO: 26: AAV2 3' ITR Sequence variant 1.
- 5 SEQ ID NO: 27: Modified VP1 subsequence variant 2 for AAV serotype 9, comprising the
PLA2 domain and flanking sequence.
- SEQ ID NO: 28: Full-length modified AAV serotype 9 capsid variant 2.
- SEQ ID NO: 29: AAV2 5' ITR Sequence variant 2.
- SEQ ID NO: 30: AAV2 3' ITR Sequence variant 2.

10 Description of the Drawings

By way of example only, embodiments are now described with reference to the accompanying drawings in which:

Figure 1 is a side view of an injection needle according to an embodiment of the present disclosure;

15 Figure 2 is a further side view of the injection needle of Fig. 1;

Figure 3 is a further side view of the injection needle of Fig. 1 with example dimensions and angles;

Figure 4 is a magnified schematic view of the distal portion and distal tip of the injection needle of Fig. 1;

20 Figure 5 is a magnified elevational front view of the distal portion and distal tip of the injection needle of Fig. 1;

Figures 6A is a magnified cross section view of the middle portion of the injection needle of Fig. 1;

25 Figure 6B is a magnified cross section view of the middle portion of an injection needle according to another embodiment of the present disclosure;

Figure 7A is a side view of an alternative injection needle design trialed for comparative purposes (Injection Needle B discussed herein);

Figure 7B is a magnified perspective view of the tip of the alternative injection needle shown in Fig. 7A;

30 Figure 8A and 8B are illustrative views of a human neck and of use of the injection needle according to embodiments of the present disclosure;

Figure 9A is a schematic illustrating a construct for simultaneous gene silencing of endogenous PABPN1 and replacement with codon optimised PABPN1 generated by subcloning two shmiRs targeting wtPABPN1 and mPABPN1 into the 3' untranslated region of the codon optimized PABPN1 transcript in between the two pAAV2 ITRs (ITRs not shown in schematic);

Figure 9B is a schematic illustrating the 'silence and replace' construct (SR-construct) designed for simultaneous gene silencing of endogenous PABPN1 and replacement with codon optimised PABPN1 generated by subcloning two shmiRs targeting wtPABPN1 and mPABPN1 (shmiR17 and shmiR13) into the 3' untranslated region of the codon optimized PABPN1 transcript in the pAAV2 vector backbone; and

Figure 10 is a posterior illustrative view of the pharyngeal muscle of a human.

Description

General Definitions

The singular forms "a", "an" and "the" include plural referents unless the context clearly dictates otherwise.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

The term "and/or", e.g., "X and/or Y" shall be understood to mean either "X and Y" or "X or Y" and shall be taken to provide explicit support for both meanings or for either meaning.

Those skilled in the art will appreciate that the present disclosure is susceptible to variations and modifications other than those specifically described. It is to be understood that the disclosure includes all such variations and modifications. The disclosure also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations or any two or more of said steps or features.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the above-described embodiments, without departing from the broad general scope of the present disclosure. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

Throughout this specification, unless specifically stated otherwise or the context requires otherwise, reference to a single step, feature, composition of matter, group of steps or

group of features or compositions of matter shall be taken to encompass one and a plurality (i.e. one or more) of those steps, features, compositions of matter, groups of steps or groups of features or compositions of matter.

5 The present disclosure is not to be limited in scope by the specific examples described herein, which are intended for the purpose of exemplification only. Functionally-equivalent products, compositions and methods are clearly within the scope of the present disclosure.

Any example of the present disclosure herein shall be taken to apply *mutatis mutandis* to any other example of the disclosure unless specifically stated otherwise.

10 Unless specifically defined otherwise, all technical and scientific terms used herein shall be taken to have the same meaning as commonly understood by one of ordinary skill in the art (for example, in cell culture, molecular genetics, immunology, immunohistochemistry, protein chemistry, and biochemistry).

15 Unless specifically defined otherwise, all technical and scientific terms used herein shall be taken to have the same meaning as commonly understood by one of ordinary skill in the art (for example, in cell culture, molecular genetics, immunology, immunohistochemistry, protein chemistry, and biochemistry).

20 Unless otherwise indicated, the recombinant DNA, recombinant protein, cell culture, and immunological techniques utilized in the present disclosure are standard procedures, well known to those skilled in the art. Such techniques are described and explained throughout the literature in sources such as, J. Perbal, A Practical Guide to Molecular Cloning, John Wiley and Sons (1984), J. Sambrook *et al.* Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press (1989), T.A. Brown (editor), Essential Molecular Biology: A Practical Approach, Volumes 1 and 2, IRL Press (1991), D.M. Glover and B.D. Hames (editors), DNA
25 Cloning: A Practical Approach, Volumes 1-4, IRL Press (1995 and 1996), and F.M. Ausubel *et al.* (editors), Current Protocols in Molecular Biology, Greene Pub. Associates and Wiley-Interscience (1988, including all updates until present), Ed Harlow and David Lane (editors) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, (1988), and J.E. Coligan *et al.* (editors) Current Protocols in Immunology, John Wiley & Sons (including all updates until
30 present).

Selected Definitions

By "RNA" is meant a molecule comprising at least one ribonucleotide residue. By "ribonucleotide" is meant a nucleotide with a hydroxyl group at the 2' position of a β -D-ribo-
35 furanose moiety. The terms include double-stranded RNA, single-stranded RNA, isolated RNA such as partially purified RNA, essentially pure RNA, synthetic RNA, recombinantly

produced RNA, as well as altered RNA that differs from naturally occurring RNA by the addition, deletion, substitution and/or alteration of one or more nucleotides. Such alterations can include addition of non-nucleotide material, such as to the end(s) of the siRNA or internally, for example at one or more nucleotides of the RNA. Nucleotides in the RNA molecules of the instant disclosure can also comprise non-standard nucleotides, such as non-naturally occurring nucleotides or chemically synthesized nucleotides or deoxynucleotides. These altered RNAs can be referred to as analogs or analogs of naturally-occurring RNA.

The term "RNA interference" or "RNAi" refers generally to RNA-dependent silencing of gene expression initiated by double stranded RNA (dsRNA) molecules in a cell's cytoplasm. The dsRNA molecule reduces or inhibits transcription products of a target nucleic acid sequence, thereby silencing the gene or reducing expression of that gene.

As used herein, the term "double stranded RNA" or "dsRNA" refers to a RNA molecule having a duplex structure and comprising an effector sequence and an effector complement sequence which are of similar length to one another. The effector sequence and the effector complement sequence can be in a single RNA strand or in separate RNA strands. The "effector sequence" (often referred to as a "guide strand") is substantially complementary to a target sequence, which in the present case, is a region of a PABPN1 mRNA transcript. The "effector sequence" can also be referred to as the "antisense sequence". The "effector complement sequence" will be of sufficient complementary to the effector sequence such that it can anneal to the effector sequence to form a duplex. In this regard, the effector complement sequence will be substantially homologous to a region of target sequence. As will be apparent to the skilled person, the term "effector complement sequence" can also be referred to as the "complement of the effector sequence" or the sense sequence.

As used herein, the term "duplex" refers to regions in two complementary or substantially complementary nucleic acids (e.g., RNAs), or in two complementary or substantially complementary regions of a single-stranded nucleic acid (e.g., RNA), that form base pairs with one another, either by Watson-Crick base pairing or any other manner that allows for a stabilized duplex between the nucleotide sequences that are complementary or substantially complementary. It will be understood by the skilled person that within a duplex region, 100% complementarity is not required; substantial complementarity is allowable. Substantial complementarity includes may include 79% or greater complementarity. For example, a single mismatch in a duplex region consisting of 19 base pairs (i.e., 18 base pairs and one mismatch) results in 94.7% complementarity, rendering the duplex region substantially complementary. In another example, two mismatches in a duplex region consisting of 19 base pairs (i.e., 17 base pairs and two mismatches) results in 89.5% complementarity, rendering the duplex region substantially complementary. In yet another example, three mismatches in a duplex region consisting of 19 base pairs (i.e., 16 base pairs

and three mismatches) results in 84.2% complementarity, rendering the duplex region substantially complementary, and so on.

The dsRNA may be provided as a hairpin or stem loop structure, with a duplex region comprised of an effector sequence and effector complement sequence linked by at least 2 nucleotide sequence which is termed a stem loop. When a dsRNA is provided as a hairpin or stem loop structure it can be referred to as a "hairpin RNA" or "short hairpin RNAi agent" or "shRNA". Other dsRNA molecules provided in, or which give rise to, a hairpin or stem loop structure include primary miRNA transcripts (pri-miRNA) and precursor microRNA (pre-miRNA). Pre-miRNA shRNAs can be naturally produced from pri-miRNA by the action of the enzymes Drosha and Pasha which recognize and release regions of the primary miRNA transcript which form a stem-loop structure. Alternatively, the pri-miRNA transcript can be engineered to replace the natural stem-loop structure with an artificial/recombinant stem-loop structure. That is, an artificial/recombinant stem-loop structure may be inserted or cloned into a pri-miRNA backbone sequence which lacks its natural stem-loop structure. In the case of stem-loop sequences engineered to be expressed as part of a pri-miRNA molecule, Drosha and Pasha recognize and release the artificial shRNA. dsRNA molecules produced using this approach are known as "shmiRNAs", "shmiRs" or "microRNA framework shRNAs".

As used herein, the term "complementary" with regard to a sequence refers to a complement of the sequence by Watson-Crick base pairing, whereby guanine (G) pairs with cytosine (C), and adenine (A) pairs with either uracil (U) or thymine (T). A sequence may be complementary to the entire length of another sequence, or it may be complementary to a specified portion or length of another sequence. One of skill in the art will recognize that U may be present in RNA, and that T may be present in DNA. Therefore, an A within either of a RNA or DNA sequence may pair with a U in a RNA sequence or T in a DNA sequence. A person of skill in the art will also recognise that a G present in RNA may pair with C or U in RNA.

As used herein, the term "substantially complementary" is used to indicate a sufficient degree of complementarity or precise pairing such that stable and specific binding occurs between nucleic acid sequences e.g., between the effector sequence and the effector complement sequence or between the effector sequence and the target sequence. It is understood that the sequence of a nucleic acid need not be 100% complementary to that of its target or complement. The term encompasses a sequence complementary to another sequence with the exception of an overhang. In some cases, the sequence is complementary to the other sequence with the exception of 1-2 mismatches. In some cases, the sequences are complementary except for 1 mismatch. In some cases, the sequences are complementary except for 2 mismatches. In other cases, the sequences are complementary except for 3 mismatches. In yet other cases, the sequences are complementary except for 4 mismatches.

The term “encoded”, as used in the context of a shRNA or shmiR of the disclosure, shall be understood to mean a shRNA or shmiR which is capable of being transcribed from a DNA template. Accordingly, a nucleic acid that encodes, or codes for, a shRNA or shmiR of the disclosure will comprise a DNA sequence which serves as a template for transcription of the respective shRNA or shmiR.

The term “DNA-directed RNAi construct” or “ddRNAi construct” refers to a nucleic acid comprising DNA sequence which, when transcribed produces a shRNA or shmiR molecule (preferably a shmiR) which elicits RNAi. The ddRNAi construct may comprise a nucleic acid which is transcribed as a single RNA that is capable of self-annealing into a hairpin structure with a duplex region linked by a stem loop of at least 2 nucleotides i.e., shRNA or shmiR, or as a single RNA with multiple shRNAs or shmiRs, or as multiple RNA transcripts each capable of folding as a single shRNA or shmiR respectively. The ddRNAi construct may be provided within a larger “DNA construct” comprising one or more additional DNA sequences. For example, the ddRNAi construct may be provided in a DNA construct comprising a further DNA sequence coding for functional PABPN1 protein which has been codon optimised such that its mRNA transcript is not targeted by shmiRs of the ddRNAi construct. The ddRNAi construct and/or the DNA construct comprising same may be within an expression vector e.g., operably linked to a promoter.

As used herein, the term “operably-linked” or “operable linkage” (or similar) means that a coding nucleic acid sequence is linked to, or in association with, a regulatory sequence, e.g., a promoter, in a manner which facilitates expression of the coding sequence. Regulatory sequences include promoters, enhancers, and other expression control elements that are art-recognized and are selected to direct expression of the coding sequence.

A “vector” will be understood to mean a vehicle for introducing a nucleic acid into a cell. Vectors include, but are not limited to, plasmids, phagemids, viruses, bacteria, and vehicles derived from viral or bacterial sources. A “plasmid” is a circular, double-stranded DNA molecule. A useful type of vector for use in accordance with the present disclosure is a viral vector, wherein heterologous DNA sequences are inserted into a viral genome that can be modified to delete one or more viral genes or parts thereof. Certain vectors are capable of autonomous replication in a host cell (e.g., vectors having an origin of replication that functions in the host cell). Other vectors can be stably integrated into the genome of a host cell, and are thereby replicated along with the host genome. As used herein, the term “expression vector” will be understood to mean a vector capable of expressing a RNA molecule of the disclosure.

A “functional PABPN1 protein” shall be understood to mean a PABPN1 protein having the functional properties of a wild-type PABPN1 protein e.g., an ability to control the site of mRNA polyadenylation and/or intron splicing in a mammalian cell. Accordingly, a

“functional PABPN1 protein” will be understood to be a PABPN1 protein which is not causative of OPMD when expressed or present in a subject. In one example, a reference herein to “functional PABPN1 protein” is a reference to human wild-type PABPN1 protein. The sequence of human wild-type PABPN1 protein is set forth in NCBI RefSeq NP_004634.

5 Accordingly, a functional human PABPN1 protein may have the functional properties in vivo of the human PABPN1 protein set forth in NCBI RefSeq NP_004634.

The term “dysphagia” as used herein describes a swallowing disorder, including an inability to swallow, difficulty in swallowing or discomfort in swallowing. The swallowing disorder may also impact speech and/or breathing of the subject suffering therefrom. In some

10 instances, the dysphagia is secondary to diagnosis with another condition or disorder e.g., OPMD. For example, the dysphagia may be secondary to genetic diagnosis of OPMD in a subject.

As used herein, the terms “treating”, “treat” or “treatment” and variations thereof, refer to clinical intervention designed to alter the natural course of the individual or cell being

15 treated during the course of clinical pathology. Desirable effects of treatment include decreasing the rate of disease progression, ameliorating or palliating the disease state, and remission or improved prognosis. Treating dysphagia may result in an improvement in strength and/or coordination of the pharyngeal muscles and a consequential improvement in swallowing in the subject. Treatment of dysphagia may also improve speech, and/or

20 breathing in the subject. In the case of treatment of dysphagia associated with OPMD, treatment may include reducing or inhibiting expression of a PABPN1 protein which is causative of OPMD in the subject and/or expressing in the subject a PABPN1 protein having the normal length of polyalanine residues. Preferably, treatment of dysphagia associated with OPMD includes reducing or inhibiting expression of the PABPN1 protein which is causative

25 of OPMD in the subject and expressing in the subject a PABPN1 protein having the normal length of polyalanine residues. Treatment of the underlying OPMD may treat the associated dysphagia. An individual is successfully “treated”, for example, if one or more of the above treatment outcomes is achieved.

As used herein, the terms “prevent”, “preventing” or similar shall be understood to

30 include hindering, reducing or eliminating the development of at least one symptom of a condition. As used in the context of dysphagia, the terms “prevent” or “preventing” shall be understood to mean hindering, reducing or eliminating the development of one or more symptoms associated with dysphagia (as described above). For example, the method of the disclosure may be used to prevent dysphagia in a subject which has been genetically

35 diagnosed with OPMD (e.g., inclusive of the PABPN1 allelic classifications: (GCN)12, (GCN)13, (GCN)14, (GCN)15, or (GCN)16), but not yet displayed physical symptoms of dysphagia. Accordingly, the method of the disclosure may be used to hinder, reduce or

eliminate the development of dysphagia in the subject, notwithstanding their genetic predisposition to OPMD and consequential dysphagia.

A "therapeutically effective amount" is at least the minimum concentration or amount required to affect a measurable improvement in the dysphagia to be treated. In accordance with an example in which the dysphagia is associated with OPMD, the therapeutically effective amount is an amount sufficient to affect a measurable improvement in one or more symptoms of OPMD e.g., dysphagia and weight loss in the subject. A measurable improvement may be determinable via a number of clinical assessment methods such as, for example: assessing pharyngeal constrictor muscle function via pharyngeal area at maximum constriction; assessing swallowing efficiency via determination of total pharyngeal residue; assessing subject-reported oropharyngeal dysphagia via the Sydney Swallow Questionnaire; and assessing swallowing capacity via the cold water timed drinking test. Exemplary clinical assessment methods which are contemplated herein for measuring improvement in one or more symptoms of OPMD are described in Example 3 of the present disclosure.

A therapeutically effective amount herein may vary according to factors such as the disease state, age, sex, and weight of the patient. A therapeutically effective amount of a composition described herein for treating OPMD and dysphagia associated with OPMD may further vary according to factors such as the ability of the shmiR, nucleic acid encoding same, ddRNAi construct, DNA construct, expression vector, or composition comprising same, to elicit a desired response in the individual and/or the ability of the expression vector to express functional PABPN1 protein in the subject. A therapeutically effective amount is also one in which any toxic or detrimental effects of the shmiR, nucleic acid encoding same, ddRNAi construct, DNA construct, expression vector, or composition comprising same, are outweighed by the therapeutically beneficial effects of the shmiR, nucleic acid encoding same, ddRNAi construct, DNA construct, expression vector, or composition comprising same, to inhibit, suppress or reduce expression of PABPN1 protein causative of dysphagia associated with OPMD considered alone or in combination with the therapeutically beneficial effects of the expression of functional PABPN1 protein in the subject.

