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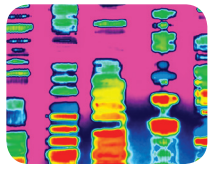

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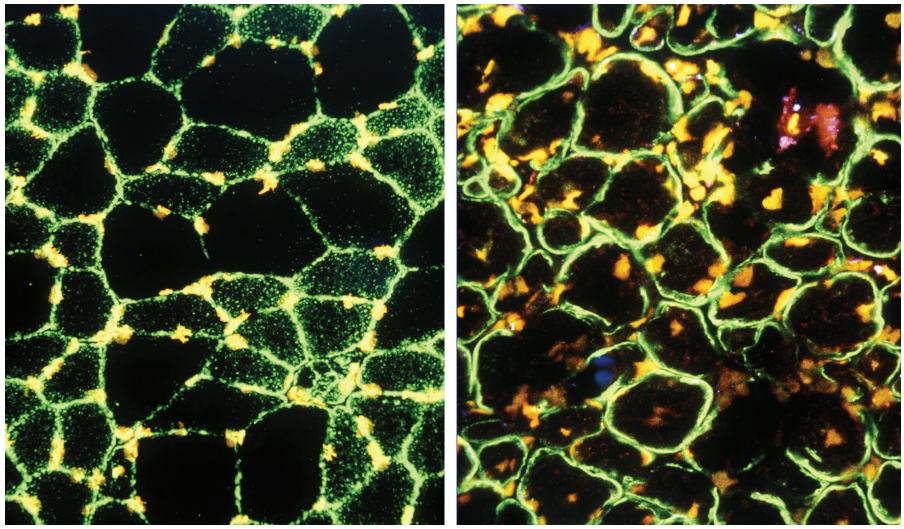
Duchenne muscular dystrophy drugs at the crossroads, as newer agents advance



Patrick Landmann / Science Source

New therapies for Duchenne muscular dystrophy attempt to restore dystrophin (green) production in muscle fibers. Dystrophin levels in normal muscle (left) and in tissue affected by the disease (right).

The first generation of exon-skipping drugs for Duchenne muscular dystrophy (DMD) has reached a critical juncture, as an FDA decision on whether to grant accelerated approval to Sarepta Therapeutics' controversial drug Exondys (eteplirsen) looms. A rival effort at BioMarin Pharmaceutical, of San Rafael, California, is now over. Biomarin withdrew a European application for Kyndrisa (drisapersen) on May 31, after unsuccessful talks with European Medicines Agency officials, and the US Food and Drug Administration (FDA) refused to approve the same drug in January. The refusals were unequivocal—drisapersen was neither safe nor effective. In the case of Sarepta's eteplirsen, however, regulatory uncertainty abounds. The Cambridge, Massachusetts–based biotech, is seeking approval with evidence from just 12 patients. "In any other therapeutic area, the agency would be within its rights to say this data set is very exciting—come back when you've conducted a trial which proves it's safe and effective," says Glyn Edwards, CEO of Abingdon, UK–based Summit Therapeutics. "The normal balance of evidence hasn't been achieved for this drug yet."

As *Nature Biotechnology* went to press, the FDA had yet to update the deadline for its verdict on eteplirsen, having delayed a decision originally due on May 26. In the meantime, it sought additional biopsy data from an ongoing phase 3 trial, suggesting that its deliberations have extended beyond the evidence aired during a recent hearing of the FDA's Peripheral and Central Nervous System Drugs Advisory Committee, which voted against eteplirsen's approval. Whatever the outcome, Sarepta and other early movers have at least managed to establish the ground rules for tackling the root cause of DMD, an X-linked condition caused by mutations in the large muscle protein dystrophin (**Table 1**). The problem at this stage in the field's development is that companies' claims made in support of their drugs are ambiguous. "The efficacy is marginal. They're just not potent enough," says Matthew Wood, professor of neuroscience at Oxford University, UK. The same applies to PTC Therapeutics, of South Plainfield, New Jersey, whose drug Translarna (ataluren) acts through a different though related mechanism—it promotes translational read-through of premature stop codons in dystrophin mRNA. The FDA refused to review its application, whereas the drug obtained conditional approval in Europe following an appeal (*Nat. Biotechnol.* **32**, 706, 2014).

