Additional file 1:
PRISMA checklist, protocol, search terms, meta-analyses on outcomes other than primary, and characteristics of excluded studies

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Appendix table 1: Characteristics of excluded studies
| Section/topic | # | Checklist item                                                                                                                                                                                                 | Reported on page # |
|---------------|---|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------|
| TITLE         |   |                                                                                                                                                                                                            |                   |
| Title         | 1 | Identify the report as a systematic review, meta-analysis, or both.                                                                                                                                          | 1                 |
| ABSTRACT      |   |                                                                                                                                                                                                            | 3-4               |
| Structured summary | 2 | Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number. |                   |
| INTRODUCTION  |   |                                                                                                                                                                                                            |                   |
| Rationale     | 3 | Describe the rationale for the review in the context of what is already known.                                                                                                                                | 6                 |
| Objectives    | 4 | Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).                                                        | 7                 |
| METHODS       |   |                                                                                                                                                                                                            |                   |
| Protocol and registration | 5 | Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.                                          | 7                 |
| Eligibility criteria | 6 | Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.                                      | 8                 |
| Information sources | 7 | Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.                                           | 8-9               |
| Section/topic                      | #  | Checklist item                                                                                                                                                                                                                                                                                                                                 | Reported on page # |
|-----------------------------------|----|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------|
| Search                            | 8  | Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.                                                                                                                                                                                                                      | Appendix 1        |
| Study selection                   | 9  | State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).                                                                                                                                                                                          | 9                  |
| Data collection process           | 10 | Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.                                                                                                                                                                              | 9                  |
| Data items                        | 11 | List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.                                                                                                                                                                                                           | 9                  |
| Risk of bias in individual studies| 12 | Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.                                                                                                                               | 10                 |
| Summary measures                  | 13 | State the principal summary measures (e.g., risk ratio, difference in means).                                                                                                                                                                                                           | 10                 |
| Synthesis of results              | 14 | Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., \( I^2 \)) for each meta-analysis.                                                                                                                                                                                                  | 11                 |
| Risk of bias across studies       | 15 | Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).                                                                                                                                                                                                  | 10                 |
| Additional analyses | 16 | Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified. | 10-11 |
|---------------------|----|---------------------------------------------------------------------------------------------------------------------------------|------|
| RESULTS             |    |                                                                                                                                 |      |
| Study selection     | 17 | Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram. | 11-12|
| Study characteristics| 18 | For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations. | Table1|
| Risk of bias within studies | 19 | Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12). | 12-13|
| Results of individual studies | 20 | For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot. | 13-16|
| Synthesis of results | 21 | Present results of each meta-analysis done, including confidence intervals and measures of consistency. | 13-16|
| Risk of bias across studies | 22 | Present results of any assessment of risk of bias across studies (see Item 15). | 12-13|
| Additional analysis | 23 | Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]). | 14-15|
| DISCUSSION          |    |                                                                                                                                 |      |
| Summary of evidence | 24 | Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers). | Table2|
| Limitations         | 25 | Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias). | 20-21|
| Conclusions | 26 | Provide a general interpretation of the results in the context of other evidence, and implications for future research. | 21 |
|---|---|---|---|
| **FUNDING** | | | |
| Funding | 27 | Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review. | 25 |

*From:* Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: [www.prisma-statement.org](http://www.prisma-statement.org).
Appendix 2: Protocol

Exon skipping for Duchenne Muscular Dystrophy (Protocol)

Yuko Shimizu-Motohashi, MD, Terumi Murakami, MD, Ph.D, En Kimura, MD, Ph.D, Hirofumi Komaki, MD, Ph.D, Norio Watanabe*, MD, Ph.D

1 Department of Child Neurology, National Center Hospital, National Center of Neurology and Psychiatry, Tokyo, Japan.
2 Department of Neurology, Higashisaitama National Hospital, 4147 Kurohama, Hasuda, Saitama 349-0196, Japan.
3 Translational Medical Center, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan.
4 Department of health Promotion and Human Behavior, Graduate School of Medicine/ School of Public Health, Kyoto University, Kyoto, Japan.

* Address correspondence to: Norio Watanabe, MD, Ph.D.
Department of health Promotion and Human Behavior, Graduate School of Medicine/ School of Public Health, Kyoto University, Kyoto, Japan
Tel.: +81-75-753-9491, Fax: +81-75-753-4641
noriowncu@gmail.com
ABSTRACT

This is the protocol for a review and there is no abstract. The objectives are as follows:

Objective

To assess whether exon skipping can positively change the clinical course of DMD patients.
BACKGROUND

Description of the condition

Duchenne muscular dystrophy (DMD) is caused by the mutation in the DMD located on Xp21 [1], which affects predominantly male individuals. It is the most common hereditary muscular disorder, estimated to affect one in 3500-6000 live male birth [2-4]. The protein product of the DMD, dystrophin, links the muscle sarcomeric structure to the extracellular matrix by constructing dystrophin–associated glycoprotein complex (DGC) [5] which confers to the strength of sarcolemma [6]. Individuals with DMD have absent or decreased level of dystrophin, manifesting walking difficulty at around age of five, and they become wheelchair bound before or during their teens [7, 8]. The distance of which a subject is able to walk in six minutes assessed by six-minute walk test (6MWT), is interpreted as the measure of physical capacity and walking function [9, 10]. In DMD, the longitudinal observational study had shown an increase in the distance of 6MWT until age of 7.5 years, followed by a decline, which became precipitous from 12.5 years to 15.5 years, when all boys were unable to perform the 6MWT [11]. Along with the motor function decline, patients also show respiratory and cardiac dysfunction owing to loss of respiratory muscle strength and cardiac tissue degeneration [12]. Preclinical cardiomyopathy becomes apparent in patients less than age 6 years [13], and respiratory function peaks out before age of 12 [14]. Without supportive care, patients typically die in their late teens or in early 20s [15]. Intervention with mechanical ventilation and cardioprotective medications have improved the survival, and patients may live to their 30s [16].

Currently, there is no curative therapy for DMD and glucocorticoids are the only medication available that slows the decline in muscle strength and function [7]. The clinical efficacy of glucocorticoids is established and is considered to be the standard therapy for patients with DMD, however, there are several concerns over the glucocorticoid therapy. One is that glucocorticoids have serious side effects in long-term usage, including weight gain, cushignoid, behavior change, growth delay, cataracts and osteoporosis [17]; second, the mechanism how glucocorticoids contribute to beneficial effects in patients with DMD is still unknown. Owing to the myriads of side effects, not all patients are eligible for receiving or continuing the glucocorticoid therapy.

Development of innovative therapies for patients with DMD is an urgent task, and several therapeutic approaches are now being investigated, including exon skipping, read through, vector-mediated gene therapy, cell transplantation, anti-inflammatory/fibrotic/oxidant drugs, myostatin pathway inhibitors, nNOS pathway enhancement, and utrophin up-regulation [18, 19]. Amongst all these approaches, exon skipping, read through and antioxidants are at the most advanced stage of clinical trials. Two major drugs evaluated for exon skipping are Drisapersen (BioMarin Pharmaceutical Inc.) and eteplirsen (Sarepta
Therapeutics), and these have been either completed or now being studied in phase 3 clinical trials (https://clinicaltrials.gov/). Ataluren, the compound for read through has also completed phase 3 study and now has an approval from European Medicines Agency (EMA) for marketing in Europe [20]. Idebenone, an antioxidant has also completed phase 3 trial [21]. Exon skipping and read through aim to treat the disease by restoring dystrophin, whereas idebenone aims to compensate for the lack of dystrophin. Each therapeutic approach bears pros and cons in their nature. Dystrophin is expressed not only in skeletal muscle but also in brain, and have been reported that significant proportion of DMD patients have cognitive and learning disabilities [22]. From the viewpoint that DMD is not only confined to the skeletal muscle but more of a systemic disorder, exon skipping or read through may have more benefit than idebenone.

The idea of exon skipping approach for DMD have emerged in 1990s [23], based on the rationale of converting the translational reading frame from out-of-frame to in-frame to produce shorter but functional dystrophin, instead of degradable dystrophin. Exon skipping can be applicable to versatile mutation types including deletion, duplication, and small mutations, theoretically applicable to up to 83% of total mutation [24], whereas read-through can treat only patients with nonsense mutation which comprises 10% of total mutation [25].

Although it is yet to be clarified whether the efficacy of exon skipping can exceed that of glucocorticoids in DMD patients, exon skipping is one of the most promising approach, with scientific rationale and accumulation of in vitro[26-28] and in vivo[29-31] study data. However, clinical trials for exon skipping have failed to demonstrate statistically significant improvement in motor function so far [32, 33]. Several factors can be thought for the reasons why it is so difficult to replicate pre-clinical research data in patients. One may be the low prevalence of DMD, and that it interferes with designing a large cohort study with sufficient power to detect the efficacy. Hence, a meta-analysis is necessary to assess the efficacy of exon skipping for patients with DMD.

To date, the results from multiple clinical trials have not been reviewed in a standardized manner. Herein, we propose to conduct a systematic review and meta-analysis on exon skipping studies in DMD to assess the potential and the limitation of exon skipping.

**Description of the intervention**

**Exon skipping**

Exon skipping induced by antisense oligonucleotides (AOs) modulates dystrophin pre-mRNA splicing process and restores the reading frame of \textit{DMD} [34]. The reading frame is converted from out-of-frame to in-frame, which generates the protein that is short but functional, instead of truncated and presumably
degradable dystrophin, and is considered to be capable of converting DMD to a milder phenotype [35]. There is a mutation hotspot in the DMD encoded by exons 43-45, and skipping of exon 51 can theoretically restore the largest subset of patients; therefore AOs targeting exon 51 were the first to be developed clinically [36]. There are several AO chemistries that have been investigated of which 2′O-methyl-phosphorothioate oligonucleotide (2′OMePS) and phosphorodiamidate morpholino oligomer (PMO) are the two major drug candidates that are currently being evaluated in advanced phase of clinical trials.

2′O-methyl-phosphorothioate oligonucleotide (2′OMePS)
The 2′OMePS chemistry has internucleotide phosphorothioate linkages, and are negatively charged. The chemistry targeting exon 51 have completed phase 3 clinical trial, and phase 1 or 2 studies targeting exon 44, 45, 53 are now being conducted (https://clinicaltrials.gov/).