As used herein, the "subject" or "patient" can be a human or non-human animal suffering from or genetically predisposed to OPMD i.e., possess a PABPN1 gene variant which is causative of OPMD. The "non-human animal" may be a primate, livestock (e.g. sheep, horses, cattle, pigs, donkeys), companion animal (e.g. pets such as dogs and cats), laboratory test animal (e.g. mice, rabbits, rats, guinea pigs, drosophila, C.elegans, zebrafish), performance animal (e.g. racehorses, camels, greyhounds) or captive wild animal. In one example, the subject or patient is a mammal. In one example, the subject or patient is a human.

The terms “reduced expression”, “reduction in expression” or similar, refer to the absence or an observable decrease in the level of protein and/or mRNA product from the target gene e.g., the PABPN1 gene. The decrease does not have to be absolute, but may be a partial decrease sufficient for there to be a detectable or observable change as a result of the RNAi effected by the shmiR, nucleic acid encoding same, ddRNAi construct, DNA construct, expression vector, or composition comprising same of the disclosure. The decrease can be measured by determining a decrease in the level of mRNA and/or protein product from a target nucleic acid relative to a cell lacking the shmiR, nucleic acid encoding same, ddRNAi construct, DNA construct, expression vector, or composition comprising same, and may be as little as 1 %, 5% or 10%, or may be absolute i.e., 100% inhibition. The effects of the decrease may be determined by examination of the outward properties i.e., quantitative and/or qualitative phenotype of the cell or organism, and may also include detection of the presence or a change in the amount of intranuclear inclusions comprised partially or completely of expPABPN1 in the cell or organism following administration of a shmiR, nucleic acid encoding same, ddRNAi construct, DNA construct, expression vector, or composition comprising same, of the disclosure.

A “delivery systems”, “delivery vectors” and similar term as used herein refer to vectors for packaging foreign genetic material, such as DNA or RNA, and which can be introduced into a cell. Delivery vectors can include viral vectors, e.g., an adeno-associated viral (AAV) vector, a retroviral vector, an adenoviral vector (AdV) and a lentiviral (LV) vector. As described herein, viral vectors can be used to deliver and express foreign genetic material in cell. Accordingly, a viral expression vector as described herein may be used as a delivery vector.

As used herein, the term “Adeno-Associated Virus” or “AAV” relates to a group of viruses within the Parvoviridae family which contain a short (approx. 4.7 kb) single-stranded DNA genome and which depend on the presence of a helper virus, such as an Adenovirus for their replication. Also contemplated by the present disclosure are vectors derived from AAV, e.g., used as gene transfer vehicles.

As used herein, the term “serotype”, as used in the context of AAV, is a distinction used to refer to an AAV having a capsid that is serologically distinct from other AAV serotypes. Serologic distinctiveness is determined on the basis of the lack of cross-reactivity between antibodies to one AAV as compared to another AAV. Such cross-reactivity differences are usually due to differences in capsid protein sequences/antigenic determinants (e.g., due to VP1, VP2, and/or VP3 sequence differences of AAV serotypes). VP1 sequences for wildtype AAVs known to infect humans are described in Chen *et al.*, (2013) *J. Vir.* 87(11):6391-6405, and include e.g., serotypes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 13). Capsid polypeptide sequences for AAV serotypes 1-13 are known in the art, for example,

AAV1 (Genbank Acc. No: AAD27757.1, GI:4689097), AAV2 (Genbank Acc. No: AAC03780.1, GP.2906023), AAV3 (Genbank Acc. No: AAC55049.1, GI: 1408469), AAV4 (Genbank Acc. No: AAC58045.1, GL2337940), AAV5 (Genbank Acc. No: AAD13756.1, GI-4249658), AAV10 (Genbank Acc. No: AAT46337.1, GL48728343), AAV11 (Genbank
 5 Acc. No: AAT46339.1, GI:48728346), AAV12 (Genbank Acc. No: ABI16639.1, GI: 112379656), or AAV13 (Genbank Acc. No: ABZ10812.1, GI: 167047087). Furthermore, the complete genomes for AAV from serotypes 1-13 are known in the art, for example, AAV1 (NCBI Reference Sequence NC_002077.1), AAV2 (GenBank Acc. No: J01901.1), AAV3 (Genbank Acc. No: AF028705.1), AAV4 (NCBI Reference Sequence: NC_001829.1), AAV5
 10 (NCBI Reference Sequence: NC_006152.1), AAV6 (GenBank: AF028704.1), AAV7 (NCBI Reference Sequence: NC_006260.1), AAV8 (NCBI Reference Sequence: NC_006261.1), AAV9 (GenBank Acc. No: AY530579.1), AAV10 (Genbank Acc. No: AY631965.1), AAV11 (Genbank Acc. No: AY631966.1) or AAV12 (Genbank Acc. No: DQ813647.1). The skilled person will also appreciate that sequence variation may exist between genomes of
 15 different strains of AAV within the same serotype, particularly where different AAVs from within the same serotype are isolated from different host species and/or different geographical locations.

As used herein in the context of an AAV, the term "viral capsid protein", "capsid protein", "capsid polypeptide" or similar relates to a polypeptide of the AAV having the
 20 activity of self-assembly to produce the proteinaceous shell of an AAV particle, also referred to as coat protein or VP protein. It is comprised of three subunits, VP1, VP2 and VP3, which are typically expressed from a single nucleic acid molecule, and which interact together to form a capsid of an icosahedral symmetry. The capsid structure of AAV is described in BERNARD N. FIELDS et al., VIROLOGY, volume 2, chapters 69 & 70 (4th ed., Lippincott-
 25 Raven Publishers).

As used herein, the term "promoter" refers generally to a DNA sequence that is involved in recognition and binding of DNA-dependent RNA polymerase and other proteins (trans-acting transcription factors) to initiate and control transcription of one or more coding sequences, and is generally located upstream of the coding sequence with respect to the
 30 direction of transcription.

A "pharyngeal muscle" as used herein refers to one or more of the group of muscles that form the pharynx. The pharyngeal muscle can include one or more of the inferior pharyngeal constrictor muscle, middle pharyngeal constrictor muscle, superior pharyngeal constrictor muscle, palatopharyngeus muscle, the salpingopharyngeus muscle, and/or the
 35 stylopharyngeus muscle.

Injection Needle

In an embodiment according to the present disclosure, and as shown in Figures 1 to 3, an injection needle 10 includes a needle cannula 20 having a proximal portion 21 having a proximal end 211, a distal portion 23 having a distal end 231 and an outlet 25 at or adjacent the distal end 231, and a middle portion 22 disposed between the proximal portion 21 and the distal portion 23. A lumen 26 extends through the proximal portion 21, the middle portion 22 and the distal portion 23 between the proximal end 211 and the distal end 231 and opening at the outlet 25. In this embodiment, a length of the proximal portion 21 is shorter than a length of the distal portion 23.

As shown in figures 1 to 3, the injection needle 10 also includes a needle hub 30 connected to the needle cannula 20, the needle cannula 20 projecting distally from the needle hub 30. The proximal end 211 of the proximal portion 21 of the needle cannula 20 is located at a boundary where the needle cannula 20 contacts and/or enters the needle hub 30. In some embodiments the needle cannula 20 may further comprise a support portion 28, represented by dotted lines in Figure 1, which extends proximally from the proximal end 211 of the proximal portion 21 of the needle cannula, and which is disposed, e.g. entirely disposed, inside the needle hub 30. The lumen 26 may further extend through the support portion 28.

The needle cannula 20 includes an inlet 24 which is at or adjacent the proximal end 211 of the proximal portion 21, or at or adjacent a proximal end 281 of the support portion 28 when present. Fluid can enter the needle cannula 20 via the inlet 24 and travel to the outlet 25, e.g., when being injected into a patient.

In this embodiment, the needle hub 30 comprises a distal end portion 31 adapted to connect to the needle cannula 20 (e.g. by having the support portion 28 of the needle cannula fixed or embedded therein), and an opposite proximal end portion 32. The proximal end portion 32 includes a screw thread 33 at an outer surface thereof, the screw thread 33 adapted to be engaged with a complimentary screw thread of additional apparatus, such as a syringe barrel, where a fluid substance (e.g. a therapeutic composition) may be stored prior to delivery via the needle cannula 20. As seen in Figures 1 and 3, the distal end portion 31 may be swaged (tapered towards a central axis).

In this embodiment, the proximal portion 21 and the distal portion 23 are each substantially straight. The middle portion 22 in this embodiment is bent, and more particularly curved, such that the distal portion 23 extends at an angle of between about 75° and 105° relative to an angle of the proximal portion 21.

A boundary between the proximal portion 21 and the middle portion 22 of the needle cannula 20 may be defined at a location at which the needle cannula 20 transitions from extending linearly at the proximal portion 21 to bending or curving at the middle portion 22. Similarly, a boundary between the middle portion 22 and the distal portion 23 of the needle cannula 20 may be defined at a location at which the needle cannula 20 transitions from

bending or curving at the middle portion 22 to extending in a linear direction at the distal portion 23.

Angle of curvature

5 The middle portion 22 of needle cannula 20 is bent and more particularly curved (e.g. arcuate) so as to assist in navigation of the anatomy at an injection site. The particular configuration has been found to be particularly suited to delivering a therapeutic composition directly to one or more pharyngeal muscles, the needle cannula being able to effectively navigate the anatomy surrounding the pharyngeal muscles.

10 As shown, for example in Figures 6A and 6B, the middle portion may be bent or curved such that the distal portion 23 extends at an angle β of between about 75° and 105° relative to the proximal portion 21. For example, as shown in the embodiment of Fig. 6B, the middle portion 22 may be bent or curved such that the distal portion 23 extends at an angle of about 105° relative to the proximal portion 21.

15 In some embodiments the distal portion 23 extends at an angle β of between about 80° and 100° relative to the proximal portion 21. In other embodiments, the distal portion 23 extends at an angle of between about 85° and 95° relative to the proximal portion 21. In other embodiments, the distal portion 23 extends at an angle of between about 87° and 93° relative to the proximal portion 21. In other embodiments, and as shown for example in Fig. 6A, the
20 distal portion 23 extends at an angle β of substantially 90° relative to the proximal portion 21.

 As indicated above, the proximal portion 21 and the distal portion 23 may each be completely straight. In other embodiments, however, the proximal portion 21 and/or distal portion 23 may have one or more curves, angles or irregularities, while still being substantially linear in comparison to the middle portion. In such circumstances, when
25 determining the angles at which the proximal portion 21 and the distal portion 23 each extend, this can be based on, respectively, a notional straight line extending between the proximal end 211 of the proximal portion 21 and a proximal end of the middle portion 22, and a notional straight line extending between a distal end of the middle portion 22 and the distal end 231 of the distal portion 23.

30 In some embodiments the proximal portion 21, middle portion 22 and distal portion 23 of needle cannula 20 extend entirely in a common plane. Alternatively, the portions may not extend entirely a common plane. For example, the needle cannula may be bent or curved three-dimensionally.

Injection Device

The present disclosure also provides an injection device 200, for example drug product injection device (DPID) 200 as shown in Fig. 8, comprising the needle 10 coupled to a syringe 100 (e.g. a pre-filled syringe).

- 5 The pre-filled syringe 100 may comprise a barrel, and a plunger received in the barrel. The syringe 100 may be pre-filled with the therapeutic composition (e.g., such as BB-301 described herein) stored in the barrel. The plunger is longitudinally slidable relative to the barrel to expel the therapeutic composition from the syringe 100.

- 10 The injection device 200 may comprise an injection volume setting mechanism operable to set and/or adjust the injection volume that is dispensed with any of the one or more injections. In some examples, the pre-filled syringe comprises the injection volume setting mechanism. In other examples, the injection volume setting mechanism may be separate from the pre-filled syringe and configured to couple to one or more components of the pre-filled syringe.

- 15 The injection volume setting mechanism may be operable to set the injection volume to between about 12.5 μ L and about 75 μ L, or between about 50 μ L and about 75 μ L. For example, the injection setting mechanism may be operable to select from a discrete set of injection volumes. The discrete set of injection volumes may comprise one or more of: about 12.5 μ L; about 25 μ L; about 50 μ L; and about 75 μ L.

- 20 The injection device may be configured to provide audible and/or tactile feedback to a user to indicate completion of an injection. For example, the plunger of the pre-filled syringe 100 may include at least one set of successive plunger formations, which provide audible and tactile feedback to a user via a series of plunger “clicks” as the therapeutic composition is expelled.

- 25 *Use of injection needle*

- The injection needle 10 of this embodiment is a pharyngeal muscle injection needle. The injection needle 10 is configured to directly inject a therapeutic composition into a pharyngeal muscle. The injection needle may be used for direct injection specifically into a pharyngeal constrictor muscle and/or any one of the following specific muscles: an inferior
30 pharyngeal constrictor muscle, a middle pharyngeal constrictor muscle, a superior pharyngeal constrictor muscle, a palatopharyngeus muscle, a salpingopharyngeus muscle, a stylopharyngeus muscle, or any combination thereof.

- In one embodiment, and as illustrated in Figs 8A to 8B, an open surgical procedure is conducted to enable direct injection into a pharyngeal muscle using the injection needle 10.
35 With reference to Fig. 8A, a first incision 511 is made, e.g., using a scalpel 504, at a first lateral side of a patient’s neck 500 to expose at least a portion 521 of at least one pharyngeal

muscle, such as a portion of an inferior pharyngeal constrictor muscle 501, a middle pharyngeal constrictor muscle 502, a superior pharyngeal constrictor muscle 503 or other pharyngeal muscle. The first lateral side may be the right or left side of the patient's neck (the left side being illustrated in Figs. 8A and 8B).

- 5 A second incision 512 (not shown) may occur on a second lateral side to expose the other side of the inferior pharyngeal constrictor muscle 501, the middle pharyngeal constrictor muscle 502, the superior pharyngeal constrictor muscle 503 or other pharyngeal muscle.

With reference to Fig. 8B, the distal portion of the needle cannula 20 of the injection needle 10 is forced to penetrate the exposed portion of the pharyngeal muscle 501, 501, 502.

- 10 The injection device 200 is operated to expel the therapeutic composition from the syringe 100, through the needle cannula 20 and directly into the pharyngeal muscles 501, 502 and 503.

This injection process may be used to treat or prevent dysphagia associated oculopharyngeal muscular dystrophy (OPMD), for example, in which case the injection
15 needle can be considered, additional or alternatively, an oculopharyngeal muscular dystrophy (OPMD) treatment injection needle. Methods of administering a therapeutic composition using the injection needle of the disclosure to treat or prevent dysphagia associated OPMD are described herein.

- 20 The injection process may be repeated on the opposite lateral side of the patient's neck, e.g. following creating of a second incision at the opposite lateral side of the patient's neck. In general, injection may be made directly into one or more locations of a pharyngeal muscle, into one or more pharyngeal muscles, into one or more sides (e.g. right side or left side) of a pharyngeal muscle, or any combination thereof. Injection at multiple locations may assist with increasing pharyngeal muscle tissue delivery. Injections may also be repeated over
25 time (e.g. weeks, months or years) to address any reduction in the effective amount of therapeutic agent over time.

Radius of curvature

As indicated, the middle portion 22 is curved.

- 30 As shown in Fig. 6A, the curved middle portion 22 has an arc length l and a substantially constant radius r . Since the proximal portion 21 extends at an angle β of substantially 90° relative to the distal portion, the arc length l in this embodiment represents a quarter of the circumference of a circle. In this embodiment, the arc length l is about 4.4 mm and the radius of curvature r is about 2.8 mm, satisfying the equation of: radius =
35 circumference/ 2π (radius of curvature $r = 4(4.4\text{mm})/2\pi = 2.8\text{mm}$).

However, as shown for example in Fig. 6B, which shows an embodiment in which the needle cannula 20 has a distal portion 23 that extends at an alternative angle β relative to the proximal portion of about 105° the radius of curvature r need not be substantially constant. Because of this potential difference in radius of curvature along the middle portion, the radius
5 of curvature may be referred to as a range of values r_v .

In some embodiments, the radius of curvature (r or r_v) of the curved middle portion 22 is between about 2.5mm and about 3.1mm. In other embodiments the radius of curvature (r or r_v) of the curved middle portion 22 is between 2.6mm and 3.0mm. In further embodiments, the radius of curvature (r or r_v) of the curved middle portion 22 is between 2.7mm and
10 2.9mm. And, in further embodiments still, the radius of curvature (r or r_v) of the curved middle portion 22 is about 2.8mm.

It will be understood by persons skilled in the art that arc length and/or radius of curvature of the needle cannula may vary to scale the needle cannula relative to the anatomy of a patient or otherwise, while retaining a constant bend angle, for example, between the
15 proximal and distal portions of the needle cannula.

Total length

As shown for example in Figure 2, the total length of needle cannula 20 can be measured as the sum of the length A of the proximal portion 21, the arc length B of the
20 middle portion 22 and the length C of distal portion 23. In the embodiment shown in Figure 3, the total length of the needle cannula may be approximately 19mm.

However, it will be understood by persons skilled in the art that the needle cannula 20 described herein may have different lengths, e.g. in order to be scaled relative to the anatomy of a patient or otherwise. In some embodiments, the total length of the needle
25 cannula is between about 10mm and about 27mm, between about 13mm and about 25mm, between about 15mm and about 23mm, between about 17mm and about 21mm or about 19mm.

Needle gauge

30 Needle gauge (G) is used to describe the size of the lumen or bore of a needle cannula. The higher the gauge, the smaller the lumen, and therefore usually the thinner the needle. Needle gauge is selected for different purposes by balancing considerations such as pain minimisation, tissue type, speed of entry of substances and effect on the integrity of contents. For example, insulin needles are commonly thin needles (29G to 31G) to minimise
35 the pain of the subcutaneous injection while thicker needles (16G to 17G) may be used for

blood donation as the increased lumen creates lower pressure on fragile red blood cells. In this regard, needle gauge may also be selected on the basis of the effect of gauge on the integrity of the needle constituents for injection, for example, a viral expression vector.

