The effects of dual-action antidepressants versus no intervention, placebo, or 'active' placebo in patients with major depressive disorder. A systematic review of randomised clinical trials with meta-analyses and trial sequential analyses

Janus Christian Jakobsen[1], Jane Lindschou Hansen[1], Signe Hellmuth[1], Anne Schou[1], Jesper Krogh[2,3], Christian Gluud[1]

1: Copenhagen Trial Unit, Centre for Clinical Intervention Research, Department 7812 Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark

2: Mental Health Centre Copenhagen, University of Copenhagen, Copenhagen Denmark,

3: Department of Endocrinology, Herlev University Hospital, Copenhagen, Denmark.

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Correspondence:

Janus Christian Jakobsen, MD

Copenhagen Trial Unit, Centre for Clinical Intervention Research, Department 7812 Rigshospitalet, Copenhagen, Denmark

Tel.: 0045 2618 6242

e-mail: jcj_ctu@icloud.com

Background

Depression

According to the WHO, major depressive disorder, i.e. unipolar depression, is the second largest healthcare problem worldwide in terms of years lived with disability (YLD) [1]. It afflicts an estimated 17% of individuals during their lifetime at tremendous cost to the individual and to society [2,3]. Roughly a third of all depressive disorders take a chronic course [4,5]. Compared to other medical disorders, depressive illness causes the most significant deterioration in individual life quality [6]. Approximately 15% of depressive patients will commit suicide over a 10 to 20 year period [7].

Antidepressant medication in general

A number of depressive patients are treated with antidepressant medication, the efficacy of which has been studied in a number of meta-analyses and systematic reviews. In their 1996 meta-analysis, Joffe et al. found medical antidepressant treatment to be significantly more effective than placebo [8]. Similarly, in 2004, Moncrieff et al. in their Cochrane systematic review found that antidepressant medication was significantly more effective than 'active' placebo [9]. 'Active' placebo is a placebo preparation that mimics the adverse reaction profile of the preparation with which it is being compared, but without having any effect on the disease. However, Moncrieff et al. also found that there is little difference between antidepressant medication versus 'active' placebo and that the efficacy of

antidepressant medication probably has been overestimated in studies where active placebo has not been used.

A review published in the New England Journal of Medicine shows that randomised trials of new antidepressants remain largely unpublished if their results are neutral or negative [10]. 94% of the published trials in the most widely used databases showed a positive effect of the newer antidepressants on depression. In the Food and Drug Administration (FDA) databases of all randomised trials submitted to the FDA, only 51% of the trials demonstrated significant positive effects from the different antidepressants. The FDA requires that information on all industry-sponsored trials must be submitted as part of the approval process; so the information from the FDA should contain information on all trials conducted prior to the approval of each drug [11]. When the unpublished trial results were added to the published ones, the updated meta-analyses showed no significant positive effects or very small significant positive effects [10]. In the majority of the trials, either no intervention or inactive placebo was involved as comparator. Similarly, a meta-analysis in which the unpublished trials were included, revealed that the six most widely used antidepressants between 1987-1999 (fluoxetine, venlafaxine, nefazodone, paroxetine, sertraline, citalopram) failed to demonstrate any significant beneficial effects on depression in patients with mild to moderate forms of the disease [11]. The results showed that significant effects from the new antidepressants were only achieved in severely depressed patients (HDRS score > 28), and that this effect was clinically small [11]. However, the meta-analysis revealed that significant effects from the new antidepressants were only achieved in severely depressed patients, and

that this effect was clinically small [11]. Furthermore, this meta-analysis did not assess the effect of antidepressants compared with 'active' placebo and did only include one of the newer dual-action antidepressants (venlafaxine).

Dual-action antidepressants

In recent years, newer 'dual-action' antidepressants have been developed [12]. These include mirtazapine, venlafaxine, duloxetine, and milnacipran. The 'dual action' refers to the effects on the two neurotransmitters serotonin and norepinephrine. Venlafaxine, duloxetine and milnacipran are serotonin-norepinephrine reuptake inhibitors (SNRIs), while mirtazapine acts on in a complex way on serotonin and norepinephrine but not through reuptake inhibition [12]. A number of Cochrane reviews have been conducted examining the effects of the dual action antidepressants milnacipran [13], duloxetine [14], and mirtazapine [15] versus other antidepressant agents. Although there might be a difference in speed of onset between the different antidepressants, none of the reviews showed any clear evidence for the overall superiority or inferiority of any of the dual-action antidepressants [13-15]. Based on the results from these reviews it is not possible to conclude if the different dual-action antidepressants are equally effective or equally ineffective.

