Natalizumab for relapsing remitting multiple sclerosis (Review)

Pucci E, Giuliani G, Solari A, Simi S, Minozzi S, Di Pietrantonj C, Galea I

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[Intervention Review]

Natalizumab for relapsing remitting multiple sclerosis

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ABSTRACT

Background

Natalizumab (NTZ) (Tysabri®) is a monoclonal antibody that inhibits leukocyte migration across the blood-brain barrier, thus reducing inflammation in central nervous system, and has been approved worldwide for the treatment of relapsing-remitting multiple sclerosis (RRMS).

Objectives

To evaluate the efficacy, tolerability and safety of NTZ in the treatment of patients with RRMS.

Search methods

We searched the Cochrane Multiple Sclerosis Group Trials Register, the Cochrane Central Register of Controlled Trials (CENTRAL, The Cochrane Library, 2010, Issue 1), MEDLINE (PubMed) and EMBASE, all up to 19 February 2010, and bibliographies of papers. Handsearching was carried out. Trialists and pharmaceutical companies were contacted. Furthermore, the websites of US Food and Drug Administration (FDA), the European Medicines Evaluation Agency (EMA) and the National Institute for health and Clinical Excellence (NICE) were also checked.

Selection criteria

All double-blind, randomised, controlled trials analysing more than a single infusion of NTZ (dosage > 3 mg/kg intravenous infusion every 4 weeks), also including its use as add-on treatment, versus placebo or other drugs in patients with RRMS. No restrictions on the basis of duration of treatment or length of follow up.

Data collection and analysis

Three reviewers independently selected articles which met the inclusion criteria. Disagreements were solved by discussion. Two reviewers independently extracted the data and assessed the methodological quality of each trial. Missing data was sought by contacting principal authors and Biogen Idec, through Biogen-Dompé Italia.

Main results

Three studies met the inclusion criteria. These included one placebo-controlled trial (942 patients) and two add-on placebo-controlled trials, i.e. one plus glatiramer acetate (110 patients) and the second plus interferon beta-1a (1171 patients).

This review assessed the efficacy, tolerability and safety of NTZ in patients with RRMS. Data was conclusive with respect to efficacy and tolerability, but not safety. As far as efficacy is concerned, the results showed statistically significant evidence in favour of NTZ for all the primary outcomes and for the secondary ones where data was available. NTZ reduced the risk of experiencing at least one new exacerbation at 2 years by about 40% and of experiencing progression at 2 years by about 25% as compared to a control group. MRI parameters showed statistical evidence in favour of participants receiving NTZ. Infusion reactions, anxiety, sinus congestion, lower limb swelling, rigors, vaginitis and menstrual disorders were reported as adverse events (AEs) more frequently after NTZ treatment. In this review NTZ was found to be well tolerated over a follow-up period of two years: the number of patients experiencing at least one AE (including severe and serious AEs) during this period did not differ between NTZ-treated patients and controls. Safety concerns have been raised about Progressive Multifocal Leukoencephalopathy (PML). In the trials included in this review, two cases of PML were encountered: one in a patient who had received 29 doses of NTZ and a second fatal case of PML in another patient after 37 doses of NTZ. Our protocol was insufficient to evaluate PML risk as well as other rare and long-term adverse events such as cancers and other opportunistic infections, which are very important issues in considering the risk/benefit ratio of NTZ.

Authors' conclusions

Although one trial did not contribute to efficacy results due to its duration, we found robust evidence in favour of a reduction in relapses and disability at 2 years in RRMS patients treated with NTZ. The drug was well tolerated. There are current significant safety concerns due to reporting of an increasing number of PML cases in patients treated with NTZ. This review was unable to provide an up-to-date systematic assessment of the risk due to the maximum 2 year-duration of the trials included. An independent systematic review of the safety profile of NTZ is warranted. NTZ should be used only by skilled neurologists in MS centres under surveillance programs.

All the data in this review came from trials supported by the Pharmaceutical Industry. In agreement with the Cochrane Collaboration policy, this may be considered a potential source of bias.

PLAIN LANGUAGE SUMMARY

The use of the monoclonal antibody Natalizumab (NTZ) in patients with relapsing remitting multiple sclerosis (RRMS)

It is currently thought that inflammation is crucial in MS, leading to a disruption in the ability of nerves to conduct impulses. NTZ is the first of a new generation of anti-inflammatory treatments for MS, which is given intravenously every 4 weeks. It is usually prescribed once other drugs have failed or when the disease is rapidly worsening.

The Authors of this review evaluated the efficacy, tolerability and safety of NTZ in patients with RRMS. Among the pertinent literature, 3 studies met the inclusion criteria of methodological quality, comprising a total of 2223 participants. The results show that NTZ treatment reduces the number of patients who experienced relapses and the number of patients who progressed at 2 years. Also Magnetic Resonance scans show evidence of a beneficial effect of NTZ on disease activity.

Although information on adverse events (AEs) was limited, as most participants were followed up for 2 years only, infusion reactions, anxiety, sinus congestion, lower limb swelling, rigors, vaginal inflammation and menstrual disorders were found to be more frequent after NTZ treatment. However, the number of patients experiencing at least one AE (including severe or serious AEs) did not differ between NTZ and control groups. On the contrary, significant safety concerns have been raised regarding Progressive Multifocal Leukoencephalopathy (PML), a rare and often fatal viral disease characterized by damage to the white matter of the brain. In the studies included in this review, PML was reported in 2 patients treated with NTZ for more than 2 years. However, our protocol was insufficient to evaluate PML risk as well as other potential rare and long-term AEs (e.g. cancers and other infections) which are important issues in considering the risk/benefit ratio of NTZ. An independent systematic review of the safety profile of NTZ is warranted. NTZ should be used only by skilled neurologists in MS centres under surveillance programs.

All the data in this review came from studies supported by the Pharmaceutical Industry. In agreement with the Cochrane Collaboration policy, this may be considered a potential source of bias.

BACKGROUND

Table 1 lists abbreviations used in the text.

Description of the condition

Multiple sclerosis (MS) is regarded as the foremost cause of non-traumatic neurologic disability in young adults (Tremlett 2010). MS is notoriously heterogeneous, both clinically and histopathologically (Lucchinetti 1996), and characterised by unpredictability from patient to patient and within a given individual over time. In most cases it begins with episodic, largely reversible neurologic dysfunction, in a pattern termed relapsing-remitting multiple sclerosis (RRMS) (Lublin 1996). A minority of patients (ranging from 10 to 20% of cases) have benign MS (as defined by no or minimal disability at 10 or 20 years), although this continues to be a controversial issue (Pittock 2007). Natural history studies show that, after about 10 years, about half of people with MS gradually develop permanent disability, which may also include acute relapses; this is known as secondary progressive MS (SPMS) (Weinshenker 1989). After a median of 15-28 years (Weinshenker 1989; Tremlett 2006) from disease onset, a disability milestone equivalent to the use of an assistive walking device is reached. There is an increased risk of death in MS (Tremlett 2010).

Magnetic resonance imaging (MRI) does provide a reflection of the underlying pathology, and it is integrated with clinical and other paraclinical diagnostic methods to facilitate the diagnosis of MS (McDonald 2001; Polman 2005). MRI parameters are used as a surrogate marker of disease activity and/or progression. MRI studies have shown that T2 lesion burden and contrast enhancing lesions are representative of the active inflammatory component which characterises the relapsing-remitting course, while their correlation with disability is poor (Filippi 2002).

The disease has an adverse and often highly debilitating impact on the quality of life (QoL) of people with MS and their families. Relapses, even when they completely remit, are associated with a level of temporary disability that disrupts working, family and social life. MS, even in its early stages, may undermine patients' confidence, restrict their activity and limit their role in society. Subtle but disabling symptoms (such as fatigue, cognitive disturbances or symptoms in the spectrum of anxiety and mood disorders), may not be easily recognised and taken into consideration.

Although the etiology is largely unknown, it is believed that MS develops in genetically predisposed individuals and that environmental factors play a central role in its pathogenesis based on immune-mediated mechanisms. It is thought that aberrant immune responses to self or foreign antigens initiate and perpetuate inflammation (Frohman 2006). The conventional hypothesis of multiple sclerosis pathogenesis is that inflammation is the primary event, leading to demyelination and subsequent axonal damage. However, the role of inflammation is complex, with both beneficial and deleterious features (Martino 2002). On the other hand, some researchers hypothesise that inflammation is not the primary pathogenic mechanism, that axonal loss occurs early and that a cryptic aetiological agent may cause axonal damage and demyelination, as well as inducing an inflammatory response, which plays a secondary role (Trapp 1998; Maggs 2004).

In summary the current predominant school of thought is that the acute inflammatory process characterises the initial stage of the illness, while progression of disability is more closely related to irreversible damage to myelin and axons. These features have important implications for therapy: strategies that target inflammation will only have a limited influence on progression once patients have entered the progressive phase of the disease.

Description of the intervention

Natalizumab (NTZ) (Tysabri®, previously labelled Antegren®; Elan Pharmaceuticals Inc., San Diego, CA, and Biogen Idec Inc., Cambridge, MA) is a recombinant humanized monoclonal antibody. It contains human IgG4$_\kappa$ framework regions and the complementary-determining regions of a murine antibody that binds to the $\alpha 4$ chain of $\alpha 4\beta 1$ integrin. The murine region may result in the generation of neutralizing antibodies or allergic reactions. The human region endows natalizumab with the effector functions of immunoglobulin subclass IgG4, which is the least immune activating amongst the human IgGs. The recommended dose of Tysabri® is 300 mg intravenous (IV) infusion every four weeks (FDA 2004).

Tysabri® was approved by Food and Drug Administration (FDA) for treatment of patients with RRMS on 23 November 2004 after priority review of 1-year data from the two ongoing SENTINEL and AFFIRM trials. Priority review and accelerated approval was determined to be appropriate because of the strength of the efficacy and safety data available at 1 year. Following the recognition of two cases of progressive multifocal leukoencephalopathy (PML) in patients who had been receiving NTZ, Biogen Idec and Elan Pharmaceuticals, in discussions with the FDA, suspended commercialization and clinical trials on 28 February 2005 and started to investigate the relationship between PML and NTZ therapy. A comprehensive clinical, radiological, and laboratory investigation of patients exposed to NTZ in clinical trials (including trials carried on in patients with Crohn Disease - CD) was completed (Yousry 2006). In addition, the 2-year results of SENTINEL and AFFIRM trials were submitted to the FDA in September 2005. On July 2006 marketing of Tysabri® resumed . In the following months, Tysabri® was gradually commercialised worldwide.

How the intervention might work

Inflammatory lesions in MS appear to arise after activated leucocytes gain access to the CNS from the circulation. Integrins on the surface of leucocytes interact with immunoglobulin superfamily

proteins on cerebral endothelial cells, facilitating diapedesis across the blood-brain barrier. Examples of integrin / immunoglobulin superfamily pairs are VLA4/VCAM1 and LFA1/ICAM1. NTZ is a monoclonal antibody against α4-integrin (part of VLA4), thus preventing interaction of VLA4- with VCAM1 and, as a consequence, blocking the transmigration of VLA4-expressing leucocytes across the blood-brain barrier (Niino 2006; Ransohoff 2007). In preclinical studies, NTZ reduced disease activity in mice with experimentally induced allergic encephalomyelitis, an animal model of MS (Yednock 1992; Kent 1995).

Why it is important to do this review

Tysabri® is available in many countries for treating RRMS. As a result of the risk of PML (Yousry 2006), it is generally recommended as second-line therapy in RRMS if a conventional DMD has failed, and in rapidly evolving severe disease (e.g. FDA 2006, EMA 2009, AIFA 2006, NICE 2007). The details of the eligibility criteria vary from country to country. A systematic review to assess the efficacy and side effect profile of NTZ is timely and important.

OBJECTIVES

The efficacy, tolerability and safety of NTZ in the treatment of people with RRMS were evaluated.

METHODS

Criteria for considering studies for this review

Types of studies

Double-blind, randomised, controlled trials (RCTs). Trials were not excluded on the basis of duration of treatment (except those involving a single infusion) or length of follow up.

Types of participants

Patients with RRMS of both gender who met the criteria of Poser (Poser 1983) for clinically definite or laboratory-supported definite MS, or the original / revised McDonald criteria (McDonald 2001, Polman 2005), aged > 17 years.

Types of interventions

NTZ (dosage > 3 mg/Kg IV infusion every 4 weeks), also as add-on treatment, versus placebo or other drug.

Types of outcome measures

Primary outcomes

We assessed the following primary outcome measures:
(1) The number of patients experiencing at least one relapse at 2 years. Definitions of relapse given in the original studies were accepted.
(2) The number of patients who progressed at 2 years. Definitions of progression given in the original studies were accepted. However, we tried to evaluate this outcome using the definition of progression as a persistent worsening of at least one point in EDSS (Kurtzke 1983), recorded outside a relapse and confirmed by a follow-up assessment at six months; a persistent half-point increase was adopted if baseline EDSS was 5.5 or worse.
(3) Mean change in Short Form 36 (SF-36) scores (Ware 1992) at 2 years. The SF-36 is a widely used, generic measure of self-reported health status that consists of 35 items investigating eight domains over the previous month: physical functioning (10 items), social functioning (2 items), physical role limitations (4 items), mental health (5 items), emotional role limitations (3 items), pain (2 items), energy/vitality (4 items), and general health (5 items); one more item (change in health over the previous year) was not used in scoring. Higher scores indicate higher QoL. The scores for the eight domains can be reduced to two composite scores, the Physical Component Summary (PCS) and the Mental Component Summary (MCS), by means of principal component analysis (Ware 1993). These summary scales are computed as standardized scores such that a mean score of 50 (standard deviation [SD], 10) corresponds to that of the general US population.
(4) The number of patients with at least one severe AE during 2 years of treatment. Many terms are used to describe harm associated with healthcare interventions, causing confusion. Thus, we define an "adverse event" as any unfavourable outcome that occurs during or after the study, whether or not related to the study drug, including an exacerbation of a preexisting condition, except for MS progression; we also include hospitalization or death (whatever the cause of both). With respect to their severity, definition of severe AE given in the original studies was accepted. If not otherwise specified, AEs were defined as severe when leading to withdrawal from the study or discontinuation of treatment without satisfying the definition of serious AE (see below for definition). It may be helpful to remember that the term "severe" refers to the intensity of a particular AE and is not synonymous with "serious", i.e. it refers to tolerability, while "serious" (see below for definition) refers to safety (e.g. a non-serious AE, such as headache, may be severe in intensity as opposed to mild or moderate) (ICH Expert Working Group 1994).
(5) The number of patients with a serious AE (no period restriction). Definitions of serious AE were those reported by the Expert Working Group of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuti-

cals for Human Use (ICH) (death, life-threatening event, hospitalisation or prolongation of existing hospitalisation, persistent or significant disability) (ICH Expert Working Group 1994), except for the fact that we did not consider permanent or significant disability caused by MS as a serious AE.

Secondary outcomes

The secondary outcome measures included:
(1) Time to progression of disability at 2 years
(2) Mean change in EDSS score at 2 years
(3) Mean change in Multiple Sclerosis Functional Composite (MSFC - Rudick 2002) at 2 years
(4) The number of patients who were unable to walk without aid (EDSS greater than 5.5) at 2 years
(5) Mean change in Modified Fatigue Impact Scale (MFIS) at 2 years (Kos 2005)
(6) Mean change in well-being as measured by a visual analog scale (VAS) at 2 years
(7) Mean change in PASAT (one of the components of MSFC), which assess cognitive function, at 2 years (Gronwall 1977)
(8) The number of patients experiencing clinically significant worsening of vision at 2 years [defined as two-line (10-letter) reductions in Sloan chart scores, sustained over 12 weeks] (Balcer 2000; Rosser 2003)
(9) The number of patients who showed at least one gadolinium-enhancing lesion at 2 years
(10) The mean change of total lesion load on T2-weighted images at 2 years
(11) The number of patients experiencing at least one AE, no matter whether mild or severe, serious or not (no period restriction)
(12) The number of patients experiencing treatment discontinuation caused by AE
(13) The number of patients experiencing a relapse in the 4 weeks after the first dose of NTZ

Search methods for identification of studies

A systematic search without language restrictions was conducted to identify all relevant published and unpublished randomised controlled trials.
For additional information about the Group's search strategy please see: Cochrane Multiple Sclerosis Group

Electronic searches

We searched the following databases

1. Cochrane Multiple Sclerosis Group Trials Register (19 February 2010)
2. The Cochrane Central Register of Controlled Trials (CENTRAL) "The Cochrane Library"(Issue 1, 2010) (Appendix 1)
3. MEDLINE (PubMed) (1966 to 19 February 2010) (Appendix 2)
4. EMBASE (EMBASE.com) (1988 to 19 February 2010) (Appendix 3)

Searching other resources

Handsearching of the references quoted or linked to the identified trials and other papers of interest, congress reports (1998 to February 2010) of the American Academy of Neurology, the American Neurological Association, the American Committee for Treatment and Research in MS, the European Committee for Treatment and Research in MS and the Italian Neurological Society. Contact with researchers who were participating in trials on NTZ; and contact with Biogen or other pharmaceutical companies.
In addition we checked the following sources for trials about NTZ: clinicaltrials.gov (www.clinicaltrials.gov); US Food and Drug Administration (FDA) (www.fda.gov), the European Public Assessment Reports from the European Medicines Evaluation Agency (EMA) (www.emea.eu) and the National Institute for health and Clinical Excellence (NICE) (www.nice.org.uk).

Data collection and analysis

Selection of studies

Three reviewers (EP, GG, AS - all MS experts), independently assessed the eligibility of articles for the review. The same reviewers independently scrutinised the full texts of the selected studies and decided which trials met the inclusion criteria. All reviewers assessing the relevance of studies knew the names of the authors, institutions, journal of publication and results when they applied the eligibility criteria. Any disagreement was resolved by discussion.

Data extraction and management

Two reviewers (EP, IG) extracted the data independently: characteristics of participants, interventions, duration of treatment, length of follow-up, outcome measures, side effects and adverse events. We sought to extract from each RCT the number of patients originally assigned to each treatment group to allow an intention-to-treat analysis, if the trial was not already presented in this way. Disagreement was resolved by discussion amongst all the reviewers. All data was registered on a collection form. Study authors were consulted to resolve controversies and clarify questions, including missing data, which were posed by the two reviewers extracting data. Similar clarifications were sought with Biogen Idec Inc. and Elan Pharmaceuticals Inc, through the Medical Direction of Biogen-Dompè Italy.

Assessment of risk of bias in included studies

The risk of bias assessment for RCTs and CCTs was performed as recommended by the Cochrane Handbook (Handbook 5 2008) using a two-part tool addressing seven specific domains: sequence generation and allocation concealment (selection bias), blinding of participants and providers (performance bias), blinding of outcome assessor (detection bias), incomplete outcome data (attrition bias), selective outcome reporting (reporting bias) and other sources of bias. The first part of the tool involves describing what was reported to have happened in the study. The second part of the tool involves assigning a judgement relating to the risk of bias for that entry, in terms of low, high or unclear risk. See Appendix 4 for details.

The domains of sequence generation, allocation concealment (avoidance of selection bias) and selective outcome reporting (avoidance of reporting bias) were addressed in the tool by a single entry for each study.

Blinding of participants, personnel and outcome assessor (avoidance of performance bias and detection bias) were considered separately for both objective and subjective outcomes.

We assessed whether included studies were in line with the CONSORT Statement (Moher 2001), a reflection of the risk for biased estimates of treatment effects (Schulz 1995; Moher 1998). Despite the controversy surrounding the importance of the CONSORT Statement (see "'Risk of bias' and 'quality'" in Cochrane Handbook for Systematic Reviews of Interventions (Higgins 2008), we felt that adherence to such a statement that aims to improve trial reporting was appropriate.

Measures of treatment effect

Data was analysed according to an intention-to-treat approach. We analysed dichotomous outcomes by calculating relative risks (RR) for each trial with the uncertainty in each trial being expressed using 95% confidence intervals (95% CI). Difference in means (MD) across trials was used for continuous outcomes.

If not available, standard deviations were obtained from sample size, mean values and p-values.

Where appropriate, we planned to calculate Number Needed to Treat (NNT), i.e. Number Needed to Benefit (NNB) or Number Needed to Harm (NNH), as follows: NNT=1/[BR(1- RR)], where BR is the baseline risk (rate of the event in the control group) and RR is the relative risk resulting from meta analysis.

Assessment of heterogeneity

The statistic I^2 was calculated for each pooled estimate, in order to assess the impact of statistical heterogeneity. I^2 may be interpreted as the proportion of total variation among effect estimates that is due to heterogeneity rather than sampling error, and it is intrinsically independent of the number of studies. When I^2 < 30% there is little concern about statistical heterogeneity Higgins 2002; Higgins 2003). We used the random-effect model to take account of the between-study variance in our findings (DerSimonian 1986). A sensitivity analysis was performed if I^2 was 30% or more, comparing results obtained via random- and fixed-effect models.

Data synthesis

We used Review Manager (RevMan) 2008 to perform meta-analyses of the included studies and displayed the results as forest plots. Descriptive analyses of included and excluded trials were also undertaken.

Subgroup analysis and investigation of heterogeneity

Subgroup analysis were planned for analysing particular AEs which were pooled with other AEs in a generic label. For example, serious AEs due to MS relapse were analysed separately from other serious AEs.

Possible sources of heterogeneity were explored by subgroup analysis where appropriate.

Sensitivity analysis

In order to incorporate assessment of risk of bias in the review process we planned to plot intervention effects estimates stratified for risk of bias for each relevant domain. In case of differences in results among studies with different risks of bias, we planned to perform sensitivity analysis excluding studies with high risk of bias.

Many issues suitable for sensitivity analysis are only identified during the review process where the individual peculiarities of the studies under investigation are identified. Thus, we retained the opportunity to carry out other sensitivity analyses during the review process that could affect the overall result and conclusions.

RESULTS

Description of studies

See: Characteristics of included studies; Characteristics of excluded studies; Characteristics of studies awaiting classification.

Results of the search

After eliminating duplicates, the number of hits retrieved by the Cochrane Multiple Sclerosis Group systematic search strategy was 251. Eleven articles were considered as potentially eligible after screening of titles and abstracts, with consensus among the reviewers. After study of the full text, with consensus among the reviewers,10 were confirmed as potentially relevant papers. The article excluded concerned the UK Antegren Study 1999.

No further studies were identified by handsearching of congress reports and other sources.

At this point, the process of linking multiple reports of the same study was carried out. Four studies were identified. Three identified studies completely satisfied the criteria for inclusion in this review. The fourth study, the INMSTG trial (INMSTG 2003), included participants affected with both RRMS and SPMS, who were randomised to two different doses of NTZ (3 or 6 mg per kilogram) or placebo. We contacted the study investigators in order to obtain data on RRMS patients in the placebo arm and in the 6 mg per kilogram arm. The INMSTG trial did not contribute to the metanalysis because we did not receive any data from the investigators; for this reason it was included in "studies awaiting classification" (see Characteristics of studies awaiting classification).

Included studies

Three studies met the inclusion criteria: one placebo-controlled trial (942 patients) (AFFIRM 2006) and two add-on placebo-controlled trials, i.e. one plus glatiramer acetate (110 patients) (GLANCE 2009) and the second plus interferon beta-1a (1171 patients) (SENTINEL 2006).

For details see "Characteristics of included studies". Baseline characteristics of participants in the studies which contributed to primary efficacy outcomes are summarised in Table 2.

Excluded studies

See "Characteristics of excluded studies".

Risk of bias in included studies

Figure 1 shows authors' judgements on each methodological quality item presented as percentages across all included studies.

Figure 1. Methodological quality graph: review authors' judgements on each methodological quality item presented as percentages across all included studies.

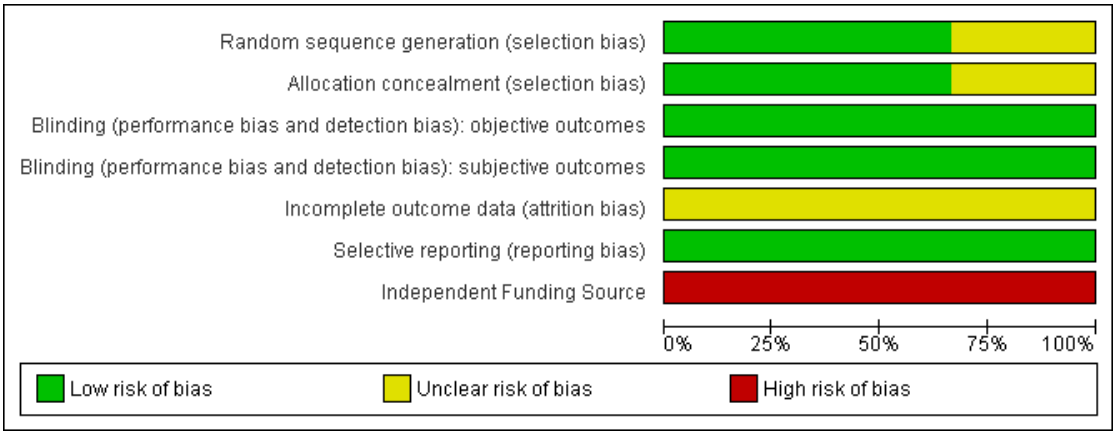

Figure 2 shows authors' judgements on each methodological quality item for each included study.

Figure 2. Methodological quality summary: review authors' judgements on each methodological quality item for each included study.

All the data of the present review came from trials supported by Biogen Idec and Elan Pharmaceuticals. All primary references of the included RCTs reported author financial disclosures. Author-industry ties were reported for 100% of the authors of the primary reference for each trial. In particular, 4 out of the 14 authors (AFFIRM 2006), 4 out of the 12 authors (GLANCE 2009) and 3 out of the 12 authors (SENTINEL 2006) were employees of Biogen Idec.

Since one of the included trials (SENTINEL 2006), which contributed to primary efficacy outcomes, was an add on study, in which the control arm was placebo plus interferon beta 1a (IFNß-1a), one possible risk was underestimation or overestimation of the effect size of NTZ efficacy due to interaction between the DMDs. Results for the NTZ group versus placebo were also reported separately. The use of GA or IFNß-1a in two of the included trials may have biased safety/tolerability outcomes because of interactions between these DMDs and NTZ.

The different durations of included trials could have been a source of bias with respect to secondary safety/tolerability outcomes. To address this concern we performed a sensitivity analysis comparing results including and excluding GLANCE 2009 for tolerability/safety outcomes, given the short period of follow-up (6 months) in this trial.

Protocol violations may affect results by introducing bias. Data about protocol violations are reported in Table 3, Table 4 and Table 5, and are mainly derived from the FDA's CDER "medical review" (FDA 2004). Unfortunately, the CDER only analysed 1-year data from the SENTINEL 2006 and AFFIRM 2006 trials. As far as this 1-year analysis is concerned, the frequency of protocol violations was considered similar for the two treatment arms in both trials.

Allocation

The AFFIRM 2006 and the SENTINEL 2006 studies had adequate sequence generation and allocation concealment. Randomisation was carried out with the use of a computer-generated schedule and a multidigit identification number, implemented by way of an interactive voice-response system. There was insufficient published information about the method of randomisation and treatment allocation of the GLANCE 2009 trial.

Blinding

All the included studies were double-blinded.

The occurrence of more frequent AEs in NTZ treated subjects (such as headache during infusion) was not of sufficiently large size to raise concerns about blinding.

A well-established pharmacodynamic feature of NTZ is the increase in number of all circulating leukocytes except neutrophils (Polman 2006 - AFFIRM 2006). The increase of leukocytes was appropriately taken in consideration in blinding procedures, at least in the AFFIRM 2006 and SENTINEL 2006 trials (see below).

Adequacy of double-blinding was appropriate in AFFIRM 2006 and SENTINEL 2006 studies. At each study site, primary and backup examining neurologists (who were not in contact with patients in any other capacity including laboratory assessments) and primary and backup treating neurologists were designated.

However, to our knowledge no analyses of the efficacy of blinding was carried out in the included studies.

Incomplete outcome data

All included studies: (a) reported the percentage of patients who dropped out from the study for each assignment group; (b) reported the percentage of patients who discontinued the treatment but continued follow up (including CONSORT flowcharts); (c) performed the analysis according to the intention-to-treat principle. However, none of the papers described how missing data was imputed for ITT analysis of primary outcomes (the rate of clinical relapse at one year and the rate of sustained progression of disability, as measured by the EDSS, at 2 years). Considering secondary outcomes, the AFFIRM 2006 and SENTINEL 2006 papers reported that missing values were imputed using the mean for the respective measures in the study population. ITT statements with no further details carry a risk of bias. Thus, after resolution of controversy among EP and SM, the methodological quality item "incomplete outcome data addressed" was judged as unclear.

It was not possible to carry out sensitivity analysis to assess the effect of patients who withdrew since raw data was not available to enable an "available case analysis". Data on "the number of patients experiencing at least one relapse" was extracted from Table 2 in Polman 2006 (AFFIRM 2006) and from Table 2 in Rudick 2006 (SENTINEL 2006). In both the tables, looking at the item "Number of relapses - no. of patients", one can see that the total number of patients in each arm is equal to the number of randomised patients (ITT populations), but it is not specified how missing values were included. As far as "the number of patients who progressed" is concerned, data was obtained from the Kaplan-Meier plots in Figure 2 in Polman 2006 (AFFIRM 2006) and in Figure 2 in Rudick 2006 (SENTINEL 2006).

The percentages of patients who withdrew were low, and similar, between NTZ treated and untreated patients, and the reasons for withdrawal were comparable.

In the AFFIRM trial, the discrepancy between the number of randomised patients (n=315) and the number of patients submitted to safety analysis (n=312) is due to the fact that three patients who were assigned to receive placebo were never treated; these patients were included in the ITT efficacy analyses but were excluded from

the safety analyses.
MRI data was not available for 8 to 9% of patients at year 2 in the AFFIRM trial (Miller 2007 - AFFIRM 2006). The main reason for missing data (>80%) was the scan not being performed because the patient withdrew from the study; in the remainder, although the patient remained in the study, the scan was either not performed, had not been received at the Central MRI Analysis Center, or had been received but was of inadequate quality for analysis (Miller 2007 - AFFIRM 2006).
The SENTINEL 2006 trial was stopped approximately one month early because of 2 reports of PML.
In the SENTINEL 2006 trial 25 patients were excluded from analysis because of irregularities in data (the original enrolled cohort was of 1196 patients while 1171 patients were included in ITT analysis). We did not analyse the effect of this exclusion but the FDA Center for drug evaluation and research (CDER) did, through a "worst case" sensitivity analyses. That analysis did not bring to light significant effects on overall study results at 1 year (FDA 2004a).

Selective reporting

We did not identify any selective reporting in all the included studies.

Other potential sources of bias

We did not find any other study characteristics that may have negatively affected the quality of the trials. Except for GLANCE 2009, papers reported that the studies were overseen by independent data and safety monitoring committees; sample size calculation was performed in all included trials.
Due to the limited number of trials available, we did not perform additional subgroup analyses.

Effects of interventions

PRIMARY EFFICACY OUTCOME MEASURES
(1) THE NUMBER OF PARTICIPANTS EXPERIENCING AT LEAST ONE RELAPSE DURING 2 YEARS OF TREATMENT
Data was available from the AFFIRM (Polman 2006, AFFIRM 2006) and SENTINEL (Rudick 2006, SENTINEL 2006) trials for a total of 2113 participants. Relapses were defined as new or recurrent neurologic symptoms not associated with fever or infection, lasting at least 24 hours, and accompanied by new, objective neurologic findings.
The pooled estimate was RR=0.57 (95% CI 0.47 to 0.69), showing statistical evidence in favour of NTZ. This result can be re-expressed as follows: NTZ (with or without IFNß-1a) reduced the risk of experiencing at least one new exacerbation at 2 years by 30% to 50% as compared to not receiving NTZ.
In order to avoid one patient from experiencing at least one relapse during two years of treatment, 3 to 5 people would have to receive NTZ (NNB=4, 95% CI 3 to 5).
There was heterogeneity between the trials (I^2=71%), but no difference was found between fixed- and random-effect models.
(2) THE NUMBER OF PATIENTS WHO PROGRESSED AT 2 YEARS
Data was available from the AFFIRM (Polman 2006, AFFIRM 2006) and SENTINEL (Rudick 2006, SENTINEL 2006) trials for a total of 2113 participants. Definitions of progression given in the original studies were used: in both studies sustained progression of disability was defined as an EDSS increase of 1.0 or more from a baseline score of 1.0 or more, or an EDSS increase of 1.5 or more from a baseline score of 0 that was sustained for 12 weeks (progression could not be confirmed during a relapse).
The pooled estimate was RR=0.74 (95% CI 0.62 to 0.89), showing statistical evidence of efficacy of NTZ in reducing the number of patients who progressed. In other words, NTZ (with or without IFNß-1a) reduced the risk of experiencing progression at 2 years by 10% to 40% as compared to a control group not receiving NTZ.
The number of patients needing treatment with NTZ in order to avoid progression in one patient at 2 years is 10 (NNB=10, 95% CI 7 to 23).
There was heterogeneity between the trials (I^2=52%), but this was not confirmed in the sensitivity analysis (data not shown).
(3) MEAN CHANGE IN SF-36 SCALE SCORES AT 2 YEARS
The PCS and the MCS were analysed separately. Data was available from the AFFIRM and SENTINEL trials (Rudick 2007, SENTINEL 2006, AFFIRM 2006) for a total of 2113 patients.
The mean difference in PCS mean change between the NTZ and control groups favoured NTZ treated patients (MD=1.98, 95% CI 1.05 to 2.91, p<0.0001). There was no statistical evidence of heterogeneity (I^2=0).
The mean difference in MCS mean change between NTZ and control groups showed a difference favouring NTZ treated patients (MD=1.38, 95% CI 0.33 to 2.42, p=0.01). There was no statistical evidence of heterogeneity (I^2=0).
SECONDARY EFFICACY OUTCOME MEASURES
The following measures were planned as secondary efficacy outcome measures in the protocol:
(1) TIME TO PROGRESSION IN DISABILITY AT 2 YEARS
No data was available despite contacting authors and sponsor.
(2) MEAN CHANGE IN EDSS SCORE AT 2 YEARS
No data was available despite contacting authors and sponsor.
(3) MEAN CHANGE IN MSFC AT 2 YEARS
No data was available despite contacting authors and sponsor.
(4) THE NUMBER OF PATIENTS WHO WERE UNABLE TO WALK WITHOUT AID (EDSS > 5.5) AT 2 YEARS
No data was available despite contacting authors and sponsor.
(5) MEAN CHANGE IN MFIS AT 2 YEARS
No data was available despite contacting authors and sponsor.

(6) MEAN CHANGE IN WELL-BEING AS MEASURED BY A VAS AT 2 YEARS

Data was only available from the AFFIRM trial (Rudick 2007, AFFIRM 2006) for a total of 942 patients.

