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Ataluren and similar compounds (specific therapies for premature termination codon class I mutations) for cystic fibrosis (Review)

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[Intervention Review]

Ataluren and similar compounds (specific therapies for premature termination codon class I mutations) for cystic fibrosis

Aisha A Aslam[1], Colin Higgins[2], Ian P Sinha[1], Kevin W Southern[1]

[1]Department of Women's and Children's Health, University of Liverpool, Liverpool, UK. [2]Alder Hey Children's NHS Foundation Trust, Liverpool, UK

Contact address: Kevin W Southern, Department of Women's and Children's Health, University of Liverpool, Alder Hey Children's NHS Foundation Trust, Eaton Road, Liverpool, L12 2AP, UK. kwsouth@liv.ac.uk.

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ABSTRACT

Background

Cystic fibrosis is a common life-shortening genetic disorder in the Caucasian population (less common in other ethnic groups) caused by the mutation of a single gene that codes for the production of the cystic fibrosis transmembrane conductance regulator protein. This protein coordinates the transport of salt (and bicarbonate) across cell surfaces and the mutation most notably affects the airways. In the lungs of people with cystic fibrosis, defective protein results in a dehydrated surface liquid and compromised mucociliary clearance. The resulting thick mucus makes the airway prone to chronic infection and inflammation, which consequently damages the structure of the airways, eventually leading to respiratory failure. Additionally, abnormalities in the cystic fibrosis transmembrane conductance regulator protein lead to other systemic complications including malnutrition, diabetes and subfertility.

Five classes of mutation have been described, depending on the impact of the mutation on the processing of the cystic fibrosis transmembrane conductance regulator protein in the cell. In class I mutations, the presence of premature termination codons prevents the production of any functional protein resulting in a severe cystic fibrosis phenotype. Advances in the understanding of the molecular genetics of cystic fibrosis has led to the development of novel mutation-specific therapies. Therapies targeting class I mutations (premature termination codons) aim to mask the abnormal gene sequence and enable the normal cellular mechanism to read through the mutation, potentially restoring the production of the cystic fibrosis transmembrane conductance regulator protein. This could in turn make salt transport in the cells function more normally and may decrease the chronic infection and inflammation that characterises lung disease in people with cystic fibrosis.

Objectives

To evaluate the benefits and harms of ataluren and similar compounds on clinically important outcomes in people with cystic fibrosis with class I mutations (premature termination codons).

Search methods

We searched the Cochrane Cystic Fibrosis Trials Register which is compiled from electronic database searches and handsearching of journals and conference abstract books. We also searched the reference lists of relevant articles. Last search of Group's register: 24 October 2016.

We searched clinical trial registries maintained by the European Medicines Agency, the US National Institutes of Health and the WHO. Last search of clinical trials registries: 28 November 2016.

Selection criteria

Randomised controlled trials of parallel design comparing ataluren and similar compounds (specific therapies for class I mutations) with placebo in people with cystic fibrosis who have at least one class I mutation. Cross-over trials were reviewed individually to evaluate whether data from the first treatment arm could be included. We excluded trials that combined therapies for premature termination codon class I mutations with other mutation-specific therapies.

Data collection and analysis

The authors independently assessed the risk of bias and extracted data from the included trial; they contacted trial authors for additional data.

Main results

Our searches identified 28 references to eight trials; five trials were excluded (three were cross-over and one was not randomised and one did not have relevant outcomes), one cross-over trial is awaiting classification pending provision of data and one trial is ongoing. The included parallel randomised controlled trial compared ataluren to placebo for a duration of 48 weeks in 238 participants (age range 6 to 53 years) with cystic fibrosis who had at least one nonsense mutation (a type of class I mutation).

The quality of evidence and risk of bias assessments for the trial were moderate overall. Random sequence generation, allocation concealment and blinding of trial personnel were well-documented; participant blinding was less clear. Some participant data were excluded from the analysis. The trial was assessed as high risk of bias for selective outcome reporting, especially when reporting on the trial's post hoc subgroup of participants by chronic inhaled antibiotic use.

The trial was sponsored by PTC Therapeutics Incorporated with grant support by the Cystic Fibrosis Foundation, the Food and Drug Administration's Office of Orphan Products Development and the National Institutes of Health (NIH).

The trial reported no significant difference between treatment groups in quality of life, assessed by the Cystic Fibrosis Questionnaire-Revised respiratory domain score and no improvement in respiratory function measures (mean difference of relative change in forced expiratory volume at one second 2.97% (95% confidence interval -0.58 to 6.52)). Ataluren was associated with a significantly higher rate of episodes of renal impairment, risk ratio 17.70 (99% confidence interval 1.28 to 244.40). The trial reported no significant treatment effect for ataluren for the review's secondary outcomes: pulmonary exacerbation; computerised tomography score; weight; body mass index; and sweat chloride. No deaths were reported in the trial.

A post hoc subgroup analysis of participants not receiving chronic inhaled tobramycin (n = 146) demonstrated favourable results for ataluren (n = 72) for relative change in % predicted forced expiratory volume at one second and pulmonary exacerbation rate. Participants receiving chronic inhaled tobramycin appeared to have a reduced rate of pulmonary exacerbation compared to those not receiving chronic inhaled tobramycin. This drug interaction was not anticipated and may affect the interpretation of the trial results.

Authors' conclusions

There is currently insufficient evidence to determine the effect of ataluren as a therapy for people with cystic fibrosis with class I mutations. Future trials should carefully assess for adverse events, notably renal impairment and consider the possibility of drug interactions. Cross-over trials should be avoided given the potential for the treatment to change the natural history of cystic fibrosis.

PLAIN LANGUAGE SUMMARY

Ataluren and similar compounds (specific therapies for premature termination codon class I mutations) for cystic fibrosis

Review question

What are the effects of ataluren and similar compounds (specific therapies for premature termination codon (class I) mutations) on clinical outcomes (quality of life, lung function and adverse effects) in people with cystic fibrosis?

Background

In people with cystic fibrosis, the gene encoding a protein called the cystic fibrosis transmembrane conductance regulator is faulty. In particular, this affects the airways, which become dehydrated making it difficult to clear thick mucus, which in turn leads to progressive lung infection and damage and a reduction in life expectancy. Ataluren and similar compounds can mask the abnormal gene sequence and may be able to restore production of the faulty protein in people with certain cystic fibrosis mutations (premature termination codon (class I) mutations). These treatments aim to help the airways retain more water allowing them to clear the mucus better, so that these people develop fewer lung infections.

Search date

The evidence is up to date as of 24 October 2016.

Study characteristics

We found one trial (238 people took part) comparing ataluren to placebo (a dummy treatment with no active medication). The trial lasted 48 weeks and included both males and females aged six years and older. Everyone taking part had at least one copy of a nonsense mutation (a type of class I mutation that causes cystic fibrosis).

Key Results

In those people who took ataluren, there was no improvement in clinical outcomes such as quality of life, lung function, exacerbations (flare up of disease), sweat chloride (salt) levels or weight. The trial found that kidney damage was more common in people who took ataluren. The trial investigators then analysed the results in a way that they hadn't planned originally and looked at how ataluren or placebo affected people depending on whether they were using inhaled tobramycin on a long-term basis or not. They found that amongst those not taking inhaled tobramycin, lung function declined at a slower rate and there were fewer exacerbations in the ataluren group compared to the placebo group.

We have not found enough high-quality evidence currently to determine the effect of ataluren for treating people with cystic fibrosis. We recommend that future trials are designed and reported clearly so that their results can be included in a systematic review.

Quality of the evidence

We judged the quality of the evidence was moderate with uncertainty due to how widely the results varied between participants. We are satisfied that everyone taking part had an equal chance of being in either group (ataluren or placebo) and that no one could work out which group the next person would be put into, so that healthier people did not receive the treatment and make the results seem better. We believe that the clinicians running the trial and those taking part in the trial did not know which treatment each person was receiving. We have some concerns on the emphasis the investigators have placed on the results of a comparison they had not planned (use of long-term inhaled tobramycin). Unfortunately, the trial did not report all their results clearly; sometimes they did not report them in a way that we could use in the review and sometimes they did not report the data at all. This affected the certainty with which we judged the overall results.

Trial Funding Sources

The trial was sponsored by PTC Therapeutics Incorporated. The Cystic Fibrosis Foundation, the Food and Drug Administration's Office of Orphan Products Development and the National Institutes of Health (NIH) also supported the trial.

SUMMARY OF FINDINGS

Summary of findings for the main comparison.

Ataluren compared with placebo for people with CF with class I mutations (PTCs): long-term outcomes

Patients or population: adults and children with CF with at least one class I mutation (PTC)

Settings: outpatient

Intervention: oral ataluren

Comparison: oral placebo

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Placebo	Ataluren				
Health Related Quality of Life Scale: age appropriate versions of CFQ-R questionnaire Follow up: 48 weeks	No significant differences were found between treatment groups for the CFQ-R.			232 (1 study)[1]	⊕⊕⊕⊖ **moderate[2]**	Data provided by investigators were analysed via a MMRM analysis
Respiratory function: **% predicted FEV_1 (relative change from baseline)** Follow up: 48 weeks	The mean (SD) relative change from baseline in the placebo group was -5.5% (12.56%).	The mean (SD) relative change from baseline in the the ataluren group was -2.53% (13.25%).	MD 2.97% (95% CI -0.58 to 6.52%).	232 (1 study)	⊕⊕⊕⊕ **high**	Data provided by investigators were analysed via a MMRM analysis.
Adverse events relating to treatment Scale: mild, moderate, severe Episodes of oropharyngeal pain	14/118 placebo participants experienced this adverse event.	4/120 ataluren participants experienced this adverse event.	RR 0.28 (95% CI 0.10 to 0.83) (P = 0.02).	238 (1 study)	⊕⊕⊕⊖ **moderate[3]**	Adverse events were graded by severity (1 - 4); however, details of criteria for grade 1-3 classifications were not reported. Adverse events occurring in more than 10% of participants was reported. There was no significant difference between treatment groups for all other recorded adverse events.

Adverse events relating to treatment Scale: mild, moderate, severe Episodes of "acute kidney injury"	1/118 placebo participants experienced this adverse event.	18/120 ataluren participants experienced this adverse event.	RR 17.70 (95% CI 2.40 to 130.47) (P = 0.005).	238 (1 study)	⊕⊕⊕⊖ **moderate[3]**	Adverse events were graded by severity (1 - 4); however, details of criteria for grade 1-3 classifications were not reported. Adverse events occurring in more than 10% of participants was reported. There was no significant difference between treatment groups for all other recorded adverse events.
Pulmonary exacerbations - protocol-defined (modified Fuchs' criteria) Scale: rate Follow up: 48 weeks	The mean (SD) protocol-defined pulmonary exacerbation rate in the placebo group was 1.78 (2.15).	The mean (SD) protocol-defined pulmonary exacerbation rate in the ataluren groups was 1.42 (2).	MD -0.36 (95% CI -0.89 to 0.17).	232 (1 study)	⊕⊕⊕⊖ **moderate[2]**	Data provided by investigators were analysed via a MMRM analysis.
Nutrition and growth Change in: a) weight (kg) b) BMI (kg/m^2) c) height (m) Follow up: 48 weeks	No significant differences between treatment groups were found for the change in body weight and BMI.			232 (1 study)[1]	⊕⊕⊕⊖ **moderate[2]**	Data provided by investigators were analysed via a MMRM analysis.
Sweat chloride level - change from baseline Scale: mmol/L Follow up: 48 weeks	The mean (SD) change from baseline in the placebo group was -0.6 (10.27).	The mean (SD) change from baseline in the ataluren group was -1.3 (8.94).	MD -0.70 mmol/L (95% CI -3.37 to 1.97).	194 (1 study)	⊕⊕⊕⊖ **moderate[2]**	Data provided by investigators were analysed via a MMRM analysis.
Mortality rate	The trial reported zero deaths in both treatment groups.			232 (1 study)	⊕⊕⊕⊖ **moderate[4]**	

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CF: cystic fibrosis; **CFQ-R**: cystic fibrosis questionnaire - revised; **CI:** confidence interval; **FEV$_1$**: forced expiratory volume at one second; **ITT**: intention to treat; **MD:** mean difference; **MMRM:** mixed model repeated measures analysis - based on the average effect across all post-baseline visits; **PTC**: premature termination codon; **RR:** risk ratio; **SD**: standard deviation.

GRADE Working Group grades of evidence
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.

Very low quality: We are very uncertain about the estimate.

1. Stated that 238 participants were randomised, 232 in ITT population and 203 completed the study. Unclear how many participants were evaluated for the outcome.
2. Evidence downgraded due to imprecision; study not powered to assess significant change in the outcome.
3. The power of the study to reliably report adverse events (acute kidney injury) was low, reflected in the wide CI for RR.
4. Evidence downgraded due to imprecision; no deaths were reported in the trial, therefore a relative comparison between groups cannot be made.

BACKGROUND

A glossary of terms specific to this review can be found in the appendices (Appendix 1); a more general glossary of terms used in Cochrane systematic reviews can be accessed at Cochrane Glossary.

Description of the condition

Cystic fibrosis (CF) is a common life-shortening genetic disorder in the Caucasian population (less common in other ethnic groups). In the UK around 1 in 3000 newborn infants are affected and in 2013 median predicted survival was reported as just over 36 years of age (UK CF Trust 2014; Welsh 2001). The condition is caused by the mutation of a single gene, which codes for the production of the cystic fibrosis transmembrane conductance regulator (CFTR) protein (Welsh 2001). After transcription (being produced in the centre of the cell), the protein is transported to the cell membrane, where it has an important role as a chloride channel, co-ordinating the transport of salt across cell surfaces (Boucher 2007).

Lung disease is the major cause of morbidity and mortality in CF (Davis 2006). In the airways, the CFTR protein does not function correctly resulting in increased fluid and sodium resorption away from the airway surface; in turn this leads to a dehydrated airway surface liquid and compromised clearance of these secretions (Boucher 2007). The resulting thick mucus in the airway provides a site for infection. Chronic airway infection and inflammation causes damage to the structure of the airways, which eventually results in respiratory failure.

Approximately 2000 mutations of the CFTR gene have been described. Five classes of mutation have been characterised, depending on the impact of the mutation on the processing of the CFTR protein in the cell (CFMD 2011) (Table 1).

This review is focused on class I mutations which stop the normal protein-producing mechanisms of the cell; as a result of this no significant amounts of CFTR protein are produced. The mutations change the sequence of the gene and a piece of DNA is inserted that tells the cell to stop producing the protein. The normal process of producing a protein in the cell is called transcription and this is faulty in class I mutations. There are different ways class I mutations cause this effect; they can insert a sequence of DNA that makes no sense (a nonsense mutation) or they can produce a sequence that directs the normal cellular mechanism to stop (a stop codon mutation). Collectively, these different types of mutation are known as premature termination codons (PTCs), but essentially have the same outcome of no functional protein being produced (Friedman 2014; McElroy 2013; Nicholson 2010).

Class I mutations are seen in approximately 10% of people with CF (with higher proportions seen in the Ashkenazi Jewish population) and they result in a severe CF phenotype with no significant functional protein being synthesised (Kerem 1997; McKone 2006).

Description of the intervention

Advances in the understanding of the molecular genetics of CF have led to the development of novel mutation-specific therapies. One potential strategy has been to correct class I mutations (PTCs) by using drugs that mask the abnormal gene sequence of the mutation and enable the normal cellular mechanism to read through the mutation and produce a full length of protein. The CFTR protein would then be transported normally to the cell membrane, where it might correct the CF salt transport defect.

Previous studies have demonstrated that aminoglycoside antibiotics (e.g. gentamicin) have the ability to read through class I mutations (PTCs) resulting in expression of full length CFTR protein and significant changes of the salt transporting defect towards normal (Clancy 2001; Howard 1996; Wilschanski 2003). Although gentamicin is widely used as an antibiotic for people with CF, it is not an ideal agent for long-term delivery because of concerns over renal and ototoxicity (Wilschanski 2003).

High-throughput screening has identified a molecule called ataluren (formerly PTC124), which has potential as an oral class I (PTC) therapy (McElroy 2013; Welch 2007). In the laboratory, ataluren has been shown to have the ability to enable read through of class I (PTC) mutations (Welch 2007).

How the intervention might work

By correcting the underlying molecular genetic defect, ataluren and related compounds may reverse the abnormal salt transport that characterises CF. This may restore the ability of the airway to prevent airway infection and inflammation and improve respiratory function. In addition, as a systemic treatment, ataluren may have impact on other parts of the body that are affected by the CF salt transport defect.

Why it is important to do this review

A number of different mutation-specific therapies are under investigation. These include potentiators, which improve the compromised function of CFTR that has reached the cell membrane (class III and IV mutations) and also correctors, which increase the amount of CFTR in the cell membrane (class II mutations) (Table 1). A Cochrane review has recently been published for trials assessing potentiators (Patel 2015) and a review is currently being undertaken for corrector therapies (Sinha 2014). At present, there are no known trials for therapies specific to class V mutations.