Phosphorodiamidate morpholino oligomer (PMO)
The PMO chemistry is a charge-neutral compound, and have reduced off-target effects, along with less immunoreactivity [37]. Phase 3 clinical trial is now being conducted for exon 51 skipping, along with phase 1 or 2 study for exon 45 and 53 skipping (https://clinicaltrials.gov/).

Others
The chemistries that have been investigated other than 2′OMePS and PMOs are 2′-O,4′-C-Ethylene-bridged Nucleic Acids (ENA), locked nucleic acid (LNA), peptide nucleic acid (PNA), tricyclo0DNA (tcDNA), cell-penetrating peptide-conjugated phosphorodiamidate morpholino oligomers (PPMOS), arginine-rich cell-penetrating peptide-conjugated PMOs (Pip-PMOs), and vivo-morpholinos (vPMOs) [34, 37]. LNA-modified AOs display better mismatch discrimination and high resistance against nucleases; tcDNA show enhanced target-binding affinity and improved nuclease resistance [34]; PPMOS, Pip-PMOs, vPMOs contain cell-penetrating moieties [37]. Viral vectors and synthetic vectors (liposomes, cationic peptides and polymers, protein complexes) and covalent attachment such as antibodies, peptides, lipids, carbohydrates, growth factors and vitamins have been tried to overcome the poor target-specific delivery [34] and relatively unstable skipping efficiency of AOs, which are the current hurdles of exon skipping strategy.

Drugs which improve the underlying dystrophic condition
Glucocorticoids including;
  ● Prednisolone
Why it is important to do this review
Currently there is no curative therapy for DMD patients. Exon skipping is a therapeutic approach that have been investigated for decades which now have become the strategy at the most advanced phase of clinical trials. Review of the results from clinical studies is crucial for understanding the efficacy and the limitation of exon skipping.

OBJECTIVES
To assess whether exon skipping can positively change the clinical course of patients with DMD.

METHODS
The systematic review of the literature will comply with the PRISMA statement.

Criteria for considering studies for this review
Types of studies
We will include double-blind, randomized controlled trials (RCTs) that investigate the effect of exon skipping in patients with DMD. We will also include the first phase of cross-over controlled trials. Comparison will be done between: the effects of exon skipping and placebo, both groups without glucocorticoid therapy; the effects of exon skipping and placebo, both groups with continuous glucocorticoid therapy; the effects of exon skipping and placebo, both groups with intermittent glucocorticoid therapy.

Types of participants
All patients, including children and adults of all ages, gender, race, out and inpatients, who are confirmed to have out-of-frame DMD mutations that are identified by the authors as correctable by exon skipping are eligible for the review. The genetic analysis must be confirmed by any of the published or author approved methodologies that evaluate all DMD exons, including multiplex ligation-dependent probe, comparative genomic hybridization, single condition amplification/internal primer analysis, and target resequencing.

Studies with participants who are receiving steroid therapies are eligible for this meta-analysis, but the
participants must be on steroid before the first administration of exon skipping drug.

Types of interventions

Exon skipping chemistries that will be reviewed are:

- 2’OMePS
  - Drisapersen
  - PRO044
  - PRO045
  - PRO053
- PMO
  - Eteplirsen
  - SRP-4045
  - SRP-4053
  - NS-065/NCNP-01
- Any other exon skipping chemistry if identified.

Administration plan that will be reviewed are:

- Subcutaneous, intravenous injection, and any other administration route if identified.
- Single dose, short and long term administration, and any other administration period if identified.
- Dosage ranging from low dose to high dose, and any other dosage if identified. Our preliminary investigation of previous reports[32, 38-40] suggested as below:
  - For drisapersen subcutaneous injection, the dose range will be defined as low for <5mg/kg/time, moderate for 5-10mg/kg/time and high for >10mg/kg/time
  - For drisapersen intravenous injection, the dose range will be defined as low for <1mg/kg/time, moderate for 1-2mg/kg/time and high for >2mg/kg/time.
  - For eteplirsen, the dose range will be defined as low for <20mg/kg/time, moderate for 20-40mg/kg/time and high for >40mg/kg/time
- Intermittent or continuous administration, and any other administration schedule if any identified.
- Both out and inpatient administration.
- Placebo include mannitol, phosphate-buffered saline (PBS), or any other placebo if identified.
- Steroid therapies will be accepted if both treated and control groups are receiving steroid.
We plan to analyze data for each type of intervention separately.

**Type of outcome measures**

The outcomes listed here are not eligibility criteria for this review, but are outcomes of interest within whichever studies are included.

**Primary outcome**

We will assess the data of change in six-minute walk test (6MWT) distance from the baseline obtained between 20-35 weeks after the treatment initiation as the primary outcome, and will refer it as “data of week 24”. If there are multiple data collected within the above designated weeks, then the data obtained at the week closest to week 24 will be selected for the review.

Six-minute walk test (6MWT) has been used to assess functional capacity in patients with heart and lung related diseases, and it was the primary endpoint in a study that supported regulatory approval of drug for primary pulmonary hypertension [41]. More recently, its relevance to neuromuscular diseases including DMD has been established [42].

For the participants who became non-ambulatory during the study, the 6MWT data will be recorded as 0.

**Secondary outcomes**

- Change in 6MWT distance from the baseline evaluated outside the time-line defined for the primary outcome measurement.
  - data obtained between 2-7 weeks after the treatment initiation will be represented as “data of week 4”
  - data obtained between 8-19 weeks after the treatment initiation will be represented as “data of week 12”
  - data obtained between 36-52 weeks after the treatment initiation will be represented as “data of week 48”
  - If there were multiple data collected within the above designated weeks, then the data obtained at the week closest to the representative weeks (i.e., week 4, 12, 48 after the treatment initiation) will be selected for the review.
  - Any other weeks, if identified

- The percentage of dystrophin positive fibers.
- Pulmonary function
Forced Vital Capacity (FVC), forced Expiratory Volume (FEV1), Maximum inspiratory/expiratory pressure percent predicted (MIP/MEP % predicted)

- Muscle function
  - North Star Ambulatory Assessment (NSAA) [43], timed tests (10-meter walk/run, rising from floor, stair climb) [44], DMD Functional Outcomes Questionnaire (DMD-FOS), Egen Klassification [45], Performance Upper Limb (PUL) [46], Patient Reported Outcome measure (PROM)

- Muscle strength
  - Quantitative Muscle Testing (QMT), Manual Muscle Testing (MMT), handheld myometry

- Cardiac function
  - Electrocardiography (QT interval, rhythm)
  - Echocardiography (Ejection fraction, Fraction shortening)

- Production of exon skipped mRNA in muscle biopsy
- Serum creatine kinase level
- Number of patients who dropped out from the protocol
- Number of patients who deviated from the protocol
- Number of patients who discontinued the protocol
- Number of patients who stopped to be assessed
- Adverse events
  - Injection site reaction, inflammation, gastrointestinal symptoms, hemorrhage, renal/hepatic/cardiac toxicity, hematologic abnormality, pain, and any other adverse events if identified.

- Pharmacokinetics
  - T1/2, Cmax, Ctrough, 7d, tmax, volume of distribution, clearance

- Change in the quality of life (QoL) of the individuals who received exons skipping, as well as their caregivers.
- Change in the ability of daily life (ADL) of the individuals who received exon skipping.
- Change in the amount of care provided by the caregivers of the individuals who received exon skipping.
- Change in the mental health status of the individuals who received exon skipping as well as their caregivers.
- Survival
**Search methods for identification of studies**

**Electronic searches**
We will search the Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE via Ovid (from January 1990 to the present), EMBASE via embase.com (from January 1990 to the present), ICHUSHI-web (Japan Centra Revuo Medicina), Clinical trials.gov, FDA website, EMEA website, any other clinical trial registries if identified.
The details of search strategies are shown in Appendix (CENTRAL), Appendix (MEDLINE), Appendix (EMBASE), and Appendix (ICHUSHI-web).
We will not limit our search by language or publication status. We will search both peer reviewed and non-peer reviewed publications. Studies reporting results will be searched in all Primary Registries in the WHO Registry Network and in registries approved by the International Committee of Medical Journal Editors (ICMJE), that meet the requirements of the ICMJE (WHO, 2013). We will also search any other registries, if any identified.

**Searching other resources**
We will review conference proceedings for non-published studies. We will screen bibliographies of identified manuscripts for studies not identified by the search. For large clinical trials sponsored by pharmaceutical companies known to the group, but does not appear in any of the electronically searched sites, the information will be retrieved from the pharmaceutical companies’ websites or will be directly contacted. We will also contact the researchers who are conducting RCTs of exon skipping for information on other exon skipping RCTs.

**Data collection and analysis**
Two authors will independently review the titles and abstracts identified from the register and will determine for eligibility. Two authors will obtain the full text of all potentially relevant studies for independent assessment. Two authors will independently decide which trials fit the inclusion criteria. Authors will resolve disagreements about inclusion criteria by discussion, to reach a consensus. If persistent, the disagreement will be resolved by a third author. The authorships of the studies will not be blinded prior to the assessment.

**Selection of studies**
We will select only randomized controlled trials, and cross-over trials for inclusion. In the Discussion, we
will review open studies, longitudinal observational studies and individual case reports but will only
discuss studies in which the diagnosis, intervention, pre-treatment and post-treatment states are
adequately described and in whom follow up for at least six months is available.

**Data extraction and management**

Two review authors will independently extract data onto pre-agreed data extraction forms. We will
resolve disagreements by discussion with the other authors. One author will enter data into the Cochrane
statistical software RevMan (Review Manager (RevMan) [Computer program]. Version 5.3. Copenhagen:
The Nordic Cochrane Centre, The Cochrane Collaboration, 2014.) and a second author will check data
entry. We will contact trial authors directly for any missing data.

**Assessment of risk of bias in included studies**

Two authors will independently assess included studies for risk of bias using pre-agreed criteria,
described in the *Cochrane Handbook for Systematic Reviews of Interventions* (http://handbook.cochrane.org/).
We will resolve disagreements by discussion with the other authors. We
will grade each aspect for risk of bias as high risk (‘No’), uncertain (‘Unclear’) or low risk (‘Yes’) for the
following domains:

• sequence generation;
• allocation concealment;
• blinding of participants;
• blinding of outcome assessors;
• incomplete outcome data;
• selective outcome reporting; and
• other sources of bias.

