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(54) Title: ATOVAQUONE FOR THE TREATMENT AND MANAGEMENT OF MUSCULAR DYSTROPHIES

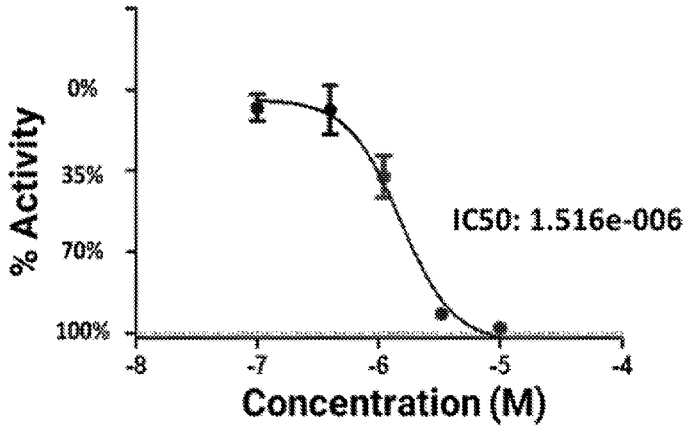

FIG. 1

(57) Abstract: Atovaquone for the treatment and management of muscular dystrophies Disclosed herein is the use of Atovaquone for the treatment or management or both of muscular dystrophy Atovaquone, according to the embodiments herein, is capable of inhibiting aryl hydrocarbon receptor (AHR), thereby upregulating utrophin levels in muscles, resulting in an overall improvement in muscle function. Embodiments herein also achieve a composition for upregulating utrophin levels in muscle cells.

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"Atovaquone for the treatment and management of muscular dystrophies"

CROSS REFERENCE TO RELATED APPLICATION

This application is based on and derives the benefit of Indian Provisional Application 202341026872, the contents of which are incorporated herein by reference.

TECHNICAL FIELD

[0001] Embodiments disclosed herein generally relate to compounds for the treatment or management of muscular dystrophy. More specifically, the invention relates to the use of Atovaquone for upregulating utrophin levels in muscle cells for the treatment of muscular dystrophy.

BACKGROUND

[0002] Muscular dystrophy (MD) refers to a group of genetic disorders characterized by the progressive degeneration and weakening of skeletal muscles. It affects approximately 1 in 5,000 males globally, with most cases diagnosed in childhood.

[0003] Duchenne Muscular dystrophy (DMD) is one of the most prevalent forms of muscular dystrophy, resulting from X-linked recessive mutations in the *dystrophin* gene. This gene encodes dystrophin, a 427-kDa cytoskeletal protein localized in the cytoplasmic surface of the muscle cell membranes. Dystrophin, along with the dystrophin-associated glycoprotein complex (DGC), functions as a key cytoskeletal integrator, essential for preserving the structural integrity of muscle membranes. It stabilizes the muscle fibers during contraction, ensuring strength, flexibility, and protection of the sarcolemma from mechanical damage.

[0004] Mutations in the *dystrophin* gene impair the production of dystrophin, leading to progressive muscle degeneration, and disruption of the neuromuscular junction organization. The absence of dystrophin also results in elevated intracellular calcium levels and excessive nitric oxide production, triggering harmful processes such as protein degradation, free radical generation, oxidative stress, inflammation, fibrosis, necrosis, and macrophage activation. These changes ultimately lead to dystrophic state of skeletal muscle, along with respiratory impairment and cardiomyopathy. Progressive muscle degeneration typically causes loss of ambulation between the ages of 8 and 12, with premature death occurring between 20 and 30 years due to respiratory and cardiac complications.

[0005] Currently, there is no cure for DMD. Management strategies primarily focus on alleviating symptoms, improving quality of life and slowing disease progression. While corticosteroids can decelerate the progression of DMD, they come with significant side effects, including weight gain, hyperglycemia, insulin resistance, Cushingoid features, short stature, behavioral changes, osteoporosis, and bone fractures.

[0006] Available therapeutic strategies for DMD primarily aim to restore dystrophin expression, primarily through gene therapy techniques. These include antisense oligonucleotide-mediated exon skipping, adeno-associated virus (AAV)-mediated mini-dystrophin gene delivery, CRISPR/Cas9 genome editing, and stop-codon suppression. However, these approaches are mutation-specific and only benefit a subset of dystrophy patients. Additionally, restoring dystrophin entails the problem of immune response against dystrophin, which can be recognized as a foreign protein by the immune system. To address this, upregulating utrophin – a non-immunogenic, autosomal homologue of dystrophin – emerges as a promising strategy for the treatment and management of muscular dystrophy.

[0007] Utrophin can be upregulated through various signaling pathways, such as AHR-ARNT, TGF-β, HDAC, GLP-1 – PGC-1α, GABPα/β and Calcineurin-NFAT. Notably, Aryl Hydrocarbon Receptor (AHR) signaling pathway plays a crucial role in muscle regeneration and repair, making it a potential target for therapeutic interventions in DMD. However, the long-term efficacy of therapies aimed at increasing utrophin levels remains uncertain and warrants further clinical investigation. For instance, Ezutromid, a small molecule drug designed to upregulate utrophin, was recently discontinued after failing to meet clinical trial endpoints, likely due to its self-limiting pharmacokinetic profile. This underscores the urgent need for more effective therapeutic strategies to upregulate utrophin in patients with muscular dystrophies.

[0008] Consequently, there exists a critical need to identify therapeutic agents that exhibit high efficacy, ease of administration, broad applicability, and excellent safety and tolerability profiles for the prevention, treatment, and management of muscular dystrophy.

OBJECTS

[0009] The principal object of the embodiments herein is to provide a compound for the treatment and management or both of muscular dystrophy.

[0010] A second object of the embodiments herein is to provide a compound that can upregulate utrophin levels in muscles.

[0011] Another object of the embodiments herein is to provide a compound that can antagonize Aryl Hydrocarbon Receptor (AHR) activation.

[0012] Another object of the embodiments herein is to provide a compound that can activate muscle regeneration and repair.

[0013] Another object of the embodiments herein is to provide a compound that can prevent or delay muscle wasting or muscle degradation.

[0014] Another object of the embodiments herein is to provide a compound capable of reducing inflammation, oxidative stress, fibrosis and necrosis in muscles.

[0015] Another object of the embodiments disclosed herein is to provide a compound that is readily available, cost-effective, user-friendly, therapeutically effective, sustainable, and has minimal side effects.

[0016] Another object of the embodiments herein is to provide a compound that confers potential protection against neuromuscular diseases.

[0017] Another object of the embodiments herein is to provide a compound for the preparation of a medicament for the treatment or management or both of muscular dystrophy through above mentioned mechanisms.

[0018] Another object of the embodiments herein is to provide a composition that can upregulate utrophin levels in muscles.

[0019] Another object of the embodiments herein is to provide a composition having an AHR antagonist activity.

[0020] Another object of the embodiments herein is to provide a composition for the treatment or management or both of muscular dystrophy.

[0021] Another object of the embodiments herein is to provide a method for the treatment or management or both of muscular dystrophy.

[0022] These and other aspects of the embodiments herein will be better appreciated and understood when considered in conjunction with the following description and the accompanying drawings. It should be understood, however, that the following descriptions, while indicating at least one embodiment and numerous specific details thereof, are given by way of illustration and not of limitation. Many changes and modifications may be made within the scope of the embodiments herein without departing from the spirit thereof, and the embodiments herein include all such modifications.

BRIEF DESCRIPTION OF FIGURES

[0023] Embodiments herein are illustrated in the accompanying drawings, and reference letters/numerals indicate corresponding parts in various figures. The embodiments herein will be better understood from the following description with reference to the following illustratory drawings. Embodiments herein are illustrated by way of examples in the accompanying drawings, and in which:

[0024] FIG. 1 presents the antagonist dose-response analyses of Human AHR for Atovaquone, according to embodiments as disclosed herein;

[0025] FIG. 2 presents the fold change in mRNA expression of utrophin in RT-PCR at different concentrations of Atovaquone, according to embodiments as disclosed herein;

[0026] FIG. 3 shows cell viability and proliferation assays at different concentrations of Atovaquone, according to embodiments as disclosed herein;

[0027] FIG. 4 displays the result of the effect of Atovaquone on normalized grip strength in D2.mdx mice, according to embodiments as disclosed herein;

[0028] FIG. 5 presents the effect of Atovaquone on hanging latency in D2.mdx mice, according to embodiments as disclosed herein; and

[0029] FIG. 6 presents the effect of Atovaquone on serum creatine kinase levels in D2.mdx mice, according to embodiments as disclosed herein.

DETAILED DESCRIPTION

[0030] The embodiments herein and the various features and advantageous details thereof are explained more fully with reference to the non-limiting embodiments that are illustrated in the accompanying drawings and detailed in the following description. Descriptions of well-known components and processing techniques are omitted so as to not unnecessarily obscure the embodiments herein. The examples used herein are intended merely to facilitate an understanding of ways in which the embodiments herein may be practiced and to further enable those of skill in the art to practice the embodiments herein. Accordingly, the examples should not be construed as limiting the scope of the embodiments herein.

[0031] For the purposes of interpreting this specification, the definitions (as defined herein) will apply and whenever appropriate the terms used in singular will also include the plural and vice versa. It is to be understood that the terminology used herein is for the purposes of describing particular embodiments only and is not intended to be limiting. The terms

"comprising", "having" and "including" are to be construed as open-ended terms unless otherwise noted.

