

active nuclear episome (extrachromosomal element). The AAV proviral genome is infectious as cloned DNA in plasmids which makes construction of recombinant genomes feasible. Furthermore, because the signals directing AAV replication, genome encapsidation and integration are contained within the ITRs of the AAV genome, some or all of the internal approximately 4.3 kb of the genome (encoding replication and structural capsid proteins, rep-cap) may be replaced with foreign DNA. The rep and cap proteins may be provided *in trans*. Another significant feature of AAV is that it is an extremely stable and hardy virus. It easily withstands the conditions used to inactivate adenovirus (56° to 65°C for several hours), making cold preservation of AAV less critical. AAV may even be lyophilized. Finally, AAV-infected cells are not resistant to superinfection.

**[0010]** There remains a need in the art for products and methods for treating muscular dystrophies including, but not limited to, FSHD. The disclosure provides a U7 small nuclear RNA (U7 snRNA) approach to inhibit DUX4 expression in muscle cells.

### **SUMMARY**

**[0011]** The disclosure provides products, methods, and uses for inhibiting DUX4 expression and for treating, ameliorating, delaying the progression of, and/or preventing a muscular dystrophy. In some examples, the muscular dystrophy is facioscapulohumeral dystrophy (FSHD).

[0011a] In one aspect, the present disclosure provides a nucleic acid encoding a U7 double homeobox 4 (DUX4) antisense ribonucleic acid (asRNA), the nucleic acid comprising (a) a nucleotide sequence comprising at least 90% identity to the sequence set forth in any one of SEQ ID NOs: 1-18; (b) the nucleotide sequence set forth in any one of SEQ ID NOs: 1-18; (c) a nucleotide sequence encoding a U7 double homeobox 4 (DUX4) antisense sequence that specifically hybridizes to a DUX4 target nucleotide sequence set forth in any one of SEQ ID NOs: 19-36; or (d) a combination of the nucleotide sequences of any one of (a) to (c).

[0011b] In another aspect, the present disclosure provides a nanoparticle, extracellular vesicle, exosome, or vector comprising the nucleic acid of the disclosure or a combination of any one or more thereof.

[0011c] In other aspect, the disclosure provides a composition comprising (a) the nucleic acid of the disclosure; (b) the nanoparticle, extracellular vesicle, exosome, or vector of the disclosure; or (c) the viral vector of the disclosure; and a pharmaceutically acceptable carrier.

[0011d] In other aspect, the disclosure provides an in vitro method of inhibiting and/or interfering with expression of a double homeobox 4 (DUX4) gene in a cell comprising contacting the cell with (a) the nucleic acid of the disclosure; (b) the nanoparticle, extracellular vesicle, exosome, or vector of the disclosure; (c) the viral vector of any one of the disclosure; or (d) the composition of the disclosure.

[0011e] In other aspect, the disclosure provides use of (a) the nucleic acid of the disclosure; (b) the nanoparticle, extracellular vesicle, exosome, or vector of the disclosure; (c) the viral vector of the disclosure; or (d) the composition of the disclosure in the preparation of a medicament for inhibiting expression of a double homeobox 4 (DUX4) gene in a cell.

[0011f] In other aspect, the disclosure provides use of (a) the nucleic acid of the disclosure; (b) the nanoparticle, extracellular vesicle, exosome, or vector of the disclosure; (c) the viral vector of the disclosure; or (d) the composition of the disclosure in the preparation of a medicament for treating or ameliorating a muscular dystrophy.

[0011g] In other aspect, the disclosure provides use of (a) the nucleic acid of the disclosure; (b) the nanoparticle, extracellular vesicle, exosome, or vector of the disclosure; (c) the viral vector of the disclosure; or (d) the composition of the disclosure in the preparation of a medicament for treating or ameliorating a cancer.

**[0012]** The disclosure provides nucleic acids, viral vectors comprising the nucleic acids which are designed to inhibit DUX4 expression, compositions and kits comprising the nucleic acids and vectors, methods for using these products for inhibiting and/or interfering with expression of a DUX4 gene in a cell, and methods for treating a subject suffering from a muscular dystrophy.

**[0013]** The disclosure provides a nucleic acid encoding a U7 double homeobox 4 (DUX4) antisense ribonucleic acid (asRNA), the nucleic acid comprising (a) a nucleotide sequence comprising at least 90% identity to the sequence set forth in any one of SEQ ID NOs: 1-18; (b) the nucleotide sequence set forth in any one of SEQ ID NOs: 1-18; or (c) a combination of the nucleotide sequences of (a) and/or (b).