**Measures of treatment effect**

Statistical methods used to measure treatment effects will be in accordance with The *Cochrane Handbook
for Systematic Reviews of Interventions* (http://handbook.cochrane.org/).

We will analyze dichotomous data as risk ratios (RR) and continuous data as mean difference, or
standardized mean difference for results across studies with outcomes that are conceptually the same but
measured in different ways. We will report these measures of effect with 95% confidence intervals (CI).
We will undertake meta-analyses, using RevMan, only where this is meaningful, that is if the treatments,
participants and the underlying clinical question are similar enough for pooling to make sense.
Unit of analysis issues
Where a single trial includes multiple trial arms, we will include only the relevant arms. If we combine two comparisons (e.g. drug A versus placebo and drug B versus placebo) in the same meta-analysis, we will halve the control group to avoid double-counting. For each participant there may be multiple observations for the same outcome.

Dealing with missing data
If necessary, we will attempt to contact trial authors for missing data including numbers of dropouts and deaths and whether or not an intention-to-treat analysis was performed.

Assessment of heterogeneity
We will assess clinical heterogeneity by judging, qualitatively, the differences between studies regarding the participants, therapies, and reporting of important study outcomes.
We will statistically test heterogeneity of intervention effects among trials using the standard Chi² statistic (P value) and the Higgins I² statistic expressed as a percentage. We will take P values of less than 0.05 as evidence of heterogeneity. We will interpret I² for heterogeneity as follows (http://handbook.cochrane.org):
• 0% to 40%: may not be important;
• 30% to 60%: may represent moderate heterogeneity;
• 50% to 90%: may represent substantial heterogeneity;
• 75% to 100%: considerable heterogeneity.
If we identify substantial unexplained heterogeneity we will report it and explore possible causes by pre-specified subgroup analysis.

Assessment of reporting biases
To detect the presence of publication bias, we will construct a funnel plot using Revman, if there are a reasonable number of studies (at least 10 in the same meta-analysis). We will use Begg’s and Egger’s tests to verify the bias [47, 48].

Data synthesis
If there is no substantial or considerable heterogeneity, we will synthesize the data in a meta-analysis using RevMan. We will perform random-effects models for comparison purposes and use the most
appropriate, depending upon the degree of heterogeneity.

**Subgroup analysis and investigation of heterogeneity**

If there are sufficient data, we plan to undertake the following subgroup analyses using the outcome:

1. steroid combination
2. non-steroid combination.
3. different types of exon skipping drugs
4. low dose administration
5. moderate dose administration
6. high dose administration

Within each group we will use the $I^2$ statistic for heterogeneity and if its value is greater than 50% we will scrutinize the trials and forest plots for differences to explain the heterogeneity. If we find no explanation, we will repeat the analysis using a random-effects model.

**‘Summary of findings’ table**

We will create a ‘Summary of findings’ table using the following outcomes:

- Change in 6MWT;
- Change in *dystrophin* gene or protein expression; and
- Adverse events.

We will use the five GRADE considerations (study limitations, consistency of effect, imprecision, indirectness and publication bias) to assess the quality of a body of evidence (studies that contribute data for the prespecified outcomes). We will use methods and recommendations described in Section 8.5 and Chapter 12 of the *Cochrane Handbook for Systematic Reviews of Interventions* [49] using GRADEpro software. We will justify all decisions to down- or upgrade the quality of studies using foot- notes and we will make comments to aid readers’ understanding of the review where necessary.

**Sensitivity analysis**

We will perform a sensitivity analysis to determine whether conclusions are robust by undertaking both fixed-effect and random effect meta-analysis. We will perform sensitivity analyses to assess the effect of including studies at high risk of bias on the change in 6MWT, and by repeating the meta-analysis excluding any studies at high risk of bias.
ACKNOWLEDGEMENTS
The work will be supported by The Clinical Research Program for Child Health and Development, AMED (No.27300101).

AMENDMENTS FROM THE PROTOCOL
Upon data extraction and management, the entered data into Revman software was double checked by a single author.

We planned to create a ‘Summary of findings’ table with outcomes including change in 6MWT, change in dystrophin gene or protein expression, and adverse events. However the primary outcomes for our study had changed according to physicians’, patients’ and their families’ opinions. Therefore the ‘Summary of findings’ was created with outcomes including change in 6MWT after 24 weeks of treatment, change in NSAA score after 24 weeks of treatment, and adverse events.

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Appendix 3: Search term for CENTRAL

#1 MeSH descriptor: [Muscular Dystrophy, Duchenne] this term only
#2 MeSH descriptor: [Muscular Dystrophies] this term only
#3 (duchenne near dystroph*):ti,ab in Trials
#4 (becker near dystroph*):ti,ab in Trials
#5 dystrophinopathy*:ti,ab in Trials
#6 MeSH descriptor: [Dystrophin] this term only
#7 (xldc or xldcm):ti,ab in Trials
#8 x linked dilated cardiomyopathy:ti,ab in Trials
#9 #1 or #2 or #3 or #4 or #5 or #6 or #7 or #8 in Trials
#10 MeSH descriptor: [Oligonucleotides, Antisense] explode all trees
#11 (((anti sense) or (antisense)) near oligo*):ti,ab in Trials
#12 MeSH descriptor: [Phosphorothioate Oligonucleotides] this term only
#13 phospho* oligonucleotide*:ti,ab in Trials
#14 exon* skip*:ti,ab in Trials
#15 2OME*:ti,ab in Trials
#16 "2O ME*":ti,ab in Trials
#17 "2 OME*":ti,ab in Trials
#18 drisapersen:ti,ab in Trials
#19 PRO044:ti,ab in Trials
#20 BMN044:ti,ab in Trials
#21 PRO045:ti,ab in Trials
#22 BMN045:ti,ab in Trials
#23 PRO051:ti,ab in Trials
#24 BMN051:ti,ab in Trials
#25 Kyndrisa:ti,ab in Trials
#26 PRO053:ti,ab in Trials
#27 BMN053:ti,ab in Trials
#28 phosphorodiamidate morpholino oligomer*:ti,ab in Trials
#29 PMO*:ti,ab in Trials
eteplirsen:ti,ab in Trials
SRP-4045:ti,ab in Trials
SRP-4053:ti,ab in Trials
AVI-4658:ti,ab in Trials
NS-065*:ti,ab in Trials
NCNP-01*:ti,ab in Trials
AON:ti,ab in Trials
AONs:ti,ab in Trials
AO:ti,ab in Trials
Aos:ti,ab in Trials
Ethylene bridged Nucleic Acid*:ti,ab in Trials
ENA*:ti,ab in Trials
DS 5141b:ti,ab in Trials
locked nucleic acid*:ti,ab in Trials
LNA*:ti,ab in Trials
MeSH descriptor: [Peptide Nucleic Acids] this term only
peptide nucleic acid*:ti,ab in Trials
PNA*:ti,ab in Trials
tricyclo DNA*:ti,ab
tcDNA*:ti,ab
MeSH descriptor: [Cell-Penetrating Peptides] this term only
cell penetrating peptide*:ti,ab in Trials
vivo morpholino*:ti,ab in Trials
#10 or #11 or #12 or #13 or #14 or #15 or #16 or #17 or #18 or #19 or #20 or #21 or #22 or
#23 or #24 or #25 or #26 or #27 or #28 or #29 or #30 or #31 or #32 or #33 or #34 or #35 or #36 or #37 or
#38 or #39 or #40 or #41 or #42 or #43 or #44 or #45 or #46 or #47 or #48 or #49 or #50 or #51 or #52 in
Trials
#9 and #53
Appendix 4: Search term for MEDLINE

1 Muscular Dystrophies/ or Muscular Dystrophy, Duchenne/
2 (duchenne adj5 dystroph$).tw.
3 (becker adj5 dystroph$).tw.
4 dystrophinopath$.mp.
5 Dystrophin/ge
6 (xldc or xldcm).tw.
7 x linked dilated cardiomyopathy.tw.
8 or/1-7
9 exp Oligonucleotides, Antisense/
10 ((anti sense or antisense) adj oligo$).tw.
11 Phosphorothioate Oligonucleotides/
12 phosphol$ oligonucleotide$.tw.
13 (exon* and skip*).tw.
14 2OME*.tw.
15 '2' O ME*.tw.
16 '2O ME*.tw.
17 '2' OME*.tw.
18 drisapersen.tw.
19 PRO044.tw.
20 BMN044.tw.
21 PRO045.tw.
22 BMN045.tw.
23 PRO051.tw.
24 BMN051.tw.
25 Kyndrisa.tw.
26 PRO053.tw.
27 BMN053.tw.
28 phosphorodiamidate morpholino oligomer$.tw.
29 PMOS.tw.
30 eteplirsen.tw.
31 SRP-4045.tw.
exp Animals/ not Humans.sh.
66  64 not 65
67  remove duplicates from 66
Appendix 5: Search term for EMBASE

#1  duchenne muscular dystrophy'/de
#2  (duchenne NEAR/5 dystroph*):ab,ti
#3  'muscular dystrophy'/de
#4  'becker muscular dystrophy'/de
#5  (becker NEAR/5 dystroph*):ab,ti
#6  'dystrophinopathy'/de
#7  dystrophinopath*:ab,ti
#8  'dystrophin'/de
#9  xldc:ab,ti OR xldcm:ab,ti OR 'x linked dilated cardiomyopathy':ab,ti
#10  #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9
#11  'antisense oligonucleotide'/exp
#12  (antisense NEAR/1 oligo*):ab,ti OR ('anti sense' NEAR/1 oligo*):ab,ti
#13  'oligonucleotide phosphorothioate'/de
#14  (phospho* NEXT/1 oligonucleotide*):ab,ti
#15  'exon skipping'/de OR (exon* NEAR/5 skip*):ab,ti
#16  2ome*:ab,ti
#17  '2 ome*':ab,ti
#18  '2 o me*':ab,ti
#19  '2o me*':ab,ti
#20  'drisapersen'/de
#21  drisapersen:ab,ti
#22  pro044:ab,ti
#23  bmn044:ab,ti
#24  pro045:ab,ti
#25  bmn045:ab,ti
#26  pro051:ab,ti
#27  bmn051:ab,ti
#28  kyndrisa:ab,ti
#29  pro053:ab,ti
#30  bmn053:ab,ti
#31  'phosphorodiamidate morpholino oligomer*':ab,ti
pmo*:ab,ti
'eteplirsen'/de
eteplirsen:ab,ti
'srp 4045*:ab,ti
'srp 4053*:ab,ti
'avi 4658*:ab,ti
'ns 065*:ab,ti
'ncnp-01*:ab,ti
aon:ab,ti
aons:ab,ti
ao:ab,ti
aos:ab,ti
'ethylene bridged nucleic acid*:ab,ti
enas:ab,ti
'ds 5141b*:ab,ti
'locked nucleic acid'/de
'locked nucleic acid*:ab,ti
lna*:ab,ti
'peptide nucleic acid'/de
'peptide nucleic acid*:ab,ti
pna*:ab,ti
'tricyclo dna*:ab,ti
tcdna*:ab,ti
'tc dna*:ab,ti
'cell penetrating peptide'/de
'cell penetrating peptide*:ab,ti
'vivo morpholino*:ab,ti