Although the current generation of disease-modifying drugs in DMD has polarized observers, the prospect of an effective therapy in DMD is becoming more realistic, as next-generation approaches, including newer exon-skipping drugs and gene therapy start to reach the clinic. The focus is on boosting—or, in the case of Summit Therapeutics, bypassing—dystrophin, a large, rod-shaped protein, which plays an important role in skeletal muscle contraction. It acts as a mechanical link between the cytoskeleton and the extracellular matrix: its C terminus binds the dystrophin-associated protein complex at the muscle cell membrane or sarcolemma; its N terminus binds the cytoskeleton through actin filaments (*Nat. Rev. Mol. Cell Biol.* **7**, 762–773, 2006). In DMD, dytrophin's absence leads to progressive muscle weakening and wasting. Most patients lose the ability to walk by about 12 years, and they also experience respiratory difficulties, curvature of the spine and cardiomyopathy. Respiratory support, steroids and cardiovascular drugs have improved average life expectancy, but it is still less than 30 years. Death usually results from cardiac or respiratory failure.

Understanding the molecular basis of Becker MD, a similar but milder condition, combined with observations during the 1990s of low levels of dystrophin synthesis in some DMD patients, helped scientists develop the exon-skipping concept (*Trends Mol. Med.* **21**, 417–426, 2015). 'Becker boys' have in-frame mutations in the *DMD* gene, which give rise to a truncated but partially functional version of dystrophin. The occasional restoration of the dystrophin reading frame during pre-mRNA splicing leads to sporadic dystrophin synthesis in some DMD patients. Drug developers have attempted to mimic—and amplify—this effect by designing antisense oligonucleotide drugs to act as molecular 'patches', which bind dystrophin pre-mRNA at precisely designated positions. The resulting steric hindrance forces the pre-mRNA splicing

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Table 1 Disease-modifying therapies for Duchenne muscular dystrophy

Developer	Molecule	Mechanism	Data	Status
Sarepta Therapeutics	Exondys (eteplirsen)	Phosphorodiamidate morpholino oligomer (PMO); facilitates skipping of exon 51 during pre-mRNA splicing	In a study 201/202 (n = 12) there was no statistically significant difference in a 6-minute walk test (6MWT) between the placebo arm and either of two dose arms (30 mg/kg, 50 mg/kg) at 24 weeks (w); after 36 months of an open-label crossover extension the 12 patients (on 30 mg/kg) had an average 151 meter (m) difference in the 6MWT vs. historically matched controls (P < 0.01)	FDA's 26 May 2016 PDUFA date postponed
PTC Therapeutics	Translarna (ataluren)	Small molecule promotes translational read-through of premature stop codons	In a phase 3 trial patients on 40 mg/kg/day had a statistically insignificant 15 m difference in 6MWT vs. placebo at 48 w (n = 228; P = 0.213); in a subgroup with baseline 6MWT performance of 300–400 m there was a 47 m difference vs. placebo (n = 99; P = 0.007)	EMA conditional approval Aug. 4, 2014; FDA refuses to file letter Feb. 22, 2016
BioMarin Pharmaceutical	Kyndrisa (drisapersen)	2′-O-methyl-Phosphorothioate antisense oligonucleotide that facilitates exon 51 skipping	6 mg/kg/week difference in 6MWT vs. placebo: phase 3, 10 m at 48 w (n = 186; P = 0.42); phase 2 35m at 24w (n = 53; P = 0.01); phase 2 27m at 24w (n = 51; p=0.07)	FDA complete response letter Jan. 14, 2016; EMA application withdrawn 31 May
Summit Therapeutics	Ezutromid	Small molecule selectively upregulates utrophin production in muscle cells	24-week biopsy data due in January 2017	48-week phase 2 trial ongoing
Nippon Shinyaku (Kyoto, Japan)	NS-065/ NCNP-01	PMO, facilitates exon 53 skipping	In an investigator-initiated trial in ten patients, dystrophin protein detected in the high-dose group	Phase 2 US IND submitted March 25, 2016

Sources: FDA, PubMed, company websites

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machinery to skip over the target exons to restore the reading frame, giving rise to partially functional dystrophin proteins (*Nucl. Acid Ther.* **24**, 37–47, 2014). The approach is highly specific. Eteplirsen targets exon 51, which restores the reading frame in patients in whom exons 45–50, 47–50, 48–50, 49–50, 50, 52 or 52–63 are deleted. Collectively they represent 13% of all patients.