5 The injection needle described herein may be used during an open surgical procedure under anaesthesia and thus pain minimisation may not be a priority consideration. During trials with different needle designs, including trials of injections into pharyngeal muscles, priority consideration was given to minimising leakage and maximising desired tissue transduction of functional virus. This was particularly important given the small volumes of drug product being delivered at each injection site. In the context of the Applicant's drug
10 product, BB-301, needle gauge was found to have an effect on the proportion of AAV that remained functional following flow of the drug product through the injection needle, presumably due to the impact of biomechanical forces (e.g., flow rate, pressure and shear stress etc) on the viral particles during injection. As a result, a needle gauge was selected which balanced the need for minimising leakage and maximising functional AAV transduced
15 into tissue on the one hand, with the need for a needle of sufficient strength to maintain its curvature and withstand mechanical deformation during use on the other hand.

In the embodiment shown in Fig 1, the needle cannula shown is 27G. In general, the needle cannula according to the present disclosure may be between about 23-31 Gauge, between about 25-29 Gauge, between about 26-28 or about 27G, or otherwise.

20 For ease of reference, the dimensions of the needle cannula described herein may be referred to relative to the Birmingham needle scale.

Nevertheless, in some embodiments, the needle cannula 20 may not be aligned to any Gauge standard.

25 *Outer and inner diameter*

In some embodiments an outer diameter 202 of the needle cannula 20 is between about 0.3mm and about 0.5mm, between about 0.35mm and about 0.45mm, between about 0.40mm and about 0.42mm, or about 0.41mm as shown in Figs. 4 and 5.

30 The outer diameter 202 of the needle cannula 20 may be substantially constant between the proximal end 211 and the distal end 231 of the cannula 20. Alternatively, although not shown, in some embodiments the outer diameter 202 of the needle cannula 20 may vary (e.g. taper) along the length of the cannula 20. The outer diameter 202 may continuously vary along the cannula 20 or vary at one or more discrete sections of the cannula 20.

In some embodiments an inner diameter 201 of the needle cannula is between about 0.1mm and 0.3mm, between about 0.15mm and 0.25mm, between about 0.20mm and 0.22mm or about 0.21mm.

5 The inner diameter 201 of the needle cannula 20 may be substantially constant between the proximal end 211 and the distal end 231 of the cannula 20. Alternatively, although not shown, in some embodiments the inner diameter 201 of the needle cannula 20 may vary (e.g. taper) along the length of the cannula 20. The inner diameter 201 may continuously vary along the cannula 20 or vary at one or more discrete sections of the cannula 20, for example.

10 In some embodiments a thickness of a wall of the needle cannula 20 is between about 0.03mm and about 0.17mm, between about 0.05mm and about 0.15mm, between about 0.07mm and about 0.13mm or about 0.1mm.

Needle volume

15 In some embodiments the injection needle comprises a dead volume of between about 0.5 μ L/25.4mm and 1.5 μ L/25.4mm. In some embodiments, the total dead volume may be between about 0.7mm³ and about 0.9mm³, between about 0.76mm³ and about 0.86mm³, between about 0.80mm³ and about 0.82mm³, or about 0.81mm³.

20 *Relative length of sections*

In some embodiments different portions of the needle cannula 20 may have different lengths.

For example, in the embodiment shown in Figures 2 and 3, the length A of the proximal portion 21 is shorter than the length C of the distal portion 23. In some
25 embodiments, a length A of the proximal portion 21 may be less than 90%, less than 80%, less than 70%, less than 60%, or less than 50% of the length C of the distal portion 23.

In some embodiments, and as shown in the embodiments of Figures 2 and 3, the length C of the distal portion 23 may be substantially the same as or greater than the sum of the length A of the proximal portion 21 and the arc length B of the middle portion 22. That is,
30 the length C of the distal portion 23 may be about 50% or more than 50% of the total length of the needle cannula 20 from the proximal end 211 of the proximal portion to the distal end 231 of the distal portion 231.

Needle tip

Preferably a distal tip 27 of the needle cannula 20 is bevelled such that the distal tip 27 is sharp, as shown for example, in Figures 1-5. Without being bound to any one theory, the Applicant believes that the inclusion of a bevelled distal tip 27 facilitates clean penetration of tissue during use, thereby minimising potential leakage of drug product from the tissue following injection and release of the drug product.

As shown in Figures 1 to 3, the orientation of the bevel of the distal tip 27 may be such that the edge of the bevel faces away from an inner bend side of the needle cannula 20 (the inner bend side generally marked by reference IB in Figure 1). For example, the edge of the bevel of the distal tip 27 may face away from a center point of curvature of the arcuate middle portion 22 (the center point of curvature generally marked by reference CP in Figure 1).

As shown in Fig. 4, preferably the distal tip comprises a long bevel. The distal tip is bevelled at an angle α of between about 5° and about 15°, between about 10° and about 12.5°, between about 11° and about 12°, or about 11.5°, for example.

As shown in Figures 2 and 3-5, the bevel length D may be between about 0.5mm and about 3.5mm, between about 1mm and about 3mm, between about 1.5mm and about 2.5mm, or about 2mm, for example.

The distal tip 27 may comprise one or more further features such as additional bevels, lancets or heels.

Needle Materials

The needle cannula 20 of the injection needle 10 described herein may comprise stainless steel which may comprise iron and chromium and/or nickel. The needle cannula 20 may comprise nickel coated stainless steel. The needle cannula may specifically comprise AISI (American Iron and Steel Institute) 304 stainless steel.

The needle cannula 20 may comprise an annealed metal, for example, to improve ductility and/or reduce brittleness. The needle cannula 20 may be annealed before or after bending or the formation of the middle portion 22.

Other materials may be suitable which maintain the integrity of the desired constituents for injection.

Kit

The present disclosure also provides a kit comprising a set of injection devices 200. The injection devices 200 may be provided in an assembled or disassembled state (e.g. with the needle 10 coupled or detached from the pre-filled syringe).

The pre-filled syringes of the injection devices may collectively contain a single therapeutic dose of the therapeutic composition for treatment of a single subject. For example, the single therapeutic dose may comprise: about 1.2×10^{13} vg/subject (low dose); about 3.6×10^{13} vg/subject (medium dose); or about 5.4×10^{13} vg/subject (high dose) of the therapeutic composition designated BB-301 as described herein.

Each of the injection devices may contain at least 200 μ L of the therapeutic composition. For example, each of the injection devices may contain between about 200 μ L and about 2000 μ L, or between about 400 μ L and about 1000 μ L, such as about 200 μ L, about 300 μ L, about 400 μ L, about 500 μ L, about 600 μ L, about 800 μ L, about 1000 μ L, about 1500 μ L or about 2000 μ L of the therapeutic composition. In some examples, the kit comprises two injection devices, each containing between about 200 μ L and about 1000 μ L of the therapeutic composition; and two injection devices, each containing between about 400 μ L and about 2000 μ L of the therapeutic composition. In some examples, the kit comprises two injection devices, each containing between about 200 μ L and about 500 μ L of the therapeutic composition; and two injection devices, each containing between about 400 μ L and about 1000 μ L of the therapeutic composition.

One or more of the injection devices may be configured to dispense the drug product in a series of injections having a 25 μ L injection volume. One or more of the injection devices may be configured to dispense the drug product in a series of injections having a 50 μ L injection volume. One or more of the injection devices may be configured to dispense the drug product in a series of injections having a 75 μ L injection volume.

Alternatively, the kit may comprise a set of the pre-filled syringes 100 and the injection needles 10 may be provided separately.

25 Administration to the pharyngeal muscle and treatment

The present disclosure also provides a method of administering a therapeutic composition to the pharyngeal muscle of a subject. The method of administration preferably comprises directly injecting the therapeutic composition into the pharyngeal muscle of the subject using a bent or curved injection needle. In a particularly preferred embodiment, the method of administration comprises directly injecting a therapeutic composition into the pharyngeal muscle of the subject using an injection needle 10 as described herein.

The pharyngeal muscle is made up of a group of muscles that form the pharynx, which is posterior to the oral cavity, determining the shape of its lumen, and which affect its sound properties as the primary resonating cavity. The pharyngeal muscles (involuntary skeletal) assist with pushing the food into the esophagus. There are two muscular layers of the pharynx: the outer circular layer and the inner longitudinal layer. The outer circular layer

includes: the inferior pharyngeal constrictor muscle, the middle pharyngeal constrictor muscle and superior pharyngeal constrictor muscle. During swallowing, these muscles constrict to propel the bolus downwards (an involuntary process). The inner longitudinal layer includes: the stylopharyngeus muscle, the salpingopharyngeus muscle, and the palatopharyngeus muscle. During swallowing, these muscles act to shorten and widen the pharynx.

The method of the disclosure may comprise administering a therapeutic composition to any one or more of the muscles within the pharyngeal muscle group. For example, the method of the disclosure may comprise directly injecting the therapeutic composition using a bent or curved injection needle into any one or more of the following specific muscles: an inferior pharyngeal constrictor muscle, a middle pharyngeal constrictor muscle, a superior pharyngeal constrictor muscle, a palatopharyngeus muscle, a salpingopharyngeus muscle, a stylopharyngeus muscle, or any combination thereof. In one example, the method comprises administering the therapeutic composition into the middle pharyngeal constrictor muscle and the inferior pharyngeal constrictor muscle.

The therapeutic composition may be administered by direct injection into the pharyngeal muscle (i.e., intramuscular injection) following an incision in a subject's neck which is sufficient to obtain direct access to at least part of the subject's pharyngeal muscle. Accordingly, the method of administration may comprise making one or more incisions (e.g., to the skin) in the subject's neck to provide direct access to the subject's pharyngeal muscle(s) and then administering the therapeutic composition by direct intramuscular injection into the pharyngeal muscle using a bent or curved injection needle (e.g., such as the injection needle 10 described herein). For example, an incision may be made to the right side of the pharyngeal area at the right side of a human or animal body, and the area sufficiently dissected to access at least the right side of one or more of the pharyngeal muscles. The needle cannula may then be inserted directly into the right side of one or more of the pharyngeal muscles. Alternatively, or additionally, an incision may be made to the left side of the pharyngeal area at the left side of a human or animal body, and the area sufficiently dissected to access the left side of one or more of the pharyngeal muscles. The needle cannula may then be inserted directly into the left side of one or more of the pharyngeal muscles.

Injection of the therapeutic composition described herein may be made directly into one or more locations of a pharyngeal muscle, into one or more pharyngeal muscles, into one or more sides (e.g. right side or left side) of a pharyngeal muscle, or any combination thereof. Injection at multiple locations may assist with increasing pharyngeal muscle tissue transduction. In one example, the therapeutic composition may be injected into the middle pharyngeal constrictor muscle and the inferior pharyngeal constrictor muscle. The direct injection may be applied into specific portions of muscles, such as the thyropharyngeus or cricopharyngeus.

Where it is desirable to inject multiple sites within the pharyngeal muscle and/or to administer multiple doses, the curved or bent injection needle (e.g., the injection needle 10 described herein) may be used with a pre-filled syringe configured to deliver multiple doses or volumes to one or more of the muscles via micro-injection.

5 In some examples, the therapeutic composition which is administered to the pharyngeal muscle using the bent or curved injection needle (e.g., the injection needle 10) in accordance with the disclosure is for treatment or prevention of dysphagia. In one example, the dysphagia is associated with oculopharyngeal muscular dystrophy (OPMD). In accordance with this example, the method may comprise administering a therapeutic composition for
10 treatment or prevention of OPMD (and thereby treat or prevent conditions associated therewith e.g., dysphagia). The therapeutic composition for treatment or prevention of OPMD may be based on a modified adeno-associated virus (AAV) delivery vector comprising a ‘silence and replace’ construct comprising (i) one or more RNAi agents targeting regions of the PABPN1 mRNA transcript which is causative of OPMD, and (ii) a PABPN1 replacement
15 construct for expression of wild-type (functional) human PABPN1 protein having a mRNA transcript which is not targeted by the RNAi agents of the disclosure. Accordingly, the method may comprise administering the modified AAV delivery vector comprising the ‘silence and replace’ construct to the subject’s pharyngeal muscle by direct injection (e.g., intramuscular injection directly into the pharyngeal muscle following an incision to the
20 pharyngeal area to provide access to the pharyngeal muscle as described herein) using a bent or curved injection needle (e.g., the injection needle 10). A therapeutic composition in accordance with this example has been previously described by the applicant in WO2017/177277, WO2018/107228, WO2019/043630, WO2020/077412 and WO2020/172720, the contents of each of which is incorporated herein in their entirety.

25 In one example, the therapeutic composition that is administered directly into the pharyngeal muscle of the subject using a curved or bent injection needle (e.g., the injection needle 10 as described herein) comprises an AAV comprising:

(a) a viral capsid protein from AAV9 comprising a modified subunit 1 (VP1) sequence wherein the amino acids at positions 26, 40, 43, and 44 are modified relative to a
30 corresponding wildtype AAV9 VP1 sequence set forth in SEQ ID NO: 17; and

(b) a ‘silence and replace’ construct comprising (i) a DNA-directed RNAi (ddRNAi) construct comprising a nucleic acid comprising a sequence which encodes a short hairpin micro-RNA (shmiR); and (ii) a PABPN1 construct comprising a nucleic acid comprising a sequence encoding a functional PABPN1 protein having a mRNA transcript which is not
35 targeted by the shmiR(s) encoded by the ddRNAi construct. For example, the modified AAV9 VP1 sequence may comprise a glutamic acid at position 26, an arginine at position 40, an aspartic acid at position 43, and a serine at position 44 relative to the AAV9 VP1 sequence set forth in SEQ ID NO: 17. For example, the modified AAV9 VP1 sequence may comprise the

following modifications A26E, Q40R, K43D, and A44S relative to the sequence set forth in SEQ ID NO: 17. In one example, the modified AAV9 VP1 sequence comprises the sequence set forth in SEQ ID NO: 27. In one example, the viral capsid protein comprises mutations A67E, Q81R, K84D and A85S with respect to the full length wild-type AAV serotype 9 capsid sequence set forth in SEQ ID NO: 19. In one example, the viral capsid protein comprises the amino acid sequence set forth in SEQ ID NO: 28.

In one example, the therapeutic composition that is administered directly into the pharyngeal muscle of the subject using a curved or bent injection needle (e.g., the injection needle 10 as described herein) comprises an AAV comprising:

(a) a viral capsid protein from AAV9 comprising a modified subunit 1 (VP1) sequence wherein the amino acids at positions 1, 26, 40, 43, and 44 are modified relative to a corresponding wildtype AAV9 VP1 sequence set forth in SEQ ID NO: 17; and

(b) a 'silence and replace' construct comprising (i) a DNA-directed RNAi (ddRNAi) construct comprising a nucleic acid comprising a sequence which encodes a short hairpin micro-RNA (shmiR); and (ii) a PABPN1 construct comprising a nucleic acid comprising a sequence encoding a functional PABPN1 protein having a mRNA transcript which is not targeted by the shmiR(s) encoded by the ddRNAi construct. For example, the modified AAV9 VP1 sequence may comprise a serine at position 1, a glutamic acid at position 26, an arginine at position 40, an aspartic acid at position 43, and a serine at position 44 relative to the AAV9 VP1 sequence set forth in SEQ ID NO: 17. For example, the modified AAV9 VP1 sequence may comprise the following modifications A1S, A26E, Q40R, K43D, and A44S relative to the sequence set forth in SEQ ID NO: 17. In one example, the modified AAV9 VP1 sequence comprises the sequence set forth in SEQ ID NO: 18. In one example, the viral capsid protein comprises mutations A42S, A67E, Q81R, K84D and A85S with respect to the full length wild-type AAV serotype 9 capsid sequence set forth in SEQ ID NO: 19. In one example, the viral capsid protein comprises the amino acid sequence set forth in SEQ ID NO: 20.

In another example, the therapeutic composition that is administered directly into the pharyngeal muscle of the subject using a curved or bent injection needle (e.g., the injection needle 10 as described herein) comprises an AAV comprising:

(a) a viral capsid protein from AAV8 comprising a modified subunit 1 (VP1) sequence wherein the amino acids at positions 1, 26, 40, 43, 44 and 64 are modified relative to a corresponding wildtype AAV8 VP1 sequence set forth in SEQ ID NO: 21; and

(b) a polynucleotide sequence comprising (i) a ddRNAi construct comprising a nucleic acid comprising a sequence which encodes a shmiR; and (ii) a PABPN1 construct comprising a nucleic acid comprising a sequence encoding a functional PABPN1 protein having a mRNA transcript which is not targeted by the shmiR(s) encoded by the ddRNAi construct. For example, the modified AAV8 VP1 sequence may comprise a serine at position

1, a glutamic acid at position 26, an arginine at position 40, an aspartic acid at position 43, a serine at position 44 and a lysine at position 64 relative to the AAV8 VP1 sequence set forth in SEQ ID NO: 21. For example, the modified AAV8 VP1 sequence may comprise the following modifications A1S, A26E, Q40R, K43D, A44S and Q64K relative to the sequence set forth in SEQ ID NO: 21. In one example, the modified AAV8 VP1 sequence comprises the sequence set forth in SEQ ID NO: 22. In one example, the viral capsid protein comprises mutations A42S, A67E, Q81R, K84D, A85S and Q105K with respect to the full length wild-type AAV serotype 8 capsid sequence set forth in SEQ ID NO: 23. In one example, the viral capsid protein comprises the amino acid sequence set forth in SEQ ID NO: 24.

10 In each of the foregoing examples, the modified viral capsid protein is a delivery vector for the polynucleotide comprising the ddRNAi construct and the PABPN1 construct. In one example, the polynucleotide sequence comprises, in a 5' to 3' direction, the ddRNAi construct and the PABPN1 construct. In another example, the polynucleotide sequence comprises, in a 5' to 3' direction, the PABPN1 construct and the ddRNAi construct.

