SPECIAL FEATURE

Myotonic dystrophy type 1 drug development: A pipeline toward the market

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Myotonic dystrophy type 1 (DM1) is a multisystemic neuromuscular genetic disease with an estimated prevalence of approximately at least half a million individuals based on its vast ethnic variation. Building upon a well-known physiopathology and several proof-of-concept therapeutic approaches, herein we compile a comprehensive overview of the most recent drug development programs under preclinical and clinical evaluation. Specifically, close to two dozen drug developments, eight of which are already in clinical trials, explore a diversity of new chemical entities, drug repurposing, oligonucleotide, and gene therapy-based approaches. Of these, repurposing of tideglusib, mexiletine, or metformin appear to be therapies with the most potential to receive marketing authorization for DM1.

Keywords: Myotonic dystrophy; Drug development; Repurposing drug; Antisense oligonucleotide; Gene therapy; Clinical trial

Introduction

The past two decades have witnessed the generation of breakthrough knowledge regarding the molecular causes and conceptual approaches to treating neuromuscular disorders. The consequence has been the approval of the first oligonucleotide-based drug treatments for Duchenne muscular dystrophy (DMD; eteplirsen and golodirsen) and spinal muscular atrophy (SMA; nusinersen) [1], and the first gene therapy (Zolgensma; SMA), which complement more traditional small-molecule therapeutic options (e.g., risdiplam; SMA) [2]. Next in line with at least one therapeutic option might be Myotonic dystrophy type 1 (DM1). DM1 is a life-threatening chronic disease with symptoms in the neuromuscular, cardiac, and central nervous systems, and compared with most neuromuscular diseases, its clinical presentation is very variable [3,4]. DM1 can affect newborns (congenital DM1) to older adults, with around at least half a million patients worldwide based on the reported prevalence estimation of 1 in 3,000–8,000 people [3,5].

A repeat length >50 units of the repetitive trinucleotide sequence (CTG)n in the 3′-untranslated region (UTR) of the DM1 protein kinase (DMPK) gene causes DM1. Mutant DMPK transcripts in skeletal muscle, heart, and brain tissue are retained in the cell nucleus in microscopically visible ribonuclear foci, which are the most prominent histopathological hallmark of the disease. CUG expansions fold into stable double-stranded stem-loop structures with U-U mismatches with a strong affinity for proteins of the Muscleblind-like (MBNL) family, which are sequestered and become depleted and unable to perform their normal function. MBNL loss of function contributes significantly to DM1 phenotypes [6]. Likewise, stress responses triggered by the toxic DMPK RNA cause the stabilization/activation of MBNL antagonists CELF1 and hnRNPA1 [6]. Together, these proteins function as developmental switches, and their imbalance results in the abnormal persistence of fetal patterns of alternative splicing in adult tissues, termed 'spliceopathy', which affects hundreds of genes, some of which explain specific symptoms in

DM1. Various signaling pathways also have a role in DM1 muscle pathogenesis, including impaired protein kinase B (AKT), activated autophagy and ubiquitin–proteasome activity, AMP-activated protein kinase (AMPK) downregulation, and activated glycogen synthase kinase 3 beta (GSK3β) [7]. Taken together, this reasonably detailed knowledge of the molecular pathophysiology of DM1, availability of cell and mouse preclinical models, and the exploration of several proof-of-concept therapeutic strategies have culminated in a promising drug development pipeline for DM1.

Drug development programs

Herein, we summarize existing drug development programs in DM1 encompassing advanced candidates from the preclinical stage to human clinical trials. Therapies are further classified into three broad categories of small molecules, oligonucleotide-based therapies, and gene therapies, encompassing mechanisms of action targeting from root cause disease events up to specific clinical symptoms (Fig. 1).

