

Effect of monoclonal antibody drug therapy on mucosal biomarkers in airway disease: A systematic review

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Abstract

Background: Monoclonal antibody therapies have a growing role in treating refractory airway disease.

Objective: The review aimed to summarize the response of respiratory mucosa to monoclonal antibody treatments in inflammatory airway conditions.

Design: We conducted a systematic review including risk of bias assessment.

Data sources: MEDLINE, EMBASE and PubMed from 1 January 2000 to 16 November 2019 were searched.

Eligibility criteria: Eligible studies assessed the immunological and histological response of airway mucosa to monoclonal antibody therapy compared with baseline or a comparison group in patients with respiratory diseases (asthma, chronic rhinosinusitis and allergic rhinitis). Any prospective interventional studies, including randomized controlled trials (RCTs) and single-arm trials, were eligible.

Results: There were 4195 articles screened, and full-text analysis produced $n = 11$ studies with extractable data. Nine were RCTs, and two were single-arm trials. These studies focused on asthma ($n = 9$ articles), chronic rhinosinusitis ($n = 1$) and allergic rhinitis ($n = 1$). Five monoclonal antibody drugs were assessed (omalizumab, mepolizumab, dupilumab, benralizumab and tralokinumab). Risk of bias was low ($n = 6$) or unclear ($n = 3$) in the RCTs and moderate in the single-arm trials. Omalizumab reduced the mucosal concentration of its target, IgE. Dupilumab reduced the concentration of one of its targets, IL-13, but not IL-4. Omalizumab, mepolizumab and benralizumab reduced tissue eosinophil cell density. Dupilumab decreased mucosal eosinophil granule proteins. Tralokinumab did not affect airway mucosa.

Conclusions: Knowledge of the expected biological response of monoclonal antibody therapy on biomarkers in disease tissue provides an important supplement to data about clinical outcomes. An understanding of the biological effect is essential to identify likely responders, reasons for treatment failure and necessary adjustments to monoclonal antibody treatment. Further investigation into the effect of monoclonal antibody therapy on disease mucosa and more precise endotyping are required to move closer to achieving personalized medicine.

KEYWORDS

asthma, biomarker, ENT, monoclonal antibody, mucosa, rhinitis

1 | INTRODUCTION

Asthma, chronic rhinosinusitis (CRS) and allergic rhinitis (AR) are common heterogeneous inflammatory conditions of the airways.¹ There has been a recent shift to group these conditions by endotype,^{2,3} defined as a distinct pathophysiological mechanism driven by unique inflammatory cells and cytokines.⁴ In a "united airway" model, asthma, CRS and AR have similar inflammatory changes and sets of endotype.^{5,6} The transcriptional profiles of upper and lower airway epithelium are similar, with only very minor deviations which reflect site-specific function.⁷

Airway disorders have been divided traditionally into two distinct endotypes based on the presence or absence of eosinophils.⁸ The current classification divides patients into type 2 or non-type 2-driven disease.⁹ The type 2 endotype is driven by type helper 2 (T_H2) cells and type 2 innate lymphoid cells (ILC2s).¹⁰ There is considerable diversity in the type 2 endotype,¹¹ with subgroups associated with increased eosinophils, driven by interleukin (IL)-5^{2,9} or immunoglobulin E (IgE), driven by IL-4 and IL-13.¹ The neutrophilic endotype represents a non-type 2 driven disease, mediated by tumour necrosis factor (TNF), IL-1, IL-6, IL-8, IL-23 and IL-17.⁹ Other endotypes include paucigranulocytic, thought to be mediated by airway hyper-responsiveness, and a mixed-granulocytic endotype, characterized by elevated eosinophils and neutrophils.⁹

Biomarkers are objective characteristics of physiological or pathogenic processes.¹² Biomarkers, such as inflammatory cells and cytokines, that drive airway disease endotypes may be measured at baseline for diagnosis and prediction of clinical response to therapy, and during treatment to monitor response.¹² Targeted therapies, mainly "biologics" in the form of monoclonal antibody (mAb) therapies, are currently available or in clinical trials for the treatment of severe asthma, CRS and AR (Table 1). Structurally based on B cell-produced antibodies, typically an IgG isotype, mAbs are engineered to bind with high specificity to designated target molecules associated with disease processes, thereby blocking the target.¹³ It is thought that clinical benefit is derived from modifying the inflammatory cascade in disease mucosa.^{14,15} Unlike corticosteroid treatment, which is a multi-targeted therapy associated with significant side effects, mAbs are a focused treatment, directed according to a specific endotype.¹⁶

Although the primary outcome of mAb trials is clinical response, understanding the effect on underlying molecular and cellular pathways by measurement of tissue biomarkers in those trials is also beneficial and may help to explain the clinical results. Numerous small-scale studies have investigated the effect of biologics in mucosal tissue, often alongside larger trials assessing clinical response. There has been no review summarizing the evidence from the trials

on mAbs in mucosal tissue. This review aims to synthesize existing literature about the response of immunological and histological features of disease mucosa to mAb therapy that targets inflammatory airway disease, including asthma, CRS and AR.

2 | METHODS

2.1 | Protocol and registration

A systematic review protocol was retrospectively registered in Review Registry (<https://www.researchregistry.com/browse-the-registry#registryofsystematicreviewsmeta-analyses/registryofsystematicreviewsmeta-analysedetails/5ebb676fb829ca001562566b/>; Registration number: reviewregistry899) and is reported in keeping with recommendations from the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.¹⁷

2.2 | Eligibility criteria

Eligible studies assessed patients of any age with respiratory diseases (including asthma, CRS and AR). The intervention was mAb therapy, compared with baseline or a comparison group treated with placebo or standard of care. The outcome of interest was the immunological and histological response in airway mucosa.