15 The polynucleotide may further comprises inverted terminal repeats (ITRs) from an AAV serotype. For example, the ITRs may flank the sequence comprising the ddRNAi construct and PABPN1 construct. In some examples, the ITRs are from an AAV2 serotype. For example, ITRs are from an AAV2 serotype may comprise a 5' ITR sequence set forth in SEQ ID NO: 29 and a 3' ITR sequence set forth in SEQ ID NO: 30. For example, ITRs are from an AAV2 serotype may comprise a 5' ITR sequence set forth in SEQ ID NO: 25 and a 3' ITR sequence set forth in SEQ ID NO: 26. However, other ITR sequence variants, including from AAV2, are known in the art and contemplated herein.

The therapeutic composition that is administered to a subject to treat or prevent dysphagia associated with OPMD in accordance with the administration method of the disclosure comprises an AAV comprising 'silence and replace' construct comprising a PABPN1 construct. In this regard, the AAV provides an agent for replacement of functional PABPN1 protein *e.g.*, to a cell or animal. The functional PABPN1 protein will not be causative of OPMD, nor will it be encoded by a mRNA transcript which is targeted by the shmiR(s) encoded by the ddRNAi construct as described herein which is also comprised within the AAV.

30 In one example, the PABPN1 construct comprises a nucleic acid *e.g.*, such as DNA or cDNA, encoding the functional PABPN1 protein. For example, the nucleic acid encoding the functional PABPN1 protein may be codon optimised *e.g.*, contain one or more degenerate or wobble bases relative to the wild type PABPN1 nucleic acid but which encodes for identical amino acids, so that the corresponding mRNA sequence coding for the functional PABPN1 protein is not recognised by the shmiR(s) encoded and expressed from the ddRNAi construct. For example, a codon optimised nucleic acid encoding the functional PABPN1 protein may comprise one or more degenerate or wobble bases relative to the wild type PABPN1 nucleic

acid within the region targeted by the one or more shmiR encoded and expressed from the ddRNAi construct. In one example, the one or more degenerate or wobble bases resides within a seed region of an effector sequence of a shmiR encoded and expressed from the ddRNAi construct.

5 In one example, a nucleic acid with the PABPN1 construct encoding the functional PABPN1 protein is codon optimised such that its corresponding mRNA sequence is not recognised by the shmiR(s) encoded and expressed from the ddRNAi construct. Preferably, the functional PABPN1 protein encoded by the codon optimised nucleic acid sequence comprises the amino acid sequence set forth in SEQ ID NO: 16 *i.e.*, the amino acid sequence of the wild-
10 type human PABPN1 protein. A skilled person will appreciate that there are a number of nucleotide sequence combinations which may be used to encode functional PABPN1 protein, and the choice of nucleotide sequence will ultimately depend on the effector sequence of the shmiR(s) encoded and expressed from the ddRNAi construct *i.e.*, such that the codon-optimised nucleic acid is not recognised by the shmiR(s). In one example, the PABPN1 construct
15 comprises a nucleic acid comprising the sequence set forth in SEQ ID NO: 15. In one example, the nucleic acid encoding the functional PABPN1 protein may also comprise a Kozak sequence.

In one example, the codon-optimised nucleic acid encoding the functional PABPN1 protein is operably-linked to a promoter suitable for expression of the functional PABPN1 protein. Promoters suitable for expression of the functional PABPN1 protein in muscle may be
20 particularly suitable. One exemplary promoter suitable for use with the nucleic acid encoding the functional PABPN1 protein is a Spc512 promoter. Another exemplary promoter suitable for use with the nucleic acid encoding the functional PABPN1 protein is a CK8 promoter. However, any suitable promoter known in the art may be used.

In one example, the PABPN1 construct and the ddRNAi construct are operably linked
25 to the same promoter within the same polynucleotide *e.g.*, they are both operably linked to a Spc512 promoter. In accordance with this example, a single promoter drives expression of the functional PABPN1 protein and the shmiRs.

The therapeutic composition that is administered to a subject to treat or prevent dysphagia associated with OPMD in accordance with the administration method of the
30 disclosure comprises an AAV comprising a 'silence and replace' construct which itself comprises a DNA-directed RNAi (ddRNAi) construct. The ddRNAi construct comprises a DNA sequence which encodes one or more short hairpin micro-RNA (shmiR) targeting the mRNA transcript of endogenous PABPN1.

The or each shmiR encoded by the ddRNAi construct comprises:
35 an effector sequence of at least 17 nucleotides in length;
an effector complement sequence;
a stem-loop sequence; and

a primary micro RNA (pri-miRNA) backbone;

wherein the effector sequence is substantially complementary to a region of corresponding length in an RNA transcript set forth in SEQ ID NO: 1 or 2. Preferably, the or each effector sequence will be less than 30 nucleotides in length. For example, a suitable effector sequence may be in the range of 17-29 nucleotides in length. In a particularly preferred example, the effector sequence will be 21 nucleotides in length. More preferably, the effector sequence will be 21 nucleotides in length and the effector complement sequence will be 20 nucleotides in length.

In one example, the shmiR encoded by the ddRNAi construct comprises an effector sequence which is substantially complementary to a region of corresponding length in an RNA transcript comprising or consisting of the sequence set forth in SEQ ID NO: 1. A shmiR in accordance with this example is also referred to herein as “shmiR13”. For example, the effector sequence may be substantially complementary to a region of corresponding length in an RNA transcript comprising or consisting of the sequence set forth in SEQ ID NO:1 and contain 4 mismatch bases relative thereto. For example, the effector sequence may be substantially complementary to a region of corresponding length in an RNA transcript comprising or consisting of the sequence set forth in SEQ ID NO: 1 and contain 3 mismatch bases relative thereto. For example, the effector sequence may be substantially complementary to a region of corresponding length in an RNA transcript comprising or consisting of the sequence set forth in SEQ ID NO: 1 and contain 2 mismatch bases relative thereto. For example, the effector sequence may be substantially complementary to a region of corresponding length in an RNA transcript comprising or consisting of the sequence set forth in SEQ ID NO:1 and contain 1 mismatch base relative thereto. For example, the effector sequence may be 100% complementary to a region of corresponding length in an RNA transcript comprising or consisting of the sequence set forth in SEQ ID NO: 1.

In one example, the shmiR encoded by the ddRNAi construct comprises an effector sequence which is substantially complementary to a region of corresponding length in an RNA transcript comprising or consisting of the sequence set forth in SEQ ID NO: 2. A shmiR in accordance with this example is also referred to herein as “shmiR17”. For example, the effector sequence may be substantially complementary to a region of corresponding length in an RNA transcript comprising or consisting of the sequence set forth in SEQ ID NO: 2 and contain 4 mismatch bases relative thereto. For example, the effector sequence may be substantially complementary to a region of corresponding length in an RNA transcript comprising or consisting of the sequence set forth in SEQ ID NO: 2 and contain 3 mismatch bases relative thereto. For example, the effector sequence may be substantially complementary to a region of corresponding length in an RNA transcript comprising or consisting of the sequence set forth in SEQ ID NO: 2 and contain 2 mismatch bases relative thereto. For example, the effector sequence may be substantially complementary to a region of corresponding length in an RNA

transcript comprising or consisting of the sequence set forth in SEQ ID NO: 2 and contain 1 mismatch base relative thereto. For example, the effector sequence may be 100% complementary to a region of corresponding length in an RNA transcript comprising or consisting of the sequence set forth in SEQ ID NO: 2.

5 In accordance with an example in which the effector sequence of the shmiR is substantially complementary to a region of corresponding length in a PABPN1 miRNA transcript described herein and contains 1, 2, 3 or 4 mismatch base(s) relative thereto, it is preferred that the mismatch(es) are not located within the region corresponding to the seed region of the shmiR i.e., nucleotides 2-8 of the effector sequence.

10 In one example, the ddRNAi construct may comprise a DNA sequence encoding a shmiR comprising: (i) an effector sequence which is substantially complementary to the sequence set forth in SEQ ID NO:3 with the exception of 1, 2, 3 or 4 base mismatches, provided that the effector sequence is capable of forming a duplex with a sequence set forth in SEQ ID NO:3; and (ii) an effector complement sequence comprising a sequence which is
15 substantially complementary to the effector sequence. For example, the shmiR encoded by the ddRNAi construct may comprise an effector sequence set forth in SEQ ID NO:4 and an effector complement sequence which is substantially complementary to the sequence set forth in SEQ ID NO:4 and capable of forming a duplex therewith. The effector complement sequence which is substantially complementary to the sequence set forth in SEQ ID NO:4
20 may be the sequence set forth in SEQ ID NO:3. A shmiR in accordance with this example is hereinafter designated "shmiR13".

In one example, the ddRNAi construct may comprise a DNA sequence encoding a shmiR comprising: (i) an effector sequence which is substantially complementary to the sequence set forth in SEQ ID NO:5 with the exception of 1, 2, 3 or 4 base mismatches,
25 provided that the effector sequence is capable of forming a duplex with a sequence set forth in SEQ ID NO:5; and (ii) an effector complement sequence comprising a sequence which is substantially complementary to the effector sequence. For example, the shmiR encoded by the ddRNAi construct may comprise an effector sequence set forth in SEQ ID NO:6 and an effector complement sequence which is substantially complementary to the sequence set forth
30 in SEQ ID NO:6 and capable of forming a duplex therewith. The effector complement sequence which is substantially complementary to the sequence set forth in SEQ ID NO:6 may be the sequence set forth in SEQ ID NO:5. A shmiR in accordance with this example is hereinafter designated "shmiR17".

In one example, at least one shmiR encoded by the ddRNAi construct is:
35 a shmiR comprising an effector sequence set forth in SEQ ID NO: 4 and an effector complement sequence set forth in SEQ ID NO: 3; or

a shmiR comprising an effector sequence set forth in SEQ ID NO: 6 and an effector complement sequence set forth in SEQ ID NO: 5.

In one particular example, the ddRNAi construct encodes a shmiR comprising an effector sequence set forth in SEQ ID NO: 4 and an effector complement sequence set forth in SEQ ID NO: 3; and a shmiR comprising an effector sequence set forth in SEQ ID NO: 6 and an effector complement sequence set forth in SEQ ID NO: 5. For example, the ddRNAi construct may encode a shmiR designated shmiR13 as described herein and a shmiR designated shmiR17 as described herein.

In one example, the or each shmiR comprises, in a 5' to 3' direction:
a 5' flanking sequence of the pri-miRNA backbone;
the effector complement sequence;
the stem-loop sequence;
the effector sequence; and
a 3' flanking sequence of the pri-miRNA backbone.

In another example, the or each shmiR comprises, in a 5' to 3' direction:
a 5' flanking sequence of the pri-miRNA backbone;
the effector sequence;
the stem-loop sequence;
the effector complement sequence; and
a 3' flanking sequence of the pri-miRNA backbone.

Suitable loop sequences may be selected from those known in the art. However, an exemplary stem-loop sequence is set forth in SEQ ID NO: 7.

In one example, the pri-miRNA backbone is a pri-miR-30a backbone. For example, the 5' flanking sequence of the pri-miRNA backbone may be the sequence set forth in SEQ ID NO: 8 and the 3' flanking sequence of the pri-miRNA backbone may be the sequence set forth in SEQ ID NO: 9.

In one example, the ddRNAi construct comprises at least two nucleic acids each encoding a shmiR, wherein each shmiR comprises an effector sequence which is substantially complementary to an RNA transcript corresponding to a PABPN1 protein which is causative of OPMD, and wherein each shmiR comprises a different effector sequence.

In one example, the ddRNAi construct encodes a shmiR comprising an effector sequence which is substantially complementary to a region of corresponding length in an RNA transcript set forth in SEQ ID NO: 1, and encodes a shmiR comprising an effector sequence which is substantially complementary to a region of corresponding length in an RNA transcript set forth in SEQ ID NO: 2. For example, the ddRNAi construct may comprise:

a nucleic acid comprising or consisting of a DNA sequence encoding a shmiR comprising an effector sequence set forth in SEQ ID NO: 4 and an effector complement sequence set forth in SEQ ID NO: 3 (shmiR13); and

- 5 a nucleic acid comprising or consisting of a DNA sequence encoding a shmiR comprising an effector sequence set forth in SEQ ID NO: 6 and an effector complement sequence set forth in SEQ ID NO: 5 (shmiR17).

- 10 An exemplary ddRNAi construct of the disclosure comprises a nucleic acid comprising or consisting of a DNA sequence set forth in SEQ ID NO: 12 (e.g., encoding shmiR13 comprising or consisting of a sequence set forth in SEQ ID NO: 10) and a nucleic acid comprising or consisting of a DNA sequence set forth in SEQ ID NO: 13 (e.g., encoding shmiR17 comprising or consisting of a sequence set forth in SEQ ID NO: 11).

- 15 In any one of the foregoing examples, the ddRNAi construct and the PABPN1 construct may be operably linked to a promoter. In one example, the ddRNAi construct and the PABPN1 construct are operably linked to the same promoter e.g., a muscle-specific promoter. The muscle-specific promoter may be a Spc512 or CK8 promoter. However, other muscle-specific promoters are known in the art and are contemplated for use in conjunction with a ddRNAi construct of the disclosure.

- 20 As described herein, the therapeutic composition that is administered to a subject to treat or prevent dysphagia associated with OPMD may comprise a single polynucleotide (or 'silence and replace' construct) comprising a ddRNAi construct and PABPN1 construct as described herein, packaged within a AAV vector for delivery. That is, the ddRNAi construct and the PABPN1 construct may be provided as a combined DNA construct (also referred to herein as a 'silence and replace' construct or SR construct), which is packaged in a modified AAV as described herein for delivery to a patient according to the administration method described herein. Exemplary 'silence and replace' constructs are described herein.

The 'silence and replace' construct may be packaged in a modified AAV as described herein for delivery to a patient.

- 30 In one example, the 'silence and replace' construct comprises, in a 5' to 3' direction, a muscle-specific promoter e.g., a Spc512 promoter, a PABPN1 construct described herein and a ddRNAi construct as described herein e.g., wherein the ddRNAi construct is positioned in the 3' untranslated region (UTR) of nucleic acid encoding the functional PABPN1 protein. A 'silence and replace' construct in accordance with this example is illustrated in Figure 9A.

An exemplary 'silence and replace' construct in accordance with this example comprises, in a 5' to 3' direction:

- 35 (a) a muscle-specific promoter e.g., Spc512;

(b) a PABPN1 construct as described herein comprising a DNA sequence encoding a functional PABPN1 protein having a mRNA transcript which is not targeted by the shmiRs encoded by the ddRNAi construct; and

- 5 (c) a ddRNAi construct of the disclosure comprising a nucleic acid comprising a DNA sequence encoding shmiR17 as described herein and a nucleic acid comprising a DNA sequence encoding shmiR13 as described herein. In accordance with this example, the 'silence and replace' construct may comprise or consist of the DNA sequence set forth in SEQ ID NO: 14. A 'silence and replace' construct in accordance with this example is illustrated in Figure 9B

10 An exemplary ddRNAi construct encoding shmiR13 and shmiR17 for inclusion in a 'silence and replace' construct of the disclosure comprises: a nucleic acid comprising or consisting of a DNA sequence encoding a shmiR (shmiR13) comprising an effector sequence set forth in SEQ ID NO: 4 and an effector complement sequence which is substantially complementary to the sequence set forth in SEQ ID NO: 4 *e.g.*, an effector complement sequence set forth in SEQ ID NO: 3; and a nucleic acid comprising or consisting of a DNA
15 sequence encoding a shmiR (shmiR17) comprising an effector sequence set forth in SEQ ID NO: 6 and an effector complement sequence which is substantially complementary to the sequence set forth in SEQ ID NO: 6 *e.g.*, an effector complement sequence set forth in SEQ ID NO: 5. For example, the ddRNAi construct in accordance with this example of the 'silence and replace' construct may comprise a nucleic acid comprising or consisting of the DNA sequence
20 set forth in SEQ ID NO: 12 (*e.g.*, encoding shmiR13 comprising or consisting of a sequence set forth in SEQ ID NO: 10), and a nucleic acid comprising or consisting of the DNA sequence set forth in SEQ ID NO: 13 (*e.g.*, encoding shmiR17 comprising or consisting of a sequence set forth in SEQ ID NO: 11).

25 An exemplary PABPN1 construct for inclusion in a 'silence and replace' construct of the disclosure comprises a codon-optimised sequence set forth in SEQ ID NO: 15 and encodes a functional PABPN1 protein set forth in SEQ ID NO: 16.

30 The skilled person will appreciate that the therapeutic composition will be formulated for delivery to a patient *e.g.*, a human patient. Accordingly, the therapeutic composition for administration to the subject in accordance with the method of the disclosure may comprise one or more pharmaceutically acceptable carriers or diluents. For example, the therapeutic composition may comprise a carrier suitable for delivery of the AAVs of the disclosure to muscle of a subject following injection thereto. Carriers suitable for formulation and delivery of AAVs are known in the art and contemplated herein. Suitable pharmaceutical carriers, as well as pharmaceutical necessities for use in pharmaceutical formulations, are described in
35 Remington: The Science and Practice of Pharmacy (formerly Remington's Pharmaceutical Sciences), Mack Publishing Co., a standard reference text in this field, and in the USP/NF.

The volume, concentration, and formulation of the therapeutic composition for administration to the subject in accordance with the method of the disclosure, as well as the dosage regimen may be tailored specifically to maximize cellular delivery while minimizing toxicity, such as an inflammatory response, with corresponding low concentrations of the active agent (e.g., the AAV vector comprising the ‘silence and replace’ construct for treatment of OPMD). An anti-inflammatory compound, such as a corticosteroid, may also be administered (e.g., orally) for systematic distribution.

In accordance with one example, the therapeutic composition for treatment of dysphagia associated with OPMD is administered in a volume ranging from about 25µl to about 75µl per injection site. For example, the therapeutic composition may be administered in a volume of about 25µl per injection site, or about 30µl per injection site, or about 35µl per injection site, or about 40µl per injection site, or about 45µl per injection site, or about 50µl per injection site, or about 55µl per injection site, or about 60µl per injection site, or about 65µl per injection site, or about 70µl per injection site, or about 75µl per injection site.