We have not identified any relevant overall systematic review using Cochrane Collaboration Methodology examining the effects of dual-action antidepressants versus no intervention, placebo, or 'active' placebo.

Methods

Objective

In a systematic review of all randomised trials we will assess the beneficial and harmful effects of the dual-action antidepressants venlafaxine, duloxetine, milnacipran, and mirtazapine versus no intervention, placebo, or 'active' placebo in the treatment of major depressive disorder using meta-analyses [16] and trial sequential analyses [17,18].

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Criteria for trials included

Trial design

Randomised clinical trials comparing venlafaxine, duloxetine, milnacipran, and mirtazapine versus no intervention, placebo, or 'active' placebo' irrespective of publication type, publication status, publication year and language. Quasi-randomised trials, e.g., trials using date of admission for allocating the participants and observational studies will be excluded for assessment of benefits, but not for harms.

Participants

Participants must be 18 years or more, and the primary diagnosis must be major depressive disorder. The diagnosis of major depression must be made based on one of the standardized criteria, such as ICD 10 [19], DSM III [20], DSM III-R [21], DSM

IV [22], or Feighner criteria [23]. Comorbidity with schizophrenia will be an exclusion criterion while comorbidity with other psychiatric diagnoses will not. Trials exclusively including participants with a somatic disease and comorbid depression, or trials on depression during or after pregnancy will be excluded.

Experimental intervention

Dual-action antidepressants: venlafaxine (serotonin noradrenalin reuptake inhibitor (SNRI)); duloxetine (serotonin noradrenalin reuptake inhibitor (SNRI)); milnacipran (SNRI), or mirtazapine (unique mechanism of antidepressive action).

Control interventions

No intervention: any control intervention with no treatment elements, e.g., 'waiting list'.

Placebo: any 'placebo' substance containing no active substance.

'Active' placebo: any active substance employed to mimic the non-specific adverse effects of taking dual action antidepressants.

Co-interventions

Trials comparing dual-action antidepressants versus no intervention, placebo, or 'active' placebo as add-on therapy to any other kind of intervention (e.g., treatment

as usual) will be included, but only if this co-intervention is described and delivered similarly in the different intervention groups.

Outcomes

We will estimate all outcomes at two time points:

- Outcome at end of treatment. Often after 6-18 weeks of treatment. The trial's choice of end of treatment will be used. This is the most important outcome measure time point in this review.
- Outcome at maximum follow-up.

Primary outcomes

- The difference between the mean values from the two intervention groups using the 17-item Hamilton Depression Rating Scale (HDRS) [24].
- The standardised mean difference [16] between the two intervention groups using the HDRS [24], any other form of the Hamilton Depression Rating Scale, Montgomery-Asberg Depression Rating Scale (MADRS) [25], and Bech's Depression Inventory (BDI) [26].
- The proportion of participants achieving remission. We have, pragmatically, defined remission as a Hamilton score of less than 8, BDI less than 10, or MADRS less than 10.
- Adverse events during the intervention period and the follow-up period. We will classify adverse events as serious and non-serious. Serious adverse events are defined as medical events that are life threatening, result in death,

disability or significant loss of function; that cause hospital admission or prolonged hospitalisation or a hereditary anomaly or foetal injury. All other adverse events (that is, events that have not necessarily had a causal relationship with the treatment, but that resulted in a change in- or cessation of the treatment) will be considered non-serious events [27].

Secondary outcomes

- Number of suicides.
- Number of suicide attempts.
- Suicide ideation (any kind of assessment used by the trialists).
- Quality of life (any assessment scale used by the trialists).

Search methods

We will search The Cochrane Library's CENTRAL, MEDLINE, EMBASE, PsycInfo, and Science Citation Index Expanded. We also searched other relevant publications for references to relevant trials **(see Search strategy).** To be able also to assess results from unpublished trials we also included trials submitted to FDA.

The timeframe for the search will be all trials published before March 29, 2012.