Small-molecule drugs

In this category, we have mainly identified repurposed pre- and investigational new drugs (INDs), which were initially developed to treat a different condition, compared with only one new chemical entity (NCE) IND with first therapeutic use in DM1. Given that drug-repurposing candidates have already demonstrated safety in humans, they can be tested in patients more quickly and at less cost and lower risk than for entirely new drugs, which explains why they are closest to reaching market authorization (Table 1).
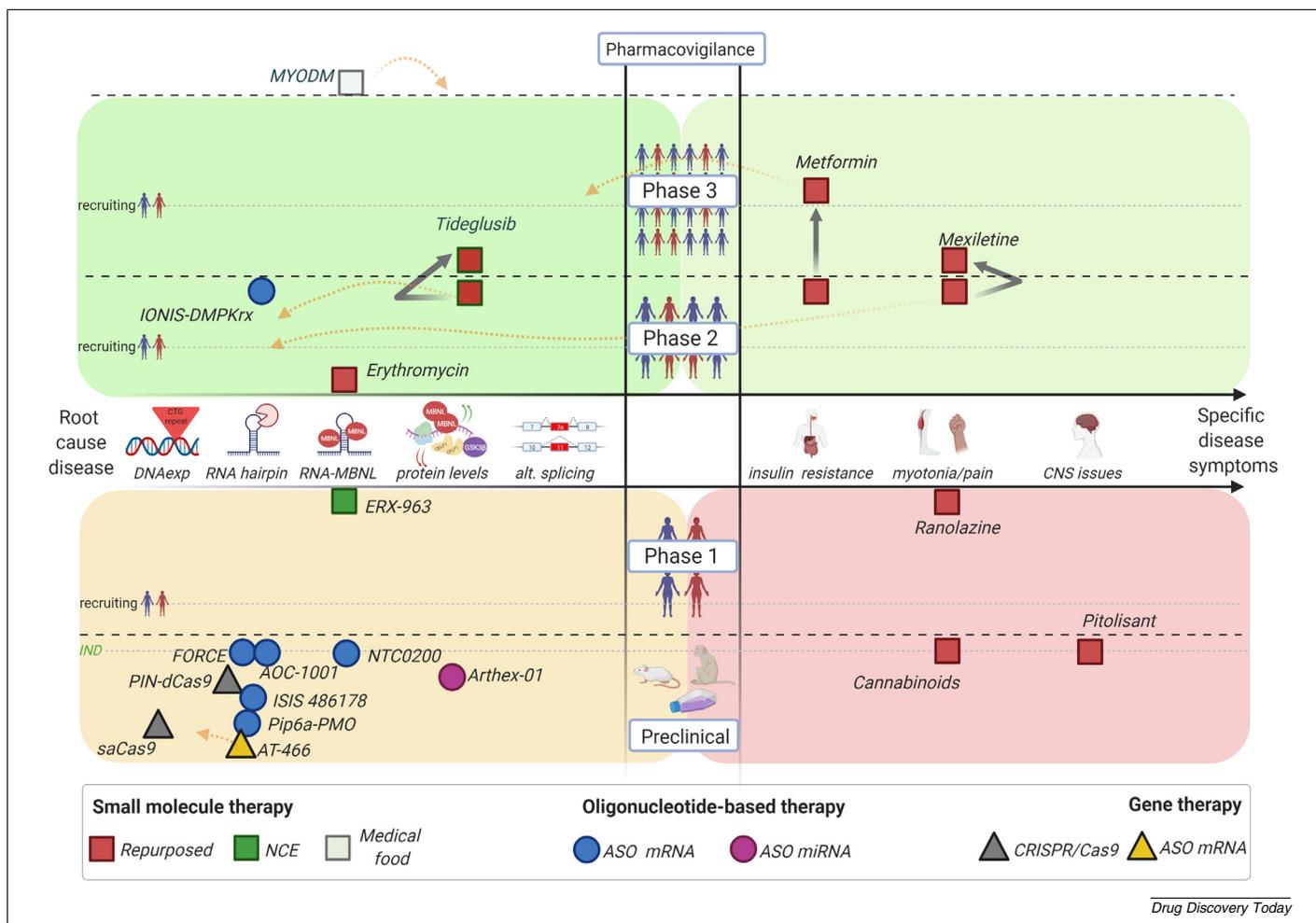
FIGURE 1

Graphical representation of the preclinical and clinical DM1 drug candidate pipeline. The middle X-axis indicates the step targeted within the DM1 physiopathological model (left side: the root causes of disease; right side: specific clinical disease symptoms), while the middle Y-axis shows the level of drug evaluation from preclinical to market authorization. Black discontinued lines separate clinical phases, while grey discontinued lines indicate human recruiting timing for each clinical phase. The green discontinued line denotes the IND milestone at the end of the preclinical evaluation phase. Drug candidates are represented in different shapes according to the type of therapy and colours for specific types of molecules. Black solid arrows display progression of those drug candidates evaluated in more than one clinical trial. Orange discontinued arrows indicate the description of additional mechanisms of action proposed for the drug candidate (see text and tables for detailed description and references) (Created with BioRender.com).