#11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19 OR #20 OR #21 OR #22 OR #23 OR #24 OR #25 OR #26 OR #27 OR #28 OR #29 OR #30 OR #31 OR #32 OR #33 OR #34 OR #35 OR #36 OR #37 OR #38 OR #40 OR #41 OR #42 OR #43 OR #44 OR #45 OR #46 OR #47 OR #48 OR #49 OR #50 OR #51 OR #52 OR #53 OR #54 OR #55 OR #56 OR #57 OR #58

#10 AND #59

'clinical trial'/de OR 'randomized controlled trial'/de OR 'randomization'/exp
AND random*:ab OR rct*:ab OR trial*:ab OR groups:ab OR placebo*:ab OR 'drug therapy'/exp

#62   #60 AND #61
#63   #62 NOT ([animals]/lim NOT [humans]/lim)
#64   #1 OR #2 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9
#65   #59 AND #64
#66   #61 AND #65
#67   #66 NOT ([animals]/lim NOT [humans]/lim)
#68   #63 AND [embase]/lim NOT [medline]/lim
#69   #67 AND [embase]/lim NOT [medline]/lim
#70   #68 NOT #69
Appendix 6: Search term for ICHUSHI-web

#1 筋ジストロフィー-Duchenne 型/TH
#2 @筋ジストロフィー/TH
#3 筋ジス/AL and (duchenne/AL or デュシェンヌ/AL or デュシャン/AL)
#4 筋ジス/AL and (becker/AL or ベッカー/AL)
#5 (Dystrophin/TH or ジストロフィン/AL)
#6 ジストロフィノパチー/AL
#7 (X 遺伝/AL or 遺伝/AL) and (心筋症-拡張型/TH or 拡張型心筋症/AL)
#8 #1 or #2 or #3 or #4 or #5 or #6 or #7
#9 "Antisense Oligonucleotides"/TH
#10 (オリゴ/AL and スクレオチド/AL) or oligonucleotide/AL or oligodeoxynucleotide/AL or oligodeoxyribonucleotide/AL
#11 (アンチセンス/AL or antisense/AL) and (オリゴ/AL or oligo/AL)
#12 "Phosphorothioate Oligonucleotides"/TH
#13 (ホスホロ/AL or phospho/AL) and ((オリゴ/AL and スクレオチド/AL) or oligonucleotide/AL or oligodeoxynucleotide/AL or oligodeoxyribonucleotide/AL)
#14 エクソン/TH
#15 エクソン/AL or エキソン/AL or exon/AL
#16 2'OMe/AL or 2'-OMe/AL or 2'O メチル/AL or 2'O-メチル/AL or 2'-O-メチル/AL or 2'-O-メチル/AL
#17 ドリサペルセン/AL or ドリサパーソン/AL or drisapersen/AL
#18 PRO044/AL
#19 BMN044/AL
#20 PRO045/AL
#21 BMN045/AL
#22 PRO051/AL
#23 BMN051/AL
#24 Kyndrisa/AL
#25 PRO053/AL
#26 BMN053/AL
#27 "Phosphorodiamidate Morpholino Oligomer"/AL
#28 PMO/AL
#29 モルフォリノ/AL or モルフォリーノ/AL
#30 エテプリルセン/AL or eteplirsen/AL
#31 SRP-4045/AL
#32 SRP-4053/AL
#33 AVI-4658/AL
#34 NS-065/AL
#35 NCNP-01/AL
#36 AON/AL
#37 AONs/AL
#38 AO/AL
#39 AOs/AL
#40 エチレン架橋核酸/AL or "ethylene bridged nucleic acid"/AL
#41 ENA/AL
#42 DS-5141b/AL
#43 "Locked Nucleic Acid"/TH
#44 (架橋/AL and 核酸/AL) or "locked nucleic acid"/AL
#45 LNA/AL
#46 "Peptide Nucleic Acids"/TH
#47 ペプチド核酸/AL or "peptide nucleic acid"/AL
#48 PNA/AL
#49 tricyclo-DNA/AL or "tricyclo DNA"/AL
#50 tcDNA/AL
#51 "Cell-Penetrating Peptides"/TH
#52 膜透過ペプチド/AL or 細胞透過ペプチド/Al or "cell penetrating peptide"/AL or "cell-penetrating peptide"/AL
#53 #9 or #10 or #11 or #12 or #13 or #14 or #15 or #16 or #17 or #18 or #19 or #20 or #21 or #22 or #23 or #24 or #25 or #26 or #27 or #28 or #29 or #30 or #31 or #32 or #33 or #34 or #35 or #36 or #37 or #38 or #39 or #40 or #41 or #42 or #43 or #44 or #45 or #46 or #47 or #48 or #49 or #50 or #51 or #52
#54 #8 and #53
#55 (#54) and (RD=ランダム化比較試験)
Appendix 7: Search terms for Primary Registries in the WHO Registry Network and in registries approved by the ICMJE

1. DMD AND exon
2. Duchenne AND exon
3. Becker AND exon
4. GSK2402968 AND DMD
5. GSK2402968 AND BMD
6. AVI-4658 AND DMD
7. AVI-4658 AND BMD
8. drisapersen AND DMD
9. drisapersen AND BMD
10. eteplirsen AND DMD
11. eteplirsen AND BMD
12. SRP-4045
13. SRP-4053
14. NS-065/NCNP01
15. PRO044
16. PRO045
17. PRO053
## Appendix 8: Adverse events (Cardiac toxicity)

### 1.5.1 etoplisken

| Study or Subgroup | Exon skipping | Placebo | Risk Ratio |
|-------------------|---------------|---------|------------|
| Mendel2012        | 1             | 3       | 1.67 [0.08, 32.75] |
| Subtotal (95% CI) | 8             | 4       | 1.67 [0.08, 33.75] |

Total events: 1

Heterogeneity: Not applicable

Test for overall effect: $Z = 0.33 (p = 0.74)$

### 1.5.2 drisapersen

| Study or Subgroup | Exon skipping | Placebo | Risk Ratio |
|-------------------|---------------|---------|------------|
| Hanigan2014       | 0             | 15      | Not estimable |
| NCT01254019       | 1             | 15      | 1.48 (0.06, 35.72) |
| NCT01462292       | 0             | 10      | Not estimable |
| Von2014           | 1             | 15      | 1.58 [0.07, 37.02] |
| Subtotal (95% CI) | 210           | 100     | 1.53 [0.16, 14.38] |

Total events: 2

Heterogeneity: $\tau^2 = 0.00$, $\chi^2 = 0.00$, df = 1 (p = 0.99); $I^2 = 0$

Test for overall effect: $Z = 0.37 (p = 0.71)$

Total (95% CI): 218 [0.26, 35.11]

### Risk Ratio

- M-H, Random, 95% CI

0.01 1 10 100

Favours exon skipping Favours placebo

Heterogeneity: $\tau^2 = 0.00$, $\chi^2 = 0.00$, df = 2 (p = 1.00); $I^2 = 0$

Test for overall effect: $Z = 0.50 (p = 0.62)$

Test for subgroup differences: $\chi^2 = 0.00$, df = 1 (p = 0.96); $I^2 = 0$
### Appendix 9: Adverse events (Gastrointestinal symptoms)

#### 1.9.1 eteplirsen

| Study or Subgroup | Exon skipping | Placebo | Risk Ratio |
|-------------------|--------------|---------|------------|
| Mendell2012       | 2            | 8       | 0.75 [0.20, 2.82] |
| Total (95% CI)    | 8            | 4       | 0.75 [0.20, 2.83] |
| Total events      | 3            | 2       |             |

Heterogeneity: Not applicable
Test for overall effect: Z = 0.42 (P = 0.67)

#### 1.9.2 drisapersen

| Study or Subgroup | Exon skipping | Placebo | Risk Ratio |
|-------------------|--------------|---------|------------|
| Hanigan2014       | 0            | 15      | Not estimable |
| NCT0254019        | 18           | 13      | 1.05 [0.59, 1.88] |
| NCT01462292       | 4            | 16      | 0.46 [0.13, 1.60] |
| Von2014           | 1            | 18      | 1.58 [0.07, 37.02] |
| Subtotal (95% CI) | 210          | 100     | 0.92 [0.55, 1.55] |

Total events: 33

Heterogeneity: Tau² = 0.00; Chi² = 1.51, df = 2 (P = 0.47); I² = 0%
Test for overall effect: Z = 0.31 (P = 0.76)

Total (95% CI): 218

Total events: 33

Heterogeneity: Tau² = 0.00; Chi² = 1.59, df = 2 (P = 0.66); I² = 0%
Test for overall effect: Z = 0.45 (P = 0.66)

Test for subgroup differences: Chi² = 0.08, df = 1 (P = 0.78); I² = 0%
Appendix 10: Adverse events (Hematologic abnormality)

### 1.13.1 ereplisen

| Study or Subgroup | Exon skipping Events | Placebo Events | Total | Weight | Risk Ratio M-H, Random, 95% CI | Risk Ratio M-H, Random, 95% CI |
|-------------------|----------------------|----------------|-------|--------|-------------------------------|-------------------------------|
| Mendil2012        | 0                    | 8              | 0     | 4      | Not estimable                 | Not estimable                 |
| Subtotal (95% CI) |                      | 8              | 4     |        |                               |                               |
| Total events      | 0                    | 0              | 0     |        |                               |                               |