Selection of trials

Two of the review authors will independently select relevant trials, based on criteria described in the above. If a trial only has been identified by one of the two, it will be discussed whether the trial should be included. If the two review authors disagree, a third review author will decide if the trial should be included. All excluded trials are entered on a list, stating the reason for exclusion.

Data extraction

The following data will be extracted from the included trials:

1. Whether the trial is published or not.
2. Choice of antidepressant.
3. Whether a placebo washout period was used in the trial before inclusion of the participants.
4. Whether the participants are elderly (any definition used by the trialists).
5. Whether the participants have drug or alcohol dependence.
6. Whether ECT was used as co-intervention.
7. Whether the participants are chronically depressed or treatment resistant depressed (any definition used by the trialists).
8. Choice of control (no intervention, placebo, or 'active' placebo).
9. Whether the participants have a high baseline depression score (HDRS ≥ 23).
10. Whether the participants have comorbid psychiatric diagnoses.

11. Whether the participants have borderline personality disorder.
12. Whether the experimental intervention is an add-on therapy on other antidepressants.
13. Whether the trial results are published or unpublished.
14. Length of intervention period and follow-up period.
15. Number of participants.
16. Distribution of age and sex.
17. Choice of outcomes (e.g., HDRS, BDI, suicides).
18. Outcomes (e.g., HDRS score, number of suicides).
19. The choice of method and an evaluation of the bias risk and choice of method (see below).

Risk of systematic error (bias)

We will use the instructions in The Cochrane Handbook for Systematic Reviews of Interventions [16] in our evaluation of the methodology and hence bias risk of the included trials. Again, two review authors will assess the included trials independent of each other. We will evaluate the methodology in respect of generation of allocation sequence, allocation concealment, blinding of participants and treatment providers, blinding of outcome assessors, incomplete outcome data, selective outcome reporting, industry funding, and other bias sources. This is done because these components enable classification of randomised trials with low risk of bias and high risk of bias. The latter trials overestimate positive intervention effects and underestimate negative effects [28-30]. We will classify the trials according to the components below:

Generating allocation sequence

‘Low risk of bias’: If randomising is performed by computer or a ‘random number table’. If the randomising is a random process, e.g., ‘heads or tails’ or a throw of a dice; and the person performing the procedure in no other way is involved in the trial.

Uncertain: If the procedure in respect of randomising is not sufficiently described.

‘High risk of bias’: If the trial uses, e.g., alternation for allocating the participants.

Allocation concealment

‘Low risk of bias’: If the allocation sequence is concealed from the investigators, treatment providers and participants, for example by central randomisation. And this procedure is described and documented.

Uncertain: If the procedure to conceal allocation is not sufficiently described.

‘High risk of bias’: If the investigators, treatment providers, and the participants are able to predict the allocation sequence. Such trials will be included only in the assessment of harms.

Blinding of participants and treatment providers

‘Low risk of bias’: If the participants and the treatment providers are blinded to treatment allocation and this is described. The placebo tablets should be identical to the antidepresssive tablets regarding appearance, colour, smell, taste, and solubility. Furthermore, the adverse reactions should be comparable to the experimental intervention, i.e., use of ‘active’ placebo.

Uncertain: If the procedure of blinding is insufficiently described or the trial use inactive placebo.

‘High risk of bias’: If blinding is not performed.

Blinding of outcome assessment

‘Low risk of bias’: If the trial investigators performing the outcome assessments, analyses and calculations are blinded to the treatment allocation and this is described. If some kind of ‘active’ placebo is used as control intervention this will be classified as ‘lowest risk of bias’.

Uncertain: If the procedure of blinding is insufficiently described or the trial use inactive placebo.

‘High risk of bias’: If blinding is not performed.

Incomplete outcome data

‘Low risk of bias’: If dropouts following randomising can be described as being similar in the two intervention groups, and if the trial allows intention-to-treat analysis [16].

Uncertain: If dropouts are not stated, or if the reasons why the participants dropped out are unclear.

‘High risk of bias’: If the pattern of dropouts can be described as being different in the two intervention groups.

Selective outcome reporting

‘Low risk of bias’: If all outcome measures are stated in the results. And the hierarchy of the outcome measures are documented in a protocol before launch of randomisation.

Uncertain: If the method of choosing outcome measures is inadequately described.

‘High risk of bias’: If there is incongruence between the original protocol and the outcome measures used in the results, or if not all of the outcome measures are stated.