Levels of GSK3β are elevated in DM1 skeletal muscles [8], leading to the proposal that resulted in a new therapeutic target to prevent myopathy in DM1 [9]. **Tideglusib** is a marine-derived GSK3β inhibitor initially developed to treat Alzheimer's disease [10]; it causes a significant correction of GSK3β and CELF1 levels and reduces expression of toxic *DMPK* RNA in normal and congenital DM1 (CMD) myoblasts [11]. GSK3β is abnormally high in the HSA^{LR} mouse model (Box 1), and tideglusib was found to improve functional parameters, such as muscle weakness and myotonia; in the muscles and brain of the DMSXL mouse (Box 1), as a model of CMD, the drug improved postnatal survival, weight, and neuromotor activity [8,11]. These results supported the potential of tideglusib to target early events of the disease in congenital and adult forms of DM1 and on skeletal muscle and brain defects via multiple pathways. Orally administered tideglusib, developed by AMO-Pharma, has already completed Phase II trials on patients with CMD and childhood-onset DM1, where most patients reported improved CNS and clinical neuromuscular symptoms [12]. Given the favorable pharmacokinetic (PK) and clinical risk/benefit profiles, an extended Phase II/III clinical trial has been announced.

Inhibition of the interaction between the toxic CUG hairpin and the MBNL1 splicing factor is one of the most common ongoing therapeutic avenues in DM1 [13]. The most-advanced lead compounds, **MYD-0124** (erythromycin) and **ERX-963**, can bind to the hairpin RNA structure with high selectivity, decreasing foci formation and rescuing mis-splicing in DM1 cell and mouse models. A Phase II clinical trial sponsored by Osaka University Hospital is currently testing the oral administration of erythromycin in adult patients with DM1 [14], uncovered as an anti-DM1 candidate after a targeted screen for RNA-binding potential of US Food and Drug Administration (FDA)-approved antibiotics [15]. **ERX-963** is a rationally designed NCE that can strongly bind pathogenic expanded CUG repeats (CUGexp) but not normal repeats, limiting off-target effects [16] (Expansion Therapeutics, www.expansionrx.com/dm1/#research). ERX-963 recently finished a Phase I clinical trial in adult patients with DM1, according to the sponsor Expansion Therapeutics.

Among candidates targeting specific disease symptoms, the most advanced is **metformin**, a first-line agent for type 2 diabetes mellitus. The drug has been suggested to treat the insulin resistance phenotype in patients with DM1 [17] and promote alternative splicing correction via AMPK-dependent and independent mechanisms [18]. More recently, metformin was also reported to improve mitochondrial dysfunction and impaired metabolism in DM1 fibroblasts and prevent cancer risk, thus suggesting ample therapeutic benefits in DM1 [19,20]. A completed Phase II clinical trial assessed oral metformin, revealing a signif-

TABLE 1

Myotonic dystrophy drug candidates currently in clinical trials.