In another example, the therapeutic composition may be administered in a volume ranging from about 50µl to about 75µl per injection site. For example, the therapeutic composition may be administered in a volume of about 50µl per injection site, or about 55µl per injection site, or about 60µl per injection site, or about 65µl per injection site, or about 70µl per injection site, or about 75µl per injection site.

The volume of therapeutic composition administered per injection site may be the volume of a single injection, or the cumulative volume of a plurality of injections administered at the one injection site. For example, an injection volume of about 75 µL per injection site may be administered as three injections of 25 µL per injection site, or as one injection of 25 µL and one injection of 50 µL.

In each of the foregoing examples describing administration of the therapeutic composition at multiple injection sites, the total dose administered to the subject in a single treatment may be about $1.5E^{+12}$ vg to about $9.5E^{+13}$ vg. For example, the total dose of the therapeutic composition administered to the subject may be between about $5.0E^{+12}$ vg to about $6.0E^{+13}$ vg. For example, the total dose of the therapeutic composition administered to the subject may be between about $1.0E^{+13}$ vg to about $5.5E^{+13}$ vg. In some examples, the total dose of the therapeutic composition administered is about $1.0E^{+13}$ vg/subject. In some examples, the total dose of the therapeutic composition administered is about $1.2E^{+12}$ vg/subject. In some examples, the total dose of the therapeutic composition administered is about $3.6E^{+13}$ vg/subject. In some examples, the total dose of the therapeutic composition administered is about $5.4E^{+13}$ vg/subject.

Accordingly, where the therapeutic composition is administered in a volume ranging from about 25µl to about 75µl per injection site, the therapeutic composition may be

administered at a concentration between about 1.0×10^{12} vg/mL and about 1.0×10^{15} vg/mL. For example, the concentration of the therapeutic composition which is administered to the subject may be between about 5.0×10^{12} vg/mL and about 1.0×10^{14} vg/mL, such as between about 5.0×10^{12} vg/mL and 5.0×10^{13} vg/mL.

5 As described herein, it is desirable to inject the therapeutic composition at multiple sites within the pharyngeal muscle group and/or to administer multiple doses to one or more of the muscles within the pharyngeal muscle group, including administering the therapeutic composition to one or more locations of a pharyngeal muscle, to one or more pharyngeal muscles, to one or more sides (e.g. right side or left side) of a pharyngeal muscle, or any
10 combination thereof. Injection at multiple locations may assist with improving delivery to pharyngeal muscle tissue. For example, the method may comprise injecting the therapeutic composition at about 2 to about 30 or more sites within a pharyngeal muscle. In accordance with an example in which the multiple injection sites are split between the left and right sides of the median raphe of the pharyngeal muscle, the method may comprise injecting the
15 therapeutic composition at about 1 to about 15 or more sites (e.g., 1, or 2, or 3, or 4, or 5, or 6, or 7, or 8, or 9, or 10, or 11, or 12, or 13, or 14, or 15 or more sites) within the muscle on the left side of median raphe of the pharyngeal muscle and about 1 to about 15 or more sites (e.g., 1, or 2, or 3, or 4, or 5, or 6, or 7, or 8, or 9, or 10, or 11, or 12, or 13, or 14, or 15 or more sites) within the muscle on the right side of median raphe of the pharyngeal muscle. For
20 example, the method may comprise injecting the therapeutic composition at about 1 to about 15 or more sites (e.g., 1, or 2, or 3, or 4, or 5, or 6, or 7, or 8, or 9, or 10, or 11, or 12, or 13, or 14, or 15 or more sites) on the left side of median raphe of the thyropharyngeal (TP) muscle and about 1 to about 15 or more sites (e.g., 1, or 2, or 3, or 4, or 5, or 6, or 7, or 8, or 9, or 10, or 11, or 12, or 13, or 14, or 15 or more sites) within the muscle on the right side of median
25 raphe of the thyropharyngeal (TP) muscle. Alternatively, or in addition, the method may comprise injecting the therapeutic composition at about 1 to about 15 or more sites (e.g., about 4 sites) on the left side of median raphe of the hypopharyngeal (HP) muscle and about 1 to about 15 or more sites (e.g., about 4 sites) within the muscle on the right side of median raphe of the hypopharyngeal (HP) muscle. In accordance with one particular example in
30 which the therapeutic composition is administered to the HP and TP muscles, the composition may be injected at 4 sites on the left side of median raphe of the HP muscle, at 4 sites on the right side of median raphe of the HP muscle, at 8 sites on the left side of median raphe of the TP muscle, and at 8 sites on the right side of median raphe of the TP muscle.

 The method may also comprise injecting the therapeutic composition multiple times
35 to more than one pharyngeal muscle. Different pharyngeal muscles are described and contemplated herein. However, in one example, the therapeutic composition may be injected at multiple sites into the middle pharyngeal constrictor muscle and at multiple sites into the

inferior pharyngeal constrictor muscle. The direct injection may also be applied to multiple sites of other pharyngeal muscles, such as the thyropharyngeus or cricopharyngeus.

As described herein, the therapeutic composition may be administered by direct injection into the pharyngeal muscle (i.e., intramuscular injection) following an incision in a subject's neck which is sufficient to obtain direct access to at least part of the subject's pharyngeal muscle. Accordingly, the method of treating dysphagia associated with OPMD in a subject suffering from OPMD may comprise making one or more incisions (e.g., to the skin) in the subject's neck to provide direct access to the subject's pharyngeal muscle(s) and then administering the therapeutic composition as described herein by direct intramuscular injection into the pharyngeal muscle using a bent or curved injection needle (e.g., such as the injection needle 10 described herein). For example, an incision may be made to the right side of the pharyngeal area at the right side of a human or animal body, and the area sufficiently dissected to access at least the right side of one or more of the pharyngeal muscles. The needle cannula may then be inserted directly into the right side of one or more of the pharyngeal muscles. Alternatively, or additionally, an incision may be made to the left side of the pharyngeal area at the left side of a human or animal body, and the area sufficiently dissected to access to the left side of one or more of the pharyngeal muscles. The needle cannula may then be inserted directly into the left side of one or more of the pharyngeal muscles.

20

Examples

EXAMPLE 1: DYE STUDY

In this study, the inventors evaluated (1) the maximal volume that can be injected into hypopharyngeal and thyropharyngeal muscles in Beagle dogs without observable leakage of the injected material, using Trypan blue (Gibco #15250-061), and (2) different injection needles to identify a delivery device suitable for delivery of the applicant's OPMD 'silence and replace' therapeutic agent "BB-301" without observable leakage of the therapeutic composition. Beagle dogs were selected as an animal model due to comparable muscles and muscles sizes to humans.

30

Animals

Two female beagles, Rosette and Rillette, aged 4 years 10 months and 4 years 9 months respectively, were included in this study, which took place at the Boisbonne Center (large animal facility) in June 2020. Both animals were sacrificed at the completion of the study.

35

Needles

In this study, two different injection needles, “Needle A” and “Needle B” were evaluated for their ability to administer Trypan blue to the hypopharyngeal, thyropharyngeal and cricopharyngeal muscles of the animals, and to affect delivery of the intended volumes of Trypan blue without the observation of post-injection leakage of Trypan blue from the injected muscles.

Needle A was configured generally in accordance with the injection needle 10 described herein, and included a 3Dose Next Generation BoNT dosing syringe from VlowMedical (Cat No. 3DOSE-GREEN-125) coupled with a 27G, 19 mm needle, having a bent, arcuate, middle portion, such that proximal and distal portions of the needle extend at 90 degrees to each other.

Needle B also included a 3Dose Next Generation BoNT dosing syringe from VlowMedical (Cat No. 3DOSE-GREEN-125), but coupled with a 25G, 19-22 mm nucleous hydrolysis needle from Accuspire (Cat No. J075-25G), generally as illustrated in Figs. 7A and 7B.

Figure 7A shows an injection needle 400, configured generally in accordance with Needle B, comprising a needle cannula 420 and hub 430. As shown in Figure 7A, the needle cannula 420 has a substantially straight portion proximal the hub (proximal portion 421), while a middle portion 422 and a distal portion 423 are curved. It can be seen that a radius of curvature is large and an angle of curvature θ between the proximal and distal portions 421, 423 is approximately 160° . Figure 7B shows that distal portion 423 of cannula 420 has a flattened oval shaped tip 427.

*Surgery and dye injection**“Rillette”*

Surgical procedures were conducted under general anesthesia with appropriate analgesia employed, and all surgical procedures were performed by a veterinary surgeon. A single incision was performed at the level of the right side of the pharyngeal area, and the area was dissected to provide access to the sections of the HP and TP muscles on the right side of the median raphe. Upon exposure of the HP and TP muscle sections on the right side of the median raphe, Trypan blue dye was administered at 75 μ l per injection via multiple direct intramuscular injections (for 4, 5 or 9 discrete injection locations) using a 3 Dose Unit Injector device (VlowMedical, Netherlands) to control the volume of each injection. Dye was aspirated into the micro-injection syringe using a 21G, 2-inch needle from BD (Cat # 301155), and injections were performed using 27G, 19 mm needles, angled at 90 degrees from Hamilton (p/n 10101008, N (27/19/4x2/A90). A surgical microscope was utilized to perform all injections.

A summary of the Trypan blue dye injections performed into the HP and TP muscle sections on the right side of the median raphe and the cricopharyngeal (CP) muscle is presented in **Table 1** below.

5 Post-dye administration, the muscle sections were evaluated for leakage of dye by visual inspection.

10 Approximately 5 minutes post-injection, the surgical incision was closed with staples, and the dog was turned to access the left side of the pharyngeal area. A single incision was then performed at the level of the left side of the pharyngeal area, and the area was dissected to provide access to the sections of the HP and TP muscles on the left side of the median raphe. Upon exposure of the HP and TP muscles on the left side of the median raphe, Trypan blue dye was administered via multiple direct intramuscular injections (25 µl, 50 µl or 75 µl per injection for 1, 2 or 4 discrete injection locations) using a 3 Dose Unit Injector device (VlowMedical, Netherlands) to control the volume of each injection. Dye was aspirated into the micro-injection syringe using a 21G, 2-inch needle from BD (Cat # 301155), and injections were performed using 27G, 19 mm needles, angled at 90 degrees from Hamilton (p/n 10101008, N (27/19/4x2/A90) or 25G, 22 mm nucleous hydrolysis needles from Accuspire (Cat # J075-25G). A surgical microscope was utilized to perform all injections.

20 A summary of the Trypan blue dye injections performed into the HP and TP muscle sections on the left side of the median raphe and the CP muscle is presented in **Table 1** below.

Post-dye administration, the muscle sections were evaluated for leakage of dye by visual inspection.

25 Approximately 5 minutes post-injection, the surgical incision was closed with staples, and whole blood samples were obtained on EDTA-coated tubes (total of 20 mL aliquoted in 2 x 10 mL and frozen at <-70°C).

Subsequently, the dog was sacrificed by lethal intravenous injection of Pentobarbital at a dose of 1 mL/kg (180 mg/kg).

Table 1 - Dye Injection performed on “Rillette”

Muscle	Position	Needle	Volume per site (µl)	No. of injections	Comments and Results
TP	Right	A	75	9	No major leakage. Lateral TP not totally stained by the dye
HP	Right	A	75	5	No major leakage. Whole HP stained by the dye.
CP	Right	A	75	4	Muscle was accessible and tested for injection. No major leakage.
TP	Left	B	25	2	High leakage via the puncture sites. Whole TP stained by the dye.
TP	Left	B	50	2	High leakage via the puncture sites. Whole HP stained by the dye.
HP	Left	B	50	2	High leakage via the puncture sites. Whole HP stained by the dye.
HP	Left	A		4	No leakage. Whole HP stained by the dye.
CP	Left	A		2	Muscle was accessible and tested for injection. No major leakage.

HP: hypopharyngeal muscle. TP: thyropharyngeal muscle. CP: cricopharyngeal muscle.

- 5 Following animal sacrifice, the entire pharyngeal area (including trachea and esophagus + the tongue) was dissected.

Left and right sections of the HP and TP muscles, the CP muscle and the Lateral Digital Extensor muscles were finely dissected, measured and weighed (length, width, depth). Each muscle was, then, placed flatly into a histological cassette, frozen directly in liquid
10 nitrogen, and then stored at <-70°C. Of note, it was not possible to mark the left and right sections of the muscles. The characteristics of each of the muscle samples are summarized in **Table 3** below.

A liver sample was also harvested, cut into small pieces, and placed in a total of 15 DNase-free, RNase-free and PCR clean microtubes, frozen directly in liquid nitrogen, and
15 then stored at <-70°C.

The following observations were made:

Except for the right section of the TP, which was only partially stained, the TP, HP and CP muscle sections were completely stained with Trypan blue dye.

After evaluating the muscles of the pharyngeal area in their entirety, some remaining
20 dye staining was observed at the level of cartilage and also at the level of the esophagus wall. These observations could be explained by the higher injection volumes of Trypan blue dye

employed for RILLETTE relative to the volumes administered to ROSETTE in addition to the enhanced level of dye leakage observed for RILLETTE relative to that recorded for ROSETTE.

5 “Rosette”

Surgical procedures were conducted under general anesthesia with appropriate analgesia employed, and all surgical procedures were performed by a veterinary surgeon.

10 A single incision was performed at the level of the left side of the pharyngeal area, and the area was dissected to provide access to the sections of the hypopharyngeal (HP) and thyropharyngeal (TP) muscles on the left side of the median raphe.

Upon exposure of the HP and TP muscle sections on the left side of the median raphe, Trypan blue dye was administered at 50 µl per injection via multiple direct intramuscular injections (for 4 or 8 discrete injection locations) using a 3 Dose Unit Injector device (VlowMedical, Netherlands) to control the volume of each injection. Dye was
15 aspirated into the micro-injection syringe using a 21G, 2-inch needle from BD (Cat # 301155), and intramuscular injections were performed using 27G, 19 mm needles, angled at 90 degrees from Hamilton (p/n 10101008, N (27/19/4x2/A90). A surgical microscope was utilized to perform all injections.

20 A summary of the Trypan blue dye injections performed into the HP and TP muscle sections on the left side of the median raphe is presented in **Table 2** below.

Post-dye administration, the muscle sections were evaluated for leakage of dye by visual inspection.

Approximately 5 minutes post-injection, the surgical incision was closed with staples, and the dog was turned to access the right side of the pharyngeal area.

25 A single incision was then performed at the level of the right side of the pharyngeal area, and the area was dissected to provide access to the sections of the HP and TP muscles on the right side of the median raphe.

Upon exposure of the HP and TP muscle sections on the right side of the median raphe, Trypan blue dye was administered via multiple direct intramuscular injections (50 µl or
30 100 µl per injection for 2, 3 or 4 discrete injection locations) using a 3 Dose Unit Injector device (VlowMedical, Netherlands) to control the volume of each injection. Dye was aspirated into the micro-injection syringe using a 21G, 2-inch needle from BD (Cat # 301155), and injections were performed using 27G, 19 mm needles, angled at 90 degrees from Hamilton (p/n 10101008, N (27/19/4x2/A90). A surgical microscope was utilized to
35 perform all injections.

A summary of the Trypan blue dye injections performed into the HP and TP muscle sections on the right side of the median raphe and the cricopharyngeal (CP) muscle is presented in **Table 2** below.

5 Post-dye administration, the muscle sections were evaluated for leakage of dye by visual inspection.

Approximately 5 minutes post-injection, the surgical incision was closed with staples, and whole blood samples were obtained on EDTA-coated tubes (total of 24 mL aliquoted in 2 x 10 mL + 1 x 4 mL and frozen at $<-70^{\circ}\text{C}$).

10 Subsequently, the dog was sacrificed by lethal intravenous injection of Pentobarbital at a dose of 1 mL/kg (180 mg/kg).

Following animal sacrifice, the entire pharyngeal area (including trachea and esophagus + the tongue) was dissected.

15 Left and right sections of the HP and TP muscles, as well as the Lateral Digital Extensor muscles, were finely dissected, measured and weighed (length, width, depth). Each muscle was, then, placed flatly into a histological cassette, frozen directly in liquid nitrogen, and then stored at $<-70^{\circ}\text{C}$. Of note, it was not possible to mark the left and right sections of the muscles. The characteristics of each of the muscle samples are summarized in **Table 4** below.

20 A liver sample was also harvested, cut into small pieces, and placed in a total of 15 DNase-free, RNase-free and PCR clean microtubes, frozen directly in liquid nitrogen, and then stored at $<-70^{\circ}\text{C}$.

The following observations were made:

The entire HP and TP muscle sections were completely stained with the Trypan blue dye.

25 The left CP muscle section was not stained, demonstrating that there was no leakage between the distinct muscles of the pharyngeal anatomical region and that the diffusion of dye was limited to the injected pharyngeal muscles.

30 After evaluating the muscles of the pharyngeal area in their entirety, some remaining dye staining was observed only at the level of cartilage. No dye staining was observed at the levels of the other tissues, including the esophagus and trachea.

Table 2 - Dye Injection performed in the dog 2.

Muscle	Position	Needle	Volume per site (µl)	No. of injections	Results
TP	Left	A	50	8	The needle is adapted. No major leakage. Whole TP stained by the dye
HP	Left	A	50	4	No major leakage. Whole HP stained by the dye.
TP	Right	A	50	3	No major leakage. Whole TP stained by the dye
TP	Right	A	100	3	High leakage via the puncture sites. Whole TP stained by the dye
HP	Right	A	100	4	High leakage via the puncture sites. Whole HP stained by the dye
CP	Right	A	50	2	Muscle was accessible and tested for injection. Few leakages.

HP: hypopharyngeal muscle, TP: thyropharyngeal muscle. CP: cricopharyngeal muscle.

