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The myotonic dystrophy type 1 drug development pipeline: 2022 edition

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The beginning of the 20th decade has witnessed an increase in drug development programs for myotonic dystrophy type 1 (DM1). We have collected nearly 20 candidate drugs with accomplished preclinical and clinical phases, updating our previous drug development pipeline review with new entries and relevant milestones for pre-existing candidates. Three interventional first-in-human clinical trials got underway with distinct drug classes, namely AOC 1001 and DYNE-101 nucleic acid-based therapies, and the small molecule pitolisant, which joins the race toward market authorization with other repurposed drugs, including tideglusib, metformin, or mexiletine, already in clinical evaluation. Furthermore, newly disclosed promising preclinical data for several additional nucleic-acid therapeutic candidates and a CRISPR-based approach, as well as the advent into the pipeline of novel therapeutic programs, increase the plausibility of success in the demanding task of providing valid treatments to patients with DM1.

Keywords: myotonic dystrophy; CTG repeat expansion; drug development; small molecule; drug repurposing; nucleic acids therapeutics; gene therapy; clinical trial

Introduction

DM1 (OMIM 160900) is a currently incurable genetic disease displaying highly variable multisystem symptoms. Disease severity is linked to the progressive loss of locomotor and cognitive functions because of skeletal muscle and central nervous system (CNS) degeneration and is life-threatening when negatively influencing cardiac or respiratory muscles [1]. Patients with DM1 also have a significantly impaired quality of life and reduced life expectancy [1,2]. Importantly, DM1 overall prevalence was estimated at 1 in 8000–10 000, but a recent population-wide screening estimated that the disease burden could be much higher, finding a mutation prevalence of 4.8 in 10 000 individuals, making it one of the most common rare diseases [3,4].

Mechanistically, a CTG repeat expansion above 50 units in the 3′-untranslated region (UTR) of the gene encoding DM1 protein kinase (*DMPK*) is the recognized mutation causing DM1 [5]. Since its discovery-three decades ago, intensive characterization of disease pathways has identified crucial therapeutic targets central to drug screening and development programs. A paradigmatic example was the evidence for nuclear accumulation as discrete aggregates, so-called 'RNA foci', of CUG repeat secondary structures of mutant *DMPK* transcripts driving a pathogenic RNA gain-of-function mechanism, which has been a classic outcome measure in drug discovery campaigns [6]. These RNA foci are located at the periphery of nuclear speckles, membrane-less structures enriched with small nuclear ribonucleoproteins, including spliceosome assembly factors and many other transcription and splicing regulators [7]. RNA foci are the cytological hallmarks of molecular events that dysregulate essential proteins, such as Muscleblind-like 1 and 2 (MBNL1/2) factors

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and CUGBP Elav-Like Family Member 1 (CUGBP1 or CELF1). MBNL1/2 and CELF1 dysregulation directly causes the spliceopathy observed in DM1, that is, the changes in the alternative splicing of hundreds of genes that explain, at least in part, several characteristic DM1 symptoms [8,9]. MBNL1/2 loss of function is well recognized as robustly contributing to DM1 phenotypes because this family of proteins is directly sequestered by CUG repeat RNA and depleted to perform its normal functions [10]. Simultaneously, stress responses triggered by toxic *DMPK* RNA cause the stabilization/activation of MBNL1/2 antagonists CUGBP1 and hnRNPA1 [10]. Recently, miRNA dysregulation was suggested to contribute to MBNL1/2 protein depletion because one of its natural repressors, miR-218, is overexpressed in DM1 samples [11,12]. Signaling pathways, such as 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), further explain DM1 muscle pathogenesis [13]. As a consequence of reasonably well-known molecular causes of disease, the exploration of distinct proof-of-concept therapeutic strategies has resulted in the set-up and execution of numerous drug search programs, currently providing a large drug development pipeline for DM1 (reviewed in [14–16]).

Drug development programs

Here, we summarize the most relevant advances regarding drug development programs in DM1 compared with our previous review [17], defining as a program any company or academic institution actively working toward regulatory preclinical and clinical stages of a drug candidate. The classification of therapeutic programs in the main text is updated into three broad categories: small molecules, nucleic acid therapeutics, and genome/transcriptome engineering, which range from drug candidates able to target the root cause of disease events to other strategies only relieving specific clinical symptoms (Figure 1).

Small molecules

A series of candidate repurposed drugs are in preclinical and clinical evaluation in DM1. Starting from known pharmacokinetics (PK) and pharmacodynamics (PD) profiles in humans, repurposed drugs can be tested in patients more quickly and at lower cost and risk compared with entirely new drugs. This explains why they have taken the lead as the type of molecules closest to reaching market authorization (Table 1). Despite disappointing clinical results for **ranolazine** and **flumazenil** [18,19], a sustained progression is observed for other repurposed drugs already in long-term clinical development programs (Figure 1).

One of the most advanced in development is **tideglusib (AMO-02)**, a marine-derived GSK3β inhibitor initially developed to treat Alzheimer's disease [20], which rescues abnormally high GSK3β levels, and normalizes CUGBP1 downstream myogenic targets, such as Dcx and Rbm45 in different disease models [21,22]. Tideglusib treatment also reduced the amount of toxic *DMPK* RNA in normal and congenital DM1 (CMD) myoblasts [21,22]. At the functional level, tideglusib significantly improved *in vivo* parameters, such as muscle weakness and myotonia, in the HSA^{LR} mouse model. The drug also improved postnatal survival, weight, and neuromotor activity in the muscles and brain of DMSXL mice, a model of CMD [22]. Sponsored by AMO-Pharma, ongoing clinical trials are targeting congenital and adult forms of DM1. Completion of the Phase II trial (NCT02858908) demonstrated drug safety when orally administered to patients with CMD and childhood-onset DM1, improvements in CNS and clinical neuromuscular symptoms in most patients, and a favorable PK profile [23]. Tideglusib is being evaluated in the REACH-CDM Phase II/III pivotal trial, targeted to children and adolescents with CMD, and is being conducted at 11 clinical centers globally (NCT03692312, Table 1). An open-label extension was recently announced (NCT05004129, Table 1) for the cohort completing the previous Phase II/III trial.