Mean difference between NTZ and control groups favoured NTZ treatment (MD=6.40, 95% CI 1.76 to 11.04, p= 0.007).

(7) MEAN CHANGE IN PASAT AT 2 YEARS

No data was available despite contacting authors and sponsor.

(8) THE NUMBER OF PATIENTS EXPERIENCING CLINICALLY SIGNIFICANT WORSENING OF VISION AT 2 YEARS

Clinically significant worsening of vision was defined as two-line (10-letter) reductions in Sloan chart scores, sustained over 12 weeks (Balcer 2000, Rosser 2003). Data from AFFIRM and SENTINEL trials was published in Balcer 2007 (SENTINEL 2006 AFFIRM 2006) but it could not be extracted in a form suitable for the meta-analysis. Data was requested from the authors and sponsor, but no response was received.

(9) THE NUMBER OF PATIENTS WHO SHOWED AT LEAST ONE GADOLINIUM-ENHANCING LESION AT 2 YEARS

Data was available from the AFFIRM (Polman 2006, AFFIRM 2006) and the SENTINEL (Rudick 2006, SENTINEL 2006) trials for a total of 2113 participants.

The number of patients with at least one Gadolinium-enhancing lesion was lower in the NTZ group as compared to the control group (RR=0.12, 95% CI 0.09 to 0.17). In other words, NTZ (with or without IFNß-1a) reduces the risk of developing at least one Gadolinium-enhancing lesion at 2 years by 87%. NNB is 4 (95% CI 4 to 4). There was no statistical evidence of heterogeneity (I^2=0).

(10) THE MEAN CHANGE OF TOTAL LESION LOAD ON T2-WEIGHTED IMAGES AT 2 YEARS

Data was available from the AFFIRM trial (Miller 2007, AFFIRM 2006) for 855 patients (91% from a total of 942). Over 2 years, NTZ significantly reduced the mean change in T2 lesion volume compared with placebo (MD = -3796, 95% CI -5849.43 to -1742.97, p = 0.0003).

PRIMARY SAFETY OUTCOMES

(1) THE NUMBER OF PATIENTS WITH SEVERE AE AT 2 YEARS

Here we report the numbers of patients with a severe AE as reported in the original papers from 2110 RRMS patients over two years [Polman 2006, (AFFIRM 2006) and Rudick 2006, (SENTINEL 2006)]. Number of patients experiencing at least one severe AE did not differ between patients treated with NTZ and controls RR=0.92 (95% CI 0.81 to 1.04), with no statistical evidence of heterogeneity (I^2=0%).

(2) THE NUMBER OF PATIENTS WITH SERIOUS AE

Serious AE were collected from SENTINEL (Rudick 2006, SENTINEL 2006), AFFIRM (Polman 2006, AFFIRM 2006) and GLANCE (Goodman 2009, GLANCE 2009) trials.

Pooled estimate showed that serious AEs in the NTZ group (227/1271, or 18%) were less common than in the control group (199/949, or 21%): RR=0.83 (95% CI 0.70 to 0.98). No statistical evidence of heterogeneity was found (I^2=0%). Since serious AEs prevailed in the control group. NNH was not calculated.

A sensitivity analysis excluding the GLANCE trial which was characterised by lower occurrence of serious AEs (2% in the NTZ arm and 4% in the control arm over 6 months, vs. 18% and 21% [SENTINEL 2006] and 19% and 24% [AFFIRM 2006], respectively, over 2 years) did not change findings: RR=0.83 (95% CI 0.70 to 0.99).

Two cases of PML were included in the serious AEs. They were two participants in the SENTINEL trial who were diagnosed as having PML after their completion of the two-year study (after 29 and 37 doses of NTZ respectively) (Langer-Gould 2005; Kleinschmidt-DeMasters 2005).

The most common serious AE was a relapse of MS, which was significantly more frequent in controls than in NTZ-treated patients (RR= 0.50, 95% CI 0.37 to 0.68). We speculated that MS relapses were considered as serious AEs because they resulted in hospitalisation (unofficial communication from the Medical Direction of Biogen-Dompé Italia in May 2009). When recalculating serious AEs without including MS relapses, we did not find any statistical difference between NTZ and control groups (RR=1.13, 95% CI 0.90 to 1.43).

Death occurred in 3 patients in the SENTINEL trial: 2 were assigned to IFNß-1a alone and one was the fatal case of PML after 37 doses of NTZ (Kleinschmidt-DeMasters 2005). Two deaths occurred in the AFFIRM study, both in the NTZ group. One patient died of malignant melanoma. A second patient, a 49 year-old woman, died of alcohol intoxication (Polman 2006, AFFIRM 2006) (a suicide was suspected - unofficial communication from the Medical Direction of Biogen-Dompé Italia in May 2009).

SECONDARY SAFETY OUTCOMES

We assessed the following secondary safety outcome measures at any time of follow-up:

(1) THE NUMBER OF PATIENTS EXPERIENCING AT LEAST ONE AE

In SENTINEL (Rudick 2006, SENTINEL 2006), AFFIRM (Polman 2006, AFFIRM 2006) and GLANCE (Goodman 2009, GLANCE 2009) trials, the numbers of patients who reported at least one AE were given.

Number of patients experiencing at least one AE did not differ between patients treated with NTZ and controls [RR=1.00 (95% CI 0.99 to 1.01)], with no statistical evidence of heterogeneity (I^2=0%).

As far as the type of AE is concerned, we report those AE which were significantly different between NTZ-treated and placebo-treated patients, as follows:

- In the SENTINEL study, anxiety was statistically more frequent in patients treated with NTZ than in patients who were not (RR=1.49, 95% CI 1.05 to 2.12). Data on anxiety was not

reported in the AFFIRM and GLANCE papers.

- In the SENTINEL study, "sinus congestion" was statistically more frequent in patients treated with NTZ than in patients who were not (RR=2.03, 95% CI 1.15 to 3.59). In the AFFIRM and GLANCE studies, the term "sinus congestion" was not reported among the AEs.
- In the SENTINEL study, "peripheral edema" was statistically more frequent in patients treated with NTZ than in patients who were not (RR=4.78, 95% CI 2.00 to 11.42). In the AFFIRM and GLANCE papers, the authors did not report the term "peripheral edema" among the AEs.
- "Rigors" were statistically more frequent in patients treated with NTZ than in patients who were not (RR=3.54, 95% CI 1.16 to 10.83). In the SENTINEL trial (Rudick 2006, SENTINEL 2006) the term "rigors" was not mentioned among the AEs.
- In the AFFIRM study, "vaginitis" was statistically more frequent in women treated with NTZ than in those who were not (RR=1.65, 95% CI 1.01 to 2.71). In the SENTINEL (Rudick 2006, SENTINEL 2006) and GLANCE (Goodman 2009, GLANCE 2009) studies the term "vaginitis" was not reported among the AEs.
- In the AFFIRM study, menstrual disorders were statistically more frequent in women treated with natalizumab than in those who were treated with placebo (RR=1.89, 95% CI 1.09 to 3.29). In the SENTINEL (Rudick 2006, SENTINEL 2006) and GLANCE (Goodman 2009, GLANCE 2009) trials menstrual disorders were not reported among the AEs.
- In the SENTINEL, AFFIRM and GLANCE trials, the numbers of patients who suffered from "infusion reactions" and "hypersensitivity reactions" (HSRs) were reported. "Infusion reactions" were defined as any event that occurred within two hours after the start of the infusion. "Infusion reactions" were more frequent in NTZ-treated patients than in controls (RR= 1.24, 95% CI 1.05 to 1.47). The most common "infusion reaction" was headache. However, when only headache was analysed, no statistical significant difference was found between NTZ-treated participants and controls. HSRs, which are a major concern among clinicians, were more frequent in NTZ-treated patients than in controls but this was not statistically significant (RR=3.43, 95% CI 0.33 to 36.07). The term "HSRs" included all conditions defined as "hypersensitivity", "allergic reaction", "anaphylactic/anaphylactoid reaction", "urticaria", "allergic dermatitis", or "hives". There was, however, heterogeneity among the trials (I^2=65%), and when repeating the comparison with the fixed model, a statistically significant difference was found. Since the most likely source of heterogeneity was the GLANCE trial, the comparison was repeated without the GLANCE data. In this comparison, HSRs were significantly more frequent in NTZ-treated patients without evidence of heterogeneity (I^2 <30% using either the random-effect or fixed-effect methods). No cardiovascular or respiratory compromise was associated with any of these events classified as HSRs, except for one patient who received epinephrine and one patient who required supplemental oxygen (both in the AFFIRM 2006 trial, Polman 2006, Phillips 2006). In the AFFIRM study two NTZ patients were re-dosed after experiencing a hypersensitivity reaction (protocol violation); thus, a total of 27 HSRs were observed in 25 NTZ patients (Phillips 2006, AFFIRM 2006).

Since clinically significant liver injury has been reported in patients treated with NTZ in the post-marketing setting (Francis 2008, US FDA 2008), we looked at liver-function tests. The number of patients experiencing an abnormality in liver-function tests was only available in the AFFIRM study (Polman 2006, AFFIRM 2006), without evidence of statistically significant differences between NTZ and placebo groups (RR=1.29, 95% CI 0.67 to 2.47). In the SENTINEL study (Rudick 2006, SENTINEL 2006) it is stated that "no increase in the incidence of chemical abnormalities, including the results of liver-function tests, was observed with combination therapy"; the same applies for the GLANCE trial (Goodman 2009, GLANCE 2009).

Finally, we pooled data regarding the number of patients with at least one "infection" (irrespective of infection type) and found no evidence of differences between NTZ and placebo groups (RR= 1.01, 95% CI 0.97 to 1.06; I^2=0%).

(2) THE NUMBER OF PATIENTS EXPERIENCING TREATMENT DISCONTINUATION CAUSED BY AE

Data was available for the SENTINEL (Rudick 2006, SENTINEL 2006), AFFIRM (Polman 2006, AFFIRM 2006) and GLANCE (Goodman 2009, GLANCE 2009) trials. The pooled estimate was RR= 1.14 (95% CI 0.82 to 1.59), showing no statistical difference in the rate of discontinuation between patients who took NTZ and those who did not; there was no statistical evidence of heterogeneity (I^2=0%). It is important to mention that we encountered difficulty in extracting raw data for this outcome; differences between the review authors were resolved by discussion.

(3) THE NUMBER OF PATIENTS EXPERIENCING A RELAPSE IN THE 4 WEEKS AFTER THE FIRST DOSE OF NTZ

No data was available despite contacting the authors and sponsor.

DISCUSSION

Summary of main results

Our results show statistical evidence in favour of NTZ for all the primary efficacy outcome measures and for those secondary efficacy measures for which data was available.

NTZ (with or without IFNß-1a once a week) reduced the risk of experiencing at least one new exacerbation at 2 years by about 40% and the risk of experiencing progression at 2 years by about 25%

as compared to a control group. To reduce the risk of experiencing at least one new exacerbation, 3 to 5 patients need to receive NTZ (with or without IFNß-1a once a week) (NNB=4; 95% CI 3 to 5). The number of patients needing treatment with NTZ in order to prevent progression in one patient at 2 years is 7 to 23 (NNB= 10; 95% CI 7 to 23). It is noteworthy that the effect size is high for both primary outcomes, with NNB of 4 and 10. Confidence intervals show that the estimate is very precise for the risk of at least one new exacerbation, though less so for progression.

NTZ therapy results in significant HRQoL benefits. Both PCS and MCS mean changes favour NTZ-treated patients.

Available data was not suitable for metanalysis of the following secondary efficacy outcome measures: time to progression at 2 years; mean change in EDSS score at 2 years; mean change in MSFC at 2 years; the number of patients who were unable to walk without aid (EDSS > 5.5) at 2 years; mean change in PASAT at 2 years.

Available data was also not suitable for metanalysis of the mean change in MFIS at 2 years. There are some trials included in the trial register ClinicalTrials.gov (accessed 1 April, 2010) aimed at evaluating NTZ for the relief of MS-related fatigue through the MFIS (ENER-G study http://clinicaltrials.gov/ct2/show/NCT00464074; TYNERGY study - http://clinicaltrials.gov/ct2/show/NCT00884481 and http://clinicaltrials.gov/ct2/show/NCT00966797); however these are open label trials with an observation period of less than 2 years. An open label trial indicated significant improvement in MFIS scores after 6 months therapy with NTZ compared to baseline (Putzki 2009). Moreover, preliminary results indicate that MFIS scores decreased (improved) significantly after the third NTZ infusion follow-up, compared to baseline, in an open study of 186 MS patients (Stephenson 2009).

We were unable to extract data from the paper by Balcer 2007, which reported results from the AFFIRM 2006 and SENTINEL 2006 trials, in order to assess the number of patients experiencing clinically significant worsening of vision at 2 years. This paper demonstrated reduction in visual loss, as assessed by low-contrast acuity testing, after NTZ treatment.

MRI parameters show statistical evidence in favour of participants receiving NTZ versus controls.

Our analysis indicated that NTZ is well tolerated and safe over a period of up to 2 years.

Serious AEs were less probable in the NTZ group than in the control group. Since this could be due to the fact that the most common serious AE in included trials was a MS relapse, which was significantly more frequent in controls than in NTZ-treated patients, MS relapses were excluded from the analysis and no difference in serious AEs was found.

We did not find evidence of potential liver injury with NTZ. This problem raised interest in 2008 when a FDA safety review of NTZ identified four cases of serious hepatic injury (http://www.fda.gov/cder/dsn/2008˙spring/postmarketing.htm#natalizumab). None of the cases resulted in death or liver transplant. As a consequence, the "*Warnings and Precautions*" section of the product labelling was updated to reflect this new safety information. Currently, monitoring liver enzymes is recommended before and during treatment with NTZ.

In the NTZ arm of the AFFIRM study, one patient died of malignant melanoma. He had a history of malignant melanoma and had noted a new lesion at the time of receiving the first dose of NTZ. In the literature, other cases of possible association between NTZ and melanoma have been reported (Mullen 2008; Bergamaschi 2009; Ismail 2009; Laroni 2010). Whether this association is real or coincidental remains to be seen.

About PML and other safety issues see "Overall completeness and applicability of evidence".

Not surprisingly, the PML cases have sensitized the neurologic community to potential risks of NTZ, and this could result in over-reporting of concomitant medical problems as potential AE in NTZ-treated patients.

The number of patients experiencing at least one severe AE during 2 years did not differ between patients treated with NTZ and controls. NTZ may cause HSRs and acute infusion reactions (Cohen 2010) but data emerging from this review is not alarming. Similar to other protein-based therapies, NTZ may trigger these events primarily in the first months of treatment. Most HSRs seem to occur during the second infusion (Berger 2009; Phillips 2006, AFFIRM 2006). HSRs and infusion-related AEs have been correlated with incidence of anti-NTZ antibodies (Calabresi 2007; Cohen 2010). However, some cases of delayed allergic reactions were reported or hypothesised in the absence of anti-NTZ antibodies (Krumbholz 2007; Hellwig 2008; Cohen 2009; Zephir 2009; Killestein 2009).

It was difficult to calculate the number of patients who discontinued the interventions because of AEs in the AFFIRM and SENTINEL trials. Reasons for discontinuation from the study interventions were not available for the patients who "discontinued study drug but completed follow-up" in the "participant flow" figures in the papers Polman 2006 (AFFIRM 2006 - Figure1, page 906) and Rudick 2006 (SENTINEL 2006 - Figure 1, page 915). Therefore, the numbers used in the metanalysis were drawn from percentages reported in the text. No statistically significant difference in rate of discontinuation because of AEs was found between patients who took NTZ and those who did not. It is worth mentioning that patients who experienced any HSR (irrespective of severity) were required by the protocol to discontinue the study drug in the AFFIRM and SENTINEL trials (Biogen Idec and Elan Pharmaceuticals 2006).

A well-established pharmacodynamic feature of NTZ is the increase in number of circulating leukocytes. Counts return to baseline levels when NTZ is discontinued. Thus, the increase in number of leukocytes was not reported among laboratory AEs.

No data was available regarding the number of patients experiencing a relapse in the 4 weeks after the first dose of NTZ. We included this outcome to assess the first-dose paradoxical reaction hypoth-

esised in some reports (Centonze 2008; Haupts 2008; Haartsen 2009; Rinaldi 2009). It was speculated that NTZ can promote the release of inflammatory mediators from lymphocytes present in the CNS (Centonze 2008) or modify the regulatory network in the brain (Rinaldi 2009) at the time of the first infusion, thus favouring the clinical manifestation of a pre-existing active lesion. A RCT (O'Connor 2004) aimed at evaluating the effects of a single dose of IV NTZ 1 or 3 mg/kg (n=117) or placebo (n=63) within 96 hours of the onset of a MS relapse, found that NTZ did not effect the short-term clinical course of patients during acute relapses; a significant decrease in Gd-enhancing lesion volume was observed at 1 and 3 weeks after treatment. Moreover, there were no differences in the NTZ versus placebo groups in the occurrence of new acute relapses during a 14-week follow-up period.

Overall completeness and applicability of evidence

The review's aim was to assess NTZ's efficacy, tolerability and safety in patients with RRMS. In our view this review has reliably assessed efficacy and short- and 2 year long-term tolerability and safety.

As far as assessment of progression is concerned, included studies required only 12 weeks of sustained EDSS worsening to classify patient outcome as progression. As stated in the review protocol, the preferred definition of progression included confirmation of sustained EDSS increase at 6 months; one cannot exclude that some patients classified as developing progression may merely have experienced a prolonged but still reversible disability after a relapse and not an actual progression (Ebers 2008).

A limitation in the analysis of the PCS and MCS components of SF-36 is the clinical significance of measured changes. A change of 5.0 points (SD, 0.5) is considered a clinically meaningful difference in a reference population of disease-free individuals (Norman 2003).

Tysabri® is available in many countries for treating RRMS. It has marketing authorisation or recommendation as a single disease-modifying therapy in patients with highly active RRMS or patients who have had an inadequate response to, or are unable to tolerate, other current multiple sclerosis therapies (FDA 2006, EMA 2009, AIFA 2006, NICE 2007). The definition of highly active RRMS differs slightly from country to country, but it can be said to include: (i) failure to respond to a full and adequate course of an interferon beta (IFNß - different types) or GA, with at least one significant relapse in the previous year of therapy, and at least 9 T2-hyperintense lesions on cranial MRI or at least one gadolinium-enhancing lesion; (ii) previously untreated patients with rapidly evolving severe RRMS defined by two or more disabling relapses in 1 year, and 1 or more gadolinium-enhancing lesions on brain MRI or a significant increase in T2 lesion load compared with a previous MRI (EMA 2009, AIFA 2006, NICE 2007). In any case, the variable licensed indications all over the world for the use of NTZ represents a compromise between the need to provide a more effective therapy for RRMS, the risk of PML and the cost of implementation (Giovannoni 2007).

This review did not evaluate the question whether the clinical efficacy of NTZ in the suboptimal therapy group can be considered to be fully established. However, study populations included patients with active MS, with clinical and MRI features similar to those included in prescribing recommendations (even with some differences from country to country). As an example 38% of patients in the combined SENTINEL 2006 and AFFIRM 2006 cohort had two or more relapses in the preceding year and 41% had at least one gadolinium enhancing lesion on brain MRI. A post hoc analysis of the AFFIRM study (Havrdova 2009, AFFIRM 2006) showed that NTZ was superior to placebo (absence of disease activity on combined clinical and radiological measures defined as no relapse, no progression of disability sustained for 3 months, no Gd+ lesion, and no new or enlarging T2-hyperintense lesions) in both highly active ($\geq$ 2 relapses in the year before entry and $\geq$ 1 Gd+ lesion at entry) and non-highly active subgroups. Another post hoc analysis of SENTINEL and AFFIRM studies (Hutchinson 2009) found that NTZ reduced the annualised relapse rate and the risk of sustained disability progression regardless of baseline disease or demographic characteristics, except for the subgroup of patients with less than 9 T2 lesions at baseline. The number of subjects in the subgroup of patients with less than 9 T2 lesions at baseline is so small (8% of the total population) that any speculation about this result is futile in our opinion.

The primary outcomes of this review were set at 2 years of treatment. No data from RCTs is available for longer treatment duration. However, data from real-world cohorts of RRMS patients who received more than 36 infusions confirm the efficacy of NTZ throughout the 3 years of treatment (O'Connor 2010, Horga 2010, Sangalli 2010). Another open question is the duration of NTZ treatment and the effects of discontinuation. Two studies report that patients who stopped NTZ experienced significant clinical relapses and radiologic worsening within 6 months (West 2010; Killestein 2010). An immune reconstitution inflammatory syndrome (IRIS) - like rebound of inflammatory MS activity after discontinuation is also reported (Miravalle 2011).

What about early treatment? This question was not directly addressed but we can report that patients who participated in AFFIRM 2006 had a median disease duration of 5 yrs (range 0-34 yrs); 4% were diagnosed as having MS after a single clinical attack according to McDonald criteria. As protocol violations, 13 patients (1.4 % of the total population) in the AFFIRM study were included even if only Clinically Isolated Syndrome (CIS) criteria were satisfied (McDonald 2001). SENTINEL 2006 patients had longer median disease duration (7 yrs; range 1-34 yrs). Thus, one can conclude that NTZ was poorly studied as an early treatment option in RRMS in these studies.

A limitation of this review's external validity is the previous use of DMDs, including immunosuppressants such as cyclophospha-

mide, mitoxantrone, cyclosporine, azathioprine and methotrexate (some of which are largely used off label in several countries). Only 55% of the combined SENTINEL 2006 and AFFIRM 2006 cohort (all patients in SENTINEL) had a duration ≥ 10 months of IFNß therapy before the first dose of NTZ. Precise data on immunosuppressant use prior to inclusion in the trials was not available. Exclusion criteria included treatment with cyclophosphamide or mitoxantrone within the previous year or treatment with cyclosporine, azathioprine or methotrexate within the previous 6 months (Polman 2006, AFFIRM 2006) or with an approved disease-modifying therapy other than IFNβ-1a intramuscularly once weekly within the 12-month period before randomisation (Rudick 2006, SENTINEL 2006). In practice a large number of patients are treated with NTZ after the use of other DMDs, including immunosuppressants and this fact may have an impact on tolerability and safety issues.

Because of the demographic characteristics of the participants in the trials included, the results of this review cannot be considered valid for pediatric RRMS patients (for pediatric use see Ghezzi 2010) and for people with RRMS aged over 55 years or with EDSS>5.

Pregnancy or conception planning were exclusion criteria in the RCTs; hence teratogenicity and/or safety of NTZ in pregnancy were not addressed. We can only quote published data on 98 pregnancies, from the TOUCH and TYGRIS studies, showing that exposure to NTZ had no negative effect on pregnancy outcomes (Bozic 2007). Since there is lack of data, NTZ is contraindicated during pregnancy and there is a pregnancy exposure registry for Tysabri sponsored by Biogen Idec & Elan Pharmaceuticals (http://clinicaltrials.gov/ct2/show/NCT00472992 accessed 9 April 2010).

This review did not intend to evaluate the impact of anti-NTZ antibodies, which develop persistently in about 6% of treated patients; they are correlated with a higher incidence of AEs, as above mentioned, as well as reduced efficacy (Calabresi 2007).

This review did not compare the efficacy of NTZ with other currently available DMDs in RRMS. Add-on studies are not as directly informative as monotherapy, however they provide evidence of efficacy in a well defined setting. If we assume that IFNβ-1a and NTZ do not interact in a synergistic manner, one might infer from the results of the SENTINEL trial that NTZ appears more efficacious than IFNß-1a once a week (i.e. Avonex) after 2 years of treatment. What DMDs are available as alternatives to Tysabri® in RRMS? In patients with suboptimal response to first line options, it is acceptable to increase the dose if applicable or switch to alternative first line treatments: e.g. high-dose IFNß if on low-dose IFNß treatment (Panitch 2002; Sharief 2003; Schwid 2005); GA in place of IFNß (Caon 2006; Zwibel 2006) or vice versa. In Italy, and also in other countries, azathioprine and cyclophosphamide are used off-label in MS. Mitoxantrone is approved for MS treatment world-wide (with some differences from country to country), but it is cardiotoxic (Ghalie 2002) and the risk for therapy-related leukaemia is increasingly reported (Straffi 2010). Cross-trial comparisons are very controversial (Freedman 2008; Goodin 2008a; Klawiter 2009). Earnshaw and collaborators, evaluating data for clinical trials and long-term clinical assessments thereafter, found that GA or NTZ in RRMS patients is associated with increased benefits compared with symptom management, albeit at higher costs, and that long-term lifetime cost effectiveness is similar for NTZ and GA (Earnshaw 2009). The best way to avoid any bias is to perform direct head-to-head comparisons of therapies in the same population with adequate randomisation and allocation concealment, clinically meaningful outcomes and statistical power.

Another issue is how well current clinical and MRI criteria for disease activity are measures of response to a particular therapy. Future trials should deal with these issues. The trial entitled "A Multicenter, Randomized, Rater-Blind, Parallel-Group, Active-Controlled Study to Evaluate the Effects of Switching Therapy (Glatiramer Acetate or Interferon ß-1a) to NTZ in Subjects With Relapsing Remitting Multiple Sclerosis (SURPASS)" intends to study the outcome of switching to Tysabri® in subjects with active RRMS despite receiving GA or IFNß-1a (Rebif®) for at least 12 months (http://clinicaltrials.gov/ct2/show/NCT01058005 accessed 9 April 2010). Findings from such a study will provide validation of proposed "activity" criteria, information about the relative benefits of different treatment options with a significant advancement toward optimising treatment in a high-risk MS patient population. IQUALYSEP is a randomised parallel single blind cost-effectiveness trial comparing three years NTZ treatment versus 6 months mitoxantrone treatment followed by immunomodulators for 2.5 years in RRMS defined as "aggressive" (1 or more disabling relapses during the 12 months before inclusion; EDSS between 2 and 5) (http://clinicaltrials.gov/ct2/show/NCT01065727 accessed 9 April 2010).

A major limit of this review is the inability to provide an up-to-date systematic assessment of long-term safety. Our protocol was insufficient to evaluate rare and long-term AEs such as PML, cancers and other opportunistic infections, which are very important issues in risk/benefit balancing.

PML is a demyelinating infectious CNS disease, usually observed in immunodeficient patients, especially in AIDS patients, caused by the human polyomavirus JC virus, a common and widespread virus infecting humans (Koralnik 2004; Tan 2010). JC virus has been identified in human post mortem brain samples from immunological normal individuals without PML (White 1992; Mori 1992). The pathogenesis of PML in patients receiving NTZ is complex and not fully understood. PML causes death or severe disability, either directly or as a result of IRIS. This syndrome is an inflammatory response to JC virus associated with a rapid recovery of the immune system after a period of immunosuppression. Therefore, although a cellular immune response directed against the JC virus is beneficial, a rapid global recovery of the immune system might not always be favourable (Tan 2010). The issue of

PML in MS therapies is not restricted to NTZ. Rituximab is a potentially effective approach in the treatment of RRMS (Hauser 2008). PML occurred during rituximab treatment for hematologic malignancies or autoimmune diseases (e.g. Rheumatoid Arthritis, Systemic Lupus Erythematosus) (Biogen Idec Inc 2008). Azathioprine has been associated with PML in some case reports in different diseases (Schneider 1991, White 2002, Pagnoux 2003, Gedizlioglu 2009). PML has also been described in a NTZ-treated patient with Crohn's disease (Van Assche 2005). NTZ has been approved for CD in USA in January 2008. To our knowledge, there have been no postmarketing reports of PML in patients treated with Tysabri® for CD. Less than 2% of Tysabri® use in the U.S. has been in patients with CD (US FDA 2009). The FDA, EMEA, Biogen Idec & Elan Pharmaceuticals continue to receive reports of PML in MS patients treated with Tysabri®. The last update obtained from Biogen Idec and Elan Pharmaceuticals dates December 2, 2010 (see http://www.biogenidec.ch accessed 31 December, 2010) when the number of patients with PML was 79 (34 in USA, 40 in Europe and 5 in the rest of the world) of which 16 died (20%), out of 75500 patients exposed to Tysabri® (exposure as of September 30, 2010). Surviving PML patients have varying levels of disability, ranging from severe to mild. The risk for developing PML in a patient treated with NTZ, initially estimated to be 1:1000 at around 18 months (Yousry 2006), appears now to increase with the number of Tysabri infusions received. On the basis of the total number of patients treated with NTZ and the number of infusions, at 2 December 2010, Biogen Idec & Elan Pharmaceuticals provided the following treatment epoch risk of PML: ≤ 12 infusions = 0.01 per 1000 patients (95% CI = 0 to 0.07 per 1000); 13-24 infusions = 0.38 per 1000 patients (95% CI = 0.23 to 0.60 per 1000); 25-36 infusions = 1.48 per 1000 patients (95% CI = 1.08 to 1.97 per 1000) and the risk does not seem to increase further after 36 infusions (see http://www.biogenidec.ch accessed 31 December, 2010). It must be noted that around 31000 patients were exposed ≥24 months and 12500 ≥36 months to Tysabri® (even this duration may not fully reflect treatment interruptions that may have happened) (see http://www.biogenidec.ch accessed 31 December, 2010). Accumulating experience indicates that a history of prior immunosuppressant use is a risk factor for PML, which appears to be independent of treatment duration. In terms of geographical distribution, the possible higher risk of PML in Europe might be due to greater use of immunosuppressant therapies for MS (Clifford 2010). In February 2010, the FDA provided a safety update that noted an increased risk of getting PML with increasing number of infusions, with the overall worldwide cumulative rate of PML in patients who have received at least 24 infusions estimated as 1.3 cases of PML per 1000 patients. The agency concluded that the benefits of the medicine continue to outweigh the risks (US FDA 2010). The European Medicines Agency (EMA) provided safety updates along similar lines (EMA 2010). Currently, there are no established interventions that can reliably prevent or adequately treat PML, though some are reported (Wenning 2009; Clifford 2010; Warnke 2010). Large-scale, prospective clinical studies are currently under way to determine whether a new JC virus assay will help clinicians predict which patients are most at risk for PML (STRATIFY-1 and STRATIFY-2). According to Thompson 2008, more than a sevenfold increase in actual risk of PML was required to decrease NTZ's health gain below that of IFNß-1a (Rebif® 44 μg).

Some controversial safety concerns have been raised in the last few years about opportunistic infections other than PML as a result of prolonged suppression of immunosurveillance of the CNS and other tissues: severe cutaneous Candida infection (Gutwinski 2010), ocular toxoplasmosis (Zecca 2009) and severe herpetic infections (Ransohoff 2007). There was one fatal case of herpes simplex encephalitis (that occurred 3 months after a single dose of NTZ in a patient previously treated with the maximum lifetime dose of mitoxantrone) and one case of herpes simplex meningitis (that developed several hours after a single dose of NTZ) in the post-marketing MS setting (Biogen Idec and Elan Pharmaceuticals 2006). It is currently too difficult to draw conclusions on the causation of these AEs. A single case of cryptosporidial gastroenteritis occurred during the AFFIRM study (the event was considered resolved 70 days after the symptoms first started - Biogen Idec and Elan Pharmaceuticals 2006); this infection may also occur in immunocompetent hosts and, in general, it is a self-limited illness with an average time to recovery ranging from several days up to 5 weeks (Leav 2003).

Other infections (more or less classifiable as opportunistic or atypical) have been reported in CD patients treated with NTZ, including one case each of fatal Pneumocystis carinii pneumonia, fatal Pulmonary aspergillosis, Mycobacterium avium complex pneumonia, cytomegalovirus colitis, cytomegalovirus hepatitis, primary varicella pneumonia, Burkholderia cepacia pneumonia, tubercle bacillus peritonitis, cavitating pneumonia with lung abscess and Candida sepsis (Tysabri CD Briefing Book). It seems that more patients with CD experienced opportunistic or atypical infections than MS patients during NTZ treatment.

A possible association of NTZ treatment with lymphoma has been postulated. Two cases of primary CNS lymphoma were reported in MS patients: one was Epstein Barr virus negative (Schweikert 2009); for the other case, Epstein Barr virus staining results were not available (Bozic 2009). A systemic B-cell lymphoma has been reported in the ENCORE trial in a patient with CD after NTZ treatment (Targan 2007).

Dysimmune disorders reported in relation to NTZ therapy have been described, including autoimmune thrombocytopenia (Jones 2008) and autoimmune haemolytic anaemia (Outteryck 2009).

As a result of these concerns, mostly PML risk, NTZ is recommended in many countries as second-line therapy if conventional immunomodulatory agents have failed or as first line therapy in severe relapsing disease, as mentioned above (FDA 2004 and 2006, EMA 2009, AIFA 2006, NICE 2007). Immune com-

petence is crucial before NTZ initiation. Expert recommendations (Gold 2007; Kappos 2007) includes: neutrophils > 1500 cells/ml, lymphocytes > 1000 cells/ml, CD4+ cells > 500 cells/ml and CD8+ cells > 250 cells/ml; no history of opportunistic infections in the previous 3-6 months; immunosuppressive drugs (except for standard steroid therapy for relapse) should be discontinued at least 6 months before; immunomodulatory agents are contraindicated if concomitant and should be discontinued at least 6 weeks before. The contraindication of simultaneous treatment with immunomodulatory agents and NTZ emerged from the fact that the first 2 cases of PML in MS occurred in patients who were part of the combination treatment arm of the SENTINEL 2006 trial. Beyond these guidelines and recommendations, an important unknown faces the clinician: once a patient is started on NTZ, it is currently unclear how duration of treatment with NTZ impacts the risk-benefit ratio of this drug. The safety issue of NTZ treatment is managed through active post-marketing surveillance programs that are currently being undertaken in several countries. There is the Tysabri Global Observational Program in Safety (TYGRIS) which is a voluntary 5000-patient registry cohort in North America (http://clinicaltrials.gov/ct2/show/NCT00477113) and the rest of world (http://clinicaltrials.gov/ct2/show/NCT00483847) with 5-year follow-up for infections requiring hospitalisation, cases of PML, malignancies, and all AEs that are serious or medically significant. The "Tysabri Outreach: Unified Commitment to Health" (TOUCH®) is a mandatory prescribing program started by Biogen Idec and Elan Pharmaceuticals in USA with the help of the Food and Drug Administration (FDA) and it is based on the restriction of NTZ prescription to physicians participating in this risk management program (http://www.tysabry.com; http://www.fda.gov/cder/drug/infopage/natalizumab/RiskMAP.pdf). Reports from the TYGRIS and TOUCH® studies show that the benefit-risk profile of NTZ remains favourable for patients with RRMS (Bozic 2009a). Tysabri Observational Program (TOP) has been planned as an observational program in Europe, Australia and Canada with the primary aim of assessing long-term safety of 5,000 patients treated with Tysabri in RRMS in the post-marketing setting (http://clinicaltrials.gov/ct2/show/NCT00493298). NTZ is under intensive monitoring by the Medicines and Healthcare products Regulatory Agency (MHRA) accredited by the United Kingdom National Health Service to provide drug safety updates (http://www.mhra.gov.uk). Other examples are the Danish Multiple Sclerosis Treatment Register (Oturai 2009), the Swedish Multiple Sclerosis registry (Piehl 2010), the Australian Prescribing Program (TAPP), and the Italian Agenzia Italiana del Farmaco (AIFA) pharmacovigilance electronic program (www.http://aifa-neuro.agenziafarmaco.it; Mancardi 2010). Moreover, STRATA is an ongoing open-label, multinational study evaluating the long-term safety of Tysabri in participants who completed AFFIRM, SENTINEL or GLANCE trials and a dosing suspension safety evaluation, with no history of anti-NTZ antibodies (O'Connor 2010).