Phase	Clinical trial	Status	Clinical trial information	Results
Mexiletine (repurposed small molecule): Orphan Drug Designation by FDA and EMA				
III	NCT04624750	Not yet recruiting: 2020–2024	Safety, efficacy, and steady-state PK of mexiletine in paediatric patients with myotonic disorders. Sponsor: Lupin, Ltd	Not posted
II	NCT01406873	Completed: 2011–2018	Effects of mexiletine on ambulation, myotonia, muscle function, strength, pain, gastrointestinal functioning, cardiac conduction, and quality of life in DM1. Sponsor: University of Rochester	[24]
Metformin (repurposed small molecule)				
III	2018–000692-32	Ongoing: 2019–2021	Efficacy of metformin on motility and strength in DM1. Sponsor: Tor Vergata	Not posted
II	2013–001732-21	Completed: 2013–2017	Randomized, double-blind, placebo-controlled Phase II study of metformin in patients with DM1. Sponsor: Centre d'Etude des Cellules Souches/Istem	[21]
Tideglusib (repurposed small molecule): Orphan Drug Designation by FDA				
II/III	NCT03692312	Not yet recruiting: 2020–2022	Randomized, multicenter, double-blind, placebo-controlled, Phase II/III study of patients (aged 6–16 years) with congenital DM1. Sponsor: AMO Pharma Ltd	Not posted
II	NCT02858908	Completed: 2016–2018	Safety, efficacy and PK of tideglusib in treatment of adolescents and adults with congenital and juvenile-onset DM1. Sponsor: AMO Pharma Ltd	[12]
Erythromycin (repurposed small molecule)				
II	jRCT2051190069	Recruiting: 2019–ongoing	Blinded, placebo-controlled study to assess the safety, tolerability, and efficacy of MYD-0124 in adult patients with DM1. Sponsor: Hospital Osaka University	Not posted
IONIS-DMPKRx (ISIS 598769) (ASO)				
I/II	NCT02312011	Completed: 2014–2016	Safety, tolerability, and PK of multiple escalating doses of ISIS 598769 administered subcutaneously to adult patients with DM1. Sponsor: IONIS-Biogen	Not posted
ERX-963 (new chemical small molecule)				
I	NCT03959189	Completed: 2019–2020	Safety, tolerability, and potential reduction of excessive daytime sleepiness/hypersomnia and improvement of cognitive function in patients with DM1. Sponsor: Expansion Therapeutics, Inc.	Not posted
Ranolazine Ralexa^{TM} (repurposed small molecule)				
I	NCT02251457	Completed: 2014–2017	Preliminary data to determine safety and efficacy of ranolazine on symptoms of DM1. Sponsor: Ohio State University/Gilead Sciences	[28]
Caffeine and theobromine formulation MYODM^{TM} (natural compounds)				
N/A	NCT04634682	Recruiting: 2020–2021	Effect of food supplement MYODM^{TM} on excessive daytime sleepiness and quality of life in adults with DM1. Sponsor: Myogem Health Company, S.L.	Not posted

[a]N/A, not applicable.

> ## Box 1 Mouse preclinical models.
>
> ### HSA^{LR} mouse model
> The HSA^{LR} mouse model is a homozygous transgenic mouse that includes a genomic fragment of the human skeletal actin gene to express 250 CTG repeats in the 3′-UTR of the gene. It shows myotonia and muscle weakness, centrally located nuclei in muscle fibers, and alternative splicing defects [64].
>
> ### DM200 mouse model
> DM200 mice overexpress a GFP-DMPK 3′-UTR (CTG)200 transgene carrying a DMPK 3′-UTR with 200 CTG repeats under the control of a doxycycline-responsive human DMPK promoter. The model reproduces myotonic dystrophy cardinal features, including myotonia, cardiac conduction abnormalities, histopathology, and RNA splicing defects [65].
>
> ### DMSXL mouse model
> The DMSXL mouse model is a homozygous mouse line carrying >700 CTG repeats, derived from the DM300–328 transgenic mouse line, which carries 45 kb of human genomic sequence from the DM1 locus [66]. The model shows mild splicing defects but ribonuclear foci, growth retardation, histology alterations, muscle weakness and atrophy, and myotonia in skeletal muscles [67,68]. It has been proposed as a study model for brain function alterations typical of DM1 [69].

icant improvement in mobility in a 6-min walk test (6MWT) associated with an increase in total mechanical power and a suggestive effect on gait parameters [21]. These encouraging results supported a replication study in a multicenter Phase III clinical trial sponsored by the Tor Vergata University of Rome.

Additional molecules in development address the symptomatic management of DM1 features, such as myotonia, chronic pain, or daytime sleepiness, namely **mexiletine**, **ranolazine**, **cannabinoids** and **pitolisant**.

Mexiletine is an antiarrhythmic medicine used to reduce or prevent myotonia through blocking sodium channels involved in the contraction and relaxation of muscles [22]. Mexiletine was orally administered to evaluate the managing of myotonia symptoms in adult patients with DM1 in two Phase II clinical trials [23,24] that reached similar results indicating a positive effect on handgrip myotonia in ambulatory patients but no significant benefit on 6MWT. Given that mexiletine was safe over a 9-year follow-up study [25], Lupin Ltd is evaluating this drug in a Phase III clinical trial in children and adolescent patients with DM1 under the name NaMuscla, already approved for nondystrophic myotonic disorders [26]. Interestingly, mexiletine has been reported to downregulate DMPK mRNA levels, pointing to additional activity through the DM1 disease pathway [27].