The situation is similar for **mexiletine (NaMuscla)**, an antiarrhythmic medicine used to reduce or prevent myotonia by blocking sodium channels involved in the contraction and relaxation of muscles [24]. Two Phase II trials, in which mexiletine was orally administered to evaluate management of myotonia symptoms in adult patients with DM1, were completed and reached similar results indicating a positive effect in handgrip myotonia in ambulatory patients but no significant benefit on the 6-min walk test (6MWT) [25,26]. Lupin is evaluating this drug through two Phase III clinical trials. One will recruit children and adolescents with DM1 (NCT04624750), whereas a second (NCT04700046) will involve adults with DM1 ($N > 150$) or DM2 ($N = 16$) (Table 1). Mexiletine has shown the ability to downregulate *DMPK* mRNA levels, pointing to additional activity through the DM1 disease pathway [27]. Together with recent data demonstrating long-term drug efficacy and cardiac safety for the treatment of myotonia in patients with myotonic dystrophies, the mexiletine drug-like profile has been strengthened for chronic treatment of patients with DM1 [28].

Additional recent advances in the DM1 pipeline led to **pitolisant** entering clinical evaluation. Pitolisant is a stimulant drug that antagonizes histamine H3 receptors to treat excessive daytime sleepiness in patients with narcolepsy [29]. Sponsored by Harmony Biosciences, the drug is under evaluation in a Phase II clinical trial to target the same symptom in patients with DM1 (NCT04886518) (Table 1). Excessive daytime sleepiness is a DM1 clinical hallmark in most patients and one of the most frequent non-muscular symptoms contributing to a reduced quality of life [30].

Also appealing is the clinical path of **metformin** (Table 1). Metformin is a biguanide antidiabetic drug with the ability to rescue multiple phenotypes of DM1 (reviewed in [31]). A first small Phase II clinical trial explored the effects of metformin oral administration on mobility in 40 patients with DM1 (2013-001732-21) [32]. The distance walked during the 6MWT was the primary outcome, but other functional capacities were also assessed. More than 50% of patients who completed the 1-year study displayed statistically significant improvements in the final distance walked (DM1: 29 m versus placebo: 14 m) and total mechanical power during gait. However, the drug did not significantly improve myotonia or muscle strength. A replication study sponsored by the Tor Vergata University of Rome in a multicenter Phase III clinical trial (2018-000692-32) is ongoing, with