[0032] The words/phrases "exemplary", "example", "illustration", "in an instance", "and the like", "and so on", "etc.", "etcetera", "e.g.,", "i.e.," are merely used herein to mean "serving as an example, instance, or illustration." Any embodiment or implementation of the present subject matter described herein using the words/phrases "exemplary", "example", "illustration", "in an instance", "and the like", "and so on", "etc.", "etcetera", "e.g.,", "i.e.," is not necessarily to be construed as preferred or advantageous over other embodiments. The terms "comprising", "having" and "including" are to be construed as open-ended terms unless otherwise noted. The terms "individual", or "patient" or "subject" or "cell-line" are used herein interchangeably.

[0033] It should be noted that elements in the drawings are illustrated for the purposes of this description and ease of understanding of aspects of the embodiments as disclosed herein. The accompanying drawings are used to help easily understand various technical features and it should be understood that the embodiments presented herein are not limited by the accompanying drawings. As such, the present disclosure should be construed to extend to any modifications, equivalents, and substitutes in addition to those which are particularly set out in the accompanying drawings and the corresponding description. Usage of words such as first, second, third etc., or I, II, III, etc., to describe components/elements/steps is for the purposes of this description and should not be construed as sequential ordering/placement/occurrence unless specified otherwise.

[0034] Embodiments herein disclose the use of Atovaquone for the treatment or management or both of muscular dystrophy. The inventors of this application have shown that inhibiting the Aryl Hydrocarbon Receptor (AHR) signaling pathway by antagonizing AHR can upregulate utrophin levels *in vitro* in mouse skeletal muscle cell lines, as well as *in vivo* in mouse D2-mdx mouse model of DMD, resulting in an overall improvement in muscle function. The inventors have illustrated, for the first time, that AHR antagonist, Atovaquone, typically used for the treatment of protozoal infections, can be repurposed to upregulate utrophin levels in muscle cells, and can be used for the treatment or management of muscular dystrophy. Embodiments herein also achieve a composition for upregulating utrophin levels in muscle cells. The composition, according to the embodiments herein, includes at Atovaquone or its pharmaceutically acceptable salts, solvates or analogues thereof, and at least one pharmaceutically acceptable excipient. It is also within the scope that the invention may or may

not have additional additive selected from carriers, solvents, stabilizers, or suspensions with the composition.

[0035] The term "muscular dystrophy" refers to genetically and clinically heterogeneous group of rare neuromuscular diseases caused by mutations in the dystrophin gene, dysferlin gene and associated glycoprotein complex (DAPC/DGC). Muscular dystrophy, as used herein, encompass different categories of muscular dystrophies including, but not limited to, dystroglycanopathy, dysferlinopathy and dystrophinopathy.

[0036] Dystroglycanopathy is a collective term referring to muscular dystrophies with abnormal glycosylation of α-dystroglycan (DG), a glycoprotein that interacts with dystrophin or mutations of genes related to Dystroglycan protein complex (DAPC/DGC). Dystroglycanopathy exhibit a broad clinical spectrum, ranging from severe congenital muscular dystrophies, to mild ones, including Fukuyama Congenital muscular dystrophy (FCMD), Myotonic muscular dystrophy, Facioscapulohumeral muscular dystrophy (FSHD1/2), Congenital muscular dystrophy (CMD1C), Limb-girdle muscular dystrophy (LGMD's around 32 variants including LGMDR9/LGMD2I), Emery-Dreiffus muscular dystrophy (EDMD), Muscle-Eye-Brain disease (MEB), Walker-Warburg syndrome (WWS), Calpainopathies or LGMD2A and Oculopharyngeal muscular dystrophy.

[0037] Dystrophinopathy covers a spectrum of X-linked muscle disease ranging from mild to severe that includes Duchenne muscular dystrophy, Becker muscular dystrophy, and DMD-associated dilated cardiomyopathy (DCM).

[0038] Dysferlinopathy is a disease caused by dysferlin deficiency due to mutations in the DYSF gene. Dysferlin is a membrane protein in the sarcolemma and is involved in different functions, such as membrane repair and vesicle fusion, T-tubule development and maintenance, Ca2+ signaling, and the regulation of various molecules. Dysferlinopathy includes Miyoshi Myopathy type 1 (MMD1) and Limb–Girdle Muscular Dystrophy R2 dysferlin-related (LGMDR2). Accordingly, the compounds of the present invention can be used for the treatment or management or both of muscular dystrophy including, but not limited to, Fukuyama Congenital muscular dystrophy (FCMD), Myotonic muscular dystrophy, Facioscapulohumeral muscular dystrophy (FSHD1/2), Congenital muscular dystrophy (CMD1C), Limb-girdle muscular dystrophy, Emery-Dreiffus muscular dystrophy (EDMD), Muscle-Eye-Brain disease (MEB), Walker-Warburg syndrome (WWS), Calpainopathies or LGMD2A, Oculopharyngeal muscular dystrophy, Duchenne muscular dystrophy, Becker muscular dystrophy, DMD-associated dilated cardiomyopathy (DCM), Miyoshi Myopathy

type 1 (MMD1), and Limb–Girdle Muscular Dystrophy R2 dysferlin-related (LGMDR2) muscular dystrophy.

[0039] In one embodiment, the muscular dystrophy is Duchenne Muscular Dystrophy (DMD). In another embodiment, the muscular dystrophy is Becker muscular dystrophy (BMD). Both DMD and BMD are characterized by progressive muscle weakness and skeletal degeneration. In DMD patients, dystrophin is virtually absent, whereas BMD patients have 10% to 40% of the normal amount. The increased permeability of the sarcolemma caused by dystrophy often leads to the release of creatine kinase (CK) from muscle fibers. Therefore, an increased level of serum CK is the hallmark of muscle damage. In patients with DMD, CK is markedly elevated compared with the normal range, which has diagnostic value.

[0040] Muscular dystrophy, as used herein, also includes atrophy characterized by muscle degeneration or loss of mass often attributed to aging or various diseases such as polio, severe malnutrition, nerve injuries or other neurogenic disorders. Dystrophy typically stems from genetic mutations and entails severe weakness due to insufficient muscle proteins, often with visible muscle weakness and wasting. While atrophy can be mitigated through exercise and lifestyle adjustments, dystrophy, being genetic in nature, is irreversible.

[0041] The aryl hydrocarbon receptor (AHR) is a ligand-dependent transcription factor, which responds to a wide range of exogenous and endogenous ligands with the induction or repression of a variety of genes. The best-characterized ligands for AHR include, but are not limited to, toxic halogenated aromatic hydrocarbons (HAHs), such as the polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls, and numerous polycyclic aromatic hydrocarbons (PAHs) and PAH-like chemicals, such as benzo(a)pyrene, 3-methylcholanthrene, and beta-naphthoflavone (BNF). Upon ligand binding, AHR dimerizes with the aryl hydrocarbon receptor nuclear translocator (ARNT), leading to the transcriptional activation of various xenobiotic metabolizing enzymes, such as cytochrome P4501A1 and glutathione-S-transferase. AHR is a pivotal determinant in human physiology and also in the incidence, onset, and progression of pathophysiological processes, including carcinogenesis, inflammation, infection, diabetes, and cardiovascular diseases. The ligand-binding pocket of AHR is extremely promiscuous which can bind to diverse small molecule agonists and antagonists, with agonists inducing nuclear translocation of the receptor and increased transcription of AhR-responsive genes.

[0042] The term “AHR antagonists”, as used herein, refers to small molecules that interfere with or prevent the binding of ligands to the AHR, thereby inhibiting the AHR

signalling pathway. A compound can antagonize AHR activation either through direct mechanisms, such as competitive binding to the AHR ligand-binding site, or through indirect mechanisms that do not involve direct AHR binding. These indirect processes include, but are not limited to, inhibiting proteins involved in nuclear import, binding to AHR-associated chaperone proteins, inhibiting kinases involved in phosphorylation events, and promoting protein degradation. Examples of AHR antagonists include, but are not limited to, 3′-methoxy-4′-nitroflavone (3M4NF), 3,3′-diindolylmethane (DIM), epigallocatechin gallate (EGCG), Ezutromid, CH223191, ANF, StemRegenin 1 (SR-1), Flavonoids, Catechins, Curcumin, Resveratrol, and Lutein. AHR antagonists have been shown to upregulate utrophin, indicating that this pathway, currently being investigated for other clinical applications such as oncology and rheumatoid arthritis, could also be utilized in future Duchenne muscular dystrophy (DMD) therapies. Several lines of evidence suggest that Ezutromid, the first-in-class utrophin modulator discontinued after phase 2 clinical trials, can bind to AHR with an apparent Kd of 50 nM and acts as an AHR antagonist.

[0043] Utrophin upregulation, as used herein, refers to a therapeutic approach for Duchenne muscular dystrophy (DMD) aimed at increasing the expression of utrophin, an autosomal paralog of the dystrophin protein. Utrophin, also known as dystrophin-related protein (DRP), can serve as a functional substitute, compensating for the absence or reduction of dystrophin. Utrophin is a member of the spectrin superfamily and shares significant sequence similarity and functional motifs with dystrophin, including the ability to bind the same dystrophin-associated glycoprotein complex. Utrophin is highly expressed in fetal tissue but is developmentally downregulated in adults. The decline in utrophin levels has been shown to coincide with the onset of muscle necrosis in the *mdx* mouse model of DMD. Restoring the expression of either truncated or full-length utrophin significantly improves the *mdx* mouse phenotype, supporting the case for pharmacological upregulation of endogenous utrophin as a promising therapeutic strategy for DMD.