It is important that the randomised controlled trials (RCTs) assessing ataluren or similar compounds are critically appraised. This will allow the analysis of the data outlining the benefits and harms of these therapies in people with CF. It is important that funding bodies have a clear evidence-base on which to assess novel mutation-specific CF therapies. It is likely that these therapies will represent a significant healthcare resource. In addition, a critical appraisal of included trials will help inform future trial design.

OBJECTIVES

To evaluate the benefits and harms of ataluren and similar compounds on clinically important outcomes in people with CF with class I mutations (PTCs).

METHODS

Criteria for considering studies for this review

Types of studies

We included randomised controlled trials (RCTs) of parallel design (published or unpublished). We excluded quasi-RCTs due to a greater risk of selection bias. Cross-over trials were reviewed individually to evaluate if data from the first treatment arm may

be included; however, since these therapies aim to correct the underlying gene defect, there is significant potential for longer-term impact on outcomes and this is not an ideal trial design.

Types of participants

We included trials involving children or adults with CF, of any severity, as confirmed either by the presence of two disease-causing mutations, or by a combination of a positive sweat test and recognised clinical features of CF. We only included trials in which participants had at least one PTC (nonsense or stop codon) mutation.

Types of interventions

We included trials in which ataluren (or similar compounds for PTC class I mutations) was compared with either placebo or another intervention. We excluded trials that combined therapies for PTC class I mutations with other mutation-specific therapies. No trials comparing different dosing regimens of these therapies were eligible for inclusion based on the aforementioned criteria. In future updates, we will include trials comparing different dosing regimens of therapies for PTC class I mutations if we identify such trials.

Types of outcome measures

Primary outcomes

1. Quality of life (QoL) (measured using validated quantitative scales or scores (e.g. Cystic Fibrosis Questionnaire- Revised (CFQ-R) (Quittner 2009))
   a. total QoL score
   b. different sub-domains
2. Respiratory function measures (litres or per cent (%) predicted for age, sex and height)
   a. forced expiratory flow rate at one second (FEV_1) (relative change from baseline)
   b. FEV_1 (absolute values)
   c. forced vital capacity (FVC) (absolute values and relative change from baseline)
3. Adverse events
   a. graded by review authors as mild (therapy does not need to be discontinued)
   b. graded by review authors as moderate (therapy is discontinued, and the adverse effect ceases)
   c. graded by review authors as severe (life-threatening or debilitating, or which persists even after treatment is discontinued)

Secondary outcomes

1. Survival
   a. time to event (death or lung transplant)
   b. mortality rate
2. Hospitalisation
   a. number of days
   b. number of episodes
   c. time to next hospitalisation
3. School or work attendance (i.e. number of days missed)
4. Extra courses of antibiotics (measured as time-to the next course of antibiotics and the total number of courses of antibiotics)
   a. oral
   b. intravenous
   c. inhaled
5. Pulmonary exacerbations (either physician- or protocol-defined)
6. Radiological measures of lung disease (assessed using any scoring system)
   a. chest radiograph score
   b. computerised tomogram (CT) score
7. Acquisition of respiratory pathogens
   a. *Pseudomonas aeruginosa* (*P aeruginosa*)
   b. *Staphylococcus aureus* (*S aureus*)
   c. *Haemophilus influenzae* (*H influenzae*)
   d. other significant pathogens
8. Eradication of respiratory pathogens (as defined by trial authors)
   a. *P aeruginosa*
   b. *S aureus*
   c. *H influenzae*
   d. other significant pathogen
9. Nutrition and growth (measured as relative change from baseline) (including z scores or centiles)
   a. weight
   b. body mass index (BMI)
   c. height
10. Sweat chloride (change from baseline) as a measure of CFTR function in sweat glands
11. Cost effectiveness (cost utility assessed as comparison of impact on quality of adjusted life years)

Search methods for identification of studies

Electronic searches

We identified relevant studies from the Cochrane Cystic Fibrosis and Genetic Disorders Group's Cystic Fibrosis Trials Register using the term: 'drugs that correct stop codon mutations'.

This register has been compiled from electronic searches of the Cochrane Central Register of Controlled Trials (CENTRAL) (updated each new issue of the Cochrane Library), weekly searches of MEDLINE, a search of Embase to 1995 and the prospective handsearching of two journals - *Pediatric Pulmonology* and the *Journal of Cystic Fibrosis*. Unpublished work is identified by searching the abstract books of three major cystic fibrosis conferences: the International Cystic Fibrosis Conference; the European Cystic Fibrosis Conference and the North American Cystic Fibrosis Conference.

For full details of all searching activities for the register, please see the relevant sections of the Cochrane Cystic Fibrosis and Genetic Disorders Group Module.

Date of the most recent search: 24 October 2016.

We also searched clinical trial registries maintained by the European Medicines Agency (www.clinicaltrialsregister.eu), the US National Institutes of Health (clinicaltrials.gov) and the WHO (www.who.int/ictrp/en/).

Date of the most recent search: 28 November 2016.

Searching other resources

We screened references of included trials to identify other potentially relevant trials. We also contacted authors of included trials, leaders in the field, and companies known to be developing and investigating therapies for PTC class I mutations, to identify any trials which may have been missed by these searches.

Data collection and analysis

Selection of studies

Two authors (AA and CH) independently assessed the suitability of each trial identified by the outlined search. If any disagreement had arisen on the suitability of a trial for inclusion in the review, we would have attempted to reach a consensus by discussion, failing which a third author (KWS) would have arbitrated.

Data extraction and management

Two authors (AA and CH) independently extracted relevant data from the only included trial using a standardised data extraction form. If any disagreement had arisen on data extraction, we would have attempted to reach a consensus by discussion, failing which a third author (KWS) would have arbitrated.

We intended to extract QoL scores ideally as relative change from baseline ((measurement at end of treatment - measurement at baseline) / measurement at baseline) x 100); however, data were not reported for this analysis and instead we extracted the absolute change from baseline. We extracted data for FEV_1 (relative change from baseline). The mean and standard error for the mean for relative change from baseline in FEV_1 and FVC were reported at 8, 16, 24, 32, 40 and 48 weeks through graphical representation and two reviewers (AA and CH) independently estimated these data. Regarding adverse events, we extracted data for severe adverse events (i.e. participants who required discontinuation of therapy). The trial report presented data for adverse events occurring in more than 10% of participants; however, the report did not provide information regarding whether treatment required interruption or not. Furthermore, the report presented data as the number of each adverse event occurring (where a participant may have had more than one adverse event), which prevented extraction of the number of participants who experienced an adverse event.

With regards to the secondary outcome, pulmonary exacerbation, we extracted the rate of pulmonary exacerbation and noted both protocol-defined and physician-defined rates. For the outcome, nutrition, the trial investigators reported insufficient data for change from baseline in each treatment arm and instead we extracted the difference in change in body weight and BMI between ataluren and placebo. For total lung CT score and sweat chloride concentration, we extracted the absolute change from baseline.

In future updates, if trials are included with different dosing regimens, we will combine results of all dosing regimens together in a single analysis and subsequently undertake a subgroup analysis to assess the doses individually. We planned to report data as immediate (up to and including one month), medium term (over one month and up to six months) and long term (over six months). We extracted data for up to 48 weeks and estimates at 8, 16, 24, 32 and 40 weeks (when presented) for selected outcomes. As only one trial met the inclusion criteria for the review, we were unable to combine results of multiple trials. We plan to do this in future when more trials are available for inclusion.

Assessment of risk of bias in included studies

Two authors (AA and CH) independently assessed the risk of bias for the included trial using the Cochrane risk of bias tool (Higgins 2011a). This included assessment of the following methodological aspects of the trial:

1. procedure for randomisation (selection bias);
2. allocation concealment (selection bias);
3. masking (blinding) of the intervention from participants, clinicians, and trial personnel evaluating outcomes (performance bias);
4. missing outcome data (attrition bias);
5. selective outcome reporting (reporting bias);
6. other sources of bias (e.g. the influence of funding sources or industry on trial characteristics and presented results).

We assessed whether all participants were included in an intention-to-treat analysis, regardless of whether they completed the treatment schedule or not. If disagreement had arisen on the assessment of risk of bias, we would have attempted to reach a consensus by discussion, failing which a third author (KWS) would have arbitrated.

Measures of treatment effect

For binary outcomes, we calculated the treatment effect for each outcome using the risk ratio (RR) and 95% confidence intervals (CIs); but (in a post hoc change) for the analysis of individual adverse events we have used 99% CIs as the type I error rate (e.g. false positive) is greatly inflated with a large number of tests. For continuous outcomes, we calculated the mean change from baseline and standard deviation (SD) for each group. We converted any reported standard errors to SDs. We calculated the mean difference (MD) and 95% CIs. For the eight-weekly estimates of the relative change from baseline in FEV_1 (as well as the subgroup analysis by chronic inhaled tobramycin use for this outcome) and FVC, the trial report did not state the number of participants for each time point. For these estimated data, as the withdrawal rate was 14% in the ataluren group and 11% in the placebo group, we felt it appropriate to use the respective ITT population for each treatment group and subgroup. For QoL, CFQ-R was the questionnaire used and we intended to calculate the MD and 95% CIs; however, insufficient data were reported in the trial. Inclusion of a single trial prevented analysing pooled estimates.

In the included trial, investigators analysed continuous outcomes over 48 weeks via a mixed model repeated measures analysis (MMRM) based on the average effect across all post-baseline visits (Kerem 2014). Such an analysis is longitudinal and uses all available data at every visit. We have presented week-48 data for relative change in FEV_1, pulmonary exacerbation rate, total lung CT scores and change in sweat chloride concentration from the MMRM model for the effect at 48 weeks derived from the treatment by visit term in the model (so data presented as 'up to 48 weeks' are the estimates from the model at the 48-week time point). It also allows for covariate adjustment (baseline value, treatment, visit, treatment-by-visit interaction, baseline-by-visit interaction) and stratification (inhaled antibiotics (yes or no), baseline age (under 18 versus 18 years and over), and baseline % predicted FEV_1 (40% up to 65% versus 65% and up to 90%)).

In future updates, if we identify any trials which report time-to-event outcomes, we will use measures of survival analysis, and report hazard ratios (HR) and 95% CIs between different arms of the trial. Futhermore, if different trials do not report change data, but instead present absolute post-treatment data without baseline data (so it is not possible to calculate change data) we will use absolute post-treatment data instead of change from baseline. However, if the report presents baseline and post-treatment data for any outcome, we will calculate SDs for the change from baseline, for example if the CI is available. When there is not enough information available to calculate the SDs for the changes, we aim to impute them from other trials in the review where the data are available and trials are similar (i.e. whether the trials used the same measurement scale, had the same degree of measurement error and had the same time periods between baseline and final value measurement). If neither of these methods are possible, we will impute a change-from-baseline SD from another trial, making use of an imputed correlation co-efficient (following methods described in chapter 16 in the *Cochrane Handbook of Systematic Reviews of Interventions* (Higgins 2011b).

Unit of analysis issues

Within this review, we only included results from RCTs of parallel design in which randomised the individual trial participants. We also reviewed cross-over trials on an individual basis; however, we could not extract data from the first arm and hence we excluded them. We did not identify any cluster RCTs. In future updates, we will exclude cluster RCTs, as this is not an appropriate study design for this type of intervention.

Dealing with missing data

In order to allow an intention-to-treat analysis, we extracted data on the number of participants with each outcome event, by allocated treated group, irrespective of compliance and whether or not the participant was later thought to be ineligible or otherwise excluded from treatment or follow up. We recorded the number of participants with outcome data and checked if this was consistent with the number of originally randomised participants. If any data were missing or unclear, we contacted the primary investigators for clarification. Specifically we have contacted the authors of one excluded cross-over trial on two occasions asking if data are available from the first arm of the trials and if so requesting that the investigators share these with us (Pradal 2002). To date we have not received a response from the authors; however, if the first-arm data from these trials are made available to us in the future we will re-assess this trial for inclusion. We also contacted the authors of a second cross-over trial and were informed that the data are held by PTC Therapeutics and we are contacting the company for further clarification; this trial is currently listed as 'Awaiting assessment' (Sermet-Gaudelus 2010).

Assessment of heterogeneity

Since we only included a single trial, we did not need to assess heterogeneity. If we are able to include more trials in future updates of the review, we will assess heterogeneity through a visual examination of the combined data presented in the forest plots, and by considering the I^2 statistic (Higgins 2003) together with Chi^2 values and their CIs (Deeks 2011). This reflects the likelihood that variation of results across trials are due to heterogeneity rather than chance, and we will interpret this statistic using the following simple classification:

- 0% to 40%: might not be important;
- 30% to 60%: may represent moderate heterogeneity;
- 50% to 90%: may represent substantial heterogeneity;
- 75% to 100%: considerable heterogeneity.

Assessment of reporting biases

In order to identify selective outcome reporting, where possible, we compared outcomes defined in the protocol with those reported in the full publication. We explored the protocol of the identified trial found on the aforementioned clinical trials registries for evidence of selective reporting bias. In future, if the protocol for an included trial is not available publicly we will contact the primary investigators, corresponding author(s), or relevant pharmaceutical company for a copy. We also compared outcomes listed in the 'Methods' section of the final paper with those presented in the 'Results' section. For negative findings that were reported either only partially, or not at all, we contacted primary investigators for these data, which included: CFQ-R score; weight; BMI; relative change in FVC; adverse events that lead to interruption of treatment; grading classification of adverse events; hospitalisations; extra courses of antibiotics; and disruptions to school or work attendance. We did not receive a reply.

We planned to assess publication bias by constructing and assessing the symmetry of a funnel plot. This would have been possible if we included more than 10 trials in the review.

Data synthesis

As only one trial was eligible for inclusion, we were not able to conduct a meta-analysis. We have presented the results in graphs using the fixed-effects model. In future updates, if we are able to include further trials, we aim to use a random-effects model to analyse the data, regardless of the value of the I^2 statistic.

Subgroup analysis and investigation of heterogeneity

We planned to investigate any heterogeneity that we identified using subgroup analyses of potential confounding factors, if sufficient numbers (at least 10 trials) were available. For this review, we planned that these confounding factors would be:

- age (children (defined as younger than 18 years of age) versus adults);
- gender;
- intervention used.

We did not plan any subgroup analyses by antibiotic use; however, the included trial presented a post hoc subgroup analysis by inhaled tobramycin use, which we have reported in the Results section below. An ongoing trial excludes participants who are using long-term inhaled aminoglycosides within the preceding four months. This difference in participant baseline characteristics is also a potential confounding factor for future updates.

The analysis in this review is based on aggregate data as we have no individual participant data. We will incorporate such analysis in future updates if these data are available.

Sensitivity analysis

If we had been able to combine a sufficient number of trials (at least 10), we planned to examine the impact of risk of bias on the results

examined by comparing meta-analyses including and excluding trials with concerns of high risk of selection or reporting bias due to issues relating to randomisation, allocation concealment, or masking of interventions from participants or trial personnel.

Summary of findings and quality of evidence (GRADE)

We specified in our protocol that we would present three tables: the first for outcomes measured up to one month; the second for outcomes between one and six months; and the third for longer term outcomes over six months. We felt that data from previous trials and reviews (Sinha 2014; Patel 2015) highlight the potential for a rapid response to this type of intervention and planned to present these in the first table, but were also interested in illustrating evidence of a sustained response in the short to medium term, as we feel this is important for stakeholders and aimed to present such data in the second table. At present, data for short- and medium-term outcomes are not available, therefore we have presented a summary of findings table for longer-term outcomes.

As planned, we have presented the following outcomes in a summary of findings table: QoL, respiratory function measures (FEV_1), adverse events, pulmonary exacerbations; nutrition and growth; and sweat chloride level. In a post hoc change we have also added the outcome of mortality.

We determined the quality of the evidence using the GRADE approach; and downgraded evidence in the presence of a high risk of bias in at least one trial, indirectness of the evidence, unexplained heterogeneity or inconsistency, imprecision of results, high probability of publication bias. We downgraded the evidence by one level if the limitation was considered to be serious and by two levels if very serious. Under the GRADE approach, evidence may have been upgraded if a large treatment effect was demonstrated with no obvious biases or if a dose-response effect exists.