Thus, unlike mitoxantrone, for which the actual risks of serious cardiotoxicity and leukaemia only became apparent as case series were published, NTZ has a robust postmarketing safety monitoring program involving a large number of NTZ-treated MS patients worldwide.

From the Cochrane Collaboration point of view, in light of these uncertainties, an independent systematic updated review of the safety profile of NTZ is warranted. Such a review for AEs should be based on different eligibility criteria for selecting studies. The use of different eligibility criteria specifically addresses the problem that RCTs are insufficient to evaluate rare and long-term AEs. AEs may be studied across different indications such as CD, Rheumatoid Arthritis and rare neurological diseases (eg multifocal motor neuropathy - Raji 2009; Susac syndrome - Lee 2009).

Quality of the evidence

The two larger included studies were classified as having good methodological quality. This is in line with the fact that they also satisfy definition of Class I studies according to the system used by the American Academy of Neurology for therapeutic interventions (Goodin 2008). The quality of evidence for each of the primary outcome measures of the review was good, even if bias could not be definitively excluded (see "Potential biases in the review process"). The two MRI-based secondary outcomes and the other available secondary outcomes confirmed superiority of NTZ treatment, allowing a robust conclusion regarding the main objectives of the review.

Showing both absolute and relative measures for each outcome is a more transparent evaluation of data, considering the different weight that several variables (e.g. frequency of the events studied, baseline patient characteristics, and so on) have on such measures. We accomplished such a need by reporting both RR and NNB values.

Potential biases in the review process

All relevant studies were identified. The International Natalizumab Multiple Sclerosis Trial Group (INMSTG 2003) was a "study awaiting classification" but we hope to obtain data, from authors or sponsors, for inclusion in pooled data of safety/tolerability outcomes in an updated future version of this review. Seemingly, the exclusion of this data does not effect results on safety/tolerability.

Single dose trials and trials using dose $\leq$ 3mg/kg were not included in the present review (Table 6), since these are dosages significantly lower than the dosage approved in clinical practice.

Our review is not able to provide data about the possible biasing effect of protocol violations on the results. During the US FDA evaluation process of NTZ in RRMS, the CDER judged the vio-

lations as minor, stating that they "would not be expected to affect the results directionally" (FDA 2004 - page 38).

We found heterogeneity for the 2 primary outcomes "number of patients who experienced a relapse at 2 yrs" and "number of patients who experienced progression at 2 yrs", but sensitivity analysis did not change conclusions. The heterogeneity may be clearly ascribed to the fact that the SENTINEL 2006 trial was an add-on study, with an active treatment control group. Also, AFFIRM 2006 and SENTINEL 2006 populations differed from each other. SENTINEL 2006 had to meet the inclusion criterion of breakthrough disease while on IFNß-1a IM monotherapy. On the other hand, patients who had received treatment with IFNß or GA for more than six months were excluded in AFFIRM 2006.

The type of ITT analysis was not described in the papers reporting included trials. ITT statements with no further details carry an unclear risk of bias.

All the data of the present review was from trials supported by Biogen Idec and Elan Pharmaceuticals. In agreement with Cochrane Collaboration policy, this may be considered as a potential risk of bias. We included Table 6 for the sake of completeness in reporting features of excluded trials which were sponsored.

Agreements and disagreements with other studies or reviews

Our review is in agreement with other papers which reviewed NTZ (Hutchinson 2007; Yaldizli 2009; Coyle 2009) and with the Report of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology (Goodin 2008). Of notable mention is the post-hoc reassessment of data from the AFFIRM trial made by Havrdova 2009 (AFFIRM 2006) which used as outcome the proportion of patients with absence of disease activity (defined as no relapses, no sustained disability progression, no gadolinium-enhancing lesions, no new or enlarging T2-hyperintense lesions on cranial MRI). This type of outcome could be an increasingly attainable goal in RRMS treatment and could be of interest for future Cochrane reviews.

Several real-life experiences confirm the efficacy and tolerability of NTZ in RRMS (Mancardi 2010, Piehl 2010, Putzki 2010, Putzki 2010a, Belachew 2010, Outteryck 2010, Ghezzi 2010).

Additional comments

Arriving at the best treatment option for an individual MS patient needs an open and realistic discussion of MS natural history, the therapeutic benefits and risks of each available DMT (paying attention to the insurmountable cross-trial comparison issues), and the patient's own disease history. It is ultimately the patient's decision to refuse or accept the relevant risks related to a treatment. People tend to underestimate common risk and overestimate rare risk. They respond to risks primarily on the basis of emotion rather than facts. They seem to be risk averse when faced with medical interventions, and want information on even the rarest of adverse events (Moore 2008). Denial and other defence mechanisms may make them less capable of weighing pros and cons to a treatment like NTZ. Treatment decisions are based on facts and emotions, both of which may be manipulated. Many factors contribute to an incomplete understanding of the available evidence on risk by both patients and health professionals (Moore 2008). Moreover, studies have revealed communication and information deficits in the context of MS care (Freeman 2000, Vickrey 2000, Heesen 2004, Solari 2007) and heterogeneity in patients' preference in their involvement in decisions regarding their care. As an example, data on Italian MS patients indicates a preference for information but less involvement in decision-making (i.e. more passive role) in more than 30% of cases (Giordano 2008). These preferences vary from country to country, for example comparing Italian with German MS patients (Giordano 2008). A passive attitude may create a marked difficulty during the decision-making process about a treatment like NTZ. On the contrary, a more active role may be associated with the fact that patients were willing to accept a higher risk of PML than neurologists, and more willing to continue treatment with NTZ as reported by Heesen 2010. Open information about treatment-related risks was appreciated by German MS patients and considered important in supporting shared decision making (Heesen 2010). Although evidence-based medicine strives to reduce medical decision-making to standardized, codified recommendations, it is the judgment of the neurologist and the personal choice of the patient that ultimately determine the treatment plan. Whereas all therapeutic decisions are ultimately in the hands of the patients, their decisions are heavily influenced by the manner in which clinicians present the choices to them. Although the first precept may be "do no harm," if this was taken too far to mean that the risks of all complications should be eliminated, we would be discouraging interventions that on average produce benefits.

More studies about patients' preferences, physicians' communication skills, methodology to enhance communication and patients' evaluation of treatment efficacy and risks are needed. This is not restricted to NTZ, since it is clear that all future therapies that effectively act on the immune system will similarly require analysis of risks and prolonged postmarketing surveillance to determine their safety profiles. As the treatment paradigm of MS evolves and newer agents become available, making treatment decisions and providing skilled guidance for patients will become more challenging for neurologists and health service providers.

AUTHORS' CONCLUSIONS

Implications for practice

In patients with RRMS, we found a consistent positive effect of NTZ in reducing relapses and disability at 2 years. NTZ was well tolerated but information on the frequency and nature of AEs was limited to a short follow-up period. There are significant safety

concerns about PML which is increasingly reported in the post-marketing setting. NTZ should be used only by skilled neurologists in MS centres under national or international surveillance programs.

Implications for research

Because of safety concerns and the substantial expense related to NTZ, future research should be aimed at:

- further study of the use of NTZ in suboptimal therapy RRMS patients;
- comparing benefit/risk and cost-effectiveness profiles between NTZ and current agents in naive RRMS patients through head-to-head trials;
- comparing benefit/risk and cost-effectiveness profiles through head to head trials between NTZ and new emerging agents in RRMS;
- development and validation of clinical and MRI criteria for reliably assessing disease activity in treatment trials;
- cohort studies investigating predictive parameters of long-term NTZ effectiveness in clinical practice;
- exploring the possible detrimental first-dose effect on relapse, including its prevention;
- investigating and optimising the use of NTZ as induction therapy, with the minimum number of infusions to reduce the risk of PML;
- further study of the risk of PML, other opportunistic infections and cancer during NTZ treatment, evaluating risk threshold on the basis of the number of infusions;
- identifying risk factors for PML at baseline and during treatment;
- optimising best practice to monitor patients during NTZ therapy to facilitate early diagnosis of PML;
- further study on the optimum duration of NTZ treatment;
- assessing the best options for treating PML and IRIS in MS patients exposed to NTZ;
- evaluating the cost-effectiveness of increasing the current 4 week time interval between infusions;
- enhancing strategies for decision making and patient empowerment.

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* *Indicates the major publication for the study*

CHARACTERISTICS OF STUDIES

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

AFFIRM 2006

Methods	Phase 3, multicentre, randomised, double-blind, placebo controlled trial
Participants	99 centers in Europe, North America, Australia, and New Zealand enrolled 942 patients recruited from November 6, 2001 to January 31, 2005. Inclusion criteria: age=18-50 years, diagnosis of RRMS (McDonald criteria), EDSS=0-5.0; at least one medically documented relapse within the 12 months before the study began Exclusion criteria: a relapse within 50 days before the administration of the first dose of the study drug; treatment with cyclophosphamide or mitoxantrone within the previous year, or treatment with IFNß, GA, cyclosporine, azathioprine, methotrexate, or intravenous immune globulin within the previous 6 months; treatment with IFNß, GA, or both for more than six months
Interventions	Patients were randomly assigned in a 2:1 ratio to receive either NTZ (at a dose of 300 mg) or placebo by intravenous infusion every 4 weeks for up to 116 weeks
Outcomes	Primary endpoints: rate of clinical relapse at 1 year; rate of sustained progression of disability at 2 years, as measured by EDSS, defined as an increase of 1.0 or more on the EDSS from a baseline score of 1.0 or more or an increase of 1.5 or more from a baseline score of 0, that was sustained for 12 weeks (progression could not be confirmed during a relapse). Secondary endpoints: different MRI parameters at 1 and 2 years; the proportion of relapse free patients at 1 year; rate of clinical relapse at 2 years; progression of disability at 2 years, as measured by MSFC. Tertiary endpoints: Visual function testing (Sloan charts) ; Physical Component Summary (PCS) and Mental Component Summary (MCS) from SF-36; Subject Global Assessment Visual Analog Scale
Notes	3 patients who were assigned to receive placebo were never treated; these patients were included in the intention-to-treat efficacy analyses but were excluded from the safety analyses Binding antibodies against NTZ were assessed.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	quote "Patients were randomly assigned in a 2:1 ratio to treatment that was stratified according to study site in blocks of three (two active, one placebo) with the use of a computer-generated block randomisation schedule"
Allocation concealment (selection bias)	Low risk	quote: "a multi digit identification number, implemented by an interactive voice-response system was used "

AFFIRM 2006 (*Continued*)

Blinding (performance bias and detection bias) objective outcomes	Low risk	quote: "All study personnel, patients, sponsor personnel involved in the conduct of the study, and the investigator advisory committee were unaware of treatment assignments throughout the study"
Blinding (performance bias and detection bias) subjective outcomes	Low risk	quote: "All study personnel, patients, sponsor personnel involved in the conduct of the study, and the investigator advisory committee were unaware of treatment assignments throughout the study"
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	quote: "8 percent of patients in the NTZ group and 10 percent of those in the placebo group) withdrew from the study. Thirty-nine patients discontinued the study drug but completed follow-up (a total of 4 percent, including 4 percent of patients in the NTZ group and 5 percent of those in the placebo group)". "All analyses followed the intention-to-treat principle." A CONSORT flowchart is shown. However, the AFFIRM Authors did not report how the outcomes for patient withdrawals were assigned in the ITT analysis
Selective reporting (reporting bias)	Low risk	No selective reporting was identified.
Independent Funding Source	High risk	Supported by Biogen Idec and Elan Pharmaceuticals. Data were analysed by Biogen Idec and Elan Pharmaceuticals.

GLANCE 2009

Methods	Phase 2, multicentre, randomised, double-blind, add-on, placebo-controlled, parallel-group study
Participants	110 patients from 25 centres in US and Canada (between June 17, 2003 and March 23, 2004. Eligible patients: aged 18-55 years, diagnosis of RRMS (McDonald criteria), EDSS = 0-5.0, treatment with GA for at least 12 months before randomisation, one or more relapses during that time. Exclusion criteria: diagnosis of progressive MS, MS relapse within 50 days before randomisation, clinically significant infectious illness within 30 days of randomisation, abnormal laboratory results (or history thereof) indicative of any major organ system disease precluding administration of NTZ or GA, history of severe allergic or anaphylactic reactions, known drug hypersensitivity, or history of malignancy (excluding nonmetastatic basal cell carcinoma). Women who were pregnant, at risk of or planning to become pregnant, or breast-feeding were excluded

GLANCE 2009 (*Continued*)

Interventions	IV NTZ 300 mg or placebo once every 4 weeks plus GA 20 mg subcutaneously once daily for 24 weeks
Outcomes	Primary endpoint: rate of development of new active lesions on cranial MRI. Secondary endpoints: AEs
Notes	Aims: safety and tolerability data. The main hypothesis was that, because the proposed mechanism of action of GA requires cellular entry into the brain, NTZ might impair rather than enhance the efficacy of GA Binding antibodies against NTZ were assessed.

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	quote:"Patients were randomly assigned 1: 1 to receive IV NTZ 300 mg or placebo" However, the investigators did not describe a random component in the sequence generation process
Allocation concealment (selection bias)	Unclear risk	unclear
Blinding (performance bias and detection bias) objective outcomes	Low risk	quote:"All study personnel, patients, and sponsor personnel involved in study conduct were blinded to treatment assignments"
Blinding (performance bias and detection bias) subjective outcomes	Low risk	quote: "All study personnel, patients, and sponsor personnel involved in study conduct were blinded to treatment assignments"
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	A CONSORT flowchart is shown. However, the GLANCE Authors did not report how the outcomes for patient withdrawals were assigned in the ITT analysis
Selective reporting (reporting bias)	Low risk	No selective reporting was identified.
Independent Funding Source	High risk	This study was supported by Biogen Idec, Inc. and Elan Pharmaceuticals, Inc

SENTINEL 2006

Methods	Phase 3, multicentre, randomised, double-blind, add-on, placebo controlled trial
Participants	124 centers in Europe and US enrolled 1196 patients beginning on January 14, 2002 up to February 28, 2005 (planned May 31, 2005). Inclusion criteria: age=18-55 years; diagnosis of RRMS (McDonald criteria), EDSS=0-5.0; at least one relapse within the 12 months before randomisation; treatment with IFNß-1a im for at least 12 months before randomisation. Exclusion criteria: a relapse within 50 days before randomisation; treatment with an approved disease-modifying therapy other than IFNß-1a im once weekly within the 12-month period before randomisation
Interventions	Patients were randomly assigned, in a 1:1 ratio, to receive 300 mg of NTZ (589 patients) or placebo (582 patients) intravenously every 4 weeks in addition to IFNß-1a (Avonex, Biogen Idec) at a dose of 30 μg intramuscularly once weekly for up to 116 weeks
Outcomes	Primary endpoints: rate of clinical relapse at 1 year; rate of sustained progression of disability at 2 years, as measured by EDSS, defined as an increase of 1.0 or more on the EDSS from a baseline score of 1.0 or more or an increase of 1.5 or more from a baseline score of 0, that was sustained for 12 weeks (progression could not be confirmed during a relapse) Secondary endpoints: different MRI parameters at 1 and 2 years; the proportion of relapse free patients at 1 year; rate of clinical relapse at 2 years; progression of disability at 2 years, as measured by MSFC Tertiary endpoints: Visual function testing (Sloan charts) ; Physical Component Summary (PCS) and Mental Component Summary (MCS) from SF-36
Notes	One center with 25 patients was excluded before unblinding owing to irregularities in data. Thus, the number of patients included in data analysis was 1171 Following the recognition of two cases of PML in patients who had been receiving NTZ in combination with IFNß-1a (Avonex®) for over 2 years, Biogen Idec and Elan Pharmaceuticals, in discussions with FDA, suspended commercialisation and clinical trials on 28 February 2005 Binding antibodies against NTZ were assessed.

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	quote: "Randomization was stratified according to study site in blocks of four (two active and two placebo) with the use of a computer-generated schedule"
Allocation concealment (selection bias)	Low risk	quote: "a multidigit identification number, implemented by an interactive voice-response system was used "
Blinding (performance bias and detection bias) objective outcomes	Low risk	quote: "All study personnel, patients, sponsor personnel involved in the conduct of the study, and members of the investigator ad-

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		visory committee were blinded to the treatment assignments throughout the study"
Blinding (performance bias and detection bias) subjective outcomes	Low risk	quote: "All study personnel, patients, sponsor personnel involved in the conduct of the study, and members of the investigator advisory committee were blinded to the treatment assignments throughout the study"
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	quote: "12 percent of the group assigned to IFNß-1a plus NTZ and 16 percent of the group assigned to IFNß-1a alone) withdrew from the study". "5 percent of the combination-therapy group and 6 percent of the group assigned to IFNß-1a alone discontinued the study drug but completed follow-up ". " All analyses followed the intention-to-treat principle." A CONSORT flowchart is shown. However, the SENTINEL authors did not report how the outcomes for patient withdrawals were assigned in the ITT analysis
Selective reporting (reporting bias)	Low risk	No selective reporting was identified.
Independent Funding Source	High risk	Supported by Biogen Idec and Elan Pharmaceuticals.

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

Study	Reason for exclusion
UK Antegren Study 1999	Dosage: 3 mg/kg.

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

INMSTG 2003

Methods	Randomized, double-blind trial, placebo-controlled.
Participants	213 patients with RRMS or relapsing SPMS.
Interventions	Three arms: (i) 3 mg of intravenous NTZ per kilogram of body weight (N=68), (ii) 6 mg per kilogram (N=74), (iii) placebo (N=71) every 28 days for 6 months

INMSTG 2003 (*Continued*)

Outcomes	The primary end point was the number of new brain lesions on monthly gadolinium-enhanced MRI during the six-month treatment period. Other MRI outcomes included the number of persistent enhancing lesions; the volume of enhancing lesions; the number of new active lesions (the number of new enhancing lesions plus the number of new or newly enlarging, nonenhancing lesions on T2-weighted MRI); and the number of scans showing one or more new enhancing lesions. Secondary and tertiary clinical end points included the frequency of relapse, EDSS changes, and patients' own assessments of well-being
Notes	Since the trial duration was 6 months, this study was included with the sole aim of assessing tolerability/safety data. Data on RRMS only in the 6 mg per kilogram arm are pending.

DATA AND ANALYSES

Comparison 1. Primary Efficacy Outcome (Natalizumab vs Control)

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 N of pts with at least one relapse at 2 yrs	2	2113	Risk Ratio (M-H, Random, 95% CI)	0.57 [0.47, 0.69]
1.1 Natalizumab vs Placebo	1	942	Risk Ratio (M-H, Random, 95% CI)	0.51 [0.44, 0.61]
1.2 Natalizumab + IFN vs IFN	1	1171	Risk Ratio (M-H, Random, 95% CI)	0.62 [0.55, 0.70]
2 N of pts who progressed at 2 yrs	2	2113	Risk Ratio (M-H, Random, 95% CI)	0.74 [0.62, 0.89]
2.1 Natalizumab vs Placebo	1	942	Risk Ratio (M-H, Random, 95% CI)	0.67 [0.55, 0.81]
2.2 Natalizumab + IFN vs IFN	1	1171	Risk Ratio (M-H, Random, 95% CI)	0.80 [0.69, 0.93]
3 PCS Change in Short Form (SF-36) follow up 2 years	2	2113	Mean Difference (IV, Random, 95% CI)	1.98 [1.05, 2.91]
3.1 Natalizumab vs Placebo	1	942	Mean Difference (IV, Random, 95% CI)	2.01 [0.48, 3.54]
3.2 Natalizumab + IFN vs IFN	1	1171	Mean Difference (IV, Random, 95% CI)	1.96 [0.79, 3.13]
4 MCS Change in Short Form (SF-36) follow up 2 years	2	2113	Mean Difference (IV, Random, 95% CI)	1.38 [0.33, 2.42]
4.1 Natalizumab vs Placebo	1	942	Mean Difference (IV, Random, 95% CI)	2.53 [0.00, 5.06]
4.2 Natalizumab + IFN vs IFN	1	1171	Mean Difference (IV, Random, 95% CI)	1.14 [-0.00, 2.28]

Comparison 2. Secondary Efficacy Outcome (Natalizumab vs Control)

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Change in Well-being (VAS) at 2 yrs	1	942	Mean Difference (IV, Random, 95% CI)	6.4 [1.76, 11.04]
1.1 Natalizumab vs Placebo	1	942	Mean Difference (IV, Random, 95% CI)	6.4 [1.76, 11.04]
2 Gd-enhacing lesion (at least one) at 2 yrs	2	2113	Risk Ratio (M-H, Random, 95% CI)	0.12 [0.09, 0.17]
2.1 Natalizumab vs Placebo	1	942	Risk Ratio (M-H, Random, 95% CI)	0.11 [0.07, 0.17]
2.2 Natalizumab + IFN vs IFN	1	1171	Risk Ratio (M-H, Random, 95% CI)	0.14 [0.09, 0.22]
3 Change of MRI T2 total lesion load at 2 yrs	1	855	Mean Difference (IV, Random, 95% CI)	-3796.20 [-5849.43, -1742.97]
3.1 Natalizumab vs Placebo	1	855	Mean Difference (IV, Random, 95% CI)	-3796.20 [-5849.43, -1742.97]

Comparison 3. Primary Safety Outcome (Natalizumab vs Control)

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 N of pts with Severe AE over 2 yrs	2	2110	Risk Ratio (M-H, Random, 95% CI)	0.92 [0.81, 1.04]
1.1 Natalizumab vs Placebo	1	939	Risk Ratio (M-H, Random, 95% CI)	0.85 [0.68, 1.08]
1.2 Natalizumab + IFN vs IFN	1	1171	Risk Ratio (M-H, Random, 95% CI)	0.95 [0.81, 1.10]
2 N of pts with Serious AE (irrespective of treatment duration)	3	2220	Risk Ratio (M-H, Random, 95% CI)	0.83 [0.70, 0.98]
2.1 Natalizumab vs Placebo	1	939	Risk Ratio (M-H, Random, 95% CI)	0.79 [0.61, 1.02]
2.2 Natalizumab + IFN vs IFN	1	1171	Risk Ratio (M-H, Random, 95% CI)	0.87 [0.69, 1.09]
2.3 Natalizumab + GA vs GA	1	110	Risk Ratio (M-H, Random, 95% CI)	0.5 [0.05, 5.36]
3 N of pts with serious AE (irrespective of treatment duration - MS relapses excluded)	3	2220	Risk Ratio (M-H, Random, 95% CI)	1.13 [0.90, 1.43]
3.1 Natalizumab vs Placebo	1	939	Risk Ratio (M-H, Random, 95% CI)	1.19 [0.81, 1.73]
3.2 Natalizumab + IFN vs IFN	1	1171	Risk Ratio (M-H, Random, 95% CI)	1.10 [0.81, 1.49]
3.3 Natalizumab + GA vs GA	1	110	Risk Ratio (M-H, Random, 95% CI)	1.0 [0.06, 15.59]

Comparison 4. Secondary Safety Outcome (Natalizumab vs Control)

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 N of pts with at least one AE (irrespective of treatment duration)	3	2220	Risk Ratio (M-H, Random, 95% CI)	1.00 [0.99, 1.01]
1.1 Natalizumab vs Placebo	1	939	Risk Ratio (M-H, Random, 95% CI)	0.99 [0.96, 1.02]
1.2 Natalizumab + IFN vs IFN	1	1171	Risk Ratio (M-H, Random, 95% CI)	1.00 [0.99, 1.01]
1.3 Natalizumab + GA vs GA	1	110	Risk Ratio (M-H, Random, 95% CI)	0.98 [0.88, 1.10]
2 Treatment Discontinuation caused by AE (irrespective of treatment duration)	3	2220	Risk Ratio (M-H, Random, 95% CI)	1.14 [0.82, 1.59]
2.1 Natalizumab vs Placebo	1	939	Risk Ratio (M-H, Random, 95% CI)	1.58 [0.84, 2.97]
2.2 Natalizumab + IFN vs IFN	1	1171	Risk Ratio (M-H, Random, 95% CI)	1.01 [0.68, 1.50]
2.3 Natalizumab + GA vs GA	1	110	Risk Ratio (M-H, Random, 95% CI)	1.0 [0.06, 15.59]

Comparison 5. Adverse Event Analysis

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Headache	3	2220	Risk Ratio (M-H, Random, 95% CI)	1.08 [0.97, 1.20]
1.1 Natalizumab vs Placebo	1	939	Risk Ratio (M-H, Random, 95% CI)	1.15 [0.95, 1.39]
1.2 Natalizumab IFN vs IFN	1	1171	Risk Ratio (M-H, Random, 95% CI)	1.05 [0.92, 1.19]
1.3 Natalizumab + GA vs GA	1	110	Risk Ratio (M-H, Random, 95% CI)	1.13 [0.63, 2.03]
2 Pain in arms or legs - Arthralgia	3	2220	Risk Ratio (M-H, Fixed, 95% CI)	1.17 [0.98, 1.40]
2.1 Natalizumab vs Placebo	1	939	Risk Ratio (M-H, Fixed, 95% CI)	1.35 [0.98, 1.85]
2.2 Natalizumab + IFN vs IFN	1	1171	Risk Ratio (M-H, Fixed, 95% CI)	1.05 [0.85, 1.31]
2.3 Natalizumab + GA vs GA	1	110	Risk Ratio (M-H, Fixed, 95% CI)	5.0 [0.60, 41.42]
3 Depression	3	2220	Risk Ratio (M-H, Random, 95% CI)	1.18 [0.98, 1.41]
3.1 Natalizumab vs Placebo	1	939	Risk Ratio (M-H, Random, 95% CI)	1.18 [0.88, 1.60]
3.2 Natalizumab + IFN vs IFN	1	1171	Risk Ratio (M-H, Random, 95% CI)	1.17 [0.92, 1.47]
3.3 Natalizumab + GA vs GA	1	110	Risk Ratio (M-H, Random, 95% CI)	1.5 [0.26, 8.63]
4 Anxiety	1	1171	Risk Ratio (M-H, Random, 95% CI)	1.49 [1.05, 2.12]
4.1 Natalizumab + IFN vs IFN	1	1171	Risk Ratio (M-H, Random, 95% CI)	1.49 [1.05, 2.12]
5 Insomnia	1	1171	Risk Ratio (M-H, Random, 95% CI)	1.06 [0.82, 1.36]
5.1 Natalizumab + IFN vs IFN	1	1171	Risk Ratio (M-H, Random, 95% CI)	1.06 [0.82, 1.36]
6 Influenza Like Illness	1	1171	Risk Ratio (M-H, Random, 95% CI)	1.05 [0.83, 1.33]
6.1 Natalizumab + IFN vs IFN	1	1171	Risk Ratio (M-H, Random, 95% CI)	1.05 [0.83, 1.33]
7 Nasopharyngitis	2	1281	Risk Ratio (M-H, Fixed, 95% CI)	1.09 [0.94, 1.26]
7.1 Natalizumab + IFN vs IFN	1	1171	Risk Ratio (M-H, Fixed, 95% CI)	1.11 [0.96, 1.29]
7.2 Natalizumab + GA vs GA	1	110	Risk Ratio (M-H, Fixed, 95% CI)	0.64 [0.27, 1.52]
8 Pharyngitis	2	2110	Risk Ratio (M-H, Random, 95% CI)	1.41 [0.98, 2.04]
8.1 Natalizumab vs Placebo	1	939	Risk Ratio (M-H, Random, 95% CI)	1.20 [0.81, 1.79]
8.2 Natalizumab + IFN vs IFN	1	1171	Risk Ratio (M-H, Random, 95% CI)	1.76 [1.07, 2.90]
9 Sinusitis	2	1281	Risk Ratio (M-H, Random, 95% CI)	1.29 [0.88, 1.88]
9.1 Natalizumab + IFN vs IFN	1	1171	Risk Ratio (M-H, Random, 95% CI)	1.20 [0.93, 1.56]
9.2 Natalizumab + GA vs GA	1	110	Risk Ratio (M-H, Random, 95% CI)	2.25 [0.74, 6.87]
10 Sinus Congestion	1	1171	Risk Ratio (M-H, Random, 95% CI)	2.03 [1.15, 3.59]
10.1 Natalizumab + IFN vs IFN	1	1171	Risk Ratio (M-H, Random, 95% CI)	2.03 [1.15, 3.59]
11 Sinus Headache	1	1171	Risk Ratio (M-H, Random, 95% CI)	1.69 [0.94, 3.03]
11.1 Natalizumab + IFN vs IFN	1	1171	Risk Ratio (M-H, Random, 95% CI)	1.69 [0.94, 3.03]
12 Upper Respiratory Infection	3	2220	Risk Ratio (M-H, Random, 95% CI)	1.05 [0.87, 1.28]
12.1 Natalizumab vs Placebo	1	939	Risk Ratio (M-H, Random, 95% CI)	1.00 [0.80, 1.26]
12.2 Natalizumab + IFN vs IFN	1	1171	Risk Ratio (M-H, Random, 95% CI)	1.13 [0.76, 1.69]

12.3 Natalizumab + GA vs GA	1	110	Risk Ratio (M-H, Random, 95% CI)	1.8 [0.64, 5.03]
13 Influenza	1	1171	Risk Ratio (M-H, Random, 95% CI)	1.14 [0.87, 1.48]
13.1 Natalizumab + IFN vs IFN	1	1171	Risk Ratio (M-H, Random, 95% CI)	1.14 [0.87, 1.48]
14 Cough	1	1171	Risk Ratio (M-H, Random, 95% CI)	1.24 [0.87, 1.75]
14.1 Natalizumab + IFN vs IFN	1	1171	Risk Ratio (M-H, Random, 95% CI)	1.24 [0.87, 1.75]
15 Diarrhea	1	1171	Risk Ratio (M-H, Random, 95% CI)	1.19 [0.93, 1.53]
15.1 Natalizumab + IFN vs IFN	1	1171	Risk Ratio (M-H, Random, 95% CI)	1.19 [0.93, 1.53]
16 Nausea	2	1281	Risk Ratio (M-H, Random, 95% CI)	1.13 [0.88, 1.46]
16.1 Natalizumab + IFN vs IFN	1	1171	Risk Ratio (M-H, Random, 95% CI)	1.14 [0.87, 1.48]
16.2 Natalizumab + GA vs GA	1	110	Risk Ratio (M-H, Random, 95% CI)	1.13 [0.47, 2.70]
17 Vomiting	1	1171	Risk Ratio (M-H, Random, 95% CI)	1.40 [0.88, 2.22]
17.1 Natalizumab + IFN vs IFN	1	1171	Risk Ratio (M-H, Random, 95% CI)	1.40 [0.88, 2.22]
18 Abdominal Pain or Discomfort	2	2110	Risk Ratio (M-H, Random, 95% CI)	1.14 [0.84, 1.55]
18.1 Natalizumab vs Placebo	1	939	Risk Ratio (M-H, Random, 95% CI)	1.11 [0.74, 1.65]
18.2 Natalizumab + IFN vs IFN	1	1171	Risk Ratio (M-H, Random, 95% CI)	1.19 [0.74, 1.92]
19 Muscle Cramp	1	1171	Risk Ratio (M-H, Random, 95% CI)	1.19 [0.74, 1.92]
19.1 Natalizumab + IFN vs IFN	1	1171	Risk Ratio (M-H, Random, 95% CI)	1.19 [0.74, 1.92]
20 Myalgia	1	1171	Risk Ratio (M-H, Random, 95% CI)	1.31 [0.95, 1.81]
20.1 Natalizumab + IFN vs IFN	1	1171	Risk Ratio (M-H, Random, 95% CI)	1.31 [0.95, 1.81]
21 Seasonal Allergy	1	1171	Risk Ratio (M-H, Random, 95% CI)	1.50 [0.90, 2.51]
21.1 Natalizumab + IFN vs IFN	1	1171	Risk Ratio (M-H, Random, 95% CI)	1.50 [0.90, 2.51]
22 Peripheral Edema	1	1171	Risk Ratio (M-H, Random, 95% CI)	4.78 [2.00, 11.42]
22.1 Natalizumab + IFN vs IFN	1	1171	Risk Ratio (M-H, Random, 95% CI)	4.78 [2.00, 11.42]
23 Tremor	2	2110	Risk Ratio (M-H, Random, 95% CI)	1.42 [0.89, 2.27]
23.1 Natalizumab vs Placebo	1	939	Risk Ratio (M-H, Random, 95% CI)	1.05 [0.48, 2.29]
23.2 Natalizumab + IFN vs IFN	1	1171	Risk Ratio (M-H, Random, 95% CI)	1.69 [0.94, 3.03]
24 Flushing	1	110	Risk Ratio (M-H, Random, 95% CI)	6.00 [0.75, 48.21]
24.1 Natalizumab GA vs GA	1	110	Risk Ratio (M-H, Random, 95% CI)	6.00 [0.75, 48.21]
25 Fatigue - Myasthenia	1	939	Risk Ratio (M-H, Random, 95% CI)	1.27 [0.99, 1.64]
25.1 Natalizumab vs Placebo	1	939	Risk Ratio (M-H, Random, 95% CI)	1.27 [0.99, 1.64]
26 Urinary Urgency / Frequency	1	939	Risk Ratio (M-H, Random, 95% CI)	1.27 [0.79, 2.03]
26.1 Natalizumab vs Placebo	1	939	Risk Ratio (M-H, Random, 95% CI)	1.27 [0.79, 2.03]
27 Hypersensitivity reactions	3	2220	Risk Ratio (M-H, Random, 95% CI)	3.43 [0.33, 36.07]
27.1 Natalizumab vs Placebo	1	939	Risk Ratio (M-H, Random, 95% CI)	25.42 [1.55, 416.15]
27.2 Natalizumab + IFN vs IFN	1	1171	Risk Ratio (M-H, Random, 95% CI)	5.43 [1.21, 24.41]
27.3 Natalizumab GA vs GA	1	110	Risk Ratio (M-H, Random, 95% CI)	0.2 [0.01, 4.07]
28 Chest Discomfort	1	939	Risk Ratio (M-H, Random, 95% CI)	1.71 [0.83, 3.56]
28.1 Natalizumab vs Placebo	1	939	Risk Ratio (M-H, Random, 95% CI)	1.71 [0.83, 3.56]