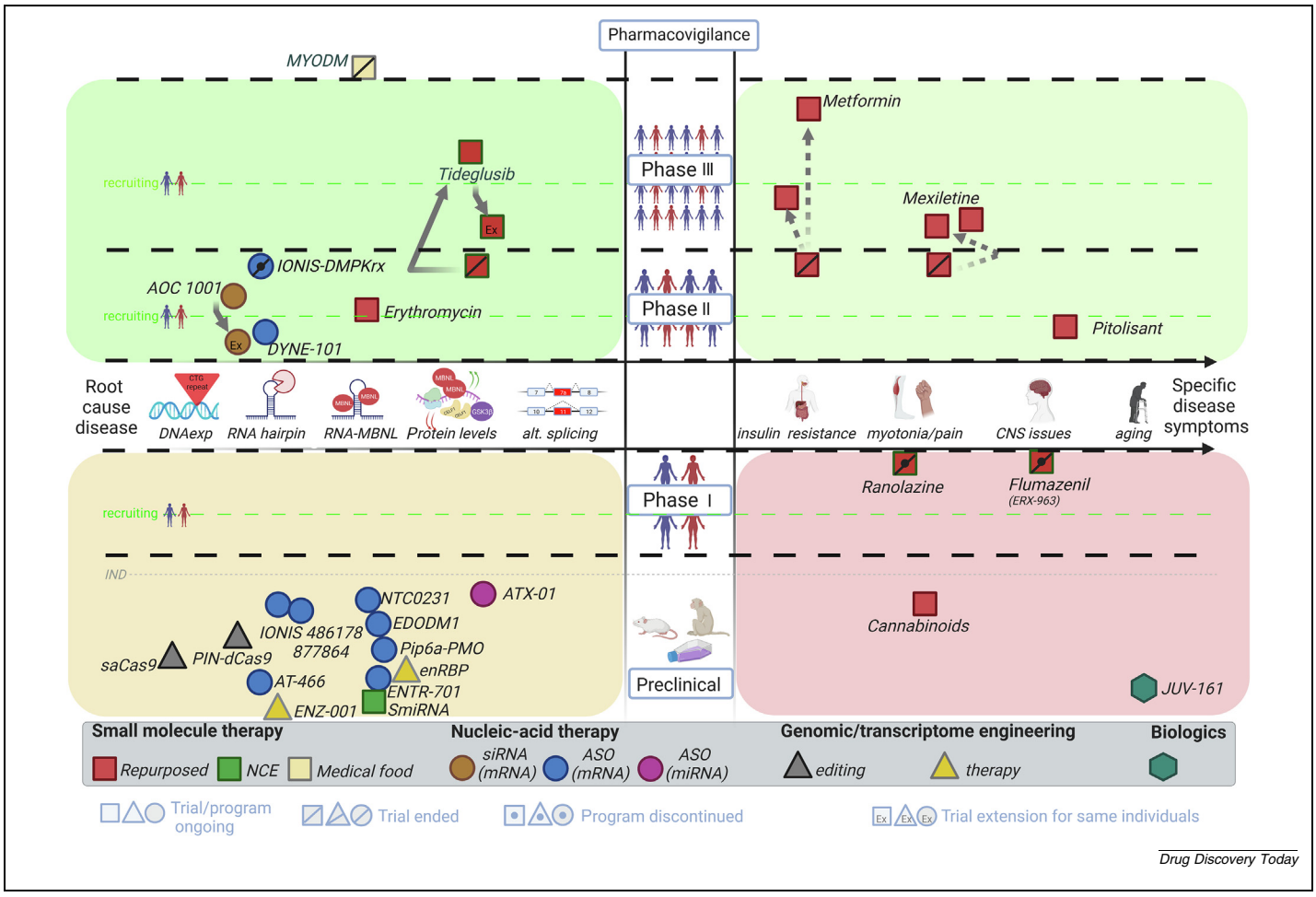

FIGURE 1

Overview of the preclinical and clinical myotonic dystrophy type 1 (DM1) drug programs. The middle *x*-axis indicates the step targeted by the candidate drug within the DM1 physiopathological cascade (left side, the root causes of disease; right side, clinical disease symptoms), whereas the middle *y*-axis shows the stage of the drug (candidate/program) evaluation from investigational preclinical to market authorization. Dashed-black lines separate the different clinical phases; dashed-green lines indicate active patient recruitment. The dashed-gray line denotes the investigational new drug (IND) milestone at the end of the preclinical evaluation phase that changes the legal status of a molecule so that it becomes a new drug subject to specific requirements of the drug regulatory system. Drug candidates are represented in different shapes according to the type of therapy and colors for specific types of molecule, as indicated. Solid shapes indicate trials/programs ongoing; shapes with a diagonal line indicate finished trials; shapes with a dot in the middle indicate discontinued drug development programs; shapes with an 'Ex' inside indicate extended clinical trials. Black arrows denote the progression of those drug candidates evaluated in more than one clinical trial (solid, the same sponsor; dotted, different sponsor). Created with BioRender (BioRender.com). Abbreviations: ASO, antisense oligonucleotide; CNS, central nervous system; NCE, new chemical entity; siRNA, small interfering RNA;

almost 200 patients with DM1 receiving metformin for the same period (1 year). The trial results are expected in 2023 and will provide evidence for an informed recommendation on using metformin in DM1. An additional Phase III was recently announced with a similar goal of evaluating the improvement of motor deficits in non-diabetic patients with DM1 (Assistance Publique – Hôpitaux de Paris, NCT05532813). However, recent studies suggest an increased birth-defect frequency in offspring from men being treated with metformin [33]. This result should be considered for further evaluation in male populations with current metformin regular prescription (diabetic) and if the drug is repurposed to new indications like DM1.

Nutritional management of DM1 based on the synergistic combination of **theobromine and caffeine** (commercialized by Myogem Health Company as **MYODM™**) has been evaluated in the clinical trial NCT04634682 in terms of its effects on the quality of life, fatigue, and hypersomnia in adult patients with DM1 (Table 1) [34,35]. The statistically significant decrease in the Epworth's sleepiness scale and increase in 6MWT results from baseline to the end of the 6-month study period substantiate that a controlled, dosed administration of MYODM™ over time provides significant improvements in the quality of life, at least in adult males with DM1 [36,37]. As a result, MYODM™ is expected to be upgraded to a food for special medical purposes [European Food Standards Agency (EFSA) classification for foods specifically intended for the dietary management of patients, under medical supervision, in the context of Article 3 of Regulation (EU, No 609/2013)] [38].

In 2016, **erythromycin (MYD-0124)** was revealed as an anti-DM1 candidate after a targeted screen for the RNA-binding