5 **Table 3: Measures performed on the muscles sampled from “RILLETTE” .**

Muscle	Position	Weight (g)	Length (mm)	Width at the middle level (mm)	Depth (mm)
TP	Left	1.74	35	12	4
HP	Left	1.34	36	12	3.5
Lateral Digital Extensor	Left	9.83	75	22	13
HP	Right	1.76	35	15	4
TP	Right	1.17	32	13	4
Lateral Digital Extensor	Right	10.31	85	22	11
CP	Right + Left	2.17	/	/	/

HP: hypopharyngeal muscle, TP: thyropharyngeal muscle.

Table 4: Measures performed on the muscles sampled from “ROSETTE” .

Muscle	Position	Weight (g)	Length (mm)	Width at the middle level (mm)	Depth (mm)
TP	Left	1.86	44	10	2
HP	Left	1.06	38	12	2
Lateral Digital Extensor	Left	8.04	95	19	9
HP	Right	1.02	29	12	3
TP (+ maybe a little piece of CP)	Right	1.50	34	20	2
Lateral Digital Extensor	Right	8.10	95	13	9

HP: hypopharyngeal muscle, TP: thyropharyngeal muscle.

5 Conclusions

From this Dye Study conducted on two Beagle dog subjects, the following information was obtained:

- No issues were encountered with the surgical approach and the dissection of the pharyngeal region. The bilateral surgical approach (on the left and right sides of the pharyngeal region) is ideally suited for use in the dosing protocol to facilitate better visualization of the discrete intramuscular injections into each section of the target pharyngeal muscles.
- As expected, the pharyngeal muscles of interest are small and very thin (approximately 4 to 5 cm², 2 to 3 mm depth).
- The VlowMedical micro-injection syringes are ideally suited to the dosing process and are very easy to use.
- It is unequivocal that Needle A is most ideally suited for the injection process. By contrast, Needle B is not sufficiently angulated or beveled. As a consequence, extensive leakage from the puncture site was observed, even when injecting only 25 μL of Trypan blue dye.
- The maximal fluid volume that can be administered per injection site without prompting significant leakage is in the range of 50 μL to 75μL. To limit the risk of leakage while removing the injection needle from the target muscle, pausing for

approximately 30 seconds at the conclusion of each injection prior to withdrawing the needle from the muscle is recommended.

- As no leakage of dye was observed upon injecting 50 μ L of Trypan blue, an injection volume of 25 μ L of dye was not evaluated. As significant leakage of dye was observed upon injecting 100 μ L of Trypan blue, an injection volume of 150 μ L of dye was not evaluated. However, an intermediate volume of 75 μ L of Trypan blue dye was evaluated. No significant leakage was observed with this intermediate volume.
- The appropriate number of total injection sites in the sections of the TP muscle on the left or the right side of the median raphe is 8 injection sites (with a total of 16 injection sites for the whole TP muscle), and the appropriate number of total injection sites in the sections of the HP muscle on the left or the right side of the median raphe is 4 injections (with a total of 8 injection sites for the whole muscle). Due to the variability in muscle sizes between dogs, the appropriate number of total injection sites could, potentially, be increased for some animals.
- The cricopharyngeal muscle was also accessible via the surgical approach.
- Observation of the diffusion of the Trypan blue dye for 30 minutes post-injection was not possible as the animal subjects had to be turned to access the contralateral pharyngeal muscles for dye injection. However, the diffusion patterns of the dye were observed following sacrifice of the animal subjects and complete dissection of the pharyngeal area. The diffusion of dye was almost complete in the injected muscles, and the diffusion of dye was essentially limited to the injected muscles.

EXAMPLE 2: PILOT DOSING STUDY FOR ‘SILENCE AND REPLACE’ CONSTRUCT BB-301

- BB-301 is a first-in-class genetic medicine employing the “silence and replace” approach for the treatment of dysphagia associated with OPMD. Specifically, BB-301 relies on the applicant’s proprietary DNA-directed RNA interference (ddRNAi) platform, which combines RNA interference (RNAi) with classical AAV-based gene therapy. The applicant’s ddRNAi platform also allows for concomitant delivery of wild type replacement genes, and these distinct genetic elements work in concert to silence the expression of disease-causing mutant genes via RNAi and to simultaneously replace the mutant genes with normal (wild type) genes to restore the natural underlying physiology of the diseased tissues.

- BB-301 and its components have been previously described by the present applicant in WO2017/177277, WO2018/107228, WO2019/043630, WO2020/077412 and WO2020/172720, the contents of each of which is incorporated herein in their entirety. BB-301 is also described herein.

BB-301 is directly injected into the pharyngeal muscles known to underlie the morbidity and mortality characterizing the natural history of dysphagia associated with OPMD. This BB-301 Pilot Dosing Study was conducted in large animal subjects, specifically Beagle dogs, to demonstrate that direct intramuscular injection of BB-301 via the use of the injection needle 10 described herein in an open surgical procedure could safely achieve the following:

- Biologically significant, highly-consistent, dose-dependent levels of BB-301 tissue transduction (i.e., delivery of the multi-functional genetic construct into the target pharyngeal muscle cells);
- Durable, broad-based, dose-dependent expression within the pharyngeal muscle cells of the three distinct genes comprising the BB-301 gene construct; and
- Durable and biologically significant levels of target gene knock-down (i.e., inhibition of the expression of the gene of interest) within the pharyngeal muscle cells.

15 *BB-301*

BB-301 incorporates Benitec's proprietary AAV9 delivery vector comprising (i) a ddRNAi construct comprising two nucleic acids encoding short hairpin microRNAs (shmiRs) designated shmiR13 and shmiR17 (described herein) which target the mRNA transcript of the mutant PABPN1 which is causative of OPMD, and (ii) a PABPN1 construct comprising a codon optimized nucleic acid encoding functional (wildtype) PABPN1 protein, wherein the mRNA corresponding to the codon optimised nucleic acids is not targeted by the shmiRs. Two distinct siRNA species (i.e. siRNA13 and siRNA17) are processed from the shmiRs, each of which is independently capable of inhibiting (i.e. "silencing") the expression of all forms of the endogenous PABPN1 protein (siRNA13 and siRNA17 silence the expression of both wild type PABPN1 [wtPABPN1] and mutant PABPN1 which is causative of OPMD). The mutant form of the PABPN1 protein underlies the development and progression of OPMD. By contrast, the codon optimised sequence within BB-301 which codes for the functional wild type version of the PABPN1 protein is unaffected by the inhibitory activities of siRNA13 and siRNA17. This codon optimized PABPN1 protein (i.e. coPABPN1) serves to replenish the endogenous form of the PABPN1 protein and to replace the mutant form of PABPN1 that underlies the development and progression of OPMD in diseased tissues.

Pilot Dosing Study for BB-301

A Pilot Dosing Study was conducted to evaluate the safety and biological activity of two concentrations of BB-301 across three different doses, as well as to optimise the method of administration for BB-301 as developed and described in Example 1 herein.

5 Briefly, two different concentrations (1.0×10^{13} vg/mL and 3.0×10^{13} vg/mL) across three distinct doses (1.0×10^{13} vg/mL, 3.0×10^{13} vg/mL with a low injection volume, and 3.0×10^{13} vg/mL with a high injection volume) were tested via direct intramuscular injection into the Hypopharyngeus (HP) muscles and the Thyropharyngeus (TP) muscles of Beagle dogs via the use of the injection needle 10 described herein via an open surgical procedure. As
10 described in Example 1, the HP muscle in Beagle dogs corresponds to the Middle Pharyngeal Constrictor muscle in Human subjects, and the TP muscle in Beagle dogs corresponds to the Inferior Pharyngeal Constrictor muscle in human subjects. Beagle dogs were also selected as a large animal model due to comparable muscles sizes to humans. The surgical procedure used to administer BB-301 to the Beagles is as described above in Example 1 using Needle A
15 (which is the injection needle 10 described herein).

BB-301 was injected only on Day 1 of the Pilot Dosing Study, and the corresponding canine pharyngeal muscles were harvested for analysis 8 weeks after delivery of BB-301.

BB-301 dosing was carried out by both a veterinary surgeon and a practicing Otolaryngologist who has extensive experience with the provision of palliative surgical care
20 for OPMD patients.

Interim data-points were collected from completed analyses of pharyngeal muscle tissues isolated from the 16 Beagle dog subjects to date (of the 24-subject study population).

It is critical to highlight the key methodological distinctions between the current BB-301 Pilot Dosing Study in Beagle dogs conducted by Benitec and the prior Beagle dog dosing
25 study carried out independently by the previous licensee of BB-301. Specifically, the BB-301 dosing study conducted by the prior BB-301 licensee employed non-ideal routes and methods of administration to the target pharyngeal muscle tissues and employed similarly limited analytical methods at the completion of the dosing phase of the study. In the current study, the inventors worked to optimize the route and method of administration of BB-301 to the
30 pharyngeal muscle (as described in Example 1) and to refine the core analytical methods employed following the completion of dosing.

Results

The results of the Pilot Dosing Study are presented in Table 5 below.

Table 5 – Results of the Pilot Dosing Study

Muscle	Dose	Pharyngeal Muscle Tissue Transduction Levels for BB-301 (copies per cell)	Gene Expression Levels for BB-301 within the Pharyngeal Muscle Tissues (copies per cell)	Wild Type PABPN1 Silencing (percentage inhibition)
HP	Low	1.7	siRNA13 – 161,358 siRNA17 – 26,652 coPABPN1 – 21	74%
HP	Intermediate	3.5	siRNA13 – 256,928 siRNA17 – 47,944 coPABPN1 – 24	80%
HP	High	7.2	siRNA13 – 374,324 siRNA17 – 57,126 coPABPN1 – 52	78%
TP	Low	2.0	siRNA13 – 195,182 siRNA17 – 40,106 coPABPN1 – 15	72%
TP	Intermediate	2.3	siRNA13 – 293,597 siRNA17 – 57,969 coPABPN1 – 43	61%
TP	High	8.6	siRNA13 – 751,484 siRNA17 – 173,211 coPABPN1 – 100	79%

5 Hypopharyngeus (HP) muscles and the Thyropharyngeus (TP) muscles.

Low dose = (1.0E+13 vg/mL)

Intermediate dose = (3.0E+13 vg/mL, low volume)

High dose = (3.0E+13 vg/mL, high volume)

10 As shown in Table 5, administration of BB-301 to the pharyngeal muscles using the open surgical procedure and injection needle 10 described herein achieved good transduction to the pharyngeal muscle across all doses and concentrations evaluated. Of particular note, there was a 248-fold improvement (+24,650%) in BB-301 transduction of the HP muscle and a 111-fold improvement (+11,027%) in BB-301 transduction of the TP muscle in the present dosing study relative to the levels of BB-301 transduction observed in the previous BB-301 preliminary study performed by the previous licensee of BB-301.

20 Collectively, these data support the conclusion that the open surgical procedure and injection needle 10 described herein are effective for administration of BB-301 (or any therapeutic) into the pharyngeal muscles, as well as demonstrating biologically significant, highly-consistent, dose-dependent levels of BB-301 tissue transduction (i.e. delivery of the multi-functional genetic construct into the target pharyngeal muscle cells). The study also showed durable, broad-based, dose-dependent expression within the pharyngeal muscle cells of the three distinct nucleic acids comprised within the BB-301 gene construct (i.e. shmiR13/siRNA13, shmiR17/siRNA17, and codon optimized PABPN1) and durable and

biologically significant levels of target gene knock-down (i.e. inhibition of the expression of the gene of interest) within the pharyngeal muscle cells.

EXAMPLE 3: FIRST-IN-HUMAN EFFICACY STUDY

5 A First-in-Human Phase 1b/2a clinical trial has been initiated for the evaluation of the safety and efficacy of BB-301 in subjects diagnosed with Oculopharyngeal Muscular Dystrophy (OPMD). An Investigational New Drug (IND) application for BB-301 was approved to proceed by the United States Food and Drug Administration (FDA) in June 2023, and a first study subject was safely dosed in the BB-301 Phase 1b/2a clinical trial
10 (NCT06185673) in November 2023. Details of NCT06185673 are available at ClinicalTrials.gov and are incorporated by reference herein.

 The clinical trial (NCT06185673) is a 3+3 dose escalation study designed to evaluate three discrete doses of BB-301 to identify the Maximum Tolerated Dose (MTD). The Phase 1b component of the study has been designed to treat between 9-18 OPMD subjects in the
15 three dose escalation cohorts. The Phase 2a component of the study has been designed to treat up to 12 OPMD subjects at the MTD. Study subjects will be monitored for 52 weeks following the administration of BB-301 to assess the efficacy of the investigational gene therapy, and safety evaluations will be conducted for 15 years following the receipt of BB-301 by each subject. The design of this first-in-human clinical trial is summarised in Table 6
20 below.

Table 6: Design of first in-human clinical trial for BB-301.

OPMD Natural History Study	Phase 1b	Phase 2a
Minimum of 6 months	About 12 months	
Comprises about 5 pre-dose clinical visits where pre-dose dysphagia assessments are conducted to identify rates of progression of dysphagia in OPMD subjects, and to determine averages and final values for each assessment.	About 9 to 18 OPMD subjects will be involved in a three dose escalation study to evaluate three discrete doses of BB-301 to identify the Maximum Tolerated Dose.	Up to 12 OPMD subjects will be treated at the Maximum Tolerated Dose.
	Postdose comparative assessments are carried out at follow-up visits for each subject at Day 90, Day 180, Day 270 and Day 360.	

To date, two subjects have progressed from the Natural History Study to the Phase 1b/2a clinical trial. At the time of writing, interim clinical safety data and interim clinical efficacy data are available for the first study subject from the first, protocol-mandated post-dosing assessment time-point which was conducted 90 days following the administration of BB-301. See *Results* section below.

OPMD Natural History Study

Phase 1b/2a clinical study eligibility requirements necessitate confirmation of a pathogenic triplet expansion of PABPN1. Prior to the conduct of direct intramuscular injections of BB-301 into the pharyngeal constrictor muscles, each study subject is required to complete a minimum of six months of evaluation in an OPMD Natural History Study during which a baseline level of dysphagia is assessed with quantitative, x-ray based, videofluoroscopic swallowing studies at five discrete time-points. Following the month-6 visit in the OPMD Natural History Study, subjects then become eligible to screen for entry into the BB-301 Phase 1b/2a clinical trial (NCT06185673). The results of each assessment performed during the OPMD Natural History Study provide a baseline against which the results of corresponding assessments may be compared following administration of BB-301 in the Phase 1b/2a clinical trial, thereby providing an indication of efficacy and safety of the BB-301 OPMD gene therapy.

Assessment criteria

Dysphagia (i.e., swallowing difficulty) is assessed at all timepoints for study subjects (i.e., during the OPMD Natural History Study and in the Phase 1b/2a clinical trial following administration of BB-301) as outlined in Tabel 7 using a variety of x-ray based techniques, clinical techniques, and subject-reported outcome measurements, each known in the art, to monitor:

1. Pharyngeal Constrictor Muscle Function (as assessed by Pharyngeal Area at Maximum Constriction)
2. Swallowing Efficiency (as assessed by Total Pharyngeal Residue)
3. Subject-Reported Oropharyngeal Dysphagia (as assessed by the Sydney Swallow Questionnaire)
4. Swallowing Capacity (as assessed by the Cold Water Timed Drinking Test)

At each assessment visit, study subjects consume Barium containing foods and liquids of various consistencies (solid food, as well as extremely thick liquid, moderately thick liquid, and thin liquids), and the swallowing process is assessed via the methods noted above.

Table 7 - Summary of assessments for the clinical development program for BB-301

Assessment methods for the OPMD Natural History Study and the BB-301 Phase 1b/2a clinical study (NCT06185673)			
Videofluoroscopic swallowing study assessments			Clinical Assessments
Global swallowing function	Pharyngeal constrictor muscle function	Swallowing efficiency	Other assessments
Dynamic imaging grade of swallowing toxicity scale	Pharyngeal area at maximum constriction Pharyngeal constriction ratio	Total pharyngeal residue Vallecular residue Pyriform sinus residue Other pharyngeal residue Normalised residue ratio scale	Clinical measurements of swallowing capacity & dysphagia (including time- based and volume-based drinking tests) Patient-reported measures of dysphagia

Pharyngeal area at maximum constriction (PhAMPC) is measured by using the length of a patient’s C2-C4 cervical vertebrae as an anatomical scalar. “Pharyngeal Area” measurement comprises the area of visible airspace or bolus at maximal constriction. Measurement occurs on the radiographic frame of tightest constriction of the pharyngeal lumen during the swallow. Normal PhAMPC values span the range of 0% to 2.2% for the characteristic food and liquids that were evaluated in the swallowing tasks discussed. PhAMPC is calculated as follows:

PhAMPC =
$$\frac{\text{Pharyngeal Area}}{(\text{C2} - \text{C4 length})^2} \times 100\%$$

Total Pharyngeal Residue is also measured using C2-C4 length to act as an anatomical scalar. Total Pharyngeal Residue measurement comprises the volume (v) of material remaining in the pharynx (res) after the first swallow of the bolus. Measurement occurs on the first radiographic frame showing pyriform sinuses (PS) at lowest position. Normal Total Pharyngeal Residue values should be close to zero. Total Pharyngeal Residue is calculates as follows:

Total Pharyngeal Residue =
$$\frac{(\text{V res.area} + \text{PS res.area} + \text{other res.area})}{(\text{C2} - \text{C4 length})^2} \times 100\%$$

Subject-Reported Oropharyngeal Dysphagia is measured using the Sydney Swallow Questionnaire (SSQ) which is a self-report inventory assessing subjective symptoms of oropharyngeal dysphagia with strong content, construct, discriminant, and predictive validity and test-retest reliability in a range of patient populations. The SSQ is a 17-item questionnaire which was developed to measure symptomatic severity of oral-pharyngeal dysphagia as reported by the affected subject. The questionnaire uses a 100mm long visual analogue scale for all but 1 question. Possible scores range from 0 to 1700, with higher scores indicating greater swallowing difficulty. Healthy subjects without dysphagia may present with a mean score of about 59.0.