Other bias sources

For profit bias

‘Low risk of bias’: If the trial is not financed by a company that might have an interest in a given result.

Uncertain: If there is no description of how the trial is financed.

‘High risk of bias’: If the trial is financed by a company that might have an interest in a given result.

Other sources of bias

If other sources of bias are evident these sources of bias will be presented and the implications will be discussed.

Overall assessment of risk of bias

A trial will be classified as ‘low risk of bias’ only if all of the bias components described in the above paragraphs are classified as ‘low risk of bias’ and. If one or more of the bias components are classified as ‘uncertain’ or ‘high risk of bias’ the trial will be classified as ‘high risk of bias’.

In case that we find no trials with low risk of bias or only find very few trials with low risk of bias, we plan to identify a group of trials with *lower* risk of bias according to the bias risk domains described in the above. This group of trials with lower risk of bias should at least include about 6% of the trial population in order to give the comparison of trials with lower risk of bias a certain power.

Assessment of reporting biases

Different types of reporting biases (e.g., publication bias, time lag bias, outcome reporting bias, etc.) will be handled following the recommendations of the Cochrane Handbook for Systematic Reviews and Interventions [16]. On all outcomes, we will test for funnel plot asymmetry when there are at least ten trials included in the meta-analysis [16]. For continuous outcomes with intervention effects measured as mean difference, the test proposed by Egger et al. [31] will be used to test for funnel plot asymmetry. We will take into account that asymmetric funnel plots are not necessarily caused by publication bias, and publication bias does not necessarily cause asymmetry in a funnel plot [31].

Statistical methods

We will undertake this meta-analysis according to the recommendations stated in The Cochrane Handbook for Systematic Reviews of Interventions [16]. In analysing the primary outcomes we will use the mean difference (MD) on the 17-item HDRS with a 95% confidence interval. We will also use the standardised mean difference with a 95% confidence interval to analyse the results from the 17-item HDRS and other forms of the HDRS, BDI, and MADRS. We will use the odds ratio (OR) with a 95% confidence interval to estimate intervention effects on dichotomous outcomes. All meta-analyses will performed both with a fixed-effect and a random-effects model [16].

The National Institute for Clinical Excellence (NICE) of the National Health Service in England has formerly defined a threshold for clinical significance as an effect size of 0.50 standardised mean difference (SMD) or a drug-placebo difference of three points on the 17-item HDRS [32]. Others have suggested and used the following 'rule of thumb': 0.2 SMD represents a small effect, 0.5 SMD a moderate effect, and 0.8 SMD a large effect [16,33]. We have chosen, as NICE has recommended and other reviewers have chosen [11,32,34], an effect size of 0.50 SMD or a drug-placebo difference of three points on the 17-item HDRS as the threshold for clinical significance.

We will also perform trial sequential analyses on the outcomes [17,18,35], in order to calculate the required information size and the cumulative Z-curve's breach of relevant trial sequential monitoring boundaries. A more detailed description of trial

sequential analysis can be found at http://www.ctu.dk/tsa/ [35]. For binary outcomes we will estimate the required information size based on the proportion of patients with an outcome in the control group, a risk ratio of 20% or as suggested by the trials with low risk of bias, an alpha of 5%, a beta of 20%, and diversity of 30% and 60%. For continuous outcomes we will estimate the required information size based on the standard deviation observed in the control group of trials with low risk of bias and a minimal relevant difference of 50% of this standard deviation, an alpha of 5%, a beta of 20%, and diversity of 30% and 60%.

Missing outcomes

Dichotomous outcomes

If missing dichotomous outcomes are not reported, we will in the primary analyses impute missing values assuming that the participants missing at follow-up have survived, had no suicide attempt, had no adverse event, and have obtained 'no remission'. Secondly, we will perform two sensitivity analyses:

1. **'Best-worst-case' scenario:** It will be assumed that all participants lost to follow-up in the experimental group have survived, had no suicide attempt, had no adverse event, and have obtained remission; and all those with missing outcomes in the control group have not survived, had a suicide attempt, had a adverse event, and have obtained 'no remission'.
2. **'Worst-best-case' scenario:** It will be assumed that all participants lost to follow-up in the experimental group have not survived, had a suicide attempt,

had a adverse event, and have obtained ‘no remission’; and that all those lost to follow-up in the control group have survived, had no suicide attempt, had no adverse event, and have obtained remission.