RESULTS

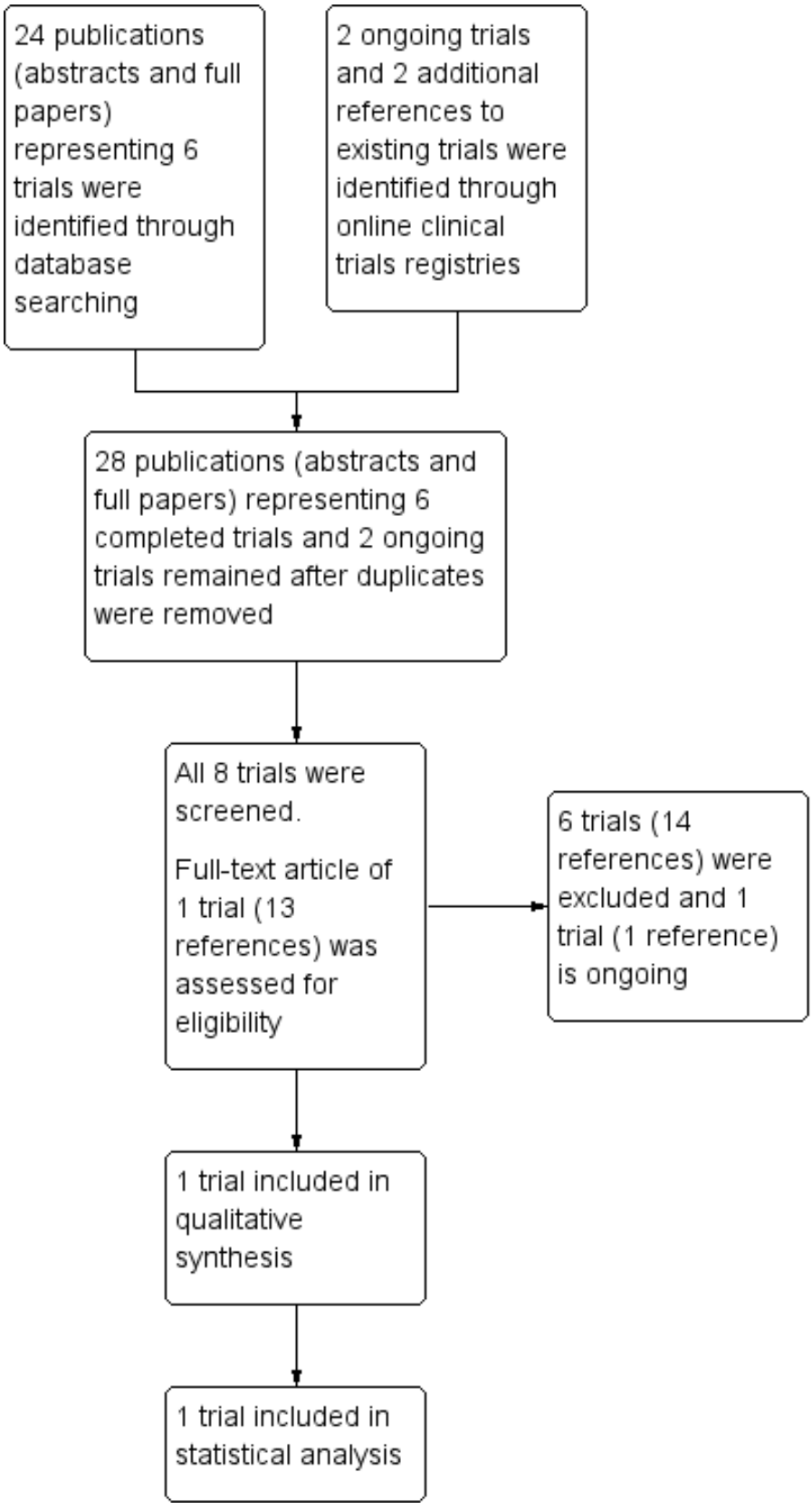
Description of studies

Please see the tables (Characteristics of included studies; Characteristics of excluded studies; Characteristics of ongoing studies).

Results of the search

The search of the Group's Cystic Fibrosis Trials Register identified a total of 24 publications (abstracts and full papers) representing six trials. The search of the online clinical trials registries identified two ongoing trials (McIntosh 2014a; McIntosh 2014b) and two additional references to trials found through the initial search of the Group's register. One trial was included (13 references) (Kerem 2014) and five trials (11 references) were excluded (Kerem 2008; McIntosh 2014b; Pradal 2002; Romano 2000; Wilschanski 2003). One cross-over trial is currently awaiting assessment until we are able to obtain clarification of data (Sermet-Gaudelus 2010) and one trial (one reference) is ongoing and potentially eligible for inclusion (McIntosh 2014a). The results of the search are displayed in the PRISMA diagram below (Figure 1). No additional trials were identified by screening references of included trials or by contacting authors of included trials, leaders in the field, and companies known to be developing and investigating ataluren and similar compounds.

Figure 1. Study flow diagram.



Included studies

We included one trial (13 references) with 238 participants; the trial was available as full text (Kerem 2014).

Trial design

The included trial was a RCT of parallel design and PTC Therapeutics Incorporated was the responsible funding body. The Cystic Fibrosis Foundation, the Food and Drug Administration's Office of Orphan Products Development and the National Institutes of Health (NIH) were also involved in providing grant support to the trial.

This phase 3 trial had two-arms and lasted 48 weeks. Participants who completed 48 weeks of treatment were entered into an ongoing open-label extension study which is planned to last up to 96 weeks (McIntosh 2014b). Participants from this extension trial are not eligible for inclusion in this review as they were not re-randomised to treatment or control.

The included trial was conducted at multiple centres, recruiting participants from 36 sites in 11 countries in North America and Europe (Kerem 2014). Outcome data were reported at time frames ranging from 8 to 48 weeks.

Participants

All 238 participants (118 male, 114 female) had a confirmed diagnosis of CF with documentation of the presence of a nonsense mutation in at least one allele of the CFTR gene. The trial recruited participants aged six years and over (mean age of 23 years). Participants were allocated in a 1:1 ratio to either intervention or placebo and were stratified according to age (under 18 years versus 18 years and over), chronic inhaled antibiotic use (yes versus no) and % predicted FEV_1 (40% up to 65% versus 65% up to 90%). All participants had a baseline FEV_1 of 40% or over for age, gender and height. The baseline characteristics presented were similar in the intervention and the placebo arms, with no statistical difference seen between the two arms (Kerem 2014).

Interventions

Ataluren (administered as a powdered oral suspension) was the intervention drug used in the included trial. The trial compared a three-times daily regimen of ataluren (10mg/kg in the morning, 10 mg/kg at mid-day and 20 mg/kg in the evening) to placebo. Participants continued on prescribed medications that were approved for CF during the trial period (Kerem 2014).

Outcomes

The primary endpoint for this trial was the relative change in % predicted FEV_1 from baseline to week 48 as assessed by spirometry. The trial used the CFQ-R respiratory domain to measure QoL and reported on the mean change in the score from baseline to week 48 in the supplementary text. The trial reported on the safety profile of ataluren and the pulmonary exacerbation rate using the modified Fuchs' definition. The change in total lung CT scores and sub-scores was presented in the trial. The trial also reported the absolute difference in change in sweat chloride concentration from baseline between the two treatment arms. The trial reported on the difference in change from baseline for BMI and body weight between the two groups. Nasal potential difference, a tertiary endpoint, was not included in this review as it is not yet a validated outcome (Kerem 2014).

Excluded studies

We excluded five trials (11 references) (Characteristics of excluded studies). One of these trials was of cross-over design and data from the first treatment arm could not be extracted independently (Pradal 2002). We have contacted the investigators of this trial requesting the relevant data, but to date it has not been made available to us. If in future, we are able to access the first-arm data, we will re-assess these trials for inclusion. One trial was excluded as it reported on the mechanism of action of the intervention and not on the clinical benefit (Wilschanski 2003).

Three additional trials were excluded: a phase II trial did not randomise participants to treatment and also entered participants into an open-label extension trial where again, participants were not randomised to control or to treatment (Kerem 2008); a second trial did not randomise to treatment and used participants homozygous for deltaF508 mutations as the control group, which was inappropriate for inclusion in this review (Romano 2000); the final trial (still ongoing) is an open-label extension of the included trial, where participants are not re-randomised and all participants receive the same treatment (McIntosh 2014b).

Studies awaiting assessment

One trial is currently listed as 'Awaiting assessment' (Sermet-Gaudelus 2010); the lead investigator has confirmed that the data belong to PTC Therapeutics Incorporated and we are contacting them for further details. Further details are available in the tables (Characteristics of studies awaiting classification).

Trial design

This is a multicentre (three centres across France and Belgium), randomised two-arm cross-over trial consisting of two cycles of 14 days on and 14 days off treatment.

Participants

The trial enrolled 30 participants with CF and nonsense mutations over the age of six years and up to the age of 18 years, with approximately equal numbers of males and females.

Interventions

The trial compared ataluren (as a powdered oral suspension) taken three times per day at either a lower dosing regimen (4 mg/kg in morning, 4 mg/kg at midday, 8 mg/kg in evening) or a higher dosing regimen (10 mg/kg in the morning, 10 mg/kg at midday, 20 mg/kg in the evening). The order of the cycles was randomised.

Outcomes

The trial's primary outcome was CFTR chloride transport (measured by nasal TEPD). Secondary outcomes included ion channel activity, proportion of cells showing atypical CFTR protein expression, disease-related clinical parameters (FEV_1, FVC, body weight), safety, compliance, pharmacokinetics and adverse events.

Ongoing studies

One trial is ongoing (McIntosh 2014a). For further details please see the table (Characteristics of ongoing studies).

Trial design

The trial is an RCT of parallel design funded by PTC Therapeutics Incorporated. This phase 3 trial has two arms and is planned to last 48 weeks (expected completion date is November 2016).

Participants

Participants aged six years and over are being recruited from 88 sites over 16 countries in North America, Europe and Australasia (expected enrolment is 208 participants). Inclusion criteria include (but not limited to): sweat chloride over 60 mEq/L; demonstration of an FEV_1 more than or equal to 40% and less than or equal to 90% of predicted; and documentation of the presence of a nonsense mutation in at least one allele of the CFTR gene. Of note, this trial states that participants who are using long-term inhaled aminoglycosides (e.g. tobramycin) or have received inhaled aminoglycosides in the four months prior to screening are to be excluded from the trial.

Interventions

Ataluren (as a powdered oral suspension) taken three times per day (10 mg/kg in the morning, 10 mg/kg at midday and 20 mg/kg in the evening) will be compared to matched placebo for 48 weeks.

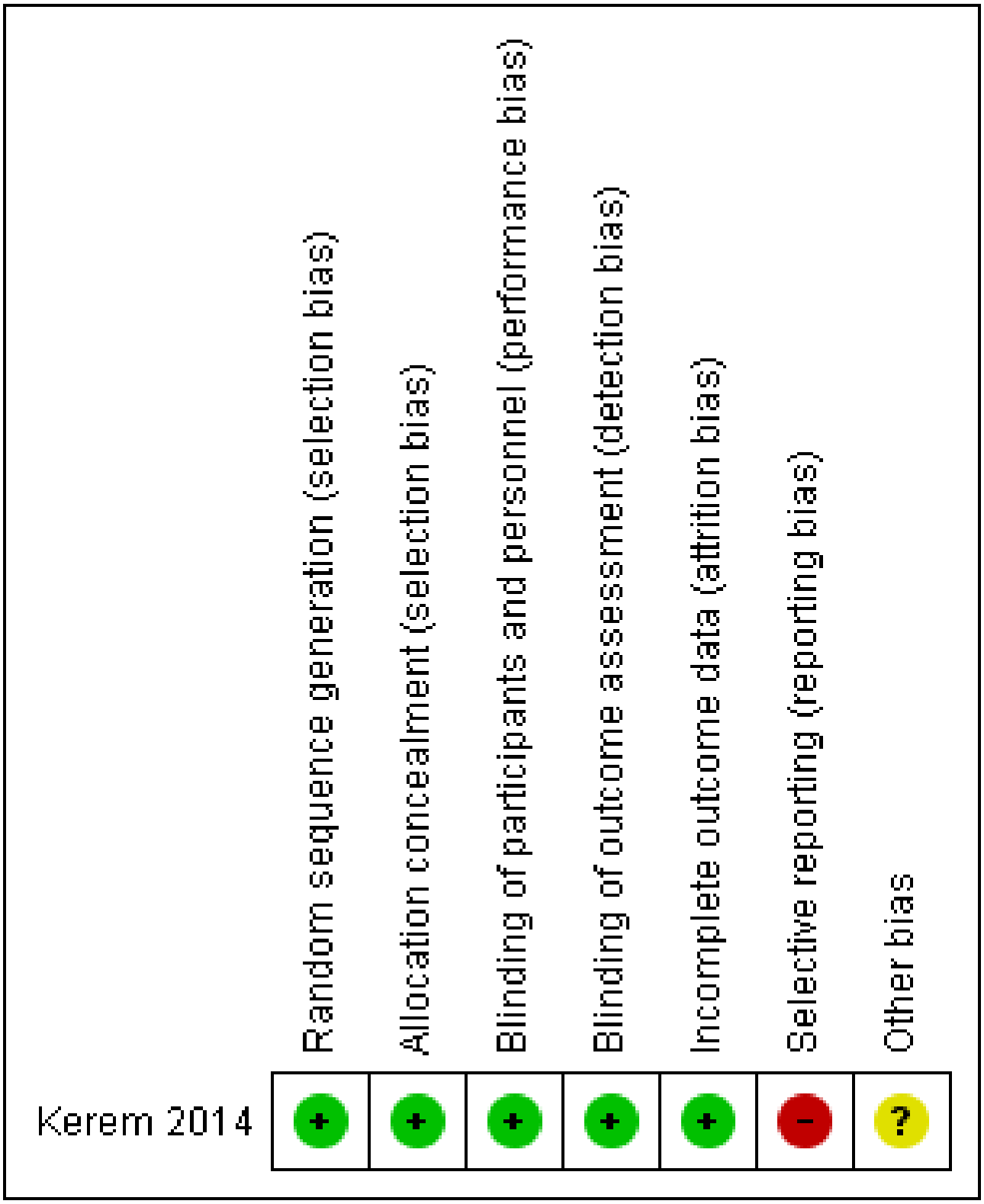
Outcomes

The primary endpoint for this ongoing trial is FEV_1 at 48 weeks assessed by spirometry. Secondary outcome measures include the rate of pulmonary exacerbations using modified Fuchs' criteria; respiratory QoL as assessed by the CFQ-R respiratory domain; and body weight and BMI.

Risk of bias in included studies

A graphical summary of the risk of bias assessments for the included trial is presented in Figure 2.

Figure 2. Risk of bias summary: review authors' judgements about each risk of bias item for each included study.



Allocation

Sequence Generation

The included trial reported that the randomisation code was externally generated using a small block size, stratified by age (under 18 years versus 18 years and over), chronic inhaled antibiotic use (yes versus no), and % predicted FEV_1 (40% up to 65% versus 65% up to 90%). We judged the trial to have a low risk of bias for sequence generation (Kerem 2014).

Allocation Concealment

Participants in the included trial were allocated to ataluren or placebo using interactive response technology according to the concealed randomisation list and we judged the risk of bias for allocation concealment to be low (Kerem 2014).

Blinding

The trial reported on blinding of trial personnel to treatment assignments including medical and ancillary staff, the trial investigators and the sponsor (Kerem 2014). The trial specifically stated that only designated personnel at the contract research organisation had access to the treatment assignments, therefore we judged the trial to have a low risk of bias with regards to blinding of personnel. The trial stated that participants were blinded to treatment assignment and that the placebo was identical in appearance to the active drug.Although it did not refer to the consistency or taste of the orally administered powder for suspension, we judge the trial to have a low risk of bias with regards to the blinding of participants (Kerem 2014).

Incomplete outcome data

Attrition was low (less than 15%) in the included trial; a total of 20 participants from the ataluren group and 14 participants from the placebo group withdrew from the trial (P = 0.36). Six participants (four from the ataluren and two from the placebo group) were excluded prior to week 8 as they did not have valid post-baseline spirometry. These participants were not included in the intention-to-treat population. There were 116 participants in the intention-to-treat population in each treatment arm. After week 8, 16 participants from the ataluren group withdrew from the trial: seven due to an adverse event; one participant was lost to follow up; nine withdrew consent; one due to investigator decision; one due to protocol non-compliance; and one withdrew for 'other' reasons. At this time, 12 participants from the placebo group withdrew from the trial: two due to an adverse event; nine withdrew consent; one due to protocol non-compliance; and two for 'other' reasons. The investigators reported the following missing values: 12% (104 out of 840) in the ataluren treatment group and 9% (73 out of 820) in the placebo group. The following missing data from the ataluren group were found by review authors: one participant's total lung CT score at week 48; and three participants change from baseline in sweat chloride concentration. In the placebo group, missing data were: one participant was reported by investigators to have a missing FEV_1 at week 48 and was excluded from the per-protocol population analysis; and seven participants change from baseline in sweat chloride concentration was found to be missing by review authors. We therefore judged there to be a low risk of bias due to incomplete outcome data.

Selective reporting

The protocol for the included trial was found through the online clinical trials registries database (NCT00803205). The following outcomes were included in the protocol, but not reported in the full publication: ataluren (PTC124) pharmacokinetics; antibiotic use and hospitalisation due to CF-related symptoms; and disruptions to school or work due to CF-related symptoms. For QoL, weight and BMI, the trial reported no significant difference in mean change from baseline between treatment arms but did not state either baseline and week 48 data, or the change from baseline in each group to allow further analysis. Regarding adverse events, the trial reported adverse events that occurred in more than 10% of the population and the adverse events that lead to discontinuation of treatment. The trial did not report whether an adverse event led to the interruption of treatment. By not reporting the aforementioned information, the trial is at high risk of selective outcome reporting.