TABLE 1

Clinical trials in DM1 from 2011 to 2022

Phase	Clinical trial	Status	Clinical trial information	Results
Mexiletine (repurposed small molecule): Orphan Drug Designation from FDA and EMA				
III	NCT04700046	Not yet recruiting; 2021–2023	Study to investigate the efficacy and safety of mexiletine in adult patients with myotonic dystrophy type 1 and type 2 (MIND). Sponsor: Lupin	Not posted
	NCT04624750	Recruiting; 2021–2024	Safety, efficacy and steady-state PK of mexiletine in pediatric patients with myotonic disorders. Sponsor: Lupin	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	[26]
Metformin (repurposed small molecule)				
III	NCT05532813	Not yet recruiting; 2022–2025	Efficacy of metformin on motility and strength in DM1. Sponsor: Assistance Publique - Hôpitaux de Paris	Not posted
	2018-000692-32	Ongoing; 2019–2022	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	[32]
Tideglusib (repurposed small molecule): Orphan Drug Designation from FDA				
II/III	NCT05004129	Enrolling by invitation; 2021–2023	Safety and efficacy of tideglusib in congenital myotonic dystrophy for children and adolescents who participated in and completed the preceding AMO-02-MD-2-003 study (Extension study). Sponsor: AMO Pharma	Not posted
	NCT03692312	Recruitment complete; 2021–2023	Randomized, multicenter, double-blind, placebo-controlled study of patients (aged 6 to 16 years) diagnosed with CDM. Sponsor: AMO Pharma	Not posted
II	NCT02858908	Trial completed; 2016–2018	Safety, efficacy, and PK of tideglusib in the treatment of adolescents and adults with congenital and juvenile-onset DM1. Sponsor: AMO Pharma	[23]
Pitolisant (repurposed small molecule)				
II	NCT04886518	Recruiting; 2021–2023	Safety and efficacy of pitolisant on excessive daytime sleepiness and other non-muscular symptoms in patients with DM1. Sponsor: Harmony Biosciences	Not posted
Erythromycin (repurposed small molecule)				
II	jRCT2051190069	Recruitment complete	Blinded, placebo-controlled study to assess the safety, tolerability, and efficacy of MYD-0124 to DM1 adult patients. Sponsor: Hospital Osaka University	Not posted
AOC 1001 (oligonucleotide-based therapy, siRNA): Orphan Drug Designation from FDA and EMA				
II	NCT05479981	Enrolling by invitation; 2022–2025	Study will continue to evaluate the safety, tolerability, PK, PD, and efficacy parameters of AOC 1001 in participants previously enrolled in randomized, placebo-controlled, First-In-Human MARINA Phase I/II clinical study. Sponsor: Avidity Biosciences	Not posted
I/II	NCT05027269	Active, not recruiting; 2021–2023	Safety, tolerability, PK, and PD parameters of single and multiple-doses of AOC 1001 administered intravenously to adult DM1 patients. Sponsor: Avidity Biosciences	Not posted
DYNE-101 (oligonucleotide-based therapy, ASO)				
I/II	NCT05481879	Recruiting; 2022–2026	*In vivo* dose-dependent correction of splicing and myotonia in HSA^{LR} model after a single low dose. DMPK mRNA reduction in mouse and nonhuman primates. FORCE™ platform. Announced first clearance of clinical trial application; Sponsor: Dyne Therapeutics	Not posted
IONIS-DMPKRx (ISIS 598769) (oligonucleotide-based therapy, ASO)				
I/II	NCT02312011	Trial completed; 2014–2016	Safety, tolerability, and PK of multiple escalating doses of ISIS 598769 administered subcutaneously to adults with DM1. Sponsor: IONIS-Biogen	Reviewed in [17]
Flumazenil (ERX-963, repurposed small molecule)				
I	NCT03959189	Trial 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	[19]
Ranolazine Ralexa™ (repurposed small molecule)				
I	NCT02251457	Trial completed; 2014–2017	Preliminary data to determine safety and efficacy of ranolazine in symptoms of DM1. Sponsor: Ohio State University/Gilead Sciences	[18]
Caffeine and theobromine formulation MYODM™ (natural compounds)				
NA	NCT04634682	Trial completed; 2020–2021	Effect of food supplement MYODM™ on excessive daytime sleepiness and quality of life in adults with DM1. Sponsor: Myogem Health Company	[36,37]

potential of US Food and Drug Administration (FDA)-approved antibiotics [39], decreasing foci formation and rescuing missplicing in DM1 cell and mouse models [39,40]. In 2019, a Phase II clinical trial sponsored by Osaka University Hospital was posted to test the oral administration of erythromycin in adults with DM1. Recently, it was announced that recruitment was complete (Table 1). In addition, **cannabinoids** (**cannabidiol** and **tetrahydrocannabinol** or CBD/THC, respectively) were suggested to treat myalgia and other muscular complaints in DM1, driven by the presence of cannabinoid receptors in muscles [41], and by modulation of both central and peripheral pain pathways [42,43]. However, a pilot study reported no clear evi-

dence to support cannabis use to treat chronic pain or myotonia in DM1 [44], and recent studies suggest that CBD does not significantly influence motor activity when administered or consumed alone [45]. Regardless, Nexien Biopharma was recently granted patent protection covering methods and compositions for treating patients with DM1 or DM2 with oral formulations of CBD and THC [46], although none of these compounds are under active clinical evaluation.

In addition, we have identified a few players firmly committed to developing investigational new chemical entities (NCE) based on innovative screening platforms (Box 1). Design Therapeutics is developing small molecule therapeutic candidates, called GeneTAC™ molecules, designed to block transcription of the expanded CTG repeat without disrupting normal *DMPK* expression. In the same area, Expansion Therapeutics is working on identifying small molecule therapeutic drugs that can selectively bind the disease-causing toxic CUG repeats in *DMPK* mRNA and liberate key sequestered proteins so that they can function correctly. No specific lead candidates have yet been announced by either company.

Nucleic acid-based therapies

Nucleic acid-based therapies (NATs) have received strong interest in the pharmaceutical industry, with 13 molecules already approved by regulatory agencies for different diseases and close to 200 in different clinical trial stages [47–49]. The success of NATs stems from their precise way of exerting pharmacological activity via Watson–Crick base pairing and from their potential to modulate gene (DNA-based) or gene products (RNA-based) traditionally considered undruggable [49]. This unique mechanism of action (MoA) has also been incorporated into the DM1 pipeline through the design of different classes of NAT approaches, mainly double-stranded small interfering RNA (siRNA) or single-stranded antisense oligonucleotides (ASOs).

The discontinuation of IONIS-DMPKrx development, the first NAT (ASO class) molecule reaching clinical trials in DM1 (reviewed in [17]) was a starting point for the current DM1 NAT pipeline (Figure 1). Here, we categorize several NATs based on the distinct carrier delivery strategies being used to overcome the intrinsically poor NAT biodistribution features [50].