Results from both scenarios will be presented in our publication.

Continuous outcomes

We will primarily use follow-up scores. If only change values are reported the results will be analysed together with follow-up scores [16]. If standard deviations (SD) are not reported the SDs will be calculated if this is possible using other data from the trial. If calculation is impossible the SDs will imputed from trials with similar characteristics [16].

Subgroup analyses

We have planned the following subgroup analyses on the primary outcomes:

1. Whether the intervention effects from trials with overall low risk of bias (or lower risk of bias) differ from trials with overall high risk of bias.
2. Whether the intervention effects from the trials using no intervention, placebo, or ‘active placebo’ differ.
3. Whether the results from trials using a placebo washout period before inclusion differ from the remaining trials.

4. Whether the intervention effects from trials assessing the effects of dual-action antidepressants in elderly depressive participants (defined by the trialists but often adults ≥ 65 years) differ from the remaining trials.
5. Whether the intervention effects from trials using ECT as co-intervention differ from the remaining trials.
6. Whether the intervention effects from trials assessing the effects of dual-action antidepressants in participants without comorbidities differ from trials including patients with drug and alcohol problems and from trials that include other psychiatric comorbidities.
7. Whether the intervention effects from trials assessing the effects from the different forms of dual-action antidepressants differ.
8. Former meta-analyses have shown that effects of antidepressive medication compared with placebo increased with increasing baseline depression severity (HDRS score) [11,34]. We will investigate whether the intervention effects from trials with participants with a baseline HDRS score of 23 or above differ from the remaining trials.
9. Whether the intervention effects from trials assessing the effects of dual-action antidepressants in chronically depressive patients or treatment resistant depression differ from the remaining trials.
10. Whether the intervention effects from trials assessing the effects of dual-action antidepressants in participants with and without borderline personality disorder differ.

11. Whether the intervention effects from trials assessing the effects of dual-action antidepressants as add-on therapy on any other antidepressant differ from the remaining trials.
12. Whether the intervention effect from the published trials differ from unpublished trials.

References

1. Vos T, Flaxman AD, Naghavi M, Lozano R, Michaud C, et al. (2013) Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 380: 2163-2196.
2. Greenberg P, Stiglin LE, Finkelstein SN, Berndt ER (1993) The economic burden of depression in 1990. Journal of Clinical Psychiatry 54: 405-418.
3. Kessler RC, McGnagle KA, Zhao S, Nelson CB, Hughes M, et al. (1994) Lifetime and 12-month prevalence of DSM - III- R psychiatric disorders in the united states: Results from the Natinal Comorbidity Survey. Archives of General Psychiatry 51: 8-19.
4. Spijker J, de GR, Bijl RV, Beekman AT, Ormel J, et al. (2002) Duration of major depressive episodes in the general population: results from The Netherlands Mental Health Survey and Incidence Study (NEMESIS). British Journal of Psychiatry 181: 208-213.
5. Arnow BA, Constantino MJ (2003) Effectiveness of psychotherapy and combination treatment for chronic depression. Journal of Clinical Psychology 59: 893-905.
6. Bech P (1999) Stress & livskvalitet (Stress & quality of life). Psykiatrifondens Forlag.
7. Fawcett J (1993) The morbitity and mortality of clinical depression. International Clinical Psychopharmacology 8: 217-220.
8. Joffe R, Sokolov S, Steiner D (1996) Antidepressant treatment of depression: a metaanalysis. Canadian Journal of Psychiatry Revue Canadienne de Psychiatrie 41: 613-616.
9. Moncrieff J, Wessely S, Hardy R (2004) Active placebos versus antidepressants for depression. Cochrane DatabaseSystRev: CD003012.
10. Turner EH, Matthews AM, Linardatos E, Tell RA, Rosenthal R (2008) Selective publication of antidepressant trials and its influence on apparent efficacy. New England Journal of Medicine 358: 252-260.
11. Kirsch I, Deacon BJ, Huedo-Medina TB, Scoboria A, Moore TJ, et al. (2008) Initial severity and antidepressant benefits: a meta-analysis of data submitted to the Food and Drug Administration. PLoS Medicine 5: e45.
12. Jain R (2004) Single-action versus dual-action antidepressants. Prim Care Companion J Clin Psychiatry 6: 7-11.
13. Nakagawa A, Watanabe N, Omori Ichiro M, Barbui C, Cipriani A, et al. (2009) Milnacipran versus other antidepressive agents for depression. Cochrane Database of Systematic Reviews: John Wiley & Sons, Ltd.
14. Cipriani A, Koesters M, Furukawa Toshi A, Nosè M, Purgato M, et al. (2012) Duloxetine versus other anti-depressive agents for depression. Cochrane Database of Systematic Reviews: John Wiley & Sons, Ltd.
15. Watanabe N, Omori Ichiro M, Nakagawa A, Cipriani A, Barbui C, et al. (2011) Mirtazapine versus other antidepressive agents for depression. Cochrane Database of Systematic Reviews: John Wiley & Sons, Ltd.
16. Higgins JPT, Green S (2011) The Cochrane Handbook for Systematic Reviews of Interventions, Version 5.1.0. The Cochrane Collaboration Available from http://www.cochrane-handbook.org.
17. Wetterslev J, Thorlund K, Brok J, Gluud C (2008) Trial sequential analysis may establish when firm evidence is reached in cumulative meta-analysis. Journal of Clinical Epidemiology 61: 64-75.