The trial investigators undertook a post hoc subgroup analysis of participants depending on their use of chronic inhaled antibiotics; however, they only reported findings for selected outcomes in that subgroup (relative change from baseline in FEV_1, absolute values for FEV_1, pulmonary exacerbation rate and total lung CT scores). The investigators selectively reported data for participants who were or were not receiving inhaled tobramycin, and did not report data for other inhaled antibiotics. Absolute values for FEV_1 at baseline and week 48 were presented for the subgroup of participants who were not receiving chronic inhaled tobramycin, highlighting favourable results for ataluren in this particular group of participants. The trial did not report complete data to allow analysis (i.e. baseline/week 48/change from baseline) for non-significant outcomes, including total lung CT scores by subgroup and relative change from baseline in FEV_1 in participants on long-term inhaled tobramycin, and instead stated that there was no significant difference between treatment arms. Due to selectively reporting the findings of the subgroup of participants who were not receiving chronic inhaled tobramycin, we judge the trial to be at high risk of bias.

Other potential sources of bias

The baseline characteristics of participants in the treatment arms was similar. Median rate of compliance was based on trial drug accountability and was reported to be approximately 90% for ataluren compared to approximately 85% for placebo (Kerem 2014).

Kerem undertook a post hoc subgroup analysis of participants by chronic inhaled antibiotic use. This was not a subgroup that we planned to report on, but we have presented the results directly from the paper within our results below. The rationale for doing this analysis in the trial is not entirely clear; the investigators hypothesised that aminoglycosides, including inhaled tobramycin, interfere with ataluren at a ribosomal level. Kerem's post hoc in vitro study demonstrated that aminoglycosides (tobramycin or gentamicin) reduce ataluren's ability to read-through the PTC, and that this effect was not seen with the other tested inhaled antibiotics, colistin or aztreonam (Kerem 2014). There is no further scientific evidence for such an effect.

Effects of interventions

See: **Summary of findings for the main comparison**

Ataluren versus placebo

Primary outcomes

1. Quality of life

a. total QoL score

The included trial did not report on total QoL scores.

b. different sub-domains

The trial reported on the CFQ-R respiratory domain scores. It did not report absolute data at baseline and week 48; however, it did report on the mean change in CFQ-R respiratory domain scores from baseline to week 48. The reported difference in the mean changes between ataluren and placebo was 2.9 for the age group 6 to 13 years age and 0.5 for participants aged 14 and over, neither of which were statistically significant (P = 0.8152 and P = 0.3723 respectively). We were unable to conduct analyses due to insufficient data provided.

2. Respiratory function measures (litres or % predicted for age, sex and height)

a. FEV_1 (relative change from baseline)

Data were available for the relative change from baseline in % predicted FEV_1 at up to 48 weeks and also in graphical format at eight-weekly intervals (8, 16, 24, 32 and 40 weeks). At up to 48 weeks, both treatment arms showed a decline in mean relative change from baseline in FEV_1, the difference of which was not significant, MD 2.97% (95% CI -0.58 to 6.52) (P = 0.10). Using data deduced from the graph (figure 2 in full paper) (Kerem 2014), at eight weeks we found a significant benefit for ataluren compared to placebo, estimated MD 3.50 (95% CI 0.86 to 6.14) (P = 0.009). At the remaining time points, we demonstrated no estimated significant difference between treatment groups (Analysis 1.1).

Subgroup analysis
In Kerem's post hoc subgroup analysis of participants by chronic inhaled antibiotic use, in the subgroup of participants who did not receive chronic inhaled tobramycin, the trial reported a significant difference in the decline in mean relative change from baseline in FEV_1 at up to 48 weeks in the ataluren arm compared to placebo, MD 5.7% (95% CI 1.71 to 9.69) (P = 0.005) (Analysis 1.2) (Kerem 2014). At 8, 32 and 40 weeks, Kerem showed an estimated significant benefit for ataluren compared to placebo, MD 5.70 (95% CI 2.21 - 9.19) (P=0.001), 5.40 (95% CI 1.08 - 9.72) (P=0.01) and 4.00 (95% CI 0.03 - 7.97) (P=0.05) respectively. There was an estimated non-significant treatment difference at weeks 16 and 24, MD 1.40 (95% CI -1.94 to 4.74) (P = 0.41) and MD 1.80 (95% -2.52 to 6.12) (P = 0.41) respectively (data estimated from figure 3 in full paper) (Analysis 1.2) (Kerem 2014).

In the subgroup of participants who were receiving chronic inhaled tobramycin, Kerem reported a non-significant treatment difference at up to 48 weeks, MD -1.60% (95% CI -8.15 - 4.95) (P = 0.63). There was an estimated significant difference at 24 weeks in the subgroup of participants receiving chronic inhaled tobramycin which favoured placebo, MD -6.80 (95% CI -12.35 to -1.25) (P = 0.02) and no estimated significant treatment difference at each of the other eight-weekly time points in this subgroup (data estimated from figure 3 in full paper) (Analysis 1.3) (Kerem 2014).

b. FEV_1 absolute values

The trial reported on FEV_1 absolute values at baseline and at week 48 and reported no significant difference in baseline FEV_1 between the ataluren and placebo treatment arms. There was no statistical difference in mean change from baseline at week 48 between treatment groups, MD 1.76% (95% -0.43 to 3.95) (P = 0.12) (Analysis 1.4) (Kerem 2014).

Subgroup analysis
In Kerem's subgroup analysis, there was a reduction in the decline in mean absolute change in FEV_1 in the ataluren group compared to the placebo group, MD 3.40% (95% CI 0.75 to 6.05) (P = 0.01) in participants not taking chronic inhaled tobramycin (Analysis 1.5) (Kerem 2014). No data for mean absolute change in FEV_1 was reported for participants who were receiving long-term inhaled tobramycin.

c. FVC (absolute values and relative change from baseline)

Estimates for mean relative change from baseline in FVC at each eight-weekly time point, including week 48, have been deduced from graphical representation (supplementary figure 1 of the paper) (Kerem 2014). There was no estimated statistical difference in the mean relative change from baseline in FVC at any time point (8, 16, 24, 32, 40 or 48 weeks), MD at 48 weeks 1.10 (95% CI -1.97 to 4.17) (P = 0.48) (Analysis 1.6) (Kerem 2014).

3. Adverse events

The investigators classified adverse events by severity as grade 1 (mild), grade 2 (moderate), grade 3 (severe) and grade 4 (life-threatening or death); however, they did not specify the criteria for grade 1 to 3 classifications. It was not possible for review authors to determine which reported adverse events they would class as mild (therapy does not need to be discontinued) or moderate (therapy is discontinued, and the adverse effect ceases). The trial reported on adverse events which required discontinuation of therapy under the heading 'severe (life-threatening or debilitating, or which persists even after treatment is discontinued)'.

In the included trial, more participants in the placebo group experienced diarrhoea, nausea, pyrexia, upper respiratory tract infections, rhinitis, pulmonary exacerbations, cough, haemoptysis and oropharyngeal pain compared to participants in the ataluren group. Abdominal pain, vomiting, sinusitis, headache and productive cough occurred more in participants in the ataluren group. Regarding statistically significant adverse events, oropharyngeal pain was less common in the ataluren group, RR 0.28 (95% CI 0.10 to 0.83). A significantly higher number of participants in the ataluren group reported episodes of acute kidney injury (includes reported terms including renal failure, acute renal failure, renal impairment, and hypercreatininaemia), RR 17.70 (99% CI 1.28 to 244.40) (Analysis 1.7) (Kerem 2014).

a. mild (therapy does not need to be discontinued)

Investigators reported fewer grade 1 (mild) adverse events in the ataluren group compared to placebo, which was not significant RR 0.88 (95% 0.49 to 1.57) (P = 0.66) (Analysis 1.8) (Kerem 2014).

b. moderate (therapy is discontinued, and the adverse effect ceases)

The trial reported a non significant difference in grade 2 adverse events between the ataluren group compared to placebo, RR 1.21 (95% 0.99 to 1.49) (P = 0.06) (Analysis 1.8) (Kerem 2014).

The trial did not report on adverse events that specifically required interruption of treatment.

c. severe (life-threatening or debilitating, or which persists even after treatment is discontinued)

The trial reported that eight participants from the ataluren group and three participants from the placebo group discontinued therapy due to adverse events. The difference between treatment groups in investigator-defined grade 3 (severe) adverse events was not significant, RR 0.62 (95% CI 0.37 to 1.03) (P = 0.07) (participants may have had more than one adverse event) (Analysis 1.8) (Kerem 2014). There were no life-threatening events reported (Kerem 2014).

Secondary outcomes

1. Survival

a. time to event (death or lung transplant)

Zero deaths were reported and the trial did not report on lung transplant (Kerem 2014).

b. mortality rate

The trial reported zero deaths in both treatment groups (Kerem 2014).

2. Hospitalisation

No data were reported for hospitalisation (Kerem 2014).

3. School or work attendance

The trial did not report on school or work attendance (Kerem 2014).

4.
Extra courses of antibiotics

No data were reported for extra courses of antibiotics (Kerem 2014).

5. Pulmonary exacerbations

This trial reported both on physician-defined (based on the individual physician's judgement) and protocol-defined pulmonary exacerbation rate (assessed using modified Fuchs' criteria). These criteria defined a pulmonary exacerbation as four of the following 12 symptoms, with or without treatment with antibiotics: change in sputum; new or increased haemoptysis; increased cough; increased dyspnoea; fatigue; temperature over 38°C; anorexia; sinus pain; change in sinus discharge; change in physical examination of the chest; decrease in pulmonary function by 10% or more from a previously recorded value; or radiographic changes indicative of pulmonary infection (Fuchs 1994). No significant treatment difference was found between ataluren and placebo for either the physician- or protocol-defined pulmonary exacerbation rate at up to 48 weeks, MD -0.02 (95% CI -0.67 to 0.63) and -0.36 (95% CI -0.89 to 0.17) respectively (Analysis 1.9) (Kerem 2014).

Subgroup Analysis

In Kerem's subgroup analysis they reported a significant reduction in the protocol defined pulmonary exacerbation rate in participants not taking chronic inhaled tobramycin, MD -0.76 (95% CI -1.51 to -0.01) compared to a higher rate in those receiving chronic inhaled tobramycin, which was not significant 0.36 (95% CI -0.28 to 1.00) (Analysis 1.10) (Kerem 2014).

Participants receiving chronic inhaled tobramycin appeared to have a reduced rate of pulmonary exacerbation compared to those not receiving chronic inhaled tobramycin, suggesting that tobramycin may have a treatment effect, possibly as a result of some correction of the underlying defect (Kerem 2014).

6. Radiological measures of lung disease

a. chest radiograph score

The included trial did not report on chest radiograph score.

b. computerised tomogram (CT) score

The included trial reported on baseline, end of treatment (approximately 48 weeks) and change from baseline in total lung CT scores and subscores. The difference in mean change in total lung CT score was not significant at up to end of treatment (approximately 48 weeks), MD -0.28 (95% CI -0.68 to 0.12) (Analysis 1.11) (Kerem 2014).

Subgroup Analysis

Kerem reported no significant difference between ataluren and placebo in changes in total lung CT scores in their subgroup analysis by inhaled tobramycin use (Kerem 2014). Data were not reported for analysis.

7. Acquisition of respiratory pathogens

The included trial did not report on this outcome.

8. Eradication of respiratory pathogens

No data was reported for eradication of respiratory pathogens.

9. Nutrition and growth

a. weight

The trial reported no statistical difference in change in body weight between ataluren and placebo over 48 weeks (difference = 0.04 kg). Insufficient data were presented for inclusion in our analysis (Kerem 2014).

b. BMI

As with weight, the trial reported no statistical difference in change in BMI between treatment groups over 48 weeks (difference = -0.108); further baseline and week 48 data were not reported to allow analysis (Kerem 2014).

c. height

Height was not reported in the trial (Kerem 2014).

10. Sweat chloride

The trial reported a non-significant difference in change from baseline in sweat chloride between ataluren and placebo at up to 48 weeks, MD-0.70 (95% CI -3.41 to 2.01) (P = 0.61) (Analysis 1.12) (Kerem 2014).

11. Cost effectiveness (cost utility assessed as comparison of impact on quality of adjusted life years)

The trial did not report on the cost of ataluren treatment for people with CF (Kerem 2014).

DISCUSSION

Summary of main results

This review examines whether specific therapies for class I mutations (premature termination codons (PTCs)) improve clinically relevant outcomes in people with cystic fibrosis (CF). Our search identified 28 references for eight trials; six trials were excluded (three cross-over trials and three non-randomised trials), one trial is ongoing and one trial was included. The included randomised controlled trial (RCT) of parallel design enrolled 238 participants aged six years and over (range 6 to 53 years old) with documentation of the presence of a nonsense mutation in at least one allele of the cystic fibrosis transmembrane conductance regulator (CFTR) gene. The trial compared 40 mg/kg/day (in divided doses) of orally administered ataluren to placebo for a duration of 48 weeks. The trial was assessed as low risk for selection and attrition bias, unclear risk for performance bias and high risk for selective reporting.

Ataluren was no better than placebo for the review's primary or secondary outcome measures at up to 48 weeks. The trial did not report a significant difference in CFQ-R respiratory domain scores between treatment groups for either the 6 to13 year-old age group or for those aged 14 years and over. There was no significant improvement in respiratory function measures (relative or absolute change in forced expiratory volume at one second (FEV_1) and relative change in forced vital capacity (FVC)) at up to 48 weeks in participants treated with ataluren. We were unable to report on mild or moderate adverse events as defined by the review's protocol. Reduced reports of oropharyngeal pain were recorded in the ataluren group, risk ratio (RR) 0.28 (95% CI 0.10 to 0.83). Ataluren was associated with a significantly higher rate of episodes of acute kidney injury (includes reported terms including renal failure, acute renal failure, renal impairment, and hypercreatininaemia) RR 17.70 (99% CI 1.28 to 244.40). The adverse events profile of ataluren should be interpreted with caution as it was based on small numbers of participants and the trial was not powered to assess this outcome.

The trial reported no significant reduction in pulmonary exacerbation rates (either physician- or protocol-defined) or sweat chloride concentration in the ataluren group compared to the placebo group. Furthermore, there was no significant difference in change in total lung computer tomography (CT) score, body weight or body mass index (BMI) over 48 weeks.

No deaths were reported in either treatment group and the length and size of the trial precluded valid assessment of the impact of ataluren on survival. An open-label extension trial of this phase 3 RCT is ongoing and although not eligible for full inclusion due to lack of randomisation and control, we aim to review results with regards to survival data.

The trial investigators undertook a post hoc subgroup analysis of participants not receiving chronic inhaled tobramycin (n = 146) which demonstrated favourable results for ataluren. They reported a reduction in the decline in FEV_1 over 48 weeks in participants treated with ataluren (n = 72, compared to placebo, n = 74, MD 5.7% (95% CI 1.71% to 9.69). Additionally, in this subgroup of participants, those treated with ataluren were seen to have significant reduction in pulmonary exacerbation rates (protocol-defined), MD -0.76 (95% CI -1.51 to -0.01).

Some aminoglycosides, including tobramycin and gentamicin, have previously been demonstrated to have the ability to mask the abnormal gene sequence, resulting in significant changes of the salt transporting defect towards normal (Clancy 2001; Howard 1996; Wilschanski 2003). The mechanism of action for such aminoglycosides is thought to be similar to that of ataluren, in that they enable read-through of class I mutations (PTCs) (Welch 2007). It is possible that Kerem's subgroup of participants receiving chronic inhaled tobramycin may have been exposed to read-through from the aminoglycoside and therefore they would not further benefit from ataluren.

Overall completeness and applicability of evidence

The included trial recruited a mixture of adults and children with CF with nonsense mutations across 36 sites internationally. The mean age was 23 years of age with 32% of participants aged under 18 years of age and the remaining participants aged 18 years and over. Nonsense mutations are a type of class I mutation (PTC) and the results can be assumed be applicable to individuals with nonsense CF mutations not included in this trial. Of note, however, is that the current trial did not enrol participants aged under six years of age, pregnant women or those with stop codon mutations (another form of class I mutations (PTCs)). There are insufficient data for outcomes which we considered important for this review: hospitalisation, school or work attendance, extra courses of antibiotics, chest radiograph score, acquisition or eradication of respiratory pathogens and cost effectiveness.

Kerem's post hoc analysis of the subgroup of participants not receiving chronic inhaled tobramycin only reported on results for specific outcomes (absolute and relative change in % predicted FEV_1 from baseline, pulmonary exacerbation rate and total lung CT scores). Results for this subgroup were not available for two out of three of this review's primary outcomes (quality of life (QoL) and adverse events).