[0044] The inventors of the present application have surprisingly found that that Atovaquone, an FDA approved oral generic drug with excellent pharmacokinetics and broader safety profile, can antagonize AHR activation resulting in an upregulation of utrophin levels in muscle cells. The inventors have shown that Atovaquone increases utrophin expression in muscle cells, resulting in an overall improvement in muscle function. Accordingly, embodiments herein disclose the use Atovaquone for the treatment or management or both of muscular dystrophy. It is also within the scope of the invention, to use salts, solvates,

derivatives, isomers, tautomeric forms, or analogues of Atovaquone. In one embodiment, the invention discloses the use of Atovaquone for the manufacture of a medicament for the treatment or management or both of muscular dystrophy.

[0045] Atovaquone or hydroxy-1,4-naphthoquinone, belonging to the class of naphthoquinones, is an antimicrobial medication typically used for the prevention and treatment of *Pneumocystis jiroveci* pneumonia (PCP) and falciparum malaria. It is an analog of both ubiquinone and lawsone and acts by interfering with the mitochondrial electron transport in susceptible organisms.

Composition

[0046] Embodiments herein also achieve a composition for upregulating utrophin levels in muscle cells. The composition, according to the embodiments herein, includes Atovaquone or its pharmaceutically acceptable salts, solvates or analogues thereof, and at least one pharmaceutically acceptable excipient.

[0047] In one embodiment, the composition contains a pharmaceutically acceptable salt of Atovaquone. Pharmaceutically acceptable salts, as used herein, refers to a salt that retains the biological effectiveness of the free acids and bases of a specified compound and that is not biologically or otherwise undesirable. Pharmaceutically acceptable salt may also refer to a salt that may have an unexpectedly superior biological efficacy or effectiveness when compared to the actual or active pharmaceutical ingredient (API) as well. According to the present invention, pharmaceutically acceptable salts are produced from acidic inorganic or organic compounds, or alkaline inorganic or organic compounds. Salts derived from inorganic bases include aluminum, ammonium, calcium, copper, ferric, ferrous, lithium, magnesium, manganic salts, manganous, potassium, sodium, zinc, and the like. Salts in the solid form may exist in more than one crystal structure and may also be in the form of hydrates. Salts derived from pharmaceutically acceptable organic non-toxic bases include salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines, and basic ion exchange resins, such as arginine, betaine, caffeine, choline, N,N-dibenzylethylene- diamine, diethylamine, 2-diethylaminoethanol, 2-dimethylaminoethanol, ethanolamine, ethylenediamine, N-ethyl-morpholine, N-ethylpiperidine, glucamine, glucosamine, histidine, hydrabamine, isopropylamine, lysine, methylglucamine, morpholine, piperazine, piperidine, polyamine resins, procaine, purines, theobromine, triethylamine, trimethylamine, tripropylamine, tromethamine, and the like. Salts from inorganic and organic acids include acetic, benzenesulfonic, benzoic, camphorsulfonic, citric, ethanesulfonic,

fumaric, gluconic, glutamic, hydrobromic, hydrochloric, isethionic, lactic, maleic, malic, mandelic, methanesulfonic, mucic, nitric, pamoic, pantothenic, phosphoric, succinic, sulfuric, tartaric, p-toluenesulfonic acid, and the like.

[0048] In one embodiment, the composition includes a solvate or analogue of Atovaquone. The term "solvate", as used herein, typically refers to a compound (or a salt thereof), in association with a solvent, such as water. Representative examples include hydrates, hemihydrates, trihydrates and the like. As used herein, the term "analogue" is typically used to denote a compound that has a chemical structure that is substantially similar to the structure of the parent compound, whilst retaining at least some of the biological function of the parent compound. Analogues also include pharmaceutically acceptable salts.

[0049] In one embodiment, the composition includes at least one pharmaceutically acceptable excipient. The term "pharmaceutically acceptable excipient", as used herein, refers to a compound or ingredient that is compatible with the other ingredients in a pharmaceutical formulation and not injurious to an intended subject when administered in normal or therapeutically effective amounts. Suitable pharmaceutically acceptable excipients will vary depending upon the dosage form chosen. In addition, suitable pharmaceutically acceptable excipients may be chosen for a particular function that they may serve in the composition. For example, certain pharmaceutically acceptable excipients may be chosen for their ability to facilitate the production of uniform dosage forms. Certain pharmaceutically acceptable excipients may be chosen for their ability to facilitate the production of stable dosage forms. Certain pharmaceutically acceptable excipients may be chosen for their ability to facilitate the carrying or transporting of the compound or compounds disclosed herein once administered to the patient from one organ, or portion of the body, to another organ, or portion of the body. Certain pharmaceutically acceptable excipients may be chosen for their ability to enhance patient compliance.

[0050] Pharmaceutically acceptable excipients include, but are not limited to fillers, extenders, diluents, wetting agents, solvents, emulsifiers, preservatives, absorption enhancers, sustained-release matrices, starches, sugars, microcrystalline cellulose, granulating agents, lubricants, binders, disintegrating agents, coloring agents, release agents, coating agents, sweetening agents, flavoring agents, perfuming agents, antioxidants, plasticizers, gelling agents, thickeners, hardeners, setting agents, suspending agents, surfactants, humectants, carriers, stabilizers, and combinations thereof. Examples of diluents include, but are not limited to calcium carbonate, calcium phosphate, calcium sulfate, cellulose acetate, dextrates,

dextrins, dextrose, ethyl cellulose, fructose, gelatin, glyceryl palmitostearate, isomalt, kaolin, lactitol, lactose, magnesium carbonate, magnesium oxide, maltodextrins, maltose, microcrystalline or powdered cellulose, polymethacrylates, pregelatinized starch, starch, sodium carbonate, sodium chloride, sorbitol or sucrose, among others, and mixtures thereof. Examples of lubricants include, but are not limited to, calcium stearate, glyceryl behenate, glyceryl palmitostearate, hydrogenated castor oil, magnesium stearate, polyethylene glycol, sodium benzoate, sodium lauryl sulfate, sodium stearyl fumarate, stearic acid, or talc, among others, and mixtures thereof. Examples of disintegrants include, but are not limited to alginic acid, crospovidone, sodium croscarmellose, sodium starch glycolate, starch, low-substituted hydroxypropyl cellulose, among others, and mixtures thereof. Examples of binding agents include, but are not limited to acacia, cellulose acetate phthalate, dextrates, dextrin, ethyl cellulose, guar gum, hydroxyethyl cellulose, hydroxyethylmethyl cellulose, hydroxypropyl cellulose, hydroxy propyl methyl cellulose methylcellulose, maltodextrin, microcrystalline cellulose, sucrose, povidone, pregelatinized starch, sodium carboxymethylcellulose, starch or stearic acid, among others, and mixtures thereof. Examples of glidants include, but are not limited to, tribasic calcium phosphate, powdered cellulose, colloidal silicon dioxide, magnesium oxide, magnesium silicate, magnesium trisilicate, silicon dioxide, or talc, among others, and mixtures thereof. Sweetening agents include, but are not limited to, sorbitol, maltitol, mannitol, dextrose, isomalt, maltose, xylitol, saccharin, sucrose, sucralose, aspartame, acesulfame potassium, or trehalose, among others, and mixtures thereof. Flavoring agents include, but are not limited to, maltol, vanillin, ethyl vanillin, menthol, citric acid, fumaric acid, ethyl maltol, tartaric acid, peppermint, artificial or natural fruit aromas, among others, and mixtures thereof. Coloring agents include, but are not limited to, curcumin, lactoflavin, iron oxides (red, yellow or black), caramel, lactoflavin phosphate, cochineal red, titanium dioxide, or carotenes, among others, and mixtures thereof.

[0051] The composition may be formulated together or separately with the pharmaceutically acceptable excipients or the carriers. Desirably, a compound of the invention and the pharmaceutically acceptable excipient or the carrier are formulated together for their simultaneous or near simultaneous administration. In one embodiment, the pharmaceutically acceptable excipient or the carrier may be formulated separately with a compound of the invention.

[0052] The concentration of Atovaquone in the composition may range from as low as 0.1% of the total amount of the composition up to as high as 100%. In some embodiments

the concentration of Atovaquone in the composition is from 1% to 90% by weight. In some embodiments the concentration of Atovaquone in the composition is from 5% to 80% by weight. In some embodiments, the concentration of Atovaquone in the composition is from 10% to 70% by weight. The exact amount will depend upon the any additional materials chosen.

[0053] Atovaquone or the composition comprising Atovaquone, according to the embodiments herein, can be administered as a monotherapy or in combination with one or more additional therapies. In one embodiment, Atovaquone or the composition including Atovaquone is administered as monotherapy. In one embodiment, Atovaquone or the composition including Atovaquone is administered as combination therapy with one or more additional therapeutic agents. Non-limiting examples of additional therapies that can be used for combination therapy include, but are not limited to, corticosteroid therapy, gene therapy, exon-skipping therapy, immunosuppressant therapy, epigenetic therapy, muscle re-generation therapy and muscle-strengthening therapy.