**[0014]** The disclosure provides a nucleic acid comprising a nucleotide sequence encoding a U7 double homeobox 4 (DUX4) antisense sequence that specifically hybridizes to a DUX4 target nucleotide sequence set forth in any one of SEQ ID NOs: 19-36, or a combination of nucleotide sequences encoding a U7 double homeobox 4 (DUX4) antisense sequence that

specifically hybridizes to a DUX4 target nucleotide sequence set forth in any one of SEQ ID NOs: 19-36.

**[0015]** In some examples, a nucleic acid of the disclosure is under the control of a promoter and therefore comprises a promoter nucleotide sequence. In some examples, the promoter is any of a U6 promoter, a U7 promoter, a tRNA promoter, a H1 promoter, a minimal CMV promoter, a T7 promoter, an EF1-alpha promoter, a Minimal EF1-alpha promoter, or a muscle-specific promoter. In some further examples, the muscle-specific promoter is wherein the muscle-specific promoter is a unc45b promoter, a tMCK promoter, a minimal MCK promoter, a CK6 promoter, a CK7 promoter, a MHCK7 promoter, or a CK1 promoter.

**[0016]** In some aspects, the disclosure includes a nanoparticle, extracellular vesicle, exosome, or vector comprising any of the nucleic acids of the disclosure or a combination of any one or more thereof. In some examples, one or more nucleic acids are combined into a single nanoparticle, extracellular vesicle, exosome, or vector. In some examples, the nanoparticle is a liposome or micelle.

**[0017]** In some aspects, the disclosure includes a vector comprising a nucleic acid of the disclosure or a combination of nucleic acids of the disclosure. Examples of the disclosure utilize vectors (for example, viral vectors, such as adeno-associated virus (AAV), adenovirus, retrovirus, lentivirus, equine-associated virus, alphavirus, pox virus, herpes virus, herpes simplex virus, polio virus, sindbis virus, vaccinia virus or a synthetic virus, e.g., a chimeric virus, mosaic virus, or pseudotyped virus, and/or a virus that contains a foreign protein, synthetic polymer, nanoparticle, or small molecule) to deliver the nucleic acids disclosed herein. Thus, in some examples, the disclosure provides a vector comprising any one of the nucleic acids of the disclosure or a combination thereof. In some examples, the vector is an adeno-associated virus (AAV) or viral vector. In some examples, the AAV lacks rep and cap genes. In some examples, the AAV is a recombinant AAV (rAAV) or a self-complementary recombinant AAV (scAAV). In some examples, the rAAV is rAAV1, rAAV2, rAAV3, rAAV4, rAAV5, rAAV6, rAAV7, rAAV8, rAAV9, rAAV10, rAAV11, rAAV12, rAAV13, rAAV-anc80, rAAV rh.74, rAAV rh.8, rAAVrh.10, or rAAV-B1. In some examples, the AAV is rAAV-9.

**[0018]** The disclosure provides a composition comprising (a) a nucleic acid as described herein or a combination of such nucleic acids; (b) a nanoparticle, extracellular vesicle, exosome, or vector as described herein; (c) a viral vector as described herein; or (d) a composition as described herein. In some examples, the composition comprises a pharmaceutically acceptable carrier.

**[0019]** The disclosure provides a method of inhibiting and/or interfering with expression of a double homeobox 4 (DUX4) gene in a cell comprising contacting the cell with (a) a nucleic acid of the disclosure or a combination thereof; (b) a nanoparticle, extracellular vesicle, exosome, or vector of the disclosure; (c) a viral vector of the disclosure; or (d) a composition of the disclosure. In some examples, the cell is in a subject. In some examples, the subject is a human subject.

**[0020]** The disclosure provides a method of treating a subject having a muscular dystrophy comprising administering to the subject an effective amount of (a) a nucleic acid of the disclosure or a combination thereof; (b) a nanoparticle, extracellular vesicle, exosome, or vector of the disclosure; (c) a viral vector of the disclosure; or (d) a composition of the disclosure. In some examples, the muscular dystrophy is facioscapulohumeral muscular dystrophy (FSHD).

**[0021]** The disclosure provides a method of treating a subject having a cancer comprising administering to the subject an effective amount of (a) a nucleic acid of the disclosure or a combination thereof; (b) a nanoparticle, extracellular vesicle, exosome, or vector of the disclosure; (c) a viral vector of the disclosure; or (d) a composition of the disclosure. In some examples, the cancer is a sarcoma.