In addition to mexiletine, ranolazine, which acts by enhancing the slow inactivation of sodium channels, is a therapeutic alternative targeting similar DM1 molecular defects, as concluded from a completed Phase I clinical trial [28].

Similar to myotonia, attempts to treat myalgia and other muscular complaints led to the evaluation of cannabinoids (**cannabidiol** and **tetrahydrocannabinol** or CBD/THC, respectively) in DM1, driven by the presence of cannabinoid receptors in muscles [29] and by modulation of both central and peripheral pain pathways [30,31]. In this regard, Nexien Bio-

pharma recently filed a patent covering methods and compositions for treating patients with myotonic dystrophy (DM1 and DM2) with oral formulations of CBD and THC [32].

On a similar theme, pitolisant, a stimulant drug that antagonizes histamine H3 receptors to treat excessive daytime sleepiness in patients with narcolepsy, will be evaluated by Harmony Biosciences to treat the same symptom in DM1. This is a clinical hallmark in most patients and one of the most frequent nonmuscular symptoms contributing to a reduced quality of life [33]. A repurposing program with a Phase II clinical trial in adult patients with DM1 was announced for the first half of 2021.

Finally, a complementary therapeutic strategy for DM1 comes from evaluating methylxanthines, natural alkaloid products commonly used as mild stimulants and bronchodilators [34]. A synergistic combination of **theobromine and caffeine** (commercialized by Myogem Health Company as **MYODM™**) showed the ability to reduce the number of foci aggregates per cell and increase MBNL1 at the transcript and protein level [35,36]. Data on *Drosophila* DM1 flies rendered a recovery of survival and cardiac and locomotor dysfunction (M. Pascual-Gilabert et al., unpublished 2021). The clinical trial NCT04634682 is evaluating the effect of MYODM™ on the quality of life, fatigue, and hypersomnia in adult patients with DM1 as a novel nutritional management strategy. MYODM™ is expected to be upgraded into a food for special medical purposes or medical food [European Food Safety Authority (EFSA) and FDA classification, respectively, for foods specifically intended for the nutritional management of patients, under medical supervision].

Oligonucleotide-based therapeutics

RNA-based therapies can target any gene in the genome and, thus, are particularly well suited to address the diversity among rare genetic diseases. The promise of oligonucleotide-based drugs is starting to be realized with the increasing pace at which these drugs reach authorization, with at least ten already approved for clinical use [2]. In DM1, chief approaches have been designing antisense oligonucleotides (ASOs) able to degrade DMPK transcripts via the activation of RNase-H machinery in the nucleus (i.e., gapmers) or able to prevent MBNL1 sequestration by CUG repeats (or displace prebound MBNL1 proteins) by an occupancy-based mechanism (i.e., mixmers) (reviewed in [37]). In contrast to their high specificity, oligonucleotide-based therapies suffer from poor delivery to the muscle tissues, which, in the case of DM1, is further aggravated because cells do not display compromised membrane integrity that could facilitate cell uptake [38].

Screening of >3000 ASOs led IONIS to identify the **IONIS-DMPKRx** (ISIS 598769) gapmer-type candidate; the first and still single ASO evaluated in a clinical trial for the treatment of DM1 [39,40]. IONIS-DMPKRx produces significant DMPK mRNA degradation through base pairing with a specific 3′-UTR gene sequence outside the repeat tract. The drug did not generate safety concerns, being well tolerated even at the highest dose tested (600 mg) (Myotonic Dystrophy Foundation Annual Conference; www.myotonic.org/digital-academy/ionis-pharmaceuticals-industry-updates-drug-development-2018-mdf-annual-conference). Still, IONIS concluded that insufficient drug reached the muscles (Muscular Dystrophy Association, Ionis Reports Set-

back on DMPKRx Program for Myotonic Dystrophy, https://strongly.mda.org/ionis-reports-setback-dmpkrx-program-myotonic-dystrophy) to elicit a significant therapeutic response on the functional and biological end-points set up in the trial (Table 1). The company announced a program to enhance the potency of future DM1 candidates in the skeletal and heart muscle through Ligand-Conjugated Antisense (LICA) technology [41].