29 Local Bleeding	1	939	Risk Ratio (M-H, Random, 95% CI)	1.58 [0.64, 3.91]
29.1 Natalizumab vs Placebo	1	939	Risk Ratio (M-H, Random, 95% CI)	1.58 [0.64, 3.91]
30 Rigors	2	1049	Risk Ratio (M-H, Random, 95% CI)	3.54 [1.16, 10.83]
30.1 Natalizumab vs Placebo	1	939	Risk Ratio (M-H, Random, 95% CI)	3.15 [0.94, 10.57]
30.2 Natalizumab GA vs GA	1	110	Risk Ratio (M-H, Random, 95% CI)	7.0 [0.37, 132.40]
31 Syncope	1	939	Risk Ratio (M-H, Random, 95% CI)	1.05 [0.48, 2.29]
31.1 Natalizumab vs Placebo	1	939	Risk Ratio (M-H, Random, 95% CI)	1.05 [0.48, 2.29]
32 Urinary Infection	2	1049	Risk Ratio (M-H, Random, 95% CI)	0.99 [0.51, 1.93]
32.1 Natalizumab vs Placebo	1	939	Risk Ratio (M-H, Random, 95% CI)	1.17 [0.88, 1.57]
32.2 Natalizumab GA vs GA	1	110	Risk Ratio (M-H, Random, 95% CI)	0.5 [0.13, 1.90]
33 Lower Respiratory Infection	1	939	Risk Ratio (M-H, Random, 95% CI)	1.06 [0.78, 1.45]
33.1 Natalizumab vs Placebo	1	939	Risk Ratio (M-H, Random, 95% CI)	1.06 [0.78, 1.45]
34 Tonsillitis	1	939	Risk Ratio (M-H, Random, 95% CI)	1.37 [0.78, 2.39]
34.1 Natalizumab vs Placebo	1	939	Risk Ratio (M-H, Random, 95% CI)	1.37 [0.78, 2.39]
35 Gastroenteritis	1	939	Risk Ratio (M-H, Random, 95% CI)	1.23 [0.81, 1.86]
35.1 Natalizumab vs Placebo	1	939	Risk Ratio (M-H, Random, 95% CI)	1.23 [0.81, 1.86]
36 Vaginitis	1	939	Risk Ratio (M-H, Random, 95% CI)	1.65 [1.01, 2.71]
36.1 Natalizumab vs Placebo	1	939	Risk Ratio (M-H, Random, 95% CI)	1.65 [1.01, 2.71]
37 Menstrual disorders	1	939	Risk Ratio (M-H, Random, 95% CI)	1.89 [1.09, 3.29]
37.1 Natalizumab vs Placebo	1	939	Risk Ratio (M-H, Random, 95% CI)	1.89 [1.09, 3.29]
38 Skin Rash	2	1049	Risk Ratio (M-H, Random, 95% CI)	1.94 [0.47, 7.99]
38.1 Natalizumab vs Placebo	1	939	Risk Ratio (M-H, Random, 95% CI)	1.23 [0.81, 1.86]
38.2 Natalizumab + GA vs GA	1	110	Risk Ratio (M-H, Random, 95% CI)	6.00 [0.75, 48.21]
39 Dermatitis	2	1049	Risk Ratio (M-H, Random, 95% CI)	2.15 [0.96, 4.85]
39.1 Natalizumab vs Placebo	1	939	Risk Ratio (M-H, Random, 95% CI)	1.82 [0.98, 3.40]
39.2 Natalizumab + GA vs GA	1	110	Risk Ratio (M-H, Random, 95% CI)	6.00 [0.75, 48.21]
40 Pruritus	1	939	Risk Ratio (M-H, Random, 95% CI)	2.07 [0.86, 5.00]
40.1 Natalizumab vs Placebo	1	939	Risk Ratio (M-H, Random, 95% CI)	2.07 [0.86, 5.00]
41 Vertigo	1	939	Risk Ratio (M-H, Random, 95% CI)	1.18 [0.67, 2.09]
41.1 Natalizumab vs Placebo	1	939	Risk Ratio (M-H, Random, 95% CI)	1.18 [0.67, 2.09]
42 Infection	3	2220	Risk Ratio (M-H, Random, 95% CI)	1.01 [0.97, 1.06]
42.1 Natalizumab vs Placebo	1	939	Risk Ratio (M-H, Random, 95% CI)	1.00 [0.93, 1.07]
42.2 Natalizumab + IFN vs IFN	1	1171	Risk Ratio (M-H, Random, 95% CI)	1.03 [0.97, 1.08]
42.3 Natalizumab + GA vs GA	1	110	Risk Ratio (M-H, Random, 95% CI)	0.92 [0.69, 1.22]
43 Infusion reactions	3	2220	Risk Ratio (M-H, Random, 95% CI)	1.24 [1.05, 1.47]
43.1 Natalizumab vs Placebo	1	939	Risk Ratio (M-H, Random, 95% CI)	1.34 [1.01, 1.77]
43.2 Natalizumab + IFN vs IFN	1	1171	Risk Ratio (M-H, Random, 95% CI)	1.20 [0.97, 1.49]
43.3 Natalizumab + GA vs GA	1	110	Risk Ratio (M-H, Random, 95% CI)	0.86 [0.31, 2.39]
44 Back Pain	1	110	Risk Ratio (M-H, Random, 95% CI)	2.25 [0.74, 6.87]
44.1 Natalizumab +GA vs GA	1	110	Risk Ratio (M-H, Random, 95% CI)	2.25 [0.74, 6.87]
45 Fall	2	2110	Risk Ratio (M-H, Random, 95% CI)	2.69 [0.32, 22.39]
46 Neoplasms	3	2220	Risk Ratio (M-H, Random, 95% CI)	0.83 [0.19, 3.66]
46.1 Natalizumab vs Placebo	1	939	Risk Ratio (M-H, Random, 95% CI)	2.49 [0.29, 21.20]
46.2 Natalizumab + IFN vs IFN	1	1171	Risk Ratio (M-H, Random, 95% CI)	0.49 [0.19, 1.31]

46.3 Natalizumab + GA vs GA	1	110	Risk Ratio (M-H, Random, 95% CI)	0.0 [0.0, 0.0]
47 Abnormal liver function tests	1	939	Risk Ratio (M-H, Random, 95% CI)	1.29 [0.67, 2.47]
47.1 Natalizumab vs Placebo	1	939	Risk Ratio (M-H, Random, 95% CI)	1.29 [0.67, 2.47]
48 Death	3	2220	Risk Ratio (M-H, Random, 95% CI)	0.92 [0.14, 6.04]
48.1 Natalizumab vs Placebo	1	939	Risk Ratio (M-H, Random, 95% CI)	2.49 [0.12, 51.75]
48.2 Natalizumab + GA vs GA	1	110	Risk Ratio (M-H, Random, 95% CI)	0.0 [0.0, 0.0]
48.3 Natalizumab + IFN vs IFN	1	1171	Risk Ratio (M-H, Random, 95% CI)	0.49 [0.04, 5.43]
49 MS relapse as a serious AE	3	2220	Risk Ratio (M-H, Random, 95% CI)	0.50 [0.37, 0.68]
49.1 Natalizumab vs Placebo	1	939	Risk Ratio (M-H, Random, 95% CI)	0.46 [0.30, 0.70]
49.2 Natalizumab + IFN vs IFN	1	1171	Risk Ratio (M-H, Random, 95% CI)	0.55 [0.36, 0.86]
49.3 Natalizumab + GA vs GA	1	110	Risk Ratio (M-H, Random, 95% CI)	0.33 [0.01, 8.01]

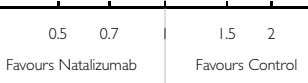
Analysis 1.1. Comparison 1 Primary Efficacy Outcome (Natalizumab vs Control), Outcome 1 N of pts with at least one relapse at 2 yrs.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 1 Primary Efficacy Outcome (Natalizumab vs Control)

Outcome: 1 N of pts with at least one relapse at 2 yrs

Study or subgroup	Experimental n/N	Control n/N	Risk Ratio M-H,Random,95% CI	Weight	Risk Ratio M-H,Random,95% CI
1 Natalizumab vs Placebo					
AFFIRM 2006	173/627	169/315		45.5 %	0.51 [0.44, 0.61]
Subtotal (95% CI)	**627**	**315**		**45.5 %**	**0.51 [0.44, 0.61]**
Total events: 173 (Experimental), 169 (Control)					
Heterogeneity: not applicable					
Test for overall effect: Z = 7.99 (P < 0.00001)					
2 Natalizumab + IFN vs IFN					
SENTINEL 2006	230/589	365/582		54.5 %	0.62 [0.55, 0.70]
Subtotal (95% CI)	**589**	**582**		**54.5 %**	**0.62 [0.55, 0.70]**
Total events: 230 (Experimental), 365 (Control)					
Heterogeneity: not applicable					
Test for overall effect: Z = 7.82 (P < 0.00001)					
Total (95% CI)	**1216**	**897**		**100.0 %**	**0.57 [0.47, 0.69]**
Total events: 403 (Experimental), 534 (Control)					
Heterogeneity: Tau² = 0.01; Chi² = 3.45, df = 1 (P = 0.06); I² =71%					
Test for overall effect: Z = 5.89 (P < 0.00001)					
Test for subgroup differences: Chi² = 3.45, df = 1 (P = 0.06), I² =71%					



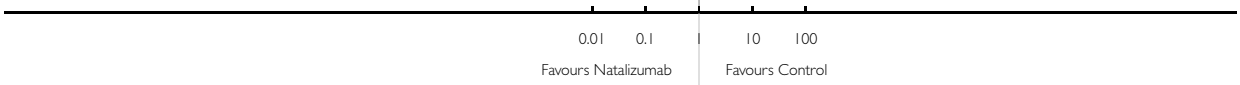
Analysis 1.2. Comparison 1 Primary Efficacy Outcome (Natalizumab vs Control), Outcome 2 N of pts who progressed at 2 yrs.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 1 Primary Efficacy Outcome (Natalizumab vs Control)

Outcome: 2 N of pts who progressed at 2 yrs

Study or subgroup	Experimental n/N	Control n/N	Risk Ratio M-H,Random,95% CI	Weight	Risk Ratio M-H,Random,95% CI
1 Natalizumab vs Placebo					
AFFIRM 2006	154/627	116/315		43.5 %	0.67 [0.55, 0.81]
Subtotal (95% CI)	**627**	**315**		**43.5 %**	**0.67 [0.55, 0.81]**
Total events: 154 (Experimental), 116 (Control)					
Heterogeneity: not applicable					
Test for overall effect: Z = 3.98 (P = 0.000068)					
2 Natalizumab + IFN vs IFN					
SENTINEL 2006	194/589	239/582		56.5 %	0.80 [0.69, 0.93]
Subtotal (95% CI)	**589**	**582**		**56.5 %**	**0.80 [0.69, 0.93]**
Total events: 194 (Experimental), 239 (Control)					
Heterogeneity: not applicable					
Test for overall effect: Z = 2.87 (P = 0.0042)					
Total (95% CI)	**1216**	**897**		**100.0 %**	**0.74 [0.62, 0.89]**
Total events: 348 (Experimental), 355 (Control)					
Heterogeneity: Tau^2 = 0.01; Chi^2 = 2.09, df = 1 (P = 0.15); I^2 =52%					
Test for overall effect: Z = 3.29 (P = 0.0010)					
Test for subgroup differences: Chi^2 = 2.09, df = 1 (P = 0.15), I^2 =52%					



Analysis 1.3. Comparison 1 Primary Efficacy Outcome (Natalizumab vs Control), Outcome 3 PCS Change in Short Form (SF-36) follow up 2 years.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 1 Primary Efficacy Outcome (Natalizumab vs Control)

Outcome: 3 PCS Change in Short Form (SF-36) follow up 2 years

Study or subgroup	Experimental N	Mean(SD)	Control N	Mean(SD)	Mean Difference IV,Random,95% CI	Weight	Mean Difference IV,Random,95% CI
1 Natalizumab vs Placebo							
AFFIRM 2006	627	0.67 (11.28)	315	-1.34 (11.26)		37.0 %	2.01 [0.48, 3.54]
Subtotal (95% CI)	**627**		**315**			**37.0 %**	**2.01 [0.48, 3.54]**
Heterogeneity: not applicable							
Test for overall effect: Z = 2.58 (P = 0.0098)							
2 Natalizumab + IFN vs IFN							
SENTINEL 2006	589	1.03 (10.2)	582	-0.93 (10.2)		63.0 %	1.96 [0.79, 3.13]
Subtotal (95% CI)	**589**		**582**			**63.0 %**	**1.96 [0.79, 3.13]**
Heterogeneity: not applicable							
Test for overall effect: Z = 3.29 (P = 0.0010)							
Total (95% CI)	**1216**		**897**			**100.0 %**	**1.98 [1.05, 2.91]**
Heterogeneity: Tau^2 = 0.0; Chi^2 = 0.00, df = 1 (P = 0.96); I^2 =0.0%							
Test for overall effect: Z = 4.18 (P = 0.000029)							
Test for subgroup differences: Chi^2 = 0.00, df = 1 (P = 0.96), I^2 =0.0%							

Analysis 1.4. Comparison 1 Primary Efficacy Outcome (Natalizumab vs Control), Outcome 4 MCS Change in Short Form (SF-36) follow up 2 years.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 1 Primary Efficacy Outcome (Natalizumab vs Control)

Outcome: 4 MCS Change in Short Form (SF-36) follow up 2 years

Study or subgroup	Experimental N	Mean(SD)	Control N	Mean(SD)	Mean Difference IV,Random,95% CI	Weight	Mean Difference IV,Random,95% CI
1 Natalizumab vs Placebo							
AFFIRM 2006	627	2 (18.67)	315	-0.53 (18.67)		17.0 %	2.53 [0.00, 5.06]
Subtotal (95% CI)	**627**		**315**			**17.0 %**	**2.53 [0.00, 5.06]**
Heterogeneity: not applicable							
Test for overall effect: Z = 1.96 (P = 0.050)							
2 Natalizumab + IFN vs IFN							
SENTINEL 2006	589	0.18 (9.98)	582	-0.96 (9.98)		83.0 %	1.14 [0.00, 2.28]
Subtotal (95% CI)	**589**		**582**			**83.0 %**	**1.14 [0.00, 2.28]**
Heterogeneity: not applicable							
Test for overall effect: Z = 1.95 (P = 0.051)							
Total (95% CI)	**1216**		**897**			**100.0 %**	**1.38 [0.33, 2.42]**
Heterogeneity: $Tau^2 = 0.0$; $Chi^2 = 0.96$, df = 1 (P = 0.33); $I^2 = 0.0\%$							
Test for overall effect: Z = 2.59 (P = 0.0096)							
Test for subgroup differences: $Chi^2 = 0.96$, df = 1 (P = 0.33), $I^2 = 0.0\%$							

Analysis 2.1. Comparison 2 Secondary Efficacy Outcome (Natalizumab vs Control), Outcome 1 Change in Well-being (VAS) at 2 yrs.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 2 Secondary Efficacy Outcome (Natalizumab vs Control)

Outcome: 1 Change in Well-being (VAS) at 2 yrs

Study or subgroup	Experimental N	Mean(SD)	Control N	Mean(SD)	Mean Difference IV,Random,95% CI	Weight	Mean Difference IV,Random,95% CI
1 Natalizumab vs Placebo							
AFFIRM 2006	627	0.2 (34.29)	315	-6.2 (34.29)		100.0 %	6.40 [1.76, 11.04]
Total (95% CI)	**627**		**315**			**100.0 %**	**6.40 [1.76, 11.04]**

Heterogeneity: not applicable

Test for overall effect: Z = 2.70 (P = 0.0069)

Test for subgroup differences: Not applicable

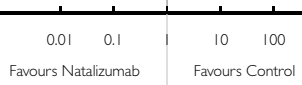
Analysis 2.2. Comparison 2 Secondary Efficacy Outcome (Natalizumab vs Control), Outcome 2 Gd-enhacing lesion (at least one) at 2 yrs.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 2 Secondary Efficacy Outcome (Natalizumab vs Control)

Outcome: 2 Gd-enhacing lesion (at least one) at 2 yrs

Study or subgroup	Experimental n/N	Control n/N	Risk Ratio M-H,Random,95% CI	Weight	Risk Ratio M-H,Random,95% CI
1 Natalizumab vs Placebo					
AFFIRM 2006	22/627	102/315		50.3 %	0.11 [0.07, 0.17]
Subtotal (95% CI)	**627**	**315**		**50.3 %**	**0.11 [0.07, 0.17]**
Total events: 22 (Experimental), 102 (Control)					
Heterogeneity: not applicable					
Test for overall effect: Z = 9.89 (P < 0.00001)					
2 Natalizumab + IFN vs IFN					
SENTINEL 2006	21/589	147/582		49.7 %	0.14 [0.09, 0.22]
Subtotal (95% CI)	**589**	**582**		**49.7 %**	**0.14 [0.09, 0.22]**
Total events: 21 (Experimental), 147 (Control)					
Heterogeneity: not applicable					
Test for overall effect: Z = 8.67 (P < 0.00001)					
Total (95% CI)	**1216**	**897**		**100.0 %**	**0.12 [0.09, 0.17]**
Total events: 43 (Experimental), 249 (Control)					
Heterogeneity: Tau^2 = 0.0; Chi^2 = 0.70, df = 1 (P = 0.40); I^2 =0.0%					
Test for overall effect: Z = 13.13 (P < 0.00001)					
Test for subgroup differences: Chi^2 = 0.69, df = 1 (P = 0.41), I^2 =0.0%					



Analysis 2.3. Comparison 2 Secondary Efficacy Outcome (Natalizumab vs Control), Outcome 3 Change of MRI T2 total lesion load at 2 yrs.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 2 Secondary Efficacy Outcome (Natalizumab vs Control)

Outcome: 3 Change of MRI T2 total lesion load at 2 yrs

Study or subgroup	Experimental N	Mean(SD)	Control N	Mean(SD)	Mean Difference IV,Random,95% CI	Weight	Mean Difference IV,Random,95% CI
1 Natalizumab vs Placebo							
AFFIRM 2006	576	-905.4 (12781.2)	279	2890.8 (15068.4)		100.0 %	-3796.20 [-5849.43, -1742.97]
Total (95% CI)	**576**		**279**			**100.0 %**	**-3796.20 [-5849.43, -1742.97]**

Heterogeneity: not applicable

Test for overall effect: Z = 3.62 (P = 0.00029)

Test for subgroup differences: Not applicable

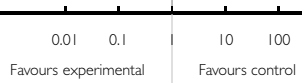
Analysis 3.1. Comparison 3 Primary Safety Outcome (Natalizumab vs Control), Outcome 1 N of pts with Severe AE over 2 yrs.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 3 Primary Safety Outcome (Natalizumab vs Control)

Outcome: 1 N of pts with Severe AE over 2 yrs

Study or subgroup	Experimental n/N	Control n/N	Risk Ratio M-H,Random,95% CI	Weight	Risk Ratio M-H,Random,95% CI
1 Natalizumab vs Placebo					
AFFIRM 2006	144/627	84/312		30.2 %	0.85 [0.68, 1.08]
Subtotal (95% CI)	**627**	**312**		**30.2 %**	**0.85 [0.68, 1.08]**
Total events: 144 (Experimental), 84 (Control)					
Heterogeneity: not applicable					
Test for overall effect: Z = 1.34 (P = 0.18)					
2 Natalizumab + IFN vs IFN					
SENTINEL 2006	206/589	215/582		69.8 %	0.95 [0.81, 1.10]
Subtotal (95% CI)	**589**	**582**		**69.8 %**	**0.95 [0.81, 1.10]**
Total events: 206 (Experimental), 215 (Control)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.70 (P = 0.48)					
Total (95% CI)	**1216**	**894**		**100.0 %**	**0.92 [0.81, 1.04]**
Total events: 350 (Experimental), 299 (Control)					
Heterogeneity: Tau^2 = 0.0; Chi^2 = 0.54, df = 1 (P = 0.46); I^2 =0.0%					
Test for overall effect: Z = 1.32 (P = 0.19)					
Test for subgroup differences: Chi^2 = 0.54, df = 1 (P = 0.46), I^2 =0.0%					



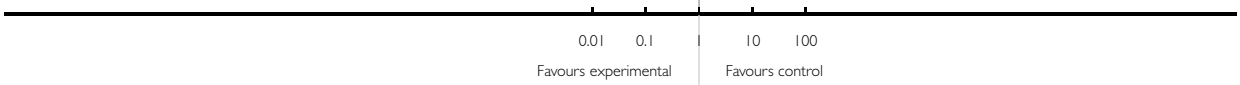
Analysis 3.2. Comparison 3 Primary Safety Outcome (Natalizumab vs Control), Outcome 2 N of pts with Serious AE (irrespective of treatment duration).

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 3 Primary Safety Outcome (Natalizumab vs Control)

Outcome: 2 N of pts with Serious AE (irrespective of treatment duration)

Study or subgroup	Experimental n/N	Control n/N	Risk Ratio M-H,Random,95% CI	Weight	Risk Ratio M-H,Random,95% CI
1 Natalizumab vs Placebo					
AFFIRM 2006	119/627	75/312		45.2 %	0.79 [0.61, 1.02]
Subtotal (95% CI)	**627**	**312**		**45.2 %**	**0.79 [0.61, 1.02]**
Total events: 119 (Experimental), 75 (Control)					
Heterogeneity: not applicable					
Test for overall effect: Z = 1.82 (P = 0.069)					
2 Natalizumab + IFN vs IFN					
SENTINEL 2006	107/589	122/582		54.2 %	0.87 [0.69, 1.09]
Subtotal (95% CI)	**589**	**582**		**54.2 %**	**0.87 [0.69, 1.09]**
Total events: 107 (Experimental), 122 (Control)					
Heterogeneity: not applicable					
Test for overall effect: Z = 1.20 (P = 0.23)					
3 Natalizumab + GA vs GA					
GLANCE 2009	1/55	2/55		0.5 %	0.50 [0.05, 5.36]
Subtotal (95% CI)	**55**	**55**		**0.5 %**	**0.50 [0.05, 5.36]**
Total events: 1 (Experimental), 2 (Control)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.57 (P = 0.57)					
Total (95% CI)	**1271**	**949**		**100.0 %**	**0.83 [0.70, 0.98]**
Total events: 227 (Experimental), 199 (Control)					
Heterogeneity: Tau^2 = 0.0; Chi^2 = 0.45, df = 2 (P = 0.80); I^2 =0.0%					
Test for overall effect: Z = 2.15 (P = 0.032)					
Test for subgroup differences: Chi^2 = 0.45, df = 2 (P = 0.80), I^2 =0.0%					



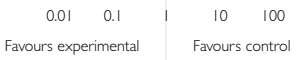
Analysis 3.3. Comparison 3 Primary Safety Outcome (Natalizumab vs Control), Outcome 3 N of pts with serious AE (irrespective of treatment duration - MS relapses excluded).

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 3 Primary Safety Outcome (Natalizumab vs Control)

Outcome: 3 N of pts with serious AE (irrespective of treatment duration - MS relapses excluded)

Study or subgroup	Experimental n/N	Control n/N	Risk Ratio M-H,Random,95% CI	Weight	Risk Ratio M-H,Random,95% CI
1 Natalizumab vs Placebo					
AFFIRM 2006	81/627	34/312		38.8 %	1.19 [0.81, 1.73]
Subtotal (95% CI)	**627**	**312**		**38.8 %**	**1.19 [0.81, 1.73]**
Total events: 81 (Experimental), 34 (Control)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.89 (P = 0.38)					
2 Natalizumab + IFN vs IFN					
SENTINEL 2006	78/589	70/582		60.5 %	1.10 [0.81, 1.49]
Subtotal (95% CI)	**589**	**582**		**60.5 %**	**1.10 [0.81, 1.49]**
Total events: 78 (Experimental), 70 (Control)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.63 (P = 0.53)					
3 Natalizumab + GA vs GA					
GLANCE 2009	1/55	1/55		0.7 %	1.00 [0.06, 15.59]
Subtotal (95% CI)	**55**	**55**		**0.7 %**	**1.00 [0.06, 15.59]**
Total events: 1 (Experimental), 1 (Control)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.0 (P = 1.0)					
Total (95% CI)	**1271**	**949**		**100.0 %**	**1.13 [0.90, 1.43]**
Total events: 160 (Experimental), 105 (Control)					
Heterogeneity: Tau^2 = 0.0; Chi^2 = 0.10, df = 2 (P = 0.95); I^2 =0.0%					
Test for overall effect: Z = 1.04 (P = 0.30)					
Test for subgroup differences: Chi^2 = 0.10, df = 2 (P = 0.95), I^2 =0.0%					



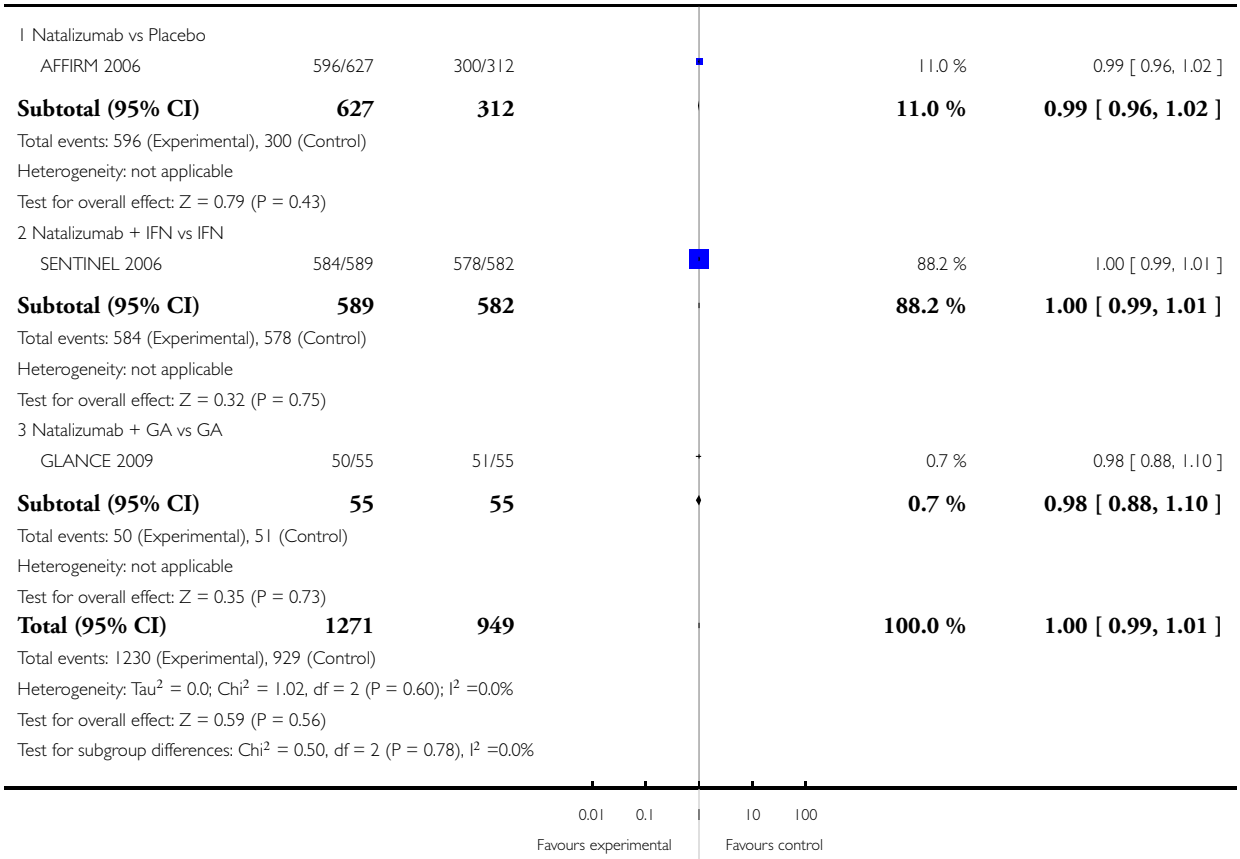
Analysis 4.1. Comparison 4 Secondary Safety Outcome (Natalizumab vs Control), Outcome 1 N of pts with at least one AE (irrespective of treatment duration).

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 4 Secondary Safety Outcome (Natalizumab vs Control)

Outcome: 1 N of pts with at least one AE (irrespective of treatment duration)

Study or subgroup	Experimental n/N	Control n/N	Risk Ratio M-H,Random,95% CI	Weight	Risk Ratio M-H,Random,95% CI
1 Natalizumab vs Placebo					
AFFIRM 2006	596/627	300/312		11.0 %	0.99 [0.96, 1.02]
Subtotal (95% CI)	**627**	**312**		**11.0 %**	**0.99 [0.96, 1.02]**
Total events: 596 (Experimental), 300 (Control)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.79 (P = 0.43)					
2 Natalizumab + IFN vs IFN					
SENTINEL 2006	584/589	578/582		88.2 %	1.00 [0.99, 1.01]
Subtotal (95% CI)	**589**	**582**		**88.2 %**	**1.00 [0.99, 1.01]**
Total events: 584 (Experimental), 578 (Control)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.32 (P = 0.75)					
3 Natalizumab + GA vs GA					
GLANCE 2009	50/55	51/55		0.7 %	0.98 [0.88, 1.10]
Subtotal (95% CI)	**55**	**55**		**0.7 %**	**0.98 [0.88, 1.10]**
Total events: 50 (Experimental), 51 (Control)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.35 (P = 0.73)					
Total (95% CI)	**1271**	**949**		**100.0 %**	**1.00 [0.99, 1.01]**
Total events: 1230 (Experimental), 929 (Control)					
Heterogeneity: $Tau^2 = 0.0$; $Chi^2 = 1.02$, df = 2 (P = 0.60); $I^2 = 0.0\%$					
Test for overall effect: Z = 0.59 (P = 0.56)					
Test for subgroup differences: $Chi^2 = 0.50$, df = 2 (P = 0.78), $I^2 = 0.0\%$					



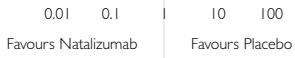
Analysis 4.2. Comparison 4 Secondary Safety Outcome (Natalizumab vs Control), Outcome 2 Treatment Discontinuation caused by AE (irrespective of treatment duration).