Even if achieved with high efficiency, exon skipping does not represent an outright cure. "The best-case scenario is you're converting the DMD phenotype to a Becker phenotype. You're slowing progression, but over time patients will still decline," says Thomas Meier, CEO of Liestal, Switzerland–based Santhera Pharmaceuticals.

Most agree the exon-skipping approach has promise, but evaluating the degree of improvement remains problematic. One approach is to quantify how much dystrophin is expressed in the muscle fibers of treated patients. But the FDA and Sarepta differed sharply on the latter's dystrophin production data. FDA reviewers called it "disappointing." "We did have data that showed we had genetic expression, and that was unequivocal," says Jerry Mendell, professor of neurology and pediatrics at Ohio State University and Nationwide Children's Hospital, in Columbus, Ohio, who led eteplirsen's clinical development. Moreover, all the boys who received the drug remain ambulatory—apart from two who lost the ability to walk shortly after the trial began. "After that no other kids lost ambulation," he says.

Other issues may have clouded the data's interpretation: the heterogeneity of DMD combined with the small scale of the trial and the use of matched external controls. Moreover, inefficient delivery of eteplirsen could have limited its effects. "The thing they could have done was increase their doses," says Wood. "I think that was an error—a big, big error. It could have made a difference." The ongoing phase 3 trial will have greater statistical power—it is recruiting about 160 patients—but it, too, is using a 30 mg/kg dose.

Meanwhile, other approaches are being actively pursued. Mendell, a pioneer of glucocorticoid therapy in DMD, is also initiating a gene therapy trial, using a truncated dystrophin protein to overcome the packaging limitations of the adeno-associated virus vector he is employing. "How that truncated version will play out in the clinic is a total, complete unknowable," he says. The 'micro-dystrophin' protein represents about 40–50% of the wild-type molecule. Evidence from nature suggests the concept is feasible—one UK group described a patient with a very mild phenotype, despite having just 46% of the wild-type protein (*Nature* **343**, 180–182, 1990).

Cambridge, Massachusetts–based Wave Life Sciences hopes to boost exon-skipping efficiency by using 'stereopure' oligonucleotides. In first-generation molecules, including drisapersen, a 2′-*O*-methyl-phosphorothioate antisense oligonucleotide, and eteplirsen, a phosphorodiamidate morpholino oligomer, the modifications introduced to increase stability and resistance to nuclease degradation also add a chiral center between each pair of neighboring residues. A 20-mer oligonucleotide such as drisapersen can give rise to 2^{19} stereoisomers, for example. "It becomes really hard to see and capture activity," CEO Paul Bolno says. "We don't know what's driving the pharmacology." Wave has developed a manufacturing platform that gives rise to a proprietary oligonucleotide chemistry. The company, which is collaborating with Oxford University's Wood in assessing its chemistry, claims to have achieved exon-skipping efficiencies of over 90% in unpublished studies—about 25-fold better than current molecules. It plans to file its first two investigational new drug applications, in DMD and Huntington's disease, this year. If improved exon-skipping efficiency translates into large increases in dystrophin, its approach could have additional therapeutic benefits. "If we get exposure to heart and diaphragm we can include other endpoints," Bolno says.

Summit Therapeutics aims to compensate for the absence of dystrophin by raising levels of a related muscle protein, utrophin, which is widely expressed during fetal development. In adults, it is found at the neuromuscular and myotendinous junctions, which link muscle to tendons and neurons, respectively, or during muscle repair. Its expression cycles on and off in DMD patients, owing to the constant damage caused by the lack of dystrophin. The concept, which is supported by evidence from *DMD* knockout mice, is not limited to specific genotypes. "It's completely independent of the other approaches and therefore highly likely to be additive if not synergistic with those," says CEO Edwards.

Further advanced is another genotype-independent approach. On May 30, Santhera filed for European approval in DMD for Raxone (idebenone) and also hopes to file in the US in 2016. A coenzyme Q10 analog, it is already approved for treating visual impairment in Leber's hereditary optic neuropathy, a maternally inherited condition caused by impaired oxidative phosphorylation. In DMD, Raxone does not restore dystrophin, but its anti-oxidant activity counters the effects of reactive oxygen species in the mitochondria, caused by excess calcium influx.

Curative therapy for DMD remains some way off, but children born with the condition now have better prospects than any who have preceded them.

Cormac Sheridan *Dublin,*