[0054] In one embodiment, combination therapy comprises administering Atovaquone or the composition comprising Atovaquone with corticosteroids. Corticosteroid therapy includes the administration of corticosteroids to delay the progression of muscular dystrophy. Examples of corticosteroids that are used in the treatment of dystrophy include, but are not limited to, Prednisone/Prednisolone, Deflazacort (an oxazoline derivative of prednisolone), Vamorolone and combinations thereof. Corticosteroids are administered by two common regimens – daily and intermittent.

[0055] In one embodiment, combination therapy comprises administering the Atovaquone or the composition comprising Atovaquone with exon-skipping therapies. Exon skipping therapy refers to the use of antisense oligonucleotides to slice out selected exons from pre-mRNA, at or next to, the mutation site, to generate a translatable transcript from the mutant of dystrophin gene. The antisense oligonucleotides (AONs) are 20 – 30 nucleotides in length, designed to target specific pre-mRNA sequences and to skip a specific DMD exon flanking the region of mutation, producing an in-frame but truncated transcript that translate a functional dystrophin protein. Examples of AON agents for exon-skipping therapy include, but are not limited to, Eteplirsen, Golodirsen, Viltolarsen, Casimersen, Drisapersen, tricyclo-DNA (tcDNA), ASO-based therapy and combinations thereof.

[0056] In one embodiment, combination therapy comprises administering Atovaquone or the composition comprising Atovaquone with epigenetic agents. Epigenetic

therapy involves utilizing small molecules or epigenetic modifiers to modify gene activity without changing the gene's coding sequence. Key epigenetic mechanisms, such as DNA methylation or histone modification, play a crucial role in regulating muscle regeneration. Epigenetic therapy includes therapeutic approaches by creating epigenetic drugs designed to target specific chromatin elements within individual signaling pathways. Examples of epigenetic drugs include, but are not limited to, Givinostat, Trichostatin A (TSA), Pan-HDAC inhibitors, HDAC6 inhibitors and combinations thereof.

[0057] In one embodiment, combination therapy comprises administering Atovaquone or the composition comprising Atovaquone with gene therapy agents. Gene therapy includes, but are not limited to, adeno-associated virus (AAV) vector-mediated gene therapy, with the micro-dystrophin gene being a preferred candidate.

[0058] It is also within the scope of the invention to use Atovaquone or the composition comprising Atovaquone in combination with muscle re-generation therapies such as AAK1 inhibitors or cAMP enhancing mechanisms, other utrophin up regulators, muscle-strengthening therapies such as aryl hydrocarbon receptor (AhR) antagonists, myostatin inhibitors, muscle Ca^{2+} overload inhibitors like P2X7 antagonist, Store Operated Calcium Entry (SOCE) / Calcium Release Activated Calcium (CRAC) channel inhibitors, anti-inflammatory agents working in the NF-kB pathway signaling targets like NF-κB inhibitors, IKK2/β inhibitors, TBK1 inhibitors, Akt-mTOR pathway inhibitors and anti-fibrotic mechanism pathway agents like TGF-β inhibitors, RIPK1/3 inhibitors, Activin receptor inhibitors, Smad2/3 inhibitors and TAK1 inhibitors and other GLP-1 agonists, and GLP-1 pathway activators.

[0059] The combination therapy is administered in a manner and at a dosage effective to increase the production of utrophin and improve muscle function and strength.

[0060] Atovaquone or the composition comprising Atovaquone of the present invention may be used in combination with one or more other drugs in the treatment, suppression or amelioration of muscular dystrophy, where the combination of the drugs together is safer or more effective than either drug alone. Such other drug(s) may be administered, by a route and in an amount commonly used therefor, contemporaneously, or sequentially with the compounds of the present invention. When a compound of the present invention is used contemporaneously with one or more other drugs, a pharmaceutical composition containing such other drugs in addition to the compound of the present invention is preferred. Accordingly, the pharmaceutical compositions of the present invention include

those that also contain one or more other active ingredients, in addition to a compound of the present invention. The combination therapy may also include therapies in which the compound of the present invention and one or more other drugs are administered on different overlapping schedules. It is also contemplated that when used in combination with one or more other active ingredients, the compounds of the present invention and the other active ingredients may be used in lower doses than when each is used singly.

[0061] Atovaquone or the composition comprising Atovaquone, according to the embodiments herein, can be formulated for administration by any suitable route, including parenteral (e.g., intravenous, intramuscular), intradermal, cutaneous, subcutaneous, oral, transdermal, transmucosal, topical, nasal, vaginal, intrathecal, epidural, ocular and rectal administration or by injection, or inhalation.

[0062] In one embodiment, Atovaquone or the composition comprising Atovaquone is in the form of a solution for parenteral, intradermal, or subcutaneous application. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include a sterile diluent such as water, saline solution, fixed oils, polyethylene glycols, glycerin, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. In one embodiment, the parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[0063] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. For intravenous administration, suitable carriers include, but are not limited to, physiological saline, bacteriostatic water, Cremophor EL.TM. (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, or liquid polyetheylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of a dispersion or by the use of surfactants. Prevention of the action of microorganisms can be achieved by incorporation of various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol,

ascorbic acid, thimerosal, and the like. It is preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, or sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate or gelatin.

[0064] In one embodiment, the sterile injectable solutions are prepared by incorporating Atovaquone in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[0065] In one embodiment, Atovaquone or the composition comprising Atovaquone is formulated as tablets, hard or soft capsules, gummy chewables, syrups, elixirs, pills, troches, lozenges, emulsions, dispersible powders or granules, liquids, gels, aqueous or oily suspensions, patches, nano formulations or other suitable forms for oral, parenteral, topical, or inhalation administration. The tablets, pills, capsules, troches and the like can include a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring. Suitable tablets may be obtained for example, by mixing Atovaquone with known excipients, for example diluents such as microcrystalline cellulose, calcium carbonate, calcium phosphate or lactose, disintegrants such as croscaramellose sodium, HPMC, sodium starch glycolate, binders such as starch or gelatin, guar gum, xanthum gum, lubricants such as magnesium stearate or talc and/or agents. In one embodiment, the shapes of the tablets include, but are not limited to, round, caplet, flat, oval and bevelled edges with and without embossing.

[0066] In one embodiment, capsules containing Atovaquone with at least one pharmaceutically acceptable excipient, is enclosed within either a hard or a soft soluble shell. The major component of a capsule shell is gelatin, while other components include water, colorants, plasticizers, such as glycerine or sorbitol, and opacifying agents. Hypromellose can alternatively be used as capsule shell material. Capsules may be prepared

by mixing the active compounds with inert carriers such as lactose or sorbitol and packing them into gelatin capsules. Capsules may be with or without imprinting.

[0067] Oily suspensions, according to the embodiments herein, may be formulated by suspending Atovaquone in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and flavoring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an antioxidant such as ascorbic acid.

[0068] Dispersible powders and granules, suitable for preparation of an aqueous suspension by the addition of water, Atovaquone in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavoring and coloring agents, may also be present.

[0069] Syrups and elixirs may be formulated with sweetening agents, for example glycerol, propylene glycol, sorbitol, or sucrose. Such formulations may also contain a demulcent, a preservative and flavoring and coloring agents.

[0070] Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated can be used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished with nasal sprays or suppositories. The compounds can be prepared in the form of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

[0071] In one embodiment, Atovaquone or the composition including Atovaquone is in the form of semisolid compositions such as creams, gels, ointments or pastes, comprising a pharmaceutically acceptable carrier or vehicle in which Atovaquone is dissolved, emulsified, dispersed or suspended.

[0072] Creams are semisolid emulsions, which can be of the oil-in-water (o/w) type or water-in-oil (w/o) type, formulated from an oil phase, an aqueous phase and an emulsifying agent. Gels are obtained from a liquid that is gelled by adding a rheological agent or a gelling agent. Ointments are semisolid fat preparations, which contain the active ingredient dissolved

or in dispersed form. Ointments can be formulated with various vehicles such as paraffin, plastibases (a mixture of polyethylene with a series of hydrocarbons) or vegetable oils. Pastes are prepared analogously to the ointments, and they show a more solid. The oily phase in oil-in-water emulsions may be a vegetable oil, for example olive oil or arachis oil, or a mineral oil, for example liquid paraffin or mixtures of these. Suitable emulsifying agents include naturally occurring gums, for example gum acacia or gum tragacanth, naturally occurring phosphatides, for example soybean, lecithin, and esters or partial esters derived from fatty acids and hexitol anhydrides, for example sorbitan monooleate, and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions may also contain sweetening and flavoring agents.

[0073] The dosage of Atovaquone or the composition including Atovaquone depends on various factors, including but not limited to metabolic stability and length of action of the compound, the route and time of administration, the rate of excretion, the duration of the treatment, severity of the condition, drug combination, the identity of any other therapeutic compounds being administered, the age, body weight, general health, sex, diet, size, and species of the subject, e.g., human patient, and like factors. In general, the dosage of Atovaquone in the present composition will be an amount which is the lowest dose effective to produce the desired effect with no or minimal side effects. The effective dose of Atovaquone may also be administered as two, three, four, five, six or more sub-doses, administered separately at appropriate intervals throughout the day. An appropriate dosage level will generally be about 10 to 1500 mg per day which can be administered in single or multiple doses. Preferably, the dosage level will be about 0.5 to about 100 mg/kg per day. A suitable dosage level may be about 0.01 to 250 mg/kg per day, about 0.05 to 100 mg/kg per day, or about 0.1 to 50 mg/kg per day. Within this range the dosage may be 0.05 to 0.5, 0.5 to 5 or 5 to 50 mg/kg per day. For oral administration, the compositions are preferably provided in the form of tablets containing 1.0 to 1000 milligrams of the active ingredient, particularly 1.0, 5.0, 10.0, 15.0. 20.0, 25.0, 50.0, 75.0, 100.0, 150.0, 200.0, 250.0, 300.0, 400.0, 500.0, 600.0, 750.0, 800.0, 900.0, and 1000.0 milligrams of the active ingredient for the symptomatic adjustment of the dosage to the patient to be treated. The compounds may be administered on a regimen of 1 to 4 times per day, preferably once or twice per day. In one embodiment, the composition is administered at a dose between 60 mg/kg per day and 120 mg/kg per day in mice. In one embodiment, the composition is administered at a dose of 100 mg/kg per day in D2.mdx mice.