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 4 Secondary Safety Outcome (Natalizumab vs Control)

Outcome: 2 Treatment Discontinuation caused by AE (irrespective of treatment duration)

Study or subgroup	Experimental n/N	Control n/N	Risk Ratio M-H,Random,95% CI	Weight	Risk Ratio M-H,Random,95% CI
1 Natalizumab vs Placebo					
AFFIRM 2006	38/627	12/312		27.5 %	1.58 [0.84, 2.97]
Subtotal (95% CI)	**627**	**312**		**27.5 %**	**1.58 [0.84, 2.97]**
Total events: 38 (Experimental), 12 (Control)					
Heterogeneity: not applicable					
Test for overall effect: Z = 1.40 (P = 0.16)					
2 Natalizumab + IFN vs IFN					
SENTINEL 2006	46/589	45/582		71.1 %	1.01 [0.68, 1.50]
Subtotal (95% CI)	**589**	**582**		**71.1 %**	**1.01 [0.68, 1.50]**
Total events: 46 (Experimental), 45 (Control)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.05 (P = 0.96)					
3 Natalizumab + GA vs GA					
GLANCE 2009	1/55	1/55		1.5 %	1.00 [0.06, 15.59]
Subtotal (95% CI)	**55**	**55**		**1.5 %**	**1.00 [0.06, 15.59]**
Total events: 1 (Experimental), 1 (Control)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.0 (P = 1.0)					
Total (95% CI)	**1271**	**949**		**100.0 %**	**1.14 [0.82, 1.59]**
Total events: 85 (Experimental), 58 (Control)					
Heterogeneity: Tau^2 = 0.0; Chi^2 = 1.37, df = 2 (P = 0.50); I^2 =0.0%					
Test for overall effect: Z = 0.78 (P = 0.44)					
Test for subgroup differences: Chi^2 = 1.37, df = 2 (P = 0.50), I^2 =0.0%					

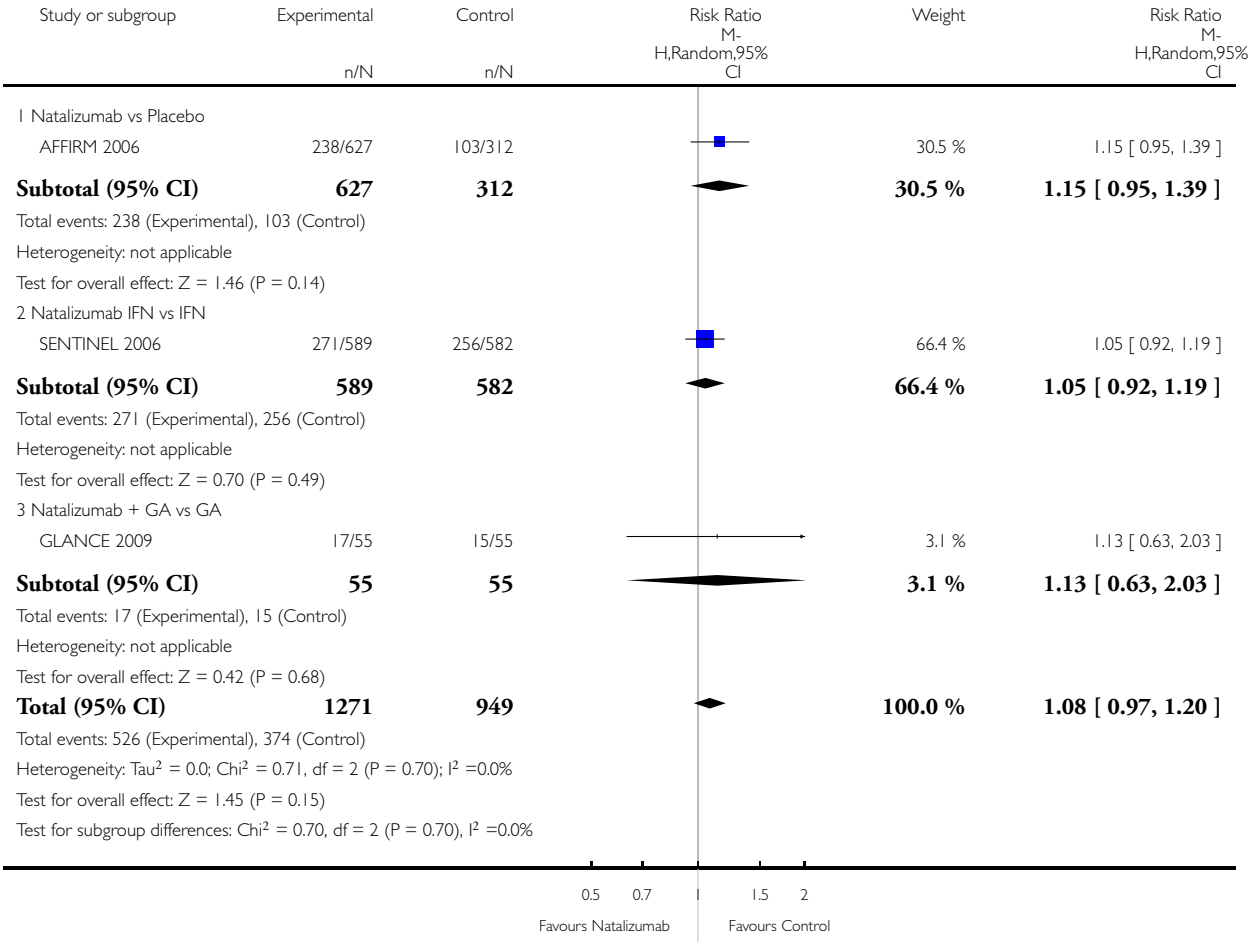


Analysis 5.1. Comparison 5 Adverse Event Analysis, Outcome 1 Headache.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 1 Headache

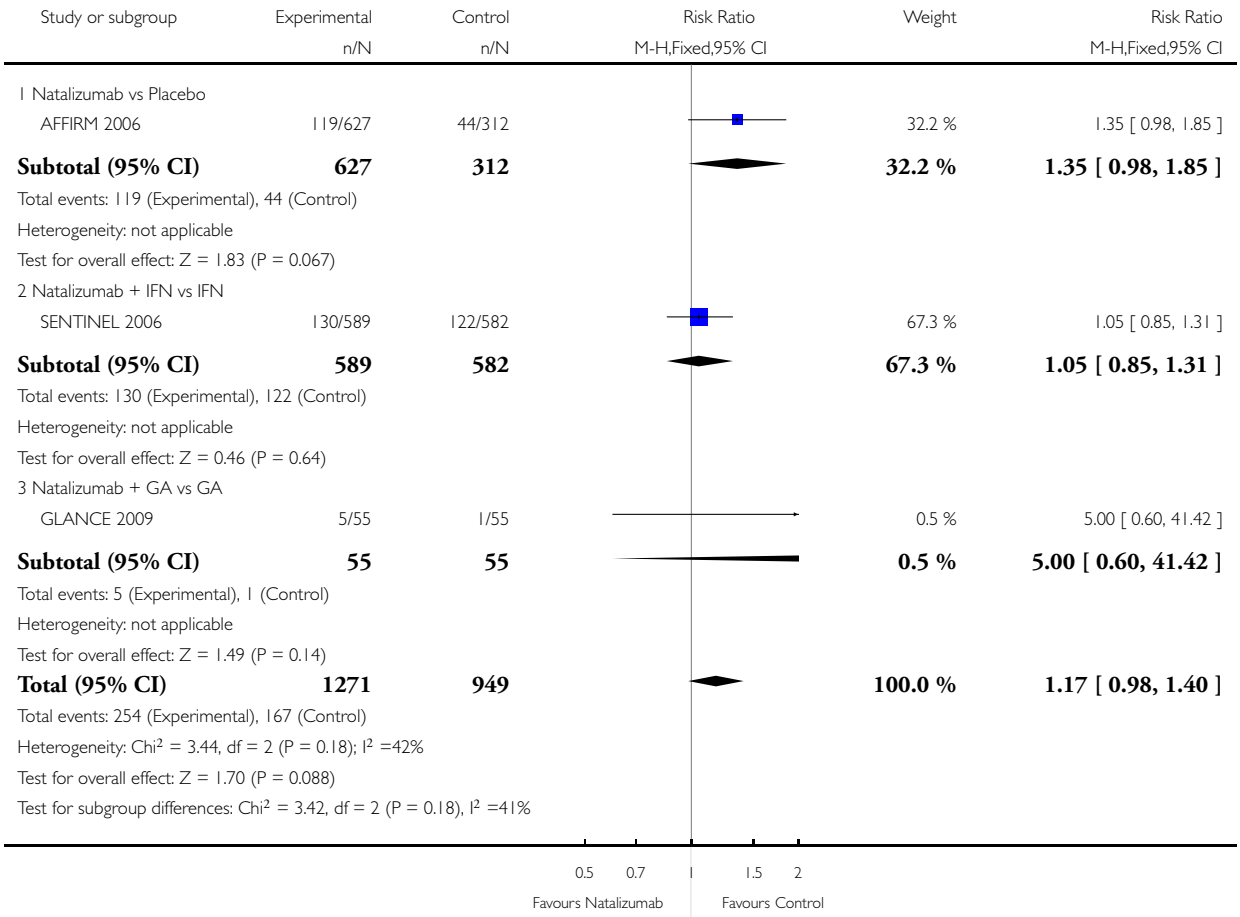


Analysis 5.2. Comparison 5 Adverse Event Analysis, Outcome 2 Pain in arms or legs - Arthralgia.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 2 Pain in arms or legs - Arthralgia

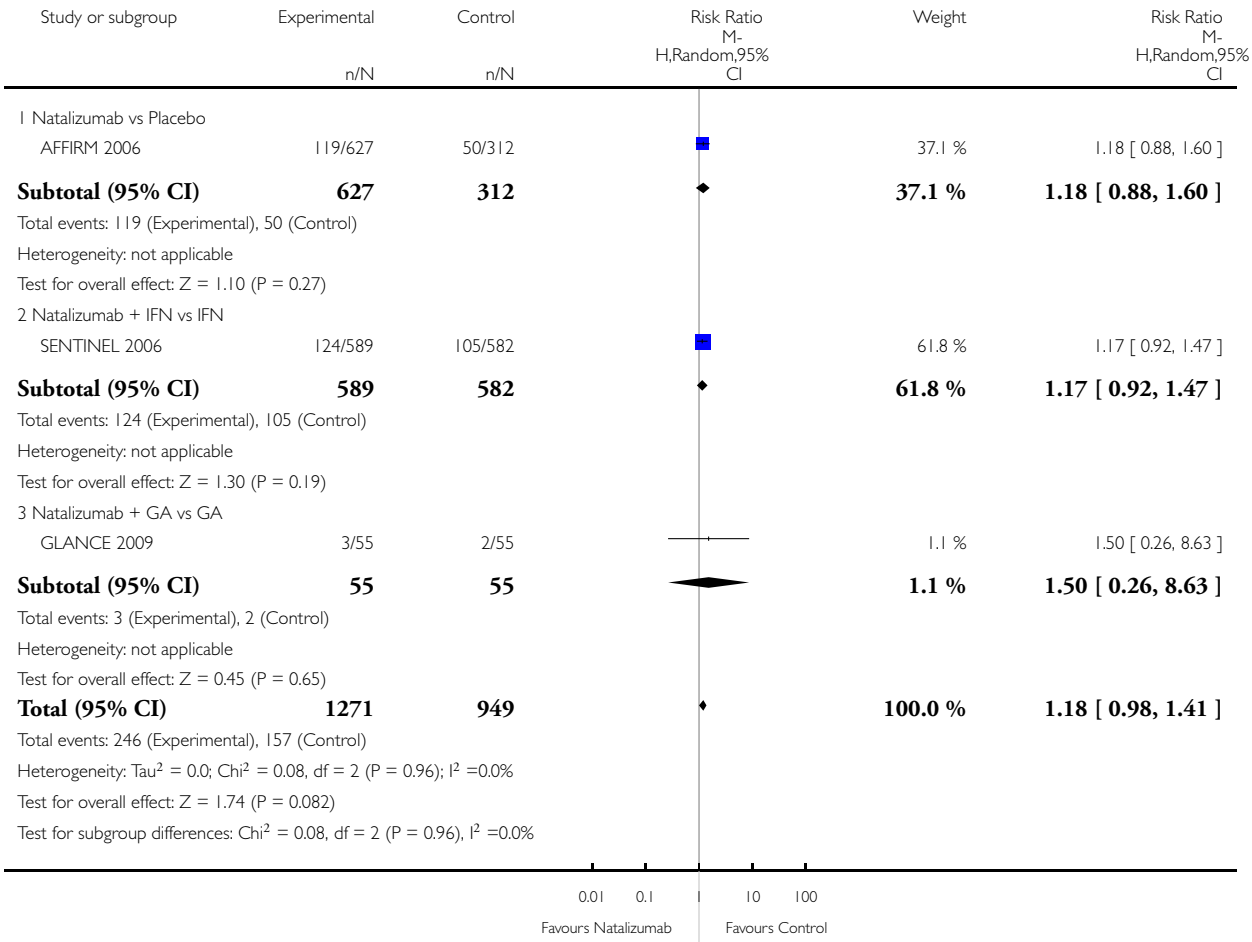


Analysis 5.3. Comparison 5 Adverse Event Analysis, Outcome 3 Depression.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 3 Depression

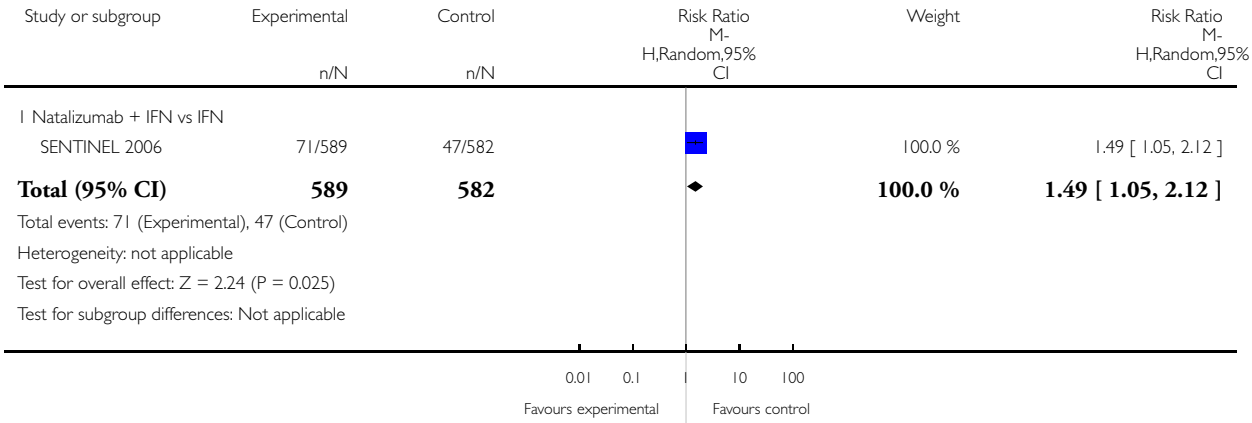


Analysis 5.4. Comparison 5 Adverse Event Analysis, Outcome 4 Anxiety.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 4 Anxiety

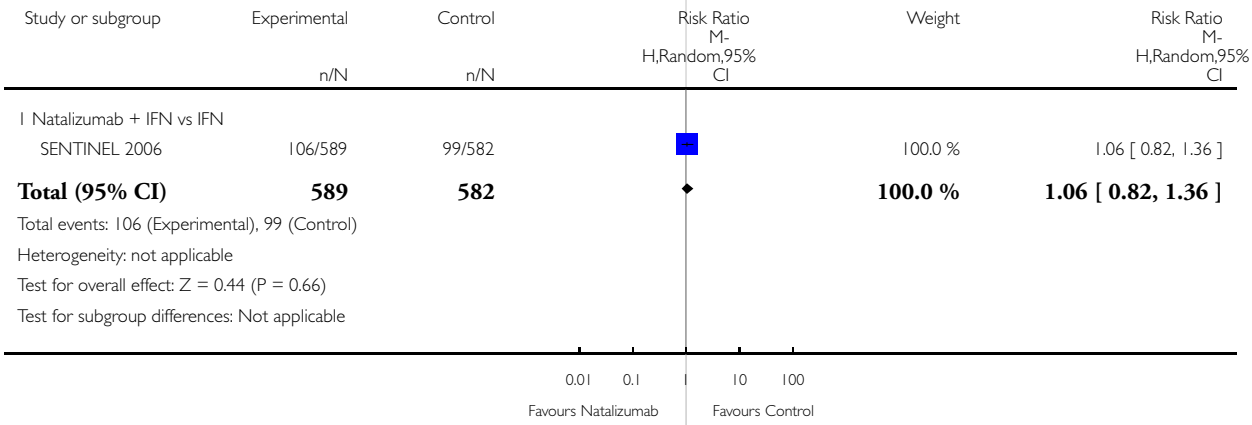


Analysis 5.5. Comparison 5 Adverse Event Analysis, Outcome 5 Insomnia.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 5 Insomnia

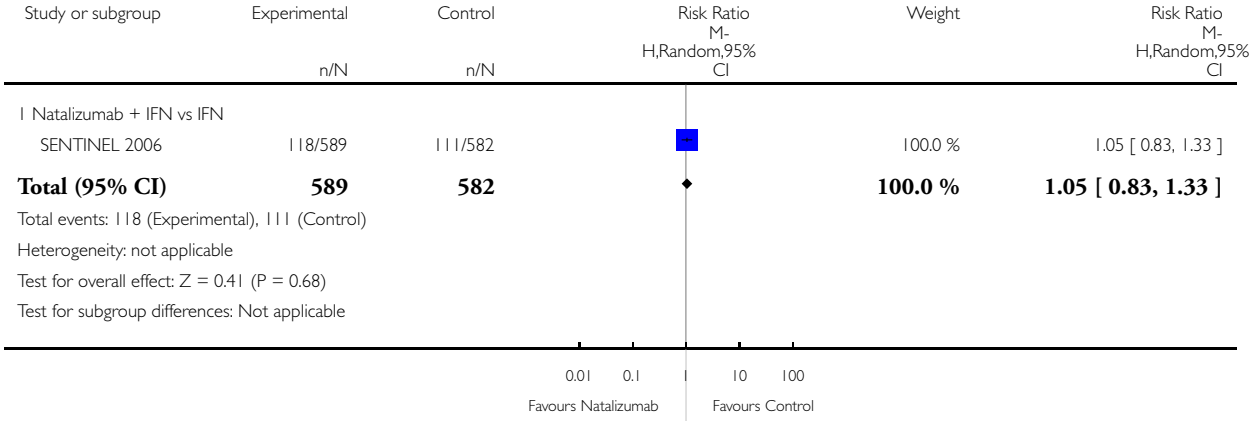


Analysis 5.6. Comparison 5 Adverse Event Analysis, Outcome 6 Influenza Like Illness.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 6 Influenza Like Illness

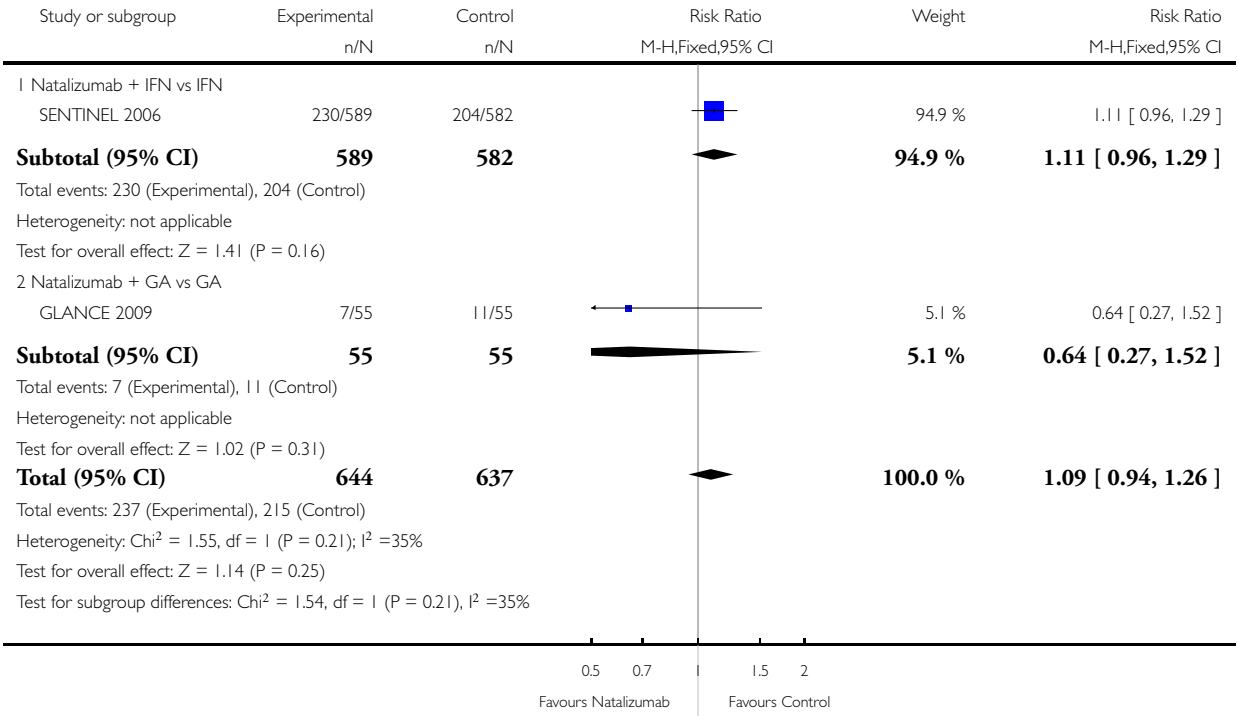


Analysis 5.7. Comparison 5 Adverse Event Analysis, Outcome 7 Nasopharyngitis.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 7 Nasopharyngitis

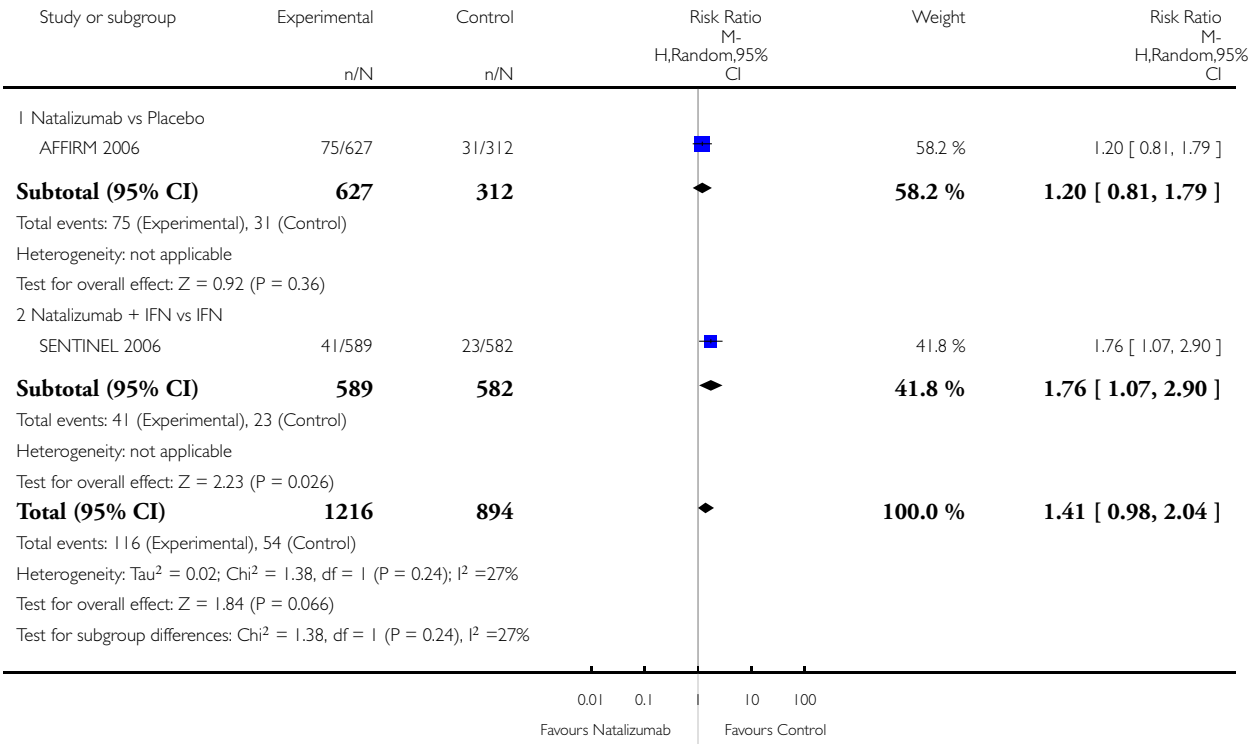


Analysis 5.8. Comparison 5 Adverse Event Analysis, Outcome 8 Pharyngitis.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 8 Pharyngitis

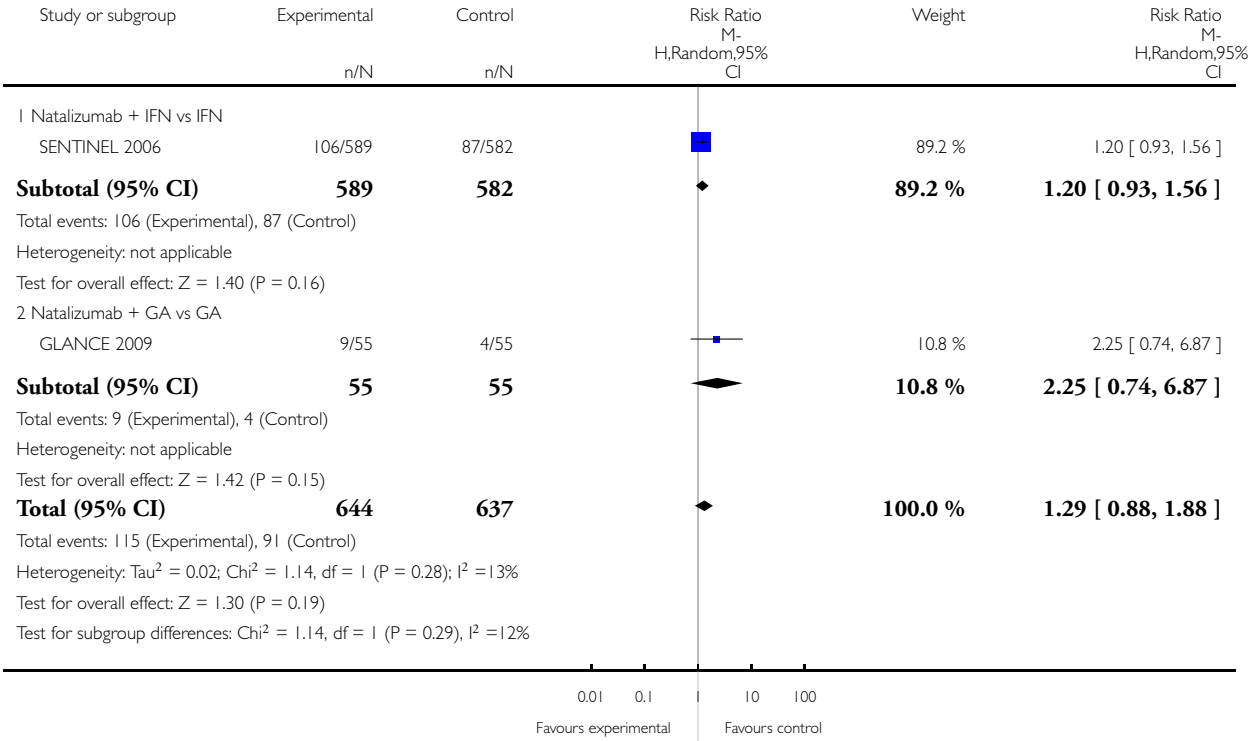


Analysis 5.9. Comparison 5 Adverse Event Analysis, Outcome 9 Sinusitis.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 9 Sinusitis

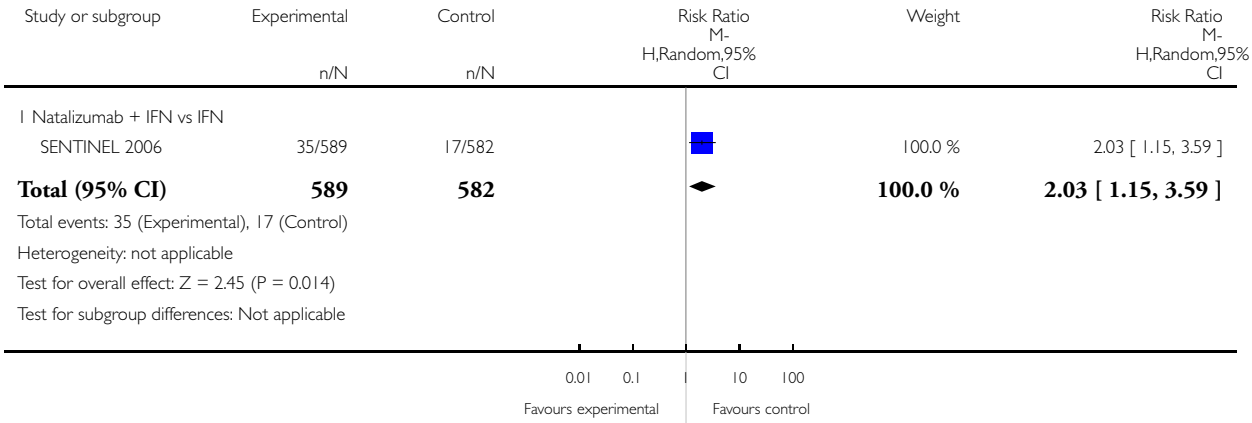


Analysis 5.10. Comparison 5 Adverse Event Analysis, Outcome 10 Sinus Congestion.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 10 Sinus Congestion

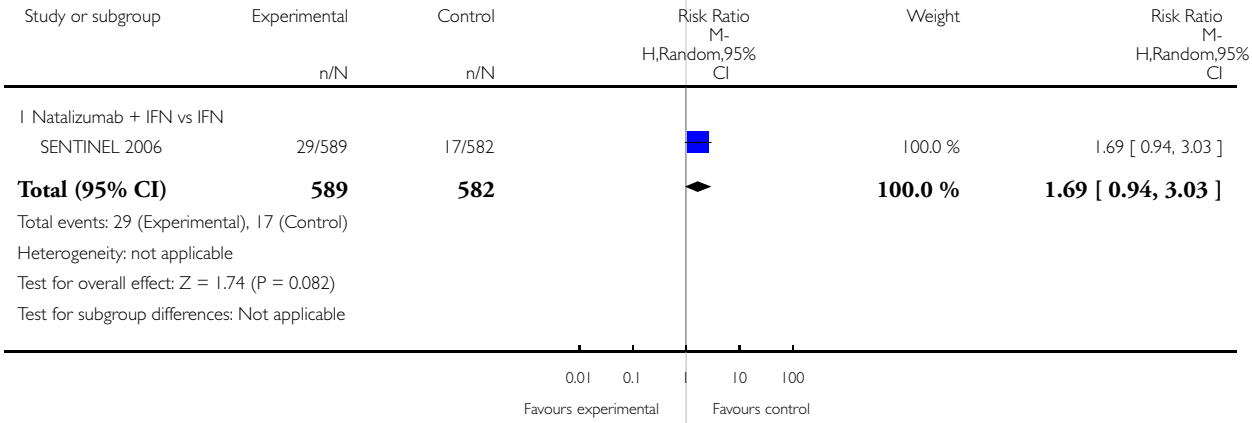


Analysis 5.11. Comparison 5 Adverse Event Analysis, Outcome 11 Sinus Headache.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 11 Sinus Headache

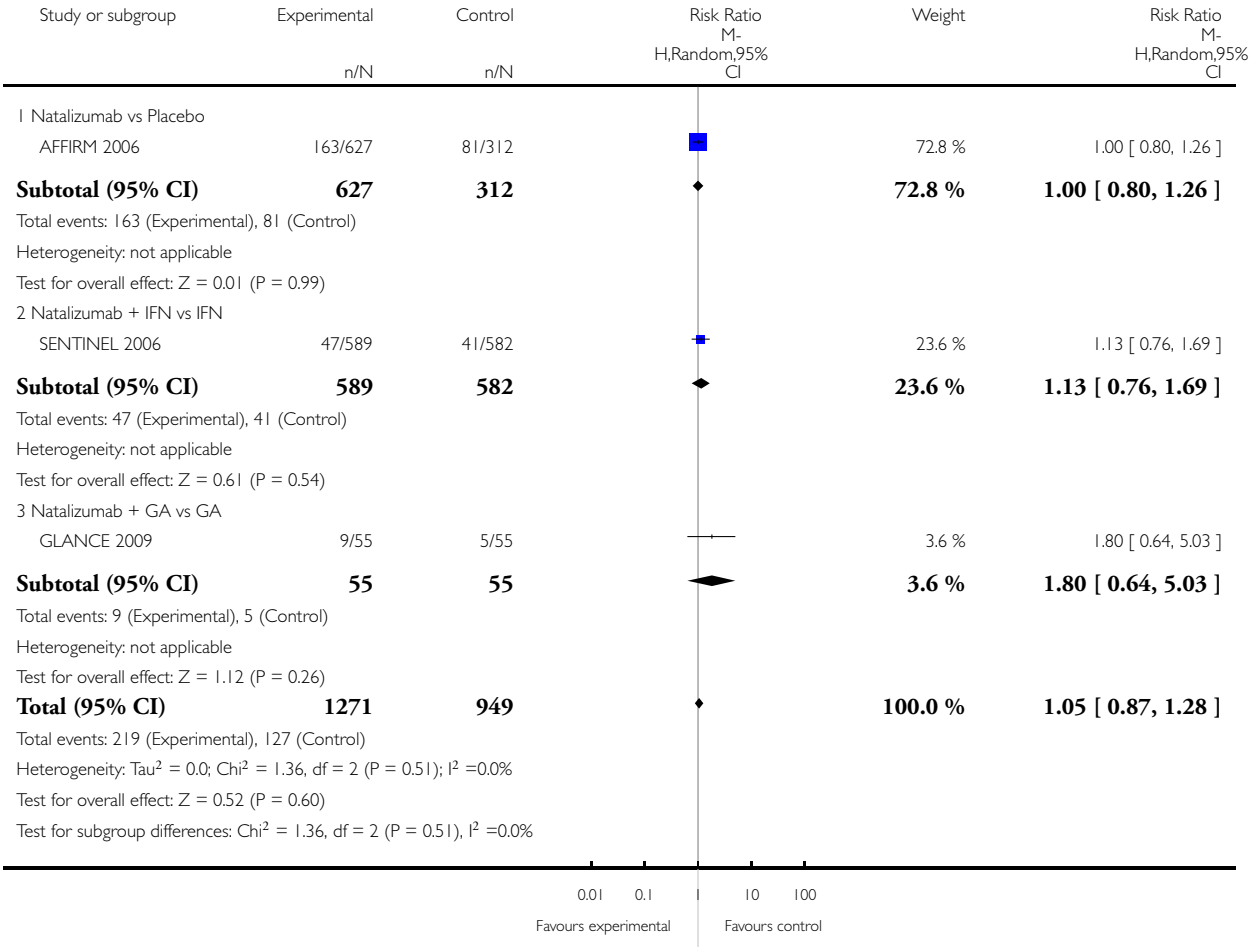


Analysis 5.12. Comparison 5 Adverse Event Analysis, Outcome 12 Upper Respiratory Infection.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 12 Upper Respiratory Infection

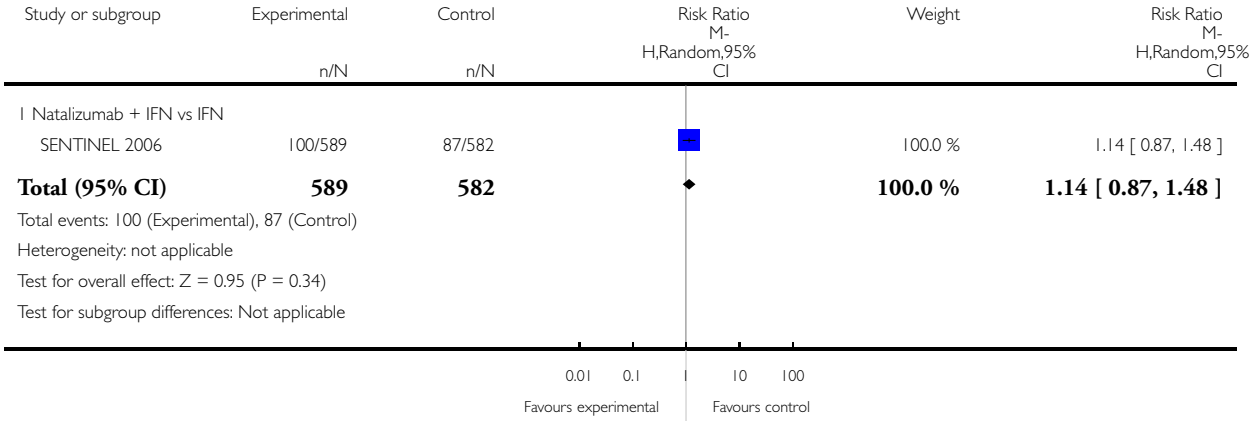


Analysis 5.13. Comparison 5 Adverse Event Analysis, Outcome 13 Influenza.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 13 Influenza

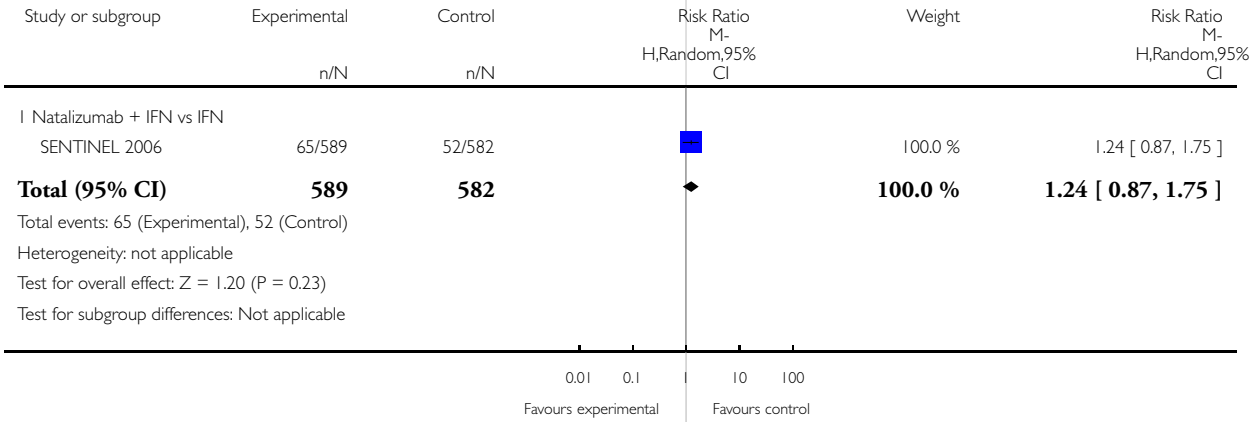


Analysis 5.14. Comparison 5 Adverse Event Analysis, Outcome 14 Cough.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 14 Cough



Analysis 5.15. Comparison 5 Adverse Event Analysis, Outcome 15 Diarrhea.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 15 Diarrhea

Study or subgroup	Experimental n/N	Control n/N	Risk Ratio M-H,Random,95% CI	Weight	Risk Ratio M-H,Random,95% CI
1 Natalizumab + IFN vs IFN					
SENTINEL 2006	112/589	93/582		100.0 %	1.19 [0.93, 1.53]
Total (95% CI)	**589**	**582**		**100.0 %**	**1.19 [0.93, 1.53]**
Total events: 112 (Experimental), 93 (Control)					
Heterogeneity: not applicable					
Test for overall effect: Z = 1.36 (P = 0.17)					
Test for subgroup differences: Not applicable					