Fatty-acid conjugation

The IONIS Pharmaceuticals pipeline currently includes **IONIS 486178**, an unconjugated gapmer type ASO comprising a central core of ten nucleotides flanked by three bases of 2′-4′-constrained ethyl (cEt)-modified nucleotides on the 5′ and 3′ ends, and targeted to the 3′-UTR of *DMPK*. IONIS 486178 effectively reduced *in vivo DMPK* expression by enhanced RNase H-mediated degradation and translated into significant therapeutic activity in skeletal muscle and heart in DMSXL and DM200 mouse models (Table 2) [51–53]. In the most recent publication for this ASO, the authors successfully targeted DM1 features linked to the CNS. Specifically, *DMPK* mRNA targeting significantly abolished aberrant foci formation, enabled nuclear redistribution of MBNL1/2 proteins, and corrected aberrant splicing events in DM1 neural cells derived from induced pluripotent stem cells. Also relevant, the authors demonstrated that intracerebroventricular injection in DMSXL mice reversed behavioral abnormalities after neonatal administration (Table 2) [54]. Interestingly, **IONIS 877864**, an ASO with the same sequence and modified nucleotides as IONIS 486178 but conjugated to a palmitate hexylamine phosphodiester (C16-HA) moiety, improved drug internalization and therapeutic activity in skeletal muscle and heart of DMSXL mice after subcutaneous administration compared with the unconjugated ASO [55]. Ligand-conjugated antisense (LICA) technology used for IONIS 877864 involves combining an ASO with a ligand with high affinity for specific cell surface receptors, looking for higher tissue specificity and increased drug uptake [56]. Unfortunately, the first results with C16-HA conjugation did not show a decrease in mutant *hDMPK* transcript levels in the mouse brain, suggesting that this modification prevents the ASO from crossing the blood–brain barrier [56].

A fatty-acid conjugation is also incorporated into **ATX-01**, an oligonucleotide-based molecule that approaches DM1 therapy from a different angle compared with other NATs. Fundamentally, given that *MBNL1* and *MBNL2* remain normal in patients with DM1, a therapeutic gene modulation approach was explored to enhance their endogenous expression to compensate for functional depletion by sequestration. The approach involves de-repression of MBNL1/2 expression by blocking natural miRNAs miR-23b and miR-218. Proof-of-concept reports with anti-miRs, a class of chemically engineered ASOs complementary to their cognate targets miR-23b or miR-218, significantly upregulated MBNL1/2 protein levels and improved molecular disease-linked phenotypes in cell models, and muscle defects in the HSA^{LR} mouse [11,12,57,58]. Furthermore, no safety concerns were observed in *in vivo* treatments, and new anti-miRNA chemistries (i.e., phosphorodiamidate morpholino oligomers; PMOs) were tested with similar results [58]. ARTHEx Biotech has led an anti-miRNA drug development program to enhance the efficacy and delivery of these molecules using its proprietary ENTRY™ platform by linking the ASO sequences to specific fatty acids. **ATX-01** lead is the improved version of the first published anti-miR-23b (Table 2). It efficiently targets different affected tissues in the disease, enhancing therapeutic efficacy and safety. ATX-01 MoA fosters higher levels of functional MBNL proteins and, therefore, a rescue of molecular and functional DM1-related phenotypes in different disease models, particularly HSA^{LR}mice [59]. During 2022, ARTHEx Biotech's lead product ATX-01 was granted orphan-drug designation (ODD) by the FDA, and the European Medicines Agency (EMA) for treating DM1, and the company announced first-in-human studies in 2023.

Monoclonal antibody conjugation

The most significant recent progression in the DM1 pipeline has been the entrance of two NAT molecules, both targeted to a non-repeat sequence of *DMPK* transcripts.

AOC 1001 entered Phase I/II trial (MARINA™ trial) evaluation at the end of 2021 (NTC05027269) (Table 1). This study evaluates the safety, tolerability, PK, and PD of single and multiple ascending doses of the drug. AOC 1001 is a conjugate of a siRNA able to reduce levels of *DMPK* RNA in skeletal, cardiac, and smooth muscles with a proprietary monoclonal antibody against the transferrin receptor 1 (TfR1) protein sponsored by Avidity Biosciences (Table 1) [60,61]. Patients with DM1 are being treated intravenously to deliver siRNA to muscle cells.