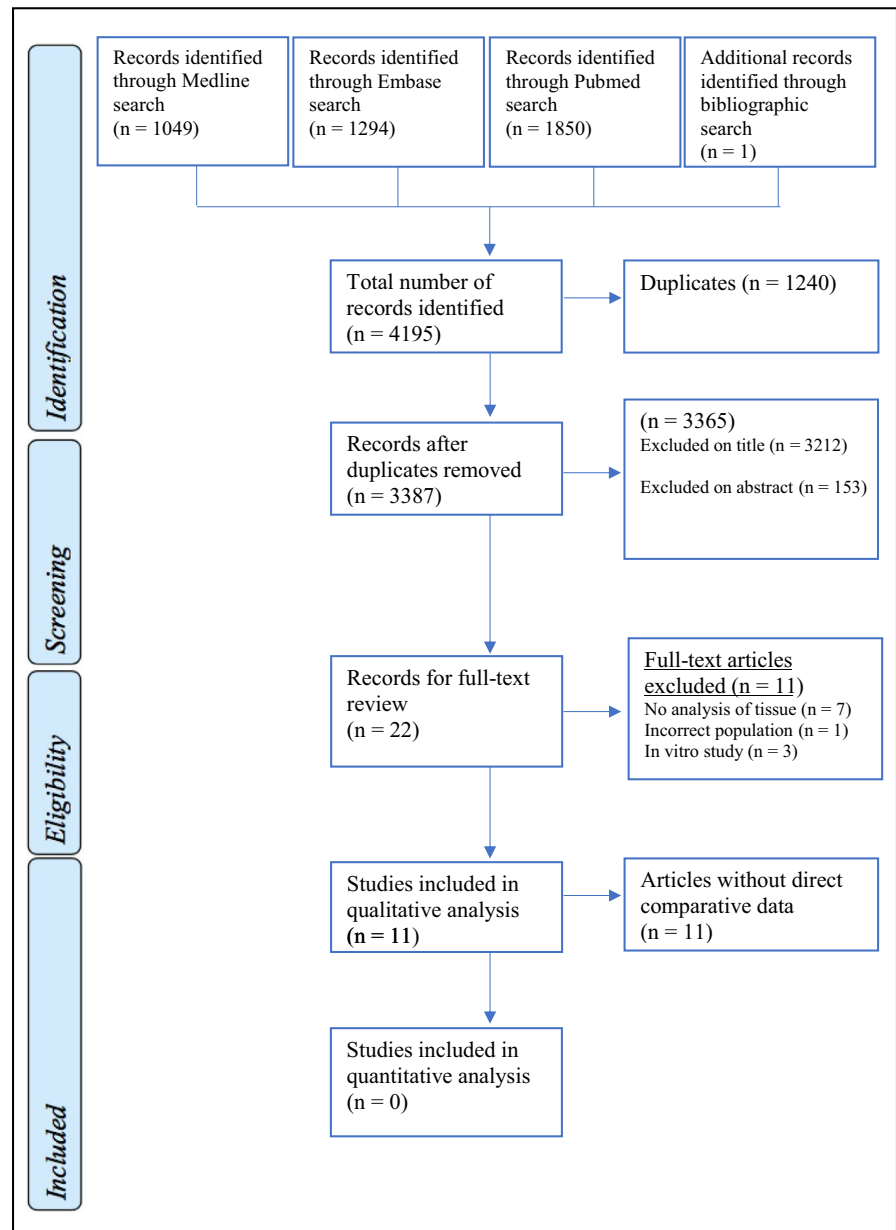
Only full-text articles published in English that reported original data were eligible. Animal studies were thus excluded. Studies which investigated any mAb therapy were included, regardless of specific agent, dose, mode of administration, frequency of administration or duration of therapy. Articles which did not analyse the effect of

TABLE 1 Monoclonal antibodies assessed for their effect on airway mucosal biomarkers

mAb name	mAb target	Disease	Studies
Omalizumab	Anti-IgE	Asthma AR CRS	21,23,28,29,31
Mepolizumab	Anti-IL-5	Asthma CRS	22,25,27
Benralizumab	Anti-IL-5R α	Asthma	26
Tralokinumab	Anti-IL-13	Asthma	30
Dupilumab	Anti-IL-4R α	Asthma CRS	24

Abbreviations: AR, allergic rhinitis; CRS, chronic rhinosinusitis; IgE, immunoglobulin E; IL, interleukin; R α , receptor- α .

FIGURE 1 Preferred reporting items for systematic reviews and meta-analyses (PRISMA)¹⁷ flow diagram of study selection process



mAbs on inflammation in respiratory mucosa, but only in locations other than respiratory mucosa (eg serum, sputum, bronchoalveolar lavage fluid), were excluded. Randomized control trials and experimental drug trials were included. Review articles and unpublished studies were excluded. Only studies published after 1 January 2000 were eligible; this date preceded publication of the initial studies on omalizumab, the first mAb identified as a novel treatment for airway diseases.¹⁸

2.3 | Information sources

The following international electronic databases covering the period 1 January 2000 up to 16 November 2019 were examined: MEDLINE, EMBASE and PubMed. The bibliographies of

identified articles were also reviewed and used as an additional data source.

2.4 | Search

A search strategy was designed to search for relevant articles. Details about the MEDLINE search strategy are presented in the supplementary material (Tables S1-S5).

2.5 | Study selection

Duplicates were removed. Titles of each reference were reviewed to ascertain whether they met the inclusion and exclusion criteria.

	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and researchers (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other biases
Plewako 2002	+	+	+	+	+	+	+
Flood-Page 2003	+	+	+	+	+	+	+
Menzies-Gow 2003	+	+	+	+	+	+	+
Djukanovic 2004	+	+	+	?	+	+	+
Van Rensen 2008	+	+	+	+	+	+	?
Laviolette 2013	+	+	+	?	+	+	+
Pillai 2016	+	+	+	+	+	+	+
Russell 2018	+	+	+	+	+	+	+
Jonstam 2019	+	+	+	+	+	+	+

FIGURE 2 Risk of bias of randomized controlled trials included in the systematic review. +, low risk of bias; -, high risk of bias; ?, unclear risk of bias

Abstracts of the remaining references were similarly screened. Full-text articles were reviewed for suitability.