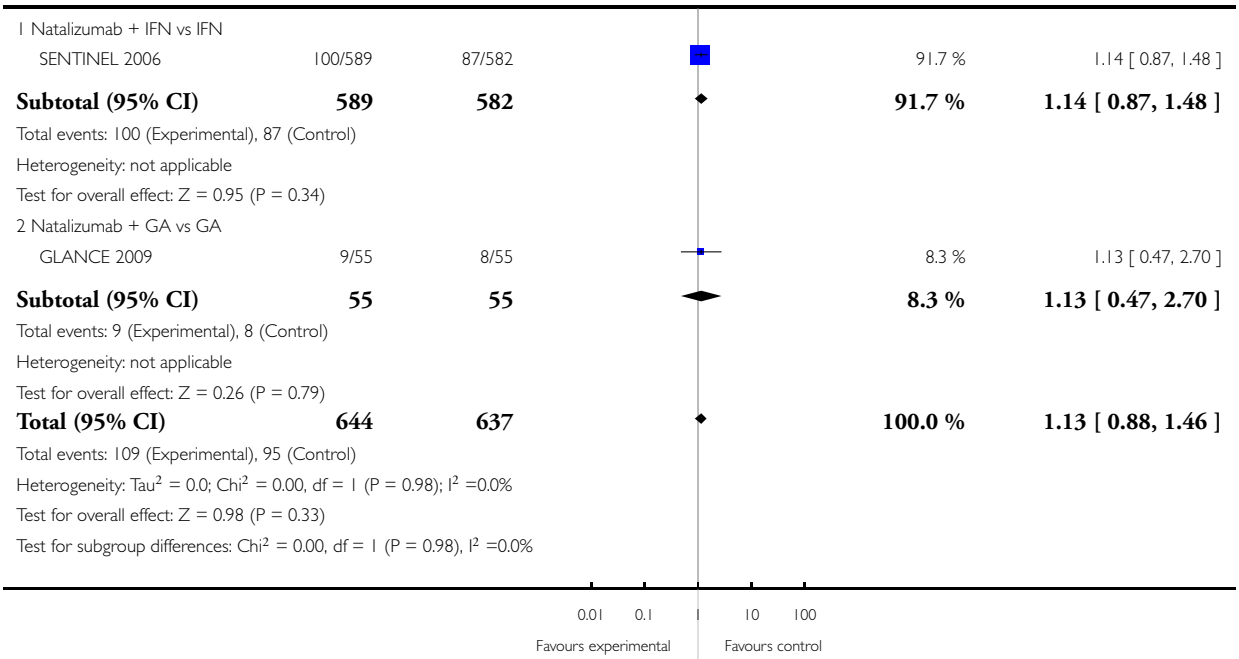
Analysis 5.16. Comparison 5 Adverse Event Analysis, Outcome 16 Nausea.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 16 Nausea

Study or subgroup	Experimental n/N	Control n/N	Risk Ratio M-H,Random,95% CI	Weight	Risk Ratio M-H,Random,95% CI
1 Natalizumab + IFN vs IFN					
SENTINEL 2006	100/589	87/582		91.7 %	1.14 [0.87, 1.48]
Subtotal (95% CI)	**589**	**582**		**91.7 %**	**1.14 [0.87, 1.48]**
Total events: 100 (Experimental), 87 (Control)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.95 (P = 0.34)					
2 Natalizumab + GA vs GA					
GLANCE 2009	9/55	8/55		8.3 %	1.13 [0.47, 2.70]
Subtotal (95% CI)	**55**	**55**		**8.3 %**	**1.13 [0.47, 2.70]**
Total events: 9 (Experimental), 8 (Control)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.26 (P = 0.79)					
Total (95% CI)	**644**	**637**		**100.0 %**	**1.13 [0.88, 1.46]**
Total events: 109 (Experimental), 95 (Control)					
Heterogeneity: Tau^2 = 0.0; Chi^2 = 0.00, df = 1 (P = 0.98); I^2 =0.0%					
Test for overall effect: Z = 0.98 (P = 0.33)					
Test for subgroup differences: Chi^2 = 0.00, df = 1 (P = 0.98), I^2 =0.0%					

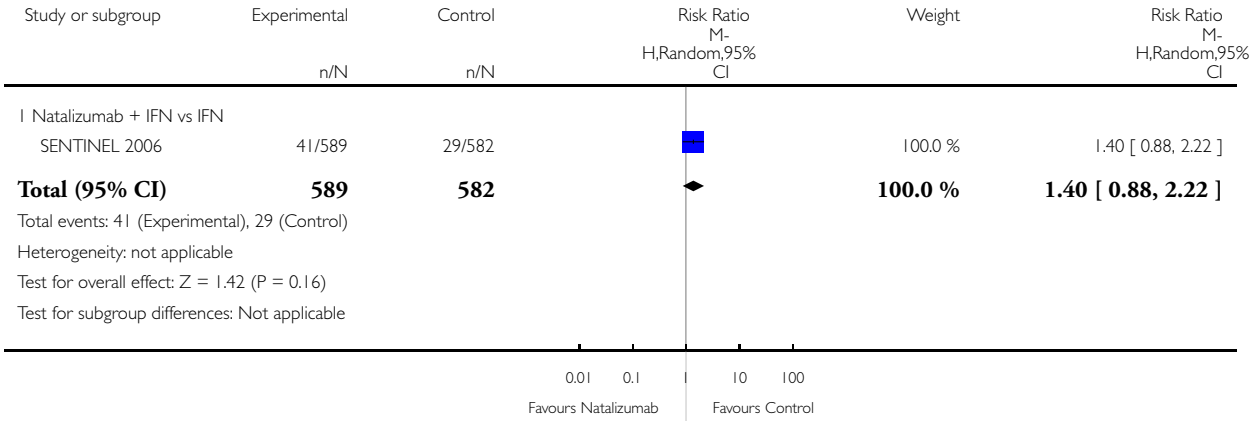


Analysis 5.17. Comparison 5 Adverse Event Analysis, Outcome 17 Vomiting.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 17 Vomiting



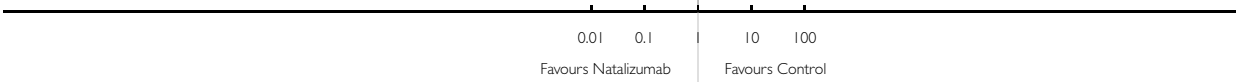
Analysis 5.18. Comparison 5 Adverse Event Analysis, Outcome 18 Abdominal Pain or Discomfort.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 18 Abdominal Pain or Discomfort

Study or subgroup	Experimental n/N	Control n/N	Risk Ratio M-H,Random,95% CI	Weight	Risk Ratio M-H,Random,95% CI
1 Natalizumab vs Placebo					
AFFIRM 2006	69/627	31/312		58.7 %	1.11 [0.74, 1.65]
Subtotal (95% CI)	**627**	**312**		**58.7 %**	**1.11 [0.74, 1.65]**
Total events: 69 (Experimental), 31 (Control)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.50 (P = 0.62)					
2 Natalizumab + IFN vs IFN					
SENTINEL 2006	35/589	29/582		41.3 %	1.19 [0.74, 1.92]
Subtotal (95% CI)	**589**	**582**		**41.3 %**	**1.19 [0.74, 1.92]**
Total events: 35 (Experimental), 29 (Control)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.72 (P = 0.47)					
Total (95% CI)	**1216**	**894**		**100.0 %**	**1.14 [0.84, 1.55]**
Total events: 104 (Experimental), 60 (Control)					
Heterogeneity: $Tau^2 = 0.0$; $Chi^2 = 0.05$, df = 1 (P = 0.82); $I^2 = 0.0\%$					
Test for overall effect: Z = 0.85 (P = 0.40)					
Test for subgroup differences: $Chi^2 = 0.05$, df = 1 (P = 0.82), $I^2 = 0.0\%$					

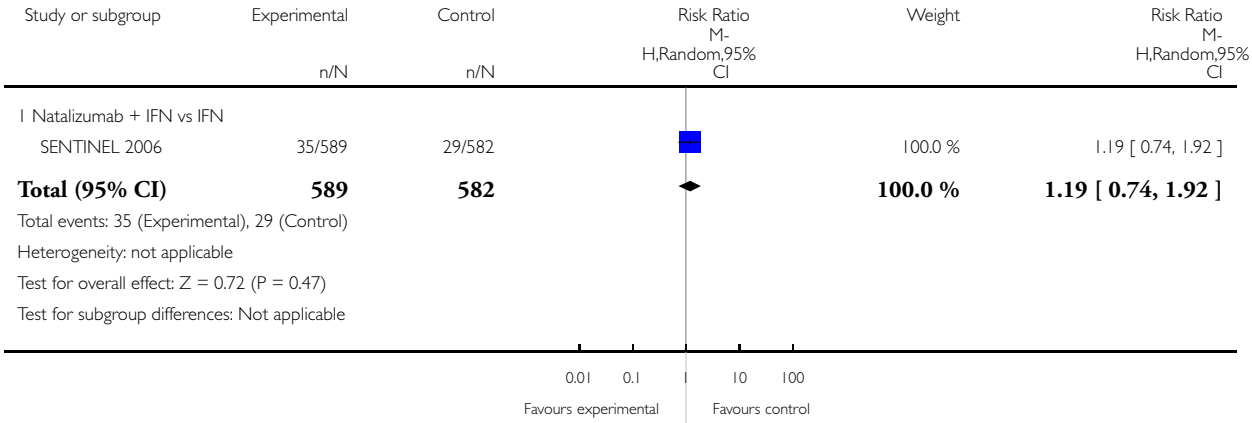


Analysis 5.19. Comparison 5 Adverse Event Analysis, Outcome 19 Muscle Cramp.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 19 Muscle Cramp

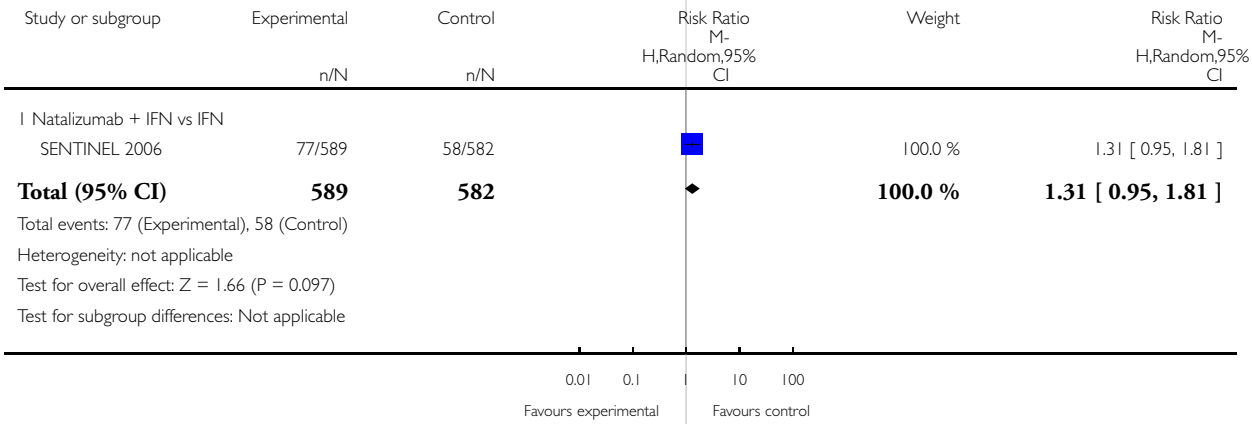


Analysis 5.20. Comparison 5 Adverse Event Analysis, Outcome 20 Myalgia.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 20 Myalgia

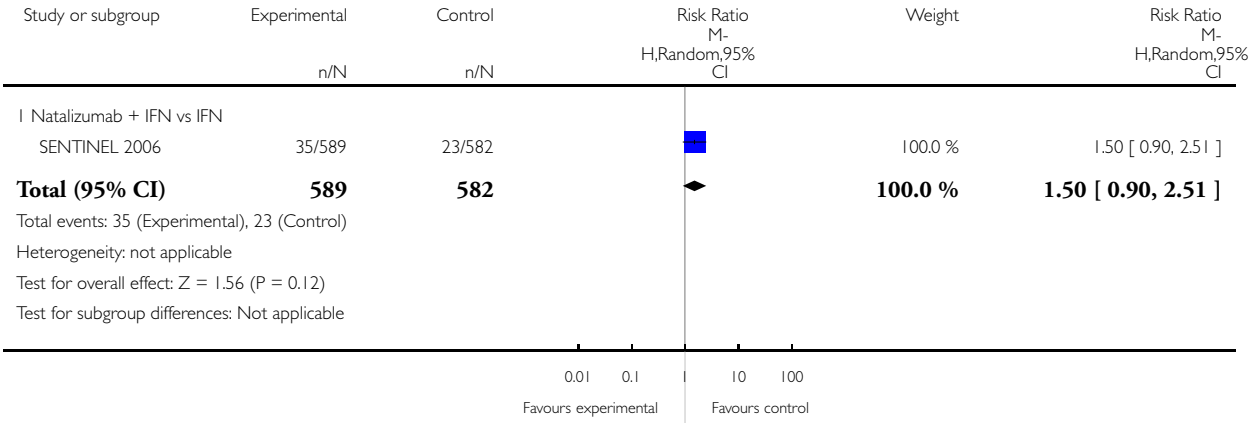


Analysis 5.21. Comparison 5 Adverse Event Analysis, Outcome 21 Seasonal Allergy.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 21 Seasonal Allergy

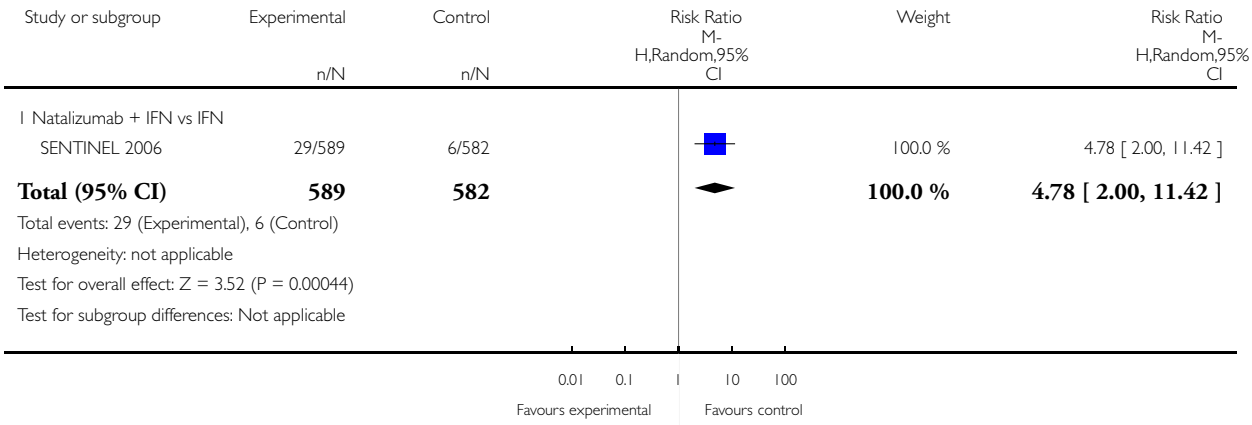


Analysis 5.22. Comparison 5 Adverse Event Analysis, Outcome 22 Peripheral Edema.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 22 Peripheral Edema



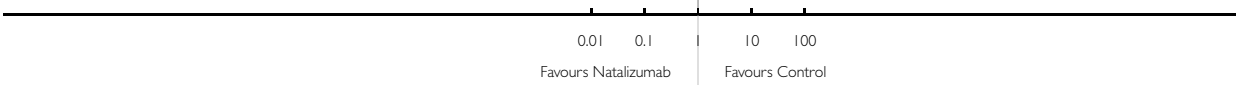
Analysis 5.23. Comparison 5 Adverse Event Analysis, Outcome 23 Tremor.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 23 Tremor

Study or subgroup	Experimental n/N	Control n/N	Risk Ratio M-H,Random,95% CI	Weight	Risk Ratio M-H,Random,95% CI
1 Natalizumab vs Placebo					
AFFIRM 2006	19/627	9/312		36.1 %	1.05 [0.48, 2.29]
Subtotal (95% CI)	**627**	**312**		**36.1 %**	**1.05 [0.48, 2.29]**
Total events: 19 (Experimental), 9 (Control)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.12 (P = 0.90)					
2 Natalizumab + IFN vs IFN					
SENTINEL 2006	29/589	17/582		63.9 %	1.69 [0.94, 3.03]
Subtotal (95% CI)	**589**	**582**		**63.9 %**	**1.69 [0.94, 3.03]**
Total events: 29 (Experimental), 17 (Control)					
Heterogeneity: not applicable					
Test for overall effect: Z = 1.74 (P = 0.082)					
Total (95% CI)	**1216**	**894**		**100.0 %**	**1.42 [0.89, 2.27]**
Total events: 48 (Experimental), 26 (Control)					
Heterogeneity: Tau^2 = 0.0; Chi^2 = 0.90, df = 1 (P = 0.34); I^2 =0.0%					
Test for overall effect: Z = 1.47 (P = 0.14)					
Test for subgroup differences: Chi^2 = 0.90, df = 1 (P = 0.34), I^2 =0.0%					

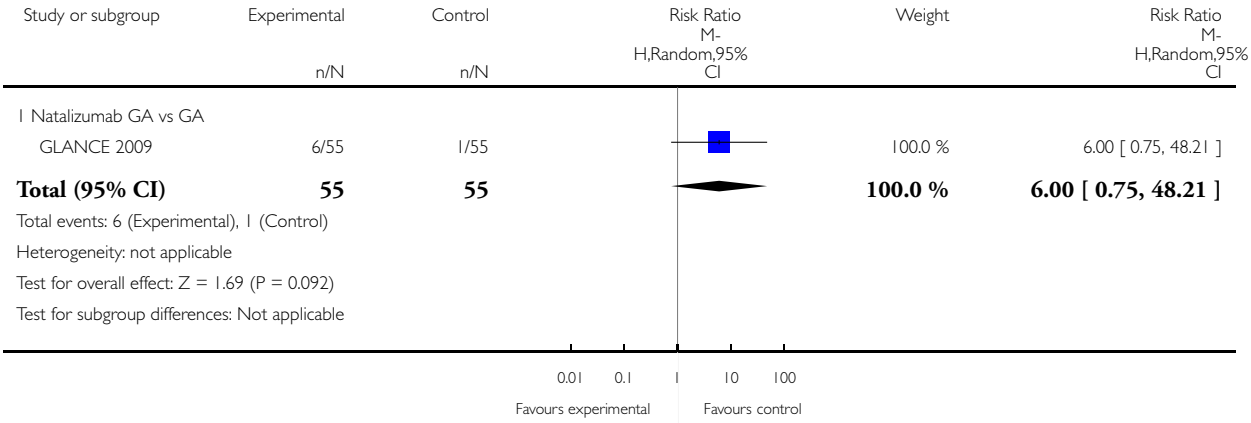


Analysis 5.24. Comparison 5 Adverse Event Analysis, Outcome 24 Flushing.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 24 Flushing

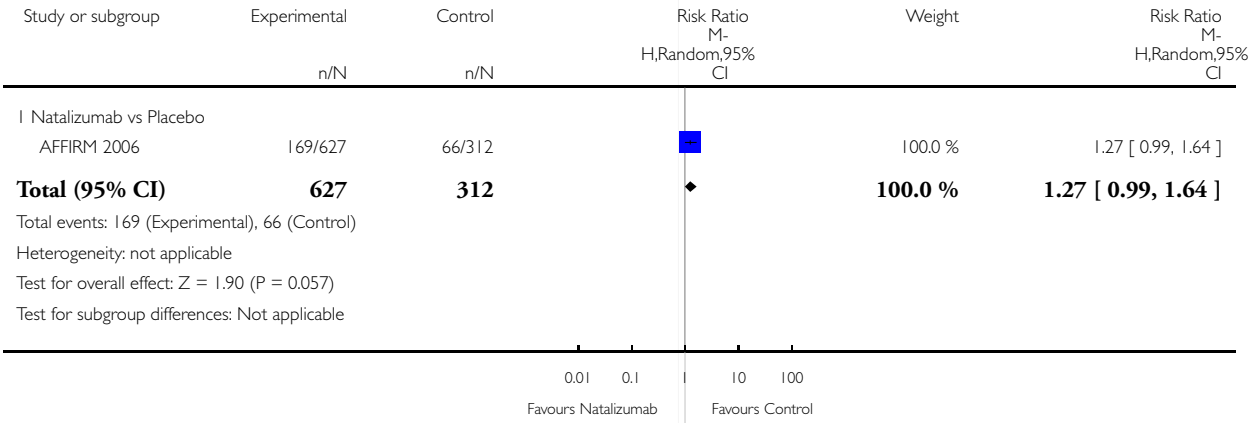


Analysis 5.25. Comparison 5 Adverse Event Analysis, Outcome 25 Fatigue - Myasthenia.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 25 Fatigue - Myasthenia

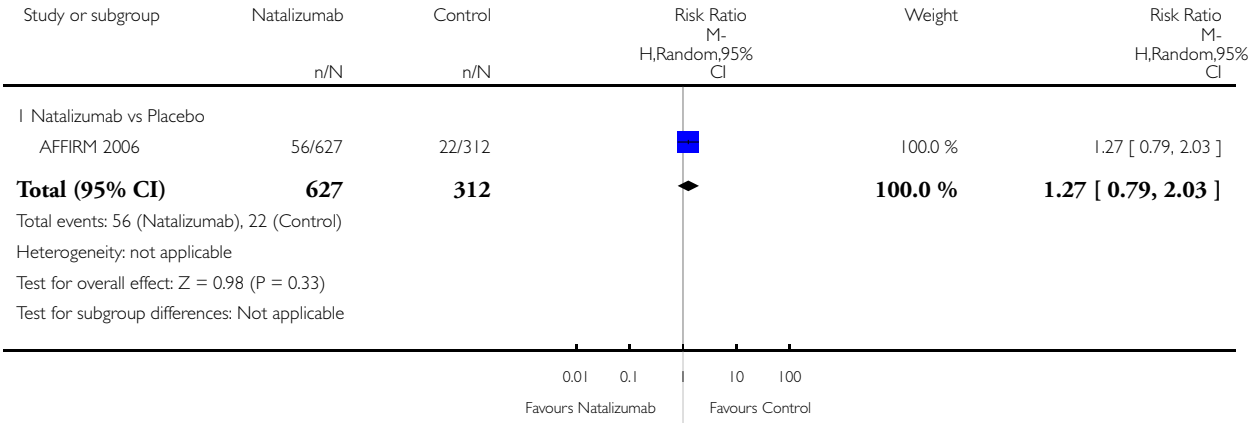


Analysis 5.26. Comparison 5 Adverse Event Analysis, Outcome 26 Urinary Urgency / Frequency.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 26 Urinary Urgency / Frequency

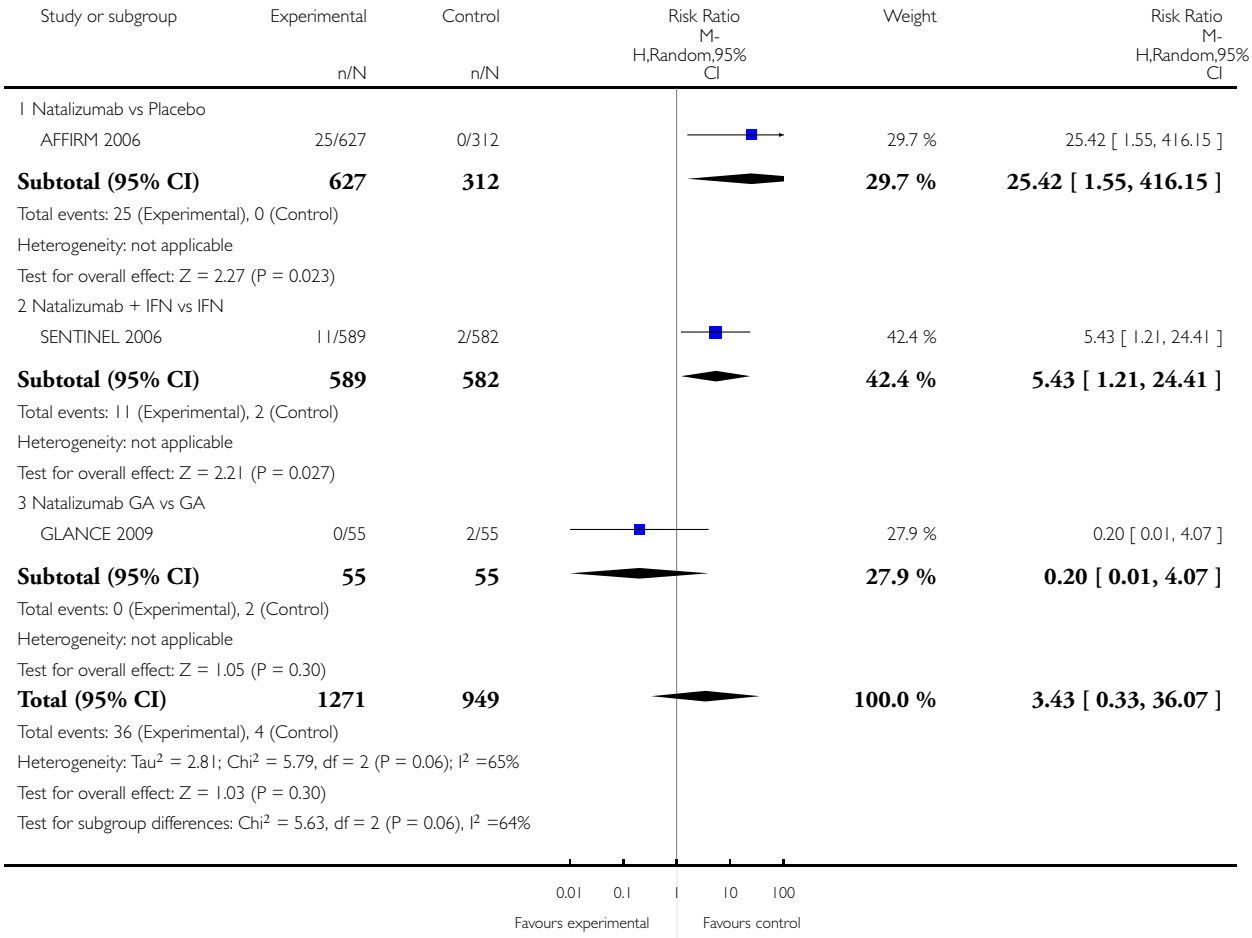


Analysis 5.27. Comparison 5 Adverse Event Analysis, Outcome 27 Hypersensitivity reactions.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 27 Hypersensitivity reactions

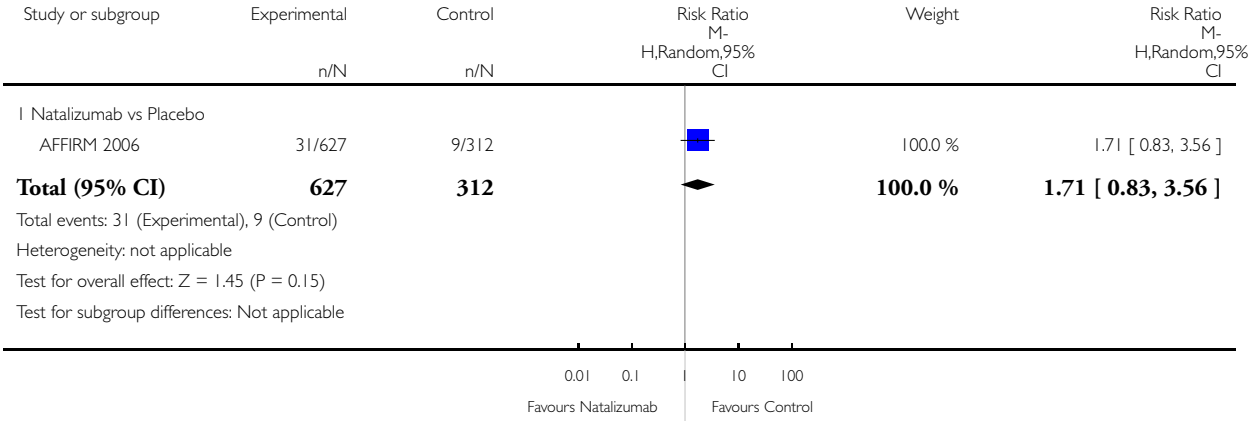


Analysis 5.28. Comparison 5 Adverse Event Analysis, Outcome 28 Chest Discomfort.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 28 Chest Discomfort

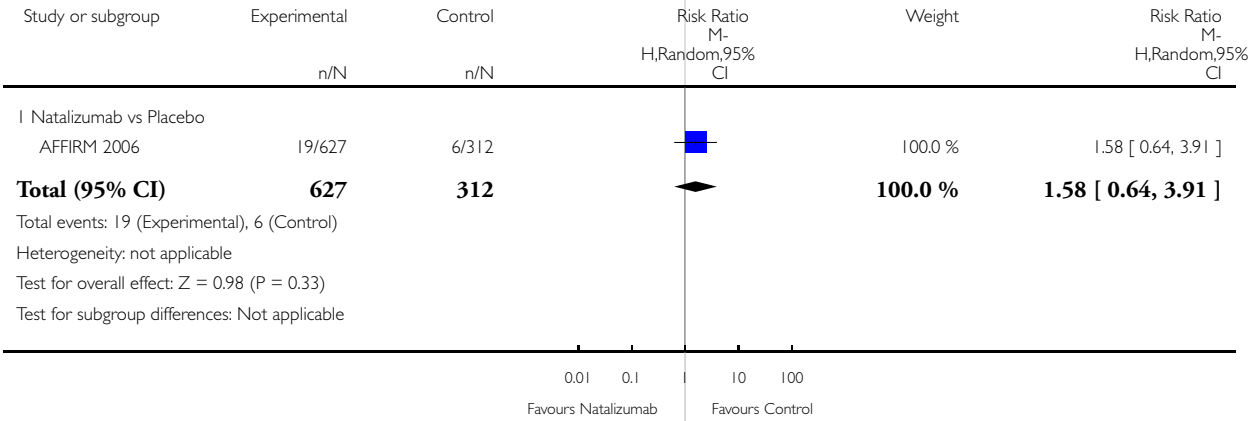


Analysis 5.29. Comparison 5 Adverse Event Analysis, Outcome 29 Local Bleeding.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 29 Local Bleeding

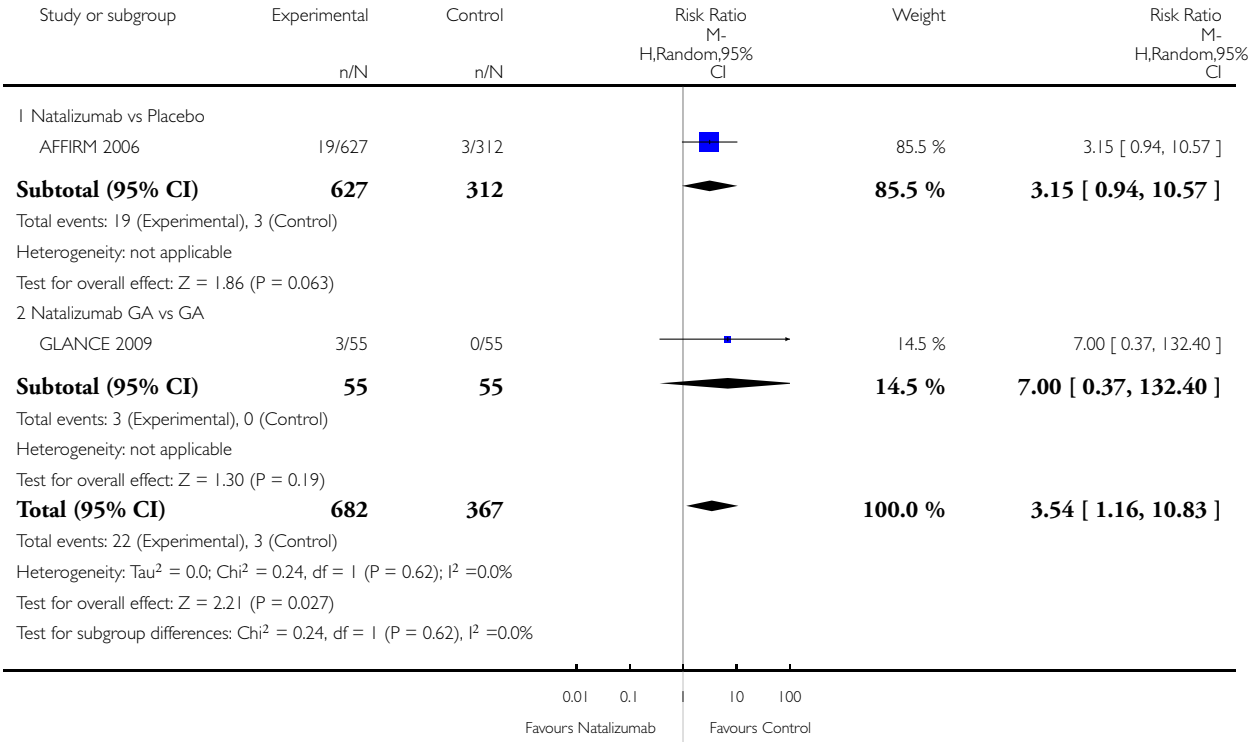


Analysis 5.30. Comparison 5 Adverse Event Analysis, Outcome 30 Rigors.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 30 Rigors

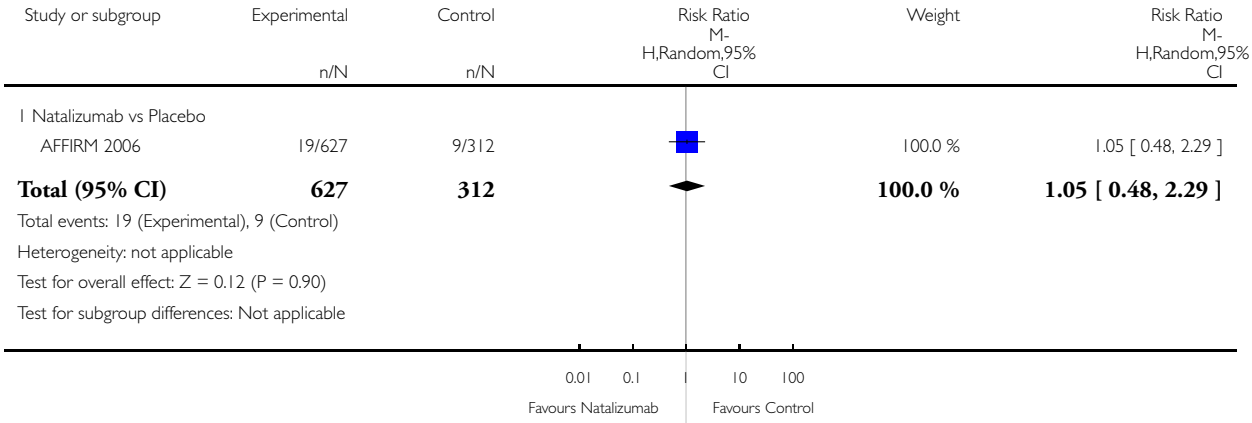


Analysis 5.31. Comparison 5 Adverse Event Analysis, Outcome 31 Syncope.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 31 Syncope