TABLE 2

Myotonic dystrophy drug candidates in preclinical stages

Preclinical status	Preclinical information	Refs
Cannabinoids CBD/THC (small molecule/repurposing)		
IND enabling phase	Management of chronic neuropathic pain, myotonia, and myalgia. A pilot survey in DM1, DM2, and congenital myotonia, with two patients per disease, and a different pilot study in a DM1 cohort of 72 participants. Sponsor: Nexien Biopharma	[43–45]
IONIS 486178 (oligonucleotide-based therapy, ASO)		
Preclinical proof of concept	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; also targets CNS phenotypes. Sponsor: Ionis Pharmaceuticals	[52–54]
IONIS 877864 (oligonucleotide-based therapy, ASO)		
Preclinical proof of concept	Systemic treatment resulted in improved drug internalization and therapeutic activity in skeletal muscle and heart of DMSXL mice compared with unconjugated IONIS 486178. Ligand-conjugated antisense (LICA) technology used. ASO unable to cross blood–brain barrier. Sponsor: Ionis Pharmaceuticals	[51,56]
Pip6a-PMO (oligonucleotide-based therapy, ASO)		
Licensed	Intravenously injected in HSA^{LR}. Nuclear foci reduction, MBNL1 redistribution, splicing, and myotonia correction. Sponsor: Oxford University	Reviewed in [17]
NTC0231 (oligonucleotide-based therapy, ASO)		
IND enabling phase	*In vitro* ability to target and open aberrant secondary RNA structure in mutant transcript, thereby displacing sequestered proteins. PATrOL™ platform. *In vivo* long-lasting correction of global levels of misspliced transcripts, broad tissue distribution, including significant accumulation in brain. Sponsor: Neubase Therapeutics	[68]
EDODM1 (oligonucleotide-based therapy, ASO)		
Preclinical proof of concept	*In vitro* ability to target CUG repeat RNA and block interaction of MBNL1 with toxic RNA. *In vivo* results displayed sustained correction of downstream missplicing events implicated in DM1 after a single dose, along with complete rescue of the myotonia in HSA^{LR} mice. Sponsor: PepGen	[69]
ENTR-701 (oligonucleotide-based therapy, ASO)		
IND enabling phase	*In vitro* ability to target CUG repeat RNA and block interaction of MBNL1 with toxic RNA. Ability to reduce nuclear foci and correct aberrant splicing events in cell (patient-derived myotubes) and murine (HSA^{LR}) DM1 models. Sponsor: Entrada Therapeutics	[70]
ATX-01 (oligonucleotide-based therapy, ASO of the antimiR type): Orphan Drug Designation from FDA and EMA		
IND enabling phase	Systemic administration 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. Definition of ATX-01 lead by ENTRY™ platform. Start of Phase I/II by 2023. Sponsor: ARTHEx Biotech.	[11,12,58,59]
AAV-PIN-dCas9 (transcriptome engineering)		
Lead optimization	Intramuscular/systemic delivery in adult and neonatal HSA^{LR}. RNA-targeting dCas9 lasted for up to 3 months. Elimination of RNA foci, reversal of splicing biomarkers, and myotonia. Under lead selection and optimization stage, in which potential selected leads A01215, A01344 and A01686 are expected to enter nonhuman primate studies to assess tolerability and biodistribution in target tissues. Sponsor: Locanabio.	[76–78]
AT466 (oligonucleotide-based therapy, ASO)		
Lead optimization	AAV delivery to overcome biodistribution limitations of ASO-based therapies. Sponsor: Audentes	[71,72]
AAV-CRISPR-SaCas9 (genome engineering)		
Preclinical proof of concept	*In vivo* genome editing for DM1: deletion of CTG repeat tract leading to reduction of nuclear foci in muscle fibers after intramuscular injection of SaCas 9 and sgRNA rAAV9 vectors in DMSXL mice. Sponsor: Genethon-INSERM	[75]
enRBP (transcriptome engineering)		
Preclinical proof of concept	*In vitro* and *in vivo* results of engineered RNA-binding protein (enRBP) to act as decoy for CUGexp, restoring MBNL1 activity and correcting transcriptomic signature of DM1. Sponsor: Sorbonne Université, Inserm, Institut de Myologie	[79]

Although this Phase I/II trial is not designed to assess functional benefit, it includes the evaluation of clinical parameters, such as measures of mobility, muscle strength, and patient-reported quality of life. The sponsor announced an extension study for patients with DM1 who complete the first clinical trial. In the new Phase II trial (MARINA-OLE™ trial), patients will receive AOC 1001 for an additional 24 months. The orphan drug designation granted by the FDA and EMA, and fast-track designation by the FDA are additional achievements in the AOC 1001 dossier. The latest report announced mid-study positive results from the MARINA™ trial. Specifically, results have shown successful targeted delivery of RNA to muscle with meaningful *DMPK* reduction in 100% of patients treated with either an AOC 1001 1 mg/kg (single dose) or 2 mg/kg (two doses) regimen. Importantly, AOC 1001 also improved alternative splicing in a key set of muscle-specific genes, and showed early signs of clinical activity (myotonia improvement) in some of the participants of the 2 mg/kg cohort [62].

A similar strategy is used for **DYNE-101** (named FORCE-DMPK candidate in [17]), sponsored by Dyne Therapeutics and developed from the proprietary FORCE™ platform leveraging the transferrin 1 receptor expression on the surface of muscle cells (Table 2) [63]. DYNE-101 involves conjugation of a proprietary ASO designed to reduce *DMPK* RNA levels in the cell nucleus by RNase H cleavage with monoclonal TfR1-binding antigen-binding fragments (Fab) for effective delivery to muscles [64]. The candidate has been reported to robustly reduce toxic *DMPK* RNA in the nucleus of different skeletal muscles and heart

of the hTfR1/DMSXL model, a novel mouse developed by Dyne that simultaneously expresses the human TfR1 and a *DMPK* gene with more than 1000 CTG repeats, stimulating the reduction of nuclear foci, correction of splicing, and reversal of myotonia. In nonhuman primates, DYNE-101 displays a favorable safety profile, being well tolerated in a non-GLP toxicology dose–range-finding study, showing no clinical signs of toxicity after repeat ascending doses, and with no effects on body weight, kidney, or liver function observed, as well as enhanced muscle distribution [65]. Dyne recently posted a Phase I/II multiple ascending dose trial (MAD) for testing the safety and tolerability of DYNE-101 after intravenous administration in patients in New Zealand (NCT05481879).