In one embodiment, the broader human dosage of the composition is 10 to 250 mg per day, administered in a single or multiple dosage regimen.

[0074] In one embodiment, Atovaquone or the composition including Atovaquone is used for the treatment or management, or both, of muscular dystrophy. Treatment or management includes inhibiting the condition, that is, arresting the development or progression of clinical symptoms, and/or relieving the condition, i.e., causing regression of clinical symptoms. The composition, according to the embodiments herein can be used to manage symptoms of muscular dystrophy such as muscle weakness and wasting and to slow disease progression. The composition can also be used to improve the quality of life in dystrophy patients. In one embodiment, the composition provides a strategy for a dystrophy-specific therapy that in principle is applicable to all patients, i.e., not limited to restricted subsets of patients having mutation-specific muscular dystrophies.

[0075] In one embodiment, Atovaquone or the composition including Atovaquone upregulates utrophin expression in muscle cells. Utrophin expression is subject to regulation at multiple steps throughout its synthesis and degradation pathways. Different approaches for modulating utrophin expression include, but are not limited to, direct mechanisms such as gene or protein replacement, and indirect ones, such as transcriptional upregulation of the utrophin promoter, post-transcriptional regulation and protein/mRNA stabilization. Utrophin can be upregulated by various signaling pathways, but not limited to, such as AHR-ARNT, TGF-β, HDAC, GLP-1 – PGC-1α, GABPα/β and Calcineurin-NFAT - mediated signaling pathways. In one embodiment, the composition upregulates utrophin expression via modulating AHR-ARNT pathway. In one embodiment, the composition inhibits inflammation, muscle atrophic factors and thereby reduce muscle wasting. In one embodiment, the composition can reduce muscle fibrosis and necrosis. In an embodiment, the composition has potential therapeutic effects in animal models and clinical trials, indicating its efficacy in humans.

[0076] The use of Atovaquone as repurposed drug for the upregulation of utrophin has several advantages. Atovaquone is a potent, well-tolerated, and orally bioavailable drug with broad applicability, excellent safety and tolerability profiles, making them suitable for long-term use. Atovaquone is also proven to be safe for long term use in pediatric (adolescents) and adult population. Atovaquone also demonstrate anti-inflammatory and anti-microbial properties.

[0077] The repurposing of Atovaquone as a drug to upregulate utrophin for the treatment of Duchenne muscular dystrophy (DMD) represents a significant breakthrough in

DMD therapeutics. This approach has the potential to reduce development time and costs, facilitating quicker access to treatment for DMD patients and providing a promising therapeutic intervention.

[0078] Embodiments herein also disclose a method for increasing expression of utrophin in a subject in need thereof, the method comprising administering to the subject, a therapeutically effective amount of Atovaquone or the composition comprising Atovaquone or its pharmaceutically acceptable salt, and at least one pharmaceutically acceptable excipient.

[0079] Embodiments herein also disclose a method for treatment of muscular dystrophy. The method, according to the embodiments herein, include administering to a subject in need of, a therapeutically effective amount of Atovaquone or the composition comprising Atovaquone or its pharmaceutically acceptable salt, and at least one pharmaceutically acceptable excipient. The term "effective" or "therapeutically effective", as used herein, refers to amount of a compound that is nontoxic, but is present in a sufficient amount to provide the desired effect at a reasonable benefit/risk ratio for attending any medical treatment. The desired effect can be alleviation of the signs, symptoms, or causes of a disease, or any other desired result in a biological symptom.

[0080] The subject is generally a mammal, preferably a human being, male or female, in whom utrophin upregulation is desired. In one embodiment, the subject includes a mammal suffering from muscular dystrophy. In one embodiment, muscular dystrophy includes Fukuyama Congenital muscular dystrophy (FCMD), Myotonic muscular dystrophy, Facioscapulohumeral muscular dystrophy (FSHD1/2), Congenital muscular dystrophy (CMD1C), Limb-girdle muscular dystrophy, Emery-Dreiffus muscular dystrophy (EDMD), Muscle-Eye-Brain disease (MEB), Walker-Warburg syndrome (WWS), Calpainopathis or LGMD2A, Oculopharyngeal muscular dystrophy, Duchenne muscular dystrophy, Becker muscular dystrophy, DMD-associated dilated cardiomyopathy (DCM), Miyoshi Myopathy type 1 (MMD1), and Limb–Girdle Muscular Dystrophy R2 dysferlin-related (LGMDR2) muscular dystrophy.

[0081] The invention is further described by reference to the following examples by way of illustration only and should not be construed to limit the scope of the embodiments disclosed herein. It will be apparent to those skilled in the art that many modifications, both to materials and methods, may be practiced without departing from the scope of the claimed embodiments.

Example 1 – AHR functional assay

[0082] 15 µl of Reporter Cell suspension is dispensed into assay plate wells and preincubated for 6 hours. Following the pre-incubation period, culture media are discarded, and 15 µl/well of the different concentrations of Atovaquone are added and incubated on the plate for 24 hrs @ 37°C in the cell culture incubator. Following 24 hr incubation, 15 µl of Luciferase Detection Reagent is added to the assay plate and incubated at room temperature for 30 minutes. The light emission intensity (in units of 'Relative Light Units'; RLU) from each assay well is quantified using a plate-reading luminometer. Appropriate controls are used to correlate the data.

[0083] The dose-response curve of Atovaquone using an AHR cell-based assay kit indicates that a concentration of 10µM, Atovaquone completely inhibits AHR (FIG. 1). The IC_{50} value of Atovaquone is measured as 1.51 µM.

Example 2 – Utrophin Upregulation by Atovaquone *in vitro*

[0084] C2C12 myoblast cells are seeded in the well plate with a growth medium (10% FBS and DMEM). After they reach 70% confluence, the cells are added to the differentiation medium (2% HS and DMEM) and differentiated for seven days. Atovaquone stock solution is made according to its molecular weight in DMSO, and all cells are treated with a final concentration of 10µM for 24 hours. RNA isolation is carried out using the Qiagen assay kit and quantified using Nanodrop RT-PCR for utrophin upregulation. Utrophin upregulation on C2C12 mouse skeletal muscle myoblasts are measured using an RT PCR test for three concentrations (10 µM, 1µM, 0.1µM) of Atovaquone.

[0085] FIG. 2 illustrates the fold change of utrophin in three different concentrations of Atovaquone. A good fold change is observed in all three concentrations indicating that Atovaquone is capable of upregulating in myoblast cells.

Example 3 – Cell viability assay

[0086] 100 µl of cell suspension is dispensed in a 96-well plate and pre-incubated for 24 hours in a humidified incubator at 37°C. Ten µl of different concentrations of Atovaquone (1.95 to 500 µM) are added to the plate. The plate is incubated for 24 hours in the incubator. Ten µl of CCK-8 solution is added to each well of the plate. The plate is incubated for 4 hours in the incubator. The absorbance at 450 nm is measured using a microplate reader. Care is taken not to introduce bubbles to the wells since they interfere with the O.D. reading. The viability of myoblast cells using the CCK8 assay is determined with C2C12 cell lines at concentrations ranging from 1.95 to 500 µM. Notably, even at the highest concentration of 500 µM, 84% of cells remained viable upon 24 h pre-incubation as shown in FIG. 3.

Example 4 – *In vivo* studies on D2.mdx mouse models

Drug Administration and Efficacy Evaluation Protocol:

[0087] All the functional experimental parameters are evaluated in mdx background wild-type mice and in D2.mdx mice (D2.B10-DMD mdx/J mice procured from Jackson laboratories (Strain # 013141), Bar Harbor, Maine (ME), USA 04609.

[0088] Atovaquone (100 mg/kg, p.o., q.d.) is orally (p.o) administered once daily (q.d.) for 14 days at a dose volume of 10 mL/kg. Atovaquone is suspended in formulation containing 0.1% Tween20 and 0.5% Carboxymethyl cellulose (CMC) is administered once daily for 14 days via the oral gavage. The wild-type control and mdx control groups received the vehicle (0.5% Carboxy methyl cellulose (CMC) containing 0.1% Tween20) alone. Body weights of the study animals are recorded before the study (pre-dose) and twice weekly/daily throughout the study. Animals are also monitored for clinical signs, mortality, and morbidity.