Heterogeneity: Not applicable

Test for overall effect: Not applicable

### 1.13.2 drisapersen

| Study or Subgroup | Exon skipping Events | Placebo Events | Total | Weight | Risk Ratio M-H, Random, 95% CI | Risk Ratio M-H, Random, 95% CI |
|-------------------|----------------------|----------------|-------|--------|-------------------------------|-------------------------------|
| Hanigan2014       | 0                    | 15             | 0     | 5      | Not estimable                 |                               |
| NCT01254019       | 1                    | 125            | 0     | 61     | 22.1% 1.48 (0.66, 3.572)       |                               |
| NCT01462292       | 0                    | 35             | 0     | 16     | Not estimable                 |                               |
| Von2014           | 2                    | 35             | 3     | 18     | 77.9% 0.34 [0.06, 1.87]        |                               |
| Subtotal (95% CI) | 210                  | 104            | 100   | 100.0% | 0.47 [0.11, 2.12]             |                               |

Total events: 3

Heterogeneity: $\tau^2 = 0.00$, $\chi^2 = 0.64$, df = 1 ($\rho = 0.43$), $I^2 = 0$

Test for overall effect: $Z = 0.98$ ($\rho = 0.33$)

### Total (95% CI)

| Study or Subgroup | Exon skipping Events | Placebo Events | Total | Weight | Risk Ratio M-H, Random, 95% CI | Risk Ratio M-H, Random, 95% CI |
|-------------------|----------------------|----------------|-------|--------|-------------------------------|-------------------------------|
|                    | 218                  | 104            | 100   | 100.0% | 0.47 [0.11, 2.12]             |                               |

Total events: 3

Heterogeneity: $\tau^2 = 0.00$, $\chi^2 = 0.64$, df = 1 ($\rho = 0.43$), $I^2 = 0$

Test for overall effect: $Z = 0.98$ ($\rho = 0.33$)

Test for subgroup differences: Not applicable
## Appendix 11: Adverse events (Hemorrhage)

| Study or Subgroup | Exon skipping | Placebo | Risk Ratio |
|-------------------|---------------|---------|------------|
| Mendel2012        | 2             | 8       | 1.00 [0.12, 8.00] |
| Subtotal (95% CI) | 8             | 4       | 1.00 [0.13, 8.00] |
| Total events      | 2             | 1       |            |

Test for overall effect: $Z = 0.00$ ($P = 1.00$)

### 1.17.2 drisapersen

| Study or Subgroup | Exon skipping | Placebo | Risk Ratio |
|-------------------|---------------|---------|------------|
| Hanigan2014       | 0             | 15      | Not estimable |
| NCT01254019       | 0             | 135     | Not estimable |
| NCT01462292       | 0             | 35      | Not estimable |
| Von2014           | 0             | 15      | Not estimable |
| Subtotal (95% CI) | 210           | 100     | Not estimable |
| Total events      | 0             | 0       |            |

Test for overall effect: Not applicable

Heterogeneity: Not applicable

### Total (95% CI)

| Total (95% CI) | Exon skipping | Placebo | Risk Ratio |
|----------------|---------------|---------|------------|
| 218            | 104           | 100.0%  | 1.00 [0.13, 8.00] |

Test for overall effect: $Z = 0.00$ ($P = 1.00$)

Test for subgroup differences: Not applicable
## Appendix 12: Adverse events (Hepatic toxicity)

| Study or Subgroup | Exon skipping | Placebo | Risk Ratio | Risk Ratio |
|-------------------|---------------|---------|------------|------------|
|                    | Events        | Total   | Weight     | M-H, Random, 95% CI | M-H, Random, 95% CI |
| Mendell2012        | 0             | 8       | 4          | Not estimable | Not estimable |
| **Subtotal (95% CI)** | **8**   | **4**   |            |              |              |
| Total events       | 0             | 0       |            |              |              |
| Heterogeneity: Not applicable |
| Test for overall effect: Not applicable |

### 1.21.1 cerlipine

| Study or Subgroup | Exon skipping | Placebo | Risk Ratio | Risk Ratio |
|-------------------|---------------|---------|------------|------------|
|                    | Events        | Total   | Weight     | M-H, Random, 95% CI | M-H, Random, 95% CI |
| Hanigan2014        | 0             | 15      | 5          | Not estimable | Not estimable |
| NCT01254019        | 1             | 125     | 42.0%      | 1.48 [0.06, 35.72] |              |
| NCT01462292        | 0             | 15      | 0          | Not estimable | Not estimable |
| Von2014            | 1             | 15      | 18         | 0.51 [0.03, 7.75] |              |
| **Subtotal (95% CI)** | **210** | **100** | **100.0%** | **0.80 [0.10, 6.32]** |              |
| Total events       | 2             | 1       |            |              |              |
| Heterogeneity: Tau² = 0.00; Chi² = 0.25; df = 1 (P = 0.62); I² = 0% |
| Test for overall effect: Z = 0.21 (P = 0.83) |

### 1.21.2 drisapersen

| Study or Subgroup | Exon skipping | Placebo | Risk Ratio | Risk Ratio |
|-------------------|---------------|---------|------------|------------|
|                    | Events        | Total   | Weight     | M-H, Random, 95% CI | M-H, Random, 95% CI |
| **Subtotal (95% CI)** | **218** | **104** | **100.0%** | **0.80 [0.10, 6.32]** |              |
| Total events       | 2             | 1       |            |              |              |
| Heterogeneity: Tau² = 0.00; Chi² = 0.25; df = 1 (P = 0.62); I² = 0% |
| Test for overall effect: Z = 0.21 (P = 0.83) |
| Test for subgroup differences: Not applicable |
Appendix 13: Adverse events (Inflammation)

| Study or Subgroup | Exon skipping | Placebo | Risk Ratio | Risk Ratio |
|-------------------|---------------|---------|------------|------------|
|                   | Events Total  | Events Total | M-H, Random, 95% CI | M-H, Random, 95% CI |
| Mendell2012       | 1 8 2 4 6.2%  | 0.25 [0.03, 2.00] |
| Subtotal (95% CI) | 8 4 6.2%      | 0.25 [0.03, 2.00] |
| Total events      | 1 2           |          |            |            |
| Heterogeneity: Not applicable |
| Test for overall effect: Z = 1.31 (p = 0.19) |

1.25.2 drisapersen

| Study or Subgroup | Exon skipping | Placebo | Risk Ratio | Risk Ratio |
|-------------------|---------------|---------|------------|------------|
|                   | Events Total  | Events Total | M-H, Random, 95% CI | M-H, Random, 95% CI |
| Hariglan2014      | 4 15 0 5 3.6%  | 3.28 [0.24, 23.70] |
| NCT01254019       | 9 15 25 61 52.4% | 0.74 [0.55, 1.11] |
| NCT01462292       | 9 15 2 16 12.2%  | 2.06 [0.50, 8.45] |
| Von2014           | 13 15 5 18 25.7% | 1.34 [0.57, 3.16] |
| Subtotal (95% CI) | 210 100 93.8% | 1.04 [0.61, 1.76] |
| Total events      | 64 32         |          |            |            |
| Heterogeneity: Tau² = 0.09; Chi² = 4.06; df = 2 (p = 0.26); I² = 26% |
| Test for overall effect: Z = 0.13 (p = 0.90) |

Total (95% CI) 218 104 100.0% 0.97 [0.56, 1.66]

| Study or Subgroup | Exon skipping | Placebo | Risk Ratio | Risk Ratio |
|-------------------|---------------|---------|------------|------------|
|                   | Events Total  | Events Total | M-H, Random, 95% CI | M-H, Random, 95% CI |
| Total events      | 65 34         |          |            |            |
| Heterogeneity: Tau² = 0.10; Chi² = 5.42; df = 4 (p = 0.25); I² = 26% |
| Test for overall effect: Z = 0.13 (p = 0.90) |
| Test for subgroup differences: Chi² = 1.69; df = 1 (p = 0.19); I² = 40.7% |
Appendix 14: Adverse events (Pain)

### 1.3.3.1 epepsen

| Study or Subgroup | Exon skipping | Placebo | Risk Ratio |
|-------------------|---------------|---------|------------|
|                   | Events | Total | Events | Total | Weight | M-H, Random, 95% CI |
| Mendel2012        | 4      | 8     | 2      | 4     | 25.0%  | 0.67 [0.27, 1.62]    |
| Subtotal (95% CI) | 8      | 8     | 4      | 4     |        | 0.67 [0.27, 1.63]    |
| Total events      | 4      |       | 3      |       |        |                      |

Heterogeneity: Not applicable
Test for overall effect: Z = 0.89 (p = 0.37)

### 1.3.3.2 drispersen

| Study or Subgroup | Exon skipping | Placebo | Risk Ratio |
|-------------------|---------------|---------|------------|
|                   | Events | Total | Events | Total | Weight | M-H, Random, 95% CI |
| Hanigan2014       | 0      | 15    | 0      | 5     |        | Not estimable        |
| NCT01254019       | 32     | 125   | 12     | 61    | 58.4%  | 1.34 [0.75, 2.41]    |
| NCT01462292       | 5      | 15    | 4      | 16    | 14.5%  | 0.57 [0.18, 1.85]    |
| Von2014           | 1      | 18    | 2      | 15    | 2.0%   | 1.58 [0.07, 37.02]   |
| Subtotal (95% CI) | 210    | 100   | 75.0%  |       |        | 1.14 [0.68, 1.92]    |
| Total events      | 39     |       | 16     |       |        |                      |

Heterogeneity: Tau^2 = 0.00; Chi^2 = 1.67, df = 2 (p = 0.42); I^2 = 0%
Test for overall effect: Z = 0.50 (p = 0.61)
Test for subgroup differences: Chi^2 = 1.04, df = 1 (p = 0.31); I^2 = 4.2%
Appendix 15: Adverse events (Others)

| Study or Subgroup | Exon skipping | Placebo | Risk Ratio |
|-------------------|---------------|---------|------------|
|                    | Events Total | Events Total | M-H, Random, 95% CI |
| Mendell2012        | 4 8           | 2 4      | 1.00 [0.20, 2.22] |
| Subtotal (95% CI)  |               |         | 1.00 [0.30, 3.32] |
| Total events       | 4 2           |         |             |
| Heterogeneity: Not applicable |
| Test for overall effect: Z = 0.00 (P = 1.00) |