2.6 | Data items and extraction

A table of characteristics of included studies was created using Excel (Microsoft version 15.33). The table documented study type, patient diagnosis, number of patients, age, mAb name, dose, route of administration, study duration, comparison treatment, tissue studied, tissue inflammatory markers investigated and method of analysis. The primary outcomes

were recorded as change in tissue immunological and histological biomarkers from baseline or in comparison with placebo or standard of care. The methodological quality of studies was assessed using the Cochrane risk of bias tool for randomized trials¹⁹; Risk Of Bias in Non-Randomized Studies—of Interventions (ROBINS-I) was used for the other studies.²⁰

3 | RESULTS

The search of MEDLINE, EMBASE, PubMed and a bibliographic search identified 4195 articles written in English, as detailed in the

TABLE 2 Characteristics of identified studies which investigate the effect of monoclonal antibody drugs on disease tissue inflammatory biomarkers in patients with inflammatory respiratory disease by drug class

Biological Investigated	Study	Study type	Diagnostic criteria	Participant number	Age (mean)	Dose and route	Study duration	Comparison
Omalizumab	Plewako, 2002	RCT	AR	30	31.6	300 mg SC at week 0 and week 4 (serum IgE ≤ 150 IU/mL), or at week 0, 3 and 6 (serum IgE > 150 IU/mL)	4 or 6	Placebo
	Djukanovic, 2004	RCT	Asthma	23	26	0.016 mg/kg/IgE (IU/mL) SC (150-300 mg every 4 wk or 225-375 mg every 2 wk)	16	Placebo
	Van Rensen, 2009	RCT	Asthma	25	20.75	0.016 mg/kg/IgE SC (150-375 mg every 2 or 4 wk)	12	Placebo
	Pillai, 2016	RCT	Asthma	18	50.25	Dose and frequency determined in standard clinical practice based on initial body weight and serum total IgE, drug administered SC	20	Placebo
	Huang, 2019	Single-arm trial	Asthma	9	56.4	0.016 mg/kg/IgE (IU/mL) SC	16	Baseline
Mepolizumab	Flood-Page, 2003	RCT	Asthma	24	30.5	750 mg IV at week 0, week 4 and week 8	20	Placebo
	Menzies-Gow, 2003	RCT	Asthma	24	30.5	750 mg IV at week 0, week 4 and week 8	20	Placebo
	Kelly, 2017	Single-arm trial	Asthma	10	25	750 mg IV single dose	20	Baseline
Benralizumab	Laviolette, 2013	RCT	Asthma	27Cohort 1: 13Cohort 2:14	38.4	Cohort 1:1 mg/kg IV single-dose Cohort 2:100 or 200 mg SC at week 0, week 4 and week 8	Cohort 1: 4Cohort 2:12	Placebo
Dupilumab	Jonstam, 2019	RCT	CRS	12	48.4	600 mg loading dose, then 300 mg SC weekly	16	Placebo
Tralokinumab	Russell, 2018	RCT	Asthma	76	48.6	Anti-IL-13: tralokinumab	300mg SC every 2 wk	Placebo

Abbreviations: AR, allergic rhinitis; IgE, immunoglobulin E; IU, international unit; IV, intravenous; mg, milligram; mL, millilitre; RCT, randomized control trial; SC, subcutaneous.

Inflammatory biomarker	Plewako, 2002	Djukanovic, 2004	Van Rensen, 2009	Pillai, 2016	Huang, 2019
Eosinophil cells	↓	↓	↓	=	NM
Total IgE	↓	↓	↓	↓	NM
High-affinity IgE receptor	=	↓	↓ ^b	NM	NM
Low-affinity IgE receptor	NM	=	=	NM	NM
CD3 T lymphocyte	NM	↓	=	NM	NM
CD4 T lymphocyte	=	↓	↓ ^b	NM	NM
CD8 T lymphocyte	=	↓	=	NM	NM
CD20 B lymphocyte	NM	↓	=	=	NM
Neutrophil	=	NM	=	NM	NM
Plasma cell	NM	NM	NM	=	NM
Plasmablast	NM	NM	NM	=	NM
Macrophage	NM	NM	=	NM	NM
Mast cell	=	=	=	=	NM
Basophil	NM	=	NM	NM	NM
Dendritic cell	NM	NM	NM	NM	NM
IL-4	NM	↓	NM	NM	NM
IL-5	NM	=	NM	NM	NM
IL-13	NM	NM	NM	NM	↓ ^b
IL-25	NM	NM	NM	NM	↓ ^b
IL-33	NM	NM	NM	NM	↓ ^b
TSLP	NM	NM	NM	NM	↓ ^b

Abbreviations: ↓, significant decrease; ↓^b, significant decrease from baseline; =, no significant change; blue box, variable studied; IgE, immunoglobulin E; IL, interleukin; NM, not measured; TSLP, thymic stromal lymphopoietin.

TABLE 3 Outcomes of published studies which investigate the effect of omalizumab on disease tissue inflammatory biomarkers in patients with inflammatory respiratory disease

PRISMA flow diagram (Figure 1). After removal of duplicates, 3387 studies remained. A title search found 175 articles. The abstract search identified 21 articles directly relating to mAbs' effect on inflammatory biomarkers in airway conditions. Those studies that did not report on the response in mucosa ($n = 7$) and were not human studies ($n = 3$) were excluded. The full-text analysis produced 11 studies with extractable data regarding the effect of mAbs on biomarkers in respiratory mucosa in inflammatory airway conditions.²¹⁻³¹ Risk of bias within the included studies was considered low^{21,22,24,27-30} or unclear^{21,26,31} in the RCTs (Figure 2) and moderate in the single-arm trials.^{23,25} The risk of bias assessments of included studies is outlined in Tables S2 and S3.

3.1 | Study characteristics

The study characteristics of the 11 articles included are described in Table 2 and Table S4. Nine trials were RCTs^{21,22,24,26-31} and two were single-arm trials.^{23,25} A total of five different mAbs were examined across these trials. The mAbs studied were omalizumab

(anti-IgE mAb),^{21,23,28,29,31} mepolizumab (anti-IL-5 mAb),^{22,25,27} benralizumab (anti-IL-5 receptor- α mAb),²⁶ dupilumab (anti-IL-4 receptor- α mAb)²⁴ and tralokinumab (anti-IL-13 mAb).³⁰ The airway conditions studied were asthma ($n = 9$),^{21,23,25-28,30,31} CRS ($n = 1$)²⁴ and AR ($n = 1$).²⁹ The median number of participants across the studies was 24 (IQR, 15-26). The minimum number of study participants was in a trial on mepolizumab ($n = 9$).²⁵ The maximum number of participants was in a trial on tralokinumab ($n = 76$).³⁰ The mAb studied in the greatest number of participants was omalizumab ($n = 105$), across five trials.^{21,23,28,29,31} The mAb studied in the least number of participants was dupilumab ($n = 12$), in a single trial.²⁴ The cumulative study participant number for the other mAbs varied (mepolizumab, $n = 34$,^{22,25,27}; benralizumab, $n = 27$ ²⁶; tralokinumab, $n = 76$ ³⁰).