Peptide backbone or conjugation

Another molecule progressing through preclinical evaluation in DM1 is **NTC0231** (NTC0200 in [17], potentially a family of similar molecules) (Table 2). This ASO was identified through the proprietary platform PATrOL™ sponsored by NeuBase Therapeutics, exploring the use of new, highly selective nucleobases able to minimize off-target effects and neutral-charged peptide nucleic acid (PNA) backbones. The resultant compound is a short, flexible, and selective PNA sequence that showed *in vitro* ability to discriminate pathogenic CUG expansions from wild-type transcripts, opening CUG RNA secondary structures in the mutant transcript and thereby releasing sequestered MBNL proteins [66]. Recently, the company announced relevant long-lasting and broad correction of misspliced transcripts in the HSA^{LR} transgenic mouse after a well-tolerated single intravenous injection of NTC0231. Broad tissue distribution was also reported in PK studies with wild-type BALB/C mice, including significant accumulation in the brain [67]. Further preclinical experiments are in progress in the DMSXL model to test molecular and functional rescue in the brain. Recent strategic restructuring news from the company put on hold the process of filing an investigational new drug (IND) application for DM1 as previously announced.

Two new companies with lead ASO compounds incorporate peptide conjugation. PepGen sponsors **EDODM1**, a peptide–conjugate oligonucleotide lead developed through its proprietary EDO™ platform that aims for optimal tissue and cell penetration for the ASOs in development (Table 2). EDODM1 hybridizes with the CUG repeat RNA and blocks the interaction of MBNL1 with the toxic RNA. The disclosed *in vivo* results support that EDODM1 administration brings about a sustained correction, up to 6 months following a single dose, of downstream missplicing events implicated in DM1, along with complete rescue of the myotonia phenotype following a single low dose in HSA^{LR} mice [68]. In addition, Entrada Therapeutics recently announced that it will submit an IND to the FDA for its first lead, **ENTR-701**, in 2023. The sponsor claims that ENTR-701 reduces nuclear foci and corrects aberrant splicing events in cell (patient-derived myotubes) and murine (HSA^{LR}) DM1 models. ENTR-701 is a PMO ASO type, conjugated to cyclic cell-penetrating peptides (cCPPs) able to block CUG repeats in an allele-specific manner, improving cellular uptake by enhancing endosomal escape [69]. Along the lines of CPP-conjugated ASOs, the **Pip6a-PMO-CAG7** molecule and derivatives, sponsored by Oxford University and involving a conjugate between a CPP moiety (arginine-rich cell-penetrating peptide, Pip6a) with a PMO oligo complementary to the CUG repeats, were licensed to PepGen (Table 2) (reviewed in [17]).

Adeno-associated virus

No new data have been released for the **AT466** candidate sponsored by Astellas Gene Therapies (Audentes Therapeutics in [17]), an ASO designed to be endogenously expressed as a recombinant adeno-associated virus (AAV). AT466 combines vectorized *DMPK* RNA knockdown and exon skipping to treat DM1, targeting specific transcript sequences [9,70,71]. The benefits of this approach in DM1 were initially disclosed with a proof-of-concept study using AAV-delivered RNAi in the HSA^{LR} mice [72].

Genome/transcriptome engineering

CRISPR/Cas9 technology has become a multifunctional platform for sequence-specific gene expression regulation and editing. Specifically, in DM1 disease models, it has been adapted for removing the expansions at the DNA level, preventing their transcription, or targeting degradation of the toxic RNA, effectively addressing the underlying DM1 etiology [15,73]. Although there has still been no clinical trial for muscular diseases, two distinct CRISPR/Cas9-based approaches in DM1 were identified in our first review (Table 2) [17], which are now progressing to preclinical stages using vectorized AAV administration.

Génethon's laboratory strategy of removing human *DMPK* CTG expansions by using recombinant **AAV vectors expressing CRISPR-Cas9** (SaCas9) components from *Staphylococcus aureus* (Sa) has not offered new data from our first statement (Table 2) [17,74]. By contrast, LocanaBio is developing **AAV-vectors encoding PIN-dCas9** (a nuclease dead Cas9 (dCas9) fused to the PIN RNA endonuclease domain) and **a single-guide RNA targeting CUG repeats** to degrade toxic CUG RNA molecules preferentially. Previously published and newly disclosed data from treated DM1 patient muscle cells and intramuscular and systemic administration in adult and neonatal HSA^{LR} mice indicate dose-dependent reduction of toxic RNA foci, redistribution of the MBNL1 factor, rescued missplicing, reduced myotonia, and muscle weakness recovery [75]. In addition, LocanaBio's data support the ability of the approach to preferentially target mutant *DMPK* RNA [76]. LocanaBio's DM1 candidates are currently at the lead selection and optimization stage, and candidate leads A01215, A01344, and A01686 have been announced to enter nonhuman primate studies to assess their tolerability and biodistribution in target tissues [77].

A conceptually new way of tackling MBNL1 sequestration is to use an engineered RNA binding domain of the protein itself (**enRBP**) to block depletion by the CUG repeat RNA. AAV-mediated expression of these decoys has been shown to rescue phenotypes in cell and mouse models of disease and could complement other gene therapy approaches in the DM1 field (Table 2) [78].

Concluding remarks and future challenges

The past two years have witnessed candidates progressing in preclinical and clinical stages, the start of two new clinical trials, extension of two already ongoing trials, and incorporation of new players in innovative drug development programs. Overall, such progress increases the chances of finally having one or more

therapeutic options available in the next few years, as has already happened in other neuromuscular diseases, such as Duchenne's muscular dystrophy or spinal muscular atrophy [79].