IONIS also reported preclinical data for **ISIS 486178** (Table 2), a different gapmer ASO designed to target the 3′-UTR region of the *hDMPK* transcript [42]. In cynomolgus monkeys, ISIS 486178 reduced *DMPK* expression by 70% in several muscles and 50% in cardiac tissue, with no skeletal muscle or cardiac toxicity after 13 weeks [42]. Importantly, this was also the first ASO reported to reach the heart with demonstrated benefit on molecular and functional alterations in the cardiac conduction defects of the DM200 mouse model (Box 1), thus suggesting ASOs as a viable option for treating cardiac pathology in DM1 [43]. This molecule also showed relevant activity in the DMSXL mouse

model in terms of body-weight increase, muscle strength, and muscle histology, whereas no overt toxicity was detected [44].

Conjugation of therapeutic ASOs to carrier delivery systems, such as peptides or antibodies, is an active field to enhance their skeletal muscles and heart uptake. In this regard, **AOC1001** is a conjugate of a small interfering (si)RNA degrading *DMPK* with a proprietary monoclonal antibody against the transferrin receptor 1 (TfR1) protein. Developed by Avidity Biosciences, the company recently stated that preclinical results confirmed the ability of AOC1001 to deliver siRNAs to muscle cells, boosting the reduction in *DMPK* mRNA levels in a durable and dose-dependent manner (Avidity Biosciences, www.aviditybiosciences.com/programs). Although details regarding the level of *DMPK* reduction, *in vivo* drug administration route, doses, or drug safety for AOC1001 have not been disclosed, Avidity has announced a Phase I/II clinical trial by the end of 2021 (Table 2).

Using a similar approach, Dyne Therapeutics is developing the **FORCE-DMPK ASO candidate**, which involves the conjugation of a proprietary ASO designed to reduce *DMPK* RNA

TABLE 2

Myotonic dystrophy drug candidates in preclinical stages of development.

Preclinical status	Preclinical information	Refs
Cannabinoids CBD/THC (small molecule/repurposing)		
IND-enabling phase	Management of chronic neuropathic pain, myotonia, and myalgia. Pilot survey in DM1, DM2, and congenital myotonia, with two patients per disease. Sponsor: Nexien Biopharma (https://nexienbiopharma.com/news)	[30–32]
Pitolisant; Wakix (small molecule/repurposing)		
IND-enabling phase: Phase II by 2021	Orally available to and able to cross the blood–brain barrier in animal models. Sponsor: Harmony Biosciences (www.harmonybiosciences.com/science)	[57]
ISIS 486178 (oligonucleotide-based therapy)		
Lead optimization	Systemic treatment resulting in myotonia and cardiac conduction improvement, correction of splicing defects, reduction in RNA foci, and redistribution of MBNL1. DMSXL and DM200 mice models. Sponsor: Ionis Pharmaceuticals	[43,44,58,59]
AOC1001 (oligonucleotide-based therapy)		
IND enabling phase: Phase I/II by 2021	Delivered to muscle cells reduces levels of *DMPK* mRNA in a durable, dose-dependent manner. AOC^{TM} platform. Sponsor: Avidity Biosciences (www.aviditybiosciences.com/)	[60]
FORCE-DMPK candidate (oligonucleotide-based therapy)		
IND enabling phase	*In vivo* dose-dependent correction of splicing and myotonia in the HSA^{LR} model after single low dose. *DMPK* mRNA reduction in mouse and nonhuman primates. FORCE^{TM} platform. Sponsor: Dyne Therapeutics (www.dyne-tx.com/pipeline/)	[61]
Pip6a-PMO (oligonucleotide-based therapy)		
Lead optimization	Intravenously injected in HSA^{LR}. Nuclear foci reduction, MBNL1 redistribution, splicing, and myotonia correction. Sponsor: Oxford University	[45]
NTC0200 (oligonucleotide-based therapy)		
IND enabling phase	*In vitro* ability to target and open up aberrant DM1-linked secondary RNA structure in mutant transcript, thereby displacing sequestered splice proteins. PATrOL^{TM} platform. Sponsor: Neubase (www.neubasetherapeutics.com/pipeline/)	[46]
Arthex-01 (oligonucleotide-based therapy)		
Lead optimization: start of Phase I/II by 2022	Subcutaneous injection in HSA^{LR}. Enhanced MBNL1/2 protein levels, recovered missplicing, muscle strength, and myotonia. Long-lasting activity of antagomiR-23b for up to 45 days. Sponsor: Arthex Biotech (https://arthexbiotech.com/pipeline/)	[49,50]
AAV-PIN-dCas9 (gene therapy)		
Lead optimization: start of IND-enabling studies by 2021	Intramuscular/systemic delivery in adult and neonatal HSA^{LR}. RNA-targeting Cas9 lasted for up to 3 months. Elimination of RNA foci, reversal of splicing biomarkers, and myotonia. Sponsor: Locanabio (https://locanabio.com/pipeline/)	[56,62]
AT-466 (gene therapy)		
Lead optimization	AAV delivery to overcome the biodistribution limitations of ASO-based therapies. Sponsor: Audentes (www.audentesbx.com/our-approach/)	[63]
AAV-CRISPR-Cas9 (gene therapy)		
Preclinical proof of concept	*In vivo* genome editing for DM1: deletion of CTG repeat tract leading to reduction in nuclear foci in muscle fibres after intramuscular injection of SaCas 9 and sgRNA rAAV9 vectors in DMSXL mice. Sponsor: Genethon-INSERM	[55]