Tissue response was defined by immunological and histological biomarkers, including inflammatory cell number, cell product deposition, cytokine concentration and other biomarkers. No biomarker was consistently reported across all 11 studies. The studied mAbs had variable effects in the inflammatory cascade in disease mucosa.

3.2 | Omalizumab: anti-IgE mAb

Five studies investigated omalizumab.^{21,23,28,29,31} Four investigated bronchial mucosa in asthma^{21,23,24,28,29,31} and one investigated nasal mucosa in AR.²⁹ Omalizumab was found to reduce the concentration of its target biomarker, IgE, in bronchial and nasal tissue, in all four studies that investigated that outcome (Table 3).^{21,28,31} Omalizumab was also associated with down-regulation of cells expressing the high-affinity IgE receptor (FcεRI) in bronchial mucosa compared with placebo²¹ and to baseline.²⁸ However, in one trial there was no change in FcεRI concentration, compared with placebo.²⁹

Additionally, omalizumab reduced indirect tissue biomarkers. In three out of four studies that assessed the effect of omalizumab on tissue eosinophil cell numbers compared with placebo, eosinophils decreased with treatment.^{21,28,31} One study found that eosinophil levels in nasal mucosa in patients with AR remained unchanged with treatment during the pollen season, compared with placebo in which eosinophils were increased.²⁹ In the same study, omalizumab stabilised the level of eosinophil granule proteins within the mucosa, compared with the placebo group in which eosinophil granule proteins increased, consistent with the increase in absolute eosinophil numbers.²⁹ Omalizumab reduced the expression of bronchial tissue cytokines, including IL-13, IL-33, IL-25 and thymic stromal lymphopoietin (TSLP) from baseline in patients who improved clinically, measured by improved asthma symptom score.²³

Several cellular biomarkers were unchanged with treatment, including neutrophils, plasma cells, plasmoblasts, mast cells, basophils and dendritic cells.^{18,25,28,29}

3.3 | Mepolizumab: anti-IL-5 mAb

Three studies assessed mepolizumab in bronchial mucosa of patients with asthma.^{22,25,27} No studies have investigated mepolizumab in

TABLE 4 Outcomes of published studies which investigate the effect of mepolizumab on disease tissue inflammatory biomarkers in patients with inflammatory respiratory disease

Inflammatory biomarker	Flood-Page, 2003	Menzies-Gow, 2003	Kelly, 2017
Eosinophil cells	↓	NM	NM
Eosinophil degranulation proteins	=	NM	↓
Progenitor cell	NM	↓ ^b	NM
CD3 T lymphocyte	=	NM	NM
Neutrophil	=	NM	NM
Mast cell	=	NM	NM
Basophil	=	NM	NM
Macrophages	=	NM	NM

Abbreviations: ↓, significant decrease; ↓^b, significant decrease from baseline; =, no significant change; blue box, variable studied; NM, not measured.

nasal mucosa. The effect of the anti-IL-5 mAb on its direct target biomarker, IL-5, has not been investigated in tissue.

Mepolizumab was found to reduce eosinophil numbers in the bronchial mucosa of asthma patients (Table 4).^{22,26} Mepolizumab also reduced the concentration of eosinophil progenitor cells in nasal mucosa.²⁷ The effect of mepolizumab on the deposition of eosinophil granule proteins in mucosa was inconsistent between studies. One study found that mepolizumab reduced the deposition of eosinophil degranulation proteins in bronchial mucosa,²⁵ but in another study mepolizumab had no effect on eosinophil degranulation proteins in mucosa.²² Mepolizumab had no effect on the mucosal cell count of CD3 T lymphocytes, neutrophils, mast cells, basophils and macrophages, compared with placebo.^{22,26}

3.4 | Benralizumab: anti-IL-5 receptor-α mAb

One study investigated benralizumab in bronchial mucosa of asthma patients.²⁶ No studies have investigated the effect of benralizumab in nasal mucosa. Benralizumab was found to reduce mucosal eosinophil numbers compared with placebo.^{22,26} The effect of benralizumab on biomarkers other than eosinophil cell count has not been investigated.

TABLE 5 Outcomes of published studies which investigate the effect of dupilumab on disease tissue inflammatory biomarkers in patients with inflammatory respiratory disease

Inflammatory biomarker	Jonstam, 2019
Eosinophil degranulation proteins	↓
Total IgE	↓ ^b
IL-1β	=
IL-4	=
IL-5	=
IL-6	=
IL-10	=
IL-13	↓ ^b
IL-17	=
IL-33	=
TNF-α	=
Eotaxin-1	=
Eotaxin-2	↓
Eotaxin-3	↓ ^b
PARC	↓
TARC	=
VEGF	=

Abbreviations: ↓, significant decrease; ↓^b, significant decrease from baseline; =, no significant change; IgE, immunoglobulin E; IL, interleukin; NM, not measured; PARC, pulmonary and activation-regulated chemokine; TARC, thymus and activation-regulated chemokine; TNF, tumour necrosis factor; VEGF, vascular endothelial growth factor.

3.5 | Dupilumab: anti-IL-4 receptor- α

One study investigated dupilumab in nasal mucosa of CRS patients.²⁴ Bronchial mucosal response to dupilumab has not been assessed.