1.41.2 drisapersen

| Study or Subgroup | Exon skipping | Placebo | Risk Ratio |
|-------------------|---------------|---------|------------|
|                    | Events Total | Events Total | M-H, Random, 95% CI |
| Hanigan2014       | 0 15          | 0 5      | Not estimable |
| NCT01254019       | 27 125        | 12 61    | 1.10 [0.60, 2.02] |
| NCT01462229       | 6 15          | 3 16     | 0.91 [0.26, 1.20] |
| Von2014           | 1 15          | 1 18     | 0.51 [0.03, 7.75] |
| Subtotal (95% CI) | 210 100       | 83.4%    | 1.03 [0.60, 1.76] |
| Total events      | 34 16         |         |             |
| Heterogeneity: Tau² = 0.00; Chi² = 0.33; df = 2 (P = 0.85); I² = 0% |
| Test for overall effect: Z = 0.11 (P = 0.91) |

Total (95% CI) 218 104 100.0% 1.03 [0.63, 1.67]

Heterogeneity: Tau² = 0.00; Chi² = 0.33; df = 2 (P = 0.95); I² = 0% |
Test for overall effect: Z = 0.10 (P = 0.92) |
Test for subgroup differences: Chi² = 0.00; df = 1 (P = 0.96); I² = 0%
Appendix 16: 6MWT at week 48 (change from baseline, meters)

| Study or Subgroup | Exon skipping | Placebo | Mean Difference |
|-------------------|---------------|---------|-----------------|
|                   | Mean  | SD   | Total | Mean  | SD   | Total | IV, Random, 95% CI | IV, Random, 95% CI |
| L412 Drisapersen   | 42.32 | 70.8053 | 117 | 52.65 | 80.0696 | 59 | 59.3% | -10.33 [-25.16, 14.70] |
| Vel2014           | -7.2  | 53.104 | 33  | 24.7  | 52.8   | 17  | 40.7% | -31.90 [-65.86, -0.94] |
| Subtotal (95% CI) | 150 | 76  | 100.0% | -19.11 [-39.88, 1.66] |
| Heterogeneity: Tau² = 26.37; Chi² = 1.13; df = 1 (P = 0.29); I² = 11% |
| Test for overall effect: Z = 1.80 (P = 0.07) |
| Total (95% CI)    | 150 | 76  | 100.0% | -19.11 [-39.88, 1.66] |
| Heterogeneity: Tau² = 26.37; Chi² = 1.13; df = 1 (P = 0.29); I² = 11% |
| Test for overall effect: Z = 1.80 (P = 0.07) |
| Test for subgroup differences: Not applicable |

For mean difference, gain of distance is shown in negative numbers and loss of distance is shown in positive numbers.
Appendix 17: Timed test at week 24, time taken for 4 stairs climb, (change from baseline, seconds)

| Study or Subgroup | Exon skipping Mean | SD Total | Placebo Mean | SD Total | Mean Difference IV, Random, 95% CI | Mean Difference IV, Random, 95% CI |
|-------------------|--------------------|---------|--------------|---------|-----------------------------------|-----------------------------------|
| Etepilinsen       |                    |         |              |         |                                   |                                   |
| Mendes2013        | 4.05               | 3.784   | -1.22        | 1.597   | 4.1% -6.07 [-0.89, 13.01]          | 1.3% 6.07 [-0.89, 13.01]          |
| Subtotal (95% CI) |                    |         |              |         |                                   |                                   |
|                   |                    | 8       | 4            | 4       |                                   |                                   |
| Heterogeneity     | Not applicable     |         |              |         |                                   |                                   |
| Test for overall effect | 2 = 1.71 (P = 0.09) |         |              |         |                                   |                                   |
| DiAspero6n       |                    |         |              |         |                                   |                                   |
| NCT01462292       | 0.1734             | 1.2345  | 0.59         | 1.2322  | 16.48 -0.02 [-1.15, 0.31]          | 16.50 -0.10 [-0.80, 0.60]         |
| Voit2014          | -0.0987            | 1.2345  | 0            | 1.2322  | 18.50 -0.10 [-0.80, 0.60]         | 18.50 -0.10 [-0.80, 0.60]         |
| Subtotal (95% CI) |                    | 70      | 34           | 34      |                                   |                                   |
| Heterogeneity Tau² | 0.06               |         |              |         |                                   |                                   |
| Test for overall effect | 2 = 0.97 (P = 0.35) |         |              |         |                                   |                                   |
| Total (95% CI)    |                    | 78      | 38           | 100.0%  | -0.17 [-0.97, 0.62]               |                                   |
| Heterogeneity Tau² | 0.26               |         |              |         |                                   |                                   |
| Test for overall effect | 2 = 0.43 (P = 0.07) |         |              |         |                                   |                                   |

Standard deviations (SDs) were not provided for Voit 2014, therefore were substituted by SDs reported in NCT01462292.
Appendix 18: Timed test at week 24, time taken for 4 stairs descent, (change from baseline, seconds)

| Study or Subgroup | Exon skipping | Placebo | Mean Difference | Mean Difference |
|-------------------|---------------|---------|-----------------|-----------------|
|                   | Mean          | SD      | Total           | IV, Random, 95% CI | IV, Random, 95% CI |
| 1,492 Drisapersen | 0.054         | 1.8454  | 35              | 0.6 1.892 16      | 48.0% -0.55 [-1.66, 0.56] |
| Voi2014           | -0.259        | 1.8454  | 35              | 0    1.892 18     | 52.0% -0.26 [-1.33, 0.81] |
| Subtotal (95% CI) | 70            |         | 34              | 100.0% -0.40 [-1.17, 0.37] |
|                   |               |         |                 | Heterogeneity: Tau² = 0.00, Chi² = 0.13, df = 1 (p = 0.71); I² = 0% |
|                   |               |         |                 | Test for overall effect: Z = 1.01 (p = 0.31) |

| Total (95% CI)    | 70            | 34      | 100.0% -0.40 [-1.17, 0.37] |
|                   |               |         | Heterogeneity: Tau² = 0.00, Chi² = 0.13, df = 1 (p = 0.71); I² = 0% |
|                   |               |         | Test for overall effect: Z = 1.01 (p = 0.31) |
|                   |               |         | Test for subgroup differences: Not applicable |

Standard deviations (SDs) were not provided for Voit 2014, therefore were substituted by SDs reported in NCT01462292.
Appendix 19: Timed test at week 24, time taken for 10 minutes walk/run, (change from baseline, seconds)

| Study or Subgroup | Exon skipping | Placebo | Mean Difference | Mean Difference |
|-------------------|---------------|---------|----------------|----------------|
|                   | Mean SD Total | Mean SD Total | IV, Random, 95% CI | IV, Random, 95% CI |
| Mendel2013        | 2.81 7.357 8 | -0.65 0.985 4 | 1.8% 2.46 [-1.73, 8.65] | |
| Subtotal (95% CI) | 8             | 4        | 1.8% 3.46 [-1.73, 8.65] | |
| Heterogeneity: Not applicable | Test for overall effect: Z = 1.31 (P = 0.19) |

1.53.2 Drisapersen

| Study or Subgroup | Exon skipping | Placebo | Mean Difference | Mean Difference |
|-------------------|---------------|---------|----------------|----------------|
|                   | Mean SD Total | Mean SD Total | IV, Random, 95% CI | IV, Random, 95% CI |
| NCT01462292       | 0.2474 0.7782 35 | -0.94 0.784 16 | 48.7% 0.29 [-0.18, 0.75] | |
| Voi2014           | -0.384 0.7782 35 | 0 0.784 18 | 45.5% -0.38 [-0.81, 0.16] | |
| Subtotal (95% CI) | 70             | 34        | 98.2% -0.05 [-0.71, 0.61] | |
| Heterogeneity: Tau^2 = 0.17; Chi^2 = 4.21, df = 1 (P = 0.04); I^2 = 76% | Test for overall effect: Z = 0.25 (P = 0.80) |
| Total (95% CI)    | 78             | 38        | 100.0% 0.01 [-0.69, 0.72] | |
| Heterogeneity: Tau^2 = 0.21; Chi^2 = 5.97, df = 2 (P = 0.05); I^2 = 56% | Test for overall effect: Z = 0.03 (P = 0.97) |
| Test for subgroup differences: Chi^2 = 1.73, df = 1 (P = 0.19); I^2 = 42.2% |

Standard deviations (SDs) were not provided for Voi 2014, therefore were substituted by SDs reported in NCT01462292.
Appendix 20: Timed test at week 24, time taken to rise from floor, (change from baseline, seconds)

| Study or Subgroup | Exon skipping | Placebo | Mean | SD | Total | Mean | SD | Total | Weight IV, Random, 95% CI | Mean Difference IV, Random, 95% CI |
|-------------------|---------------|---------|------|----|-------|------|----|-------|---------------------------|----------------------------------|
| 1.57.1 Ergilvursion |               |         | 5.125 | 10.262 | 8 | -0.7 | 1.14 | 4 | 3.2% | 5.83 [-1.37, 13.02] |                                   |
| Subtotal (95% CI) |               |         |       |     | 8 |       | 4 |   | 3.2% | 5.83 [-1.37, 13.02] |                                   |
| Heterogeneity: Not applicable |       |         |       |     |    |       |       |      | Test for overall effect: $Z = 1.59 (P = 0.11)$ |                                   |
| 1.57.2 Dripsersen |               |         | 1.7314 | 2.7573 | 35 | 1.12 | 2.8 | 16 | 46% | 0.61 [-1.04, 2.26] |                                   |
| Vool2014 |               |         | 0.109 | 2.7573 | 35 | 0 | 2.8 | 18 | 49% | 0.11 [-1.47, 1.69] |                                   |
| Subtotal (95% CI) |               |         |       |     | 70 |       | 34 |   | 96.8% | 0.35 [-0.79, 1.49] |                                   |
| Heterogeneity: $Taur^2 = 0.00; Chi^2 = 0.19, df = 1 (P = 0.67); I^2 = 0% |   |       |       |     |    |       |       |      | Test for overall effect: $Z = 0.60 (P = 0.55)$ |                                   |
| Total (95% CI) |               |         | 78 |       | 38 | 100.0% | 0.53 [-0.77, 1.82] |  |                                   |
| Heterogeneity: $Taur^2 = 0.22; Chi^2 = 2.35, df = 2 (P = 0.31); I^2 = 15% | |       |       |     |    |       |       |      | Test for subgroup differences: $Chi^2 = 2.17, df = 1 (P = 0.143), I^2 = 58.9% |                                   |