Quality of the evidence

Please see section in table (Summary of findings for the main comparison). The trial was an RCT which represents the highest quality with regards to trial design. The trial included a total of 238 participants and was well-powered for the primary outcome (relative change in % predicted FEV_1). The randomised phase of the trial lasted 48 weeks and participants have been entered into an open-label extension trial due to last 96 weeks (open-label extension results not included in this review). The overall risk of bias was judged to be moderate with well-documented random sequence generation, allocation concealment and blinding of trial personnel. Blinding of participants was less clear, as details of orally administered placebo compared to ataluren (such as taste and consistency) were not provided. The risk of selective reporting was generally high as the trial did not always report on outcomes outlined in the protocol, present data for all outcomes and report on outcomes considered important by the review authors. The trial was sponsored by the manufacturer of ataluren, PTC Therapeutics Incorportated. Some trial authors declared a financial interest: eight out of 27 authors are employees of PTC Therapeutics Incorportated and hold financial interests in the company; two authors received compensation for consultant services from PTC Therapeutics Incorportated during the trial; and four authors received compensation for travel expenses for meetings related to the trial.

Overall, we judged the quality of the evidence to be moderate (Summary of findings for the main comparison).

Potential biases in the review process

Two authors independently applied inclusion and exclusion criteria to references found through a comprehensive literature search (AA and CH). The authors then individually extracted data and assessed the risk of bias in the included trial. If any disagreement had arisen on the risk of bias of the trial, we would have attempted to reach a consensus by discussion, failing which a third author (KWS) would have arbitrated. The analysis was undertaken by one review author (AA). This methodological approach ensured that risks of bias in the review process were kept to a minimum. This review has assessed all available published trial data. Trial authors were contacted for relevant unpublished information and individual participant data. None have been made available to date. We are not aware of any unpublished trials, although we have highlighted issues with selective reporting in the included trials.

We planned to include cross-over trials if results from the first treatment arm were available. Independently assessing the two relevant cross-over trials which were identified, we were unable to extract their data and therefore contacted the investigators. We consequently listed one trial as 'Awaiting assessment' until we are able to further clarify details with PTC Therapeutics Incorporated (owners of the data) and excluded one trial as we received no response from the investigators. We are aware that this reduces the number of trials we could include in the review. We acknowledge that the primary outcome for the included trial was relative change in % predicted FEV_1 and therefore it may not be powered to detect a difference in our other primary outcomes, QoL and adverse events. Therefore, an absence of evidence of a difference in QoL or for the majority of adverse events does not rule out that ataluren may improve QoL or have a higher adverse event profile compared to placebo.

Agreements and disagreements with other studies or reviews

One trial was eligible for inclusion in this review. In future, should further trials become eligible, we will update this review. An ongoing trial states that participants who are using long-term inhaled aminoglycosides (e.g. tobramycin) or have received inhaled aminoglycosides in the four months prior to screening are to be excluded from the trial. We will assess eligibility for this trial against outlined criteria. This difference in participant baseline characteristics is a potential confounding factor for future updates.

We are not aware of any other published systematic reviews of ataluren and similar compounds (specific therapies for premature termination codon class I mutations) for CF.

AUTHORS' CONCLUSIONS

Implications for practice

There is currently insufficient evidence to determine the effect of ataluren as a therapy for people with CF with class I mutations. Ataluren was associated with a significantly higher rate of episodes of renal impairment, the degree of which requires further information.

Implications for research

It is important that future trials assess outcomes to determine the long-term use of ataluren in terms of both efficacy and tolerability. Future trials should be powered to detect a difference not only in relative change from baseline in FEV_1 but also in QoL and adverse events; outcomes of significant importance to people with CF. In particular, the clear reporting of adverse events is critical, with particular attention to episodes of renal impairment. Criteria for any grading classifications used when reporting adverse events should be clearly stated.

Given the chronic nature of CF, it is important that the burden of additional treatment to people's current regimen is examined as well as the potential impact of new therapies on adherence to existing CF medications.

Cross-over trials should be avoided given the potential for the treatment to change the natural history of CF. Furthermore, future trials should be large enough to enable detailed analysis of the effectiveness of ataluren in population subgroups, for example, adults and children.

Currently, there are no trials comparing ataluren to treatment with other compounds for class I mutations (PTCs) in CF. In particular, given the results of Kerem's subgroup analysis by inhaled antibiotic use, further work looking at any treatment differences of aminoglycosides versus ataluren in people with CF with class I mutations (PTCs) is required. At present, one ongoing study is investigating ataluren specifically in people with CF with class I mutations (PTCs) who are not on chronic inhaled aminoglycosides (McIntosh 2014a). The rationale for undertaking a trial enrolling this subgroup of participants is not clear. Further research examining the interaction of tobramycin and ataluren is required, in particular characterising the potential of tobramycin to cause read-through of PTCs.

Determinations of resource use and the cost of ataluren treatment are important and ongoing health economic evaluations are required.

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* Indicates the major publication for the study

CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Kerem 2014

Methods	2-arm RCT of parallel design. Multicentre: 36 sites. Duration: 48 weeks.
Participants	Participants with CF aged 6 years and over with a nonsense mutation in at least one allele of the CFTR gene. A total of 238 participants were enrolled into this study. **Age** Treatment group: mean (SD) 22.8 (10.18) years; median (range) 22.0 (6 - 49) years. Control group: mean (SD) 23.2 (9.32) years; median (range): 22.0 (8 - 53) years. **Age split by group** Treatment group: under 18 years n (%) 38 (32.8%); 18 years and over n (%) 78 (67.2%). Control group: under 18 years n (%) 37 (31.9%); 18 years and over n (%): 79 (68.1%). **Sex** Treatment group: n (%) 60 (51.7%) males, 56 (48.3%) females. Control group: n (%) 58 (50%) males, 58 (50%) females. **Body weight** Treatment group: mean (SD) 53.5 (13.94) kg; median (range): 54.4 (21 - 105) kg. Control group: mean (SD) 56.0 (13.15) kg; median (range): 57.2 (24 - 93) kg. **% predicted FEV_1 at baseline** Treatment group: mean (SD) 62.1 (13.62); median (range) 63.4 (38.4 - 90.3). Control group: mean (SD) 60.2 (15.14); median (range): 59.0 (36.2 - 92.6). **Sweat chloride at baseline** Treatment group: mean (SD) 100.1 (14.22); median (range) 101.5 (22.5 - 128.0). Control group: mean (SD) 96.6 (15.93): median (range): 100.0 (22.0 - 117.5). **Inhaled antibiotic use at randomisation** Total n (%): treatment group 64 (55.2%); control group 63 (53.4%). Aminoglycoside (tobramycin) n (%): treatment group 44 (37.9%); control group 42 (35.6%). Colistin n (%): treatment group 30 (25.9%); control group 22 (18.6%). Aztreonam n (%): treatment group 10 (8.3%); control group 8 (6.8%).
Interventions	Treatment group (n = 120): ataluren 3 times a day - 10 mg/kg in the morning, 10 mg/kg at midday, 20 mg/kg in the evening. Control (n = 118): placebo 3 times a day.
Outcomes	**Primary outcome measure** 1. FEV_1* **Secondary outcome measures** 1. Pulmonary exacerbation frequency*

Kerem 2014 *(Continued)*

	2. Cough frequency* 3. Respiratory health related quality of life as assessed by the CFQ-R respiratory domain* 4. FVC* 5. Safety profile* 6. Compliance with trial drug administration* 7. Ataluren (PTC124) pharmacokinetics 8. Antibiotic use and hospitalisation due to CF-related symptoms 9. Disruptions to school or work due to CF-related symptoms 10. Body weight* 11. Markers of lung inflammation 12. Lung computerized tomography CF score* 13. Nasal TEPD* 14. Sweat chloride concentration*
Notes	PTC Therapeutics Incorporated was the responsible funding body. *These outcomes are presented in the review.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	The randomisation code was externally generated using a small block size, stratified by age (<18 versus ≥18 years), chronic inhaled antibiotic use (yes versus no), and % predicted FEV_1 (40% to <65% versus ≥65% to 90%).
Allocation concealment (selection bias)	Low risk	Interactive response technology was used to allocate participants to ataluren or placebo.
Blinding of participants and personnel (performance bias) All outcomes	Low risk	All participants were blinded to treatment; details of consistency and taste of placebo have not been provided.
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Only designated personnel at the contract research organisation had access to the treatment assignments. Medical and ancillary staff, the trial investigators and the sponsor were blinded to treatment.
Incomplete outcome data (attrition bias) All outcomes	Low risk	238 participants were randomised; 120 participants to the ataluren group and 118 participants to the placebo group. 4 participants from the ataluren group and 2 from the placebo group were excluded prior to week 8 and were excluded from the ITT population. The ITT population included 116 participants in each treatment arm. 16 participants withdrew from the study in ataluren group and 12 in the placebo group. 100 participants in the ataluren group and 104 in the placebo group were included in analysis (total = 204). The review authors found minor discrepancies in the number of participants analysed for the following outcome measures: total lung CT score and sweat chloride concentration.
Selective reporting (reporting bias)	High risk	Protocol located on online trials registries database. The following outcomes were listed in the protocol but not published: 1. Ataluren (PTC124) pharmacokinetics 2. Antibiotic use and hospitalisation due to CF-related symptoms 3. Disruptions to school or work due to CF-related symptoms

Kerem 2014 *(Continued)*

		Subgroup analysis of participants not receiving chronic inhaled tobramycin showing significant benefit for ataluren were reported for FEV_1 and pulmonary exacerbations. Results for this subgroup were not reported for each outcome and results for participants on other inhaled antibiotics were not fully reported.
Other bias	Unclear risk	Similar baseline characteristics and median rate of compliance (approximately 90% for ataluren and approximately 85% for placebo); however, the rationale for the subgroup is not clear and may be flawed leading to a potential source of bias.

CF: cystic fibrosis
CFQ-R: cystic fibrosis questionnaire - revised
CFTR: cystic fibrosis transmembrane conductance regulator
FEV_1: forced expiratory volume in 1 second
FVC: forced vital capacity
ITT: intention to treat
RCT: randomised controlled trial
SD: standard deviation
TEPD: transepithelial potential difference

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Kerem 2008	Participants not controlled, randomised or blinded to treatment.
McIntosh 2014b	Not a randomised controlled trial; participants invited to single allocation safety trial after completing a previous randomised controlled trial.
Pradal 2002	Cross-over trial and data from first treatment arm not presented independently. Data requested, but no response to date.
Romano 2000	Controlled clinical trial: participants were not randomised or blinded to treatment. Controls stated to be participants homozygous for deltaF508 mutations.
Wilschanski 2003	Cross-over trial in which each participant served as their own control. Reported on mechanism of action of the intervention (nasal potential difference and immunofluorescence microscopy of primary human airway cells) which were not relevant to this review which is looking at clinical benefit.

Characteristics of studies awaiting assessment *[ordered by study ID]*

Sermet-Gaudelus 2010

Methods	Randomised controlled trial. Cross-over design. Duration: 2x 28-day cycles of 14-day on and 14-day off. Location: multicentre (3 centres - 1 in France and 2 in Belgium).
Participants	Children (at least 6 years old and no older than 18 years) with a nonsense mutation of CF. 30 participants (16 children aged 6 - 12 years and 14 adolescents aged 13 - 18 years) enrolled, no drop outs.

Sermet-Gaudelus 2010 *(Continued)*

	Age (median (range)): 12 (6 - 18) years. Gender: 16 males, 14 females.
Interventions	Ataluren in the form of vanilla flavoured granules (in aluminium foil sachets) to mix with either water, apple juice or milk to make a suspension for oral administration. Treatment taken 3x daily with dosage based on weight, but cycles had different dosing levels. Order of dose levels randomised. Cycle 1 (lower dosing regimen): 4 mg/kg in morning, 4 mg/kg at midday, 8 mg/kg in evening. Cycle 2 (higher dosing regimen): 10 mg/kg in morning, 10 mg/kg at midday, 20 mg/kg in evening.
Outcomes	Primary outcome: CFTR chloride transport (nasal TEPD). Secondary outcomes: ion channel activity, proportion of cells showing atypical CFTR protein expression, disease-related clinical parameters, safety, compliance, pharmacokinetics, FEV_1, FVC, body weight, adverse events.
Notes	Trial drug provided by PTC Therapeutics. Powered to detect change in total chloride transport.

CF: cystic fibrosis
CFTR: cystic fibrosis transmembrane conductance regulator
FEV_1; forced expiratory volume in one second
FVC: forced vital capacity
TEPD: transepithelial potential difference

Characteristics of ongoing studies *[ordered by study ID]*

McIntosh 2014a

Trial name or title	A Phase 3 Efficacy and Safety Study of Ataluren (PTC124®) in Patients With Nonsense Mutation Cystic Fibrosis
Methods	Phase 3, international, multicentre, randomised, double-blind, placebo-controlled trial of ataluren in participants with nonsense mutation cystic fibrosis not receiving chronic inhaled aminoglycosides.
Participants	Participants to be recruited from 88 sites across 16 countries in North America, Europe and Australasia. Inclusion criteria include (but not limited to): age 6 years and over; sweat chloride >60 mEq/L; demonstration of an $FEV_1 \geq 40\%$ and $\leq 90\%$ of predicted; and documentation of the presence of a nonsense mutation in at least 1 allele of the CFTR gene. Estimated enrolment: 208
Interventions	Ataluren (PTC124®) in oral powder for suspension form taken 3 times per day (10 mg/kg in the morning, 10 mg/kg at midday and 20 mg/kg in the evening for 48 weeks. Placebo.
Outcomes	**Primary outcome measure** 1. FEV_1 by spirometry **Secondary outcome measures** 1. Rate of pulmonary exacerbations (modified Fuchs' criteria) 2. Respiratory health related quality of life as assessed by the CFQ-R respiratory domain

McIntosh 2014a *(Continued)*	3. Body weight and BMI **Other outcome measures** 1. FVC and FEF25-75 by spirometry 2. Incidence and duration of pulmonary exacerbations 3. Other health related quality of life domains as assessed by the CFQ-R 4. Concentrations of liver enzyme tests (AST, ALT, GGT) 5. Fecal calprotectin level 6. New Pseudomonas aeruginosa lung infection 7. Safety profile characterized by type, frequency, severity, timing, and relationship to study drug of treatment-emergent adverse events, laboratory abnormalities, and ECG abnormalities 8. Trial drug compliance as assessed by quantification of unused trial drug 9. Trough ataluren plasma concentrations Timeframe for all outcomes measures stated to be 48 weeks.
Starting date	June 2014
Contact information	Joseph McIntosh, MD PTC Therapeutics
Notes	Estimated Study Completion Date: November 2016

ALT: alanine aminotransferase
AST: aspartate aminotransferase
BMI: body mass index
CFQ-R: cystic fibrosis questionnaire - revised
CFTR: cystic fibrosis transmembrane conductance regulator
ECG: electrocardiogram
FEF25-75: mid expiratory flow
FEV_1: forced expiratory volume in 1 second
FVC: forced vital capacity
GGT: gamma-glutamyl transpeptidase

DATA AND ANALYSES

Comparison 1. Ataluren versus placebo

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 FEV_1 - mean relative change from baseline	1		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
1.1 8 weeks	1	232	Mean Difference (IV, Fixed, 95% CI)	3.5 [0.86, 6.14]
1.2 16 weeks	1	232	Mean Difference (IV, Fixed, 95% CI)	1.5 [-1.29, 4.29]
1.3 24 weeks	1	232	Mean Difference (IV, Fixed, 95% CI)	1.5 [-1.69, 4.69]
1.4 32 weeks	1	232	Mean Difference (IV, Fixed, 95% CI)	2.8 [-0.54, 6.14]
1.5 40 weeks	1	232	Mean Difference (IV, Fixed, 95% CI)	2.3 [-1.07, 5.67]
1.6 At up to 48 weeks	1	203	Mean Difference (IV, Fixed, 95% CI)	2.97 [-0.58, 6.52]

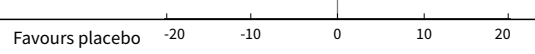
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
2 FEV_1 - mean relative change from baseline: subgroup analysis of participants NOT receiving chronic inhaled tobramycin	1		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
2.1 8 weeks	1	146	Mean Difference (IV, Fixed, 95% CI)	5.7 [2.21, 9.19]
2.2 16 weeks	1	146	Mean Difference (IV, Fixed, 95% CI)	1.4 [-1.94, 4.74]
2.3 24 weeks	1	146	Mean Difference (IV, Fixed, 95% CI)	1.80 [-2.52, 6.12]
2.4 32 weeks	1	146	Mean Difference (IV, Fixed, 95% CI)	5.4 [1.08, 9.72]
2.5 40 weeks	1	146	Mean Difference (IV, Fixed, 95% CI)	4.00 [0.03, 7.97]
2.6 At up to 48 weeks	1	146	Mean Difference (IV, Fixed, 95% CI)	5.7 [1.71, 9.69]
3 FEV_1 - mean relative change from baseline: subgroup analysis of participants receiving chronic inhaled tobramycin	1		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
3.1 8 weeks	1	86	Mean Difference (IV, Fixed, 95% CI)	0.30 [-4.02, 4.62]
3.2 16 weeks	1	86	Mean Difference (IV, Fixed, 95% CI)	0.40 [-3.92, 4.72]
3.3 24 weeks	1	86	Mean Difference (IV, Fixed, 95% CI)	-6.80 [-12.35, -1.25]
3.4 32 weeks	1	86	Mean Difference (IV, Fixed, 95% CI)	-1.5 [-7.88, 4.88]
3.5 40 weeks	1	86	Mean Difference (IV, Fixed, 95% CI)	-0.80 [-7.23, 5.63]
3.6 At up to 48 weeks	1	86	Mean Difference (IV, Fixed, 95% CI)	-1.60 [-8.15, 4.95]
4 FEV_1 - mean absolute change from baseline	1		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
4.1 At up to 48 weeks	1	203	Mean Difference (IV, Fixed, 95% CI)	1.76 [-0.43, 3.95]
5 FEV_1 - mean absolute change from baseline: subgroup of participants not receiving chronic inhaled tobramycin	1		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
5.1 At up to 48 weeks	1	125	Mean Difference (IV, Fixed, 95% CI)	3.40 [0.75, 6.05]
6 FVC - mean relative change from baseline	1		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
6.1 8 weeks	1	232	Mean Difference (IV, Fixed, 95% CI)	2.00 [-0.37, 4.37]
6.2 16 weeks	1	232	Mean Difference (IV, Fixed, 95% CI)	0.7 [-1.93, 3.33]