levels in the nucleus by RNase H cleavage with a TfR1-binding Fab for effective delivery to muscles. According to Dyne's preclinical results in mouse models, the FORCE-DMPK candidate reached potent *Dmpk* RNA knockdown in skeletal muscles (70–78%) and durable ASO muscle distribution on its proprietary wild-type hTfR1 mouse (up to 12 weeks) as well as in nonhuman primates. *Dmpk* knockdown was also observed in the heart, with an acceptable toxicity profile for blood, kidney, and liver function parameters. For therapeutic parameters, the FORCE candidate rescued mis-splicing and myotonia to near-normal levels after a single low dose in HSA[LR] mice within 14 days after administering a single low dose (R. Subramanian et al., unpublished 2021).

An alternative to enhance muscle and heart uptake of ASOs is conjugation with cell-penetrating peptides (CPPs). The **Pip6a-PMO-CAG7**, a conjugate that combines a CPP moiety (Pip6a) and an ASO with morpholino chemistry-targeting repeats, promotes an occupancy-based mechanism for MBNL1 displacement from the toxic CUG repeat. *In vivo*, CPP-conjugated CAG7 PMO displayed significant improvement of oligonucleotide delivery into the striated muscles of HSA[LR] mice following systemic administration, which promoted reversion of myotonia and splicing defects that were long-lasting and involved most of the altered transcriptome [45].

The **NTC0200** (or Compound A) preclinical candidate developed by NeuBase Therapeutics explores a peptide-nucleic acid ASO identified through the proprietary platform PATrOL™. The resultant compound is a short, flexible, and selective peptide-nucleic acid sequence proved *in vitro* to discriminate the pathogenic CUG expansions from the wild-type transcripts, opening up CUG RNA secondary structures in the mutant transcript and displacing sequestered MBNL proteins [46]. Recent communication from the company on social networks announced long-lasting correction of global levels of mis-spliced transcripts in the HSA[LR] transgenic mouse after a well-tolerated single intravenous injection.

There is ample evidence that limited MBNL protein function contributes to DM1. Given that the endogenous genes remain normal in patients with DM1, a therapeutic gene modulation approach to compensate for their depletion appears feasible. Building upon previous proof-of-concept reports [47,48], this therapeutic avenue is being tested based on the identification of miR-23b and miR-218 as natural endogenous translational repressors of *MBNL1/2* genes. Treatment with antimiRs, a class of chemically engineered ASOs complementary to their cognate target miRNA, significantly upregulated MBNL1/2 protein levels and improved molecular defects in cell models and functional muscle defects in the HSA[LR] mouse model. Additionally, no safety concerns were observed in treatments *in vivo* [49,50]. ARTHEx Biotech is working on the preclinical evaluation of the antimiR lead candidate **(Arthex-01)** against miR-23b or miR-218 with planned clinical trials by the beginning of 2022 (Table 2).