Standard deviations (SDs) were not provided for Voit 2014, therefore were substituted by SDs reported in NCT01462292.
Appendix 21: QOL (PedsQL) at week 48, parents, (change from baseline)

| Study or Subgroup | Exon skipping Mean | Exon skipping SD | Placebo Mean | Placebo SD | Weight | Mean Difference IV, Random, 95% CI | Mean Difference IV, Random, 95% CI |
|-------------------|--------------------|------------------|--------------|------------|--------|----------------------------------|----------------------------------|
| 1,612 Drisapersen | 1.19               | 11.269           | 0.11         | 11.064     | 58     | 68.1% 1.08 [-2.42, 4.58]           | 76 100.0% -0.26 [-4.09, 3.57]          |
| Ya020/14          | 0.8559             | 7.1599           | 2.08         | 11.96      | 18     | 21.9% -2.11 [-9.17, 2.94]          |                                   |
| Subtotal (95% CI) | 149                |                  |              |            | 76     | 100.0% -0.26 [-4.09, 3.57]         |                                   |

Heterogeneity: $I^2 = 2.42$, $Q I^2 = 1.38$, $df = 1$ ($p = 0.24$); $I^2 = 28\%$

Test for overall effect: $Z = 0.13$ ($p = 0.89$)

Test for subgroup differences: Not applicable

For mean difference, gain of score is shown in negative numbers and loss of score is shown in positive numbers
Appendix 22: QOL (PedsQL) at week 48, patient, (change from baseline)

| Study or Subgroup | exon skipping | Mean | SD | Total | Placebo | Mean | SD | Total | Weight | Mean Difference IV, Random, 95% CI | Mean Difference IV, Random, 95% CI |
|-------------------|---------------|------|----|-------|---------|------|----|-------|--------|---------------------------------|---------------------------------|
| 1,632 Driisapersen | -1.36         | 11.36| 114| -0.52 | 11.313  | 57   | 76.6%| 0.84 [-4.44, 2.76] |
| 2021914           | -4.17         | 8.509| 21 | -0.37 | 12.25   | 17   | 23.2%| 1.53 [-4.69, 1.63] |
| Subtotal (95% CI) | 145           | 74   | 100.0% | 1.53 [-4.69, 1.63] |
| Heterogeneity: $I^2 = 0.00, R^2 = 0.00, df = 1 (P = 0.44)$, $I^2 = 0.00$ |
| Test for overall effect: $Z = 0.95, (P = 0.34)$ |
| Total (95% CI)    | 145           | 74   | 100.0% | 1.53 [-4.69, 1.63] |
| Heterogeneity: $I^2 = 0.00, R^2 = 0.00, df = 1 (P = 0.44)$, $I^2 = 0.00$ |
| Test for overall effect: $Z = 0.95, (P = 0.34)$ |
| Test for subgroup differences: Not applicable |

For mean difference, gain of score is shown in negative numbers and loss of score is shown in positive numbers.
Appendix 23: Number of participants who withdrew from the study

| Study or Subgroup | Exon Skipping | Placebo | Risk Ratio |
|-------------------|---------------|---------|------------|
|                    | Events | Total | Events | Total | Weight | M-H, Random, 95% CI |
| **1.67.1 ephrinsen** |        |        |        |        |        |                        |
| Mendili2012       | 0       | B      | 0       | 4      | Not estimable |                        |
| Subtotal (95% CI) | 8       | 4      | Not estimable |        |        |                        |
| Total events      | 0       | 0      | Not applicable |        |        |                        |
| Heterogeneity: Not applicable |        |        |        |        |        |                        |
| Test for overall effect: Not applicable |        |        |        |        |        |                        |
| **1.67.2 drisapersen** |        |        |        |        |        |                        |
| Harigan2014       | 0       | 15     | 0       | 5      | Not estimable |                        |
| NCT01254019       | 4       | 13     | 1       | 61     | 100.0% | 1.95 [0.22; 17.09] |
| NCT01462292       | 0       | 15     | 0       | 16     | Not estimable |                        |
| Von2014           | 0       | 15     | 0       | 18     | Not estimable |                        |
| Subtotal (95% CI) | 210     | 104    | 100.0% | 1.95 | [0.22; 17.09] |                        |
| Total events      | 4       | 1      | Not applicable |        |        |                        |
| Heterogeneity: Not applicable |        |        |        |        |        |                        |
| Test for overall effect: Z = 0.60 (p = 0.55) |        |        |        |        |        |                        |

Total (95% CI) 218 104 100.0% 1.95 [0.22; 17.09]

Test for subgroup differences: Not applicable
Appendix 24: 6MWT at week 24 (change from baseline, meters), eteplirsen vs placebo

For mean difference, gain of distance is shown in negative numbers and loss of distance is shown in positive numbers.

| Study or Subgroup | Exon skipping Mean(SD) | Placebo Mean(SD) | Mean Difference IV, Random, 95% CI | Mean Difference IV, Random, 95% CI |
|-------------------|-------------------------|-----------------|-----------------------------------|-----------------------------------|
| Total             | 64.25 (62.80)           | 25.8 (61.2)     | 38.45 (35.65, 112.55)             |                                   |
| Eteplirsen        |                         |                 |                                   |                                   |
| Nandial2013       | 64.25 (62.80)           | 25.8 (61.2)     | 38.45 (35.65, 112.55)             |                                   |
|                   |                         |                 |                                   |                                   |
| Subtotal (95%) C0 | 8                       |                 |                                   |                                   |
|                   | 64.25 (62.80)           | 25.8 (61.2)     | 38.45 (35.65, 112.55)             |                                   |
|                   |                         |                 |                                   |                                   |

Test for overall effect: Z = 1.02 (P = 0.31)

Test for subgroup differences: Not applicable

Test for overall effect: Z = 1.02 (P = 0.31)
Appendix 25: 6MWT at week 24 (change from baseline, meters), drisapersen vs placebo

| Study or Subgroup | Exon skipping | Placebo | Mean Difference | Mean Difference |
|-------------------|---------------|---------|-----------------|----------------|
|                   | Mean          | SD      | Total           | Weight         | IV, Random, 95% CI |        | IV, Random, 95% CI |
| drisapersen       | 24.24         | 64.284  | 122             | 29.11          | 62.5231          | 59    | 42.8% -4.77 [-24.59, 15.05] |
| NCT01394019       | 1.39          | 41.6495 | 35              | 10.98          | 42.664           | 16    | 26.0% -9.59 [-14.64, 15.46] |
| Yeh2014           | -16.21        | 20.532  | 21              | 3.6            | 28.8             | 16    | 20.2% -12.81 [-42.37, 21.75] |
| Subtotal (95% CI) | 188           |         |                 |                |                  | 91    | 100.0% -10.62 [-23.60, 2.33] |

Heterogeneity: Tau² = 0.60, Chi² = 0.93, df = 2 (P = 0.63); I² = 0%
Test for overall effect: Z = 1.60 (P = 0.11)

Total (95% CI) 188
Heterogeneity: Tau² = 0.60, Chi² = 0.93, df = 2 (P = 0.63); I² = 0%
Test for overall effect: Z = 1.60 (P = 0.11)
Test for subgroup differences: Not applicable

For mean difference, gain of distance is shown in negative numbers and loss of distance is shown in positive numbers
Appendix 26: NSAA at week 24 (change from baseline), eteplirsen vs placebo

| Study or Subgroup | Exon skipping | Placebo | Mean Difference |
|-------------------|---------------|---------|----------------|
|                   | Mean (SD)     | Mean (SD) | Mean Difference (SD) | Weight |
| Mendell2012       | 4.15 (6.42)   | -3.3 (2.5) | 7.45 (2.36, 12.54) | 4 (100.0%) |
| Subtotal (95% CI) | 4 (100.0%)    | 7.45 (2.36, 12.54) |
| Total (95% CI)    | 8 (100.0%)    | 7.45 (2.36, 12.54) |

Heterogeneity: Not applicable
Test for overall effect: k = 2.87 (p = 0.004)

For mean difference, gain of score is shown in negative numbers and loss of score is shown in positive numbers

![Graph showing mean difference between eteplirsen and placebo](image-url)
### Appendix 27: NSAA at week 24 (change from baseline), drisapersen vs placebo

| Study or Subgroup | Exon skipping | Placebo | Mean Difference |
|-------------------|---------------|---------|-----------------|
|                   | Mean | SD | Total | Mean | SD | Total | Weight | IV, Random, 95% CI | IV, Random, 95% CI |
| 1.2.2 Drisapersen |      |    |       |      |    |       |        |                     |                     |
| NCT01462292      | 0.946 | 3.326 | 35 | 0.6 | 2.36 | 16 | 49.0% | 0.35 [-1.64, 2.33] |                     |
| Yoh2014          | -1.67 | 3.326 | 35 | 0.3 | 3.36 | 18 | 51.0% | -1.67 [-3.57, 0.23] |                     |
| Subtotal (95% CI)| 70   |     |      | 34   | 3.36 | 18 | 100.0% | -0.68 [-2.66, 1.29] |                     |
|                   |      |    |       |      |    |       |        |                     |                     |
|                   |      |    |       |      |    |       |        |                     |                     |
| Total (95% CI)   | 70   |     |      | 34   | 3.36 | 18 | 100.0% | -0.68 [-2.66, 1.29] |                     |

For mean difference, gain of score is shown in negative numbers and loss of score is shown in positive numbers.
Appendix 28: 6MWT at week 24, (change from baseline, meters), drisapersen 6mg/kg/time weekly injection