Experimental procedure and Efficacy Evaluation:

[0089] All the three groups of mice (Wild-type, DMD-Control & DMD-Atovaquone) underwent functional tests at week 0 (Basal – Day 0) and week 2 (Day 14). Muscle function is assessed through grip strength test using a Grip strength meter (Orchid Scientific, Model No.: GSM02RS, India), hanging test, in response to Vehicle and Atovaquone (100 mg/kg, p.o., q.d.) treatments. Blood samples are collected for Creatine kinase (CK) analysis on Day 14 after the chronic treatment. The efficacy of Atovaquone is evaluated by comparing the functional test parameters and serum CK levels of the treated group with that of the mdx-control group in comparison to the basal value of Wild-type group.

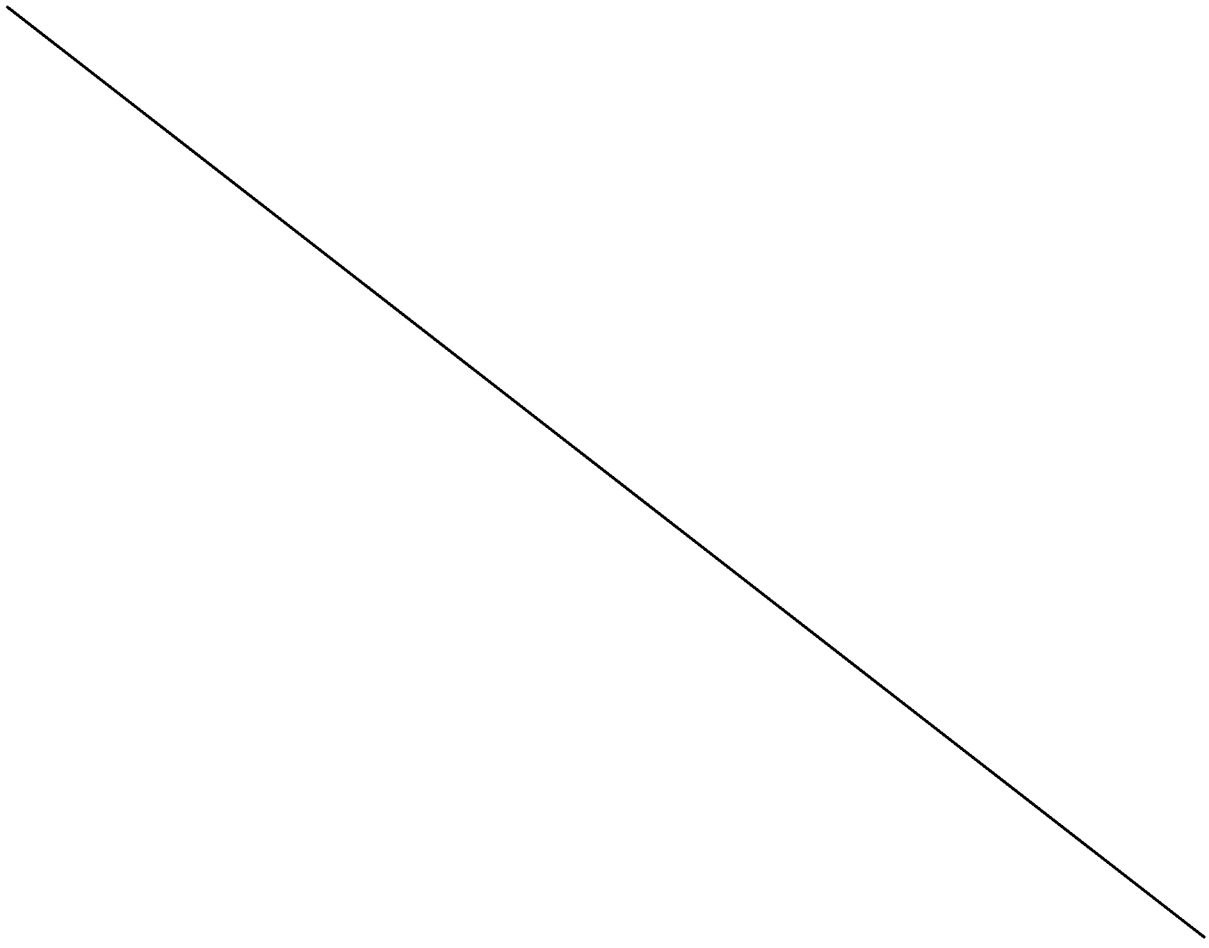
[0090] Statistical analyses are performed using GraphPad Prism 10 version software using one-way ANOVA (multiple comparisons method) followed by Bonferroni 't' test wherein the significance *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$ vs DMD-Control (Vehicle treated) are applied wherever applicable in the drawings.

[0091] FIG. 4 presents the effect of Atovaquone (100 mg/kg, p.o., q.d.) on normalized grip strength in D2.mdx mice. Atovaquone treated DMD mice could grip as well as the wild type mice with a 60% maximal possible efficacy when compared to the vehicle-treated DMD mice.

[0092] Hanging test (FIG. 5) also demonstrates that Atovaquone (100 mg/kg, p.o., q.d.) treated DMD-mice perform comparably with wild type mice, with a maximal possible efficacy of 77% recovery in 'Latency to Fall' when compared to the vehicle-treated DMD mice.

[0093] Elevated levels of Serum Creatine Kinase (CK) in blood sample are an indicative of muscle disintegration caused by muscular dystrophies. Serum Creatine Kinase level is measured on day 14 after the chronic dosing of various treatment (Wild-type – Vehicle, DMD – Vehicle & DMD – Atovaquone). FIG. 6 shows that chronic treatment with Atovaquone for 14 days / 2 weeks causes significant reductions (61%) in serum Creatine Kinase levels over 14 days of treatment.

[0094] The foregoing description of the specific embodiments will so fully reveal the general nature of the embodiments herein that others can, by applying current knowledge, readily modify and/or adapt for various applications such specific embodiments without departing from the generic concept, and, therefore, such adaptations and modifications should and are intended to be comprehended within the meaning and range of equivalents of the disclosed embodiments. It is to be understood that the phraseology or terminology employed herein is for the purpose of description and not of limitation. Therefore, while the embodiments herein have been described in terms of embodiments and examples, those skilled in the art will recognize that the embodiments and examples disclosed herein can be practiced with modification within the scope of the embodiments as described herein



CLAIMS

We Claim:

1. Use of the compound Atovaquone or its pharmaceutically acceptable salt for the preparation of a medicament for the treatment or management or both of muscular dystrophy.

2. The use as claimed in Claim 1, wherein muscular dystrophy is selected from a group consisting of Duchenne muscular dystrophy, Becker muscular dystrophy, Fukuyama Congenital muscular dystrophy (FCMD), Myotonic muscular dystrophy, Facioscapulohumeral muscular dystrophy (FSHD1/2), Congenital muscular dystrophy (CMD1C), Limb-girdle muscular dystrophy, Emery-Dreiffus muscular dystrophy (EDMD), Muscle-Eye-Brain disease (MEB), Walker-Warburg syndrome (WWS), Calpainopathis or LGMD2A, Oculopharyngeal muscular dystrophy, DMD-associated dilated cardiomyopathy (DCM), Miyoshi Myopathy type 1 (MMD1), and Limb–Girdle Muscular Dystrophy R2 dysferlin-related (LGMDR2) muscular dystrophy.

3. The use as claimed Claim 1, wherein Atovaquone is administered in combination with at least one additional therapy selected from a group consisting of corticosteroid therapy, gene therapy, exon-skipping therapy, immunosuppressant therapy, epigenetic therapy, muscle re-generation therapy and muscle-strengthening therapy.

4. The use as claimed in claim 3, wherein the corticosteroid therapy comprises administration of at least one corticosteroid selected from a group consisting of Prednisone, Prednisolone, Deflazacort, Vamorolone and combinations thereof.

5. The use as claimed in claim 3, wherein the exon-skipping therapy comprises administration of at least one agent selected from a group consisting of Eteplirsen, Golodirsen, ASO-based therapy and combinations thereof.

6. The use as claimed in claim 3, wherein the epigenetic therapy comprises of administration of at least one agent selected from a group consisting of Givinostat, Pan-HDAC inhibitors, HDAC6 inhibitors and combinations thereof.

7. The use as claimed in Claim 1, wherein the dosage of Atovaquone is in the range of 10 to 1500 mg/day, administered in a single or multiple dosage regimen.

8. The use as claimed in Claim 1, wherein Atovaquone is formulated for intravenous, intramuscular, inhalation, intradermal, cutaneous, subcutaneous, oral, transdermal, transmucosal, topical, nasal, vaginal, intrathecal, epidural, ocular or rectal administration.

9. A composition comprising the compound Atovaquone, or its pharmaceutically acceptable salt, solvates or analogues thereof, and at least one pharmaceutically acceptable excipient.

10. The composition as claimed in Claim 9, wherein the composition is administered in combination with at least one additional therapy selected from a group consisting of corticosteroid therapy, gene therapy, exon-skipping therapy, immunosuppressant therapy, epigenetic therapy, muscle re-generation therapy and muscle-strengthening therapy.

11. The composition as claimed in claim 10, wherein the corticosteroid therapy comprises administration of at least one corticosteroid selected from a group consisting of Prednisone, Prednisolone, Deflazacort, Vamorolone and combinations thereof.

12. The composition as claimed in claim 10, wherein the exon-skipping therapy comprises administration of at least one agent selected from a group consisting of Eteplirsen, Golodirsen, ASO-based therapy and combinations thereof.

13. The composition as claimed in claim 10, wherein the epigenetic therapy comprises of administration of at least one agent selected from a group consisting of Givinostat, Pan-HDAC inhibitors, HDAC6 inhibitors and combinations thereof.