Dupilumab decreased the concentration of one of its target molecules, IL-13, in nasal mucosa of patients with CRS from baseline, but did not decrease the concentration of IL-4 (Table 5).²⁴ Although dupilumab did not reduce the concentration of both its target molecules, the treatment group showed improvement in endoscopic, radiographic and quality of life measures, relative to placebo.²⁴ Dupilumab reduced IgE in nasal mucosa in CRS patients from baseline.²⁴

Additionally, dupilumab reduced other inflammatory biomarkers.^{21,23,24,28,29,31} Dupilumab treatment was associated with decreased eosinophil granule proteins, eotaxin-2 and pulmonary and activation-regulated chemokine (PARC) concentrations, compared with placebo, and decreased eotaxin-3, IgE and IL-13 from baseline.²⁴

3.6 | Tralokinumab: anti-IL-13 mAb

One study investigated tralokinumab in bronchial mucosa of asthma patients.³⁰ Tralokinumab's effect on direct target biomarkers has not been investigated in mucosa. The effect of tralokinumab on direct downstream targets, including IgE, has also not been investigated in mucosal tissue. Tralokinumab did not affect mucosal eosinophilic cell numbers, other inflammatory cell counts (neutrophils, mast cells, macrophages and CD3 T lymphocytes), nor bronchial mucosal remodelling factors (epithelial cell integrity, matrix deposition, matrix composition and reticular basement membrane thickening) compared with placebo.³⁰

4 | DISCUSSION

Advances in developing and studying biologics are occurring rapidly.³² mAbs represent an attractive alternative to systemic anti-inflammatories, including corticosteroid treatment, because of their proven clinical efficacy and favourable side effect profile. Research thus far on mAbs has focused on clinical outcome measures to assess the drugs' efficacy and safety. However, an understanding of the tissue and biomarker changes in response to mAb therapy is an important supplement to clinical data as those changes underpin the clinical response. This systematic review identified that there is an extremely small body of literature that has investigated the effect of mAbs on disease mucosa. The review highlights the current paucity of data about the effect of mAb therapy for inflammatory airway conditions on disease mucosa. It is surprising that there is so little information available on the end organ effect of the currently used mAbs. No review has previously summarized the tissue effects of mAbs on diseased respiratory mucosa. In comparison, the effect of

corticosteroid therapy on airway inflammation is well established from biopsy data.³³

With a move towards precision medicine, knowledge of the molecular and cellular biological response to therapy—locally and systemically—is a necessary adjunct to clinical measures of response. A method to identify likely responders and non-responders to existing mAb therapies is yet to be determined. Such a method would help to stratify patients with severe airway disease to select an appropriate biologic. In asthma, not all subjects treated with mAbs respond equally well, despite fairly rigid and uniform selection criteria.³⁴ For example, a subgroup of patients with severe asthma have been identified as "super-responders" to mepolizumab; elevated blood eosinophils and fewer comorbidities characterized this subgroup.³⁵ The ability to detect patients who will benefit from a particular mAb therapy over another, and also to identify likely poor responders, will improve selection of appropriate biologics and the allocation of these expensive agents. Minimally invasive markers for airway inflammation, such as fraction of exhaled nitric oxide, blood eosinophilia, IgE and serum periostin, only have moderate diagnostic utility.³⁶ For example, blood eosinophil levels are affected by other conditions³⁷ and may not reflect tissue inflammation.³⁸ Direct assessment of histological and immunological features in disease tissue offers a more sensitive and comprehensive approach. Tissue biomarker response may help to demarcate likely treatment responders from non-responders, guide individualized treatment dosages and highlight the potential need for concomitant mAb or non-mAb therapy.

Four of the five biologics, namely omalizumab (anti-IgE mAb), mepolizumab (anti-IL-5 mAb), benralizumab (anti-IL-5 receptor- α mAb) and dupilumab (anti-IL-4 receptor- α mAb), which were investigated in studies in the systematic review, have been found to be clinically efficacious in large clinical trials in the lower airway in asthma.³⁹⁻⁴⁴ Omalizumab has also been found to have clinical benefit in seasonal AR.⁴⁵ In addition, there is preliminary evidence for the efficacy of mepolizumab and dupilumab in the upper airways in CRS.⁴⁶⁻⁴⁸ The summarized data from the studies included in this review highlights that those four mAb therapies have an inhibitory effect on inflammation in the local diseased mucosa. Interestingly, while biologic agents are considered to be "targeted," summarized evidence from this review suggests that the effect of mAbs extends far beyond the direct target, to other inflammatory cells and cytokines in the inflammatory cascade. For example, while it is expected that omalizumab, an anti-IgE agent, would reduce mucosal IgE, it is rather surprising that the mAb has also been found to reduce mucosal eosinophils. Omalizumab has also been found to reduce blood eosinophils.⁴⁹ The decrease in tissue biomarker levels with clinically beneficial mAb therapy suggests that these drugs operate by reducing the level of the target directly downstream and other non-target biomarkers in respiratory mucosa. Thus, the mechanism of action of targeted therapy in disease tissue involves inhibition of multiple components of the inflammatory cascade, initiated by the blockade of a single target.

The remaining biologic, tralokinumab (anti-IL-13), did not reduce the rate of exacerbations in patients with severe refractory

asthma,^{50,51} nor improve asthma symptoms.⁵² The absence of a change in airway mucosal inflammation and remodelling by the anti-IL-13 mAb, tralokinumab, in the study by Russell et al³⁰ may suggest that neutralization of IL-13 alone may not play an important role in the suppression of inflammation in asthma. This is important as dupilumab, with both IL4 and 13 effects, shows a clear inhibitory action.

The identified studies are not without their shortcomings, including the broad inclusion criteria and absence of endotyping. Patients were recruited based on disease severity or phenotype. The studies are limited by their small cohort sizes (most with less than $n = 30$ patients), which may render the studies underpowered to detect differences in mucosal inflammation after mAb treatment. The anti-IgE mAb, omalizumab, has been studied across the largest population in asthma, CRS and AR, and thus has the strongest evidence base for changes in mucosal tissue. Another limitation pertains to flaws in biomarker analysis. Various studies assessed the effect of mAbs on diseased mucosa via immunohistochemical staining, which can be subject to human error in interpretation.^{21-23,25,26,28,29,31} Differences in methodology between studies may limit the ability to synthesize data. Additionally, moderate risk of bias was identified in the two non-randomized trials included in the review and thus caution should be taken in interpreting the results.