For mean difference, gain of distance is shown in negative numbers and loss of distance is shown in positive numbers
Appendix 29: Adverse events (Injection site reaction, drisapersen 6mg/kg/time weekly injection)

| Study or Subgroup | Exon skipping | Placebo | Risk Ratio | Risk Ratio |
|-------------------|---------------|---------|------------|------------|
|                   | Events Total  | Events Total  | M-H, Random, 95% CI | M-H, Random, 95% CI |
| 1.5.2 drisapersen |               |          |            |            |
| Hanigan 2014      | 5 6           | 1 5     | 11.8%      | 4.17 [0.70, 24.94] |
| NCT01254019       | 62 125        | 4 61    | 31.7%      | 7.56 [2.89, 19.83] |
| NCT01462292       | 9 18          | 3 16    | 25.5%      | 2.67 [0.67, 8.17]  |
| Von2014           | 9 18          | 4 18    | 30.0%      | 2.25 [0.84, 5.99]  |
| Subtotal (95% CI) | 167 100       | 100 100 | 100.0%     | 3.71 [1.93, 7.15]  |

Total events 85 12
Heterogeneity: $\hat{\tau}^2 = 0.11; \chi^2 = 3.98, df = 3 (\ P = 0.26); \ I^2 = 25\%$
Test for overall effect: $Z = 3.92 (P < 0.0001)$

Total (95% CI) 167 100 100.0%
3.71 [1.93, 7.15]

Total events 85 12
Heterogeneity: $\hat{\tau}^2 = 0.11; \chi^2 = 3.98, df = 3 (\ P = 0.26); \ I^2 = 25\%$
Test for overall effect: $Z = 3.92 (P < 0.0001)$
Test for subgroup differences: Not applicable
Appendix 30: Adverse events (Renal toxicity, drisapersen 6mg/kg/time weekly injection)

| Study or Subgroup | Exon skipping | Placebo | Risk Ratio |
|-------------------|---------------|---------|------------|
|                   | Events  Total | Events  Total | M-H  Random, 95% CI | M-H  Random, 95% CI |
| L.33.2 drisapersen| 0    6      | 0       5      | Not estimable |
| Hargami2014       | 42     125 | 11      61    71.6%   | 1.86 (1.00, 3.46) |
| NCT01254019       | 3      18   | 2       16     9.3%   | 1.33 (0.25, 7.00) |
| Von2014           | 6      18   | 3       18     17.1%   | 2.00 (0.59, 6.79) |
| Subtotal (95% CI) | 167       100  100.0% | 1.83 (1.10, 3.03) |

Total events 51 16
Heterogeneity: Tau² = 0.00; Chi² = 0.16, df = 2 (P = 0.92), I² = 0%
Test for overall effect: Z = 2.34 (P = 0.02)

Total (95% CI) 167 100 100.0% 1.83 [1.10, 3.03]

Test for subgroup differences: Not applicable
Appendix 31: 6MWT at week 24 (change from baseline, meters), fixed-effect meta-analysis

| Study or Subgroup   | Exon skipping Mean | SD | Total | Placebo Mean | SD | Total | Mean Difference IV, Fixed, 95% CI | Mean Difference IV, Fixed, 95% CI |
|---------------------|--------------------|----|-------|--------------|----|-------|-----------------------------------|----------------------------------|
| 1.1.1.1 Exon skipping |                    |    |       |              |    |       |                                   |                                  |
| Haschke (2011)      | 64.25              | 62.801 | 8     | 25.8         | 61.2 | 4     | 4.0% 38.45 [-35.65, 112.55]       |                                   |
| Subtotal (95% CI)   |                    |    |       |              |    |       |                                   |                                  |
| Haschke (2011)      | 8                  |    |       |              |    |       |                                   |                                  |
| Haschke (2011)      |                    |    |       |              |    |       |                                   |                                  |
| 1.1.2.2 Doripenem   |                    |    |       |              |    |       |                                   |                                  |
| NCT01254010         | 24.34              | 64.284 | 122 | 29.11        | 63.523 | 59 | 41.6% -4.77 [-24.59, 15.05]       |                                   |
| NCT01462292         | 1.39               | 42.6495 | 25  | 10.98        | 42.064 | 16 | 26.0% -2.59 [-24.64, 15.46]       |                                   |
| Subtotal (95% CI)   | 188                |    |       |              |    |       |                                   |                                  |
| 1.1.2 Drisapersen   |                    |    |       |              |    |       |                                   |                                  |
| NCT01254010         | -16.21             | 39.355 | 31  | 36.8         | 38.8 | 16 | 29.4% -19.81 [-33.78, 3.37]       |                                   |
| NCT01462292         |                    |    |       |              |    |       |                                   |                                  |
| Subtotal (95% CI)   | 91                 |    |       |              |    |       |                                   |                                  |
| Total (95% CI)      | 196                |    |       |              |    |       |                                   |                                  |

For mean difference, gain of distance is shown in negative numbers and loss of distance is shown in positive numbers.
Appendix 32: NSAA at week 24 (change from baseline), fixed-effect meta-analysis

For mean difference, gain of score is shown in negative numbers and loss of score is shown in positive numbers.
Appendix 33: 6MWT at week 24 (change from baseline, meters), excluding Mendell 2013 and Voit 2014 which were considered to possess high risk of bias

| Study or Subgroup | Exon skipping | Mean | SD Total | Placebo | Mean | SD Total | Weight | Mean Difference IV, Random, 95% CI | Mean Difference IV, Random, 95% CI |
|-------------------|---------------|------|----------|---------|------|----------|--------|-----------------------------------|-----------------------------------|
| 3.1.2 Dinslaken   |               |      |          |         |      |          |        |                                   |                                   |
| NCT01628692       | 24.34         | 64.284 | 122      | 29.11   | 63.5231 | 59      | 61.5%  | +4.77 [-24.59, 15.05]             |                                   |
| NCT0162290        | 1.39          | 41.6495 | 35       | 10.98   | 42.664  | 16      | 38.5%  | -0.59 [-34.64, 15.40]             |                                   |
| Subtotal (95% CI) |               | 157  | 100.0%   |         |       |          |        |                                   |                                   |
|                  |               | 157  | 100.0%   |         |       |          |        | -6.63 [-22.17, 8.92]             |                                   |

Heterogeneity Tau² = 0.00; CH² = 0.09, df = 1 (P = 0.77); I² = 0%
Test for overall effect: Z = 0.84 (P = 0.40)

For mean difference, gain of distance is shown in negative numbers and loss of distance is shown in positive numbers.
## Appendix table 1: Characteristics of excluded studies

| Study ID                  | Reason for exclusion                                      |
|---------------------------|------------------------------------------------------------|
| Nakamura 2011¹            | Not double-blind randomized-controlled trial               |
| Goemans 2011¹             | Not double-blind randomized-controlled trial               |
| Gerrald 2011¹              | Not double-blind randomized-controlled trial               |
| Cirak 2012³               | Not double-blind randomized-controlled trial               |
| Rodino-Kaplac 2013³       | Not double-blind randomized-controlled trial               |
| NCT01128855               | Secondary publication or studies of included studies⁶      |
| NCT01153932               | Secondary publication or studies of included studies⁷      |
| EUCTR2010-018412-32-NL    | Secondary publication or studies of included studies⁸      |
| EUCTR2010-018412-32-GB    | Secondary publication or studies of included studies⁸      |
| EUCTR2010-018412-32-FR    | Secondary publication or studies of included studies⁸      |
| EUCTR2010-018412-32-ES    | Secondary publication or studies of included studies⁸      |
| EUCTR2010-018412-32-DE    | Secondary publication or studies of included studies⁸      |
| EUCTR2010-018412-32-BE    | Secondary publication or studies of included studies⁸      |
| EUCTR2010-018412-32-DE    | Secondary publication or studies of included studies⁸      |
| EUCTR2010-024566-22-PL    | Secondary publication or studies of included studies⁸      |
| EUCTR2010-020069-26-NL    | Secondary publication or studies of included studies⁸      |
| EUCTR2010-020069-26-IT    | Secondary publication or studies of included studies⁸      |
| EUCTR2010-020069-26-HU    | Secondary publication or studies of included studies⁸      |
| EUCTR2010-020069-26-FR    | Secondary publication or studies of included studies⁸      |
| EUCTR2010-020069-26-ES    | Secondary publication or studies of included studies⁸      |
| EUCTR2010-020069-26-DK    | Secondary publication or studies of included studies⁸      |
| EUCTR2010-020069-26-DE    | Secondary publication or studies of included studies⁸      |
| EUCTR2010-020069-26-CZ    | Secondary publication or studies of included studies⁸      |
| EUCTR2010-020069-26-BE    | Secondary publication or studies of included studies⁸      |
| NCT01462392               | Secondary publication or studies of included studies⁸      |
| NCT01254019               | Secondary publication or studies of included studies⁸      |
| EUCTR2010-020069-26-NO    | Secondary publication or studies of included studies⁸      |
| EUCTR2014-002008-25-FR    | Secondary publication or studies of included studies⁸      |
| EUCTR2016-005000-26-NL    | Secondary publication or studies of included studies⁸      |
| EUCTR2010-024566-22-DE    | Secondary publication or studies of included studies⁸      |
| Goemans 2017⁶             | Secondary publication or studies of included studies⁸      |
| PMID:26086759             | Secondary publication or studies of included studies⁸      |
| NCT02310906               | Clinical trial registry with no result data               |
| EUCTR2014-005296-81-BE    | Clinical trial registry with no result data               |
| NCT02500381               | Clinical trial registry with no result data               |
| NCT02740972               | Clinical trial registry with no result data               |
| EUCTR2015-002069-52-BE    | Clinical trial registry with no result data               |
| EUCTR2015-002069-52-CZ    | Clinical trial registry with no result data               |
| EUCTR2015-002069-52-DE    | Clinical trial registry with no result data               |
| EUCTR2015-002069-52-ES    | Clinical trial registry with no result data               |
| EUCTR2015-002069-52-FR    | Clinical trial registry with no result data               |
| EUCTR2015-002069-52-GB    | Clinical trial registry with no result data               |

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Secondary publications of: a, Flanigan 2014; b, Voit 2014; c, Mendell 2013; d, NCT01462292; e, NCT01254019; f, Muntoni 2017

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4. Cirak S, Feng L, Anthony K, et al. Restoration of the dystrophin-associated glycoprotein complex after exon skipping therapy in duchenne muscular dystrophy. Mol Ther. 2012;20:462-67.
5. Rodino-Klapac LR. Microrna based treatment of cardiomyopathy: Not all dystrophies are created equal. J Am Heart Assoc. 2013;2.
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