18. Brok J, Thorlund K, Gluud C, Wetterslev J (2008) Trial sequential analysis reveals insufficient information size and potentially false positive results in many meta-analysis. Journal of Clinical Epidemiology: 763-769.
19. World Health O The ICD-10 Classification of Mental and Behavioral Disorders.
20. American Psychiatric A (1980) Diagnostic and Statistical Manual of Mental Disorders (DSM III). Washington, DC: Author.
21. American Psychiatric A (1987) Diagnostic and Statistical Manual of Mental Disorders (DSM III-R). Washington, DC: Author.
22. American Psychiatric A (1994) Diagnostic and Statistical Manual of Mental Disorders (DSM IV). Washington, DC: Author.
23. Feighner JP, Robins E, Guze SB, Woodruff RA, Jr., Winokur G, et al. (1972) Diagnostic criteria for use in psychiatric research. ArchGenPsychiatry 26: 57-63.
24. Hamilton M (1960) A rating scale for depression. Journal of Neurology, Neurosurgery & Psychiatry 23: 56-61.
25. Montgomery SA, Asberg M (1979) A new depression scale designed to be sensitive to change. BrJPsychiatry 134: 382-389.
26. Bech AT (1961) An inventory for measuring depression. Archives of General Psychiatry 4: 561-571.
27. Englev E, Petersen KP (2003) ICH-GCP Guideline: quality assurance of clinical trials. Status and perspectives. Ugeskrift for Laeger 165: 1659-1662.
28. Kjaergaard L, Villumsen J, Gluud C (2001) Reported methodologic quality and discrepancies between large and small randomized trials in meta-analysis. Annals of Internal Medicine 135: 982-989.
29. Gluud LL (2006) Bias in clinical intervention research. AmJEpidemiol: 493-501.
30. Lundh A, Sismondo S, Lexchin J, Busuioc OA, Bero L (2012) Industry sponsorship and research outcome. Cochrane Database Syst Rev 12: MR000033.
31. Egger M, Davey Smith G, Schneider M, Minder C (1997) Bias in meta-analysis detected by a simple, graphical test. BMJ 315: 629-634.
32. National Institute for Clinical Excellence (2004) Depression: Mangement of Depression in Primary and Secundary Care. London, England: National Institute for Clinical Excellence: 640.
33. Rimer J, Dwan K, Lawlor Debbie A, Greig Carolyn A, McMurdo M, et al. (2012) Exercise for depression. Cochrane Database of Systematic Reviews: John Wiley & Sons, Ltd.
34. Fournier JC, DeRubeis RJ, Hollon SD, Dimidjian S, Amsterdam JD, et al. (2010) Antidepressant drug effects and depression severity: a patient-level meta-analysis. JAMA 303: 47-53.
35. Thorlund K, Engstrøm J, Wetterslev J, Brok J, Imberger G, et al. (2011) User manual for trial sequential analysis (TSA). Copenhagen Trial Unit, Centre for Clinical Intervention Research, Copenhagen, Denmark Available from wwwctudk/tsa: 1-115.