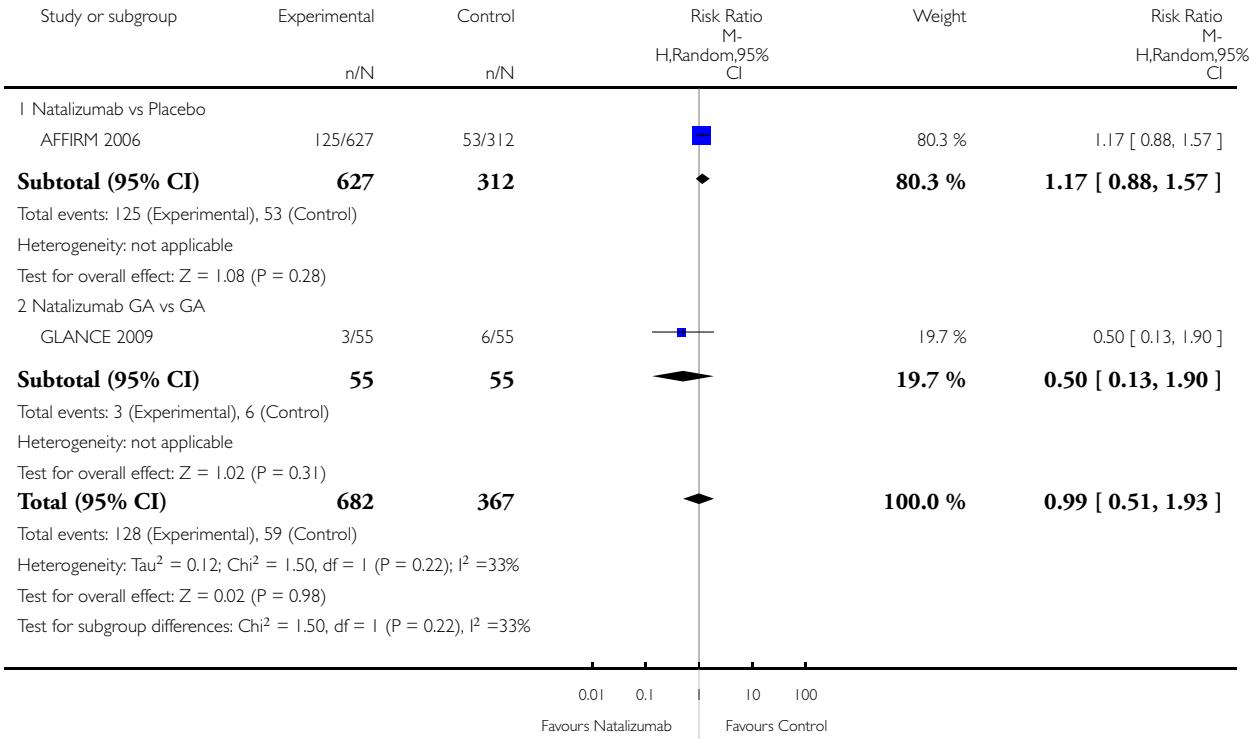


Analysis 5.32. Comparison 5 Adverse Event Analysis, Outcome 32 Urinary Infection.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 32 Urinary Infection

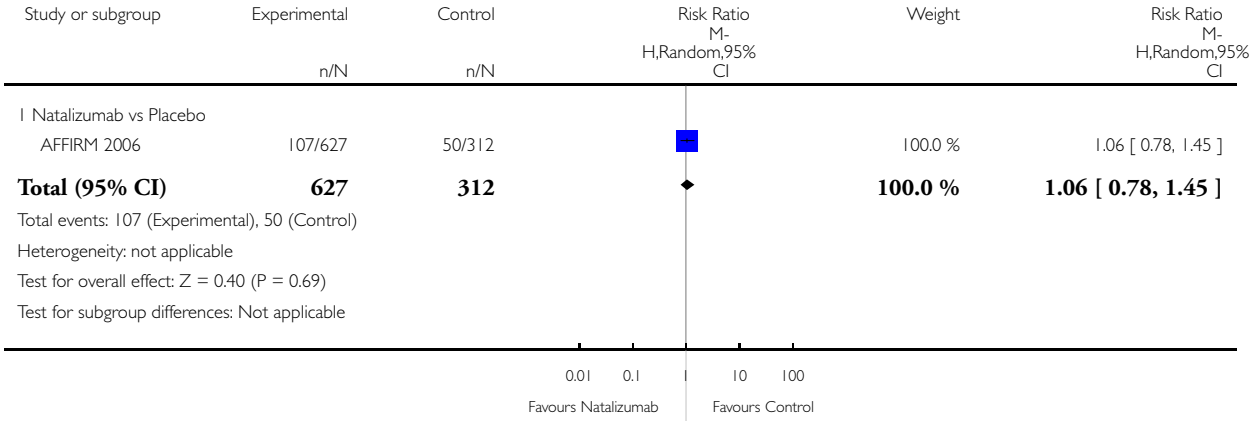


Analysis 5.33. Comparison 5 Adverse Event Analysis, Outcome 33 Lower Respiratory Infection.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 33 Lower Respiratory Infection

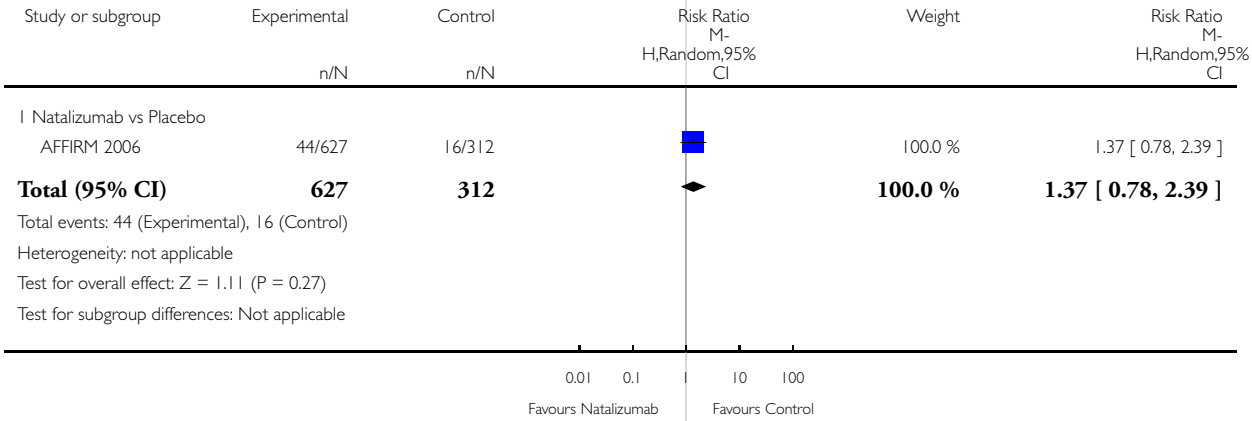


Analysis 5.34. Comparison 5 Adverse Event Analysis, Outcome 34 Tonsillitis.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 34 Tonsillitis

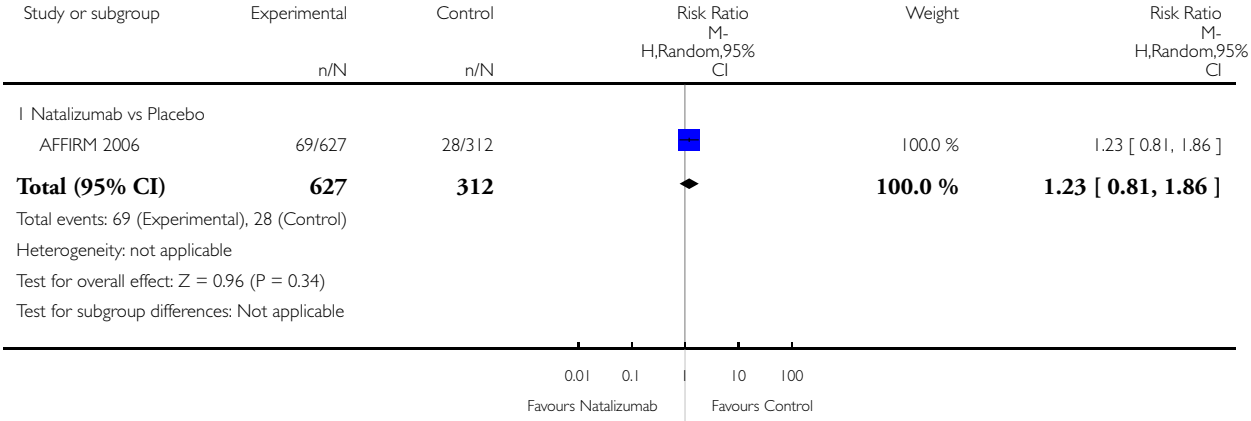


Analysis 5.35. Comparison 5 Adverse Event Analysis, Outcome 35 Gastroenteritis.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 35 Gastroenteritis

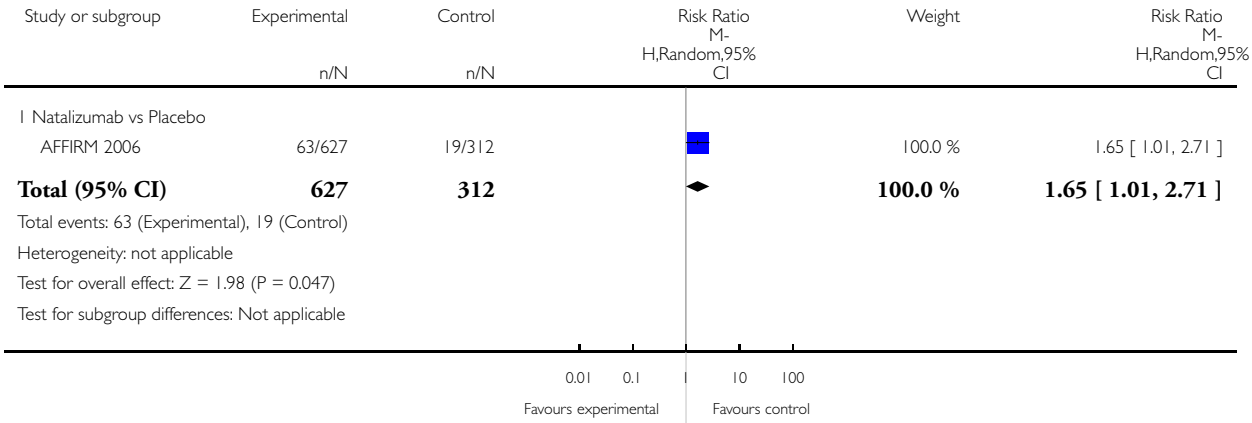


Analysis 5.36. Comparison 5 Adverse Event Analysis, Outcome 36 Vaginitis.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 36 Vaginitis

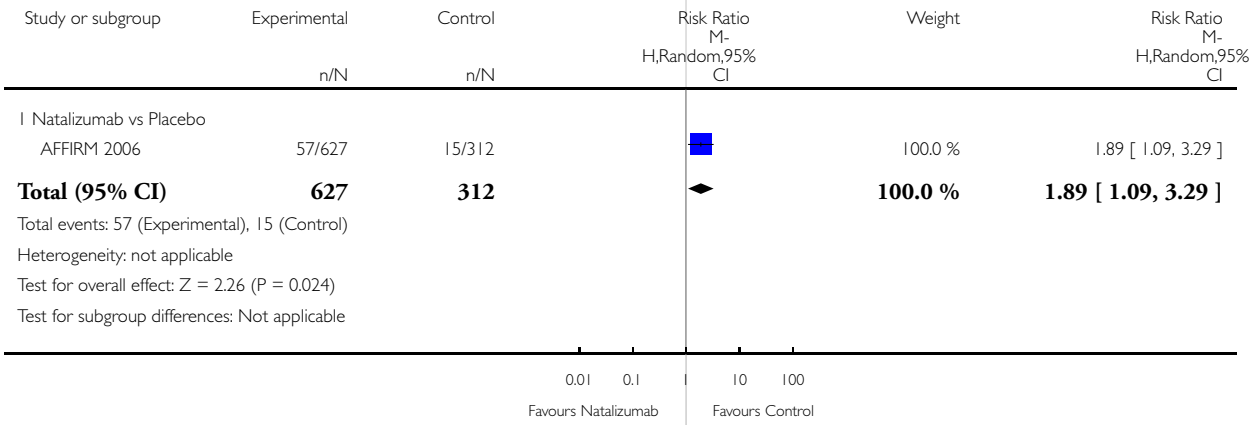


Analysis 5.37. Comparison 5 Adverse Event Analysis, Outcome 37 Menstrual disorders.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 37 Menstrual disorders

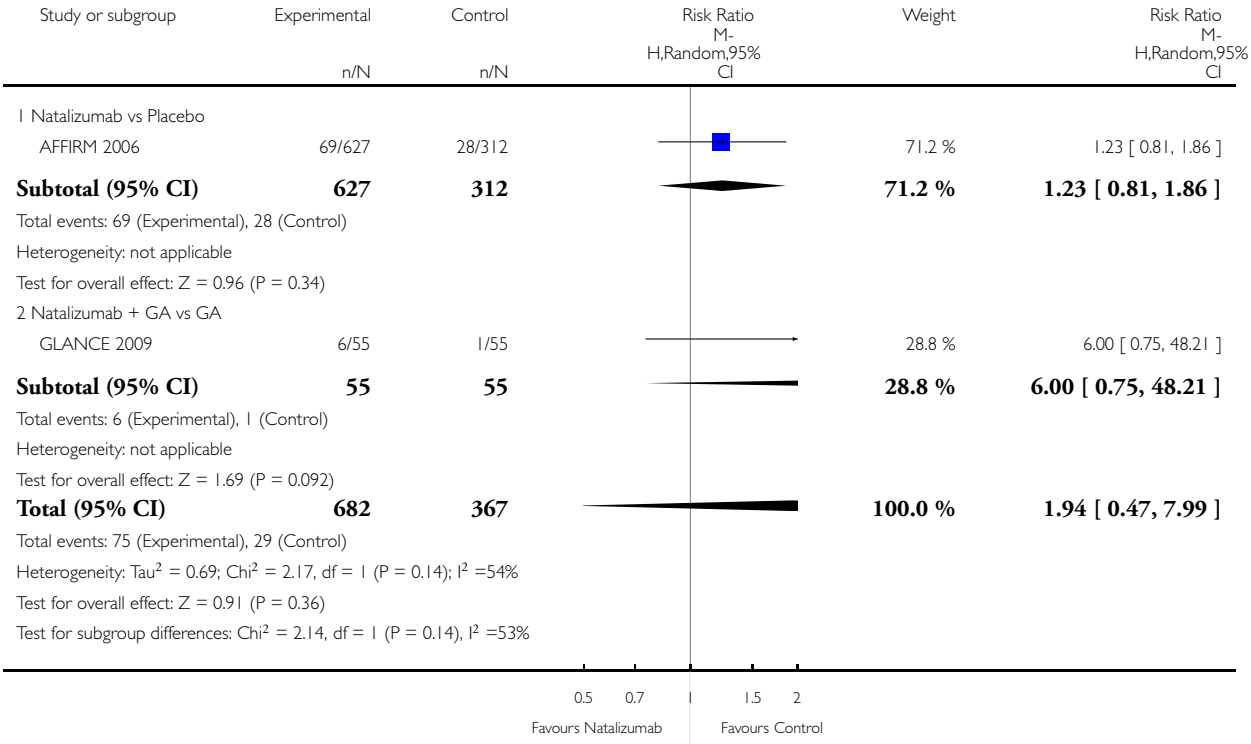


Analysis 5.38. Comparison 5 Adverse Event Analysis, Outcome 38 Skin Rash.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 38 Skin Rash

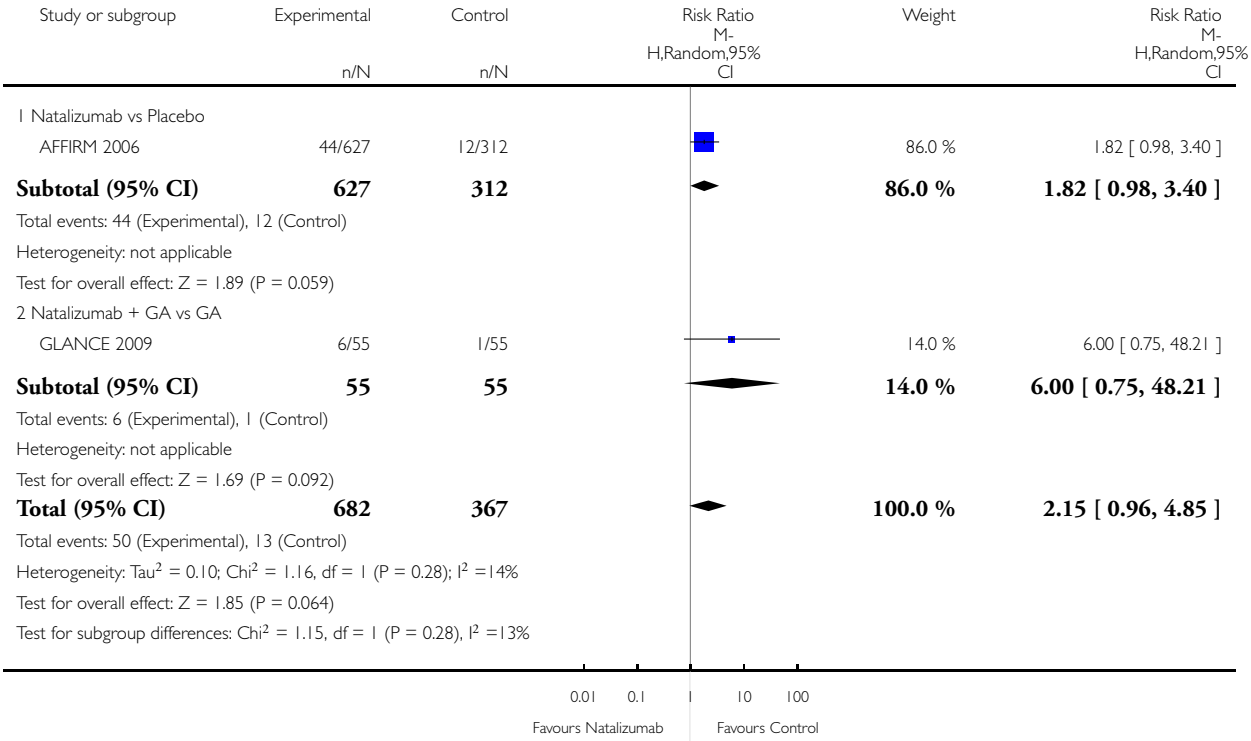


Analysis 5.39. Comparison 5 Adverse Event Analysis, Outcome 39 Dermatitis.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 39 Dermatitis



Analysis 5.40. Comparison 5 Adverse Event Analysis, Outcome 40 Pruritus.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 40 Pruritus

Study or subgroup	Experimental n/N	Control n/N	Risk Ratio M-H,Random,95% CI	Weight	Risk Ratio M-H,Random,95% CI
1 Natalizumab vs Placebo					
AFFIRM 2006	25/627	6/312		100.0 %	2.07 [0.86, 5.00]
Total (95% CI)	**627**	**312**		**100.0 %**	**2.07 [0.86, 5.00]**
Total events: 25 (Experimental), 6 (Control)					
Heterogeneity: not applicable					
Test for overall effect: Z = 1.62 (P = 0.10)					
Test for subgroup differences: Not applicable					

Analysis 5.41. Comparison 5 Adverse Event Analysis, Outcome 41 Vertigo.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 41 Vertigo

Study or subgroup	Experimental n/N	Control n/N	Risk Ratio M-H,Random,95% CI	Weight	Risk Ratio M-H,Random,95% CI
1 Natalizumab vs Placebo					
AFFIRM 2006	38/627	16/312		100.0 %	1.18 [0.67, 2.09]
Total (95% CI)	**627**	**312**		**100.0 %**	**1.18 [0.67, 2.09]**
Total events: 38 (Experimental), 16 (Control)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.58 (P = 0.56)					
Test for subgroup differences: Not applicable					

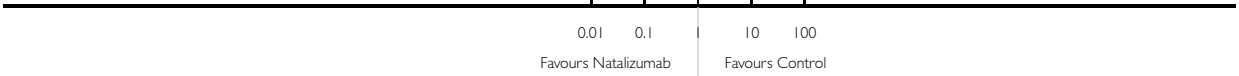
Analysis 5.42. Comparison 5 Adverse Event Analysis, Outcome 42 Infection.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 42 Infection

Study or subgroup	Experimental n/N	Control n/N	Risk Ratio M-H,Random,95% CI	Weight	Risk Ratio M-H,Random,95% CI
1 Natalizumab vs Placebo					
AFFIRM 2006	495/627	246/312		36.1 %	1.00 [0.93, 1.07]
Subtotal (95% CI)	**627**	**312**		**36.1 %**	**1.00 [0.93, 1.07]**
Total events: 495 (Experimental), 246 (Control)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.04 (P = 0.97)					
2 Natalizumab + IFN vs IFN					
SENTINEL 2006	489/589	471/582		61.7 %	1.03 [0.97, 1.08]
Subtotal (95% CI)	**589**	**582**		**61.7 %**	**1.03 [0.97, 1.08]**
Total events: 489 (Experimental), 471 (Control)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.93 (P = 0.35)					
3 Natalizumab + GA vs GA					
GLANCE 2009	33/55	36/55		2.1 %	0.92 [0.69, 1.22]
Subtotal (95% CI)	**55**	**55**		**2.1 %**	**0.92 [0.69, 1.22]**
Total events: 33 (Experimental), 36 (Control)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.59 (P = 0.55)					
Total (95% CI)	**1271**	**949**		**100.0 %**	**1.01 [0.97, 1.06]**
Total events: 1017 (Experimental), 753 (Control)					
Heterogeneity: Tau^2 = 0.0; Chi^2 = 0.79, df = 2 (P = 0.67); I^2 =0.0%					
Test for overall effect: Z = 0.67 (P = 0.50)					
Test for subgroup differences: Chi^2 = 0.77, df = 2 (P = 0.68), I^2 =0.0%					

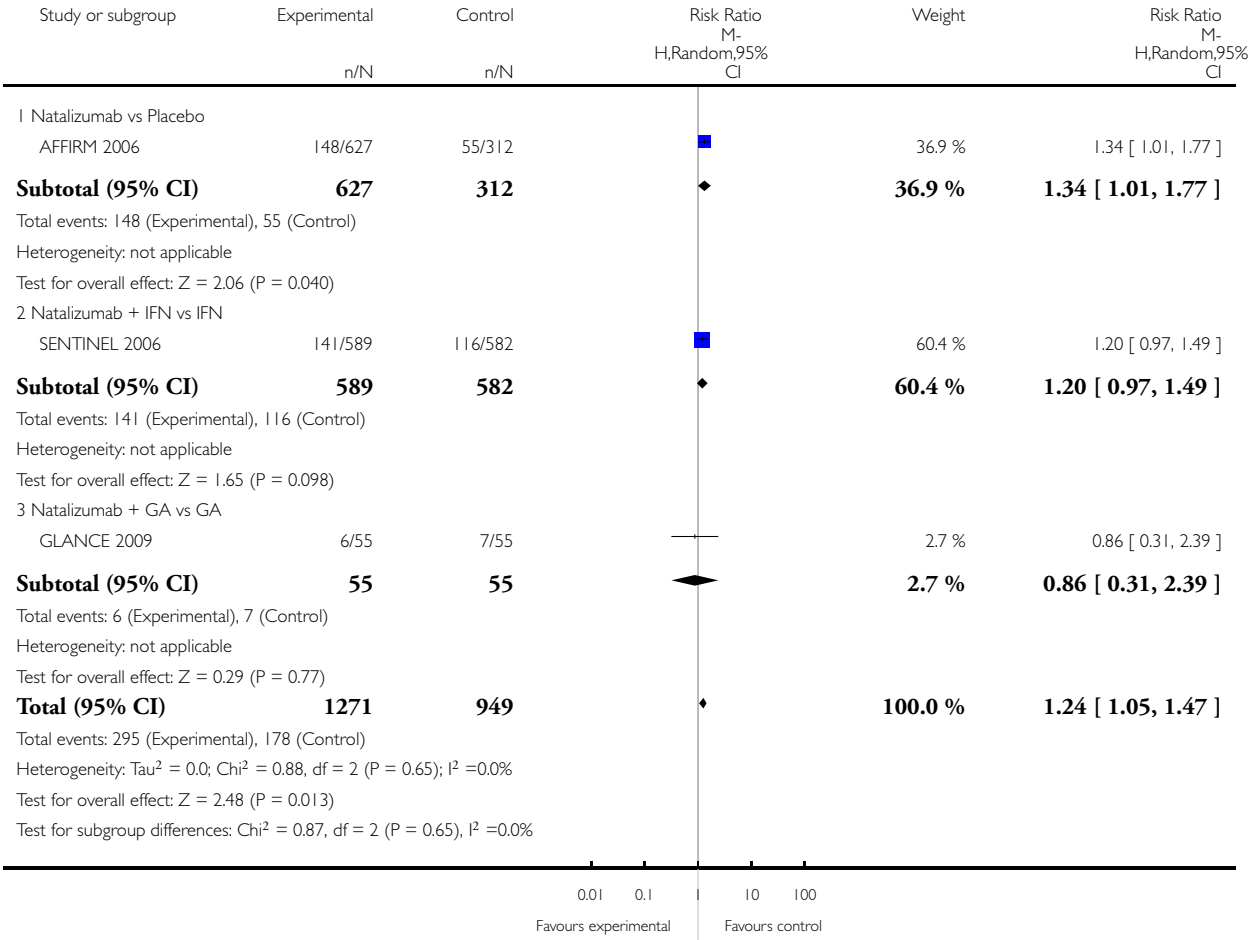


Analysis 5.43. Comparison 5 Adverse Event Analysis, Outcome 43 Infusion reactions.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 43 Infusion reactions

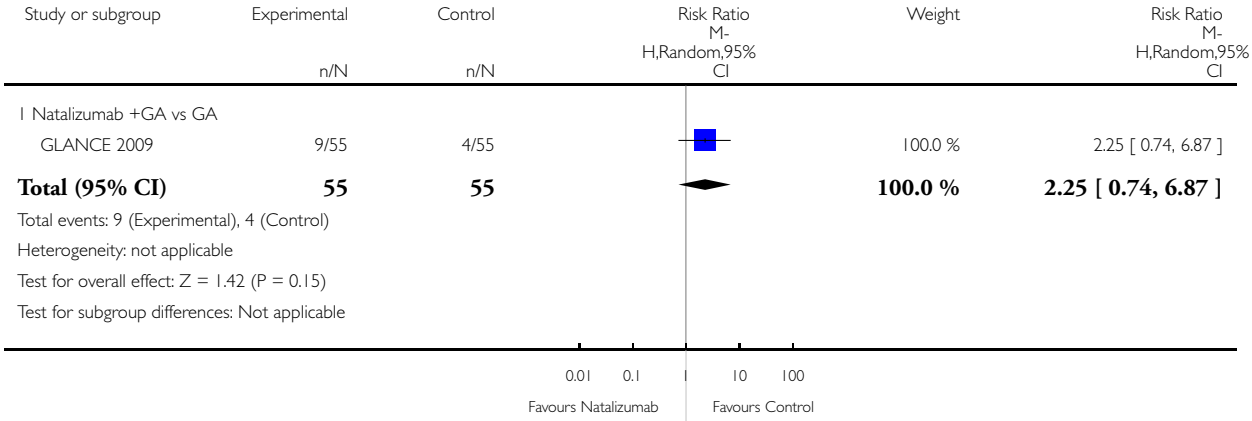


Analysis 5.44. Comparison 5 Adverse Event Analysis, Outcome 44 Back Pain.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 44 Back Pain

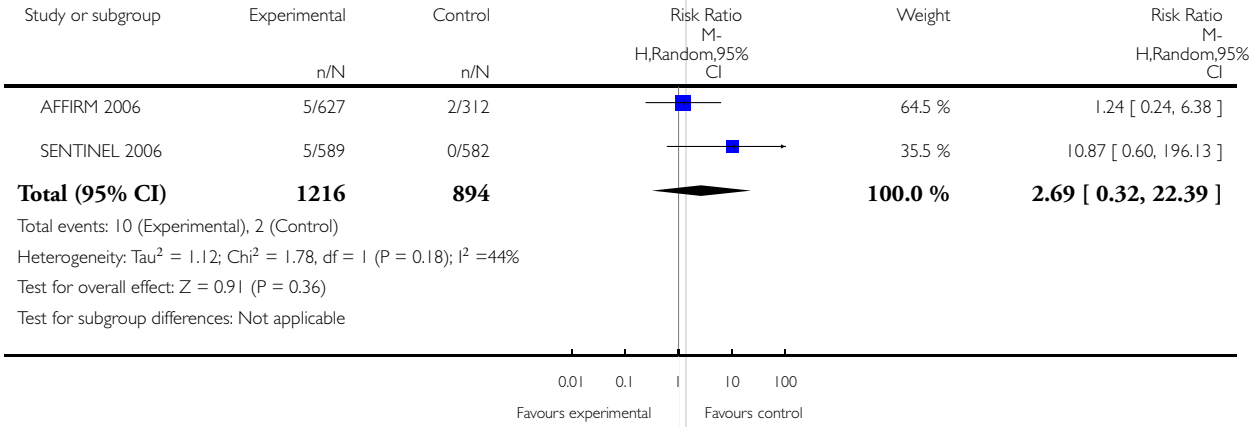


Analysis 5.45. Comparison 5 Adverse Event Analysis, Outcome 45 Fall.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 45 Fall

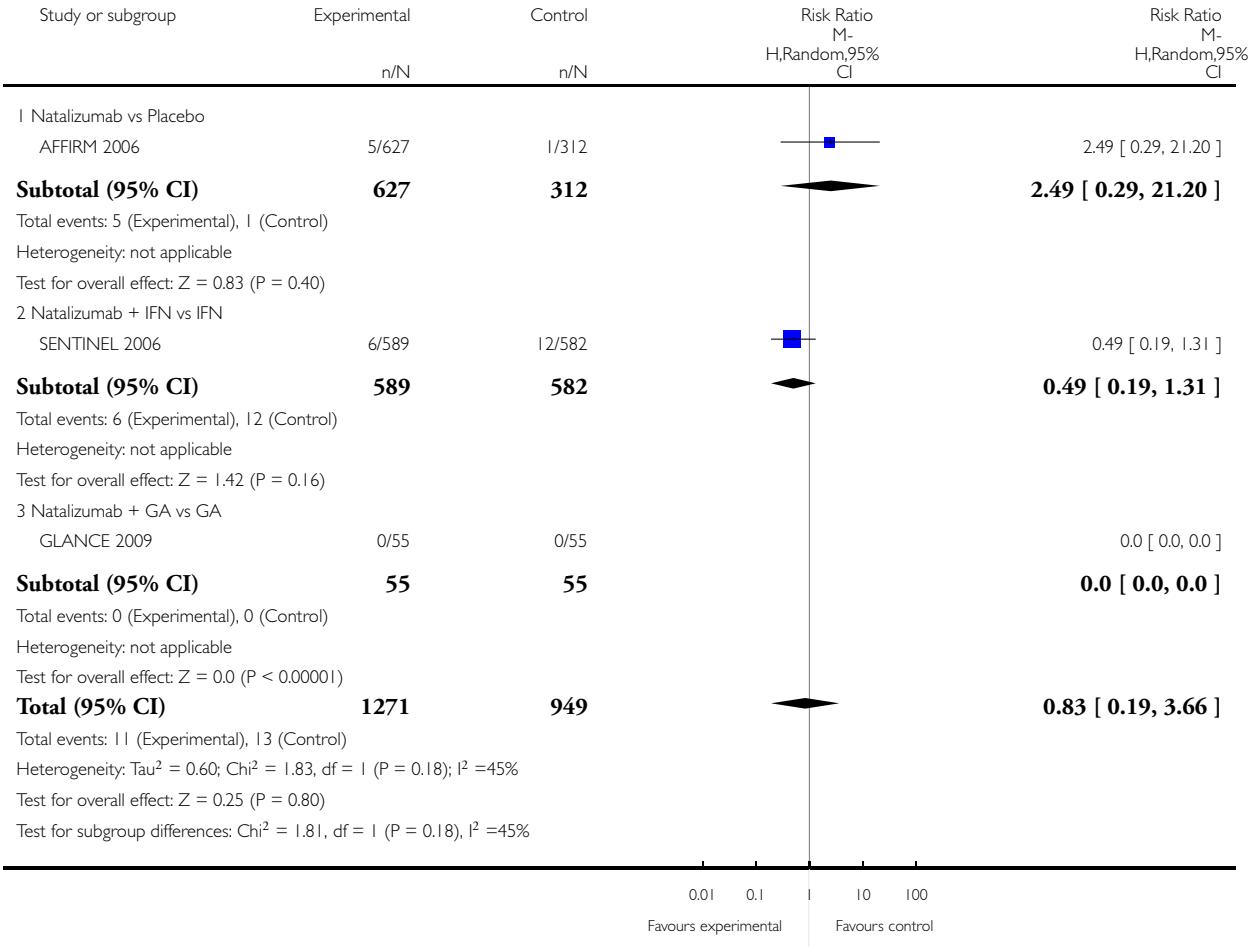


Analysis 5.46. Comparison 5 Adverse Event Analysis, Outcome 46 Neoplasms.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 46 Neoplasms

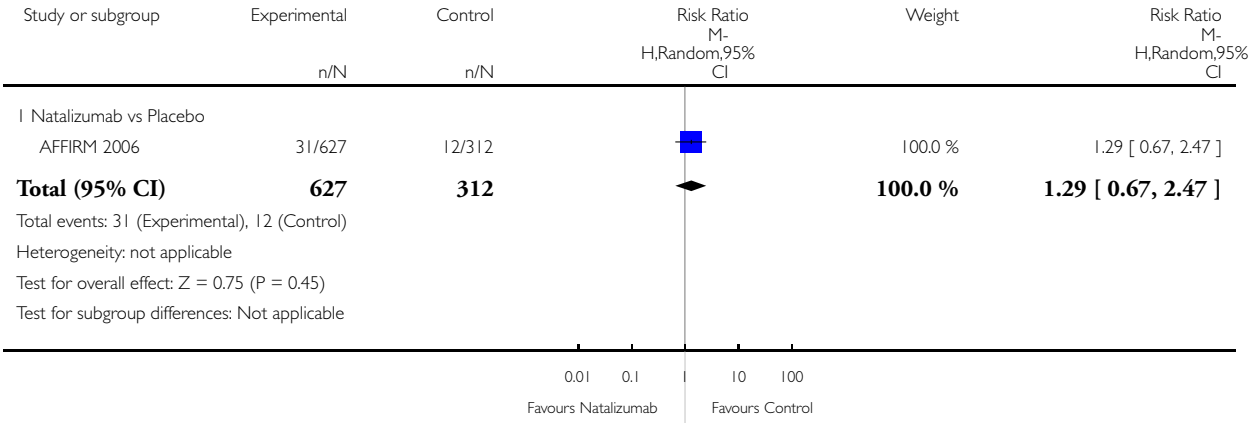


Analysis 5.47. Comparison 5 Adverse Event Analysis, Outcome 47 Abnormal liver function tests.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 47 Abnormal liver function tests