It is noteworthy that the current definition of type 2 inflammation⁹ is insufficient to reflect the diversity of pathways within the subgroup of inflammatory airway diseases. The broad selection criteria in mAb research trials that have investigated clinical and mucosal inflammatory response have meant that the evidence base to guide patient selection for biologic treatment is currently non-specific, and thus, the opportunity for personalized medicine is presently limited. The authors propose that for future research trials on biologics, a new classification of biomarkers in type 2 inflammation is adopted in which endotypes will reflect levels of mAb targets (eg IgE, IL-5, IL-4/IL-13) in disease tissue. It is acknowledged that performing lower airway mucosal biopsies on patients can be problematic due to the invasive nature of the procedure. The possibility of using nasal mucosa biopsies instead of bronchial biopsies should be considered. Although there are minor intrinsic differences between the upper and lower airways,⁷ the united airway model shows that the inflammatory changes are strongly correlated^{5,6,53}; hence, a nasal biopsy represents a suitable surrogate for the lower airway. Quantification of the concentration of inflammatory markers in nasal tissue can be done histologically or by immunoassay, as illustrated by the studies included in this systematic review.

5 | CONCLUSIONS

There has been recent growth in the number of mAb agents available for the treatment of refractory inflammatory airway diseases. It is thought that by targeting biologic cellular and cytokine pathways,

reduced inflammatory activity in disease tissue will lead to clinical improvement. This review summarizes the effects of mAb therapy on histological and immunological features in the airway mucosa of patients with asthma, CRS and AR. The review highlights that prevailing evidence about the effect of mAbs on disease mucosa is very limited and that further investigation is required.

A greater understanding of the underlying tissue response to mAb therapy has potential to be beneficial in several ways, including identifying likely responders to various mAb therapy options at baseline and guiding the choice of precision medicine according to disease endotype. In patients who do not improve with mAb therapy, measurement of the underlying tissue response to treatment may help to elucidate reasons for treatment failure and inform management decisions, such as the need for increased mAb dose or concomitant mAb or non-mAb treatment if single mAb therapy is insufficient.

In future research trials investigating mAb therapy, precise endotyping of patients, including evaluation of baseline and serial quantification of biomarker levels in biopsies, may be required for the full potential of individualized therapy in refractory airway disease to be realized.

CONFLICT OF INTERESTS

Sophie J Walter, Jacqueline Ho, Raquel Alvarado and Raewyn Campbell declare that they have no conflicts of interest and no financial disclosures. Richard J Harvey is a consultant with Medtronic, Stryker, Novartis, Meda and NeilMed pharmaceuticals. Research grant funding received from Glaxo-Smith-Kline and Stallergenes. He has been on the speakers' bureau for Glaxo-Smith-Kline, Meda Pharmaceuticals and Seqirus. Raymond Sacks is a consultant for Medtronic and is on the speaker's bureau for Meda Pharmaceuticals. Larry H Kalish is on the speakers' bureau at Mylan and Care Pharmaceuticals. Janet Rimmer has honoraria with Sanofi Aventis, Novartis, Mundipharma, BioCSL and Stallergenes.

AUTHOR CONTRIBUTIONS

All authors contributed significantly to the research and preparation, revision and final production of the manuscript and approved the final version of the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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Supplementary Table 1 Search Strategy using MEDLINE

1.	Asthma*.mp
2.	Rhinosinusitis.mp
3.	CRS.mp
4.	Rhinitis.mp
5.	“Allergic rhinitis”.mp
6.	Sinusitis.mp
7.	“Nose polyp*”.mp
8.	Allerg*.mp
9.	OR/1-8
10.	“Monoclonal antibod*”.mp
11.	Biologic*.mp
12.	Anti-Interleukin*.mp
13.	Anti-IL*.mp
14.	Mepolizumab.mp
15.	Dupilumab.mp
16.	Omalizumab.mp
17.	Benralizumab.mp
18.	Reslizumab.mp
19.	OR/10-18
20.	Biomarker*.mp
21.	Inflammat*.mp
22.	Cytokine*.mp
23.	IL*.mp
24.	Eosinophil*.mp
25.	Basophil*.mp
26.	IgE.mp
27.	Th2.mp
28.	“Type 2”.mp
29.	OR/20-28
30.	9 AND 19 AND 29
31.	Limit 30 to English language and Full Text and humans
32.	Limit 31 to 2000-Current
33.	Limit 32 to “review articles”
34.	NOT 33

Similar strategy used in Embase and PubMed.

Supplementary Table 2 Risk of bias assessment of included randomized controlled trials using Cochrane Collaboration's tool.

Trial	Bias domain	Authors' judgement	Support
Plewako, 2002	Random sequence generation (selection bias)	Low risk	Quote: "Patients were randomized to active or placebo treatment at a 2:1 ratio".
	Allocation concealment (selection bias)	Low risk	Quote: "Double-blind".
	Blinding of participants and researchers (performance bias)	Low risk	Quote: "Double-blind". Quote: "Injections were prepared by a person otherwise unconnected with the study and administered in the clinic".
	Blinding of outcome assessment (detection bias)	Low risk	Quote: "2 readers independently examined coded sections".
	Incomplete outcome data (attrition bias)	Low risk	Number of patients included in the sub-study of the larger clinical trial were disclosed. No patients were lost to follow-up.
	Selective reporting (reporting bias)	Low risk	All pre-specified outcomes were reported.
	Other bias	Low risk	Study reported that the use of "rescue medications" was similar between groups, which lowers the risk of performance bias.
	Overall risk of bias	Low risk	
Flood-Page, 2003	Random sequence generation (selection bias)	Low risk	Quote: "Randomized".
	Allocation concealment (selection bias)	Low risk	Quote: "Double-blind".
	Blinding of participants and researchers (performance bias)	Low risk	Quote: "Double-blind".
	Blinding of outcome assessment (detection bias)	Low risk	Quote: "The number of cells/mm ² was determined by counting whole sections in a blinded fashion".
	Incomplete outcome data (attrition bias)	Low risk	There was no loss to follow-up.
	Selective reporting (reporting bias)	Low risk	All pre-specified outcomes were reported.
	Other bias	Low risk	Study appears to be free of other sources of risk
	Overall risk of bias	Low risk	