14. The composition as claimed in Claim 10, wherein the dosage of the composition is in the range of 10 to 1500 mg/day, administered in a single or multiple dosage regimen.

15. The composition as claimed Claim 10, wherein the composition is formulated for intravenous, intramuscular, inhalation, intradermal, cutaneous, subcutaneous, oral, transdermal, transmucosal, topical, nasal, vaginal, intrathecal, epidural, ocular or rectal administration.

16. A method for treatment of muscular dystrophy including administering to a subject in need of, a therapeutically effective amount of the compound Atovaquone.

17. The method as claimed in Claim 16, wherein the compound Atovaquone is administered in combination with at least one additional therapy selected from a group consisting of corticosteroid therapy, gene therapy, exon-skipping therapy, immunosuppressant therapy, epigenetic therapy, muscle re-generation therapy and muscle-strengthening therapy.

18. The method as claimed in claim 17, wherein the corticosteroid therapy comprises administration of at least one corticosteroid selected from a group consisting of Prednisone, Prednisolone, Deflazacort, Vamorolone and combinations thereof.

19. The method as claimed in claim 17, wherein the exon-skipping therapy comprises administration of at least one agent selected from a group consisting of Eteplirsen, Golodirsen, ASO-based therapy and combinations thereof.

20. The method as claimed in claim 17, wherein the epigenetic therapy comprises of administration of at least one agent selected from a group consisting of Givinostat, Pan-HDAC inhibitors, HDAC6 inhibitors and combinations thereof.

21. The method as claimed in claim 16, wherein muscular dystrophy is selected from a group consisting of Duchenne muscular dystrophy, Becker muscular dystrophy, Fukuyama Congenital muscular dystrophy (FCMD), Myotonic muscular dystrophy, Facioscapulohumeral muscular dystrophy (FSHD1/2), Congenital muscular dystrophy (CMD1C), Limb-girdle muscular dystrophy, Emery-Dreiffus muscular dystrophy (EDMD), Muscle-Eye-Brain disease (MEB), Walker-Warburg syndrome (WWS), Calpainopathis or LGMD2A, Oculopharyngeal muscular dystrophy, DMD-associated dilated cardiomyopathy (DCM), Miyoshi Myopathy type 1 (MMD1), and Limb–Girdle Muscular Dystrophy R2 dysferlin-related (LGMDR2) muscular dystrophy.

22. A method for upregulating utrophin level in muscle cells including administering to a subject in need of, a therapeutically effective amount of the compound Atovaquone.

23. The method as claimed in claim 22, wherein the compound is administered in combination with at least one additional therapy selected from a group consisting of corticosteroid therapy, gene therapy, exon-skipping therapy, immunosuppressant therapy, epigenetic therapy, muscle re-generation therapy and muscle-strengthening therapy.

24. The method as claimed in claim 23, wherein the corticosteroid therapy comprises administration of at least one corticosteroid selected from a group consisting of Prednisone, Prednisolone, Deflazacort, Vamorolone and combinations thereof.

25. The method as claimed in claim 23, wherein the exon-skipping therapy comprises administration of at least one agent selected from a group consisting of Eteplirsen, Golodirsen, ASO-based therapy and combinations thereof.

26. The method as claimed in claim 23, wherein the epigenetic therapy comprises of administration of at least one agent selected from a group consisting of Givinostat, Pan-HDAC inhibitors, HDAC6 inhibitors and combinations thereof.

27. Use of the compound Atovaquone, for upregulating utrophin levels in muscle cells.

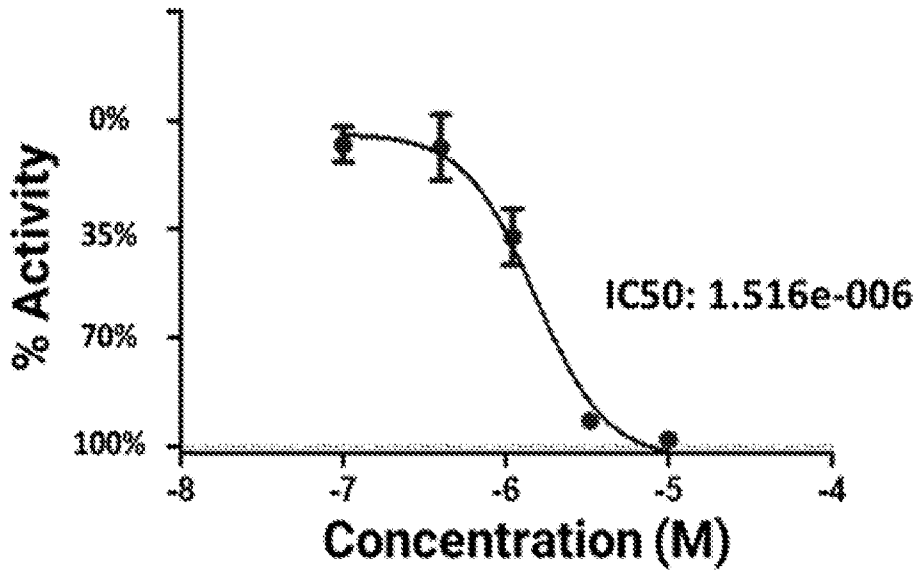

% Activity
0%
35%
70%
100%
IC50: 1.516e-006
-8
-7
-6
-5
-4
Concentration (M)

FIG. 1

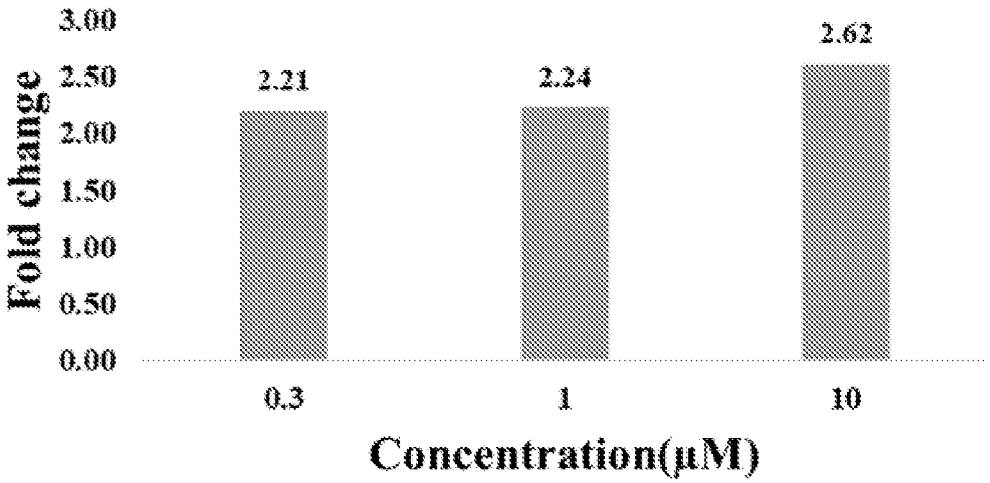

Fold change
3.00
2.50
2.00
1.50
1.00
0.50
0.00
2.21
2.24
2.62
0.3
1
10
Concentration(µM)

FIG. 2

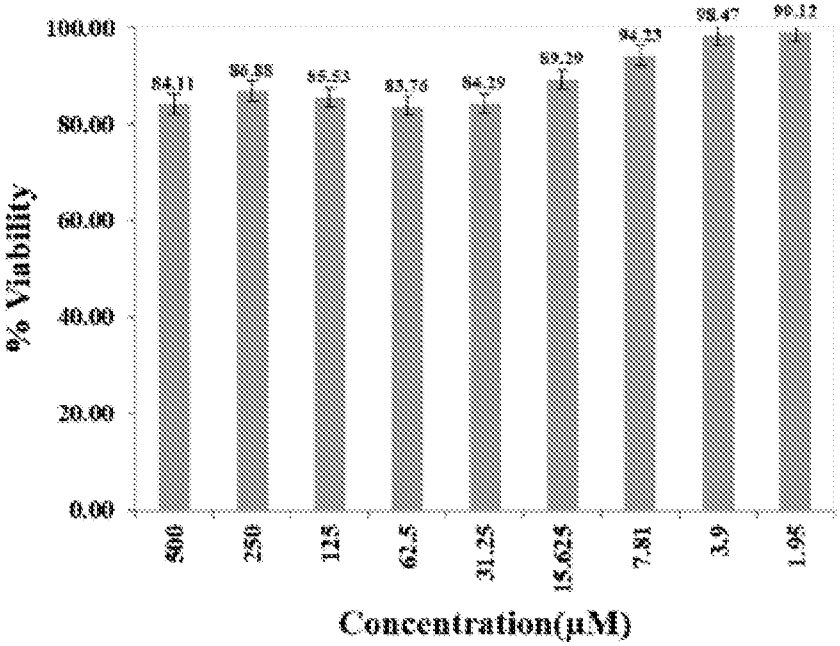

100.00
80.00
60.00
40.00
20.00
0.00
% Viability
84.11
86.88
85.53
83.76
84.29
89.29
94.23
98.47
99.12
500
250
125
62.5
31.25
15.625
7.81
3.9
1.95
Concentration(µM)