The strategy of repurposing drugs for diseases with urgent clinical needs is appealing to reduce risky evaluation steps and explains their current advantage over other strategies in DM1. However, the critical mass already generated around NATs, attacking the disease at different upstream steps, is gaining momentum, with two new molecules in clinical trials (AOC 1001 and DYNE-101), several others close to achieving this significant milestone, and relevant new players coming in (Box 1). In addition, progression of drug development programs involving NCEs able to target the disease mechanism at a very early stage (mutant DNA or RNA) is expected. The announcement of pioneering, new drug development programs in DM1 is solidly expanding the opportunities for final success in effective DM1 clinical management. Some of the examples identified and described in Box 1 are: (i) **JUV-161**, the lead compound reported by Juvena Therapeutics, which is a biologic identified through a machine learning (ML)-enhanced platform and targeting defined age-related aspects in DM1 progression (Figure 1); (ii) Faze Medicines program, which is using new screening and proteomics techniques to define promising therapeutic targets for the modulation of RNA foci; and (iii) Enzerna Biosciences gene therapy approach, working in effective targeting of **EZN-001, an** Artificial Site Specific RNA Endonuclease (ASRE), into cells to specifically destroy expanded CUG RNAs (Figure 1).

Box 1 Additional active therapeutic programs in DM1.

Juvena Therapeutics

Platform

Juvena Therapeutics is a computationally driven company discovering and developing **biologics** by mining the **secretomes** of human pluripotent stem cells through a ML-enhanced platform. Specifically, the goal is to unlock the potential of **rejuvenating proteins** for restorative tissue therapeutics. Juvena Therapeutics' platform integrates proteomics, transcriptomics, and images with a phenotypic human *in vitro* disease model screening to build a map of secreted proteins in a compounding database linking therapeutic proteins to disease indications (see Figure 1 in the main text).

DM1 program

Juvena Therapeutics announced investment for the advancement of the JUV-161 fusion protein therapeutic lead JUV-161 through initial IND-enabling studies and a pre-IND FDA meeting in 2023.

Design Therapeutics

Platform

Design Therapeutics announced development of **small molecule NCEs**, called GeneTAC^{TM} molecules (gene-targeted chimera) [88], that combine medicinal chemistry and structure–activity aspects to design targeted DNA-binding moieties connected via a linker to ligands to engage and modulate the transcriptional machinery. Thus, GeneTAC^{TM} molecules are heterobifunctional and, specifically in DM1, the DNA-binding moiety is designed to bind to the CTG repeats in the 3′-UTR of *DMPK*, linked to a ligand moiety designed to block transcription of the expanded CTG repeat without disrupting normal *DMPK* expression.

DM1 program

No candidate(s) have yet been announced.

Expansion Therapeutics

Platform

Expansion Therapeutics announced the development of **small molecule NCEs** that can selectively bind disease-causing expanded CUG repeats in *DMPK* mRNA and liberate key sequestered proteins so that they can function properly (SmiRNA platform) (see Figure 1 in the main text).

DM1 program

No candidate(s) have yet been announced, but papers on its approach defining MoA for the molecules in development for DM1 and DM2 have been published [87–89]

Faze Medicines

Platform

Faze Medicines looks for the exact identification, using screening and metabolomics approaches, of key protein components of the nuclear foci aberrantly formed in DM1 to target and dissolve them in patient cells.

DM1 program

No (type of) candidate(s) have yet been announced.

Enzerna Biosciences

Platform

Enzerna Biosciences has developed an **artificial site-specific RNA endonuclease (ASRE)** platform to define a rationally designed protein that, when expressed in cells, targets the expanded CUG repeat mRNA and destroys it. ASRE constructs are built with an RNA-binding module (PUF) that can be engineered to bind any RNA sequence of choice and an RNA-degrading module (PIN) that will destroy the RNA. Combined with gene delivery vectors, they provide a new strategy for selective degradation of pathogenic transcripts (see Figure 1 in the main text).

DM1 program

An ENZ-001 ASRE anti-DM1 construct is under development.

Importantly, recent publications anticipate an additional need for valid treatments in DM1, considering the practical difficulties encountered in similar diseases in alleviating the whole, or at least a significant amount, of multisystemic clinical presentations. This includes the concept of combining treatments, either using pharmaceutical (drug) with nonpharmaceutical (exercise or medical devices) tools [80–82], or by exploring the simultaneous use of two drugs potentially complementing their MoA [83].Instrumental for all of the above achievements are outstanding social and patient-driven initiatives around drug development news that helped position DM1, for society at large and the pharmaceutical industry, as a rare disease that will likely reach an effective treatment in the next few years. A significant example is the International Myotonic Dystrophy Awareness Day on September 15, 2022, which promoted the alliance of over 50 myotonic dystrophy organizations worldwide [84]. Another important initiative has been 'meet-DM-drug-developers', making the status of the different DM1 drug development programs affordable and understandable to patients and relatives [85], as well as the publication of the first virtual and interactive database of the current DM1 research ecosystem [86].

Data availability

Review manuscript

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Declaration of interests

The authors declare the following financial interests/personal relationships, which may be considered as potential competing interests: R.A. is an inventor on patents PCT/EP2017/073685 and EP22382493.9, currently licensed to Arthex Biotech, of which he is a co-founder and scientific consultant. R.A and M. P-G. are inventors on patents WO2016075285A1 and WO2016075288A1, currently licensed to Myogem Health Company. M.P-G. is a former employee, member of the Board of Directors, and shareholder of Myogem Health Company. A.L-C. is a member of the Arthex Board of Directors.

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POST-SCREEN (GREY)