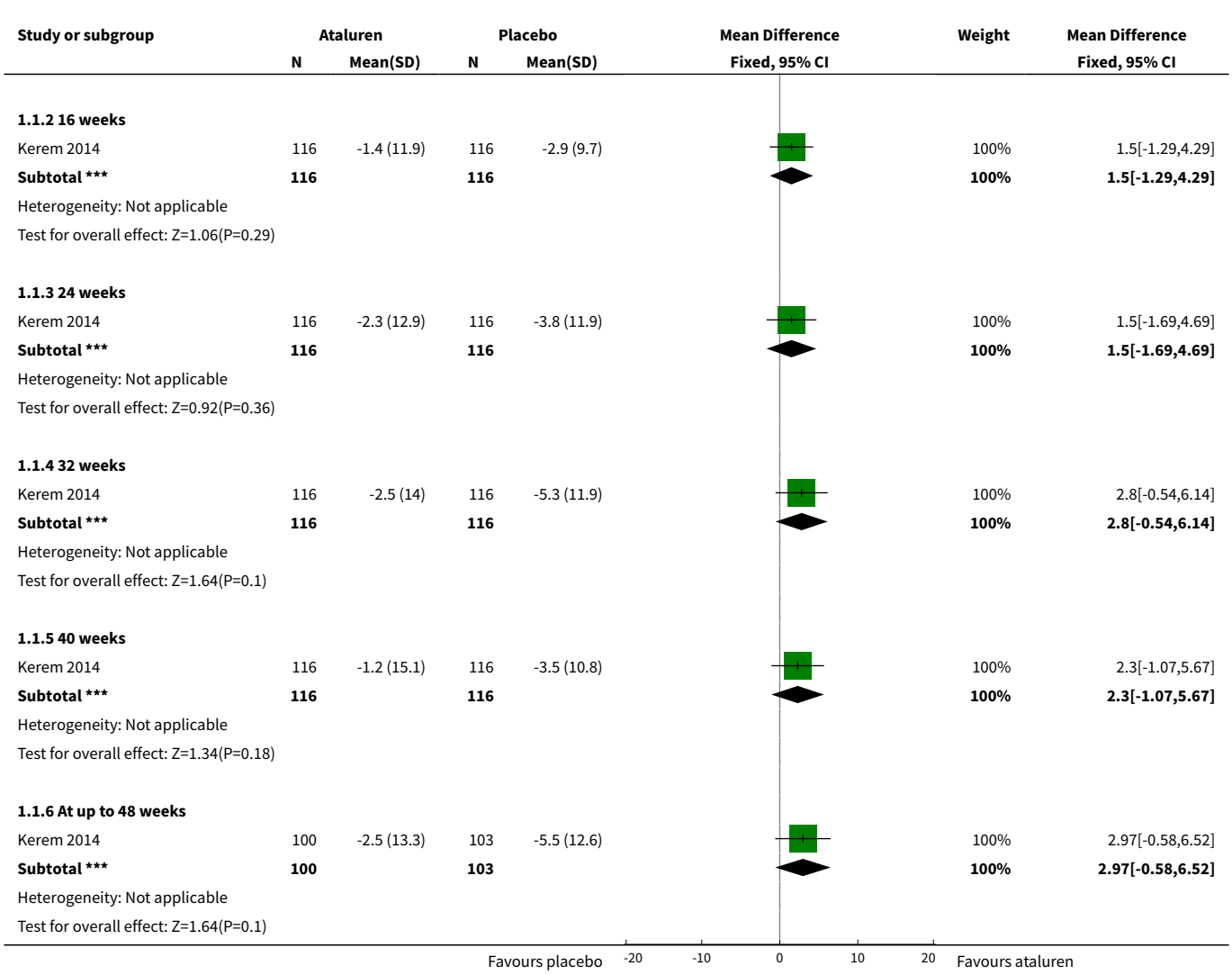
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
6.3 24 weeks	1	232	Mean Difference (IV, Fixed, 95% CI)	1.3 [-1.69, 4.29]
6.4 32 weeks	1	232	Mean Difference (IV, Fixed, 95% CI)	1.80 [-1.53, 5.13]
6.5 40 weeks	1	232	Mean Difference (IV, Fixed, 95% CI)	1.2 [-1.79, 4.19]
6.6 At up to 48 weeks	1	232	Mean Difference (IV, Fixed, 95% CI)	1.1 [-1.97, 4.17]
7 Adverse events occurring in greater than 10% of trial participants	1		Risk Ratio (M-H, Fixed, 99% CI)	Subtotals only
7.1 Diarrhoea	1	238	Risk Ratio (M-H, Fixed, 99% CI)	0.61 [0.32, 1.16]
7.2 Abdominal Pain	1	238	Risk Ratio (M-H, Fixed, 99% CI)	1.18 [0.62, 2.23]
7.3 Vomiting	1	238	Risk Ratio (M-H, Fixed, 99% CI)	1.38 [0.64, 2.98]
7.4 Nausea	1	238	Risk Ratio (M-H, Fixed, 99% CI)	0.90 [0.41, 1.96]
7.5 Pyrexia	1	238	Risk Ratio (M-H, Fixed, 99% CI)	0.84 [0.47, 1.50]
7.6 Viral Upper Respiratory Tract Infections	1	238	Risk Ratio (M-H, Fixed, 99% CI)	0.71 [0.43, 1.17]
7.7 Sinusitis	1	238	Risk Ratio (M-H, Fixed, 99% CI)	1.05 [0.53, 2.09]
7.8 Rhinitis	1	238	Risk Ratio (M-H, Fixed, 99% CI)	0.98 [0.46, 2.10]
7.9 Upper Respiratory Tract Infection	1	238	Risk Ratio (M-H, Fixed, 99% CI)	0.41 [0.15, 1.13]
7.10 Headache	1	238	Risk Ratio (M-H, Fixed, 99% CI)	1.40 [0.75, 2.65]
7.11 Acute Kidney Injury	1	238	Risk Ratio (M-H, Fixed, 99% CI)	17.7 [2.40, 130.47]
7.12 Pulmonary Exacerbation	1	238	Risk Ratio (M-H, Fixed, 99% CI)	0.96 [0.84, 1.10]
7.13 Cough	1	238	Risk Ratio (M-H, Fixed, 99% CI)	0.73 [0.47, 1.13]
7.14 Haemoptysis	1	238	Risk Ratio (M-H, Fixed, 99% CI)	0.60 [0.30, 1.22]
7.15 Productive Cough	1	238	Risk Ratio (M-H, Fixed, 99% CI)	1.07 [0.49, 2.33]
7.16 Oropharyngeal Pain	1	238	Risk Ratio (M-H, Fixed, 99% CI)	0.28 [0.10, 0.83]
8 Severity of adverse effects of therapy as graded by trial authors	1		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
8.1 Grade 1 (mild)	1	233	Risk Ratio (M-H, Fixed, 95% CI)	0.88 [0.49, 1.57]
8.2 Grade 2 (Moderate)	1	233	Risk Ratio (M-H, Fixed, 95% CI)	1.21 [0.99, 1.49]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
8.3 Grade 3 (Severe)	1	233	Risk Ratio (M-H, Fixed, 95% CI)	0.62 [0.37, 1.03]
8.4 Grade 4 (life-threatening or death)	1	233	Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
9 Pulmonary Exacerbation Rate	1		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
9.1 Physician Defined Pulmonary Exacerbation Rate	1	232	Mean Difference (IV, Fixed, 95% CI)	-0.02 [-0.67, 0.63]
9.2 Protocol Defined Pulmonary Exacerbation Rate (as defined by modified Fuchs' criteria) overall	1	232	Mean Difference (IV, Fixed, 95% CI)	-0.36 [-0.89, 0.17]
10 Protocol Defined Pulmonary Exacerbation Rate: subgroup analysis by chronic inhaled tobramycin use	1		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
10.1 Protocol defined pulmonary exacerbation rate in participants not receiving chronic inhaled tobramycin	1	146	Mean Difference (IV, Fixed, 95% CI)	-0.76 [-1.51, -0.01]
10.2 Protocol defined pulmonary exacerbation rate in participants receiving chronic inhaled tobramycin	1	86	Mean Difference (IV, Fixed, 95% CI)	0.36 [-0.28, 1.00]
11 Total Lung Computerised Tomography Score - Mean Change From Baseline	1		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
11.1 At up to 48 Weeks	1	203	Mean Difference (IV, Fixed, 95% CI)	-0.28 [-0.68, 0.12]
12 Sweat chloride level - change from baseline	1		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
12.1 At up to 48 weeks	1	194	Mean Difference (IV, Fixed, 95% CI)	-0.70 [-3.41, 2.01]

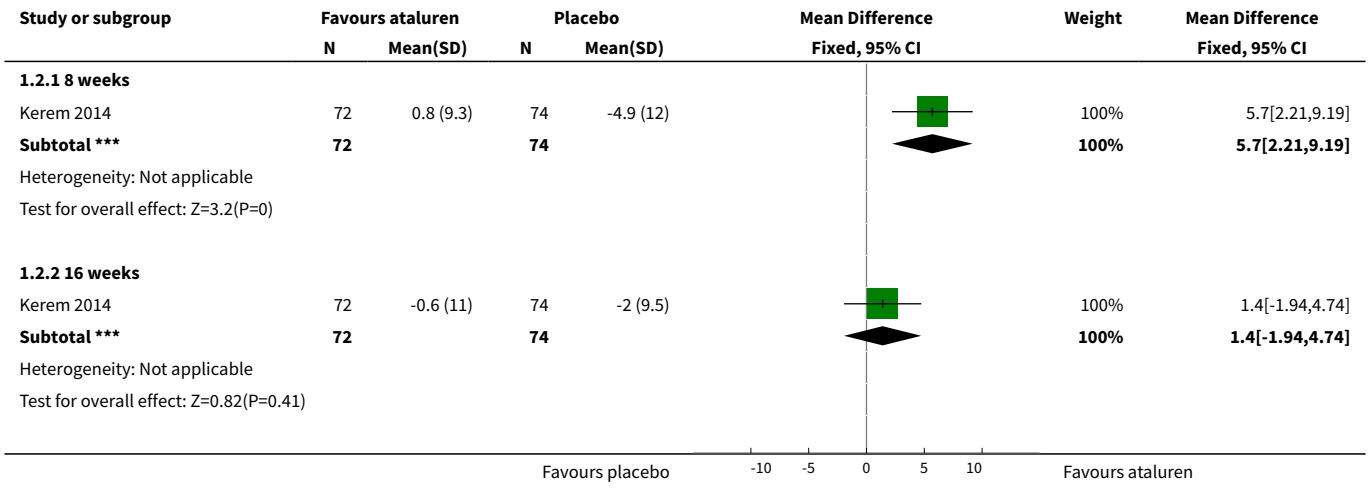
Analysis 1.1. Comparison 1 Ataluren versus placebo, Outcome 1 FEV_1 - mean relative change from baseline.

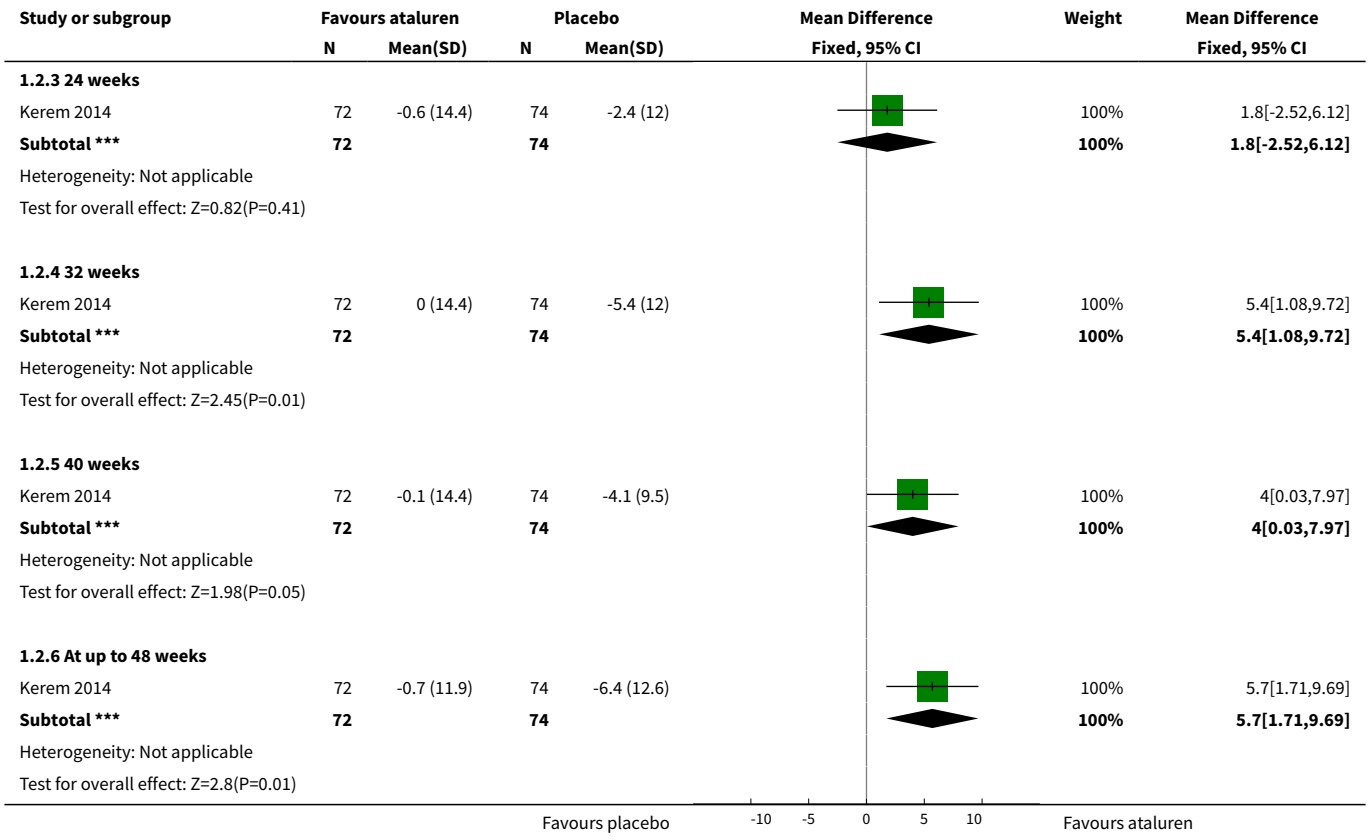
Study or subgroup	Ataluren N	Ataluren Mean(SD)	Placebo N	Placebo Mean(SD)	Mean Difference Fixed, 95% CI	Weight	Mean Difference Fixed, 95% CI
1.1.1 8 weeks							
Kerem 2014	116	-1 (9.7)	116	-4.5 (10.8)		100%	3.5[0.86,6.14]
Subtotal ***	**116**		**116**			**100%**	**3.5[0.86,6.14]**
Heterogeneity: Not applicable							
Test for overall effect: Z=2.6(P=0.01)							