FIG. 3

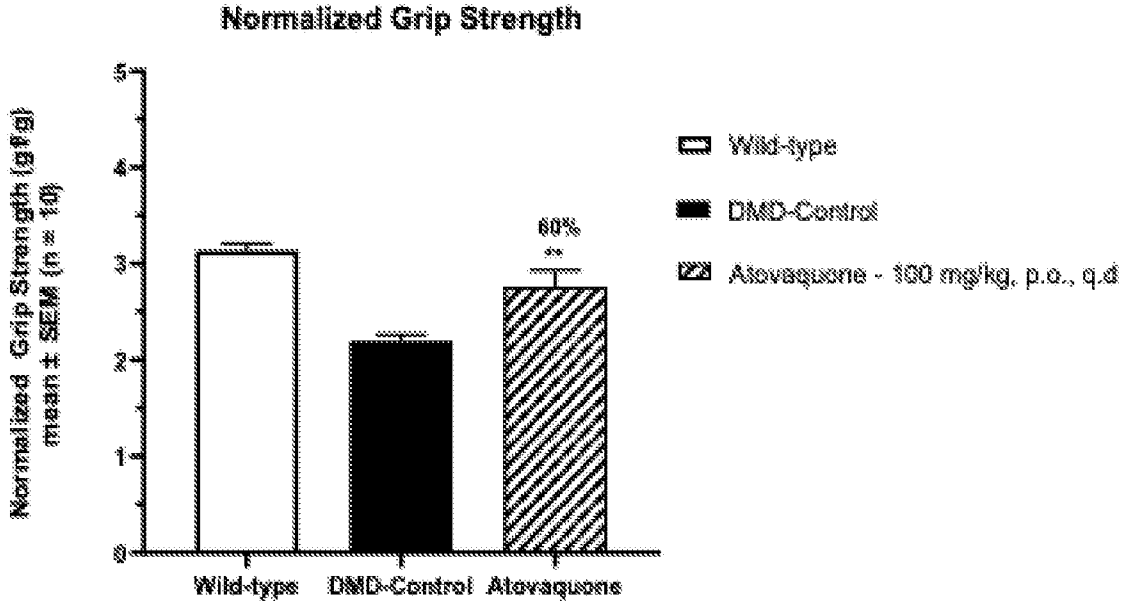

Normalized Grip Strength
Normalized Grip Strength (gf/g)
mean ± SEM (n = 10)
5
4
3
2
1
0
60%
Wild-type
DMD-Control
Atovaquone
Wild-type
DMD-Control
Atovaquone - 100 mg/kg, p.o., q.d

FIG. 4

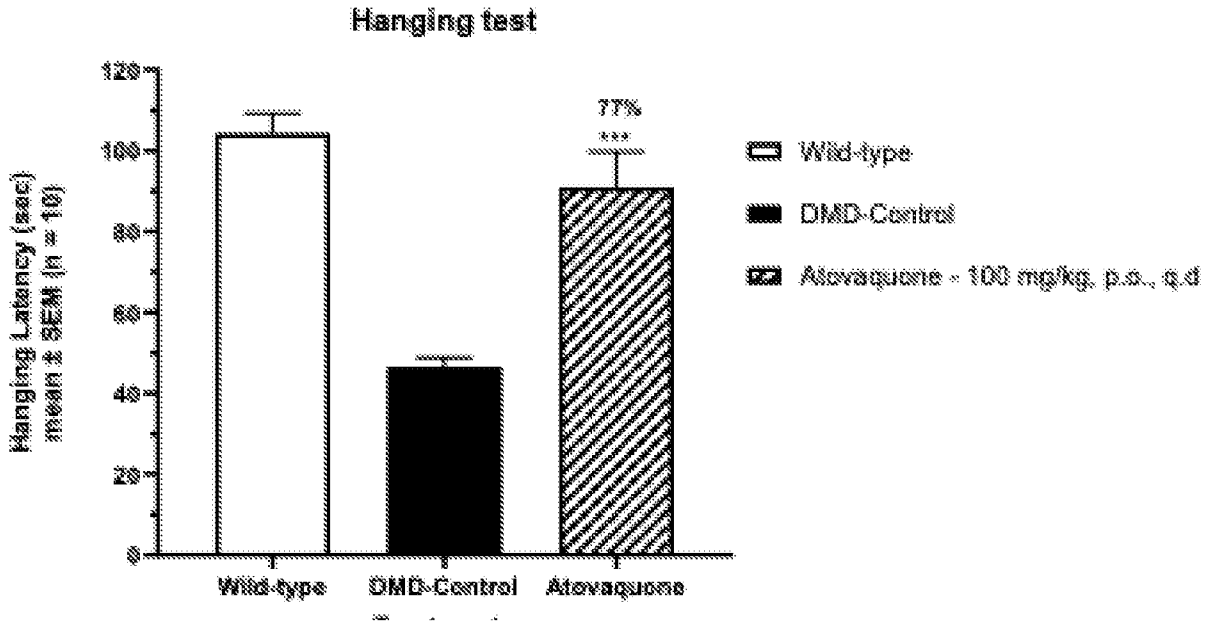

Hanging test
Hanging Latency (sec)
mean ± SEM (n = 10)
120
100
80
60
40
20
0
77%
***
Wild-type
DMD-Control
Atovaquone - 100 mg/kg, p.o., q.d
Wild-type
DMD-Control
Atovaquone

FIG. 5

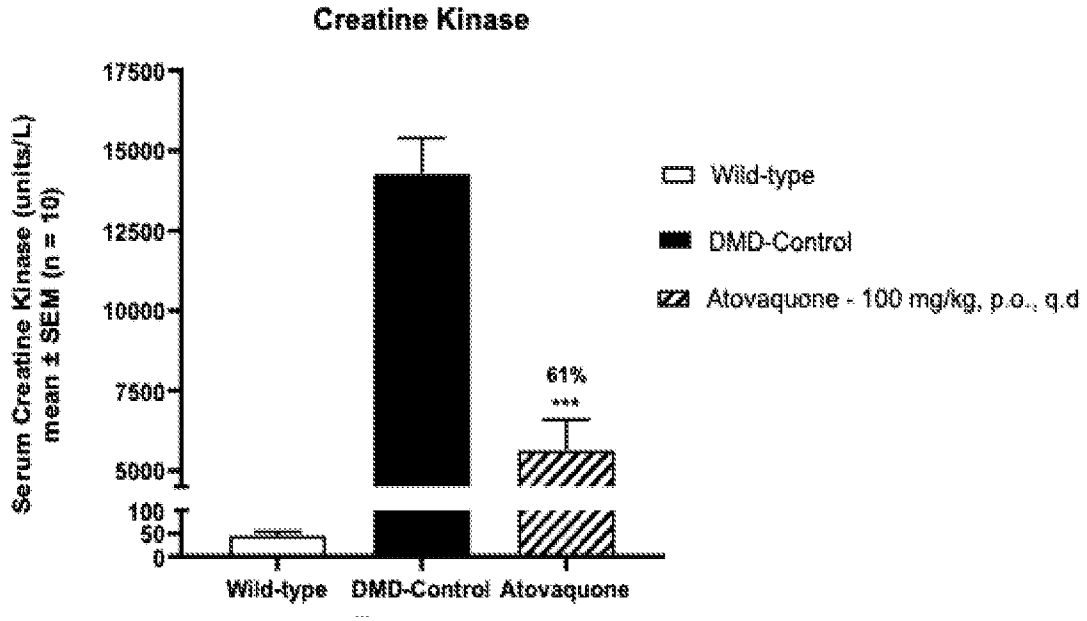

Creatine Kinase
Serum Creatine Kinase (units/L)
mean ± SEM (n = 10)
17500
15000
12500
10000
7500
5000
100
50
0
61%
***
Wild-type
DMD-Control
Atovaquone - 100 mg/kg, p.o., q.d
Wild-type
DMD-Control
Atovaquone

FIG. 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IN2024/052049

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***A61K 48/00*** (2024.01); ***A61K 31/122*** (2024.01); ***A61K 31/23*** (2024.01); ***A61P 21/00*** (2024.01); ***C07C 50/32*** (2024.01); ***C07D 209/04*** (2024.01); ***C07D 213/56*** (2024.01); ***C12N 15/11*** (2024.01)

CPC: ***A61K48/00; A61K31/122; A61K31/23; A61P21/00; C07C50/32; C07D209/04; C07D213/56; C12N15/11;*** *C12N2810/6027*

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2022/0243226 A1 (THE BROAD INSTITUTE, INC. et al.) 04 August 2022 (04.08.2022) paragraphs [0005], [0020], [0051], [0110], [0139], [0412], [0414], [0536], [0542], [0547], [0554], [0584], [0598]	1-21
Y	US 2011/0224128 A1 (WHALEN, ANNE et al.) 15 September 2011 (15.09.2011) abstract, paragraphs [0028], [0059], [0125], claim 1	1-27
Y	US RE47009 E (THE CHILDREN'S HOSPITAL OF PHILADELPHIA et al.) 28 August 2018 (28.08.2018) column 105 lines 14-18, 29-33	7, 14
Y	SOBLECHERO-MARTIN. "Utrophin modulator drugs as potential therapies for Duchenne and Becker muscular dystrophies" 711–723. Neuropathology and Applied Neurobiology. 10 May 2021; <DOI: DOI: 10.1111/nan.12735> page 712, second paragraph; page 714, fourth-fifth paragraphs; page 715, fifth paragraph; page 717, third paragraph;	22-27

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 December 2024 (19.12.2024)	**11 February 2025 (11.02.2025)**

Name and mailing address of the ISA/US	Authorized officer
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