**[0022]** The disclosure provides use of (a) a nucleic acid of the disclosure or a combination thereof; (b) a nanoparticle, extracellular vesicle, exosome, or vector of the disclosure; (c) a viral vector of the disclosure; or (d) a composition of the disclosure for the preparation of a medicament for inhibiting expression of a double homeobox 4 (DUX4) gene in a cell. In some examples, the cell is in a subject. In some examples, the subject is a human subject.

**[0023]** The disclosure provides use of (a) a nucleic acid of the disclosure or a combination thereof; (b) a nanoparticle, extracellular vesicle, exosome, or vector of the disclosure; (c) a viral vector of the disclosure; or (d) a composition of the disclosure for inhibiting expression of a double homeobox 4 (DUX4) gene in a cell. In some examples, the cell is in a subject. In some examples, the subject is a human subject.

**[0024]** The disclosure provides use of (a) a nucleic acid of the disclosure or a combination thereof; (b) a nanoparticle, extracellular vesicle, exosome, or vector of the disclosure; (c) a viral vector of the disclosure; or (d) a composition of the disclosure for the preparation of a medicament for treating or ameliorating a muscular dystrophy.

**[0025]** The disclosure provides use of (a) a nucleic acid of the disclosure or a combination thereof; (b) a nanoparticle, extracellular vesicle, exosome, or vector of the disclosure; (c) a

viral vector of the disclosure; or (d) a composition of the disclosure for treating or ameliorating a muscular dystrophy. In some examples, the muscular dystrophy is FSHD.

**[0026]** The disclosure provides use of (a) a nucleic acid of the disclosure or a combination thereof; (b) a nanoparticle, extracellular vesicle, exosome, or vector of the disclosure; (c) a viral vector of the disclosure; or (d) a composition of the disclosure for the preparation of a medicament for treating or ameliorating a cancer. In some examples, the cancer is a sarcoma.

**[0027]** The disclosure provides use of (a) a nucleic acid of the disclosure or a combination thereof; (b) a nanoparticle, extracellular vesicle, exosome, or vector of the disclosure; (c) a viral vector of the disclosure; or (d) a composition of the disclosure for treating or ameliorating a cancer. In some examples, the cancer is a sarcoma.

**[0028]** The disclosure also provides methods and uses, wherein the nucleic acid, nanoparticle, extracellular vesicle, exosome, vector, viral vector, composition, or medicament of the disclosure is formulated for intramuscular injection, transdermal transport or injection into the blood stream.

**[0029]** Further aspects and advantages of the disclosure will be apparent to those of ordinary skill in the art from a review of the following detailed description, taken in conjunction with the drawings. It should be understood, however, that the detailed description (including the drawings and the specific examples), while indicating embodiments of the disclosed subject matter, are given by way of illustration only, because various changes and modifications within the spirit and scope of the disclosure will become apparent to those skilled in the art from this detailed description.

#### **BRIEF DESCRIPTION OF THE DRAWINGS**

**[0030]** Fig. 1A-D shows that U7-asDUX4 snRNAs protect HEK293 cells from DUX4-mediated death. Fig. 1A shows U7-snRNA structure consisting of a stabilizing hairpin-loop, Sm binding region, and an antisense sequence complementary to a target site on the DUX4 pre-mRNA (see Table 1 for sequences). Fig. 1B is a schematic drawing of 18 U7-asDUX4 constructs targeting different parts of DUX4 mRNA. ATG indicates start codon. Exon 1 (Ex1) contains the entire DUX4 open reading frame, while Ex2 and Ex3 contain 3' untranslated regions (3' UTR). \*P ≤ 0.05, \*\* P ≤ 0.01, ANOVA. N=3 independent experiments performed in triplicate. Fig. 1C shows results of a Caspase-3/7 assay for apoptosis. All DUX4-targeting U7-asDUX4 snRNA constructs significantly reduced Caspase-3/7 activity except in cells treated with sequence 6. Fourteen of 18 constructs

## CLAIMS

We claim:

1. A nucleic acid encoding a U7 double homeobox 4 (DUX4) antisense ribonucleic acid (asRNA), the nucleic acid comprising
  - (a) a nucleotide sequence comprising at least 90% identity to the sequence set forth in any one of SEQ ID NOs: 1-18;
  - (b) the nucleotide sequence set forth in any one of SEQ ID NOs: 1-18;
  - (c) a nucleotide sequence encoding a U7 double homeobox 4 (DUX4) antisense sequence that specifically hybridizes to a DUX4 target nucleotide sequence set forth in any one of SEQ ID NOs: 19-36; or
  - (d) a combination of the nucleotide sequences of any one of (a) to (c).
2. The nucleic acid of claim 1 further comprising a promoter nucleotide sequence.
3. The nucleic acid of claim 2, wherein the promoter is any of a U6 promoter, a U7 promoter, a tRNA promoter, a H1 promoter, a minimal CMV promoter, a T7 promoter, an EF1-alpha promoter, a Minimal EF1-alpha promoter, or a muscle-specific promoter.
4. The nucleic acid of claim 3, wherein the muscle-specific promoter is a unc45b promoter, a tMCK promoter, a minimal MCK promoter, a CK6 promoter, a CK7 promoter, a MHCK7 promoter, or a CK1 promoter.
5. A nanoparticle, extracellular vesicle, exosome, or vector comprising the nucleic acid of any one of claims 1-4 or a combination of any one or more thereof.
6. The vector of claim 5, wherein the vector is a viral vector.
7. The viral vector of claim 6, wherein the viral vector is an adeno-associated virus (AAV), adenovirus, lentivirus, retrovirus, poxvirus, baculovirus, herpes simplex virus, vaccinia virus, or a synthetic virus.
8. The viral vector of claim 6 or 7, wherein the viral vector is an AAV.

9. The viral vector of claim 8, wherein the AAV lacks rep and cap genes.
10. The viral vector of claim 8 or 9, wherein the AAV is a recombinant AAV (rAAV) or a self-complementary recombinant AAV (scAAV).
11. The viral vector of any one of claims 8-10, wherein the AAV is rAAV1, rAAV2, rAAV3, rAAV4, rAAV5, rAAV6, rAAV7, rAAV8, rAAV9, rAAV10, rAAV11, rAAV12, rAAV13, rAAV-anc80, rAAV rh.74, rAAV rh.8, rAAVrh.10, or rAAV-B1.
12. The viral vector of any one of claims 8-11, wherein the AAV is rAAV-9.
13. A composition comprising
  - (a) the nucleic acid of any one of claims 1-4;
  - (b) the nanoparticle, extracellular vesicle, exosome, or vector of claim 5; or
  - (c) the viral vector of any one of claims 6-12; anda pharmaceutically acceptable carrier.
14. An *in vitro* method of inhibiting and/or interfering with expression of a double homeobox 4 (DUX4) gene in a cell comprising contacting the cell with
  - (a) the nucleic acid of any one of claims 1-4;
  - (b) the nanoparticle, extracellular vesicle, exosome, or vector of claim 5;
  - (c) the viral vector of any one of claims 6-12; or
  - (d) the composition of claim 13.
15. Use of
  - (a) the nucleic acid of any one of claims 1-4;
  - (b) the nanoparticle, extracellular vesicle, exosome, or vector of claim 5;
  - (c) the viral vector of any one of claims 6-12; or
  - (d) the composition of claim 13in the preparation of a medicament for inhibiting expression of a double homeobox 4 (DUX4) gene in a cell.
16. Use of
  - (a) the nucleic acid of any one of claims 1-4;
  - (b) the nanoparticle, extracellular vesicle, exosome, or vector of claim 5;

- (c) the viral vector of any one of claims 6-12; or
  - (d) the composition of claim 13
- in the preparation of a medicament for treating or ameliorating a muscular dystrophy.
17. The use of claim 16, wherein the muscular dystrophy is facioscapulohumeral muscular dystrophy.
18. Use of
- (a) the nucleic acid of any one of claims 1-4;
  - (b) the nanoparticle, extracellular vesicle, exosome, or vector of claim 5;
  - (c) the viral vector of any one of claims 6-12; or
  - (d) the composition of claim 13
- in the preparation of a medicament for treating or ameliorating a cancer.
19. The use of claim 18, wherein the cancer is a sarcoma.
20. The
- (a) nucleic acid of any one of claims 1-4;
  - (b) nanoparticle, extracellular vesicle, exosome, or vector of claim 5;
  - (c) viral vector of any one of claims 6-12;
  - (d) composition of claim 13;
  - (e) method of claim 14; or
  - (e) use of any one of claims 15-19,
- wherein the nucleic acid, nanoparticle, extracellular vesicle, exosome, vector, composition, or medicament is formulated for intramuscular injection, transdermal transport or injection into the blood stream.