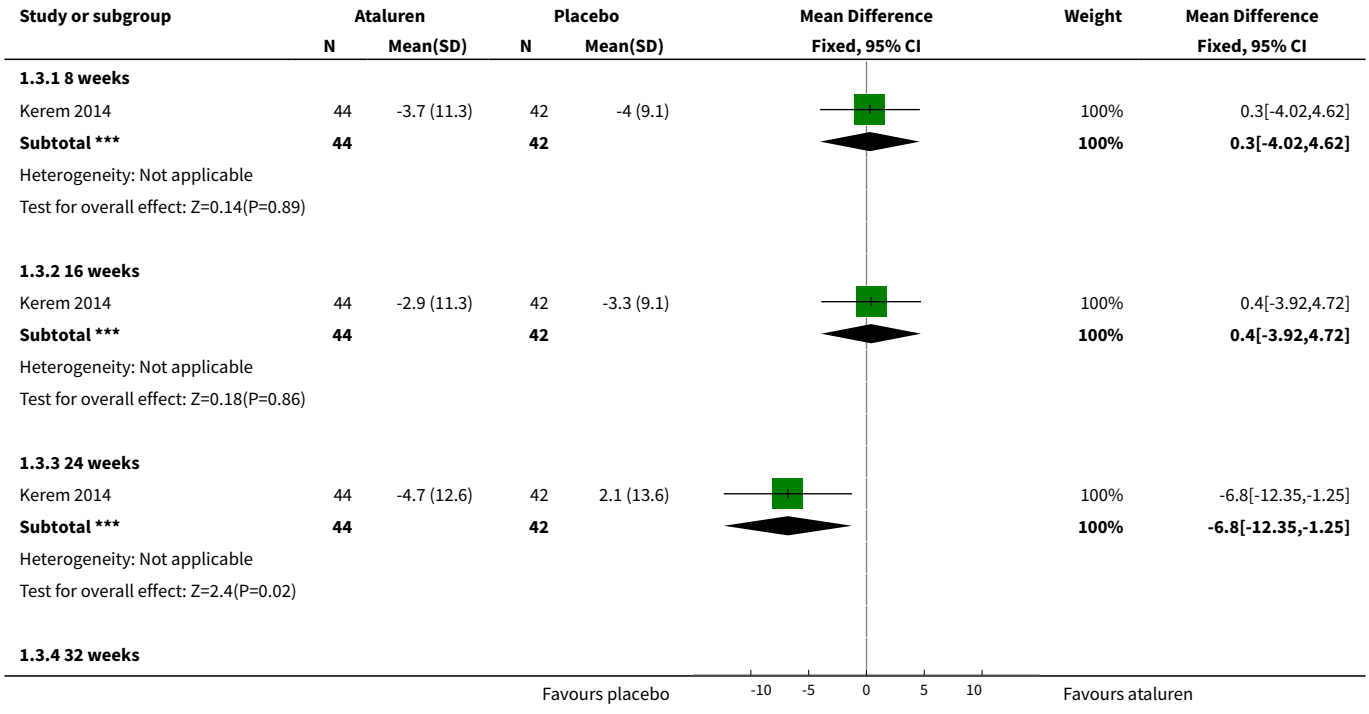


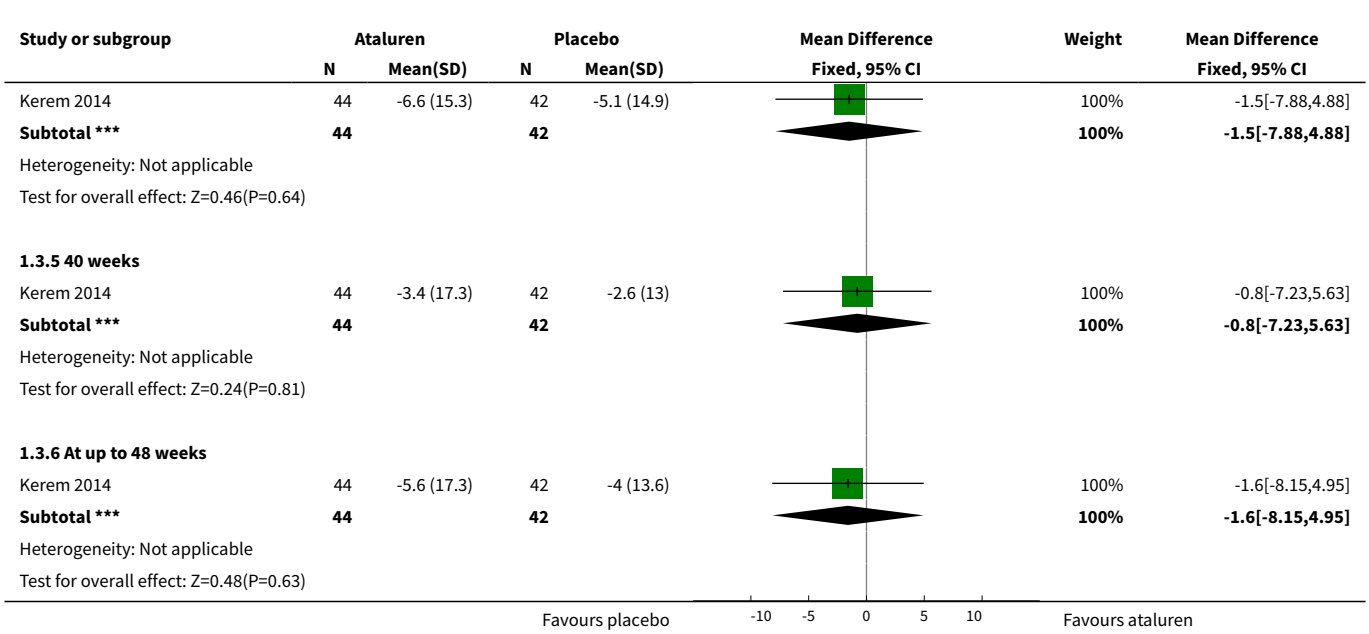
Analysis 1.2. Comparison 1 Ataluren versus placebo, Outcome 2 FEV_1 - mean relative change from baseline: subgroup analysis of participants NOT receiving chronic inhaled tobramycin.



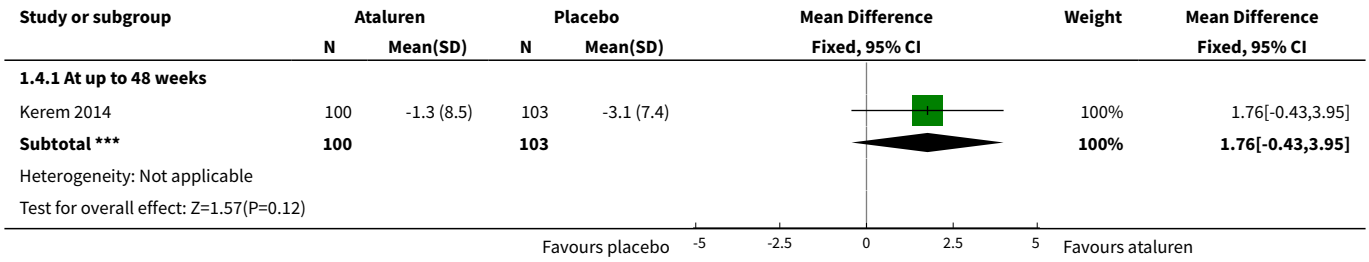


Analysis 1.3. Comparison 1 Ataluren versus placebo, Outcome 3 FEV_1 - mean relative change from baseline: subgroup analysis of participants receiving chronic inhaled tobramycin.

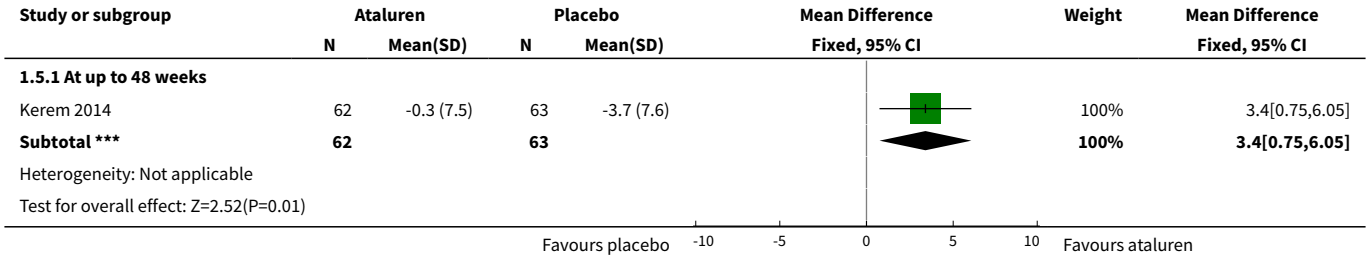




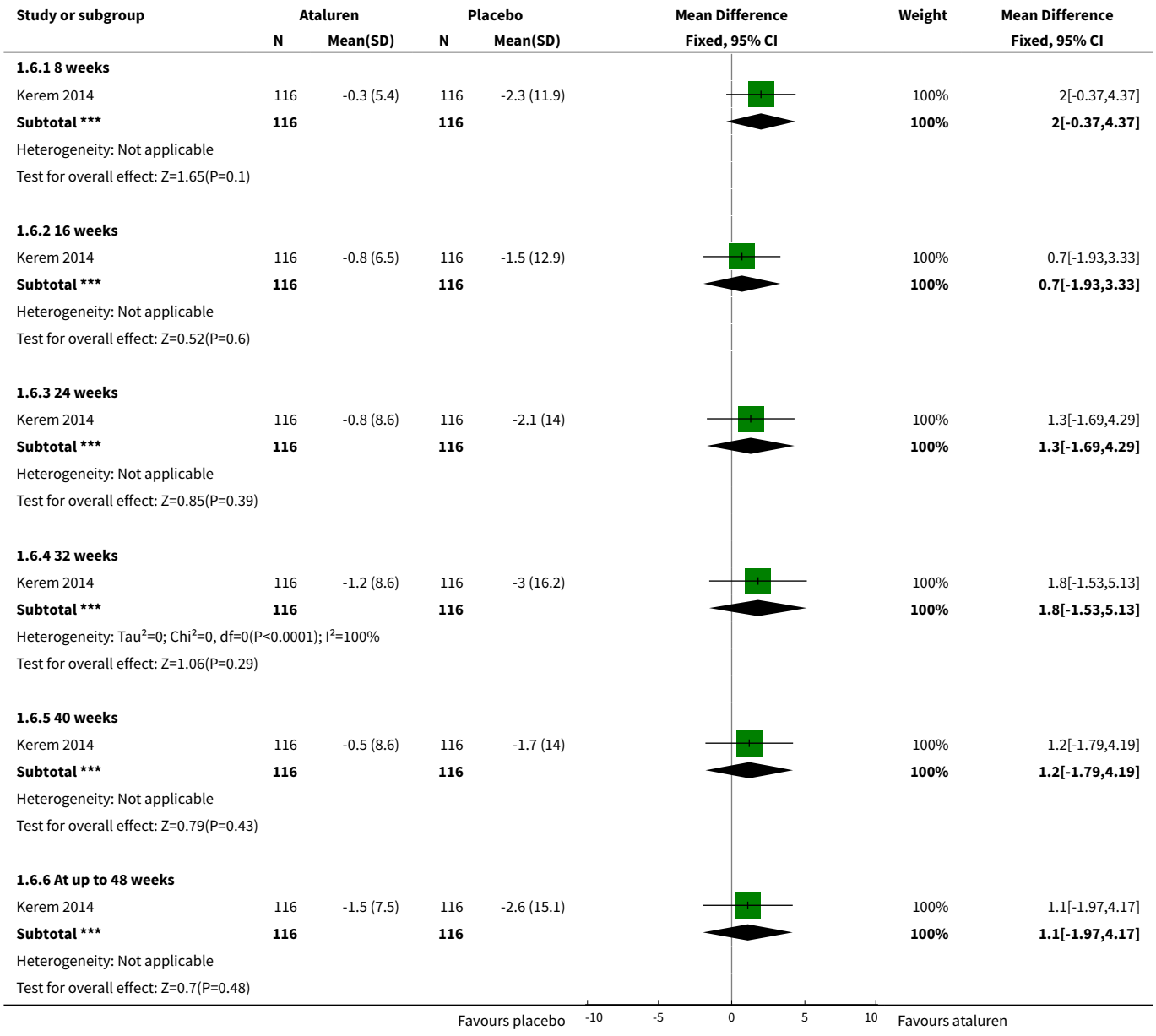
Analysis 1.4. Comparison 1 Ataluren versus placebo, Outcome 4 FEV_1 - mean absolute change from baseline.



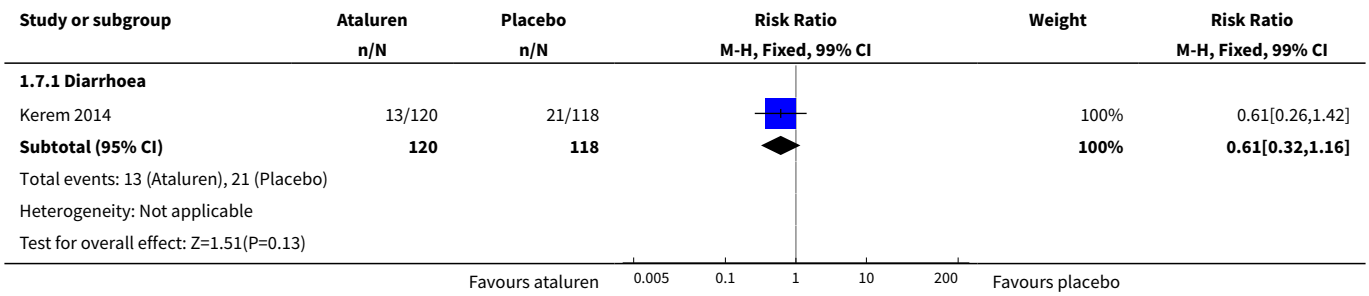
Analysis 1.5. Comparison 1 Ataluren versus placebo, Outcome 5 FEV_1 - mean absolute change from baseline: subgroup of participants not receiving chronic inhaled tobramycin.