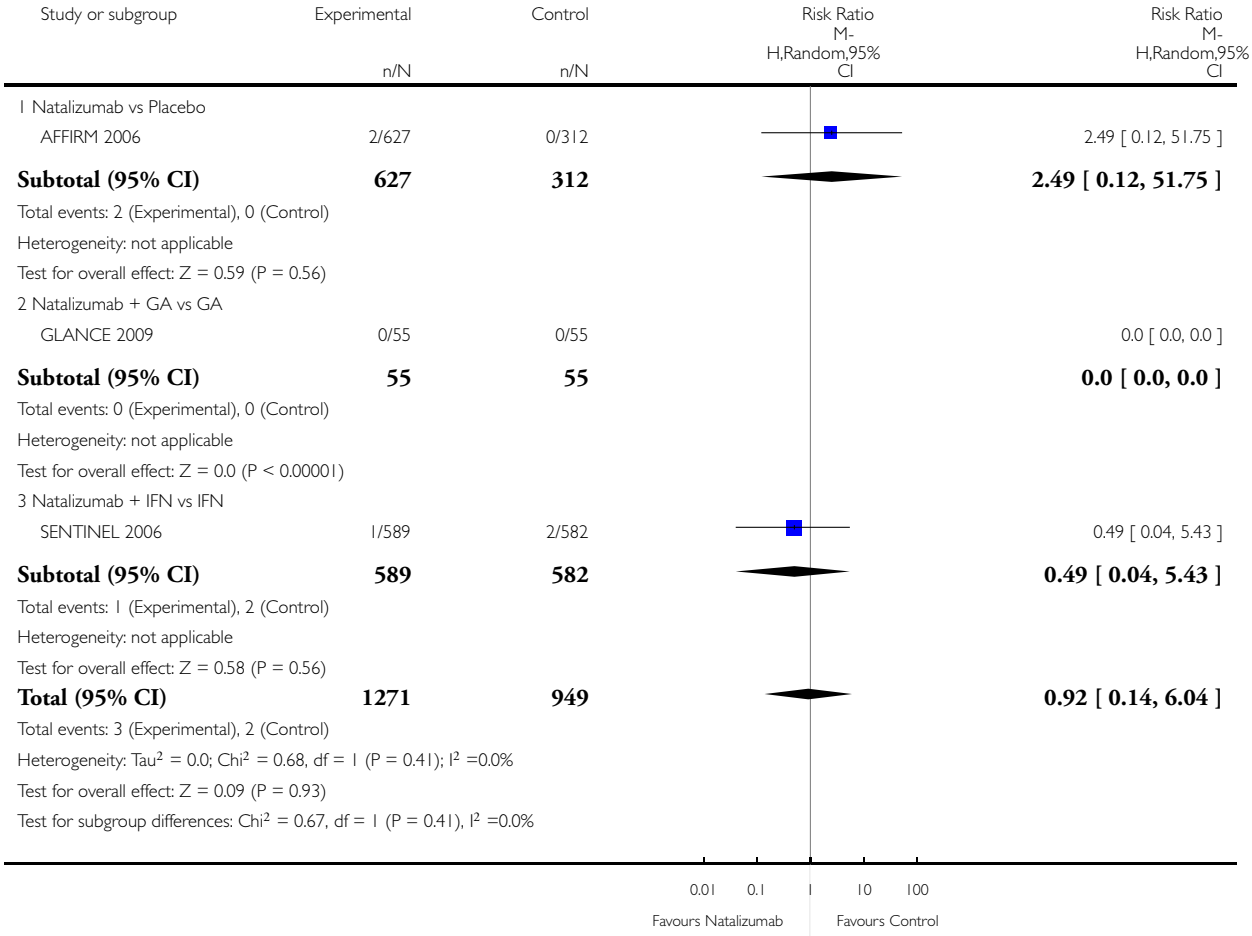


Analysis 5.48. Comparison 5 Adverse Event Analysis, Outcome 48 Death.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 48 Death

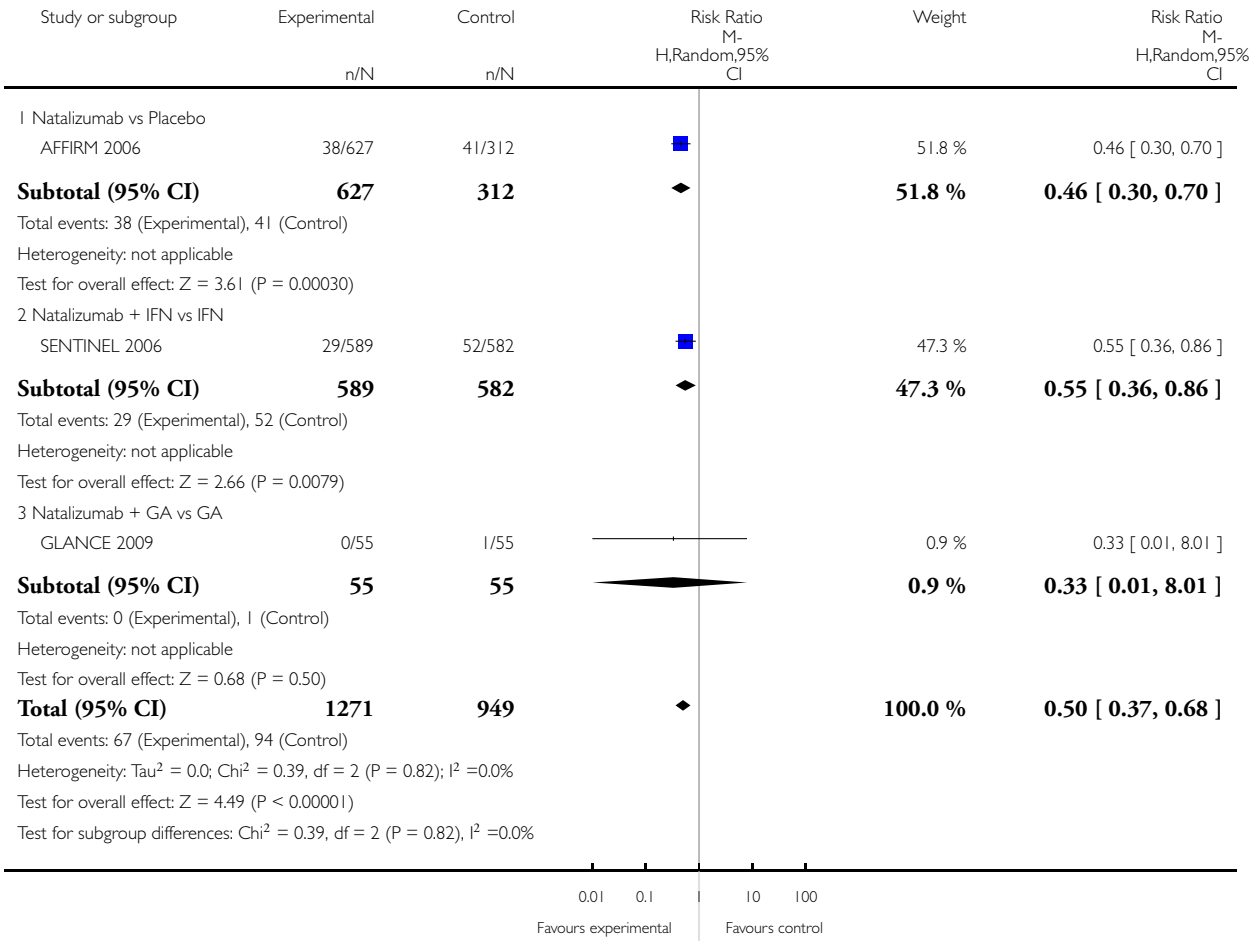


Analysis 5.49. Comparison 5 Adverse Event Analysis, Outcome 49 MS relapse as a serious AE.

Review: Natalizumab for relapsing remitting multiple sclerosis

Comparison: 5 Adverse Event Analysis

Outcome: 49 MS relapse as a serious AE



ADDITIONAL TABLES

Table 1. ABBREVIATIONS

ABBREVIATION	TERM
AIFA	Agenzia Italiana Farmaco
CD	Crohn Disease
CDER	Center for drug evaluation and research (FDA)
CI	Confidence Interval
CNS	Central Nervous System
CIS	Clinically Isolated Syndrome
DMDs	Disease-Modifying Drugs
EDSS	Expanded Disability Status Scale
EMA	European Medicines Agency
FDA	Food and Drug Administration
GA	Glatiramer Acetate (Copaxone®)
Gd+	Gadolinium enhancing lesion in MRI
HRQoL	Health Related Quality of Life
HSRs	hypersensitivity reactions
IFNß	Interferon beta
IFNß-1a	Interferon beta-1a
IgG	Immunoglobulin G
IRIS	Immune reconstitution inflammatory syndrome
ITT	intention-to-treat
IV	intravenous
MCS	Mental Component Summary (composite scores of SF-36)
MD	mean difference
MFIS	Modified Fatigue Impact Scale

Table 1. ABBREVIATIONS (*Continued*)

MSFC	Multiple Sclerosis Functional Composite
MRI	Magnetic resonance imaging
MS	Multiple Sclerosis
N	number
n.a.	not available
NICE	National Institute for Clinical Excellence
NNB	Number Needed to Benefit
NNH	Number Needed to Harm
NNT	Number Needed to Treat
NTZ	natalizumab
PASAT	Paced Auditory Serial Addition Task (one of the components of MSFC)
PCS	Physical Component Summary (composite scores of SF-36)
PML	Progressive Multifocal Leukoencephalopathy
QoL	Quality of Life
SF-36	Short Form 36
VAS	Visual Analog Scale

Table 2. Baseline patient characteristics in studies which contributed to primary efficacy outcomes (AFFIRM and SENTINEL)

Characteristic	Patients randomised to NTZ (with or without IFNß-1a) (n=1216)	Patients randomised to placebo or IFNß-1a alone (n=897)	Total (n= 2113)
Age range	18-55	19-55	18-55
Sex N of male: N of female	325:891	266:631	591:1522
Disease duration* - years (range)	1-41	1-34	

Table 2. Baseline patient characteristics in studies which contributed to primary efficacy outcomes (AFFIRM and SENTINEL) (*Continued)*

N of patients with 1 relapse in previous 1 yr (% of total)	758	537	1295 (61)
N of patients with ≥ 2 relapse in previous 1 yr (% of total)	450	353	803 (38)
N of patients with EDSS ≤ 3.5 (% of total)	1056	769	1825
N of patients with EDSS > 3.5 (% of total)	160	128	288
N of patients with 0 Gd+ (% of total)	699	544	1243
N of patients with ≥ 1 Gd+ (% of total)	511	348	859 (41)
Duration ≥ 10 months of previous IFNß-1a therapy (% of total)	589	582	1171 (55)

*Definition of disease duration (from the onset? form the diagnosis?) was not available for AFFIRM and SENTINEL trials. We used time since first MS symptoms for participants in GLANCE trial.

Table 3. Protocol violations in AFFIRM trial

Type of protocol violation	N of violations in NTZ group	N of patients with at least one protocol violation in NTZ group	N of violations in control group	N of patients with at least one protocol violation in control group	Total N of violations in all randomised patients	Total N of patients with at least one protocol violation in all randomised patients	Details	Reference
Inclusion criteria: diagnosis of relapsing multiple sclerosis according to McDonald criteria	9	9	4	4	13	13 (1.4%)	These patients only satisfied CIS criteria	Polman 2006 AFFIRM 2006

Table 3. Protocol violations in AFFIRM trial (*Continued*)

Inclusion criteria: at least one medically documented relapse within 12 months before randomisation	6	6 (1%)	6	6 (2%)	12	12 (1.3%)	No relapse in previous year	Polman 2006 AFFIRM 2006
Prohibited concomitant medication	29	22 (3.5%)	17	11 (3.5%)	46	33 (3.5%)	Only data from the first year of study is reported. Data for all the duration of the trial is not available Medications prohibited: cyclophosphamide, mitoxantrone IFNß, GA, cyclosporine, azathioprine, methotrexate, intravenous immune globulin, experimental drugs	Center for drug evaluation and research. Medical Review. November 23, 2004- Drug Approval Package - http://www.accessdata.fda.gov/drugsatfda˙docs/nda/2004/125104s000˙Natalizumab.cfm
Other eligibility criteria	58	46 (7.3%)	27	21 (6.7%)	85	67 (7.1%)	Only data from the first year of study is reported. Data for all the duration of the trial is not available	Center for drug evaluation and research. Medical Review November 23, 2004 - Drug

Table 3. Protocol violations in AFFIRM trial (*Continued*)

							No other detail is available.	Approval Package - http://www.accessdata.fda.gov/drugsatfda_docs/nda/2004/125104s000_Natalizumab.cfm
Missed, partial or incorrect dosing	323	144 (23.0%)	145	77 (24.4%)	468	221 (23.4%)	Only data from the first year of study is reported. Data for all the duration of the trial is not available No other detail is available.	Center for drug evaluation and research. Medical Review. November 23, 2004 - Drug Approval Package - http://www.accessdata.fda.gov/drugsatfda_docs/nda/2004/125104s000_Natalizumab.cfm
Efficacy evaluation not performed or not valid	104	73 (11.6%)	60	45 (14.3%)	164	118 (12.5%)	Only data from the first year of study is reported. Data for all the duration of the trial is not available No other detail is available.	Center for drug evaluation and research. Medical Review. November 23, 2004 - Drug Approval Package - http://www.accessdata.fda.gov/drugsatfda_docs/nda/2004/125104s000_Natalizumab.cfm
Safety evaluation not performed or not valid	162	103 (16.4%)	95	53 (16.8%)	257	156 (16.6%)	Only data from the first year of study is reported. Data for all the duration of	Center for drug evaluation and research. Medical Review. November

Table 3. Protocol violations in AFFIRM trial (*Continued*)

							the trial is not available No other detail is available.	23, 2004 - Drug Approval Package - http://www.accessdata.fda.gov/drugsatfda_docs/nda/2004/125104s000_Natalizumab.cfm
Outside acceptable visit window	1239	406 (64.8%)	692	218 (69.2)	1931	624 (66.2%)	Only data from the first year of study are reported. Data for all the duration of the trial are not available No other detail is available.	Center for drug evaluation and research. Medical Review. November 23, 2004 - Drug Approval Package - http://www.accessdata.fda.gov/drugsatfda_docs/nda/2004/125104s000_Natalizumab.cfm
Missed study visit	38	25 (4.0%)	22	13 (4.1%)	60	38 (4.0%)	Only data from the first year of study is reported. Data for all the duration of the trial is not available No other detail is available.	Center for drug evaluation and research. Medical Review. November 23, 2004 - Drug Approval Package - http://www.accessdata.fda.gov/drugsatfda_docs/nda/2004/125104s000_Natalizumab.cfm
Discontinuation of study treatment in patients who had HSRs	2	2	0	0	2	2 (< 1%)	Two NTZ patients were redosed after experienc-	Phillips 2006 AFFIRM 2006

Table 3. Protocol violations in AFFIRM trial (*Continued*)

							ing a hypersensitivity reaction (per protocol, study drug was to be discontinued in all patients who had HSRs).	
Missed MRI scan	n.a.	n.a.	n.a.	n.a.	n.a.	87 (9.0%)	According to Miller and collaborators the main reason for missing data (>80%) was the scan not being performed because the patient withdrew from the study; in the remainder (<20%), although the patient was still in the study, the scan was either not performed, had not been received at the Central MRI Analysis Center, or had been received but was of inadequate quality for analysis	Miller 2007 AFFIRM 2006
Other (according to the Cen-	996	401 (63.9%)	529	208 (66.0%)	1525	609 (64.6%)	Only data from the	Center for drug eval-

Table 3. Protocol violations in AFFIRM trial (*Continued*)

ter for drug evaluation and research)							first year of study is reported. Data for all the duration of the trial is not available No other detail is available.	uation and research. Medical Review. November 23, 2004 - Drug Approval Package - http://www.accessdata.fda.gov/drugsatfda_docs/nda/2004/125104s000_Natalizumab.cfm
All violations according to the Center for drug evaluation and research	2955	554 (88.4%)	1593	291 (92.4%)	4548	845	Only data from the first year of study is reported. Data for all the duration of the trial is not available	Center for drug evaluation and research. Medical Review. November 23, 2004 - Drug Approval Package - http://www.accessdata.fda.gov/drugsatfda_docs/nda/2004/125104s000_Natalizumab.cfm

Table 4. Protocol violations in GLANCE trial

Type of protocol violation	N of violations in NTZ group	N of patients with at least one protocol violation in NTZ group	N of violations in control group	N of patients with at least one protocol violation in control group	Total N of violations in all randomised patients	Total N of patients with at least one protocol violation in all randomised patients	Details	Reference
Incusion criteria: at least one medically documented relapse within	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	the minimum of N of relapses in previous 12 mo is 0 In	Goodman 2009 GLANCE 2009

Table 4. Protocol violations in GLANCE trial (*Continued*)

12 months before randomisation							the baseline characteristics of NTZ + GA group (i.e. at least one patient was included with no relapse in previous 12 mo)	
Other	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	

Table 5. Protocol violations in SENTINEL trial

Type of protocol violation	N of violations in NTZ group	N of patients with at least one protocol violation in NTZ group	N of violations in control group	N of patients with at least one protocol violation in control group	Total N of violations in all randomised patients	Total N of patients with at least one protocol violation in all randomised patients	Details	Reference
Inclusion criteria: at least one medically documented relapse within 12 months before randomisation	0	0	1	1 (< 1%)	1	1 (< 1%)	no relapse in past 12 mo.	Rudick 2006 SENTINEL 2006
Prohibited concomitant medication	69	55 (9.3%)	72	53 (9.1%)	141	108 (9.2%)	Only data from the first year of study is reported. Data for all the duration of the trial is not available Medications prohibited: any ap-	Center for drug evaluation and research. Medical Review. November 23, 2004 - Drug Approval Package

Table 5. Protocol violations in SENTINEL trial (*Continued*)

							proved disease modifying therapy other than IFNß-1a im once a week, experimental drugs	- http://www.accessdata.fda.gov/drugsatfda_docs/nda/2004/125104s000_Natalizumab.cfm
Other eligibility criteria	88	70 (11.9%)	87	66 (11.3%)	175	136 (11.6%)	Only data from the first year of study is reported. Data for all the duration of the trial is not available No other detail is available.	Center for drug evaluation and research. Medical Review. November 23, 2004 - Drug Approval Package - http://www.accessdata.fda.gov/drugsatfda_docs/nda/2004/125104s000_Natalizumab.cfm
Missed, partial or incorrect dosing	868	306 (52.0%)	918	310 (53.3%)	1786	616 (52.6%)	Only data from the first year of study is reported. Data for all the duration of the trial is not available No other detail is available.	Center for drug evaluation and research. Medical Review. November 23, 2004 - Drug Approval Package - http://www.accessdata.fda.gov/drugsatfda_docs/nda/2004/125104s000_Natalizumab.cfm
Efficay evaluation not performed or not valid	189	113 (19.2%)	197	107 (18.4%)	386	220 (18.8%)	Only data from the first year of study is reported. Data for all the	Center for drug evaluation and research. Medical Review.

Table 5. Protocol violations in SENTINEL trial (*Continued*)

							duration of the trial is not available No other detail is available.	November 23, 2004 - Drug Approval Package - http://www.accessdata.fda.gov/drugsatfda_docs/nda/2004/125104s000_Natalizumab.cfm
Safety evaluation not performed or not valid	185	101 (17.1%)	213	120 (20.6%)	398	221 (18.9%)	Only data from the first year of study is reported. Data for all the duration of the trial is not available No other detail is available.	Center for drug evaluation and research. Medical Review. November 23, 2004 - Drug Approval Package - http://www.accessdata.fda.gov/drugsatfda_docs/nda/2004/125104s000_Natalizumab.cfm
Outside acceptable visit window	1423	418 (71.0%)	1504	430 (73.9%)	2927	848 (72.4%)	Only data from the first year of study is reported. Data for all the duration of the trial is not available No other detail is available.	Center for drug evaluation and research. Medical Review. November 23, 2004 - Drug Approval Package - http://www.accessdata.fda.gov/drugsatfda_docs/nda/2004/125104s000_Natalizumab.cfm
Missed study visit	39	29 (4.9%)	54	39 (6.7%)	93	68 (5.8%)	Only data from the first year of study is re-	Center for drug evaluation and research.

Table 5. Protocol violations in SENTINEL trial (*Continued*)

							ported. Data for all the duration of the trial is not available No other detail is available.	Medical Review. November 23, 2004 - Drug Approval Package - http://www.accessdata.fda.gov/drugsatfda_docs/nda/2004/125104s000_Natalizumab.cfm
Other (according to the Center for drug evaluation and research)	1639	411 (69.8%)	1799	425 (73.0%)	3438	836 (71.4%)	Only data from the first year of study is reported. Data for all the duration of the trial is not available No other detail is available.	Center for drug evaluation and research. Medical Review. November 23, 2004 - Drug Approval Package - http://www.accessdata.fda.gov/drugsatfda_docs/nda/2004/125104s000_Natalizumab.cfm
All violations according to the Center for drug evaluation and research	4500	569 (96.6%)	4855	568 (97.6%)	9355	1137 (97.1%)		Center for drug evaluation and research. Medical Review. November 23, 2004 - Drug Approval Package - http://www.accessdata.fda.gov/drugsatfda_docs/nda/2004/125104s000_Natalizumab.cfm

Table 6. Other trials sponsored by the Pharmaceutical Industry

Study label	Phase	Aims	Population	Design	Dose regimen	N of participants	Duration	Planned N of doses	Outcome measures	Main references
200	1	Safety Tolerability	RRMS and SPMS subjects	Randomised, Double-blind, Placebo-controlled, Dose-escalation	0.03 (n=3) - 0.1 (n=3) 0.3 (n=3) 1.0 (n=6) 3.0 (n=6) mg/Kg; (placebo n=7)	28	n.a.	1	Safety Tolerability	Sheremata 1999
221	1	Safety Pharmacokinetics Pharmacodynamics	RRMS and SPMS subjects	Randomised, Placebo-controlled	1-3-6 mg/Kg	39	n.a.	1	Safety Pharmacodynamics	Center for drug evaluation and research. Medical Review - Drug Approval Package - http://www.accessdata.fda.gov/drugsatfda_docs/nda/2004/125104s000_Natalizumab.cfm study quoted by Rudick 2004
224	1	Safety Pharmacokinetics Interaction with IFNß-1a	RRMS subjects treated with intramuscular IFNß-1a	Open label	3 (n=15) - 6 (n=23) mg/Kg	38	n.a.	1	Safety Pharmacokinetics	Center for drug evaluation and research. Medical Review - Drug Approval Package - http://www.accessdata.fda.gov/drugsatfda_docs/

Table 6. Other trials sponsored by the Pharmaceutical Industry (*Continued*)

										nda/2004/125104s000˙Natalizumab.cfm Vollmer 2004
201 UK Antegren Study	2	Preliminary efficacy	RRMS and SPMS subjects	Randomised, Double-blind, Placebo-controlled	3 mg/Kg	72 (placebo n=35)	24 wks	2	MRI parameters	UK Antegren Study 1999
202 Natalizumab Multiple Sclerosis Trial	2	Preliminary efficacy (on relapse)	RRMS and SPMS subjects in relapse	Randomised, Double-blind, Placebo-controlled	1-3 mg/Kg	180	14 wks	1	MRI parameters EDSS Scripp Neurologic Rating Scale Patient's own assessment of well-being	O'Connor 2004

APPENDICES

Appendix 1. CENTRAL search strategy

#1MeSH descriptor Multiple Sclerosis explode all trees
#2MeSH descriptor Demyelinating Diseases, this term only
#3MeSH descriptor Myelitis, Transverse, this term only
#4MeSH descriptor Optic Neuritis explode all trees
#5MeSH descriptor Encephalomyelitis, Acute Disseminated, this term only
#6(multiple sclerosis)
#7(demyelinating disease*)
#8(transverse myelitis)
#9(neuromyelitis optica)
#10(optic neuritis)
#11(encephalomyelitis acute disseminated)
#12(devic)
#13(#1 OR #1 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12)
#14natalizumab OR antegren OR tysabri
#15(#13 AND #14)

Appendix 2. MEDLINE (PubMed) search strategy

((natalizumab OR antegren OR tysabri))) AND ((((“Multiple Sclerosis”[mh]) OR (“Myelitis, Transverse”[mh:noexp]) OR (“Demyelinating Diseases”[mh:noexp]) OR (“Encephalomyelitis, Acute Disseminated”[mh:noexp]) OR (“Optic Neuritis”[mh])) OR (((“multiple sclerosis”) OR (“neuromyelitis optica”) OR (“transverse myelitis”) OR (encephalomyelitis) OR (devic) OR (“optic neuritis”)) OR (“demyelinating disease*”) OR (“acute disseminated encephalomyelitis”))) AND (((randomized controlled trial[pt]) OR (controlled clinical trial[pt]) OR (randomized[tiab]) OR (placebo[tiab]) OR (drug therapy[sh]) OR (randomly[tiab]) OR (trial[tiab]) OR (groups[tiab])) NOT ((animals[mh]) NOT ((animals[mh]) AND (human[mh])))))

Appendix 3. EMBASE search strategy

(((’encephalomyelitis’/exp) OR (’demyelinating disease’/exp) OR (’multiple sclerosis’/exp) OR (’myelooptic neuropathy’/exp) OR (’multiple sclerosis’:ti,ab) OR (’neuromyelitis optica’:ab,ti) OR (encephalomyelitis:ab,ti) OR (devic:ti,ab)) AND ((’crossover procedure’/exp) OR (’double blind procedure’/exp) OR (’clinical trial’/exp) OR (’controlled clinical trial’/exp) OR (’single blind procedure’/exp) OR (’randomized controlled trial’/exp) OR (random*:ab,ti) OR (factorial*:ab,ti) OR (crossover:ab,ti) OR (cross:ab,ti AND over:ab,ti) OR (placebo:ab,ti) OR (’double blind’:ab,ti) OR (’single blind’:ab,ti) OR (assign*:ab,ti) OR (allocat*:ab,ti) OR (volunteer*:ab,ti))) AND (((’natalizumab’/exp) OR (antegren:ab,ti OR tysabri:ab,ti OR natalizumab:ab,ti))) AND [humans]/lim AND [embase]/lim

Appendix 4. Risk of bias criteria

	Item	Judgment	Description
1	Random sequence generation (selection bias)	low risk	The investigators describe a random component in the sequence generation process such as: random number table; computer random number generator; coin tossing; shuffling cards or envelopes; throwing dice; drawing of lots; minimization
		high risk	The investigators describe a non-random component in the sequence generation process such as: odd or even date of birth; date (or day) of admission; hospital or clinic record number; alternation; judgement of the clinician; results of a laboratory test or a series of tests; availability of the intervention
		unclear risk	Insufficient information about the sequence generation process to permit judgement of ‘Yes’ or ‘No’
2	Allocation concealment (selection bias)	low risk	Investigators enrolling participants could not foresee assignment because one of the following, or an equivalent method, was used to conceal allocation: central allocation (including telephone, web-based, and pharmacy-controlled, randomization); sequentially numbered drug containers of identical appearance; sequentially numbered, opaque, sealed envelopes
		high risk	Investigators enrolling participants could possibly foresee assignments because one of the following method was used: open random allocation schedule (e.g. a list of random numbers); assignment envelopes without appropriate safeguards (e.g. if envelopes were unsealed or non opaque

(*Continued*)

			or not sequentially numbered); alternation or rotation; date of birth; case record number; any other explicitly unconcealed procedure
		unclear risk	Insufficient information to permit judgement of 'Yes' or 'No'. This is usually the case if the method of concealment is not described or not described in sufficient detail to allow a definite judgement
3	Blinding of patients, provider, outcome assessor (performance bias and detection bias) Objective outcomes	low risk	Blinding of participants, providers and outcome assessor and unlikely that the blinding could have been broken; Either participants or providers were not blinded, but outcome assessment was blinded and the non-blinding of others unlikely to introduce bias No blinding, but the objective outcome measurement are not likely to be influenced by lack of blinding
		high risk	No blinding or incomplete blinding, and the outcome or outcome measurement is likely to be influenced by lack of blinding; Blinding of key study participants and personnel attempted, but likely that the blinding could have been broken; Either participants or outcome assessor were not blinded, and the non-blinding of others likely to introduce bias
		unclear risk	Insufficient information to permit judgement of 'Yes' or 'No';
4	Blinding of patients, provider, outcome assessor (performance bias and detection bias) Subjective outcomes	low risk	Blinding of participants, providers and outcome assessor and unlikely that the blinding could have been broken; Either participants or providers were not blinded, but outcome assessment was blinded and the non-blinding of others unlikely to introduce bias
		high risk	No blinding or incomplete blinding, and the outcome or outcome measurement is likely to be influenced by lack of blinding; Blinding of key study participants and personnel attempted, but likely that the blinding could have been broken; Either participants or outcome assessor were not blinded, and the non-blinding of others likely to introduce bias
		unclear risk	Insufficient information to permit judgement of 'Yes' or 'No';
5	Incomplete outcome data (attrition bias) for all outcomes except retention in treatment or drop out	low risk	No missing outcome data; Reasons for missing outcome data unlikely to be related to true outcome (for survival data, censoring unlikely to be introducing bias); Missing outcome data balanced in numbers across intervention groups, with similar reasons for missing data across groups; For dichotomous outcome data, the proportion of missing outcomes compared with observed event risk not enough to have a clinically relevant impact on the intervention effect estimate; For continuous outcome data, plausible effect size (difference in means

(*Continued*)

			or standardized difference in means) among missing outcomes not enough to have a clinically relevant impact on observed effect size; Missing data have been imputed using appropriate methods All randomized patients are reported/analyzed in the group they were allocated to by randomization irrespective of non-compliance and co-interventions (intention to treat)
		high risk	Reason for missing outcome data likely to be related to true outcome, with either imbalance in numbers or reasons for missing data across intervention groups; For dichotomous outcome data, the proportion of missing outcomes compared with observed event risk enough to induce clinically relevant bias in intervention effect estimate; For continuous outcome data, plausible effect size (difference in means or standardized difference in means) among missing outcomes enough to induce clinically relevant bias in observed effect size; 'As-treated' analysis done with substantial departure of the intervention received from that assigned at randomization;
		unclear risk	Insufficient reporting of attrition/exclusions to permit judgement of 'Yes' or 'No' (e.g. number randomized not stated, no reasons for missing data provided; number of drop out not reported for each group);
6	Selective reporting	low risk	The study protocol is available and all of the study's pre-specified (primary and secondary) outcomes that are of interest in the review have been reported in the pre-specified way; The study protocol is not available but it is clear that the published reports include all expected outcomes, including those that were pre-specified (convincing text of this nature may be uncommon)
		high risk	Not all of the study's pre-specified primary outcomes have been reported; One or more primary outcomes is reported using measurements, analysis methods or subsets of the data (e.g. subscales) that were not pre-specified; One or more reported primary outcomes were not pre-specified (unless clear justification for their reporting is provided, such as an unexpected adverse effect); One or more outcomes of interest in the review are reported incompletely so that they cannot be entered in a meta-analysis; The study report fails to include results for a key outcome that would be expected to have been reported for such a study
		unclear risk	Insufficient information to permit judgement of 'Yes' or 'No'
	Independent funding source	low risk	Not sponsored by pharmaceutical industry
		high risk	Sponsored by pharmaceutical industry

(*Continued*)

		unclear risk	Insufficient information to permit judgement of 'Yes' or 'No'

HISTORY

Protocol first published: Issue 1, 2009

Review first published: Issue 10, 2011

Date	Event	Description
13 May 2009	Amended	The section "Criteria for considering studies for this review" has been amended
10 February 2009	Amended	Search strategies
27 October 2008	Amended	Converted to new review format.

CONTRIBUTIONS OF AUTHORS

Pucci, Giuliani, Solari, Simi and Minozzi drafted the protocol.

Pucci developed the search strategy.

Pucci, Giuliani and Solari performed screening of identified trials for possible inclusion.

Pucci and Galea extracted data from trials.

Pucci, Minozzi and Giuliani assessed methodological quality of each included trial.

Di Pietrantonj checked the extraction, structured the comparisons, entered the data into Review Manager (RevMan) 2008 and performed the analysis.

Pucci and Di Pietrantonj interpreted the analysis.

Pucci, Giuliani, Solari, Simi, Galea, Minozzi and Di Pietrantonj wrote the final report.

Pucci and Di Pietrantonj will update the review.

DECLARATIONS OF INTEREST

Pucci has received funds from a non-profit association, the "Associazione Marchigiana sclerosi multipla e altre malattie neurologiche"; this association has received donations from Biogen Dompé, Merck-Serono and Bayer-Schering. In the last 5 years, Pucci has also received honoraria, reimbursement for attending congresses, and grant support for organising scientific activities from the above-mentioned drug industries and from Aventis, UCB, Lundbeck and Novartis.

Solari has received research funding from Biogen Dompé and has also received consultancy payment from Merck-Serono.

Giuliani, Di Pietrantonj, Simi, Minozzi and Galea have no conflict of interests.

SOURCES OF SUPPORT

Internal sources

- Cochrane Vaccines Field - SSEpi / SeREMI - ASL Alessandria (Local Health Unit), Italy.

External sources

- Associazione Marchigiana Sclerosi Multipla e altre malattie neurologiche, Italy.

Travel expense reimbursement for meetings among the authors. Computer and Internet costs.

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

In the protocol, we stated that "adverse event" did not include MS relapses. In the review we included MS relapses in AEs as the original trials did. Whenever possible, however, we analysed data including and excluding MS relapses.

Incidence of serious AE was moved from secondary outcomes to primary outcomes.

INDEX TERMS

Medical Subject Headings (MeSH)

Adjuvants, Immunologic [adverse effects; *therapeutic use]; Antibodies, Monoclonal [adverse effects; *therapeutic use]; Antibodies, Monoclonal, Humanized; Cell Migration Inhibition; Interferon-beta [*therapeutic use]; Multiple Sclerosis, Relapsing-Remitting [*drug therapy]; Peptides [adverse effects; *therapeutic use]; Randomized Controlled Trials as Topic

MeSH check words

Humans