Changes in assessment criteria over the course of the Natural History Study are used to determine progression of dysphagia in study subjects (if any). Average results and final results (i.e., pre-dose) are assessed in study subjects to determine baseline measurements for assessment criteria against which any changes may be assessed at Day 90, Day 180, Day 270, and Day 360 post treatment with BB-301 DP.

As discussed above, at the time of writing two subjects have completed the Natural History Study and progressed to the Phase 1b/2a clinical trial.

BB-301 Phase 1b/2a clinical trial methodology

NCT06185673 is phase 1b/2a, first-in-human, single arm, open-label, sequential, dose escalation cohort study. Subjects who have previously enrolled in the Benitec Biopharma Inc OPMD-NH-001 Natural History Study and completed at least 6 months of follow up may be eligible to participate in this interventional study.

To characterize the safety profile and to determine a Maximum Tolerated Dose (MTD)/Recommended Phase 2 Dose (RP2D) of BB-301, the Phase 1b component has been designed to include dose escalation via the enrollment of subjects into sequential dose cohorts in which BB-301 is administered as direct intramuscular injections into the Middle Pharyngeal Constrictor (MPC) muscles and the Inferior Pharyngeal Constrictor (IPC) muscles. Intramuscular injections of BB-301 follow the open surgical dissection of the pharyngeal region under general anesthesia. An independent data safety monitoring board (DSMB), operating under a charter, will review all available safety data from a cohort as it arises, along with the cumulative information available from all dosed subjects, and will issue a decision regarding the appropriateness of dose escalation, and other aspects of the safety profile of BB-301 and the conduct of the study.

The dose of BB-301 administered to the respective subjects (as illustrated in Figure 8) will be defined by the cohort assignment (Phase 1b) and the MTD/RP2D determined (Phase 2a). On Day 1, the designated dose of BB-301 will be directly injected into each

contralateral side of the MPC muscles 502 ($4 \times 50 \mu\text{L}$ or $4 \times 75 \mu\text{L}$) and the IPC muscles 501 ($8 \times 50 \mu\text{L}$ or $8 \times 75 \mu\text{L}$) following open surgical dissection of the pharyngeal region of the subject's neck 500 under general anesthesia.

- 5 To minimise generalized inflammatory responses and/or specific immunological reactions within study subjects caused by the surgical dissection, prednisone will be administered prophylactically (beginning on Day -1) and tapered over the course of 8 weeks.

Injection procedure and device operation

- 10 Unless otherwise stated, the injection procedure and device operation employed in the Phase 1b/2a clinical trial is generally consistent with Examples 1 and 2 herein.

A 3Dose™ Unit Injector micro-injection syringe from Vlow Medical (Cat No. 3DOSE GREEN-125) was selected as the injection syringe 100 (Figure 8B). In this example, the injection syringe 100 is a pre-filled, multi-injection syringe, which enables repeated administration of pre-determined, small volumes of BB-301 drug product (DP).

- 15 The syringe 100 includes an injection volume selection mechanism, enabling selection of injection volume ranges encompassing $12.5\mu\text{L}$ to $50\mu\text{L}$ per plunger “click”. The injection volume selection mechanism comprises a plunger rotatable relative to the barrel of the syringe to select one of a plurality of sets of plunger formations corresponding to the desired injection volume. The injection syringe 100 facilitated the completion of multiple injections of BB-301 DP into the targeted pharyngeal constrictor muscles and ensured that the desired total volume of BB-301 DP was delivered to the target muscles. Setting of the desired volume of BB 301 DP with each injection was achieved by selecting the appropriate plunger setting via rotation of the plunger around the central axis of the injection syringe to facilitate dispensation of either $12.5\mu\text{L}$, $25\mu\text{L}$, or $50\mu\text{L}$ of BB-301 DP with each plunger “click” of the injection syringe 100 and the completion of the total number of pre-determined injections per contralateral side of the pharyngeal constrictor muscles.

- 25 As described above, the injection syringe 100 allowed the operator to complete one or more injections of the selected volume of BB-301 DP ($12.5\mu\text{L}$, $25\mu\text{L}$, or $50\mu\text{L}$ of BB-301 DP) at any injection site within the targeted pharyngeal constrictor muscle via the use of a single “click” or multiple “clicks” of the plunger at each discrete injection site within the targeted pharyngeal constrictor muscle of the subject. Additionally, the injection syringe 100 allowed the operator to complete multiple injections of the selected volume of BB-301 DP over the surgically accessible anatomical region of a pharyngeal constrictor muscle via the targeting of discrete injection sites distributed evenly over the plane of the muscle.

- 30 The intramuscular injections of BB-301 DP into the pharyngeal constrictor muscles were conducted (and will be conducted for future OPMD subjects) intraoperatively via the

injection syringe 100 and the injection needle 10 described herein. Specifically, Needle A described in Examples 1 and 2 herein was used as the injection needle 10 (Fig. 1). The injection syringe 100 was coupled with the injection needle 10 (i.e., Needle A described herein) to form a drug product injection device (DPID) 200 (Fig 8).

5 For the intraoperative administration of BB-301 DP to OPMD subjects currently enrolled in the Phase 1b/2a clinical trial, two DPIDs 200 have been used per pharyngeal constrictor muscle i.e., two (2) DPIDs 200 to complete the dosing of the MPC muscle 502 and two (2) DPIDs 200 to complete the dosing of the IPC muscle 501. This protocol will also be used for intraoperative administration to future OPMD subject enrolled in the Phase 1b/2a
10 clinical trial.

The total dose of BB-301 DP to be administered to each pharyngeal constrictor muscle will be delivered in a two-step process that partitions the intramuscular injections in a manner which focuses on the two separate contralateral portions of the respective pharyngeal constrictor muscles as determined by the relative anatomical position of each tissue section
15 with respect to pharyngeal raphe 506 (Fig 10). In this regard, one (1) DPID 200 will be used to complete the dosing of each contralateral side (i.e., left side and right side) of the MPC muscle 502. Similarly, one (1) DPID 200 will be used to complete the dosing of each contralateral side (i.e., left side and right side) of the IPC muscle 501. Therefore, the completion of the planned injections of the pharyngeal constrictor muscles for each study
20 subject will require the use of at least four (4) DPIDs 200 per subject.

Figure 10 illustrates the specific anatomical sites that are injected with BB-301 DP, including the left and right components of the MPC muscle 502 and the left and right components of the IPC 501 muscle. Specifically Figure 10 shows a posterior view of the pharyngeal muscles of patient's neck 500 including inferior pharyngeal constrictor muscle
25 501, middle pharyngeal constrictor muscle 502, superior pharyngeal constrictor muscle 503. Figure 10 also shows pharyngeal raphe 506 separating left and right components of the pharyngeal constrictor muscles 501, 502 and 503.

The final dose of BB-301 DP administered to each study subject is determined according to the subject's cohort assignment (as discussed below).

30

Administration procedure

'Subject 1' and 'Subject 2' were intraoperatively administered BB-301 DP in the Phase 1b dose escalation study according to the following general administration procedure. The same procedure will be used for the remaining subjects that progress from the Natural
35 History Study to the Phase 1b/2b study.

Amoxicillin (1g) was delivered intravenously immediately prior to intraoperative administration of BB-301 DP (i.e., Day 1 of the Phase 1b study), and again twice daily for the first 2 days following the intraoperative administration of BB-301 DP.

Administration of BB-301 DP was performed under general anesthesia with
5 endotracheal intubation and appropriate analgesia. The patient was first disposed in supine position, after which a medium-sized bougie or a regular tracheal tube was visually guided and placed in the esophagus to facilitate preoperative identification of the pharyngo-esophageal junction (“esophageal identification tube”).

A bilateral cervicotomy of about 5 cm was performed along the anterior edge of the
10 lower third of a sternocleidomastoid muscle (first incision 511 and second incision 512). After separating the omohyoid muscle from a middle thyroid vein, an internal jugular vein and carotid were reclined laterally and larynx rotated. Blunt dissection then provided access to the posterior midline of the pharynx. Dissection of the middle thyroid vein and/or inferior thyroid artery is necessary only where access is limited. This approach allows wide exposure of the
15 posterior face of the pharynx in totality with constrictor muscles 501, 502, 503.

A cricopharyngeal muscle, the inferior pharyngeal constrictor 501 and the middle pharyngeal constrictor 502 muscles of the pharynx are widely exposed up to the level of a superior cornu of thyroid cartilage. The cricopharyngeal muscle is more or less distinct from the inferior pharyngeal constrictor muscle 501, and is identified by its annular fibers, a cricoid
20 cartilaginous ring, and the lower edge of the inferior pharyngeal constrictor 501. The more superior part of the inferior pharyngeal constrictor muscle 501 arises from a thyroid cartilage (thyropharyngeal part). It is identified between the posterior edges of the thyroid cartilage, and each half articulates with the cricoid cartilage inferiorly at a joint (cricothyroid joint), with the fibrous pharyngeal raphe 506 in the posterior median line of the pharynx. A higher
25 zone of pharyngeal muscular injection occurs at the level of a superior cornu of the thyroid cartilage.

Intramuscular injections of BB-301 DP were performed on each contralateral side (to the left and to the right of the pharyngeal raphe 506) of the inferior pharyngeal constrictor muscle 501 (Constrictor pharyngis inferior) and each contralateral side (to the left and to the
30 right of the pharyngeal raphe 506) of the middle pharyngeal constrictor muscle 502 (Musculus constrictor pharyngis medius) using the pre-filled BB-301 DPIDs 200. Each DPID 200 was filled with a final volume of the designated dose of BB-301 DP sufficient to inject one side of each pharyngeal constrictor muscle, as follows:

- One DPID 200 was filled with sufficient BB-301 DP to complete the planned
35 intramuscular injections of the left section (left, lateral to the pharyngeal raphe 506) of the middle pharyngeal constrictor muscle 502 four (4) times, with injections spread evenly throughout the tissue plane (i.e., $4 \times 50 \mu\text{L}$ or $4 \times 75 \mu\text{L}$).

- One DPID 200 was filled with sufficient BB-301 DP to complete the planned intramuscular injections of the right section (right, lateral to the pharyngeal raphe 506) of the middle pharyngeal constrictor muscle 502 four (4) times, with injections spread evenly throughout the tissue plane (i.e., $4 \times 50 \mu\text{L}$ or $4 \times 75 \mu\text{L}$).
- 5 • One DPID 200 was filled with sufficient BB-301 DP to complete the planned intramuscular injections of the left section (left, lateral to the pharyngeal raphe 506) of the inferior pharyngeal constrictor muscle 501 eight (8) times, with injections spread evenly throughout the tissue plane (i.e., $8 \times 50 \mu\text{L}$ or $8 \times 75 \mu\text{L}$).
- 10 • One DPID 200 was filled with sufficient BB-301 DP to complete the planned intramuscular injections of the right section (right, lateral to the pharyngeal raphe 506) of the inferior pharyngeal constrictor muscle 501 eight (8) times, with injections spread evenly throughout the tissue plane (i.e., $8 \times 50 \mu\text{L}$ or $8 \times 75 \mu\text{L}$).

Therefore, to treat each study subject in each cohort, the completion of the planned injections of the pharyngeal constrictor muscles will require the use of at least four (4) DPIDs
15 200 per subject.

As for the final dose of BB-301 DP administered to each study subject, this is/was determined according to the subject's cohort assignment. In this regard, OPMD subjects enrolled in the Phase 1b dose escalation study are randomised into the following cohorts:

- 20 • Cohort 1: Subjects in cohort 1 receive a fixed number of IM injections of BB-301 into the respective pharyngeal constrictor muscles on the day of dosing (as described above), with a total dose of $1.2\text{e}13$ vg/subject (low dose).
- Cohort 2: Subjects in cohort 2 receive a fixed number of IM injections of BB-301 into the respective pharyngeal constrictor muscles on the day of dosing (as described above), with a total dose of $3.6\text{e}13$ vg/subject (medium dose).
- 25 • Cohort 3: Subjects in cohort 3 receive a fixed number of IM injections of BB-301 into the respective pharyngeal constrictor muscles on the day of dosing (as described above), with a total dose of $5.4\text{e}13$ vg/subject (high dose).

Accordingly, the dose of BB-301 DP delivered to any one subject during the course of the Phase 1b dose escalation study may vary from one subject to another.

30 Following administration of BB-301 DP to the study subject, closure of the dissected tissue was performed by suture (subcutaneous suturing with 3/0 adsorbable vicryl or equivalent and cutaneous suturing with non-absorbable monofilament), with or without drainage and a dressing (or bandage) as appropriate. The esophageal identification tube was removed from esophagus and mouth; this permitted an additional check of the integrity of the
35 upper esophageal sphincter mucosa.

Post-operative in-patient care of study subjects was provided following intraoperative administration of the BB-301, including IV administration of amoxicillin 1g (e.g., Clamoxyl) twice daily on days 0-2, daily examination of the surgical site, removal of drain (if present) on day 1-3 as appropriate, and removal of cutaneous stitches on day 10-12 provided adequate healing is observed.

Study subjects were able to resume a soft diet at day 0-1.

Results

At the time of writing, clinical efficacy data is available for the first study subject ("Subject 1") from the first, protocol-mandated post-dosing assessment time-point which was conducted 90-days following the administration of BB-301.

During the OPMD Natural History Study, which represents the pre-dose observational period for each subject, Subject 1 experienced progressive worsening of dysphagia as demonstrated by the results of the videofluoroscopic swallowing studies (VFSS), the cold water timed drinking test, and the key subject-reported outcome measure (the Sydney Swallow Questionnaire). Videofluoroscopic swallowing studies represent the gold standard analytical method for the quantitative assessment of dysphagia (swallowing difficulty) in the clinical setting.

Preliminary clinical, radiographic, and subject-reported assessment results (90 days post treatment "Day 90") for Subject 1 of the BB-301 Phase 1b/2a clinical trial (NCT06185673) are summarized in **Table 8**. At Day 90, Subject 1 demonstrated improvements in key videofluoroscopic assessments which correlated with the observation of similar improvement in the key subject-reported outcome measures as compared to both the average and final values for the respective assessments completed during the pre-dose observational period. Notably, the results of many assessments completed at Day 90 demonstrated improvements over the initial measurements assessed at the subject's first visit for the OPMD Natural History Study which occurred more than 12 months prior to the Day 90 assessment.

The most significant improvements at Day 90 were observed for swallowing tasks centered on the evaluation of pharyngeal constrictor muscle function and swallowing efficiency in the context of the consumption of thin liquids, solid foods and thick, non-solid foods (e.g., yogurt or pudding) (see **Table 8**). The improvements correlated with an improvement in the key subject-reported outcome measure the Sydney Swallow Questionnaire, indicating an improvement in swallowing function as reported by Subject 1 (see **Table 8**).

Table 8 - Summary of swallowing studies results for Subject 1

Part A – Radiographic assessments of pharyngeal area at maximum constriction to determine pharyngeal constrictor muscle function during swallowing.							
Barium containing items	Pre-Dose Period OPMD Natural History Study		Post-Dose Period Phase 1b/2a BB-301 dosing study Day 90 PhAMPC during swallowing	Improvement in pharyngeal closure during swallowing after BB-301 dose			
	<i>Average pre-dose PhAMPC during swallowing</i>	<i>Final pre-dose PhAMPC during swallowing</i>		<i>Average pre-dose PhAMPC minus Day 90 PhAMPC</i>	<i>Reduction in PhAMPC from average pre-dose</i>	<i>Final pre-dose PhAMPC minus Day 90 PhAMPC</i>	<i>Reduction in PhAMPC from final pre-dose</i>
<i>Thin liquid</i>	9.1	15.8	3.9	- 5.2	- 57.1 %	- 11.9	- 75.3 %
<i>Moderately thick liquid</i>	20.0	22.3	14.8	- 5.2	- 26.0 %	- 7.5	- 33.6 %
<i>Extremely thick liquid</i>	21.8	24.2	18.5	- 3.3	- 15.1 %	- 5.7	- 23.6 %
<i>Solid food</i>	18.0	19.2	10.9	- 7.1	- 39.4 %	- 8.3	- 43.2 %
Part B - Radiographic assessments of pharyngeal residue (i.e. food or liquid material) remaining post-swallow to determine swallowing efficiency.							
Barium containing items	Pre-Dose Period OPMD Natural History Study		Post-Dose Period Phase 1b/2a BB-301 dosing study Day 90 pharyngeal residue remaining post-swallow	Reduction in post-swallow pharyngeal residue after BB-301 dose			
	<i>Average pharyngeal residue remaining post-swallow</i>	<i>Final pharyngeal residue remaining post-swallow</i>		<i>Average pre-dose residue minus Day 90 residue</i>	<i>Reduction in residue from average pre-dose</i>	<i>Final pre-dose residue minus Day 90 residue</i>	<i>Reduction in residue from final pre-dose</i>
<i>Thin liquid</i>	4.9	7.7	0.9	- 4.0	- 81.6 %	- 6.8	- 88.3 %
<i>Moderately thick liquid</i>	18.2	11.4	11.0	- 7.2	- 39.6 %	- 0.4	- 3.5 %
<i>Extremely thick liquid</i>	21.5	22.5	11.9	- 9.6	- 44.7 %	- 10.6	- 47.1 %
<i>Solid food</i>	19.0	17.3	12.3	- 6.7	- 35.3 %	- 5.0	- 28.9 %

Table 8 (continued) - Summary of swallowing studies results for Subject 1

Part C- Subject reported outcome measure: Sydney Swallow Questionnaire “SSQ” (17 Question self-report inventory assessing subjective symptoms of oropharyngeal dysphagia.							
	Pre-Dose Period OPMD Natural History Study		Post-Dose Period Phase 1b/2a BB-301 dosing study	Reduction in SSQ score after BB-301 dose			
	<i>Average pre-dose</i>	<i>Final pre-dose</i>	<i>Day 90</i>	<i>Average pre-dose score minus Day 90 score</i>	<i>Reduction in score from average pre-dose</i>	<i>Final pre-dose score minus Day 90 score</i>	<i>Reduction in score from final pre-dose</i>
<i>SSQ score</i>	1,136	1,264	935	- 201	- 17.7 %	- 329	- 26.0 %
Part D – Cold water timed drinking test: Time in seconds the subject requires to consume 80mL of cold water.							
	Pre-Dose Period OPMD Natural History Study		Post-Dose Period Phase 1b/2a BB-301 dosing study	Reduction in drinking time after BB-301 dose			
	<i>Average pre-dose drinking time (seconds)</i>	<i>Final pre-dose drinking time (seconds)</i>	<i>Day 90 time drinking time (seconds)</i>	<i>Average pre-dose drinking time minus Day 90 time</i>	<i>Reduction in drinking time from average pre-dose</i>	<i>Final pre-dose drinking time minus Day 90 residue</i>	<i>Reduction in drinking time from final pre-dose</i>
<i>Cold water</i>	30	28	26	- 4.0	- 13.3 %	- 2.0	- 7.1 %

Conclusions from the first in-human efficacy study

- As expected, Subject 1 experienced disease progression during their enrolment in the
- 5 Natural History Study. However, after dosing with BB-301 DP using the devices and methods described herein, dysphagia improved markedly in Subject 1. The dose of BB-301 was sufficiently biologically active to facilitate a benefit in Subject 1 and these benefits were visible at the first follow-up assessments conducted at Day 90 post-dose. BB-301 did not cause any serious adverse events in Subject 1.