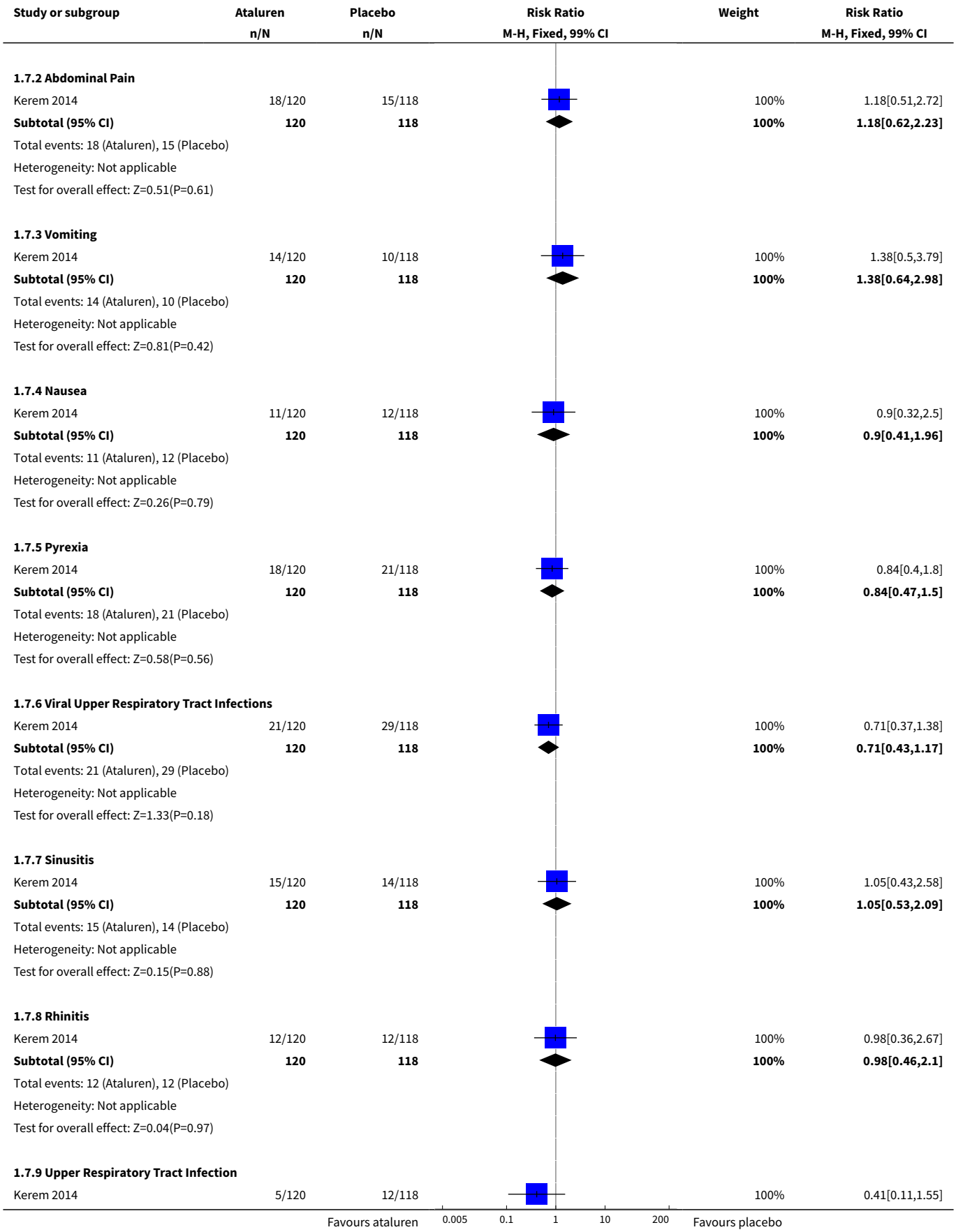


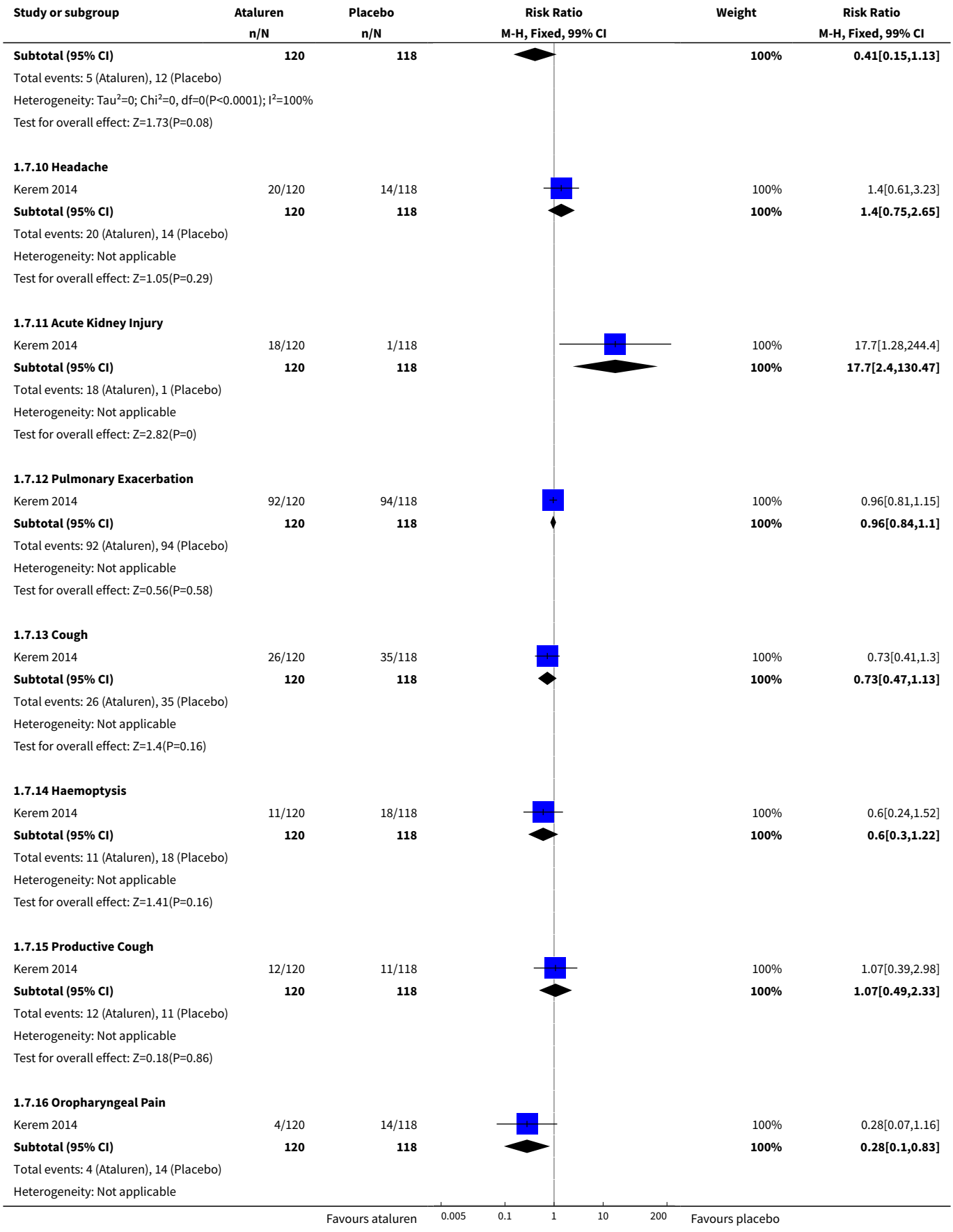
Analysis 1.6. Comparison 1 Ataluren versus placebo, Outcome 6 FVC - mean relative change from baseline.



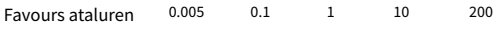
Analysis 1.7. Comparison 1 Ataluren versus placebo, Outcome 7 Adverse events occurring in greater than 10% of trial participants.





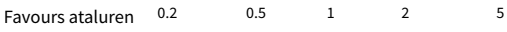


Study or subgroup	Ataluren n/N	Placebo n/N	Risk Ratio M-H, Fixed, 99% CI	Weight	Risk Ratio M-H, Fixed, 99% CI
Test for overall effect: Z=2.3(P=0.02)					



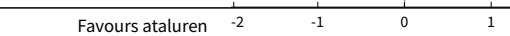
Analysis 1.8. Comparison 1 Ataluren versus placebo, Outcome 8 Severity of adverse effects of therapy as graded by trial authors.

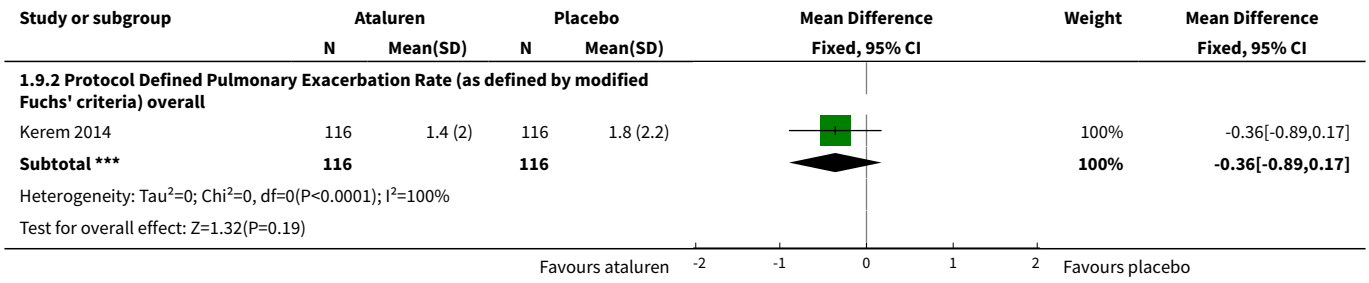
Study or subgroup	Ataluren n/N	Placebo n/N	Risk Ratio M-H, Fixed, 95% CI	Weight	Risk Ratio M-H, Fixed, 95% CI
1.8.1 Grade 1 (mild)					
Kerem 2014	18/118	20/115		100%	0.88[0.49,1.57]
Subtotal (95% CI)	**118**	**115**		**100%**	**0.88[0.49,1.57]**
Total events: 18 (Ataluren), 20 (Placebo)					
Heterogeneity: Tau^2=0; Chi^2=0, df=0(P<0.0001); I^2=100%					
Test for overall effect: Z=0.44(P=0.66)					
1.8.2 Grade 2 (Moderate)					
Kerem 2014	81/118	65/115		100%	1.21[0.99,1.49]
Subtotal (95% CI)	**118**	**115**		**100%**	**1.21[0.99,1.49]**
Total events: 81 (Ataluren), 65 (Placebo)					
Heterogeneity: Not applicable					
Test for overall effect: Z=1.89(P=0.06)					
1.8.3 Grade 3 (Severe)					
Kerem 2014	19/118	30/115		100%	0.62[0.37,1.03]
Subtotal (95% CI)	**118**	**115**		**100%**	**0.62[0.37,1.03]**
Total events: 19 (Ataluren), 30 (Placebo)					
Heterogeneity: Not applicable					
Test for overall effect: Z=1.84(P=0.07)					
1.8.4 Grade 4 (life-threatening or death)					
Kerem 2014	0/118	0/115			Not estimable
Subtotal (95% CI)	**118**	**115**			**Not estimable**
Total events: 0 (Ataluren), 0 (Placebo)					
Heterogeneity: Not applicable					
Test for overall effect: Not applicable					



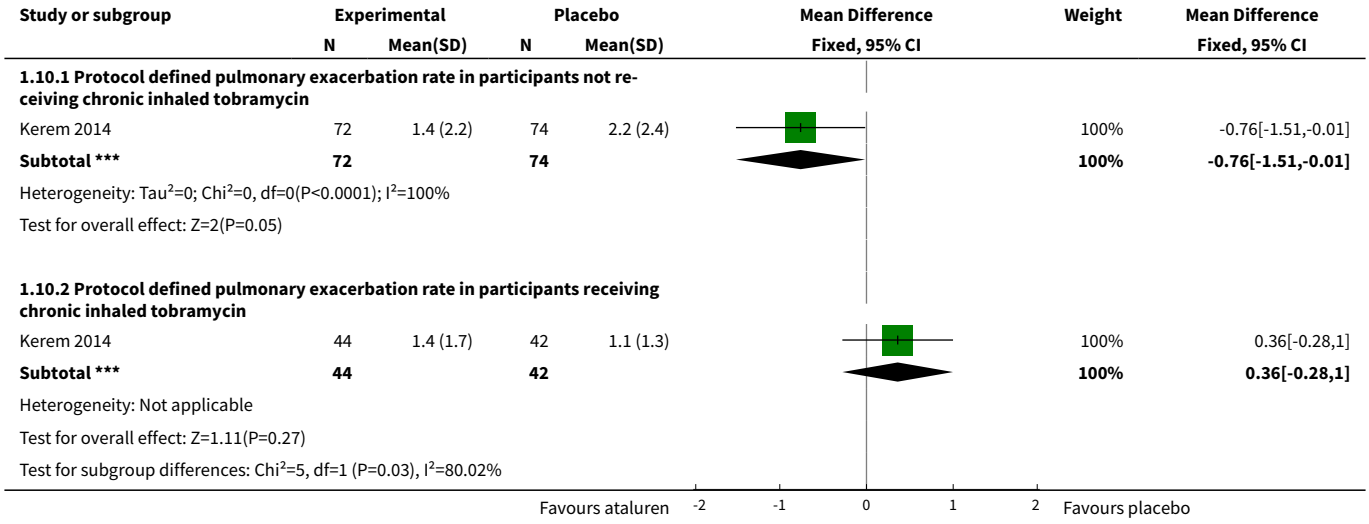
Analysis 1.9. Comparison 1 Ataluren versus placebo, Outcome 9 Pulmonary Exacerbation Rate.

Study or subgroup	Ataluren N	Ataluren Mean(SD)	Placebo N	Placebo Mean(SD)	Mean Difference Fixed, 95% CI	Weight	Mean Difference Fixed, 95% CI
1.9.1 Physician Defined Pulmonary Exacerbation Rate							
Kerem 2014	116	2.3 (2.4)	116	2.3 (2.7)		100%	-0.02[-0.67,0.63]
Subtotal ***	**116**		**116**			**100%**	**-0.02[-0.67,0.63]**
Heterogeneity: Not applicable							
Test for overall effect: Z=0.06(P=0.95)							

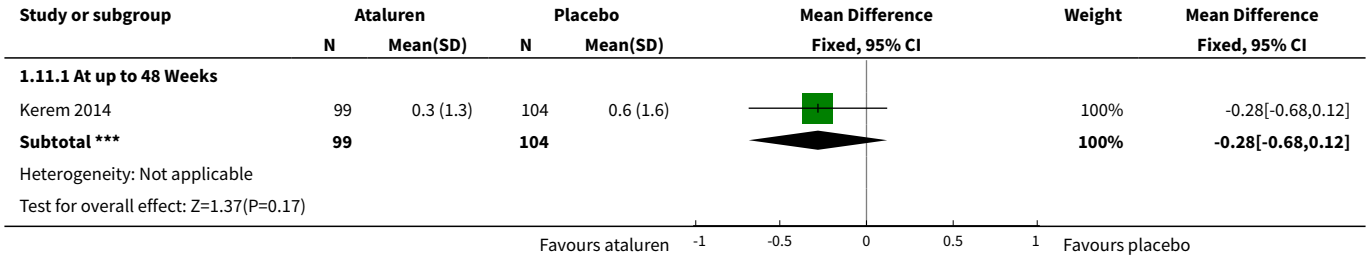




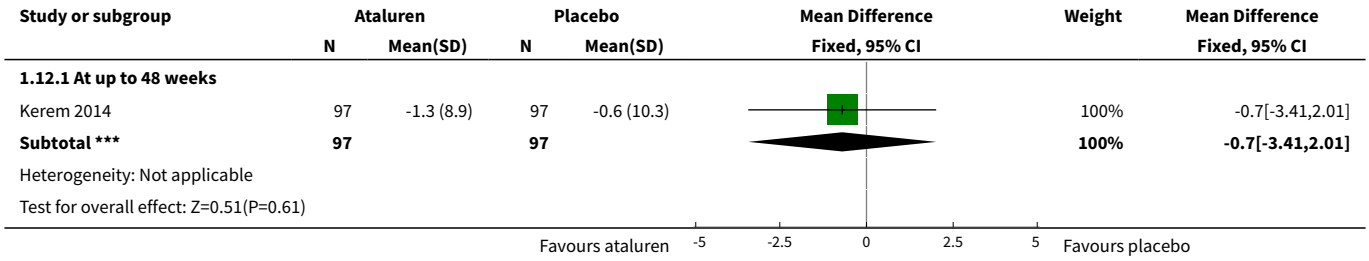
Analysis 1.10. Comparison 1 Ataluren versus placebo, Outcome 10 Protocol Defined Pulmonary Exacerbation Rate: subgroup analysis by chronic inhaled tobramycin use.



Analysis 1.11. Comparison 1 Ataluren versus placebo, Outcome 11 Total Lung Computerised Tomography Score - Mean Change From Baseline.



Analysis 1.12. Comparison 1 Ataluren versus placebo, Outcome 12 Sweat chloride level - change from baseline.



ADDITIONAL TABLES

Table 1. Classes of mutations affecting CFTR production, structure, and function

Class	Example mutation	Impact on CFTR structure and function
I	G542X	Synthesis of CFTR is critically impaired, and no functional protein is produced. This is due to the presence of a premature termination codon in the nucleotide sequence. Individuals have no CFTR function.
II	phe508del (ΔF508)	A full length of CFTR is produced, but this is structurally abnormal and destroyed by the cell before it reaches the cell membrane. This is called a defect in the intracellular trafficking pathway. Individuals have no CFTR function under normal conditions.
III	G551D	CFTR is produced and embedded in the cell membrane, but the chloride channel does not respond ('switch on') to normal stimulation from the cell. This means there is no significant ion transport across the protein. Individuals have no CFTR function.
IV	R347P	CFTR is transported to the outer cell membrane, and responds to normal stimulation, but functions at a low level because chloride ions do not cross the channel appropriately. Individuals have some residual CFTR function.
V	A455E	Normal CFTR is produced, but the amount of protein is reduced. Individuals have some residual CFTR function.

CFTR: cystic fibrosis transmembrane conductance regulator

APPENDICES

Appendix 1. Glossary

Term	Explanation
CFTR (cystic fibrosis transmembrane regulator)	A protein which is in the outer membrane of cells. It works by regulating transport of salt in and out of the cell. Problems with the amount of CFTR in the cell membrane, its structure, or the manner in which it functions can lead to altered transport of salt across the cell membrane. In the lungs of people with CF, these problems cause thick airway secretions. Abnormalities of CFTR can also lead to problems in other organs.

(Continued)

CFTR correctors	Drugs or chemicals which work by increasing the amount of CFTR in the cell membrane.
CFTR potentiators	Drugs or chemicals which increase the effectiveness of CFTR at transporting salt across the cell membrane.
Nonsense mutation	A sequence of DNA that makes no sense resulting in no functional protein being produced. This is a type of class I mutation (PTC) causing CF.
Phenoytype	The clinical picture resulting from inherited information.
Premature Termination Codon	Also known as a class I mutation; includes nonsense and stop codon mutations.
Stop codon mutation	A sequence that directs the normal cellular mechanism to stop resulting in no functional protein being synthesised. This is a type of class I mutation (PTC) causing CF.
Transcription	The first step in protein synthesis. Information in a strand of DNA is copied into a code for the protein which is then used for the construction of a protein molecule. The process of transcription is faulty in people with CF with class I mutations.

CONTRIBUTIONS OF AUTHORS

Roles and responsibilities	
TASK	WHO UNDERTOOK THE TASK?
Protocol stage: draft the protocol	AA with comments from all
Review stage: select which trials to include (2 + 1 arbiter)	AA and CH (KS to arbitrate)
Review stage: extract data from trials (2 people)	AA and CH
Review stage: enter data into RevMan	AA
Review stage: carry out the analysis	AA
Review stage: interpret the analysis	AA
Review stage: draft the final review	AA with comments from all
Update stage: update the review	AA, IS and KS

DECLARATIONS OF INTEREST

There are no declarations of interest for any of the authors.

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DIFFERENCES BETWEEN PROTOCOL AND REVIEW

For the analysis of individual adverse events we have used 99% CIs rather than 95% CIs as the type I error rate (e.g. false positive) is greatly inflated with a large number of tests.

INDEX TERMS

Medical Subject Headings (MeSH)

Anti-Bacterial Agents [therapeutic use]; Codon, Nonsense [*drug effects]; Cystic Fibrosis [*drug therapy] [*genetics]; Disease Progression; Oxadiazoles [adverse effects] [*therapeutic use]; Quality of Life; Randomized Controlled Trials as Topic; Tobramycin [therapeutic use]

MeSH check words

Adolescent; Adult; Child; Female; Humans; Male; Middle Aged