Menzies-Gow, 2003	Random sequence generation (selection bias)	Low risk	Quote: “Randomized”
	Allocation concealment (selection bias)	Low risk	Quote: “Double-blind”.
	Blinding of participants and researchers (performance bias)	Low risk	Quote: “Double-blind”.
	Blinding of outcome assessment (detection bias)	Low risk	Quote: “The number of cells/mm ² was determined by counting whole sections in a blinded fashion”.
	Incomplete outcome data (attrition bias)	Low risk	There was no loss to follow-up.
	Selective reporting (reporting bias)	Low risk	All pre-specified outcomes were reported.
	Other bias	Low risk	Study appears to be free of other sources of risk
	Overall risk of bias	Low risk	
Djukanovic, 2004	Random sequence generation (selection bias)	Unclear risk	Quote: “Randomized (1:1) into the study”
	Allocation concealment (selection bias)	Low risk	Quote: “Double-blind”.
	Blinding of participants and researchers (performance bias)	Low risk	Quote: “Double-blind”.
	Blinding of outcome assessment (detection bias)	Unclear risk	No description.
	Incomplete outcome data (attrition bias)	Low risk	Reason for subject withdrawal was given. Quote: “1 subject treated with omalizumab was withdrawn because of a positive pregnancy test while another subject receiving placebo was lost to follow-up”. Select participants could not be included in biopsy analysis. However, reason for exclusion was based on pre-specified methodological criteria and numbers were similar between active and placebo groups.
	Selective reporting (reporting bias)	Low risk	All pre-specified outcomes were reported.
	Other bias	Low risk	Study appears to be free of other sources of risk
	Overall risk of bias	Low risk	
Van Rensen, 2008	Random sequence generation (selection bias)	Unclear risk	Randomized
	Allocation concealment (selection bias)	Low risk	Quote: “Double-blind”.

	Blinding of participants and researchers (performance bias)	Low risk	Quote: "Double-blind". Quote: "A research nurse who was not involved in any other measurement of the study administered the subcutaneous anti-IgE or placebo".
	Blinding of outcome assessment (detection bias)	Low risk	Quote: "All biopsies were coded and sections analysed in a blinded fashion, using a fully automated image analysis system".
	Incomplete outcome data (attrition bias)	Low risk	Loss to follow-up of one patient was reported. Quote: "From one patient in the placebo group, no biopsies were obtained during the second bronchoscopy".
	Selective reporting (reporting bias)	Low risk	All pre-specified outcomes were reported.
	Other bias	Unclear risk	Other medication use was not reported, thus level of performance bias is unknown.
	Overall risk of bias	Unclear risk	
Laviolette, 2013	Random sequence generation (selection bias)	Low risk	Quote: "Randomized" Quote: "Group assignment was determined by using block randomization through interactive voice-response system".
	Allocation concealment (selection bias)	Low risk	Quote: "Double-blind".
	Blinding of participants and researchers (performance bias)	Low risk	Quote: "Double-blind".
	Blinding of outcome assessment (detection bias)	Unclear risk	No description.
	Incomplete outcome data (attrition bias)	Low risk	Quote: "All subjects were included in all analyses"
	Selective reporting (reporting bias)	Low risk	All pre-specified outcomes were reported.
	Other bias	Low risk	Study appears to be free of other sources of risk.
	Overall risk of bias	Unclear risk	
Pillai, 2016	Random sequence generation (selection bias)	Low risk	Quote: "Randomized (1:1)"
	Allocation concealment (selection bias)	Low risk	Quote: "Double-blind". Quote: "Allocated by the hospital pharmacy using randomization tables with the patient and attending physician blinded".

	Blinding of participants and researchers (performance bias)	Low risk	Quote: "Double-blind".
	Blinding of outcome assessment (detection bias)	Low risk	Quote: "Biopsy sections were analysed objectively by operators ignorant of their provenance".
	Incomplete outcome data (attrition bias)	Low risk	Loss to follow-up of one patient was reported. Quote: "One patient allocated to placebo and two to omalizumab withdrew prematurely"
	Selective reporting (reporting bias)	Low risk	All pre-specified outcomes were reported.
	Other bias	Low risk	Study appears to be free of other sources of bias. Quote: "Patients encouraged to comply with their usual medication", and other medication was (B2 agonists and oral prednisolone) reported.
	Overall risk of bias	Low risk	
Russell, 2018	Random sequence generation (selection bias)	Low risk	Quote: "We randomly assigned participants (1:1). Randomization codes, prepared by an internal AstraZeneca computerized system, were assigned sequentially as participants become eligible for randomization"
	Allocation concealment (selection bias)	Low risk	Quote: "Double-blind". Quote: "Participants and study personnel were masked to treatment allocation... "because tralokinumab and placebo are visually distinct, they were handled and administered as pre-filled syringes by separate unmasked site staff, who were not involved in management of trial participants. The packaging and labelling of both study drugs was consistent".
	Blinding of participants and researchers (performance bias)	Low risk	Quote: "Double-blind".
	Blinding of outcome assessment (detection bias)	Low risk	Quote: "Investigators were masked".
	Incomplete outcome data (attrition bias)	Low risk	Quote: "All participants that completed the study successfully underwent baseline and end-of-treatment bronchoscopy. The biopsies obtained were of sufficient quality for analysis".

	Selective reporting (reporting bias)	Low risk	All pre-specified outcomes were reported. Trial funding by pharmaceutical group and participation in study design, data interpretation and writing of the report.
	Other bias	Low risk	Study appears to be free of other sources of risk.
	Overall risk of bias	Low risk	
Jonstam, 2019	Random sequence generation (selection bias)	Unclear risk	Quote: "Patients were randomly allocated (1:1)"
	Allocation concealment (selection bias)	Low risk	Quote: "Double-blind".
	Blinding of participants and researchers (performance bias)	Low risk	Quote: "Double-blind".
	Blinding of outcome assessment (detection bias)	Low risk	Objective methodology used to measure cytokine levels in tissue.
	Incomplete outcome data (attrition bias)	Low risk	Loss to follow-up for second biopsy was reported.
	Selective reporting (reporting bias)	Low risk	All pre-specified outcomes were reported.
	Other bias	Unclear risk	Study appears to be free of other sources of risk.
	Overall risk of bias	Unclear risk	