Gene therapy approaches

The most straightforward approach to overcome the limited biodistribution of AONs is to promote its endogenous expression using gene therapy vectors. Adeno-associated viruses (AAVs) are being evaluated for drug development in several muscular dystrophies [51], and with recent success with Zolgensma gene replacement approach in SMA. Benefits of this avenue in DM1 were initially disclosed with a proof-of-concept approach for an AAV-delivered RNAi in the HSA[LR] mice [52]. Audentes is taking a similar approach with the AT466 candidate, a vectorized ASO-like design targeting the reduction of toxic *DMPK* RNA levels in cells from patients with DM1 by RNA degradation or exon skipping (or both simultaneously) mechanisms of action, both clinically already validated in studies with ASOs (Audentes, www.audentestx.com/innovative-therapies). Preclinical studies are underway to determine the optimal construct for AT466 (Table 2).

CRISPR/Cas9 genetic scissors have also emerged as innovative approaches to treat DM1 by targeting the removal of the expansions at the DNA level, preventing their transcription, or targeting degradation of the toxic RNA, therefore effectively targeting the underlying DM1 etiology [53,54]. The fundamental requirement for genome editing by CRISPR is a purified Cas9 nuclease and a guide RNA (gRNA) targeting the desired genome sequence with an adjacent protospacer motif (PAM sequence). The endonuclease cuts both strands of the chromosomal DNA, making genome modifications possible through nonhomologous end joining or homologous-driven repair.

More recently, CRISPR-Cas9 has emerged as a multifunctional platform for sequence-specific gene expression regulation. Engineered nuclease-deficient Cas9, termed dCas9, when fused to effector domains with distinct regulatory functions, enables the CRISPR-Cas9 system to be repurposed as a general platform for RNA-guided DNA targeting without cleavage activity. Two approaches (Table 2) are moving through preclinical stages, making use of vectorized AAV administration.

The approach reported by Lo Scrudato et al. [55] at Généthon demonstrated the excision of long CTG repeats and reduced pathological RNA foci within tibialis anterior muscle in the DMSXL mouse model after a single intramuscular injection of recombinant AAV vectors expressing CRISPR-SaCas9 components.

On a related approach, but targeting the toxic RNA molecules, Locanabio is developing AAV-vectors encoding PIN-dCas9 (a dCas9 fused to the PIN RNA endonuclease) and a single-gRNA targeting CUG repeats, which, in intramuscular and systemic administration in adult and neonatal HSA[LR] mice, showed up to 3 months of expression of the RNA-targeting Cas9. The treatment eliminated RNA foci, redistributed the MBNL1 factor, reversed dysregulated splicing biomarkers and transcriptional profiles, and ameliorated myotonia in skeletal muscle without significant toxicity signs, thus demonstrating potential clinical utility [56]. Future studies in large animals will be needed to assess further the safety, dose levels, and immunosuppression regimens required for safe and long-term treatments.

Concluding remarks and future challenges

We anticipate that patients with DM1 will have at least one therapeutic option targeting the genetic cause of the disease within the next few years. To reach this goal, it is imperative to maintain ongoing close collaboration between the academic and pharma-

POST-SCREEN (GREY)

ceutical sectors and to keep developing new and better preclinical models for hypothesis testing and drug evaluation. In this regard, it is vital to develop human 3D skeletal muscle models using tissue engineering to overcome the numerous limitations of 2D cultured myoblasts. These bioengineered microtissues would better recapitulate structural and molecular DM1 phenotypes as the gene expression changes associated with the CUG toxic RNA, and provide platforms to test drug activity and uptake under conditions closer to *in vivo*. Additional mammal models outside the murine paradigm would also significantly speed up preclinical validation in support for clinical programs. Overall, over the past decade, the DM1 community has smoothly transitioned from basic to translational research with the common goal of improving the lives of patients.

Note added in proofs

Since the information described in this paper was completed and accepted for publication, Expansion Therapeutics has disclosed the name of the **ERX-963** lead compound as flumazenil (online talk: https://youtu.be/Nf7AUdTvC9k). Instead of a new chemical entity able to bind the CUG expanded repeat, as described through the paper and suggested on the previous limited available data, **ERX-963** is a repurposed drug targeting the GABA receptor in the brain that showed no significant improvement in DM1 daytime sleepiness through the NCT03959189 clinical trial.

Declaration of interest

Acknowledgments

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