Based on the outcome of the dose escalation Phase 1b study for all subjects, the maximum tolerated dose (MTD)/Recommended Phase 2 Dose (RP2D) will be determined for the dose expansion Phase 2a study.

- 5 It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the above-described embodiments, without departing from the broad general scope of the present disclosure. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

CLAIMS

1. An injection needle comprising:
 - a needle cannula comprising:
 - a proximal portion having a proximal end,
 - a distal portion having a distal end,
 - a middle portion disposed between the proximal portion and distal portion, and
 - a lumen extending through the proximal portion, the middle portion and the distal portion between the proximal end of the proximal portion and the distal end of the distal portion, the lumen opening at an outlet at or adjacent the distal end of the distal portion;
 - wherein the proximal portion and the distal portion are each substantially straight and wherein the middle portion is curved such that the distal portion extends at an angle of between 75° and 105° relative to the proximal portion; and
 - wherein the length of the proximal portion is shorter than the length of the distal portion.
2. The injection needle of claim 1, wherein the distal portion extends at an angle of between about 85° and 95° relative to the proximal portion.
3. The injection needle of claim 1, wherein the distal portion extends at an angle of about 90° relative to the proximal portion.
4. The injection needle of claim 1 or claim 2, wherein the radius of curvature of the middle portion is between about 2.5mm and 3.1mm.
5. The injection needle of any one of claims 1 to 4, wherein the proximal portion has a length that is less than 90%, less than 80%, less than 70%, or less than 60%, or less than 50% of a length of the distal portion.
6. The injection needle of any one of claims 1 to 5, wherein the length of the distal portion is greater than the sum of the length of the proximal portion and a length of the middle portion.

7. The injection needle of any one of claims 1 to 6, wherein the total length of the needle cannula is between about 10mm and 27mm.
8. The injection needle of any one of claims 1 to 7, wherein the length of the distal portion is between about 7mm and about 15mm.
9. The injection needle of any one of claims 1 to 8, wherein the length of the middle portion is between about 3mm and about 6mm.
10. The injection needle of any one of claims 1 to 9, wherein the length of the proximal portion is between about 2mm and about 6mm.
11. The injection needle of any one of claims 1 to 10, wherein:
 - the distal portion extends at an angle of between about 85° and 95° relative to the proximal portion; and
 - a radius of curvature of the middle portion is between about 2.5mm and 3.1mm.
12. The injection needle of any one of claims 1 to 11, wherein:
 - the proximal portion has a length that is less than 70% of a length of the distal portion;
 - the length of the distal portion is greater than the sum of the length of the proximal portion and the length of the middle portion, optionally an arc length of the middle portion; and
 - the total length of the needle cannula is between about 10mm and 27mm.
13. The injection needle of any one of claims 1 to 12, wherein:
 - the distal portion extends at an angle of between about 85° and 95° relative to the proximal portion;
 - the length of the distal portion is between about 7mm and 15mm; and
 - the length of the proximal portion is between about 2mm and 6mm.
14. The injection needle of any one of claims 1 to 13, wherein the needle cannula is a 26-28 Gauge.

15. The injection needle of any one of claims 1 to 14, wherein an outer diameter of the needle cannula is between about 0.3mm and about 0.5mm.
16. The injection needle of any one of claims 1 to 15, wherein an inner diameter of the needle cannula is between about 0.1mm and about 0.3mm.
17. The injection needle of any one of claims 1 to 16, wherein a thickness of a wall of the needle cannula is between about 0.03mm and about 0.17mm.
18. The injection needle of any one of claims 1 to 17, wherein the needle cannula comprises a dead volume of between about 0.5 μ L/25.4mm and about 1.5 μ L/25.4mm.
19. The injection needle of any one of claims 1 to 18, wherein the needle cannula comprises an outlet that is at or adjacent the distal end of the distal portion.
20. The injection needle of any one of claims 1 to 19, wherein the needle cannula comprises a bevel formed at a distal end of the needle, wherein the bevel includes a bevelled edge that faces away from an inner bend side of the needle cannula.
21. The injection needle of any one of claims 1 to 20, wherein the distal tip is bevelled at an angle of between about 5° and about 15°.
22. The injection needle of any one of claims 1 to 21, wherein a bevel length is between about 0.5mm and about 3.5mm.
23. The injection needle of any one of claims 1 to 22, wherein the outlet of the needle cannula is located at the bevelled edge.
24. The injection needle of any one of claims 1 to 23, wherein injection needle further comprises a needle hub connected to the needle cannula, wherein the needle cannula projects distally from the needle hub
25. The injection needle of claim 24, wherein the needle cannula further comprises a support portion and wherein the support portion disposed inside the needle hub.

26. The injection needle of any one of claims 1 to 25, wherein the needle cannula comprises an inlet at a proximal end of the support portion.
27. The injection needle of any one of claims 24 to 26, wherein the needle hub has a distal portion adjacent the proximal end of the proximal portion of the needle cannula, and wherein the distal portion of the needle hub is swaged.
28. The injection needle of any one of claims 1 to 27, wherein injection needle comprises an inlet at or adjacent the proximal end of the proximal portion.
29. Use of an injection needle of any one of claims 1 to 28 to directly inject a therapeutic composition into a pharyngeal muscle of a subject.
30. The use of claim 29, wherein the injection needle is used to directly inject a therapeutic composition into the pharyngeal muscle following an incision in a subject's neck to obtain direct access to the pharyngeal muscle.
31. The use of claims 29 or 30, wherein the injection needle is used to directly inject a therapeutic composition into a pharyngeal constrictor muscle of a subject.
32. The use of any one of claims 29 to 31, wherein the pharyngeal muscle comprises one or more of an inferior pharyngeal constrictor muscle, a middle pharyngeal constrictor muscle, a superior pharyngeal constrictor muscle, a palatopharyngeus muscle, a salpingopharyngeus muscle, a stylopharyngeus muscle, or any combination thereof.
33. The use any one of claims 29 to 32, wherein the therapeutic composition is effective to treat oculopharyngeal muscular dystrophy (OPMD) and the injection needle is used to treat or prevent dysphagia associated with oculopharyngeal muscular dystrophy (OPMD) in a subject.
34. A method of forming an injection needle, wherein the method comprises:
 - providing a needle cannula comprising a proximal end, a distal end, and a lumen extending through the needle cannula between the proximal end and the distal end; and
 - bending the needle cannula such that the needle cannula comprises a substantially straight proximal portion having the proximal end, a substantially straight

distal portion having the distal end, and a middle portion disposed between the proximal portion and the distal portion, the middle portion being curved such that the distal portion extends at an angle relative to the proximal portion.

35. The method of claim 34, wherein is curved such that the distal portion extends at an angle of between 75° and 105° relative to the proximal portion.
36. The method of claim 34 or 35, wherein the length of the proximal portion is shorter than the length of the distal portion.
37. The method of any one of claims 34 to 36, wherein the injection needle that is formed is the injection needle of any one of claims 1 to 28.
38. A method of administering a therapeutic composition into a pharyngeal muscle of a subject, comprising directly injecting the therapeutic composition into the pharyngeal muscle of the subject using a bent or curved injection needle.
39. The method of claim 38, wherein the bent or curved injection needle is an injection needle comprising:
 - a proximal portion having a proximal end,
 - a distal portion having a distal end,
 - a middle portion disposed between the proximal portion and distal portion, and
 - a lumen extending through the proximal portion, the middle portion and the distal portion between the proximal end of the proximal portion and the distal end of the distal portion, the lumen opening at an outlet at or adjacent the distal end of the distal portion;wherein the proximal portion and the distal portion are each substantially straight and wherein the middle portion is curved such that the distal portion extends at an angle relative to the proximal portion,
 - optionally wherein the injection needle is an injection needle of any one of claims 1 to 28.
40. The method of claims 38 or 39, wherein the therapeutic composition is administered into one or more pharyngeal muscles selected from: an inferior pharyngeal constrictor muscle, a middle pharyngeal constrictor muscle, a superior pharyngeal constrictor

muscle, a palatopharyngeus muscle, a salpingopharyngeus muscle, a stylopharyngeus muscle, and any combination thereof.

41. The method of any one of claims 38 to 40, comprising administering the therapeutic composition into the middle pharyngeal constrictor muscle and the inferior pharyngeal constrictor muscle.
42. The method of any one of claims 38 to 41, wherein the therapeutic composition is administered into the pharyngeal muscle by direct injection following an incision in a subject's neck which is sufficient to provide direct access to at least part of the subject's pharyngeal muscle.
43. The method of claim 42, comprising making one or more incisions in the subject's neck to provide direct access to at least part of the subject's pharyngeal muscle and then administering the therapeutic composition by direct intramuscular injection into the pharyngeal muscle.
44. The method of any one of claims 38 to 43, wherein the therapeutic composition is effective to treat or prevent dysphagia associated with oculopharyngeal muscular dystrophy (OPMD).
45. The method of any one of claims 38 to 44, wherein the therapeutic composition comprises a viral delivery vector comprising a 'silence and replace' construct comprising:
 - (i) a DNA-directed RNAi (ddRNAi) construct comprising a nucleic acid comprising a sequence which encodes a short hairpin micro-RNA (shmiR); and
 - (ii) a PABPN1 construct comprising a nucleic acid comprising a sequence encoding a functional PABPN1 protein having a mRNA transcript which is not targeted by the shmiR(s) encoded by the ddRNAi construct.
46. The method of claim 45, wherein the viral delivery vector is an adeno-associated virus (AAV) comprising:
 - (i) a viral capsid protein from AAV9 comprising a modified subunit 1 (VP1) sequence wherein the amino acids at positions 26, 40, 43, and 44 are modified relative to a corresponding wildtype AAV9 VP1 sequence set forth in SEQ ID

- NO: 17, optionally wherein the viral capsid protein comprises the amino acid sequence set forth in SEQ ID NO: 27; or
- (ii) a viral capsid protein from AAV8 comprising a modified subunit 1 (VP1) sequence wherein the amino acids at positions 1, 26, 40, 43, 44 and 64 are modified relative to a corresponding wildtype AAV8 VP1 sequence set forth in SEQ ID NO: 21.
47. The method of claim 45, wherein:
- (i) the AAV comprises a viral capsid protein from AAV9 comprising mutations A67E, Q81R, K84D and A85S with respect to the full length wild-type AAV serotype 9 capsid sequence set forth in SEQ ID NO: 19, optionally wherein the viral capsid protein comprises the amino acid sequence set forth in SEQ ID NO: 28; or
- (ii) the AAV comprises a viral capsid protein from AAV8 comprising mutations A42S, A67E, Q81R, K84D, A85S and Q105K with respect to the full length wild-type AAV serotype 8 capsid sequence set forth in SEQ ID NO: 23, optionally wherein the viral capsid protein comprises the amino acid sequence set forth in SEQ ID NO: 24.
48. The method of any one of claims 45 to 47, wherein the PABPN1 construct comprises nucleic acid molecule encoding functional PABPN1 protein is codon optimised such that its corresponding mRNA sequence is not recognised by the shmiR(s) encoded and expressed from the ddRNAi construct, optionally wherein the codon-optimised nucleic acid comprises the sequence set forth in SEQ ID NO: 15 and encodes the amino acid sequence set forth in SEQ ID NO: 16.
49. The method of any one of claims 45 to 48, wherein the ddRNAi construct comprises a DNA sequence encoding a shmiR comprising an effector sequence which is substantially complementary to the sequence set forth in SEQ ID NO:1 and/or an effector sequence which is substantially complementary to the sequence set forth in SEQ ID NO:2.
50. The method of claim 49, wherein the ddRNAi construct comprises:
- (i) a DNA sequence encoding a shmiR comprising an effector sequence set forth in SEQ ID NO: 4 and an effector complement sequence set forth in SEQ ID NO: 3 (shmiR13); and a DNA sequence encoding a shmiR comprising an

- effector sequence set forth in SEQ ID NO: 6 and an effector complement sequence set forth in SEQ ID NO: 5 (shmiR17); and/or
- (ii) a nucleic acid comprising or consisting of a DNA sequence set forth in SEQ ID NO: 12 (shmiR13) and a nucleic acid comprising or consisting of a DNA sequence set forth in SEQ ID NO: 13 (shmiR17).
51. The method of any one of claims 45 to 50, wherein the therapeutic composition for treatment of OPMD is administered in a volume ranging from about 25µl to about 100µl per injection site.
52. The method of any one of claims 45 to 51, wherein the therapeutic composition for treatment of OPMD is administered to the subject at a concentration between about 1.0+E12 vg/mL and about 1.0+E14 vg/mL, optionally wherein the concentration of the therapeutic composition is between about 5.0+E12 vg/mL and about 5.0+E13 vg/mL, optionally wherein the concentration of the therapeutic composition is between about 1.0+E13 vg/mL and 3.0+E13 vg/mL.
53. The method of any one of claims 44 to 52, wherein comprising administering the therapeutic composition to one or more locations of a pharyngeal muscle, to one or more pharyngeal muscles, to one or more sides of a pharyngeal muscle, or any combination thereof.
54. An injection device comprising:
- (i) a pre-filled syringe containing a therapeutic composition which is effective for treatment or prevention of dysphagia associated with OPMD; and
- (ii) the injection needle of any one of claims 1 to 28, coupled to the pre-filled syringe.
55. The injection device of claim 54, wherein the therapeutic composition comprises an AAV9 vector comprising:
- (a) a viral capsid protein from AAV9 comprising mutations A67E, Q81R, K84D and A85S determine relative to the full length wild-type AAV9 capsid sequence set forth in SEQ ID NO: 19, optionally wherein the viral capsid protein comprises the amino acid sequence set forth in SEQ ID NO: 28; and
- (b) a silence and replace construct comprising:

- (i) a muscle-specific promoter (optionally wherein the promoter is a Spc512 muscle-specific promoter);
 - (ii) a DNA-directed RNAi (ddRNAi) construct comprising: a nucleic acid comprising or consisting of a sequence which encodes shmiR13 as described herein (optionally the DNA sequence set forth in SEQ ID NO: 12); and a nucleic acid comprising or consisting of a sequence which encodes shmiR17 as described herein (optionally the DNA sequence set forth in SEQ ID NO: 13); and
 - (iii) a PABPN1 construct comprising a nucleic acid sequence encoding a functional PABPN1 protein comprising the amino acid sequence set forth in SEQ ID NO: 16, wherein the nucleic acid sequence encoding the functional PABPN1 protein is a DNA sequence which is codon-optimised such that its corresponding mRNA sequence is not targeted by the shmiR(s) encoded by the ddRNAi construct (optionally wherein the codon-optimised nucleic acid comprises the sequence set forth in SEQ ID NO: 15);
wherein the muscle-specific promoter is operably-linked to the ddRNAi construct and the PABPN1 construct.
56. The injection device of claim 54 or claim 55, wherein the injection device is configured to deliver the therapeutic composition from the pre-filled syringe in a series of one or more injections, each injection having a pre-determined injection volume.
57. The injection device of claim 56, comprising an injection volume setting mechanism operable to set the injection volume.
58. The injection device of claim 57, wherein the injection volume setting mechanism, is operable to set the injection volume to between about 12.5 μ L and about 75 μ L.
59. The injection device of claim 58, wherein the injection setting mechanism is operable to select from a set of discrete injection volumes.
60. The injection device of claim 59, wherein the set of discrete injection volumes comprises at least: 25 μ L and 50 μ L.

61. A kit comprising a set of two or more injection devices according to any one of claims 54 to 60, wherein the prefilled syringes of the injection devices collectively contain a single therapeutic dose of the therapeutic composition for treatment of a single subject.
62. The kit of claim 61 comprising four of the injection devices.
63. The kit of claim 62, wherein the injection devices contain at least 200 μL of the therapeutic composition, such as between about 200 μL and about 2000 μL of the therapeutic composition.
63. The kit of claim 63, comprising:
 - (i) two injection devices containing between about 200 μL and about 500 μL of the therapeutic composition; and
 - (ii) two injection devices containing between about 400 μL and about 1000 μL of the therapeutic composition.
64. The kit of claim 62, wherein one or more of the injection devices is configured to dispense the therapeutic composition in a series of injections each having a 50 μL injection volume and/or wherein one or more of the injection devices is configured to dispense the therapeutic composition in a series of injections each having a 75 μL injection volume.
65. The kit of any one of claims 61 to 64, wherein the single therapeutic dose of the therapeutic composition is:
 - 1.2e13 vg/subject of the therapeutic composition;
 - 3.6e13 vg/subject of the therapeutic composition; or
 - 5.4e13 vg/subject of the therapeutic composition.