Supplementary Table 3 Risk of bias assessment of non-randomized studies using “Risk of Bias in Non-Randomized Studies – of Interventions” (ROBINS-I)

Trial	Bias	Authors’ judgement	Support
Kelly, 2017	Pre-intervention		
	Bias due to confounding	Low risk	Selection criteria of patients was clearly outlined and would reduce counfounding. Quote: “Participants had not received corticosteroids or leukotriene inhibitors within one month of screening, omalizumab within nine months of screening, an investigational drug within 1 month of screening, did not have any prior treatment with an anti-IL-5 monoclonal antibody, and were not on medications that would affect the outcome of the study. Participants did not a have major health problem or previous malignancy, were not pregnant, had safety lab assessments within normal ranges, had not smoked within the last year or had a smoking history of >5 pack years”.
	Bias in selection of participants to the study	Low risk	Patients were recruited based on objective clinical characteristics.
	At intervention		
	Bias in classification of interventions	N/A	N/A Single-arm study; all patients received intervention.
	Post-intervention		
	Bias due to deviations from intended interventions	Low risk	According to pre-specified methods, “one participant required a second dose of mepolizumab”.
	Bias due to missing data	Low risk	All patients completed the study, where appropriate, according to pre-specified criteria.
	Bias in measurement of outcomes	Moderate risk	For the immunohistochemistry outcome measure, a “semiquantitative scoring system” was used, which may be subject to subjective bias.
	Bias in selection of reported result	Low risk	All pre-specified outcomes were reported.
	Overall risk of bias	Moderate risk	

Huang, 2019	Pre-intervention		
	Bias due to confounding	Low risk	Quote: “All antiasthma medications were continuously used without any change throughout treatment”, which protected against potential confounding. Smoking may have been a confounding factor.
	Bias in selection of participants to the study	Low risk	Participants were “prospectively” recruited from a single centre. Quote: “All the patients in this study were regularly followed up at our clinic for more than 3 years”.
	At intervention		
	Bias in classification of interventions	N/A	N/A Single-arm study; all patients received intervention.
	Post-intervention		
	Bias due to deviations from intended interventions	Moderate risk	There were no deviations from intended interventions. However, the “Responder” group had significantly greater use of oral corticosteroids compared to the mild asthma control group.
	Bias due to missing data	Moderate risk	Post-treatment biopsy was not performed in all patients included in the study.
	Bias in measurement of outcomes	Moderate risk	Post-intervention definition of responders versus non-responders was based on a patient-reported outcome measure, and while validated, may introduce subjective bias. Quote: “Those patients who achieved an increase in ACT score more than 3 after omalizumab treatment were categorized as omalizumab responders (Responder); those who failed to improve their asthma control (increase in ACT scores <3) were categorized as omalizumab nonresponders (Non-Responder).
	Bias in selection of reported result	Low risk	All pre-specified outcomes were reported.
	Overall risk of bias	Moderate risk	

Supplementary Table 4 Type of tissue biopsied, inflammatory markers investigated and method of investigation from included studies.

Biological Investigated	Study	Tissue biopsied	Inflammatory markers investigated	Method
Omalizumab	Plewako, 2002	Nasal biopsy	Inflammatory cell and product: Eosinophil Lymphocyte (CD4, CD8) Neutrophil Mast cell IgE high affinity receptor	IHC
	Djukanovic, 2004	Bronchial biopsy	Inflammatory cell and product: Eosinophil IgE IgE high affinity receptor Lymphocyte (CD3, CD4, CD8), Mast cell Basophil Cytokine: IL-4 IL-5	IHC
	Van Rensen, 2009	Bronchial biopsy	Inflammatory cell and product: Eosinophil IgE IgE high affinity receptor IgE low affinity receptor Lymphocyte (CD3, CD4, CD8) Mast cell Macrophage Neutrophils	IHC
	Pillai, 2016	Bronchial biopsy	Inflammatory cell and product: Eosinophil IgE Mast cell Lymphocyte (CD20) Plasma cell Plasmablast	IHC IF
	Huang, 2019	Bronchial biopsy	Inflammatory cell and product: Eosinophil Neutrophil Cytokine: IL-4 IL-5 IL-9 IL-13 IL-25 IL-33 TSLP	PCR IHC Western blotting
Mepolizumab	Flood-Page, 2003	Bronchial biopsy	Inflammatory cell and product:	IHC

			Eosinophil Eosinophil granule protein (MBP) Lymphocyte (CD3) Neutrophil Mast cell Basophil Macrophages	
	Menzies-Gow, 2003	Bronchial biopsy	Inflammatory cell and product: Eosinophil progenitor cell	IHC
	Kelly, 2017	Bronchial biopsy	Inflammatory cell and product: Eosinophil granule protein (EPX)	IHC
Benralizumab	Laviolette, 2013	Bronchial biopsy	Inflammatory cell and product: Eosinophil	IHC
Dupilumab	Jonstam, 2019	Nasal biopsy	Inflammatory cell and product: Eosinophil granule protein IgE Cytokine: IL-4 IL-5 IL-6 IL-10 IL-13 IL-17 IL-33 TNF- α Eotaxin-1 Eotaxin-2 Eotaxin-3 TARC PARC	Multiplex immunoassay
Tralokinumab	Russell, 2018	Bronchial biopsy	Inflammatory cell and product: Eosinophil Neutrophils Lymphocyte (CD3) Macrophages Mast cells Tissue remodelling factors: RBM thickness Intact epithelium Partially intact epithelium Denuded epithelium Epithelial MUC5AC Periostin Collagen type IV Fibronectin Tenascin TGF- β	IHC

IgE = immunoglobulin E; IL = interleukin; TSLP = thymic stromal lymphopoietin; MBP = major basic protein; EPX = eosinophil peroxidase; TARC = thymus and activation-regulated chemokine; PARC = pulmonary and activation-regulated chemokine ; TNF = tumour necrosis factor; RBM = reticular basement membrane ; MUC5AC = mucin-5AC ; TGF = transforming growth factor; IHC = immunohistochemistry; IF = immunofluorescence ; PCR = polymerase chain reaction

Supplementary Table 5 Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	5
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	6, 18
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	19
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6

Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	n/a
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	n/a

