SUPPLEMENTARY FILES

Supplementary file 1 (S1): Search strategy. The following search strategy was developed for PubMed, and applied in all databases taking into account the terminological and technical differences between these databases (Medline (OVID-version), Embase (OVID-version), Web of Science, Cochrane Library, CINAHL, Academic Search Premier and Science Direct; details available from the authors).

("Arthritis, Experimental"[Mesh] OR "experimental arthritis"[tw] OR "Collagen-Induced Arthritis"[tw] OR "Collagen Arthritis"[tw] OR "Adjuvant-Induced Arthritis"[tw] OR "Adjuvant Arthritis"[tw] OR (("Arthritis, Rheumatoid"[Mesh:NoExp] OR "Rheumatoid Arthritis"[tw] OR Rheumatoid Arthrit*[tw]) AND ("Murinae"[mesh] OR "mouse"[tw] OR "mice"[tw] OR "rat"[tw] OR "rats"[tw] OR "Guinea Pigs"[Mesh] OR "Guinea Pigs"[tw] OR "Guinea Pig"[tw] OR "Rabbits"[Mesh] OR "Rabbits"[tw] OR "Rabbit"[tw]))) AND ("Methotrexate"[mesh] OR "Amethopterin"[tw] OR "Methotrexate"[tw] OR "Mexate"[tw] OR "Leflunomide"[supplementary concept] OR "N-(4-trifluoromethyphenyl)-5-methylisoxazole-4-carboxamide"[tw] OR "HWA 486"[tw] OR "HWA-486"[tw] OR "SU101"[tw] OR "Arava"[tw] OR "Leflunomide"[tw] OR "Cyclosporine"[mesh] OR "Cyclosporin"[tw] OR "Ciclosporine"[tw] OR "Neoral"[tw] OR "CyA-NOF"[tw] OR "CyA NOF"[tw] OR "Sandimmune"[tw] OR "Sandimmun"[tw] OR "CsA-Neoral"[tw] OR "CsA Neoral"[tw] OR "CsANeoral"[tw] OR "OL 27-400"[tw] OR "OL 27 400"[tw] OR "OL 27400"[tw] OR "Sulfasalazine"[mesh] OR "Sulfasalazine"[tw] OR "Salicylazosulfapyridine"[tw] OR "Sulphasalazine"[tw] OR "Salazosulfapyridine"[tw] OR "Colo-Pleon"[tw] OR "Colo Pleon"[tw] OR "Pleon"[tw] OR "ratio-Sulfasalazine"[tw] OR "Ulcol"[tw] OR "Ucine"[tw] OR "Azulfidine"[tw] OR "Azulfadine"[tw] OR "Salazopyrin"[tw] OR "Pyralin EN"[tw] OR "Asulfidine"[tw] OR "Sulfasalazin"[tw] OR "Azathioprine"[mesh] OR "Azatioprine"[tw] OR "Azothioprine"[tw] OR "Imurel"[tw] OR "Imuran"[tw] OR "Immuran"[tw] OR "Hydroxychloroquine"[mesh] OR "Hydroxychloroquine"[tw] OR "Oxychlorochin"[tw] OR "Oxychloroquine"[tw] OR "Hydroxychlorochin"[tw] OR "Plaquenil"[tw] OR "TNFR-Fc Fusion protein"[supplementary concept] OR "TNFR-Fc Fusion protein"[tw] OR "TNR-001"[tw] OR "TNR 001"[tw] OR "TNT receptor fusion protein"[tw] OR "TNTR-Fc"[tw] OR "TNF receptor type II-IgG fusion protein"[tw] OR "Recombinant human dimeric TNF receptor type II-IgG fusion protein"[tw] OR "Enbrel"[tw] OR "Etanercept"[tw] OR "Interleukin 1 receptor antagonist protein"[mesh] OR "Interleukin 1 receptor antagonist protein"[tw] OR "Urine-Derived IL1 Inhibitor"[tw] OR "Urine Derived IL1 Inhibitor"[tw] OR "IL1 Febrile Inhibitor"[tw] OR "Urine Interleukin 1 Inhibitor"[tw] OR "Urine IL-1 Inhibitor"[tw] OR "IL-1Ra"[tw] OR "Antril"[tw] OR "Kineret"[tw] OR "Anakinra"[tw] OR "Abatacept"[supplementary concept] OR "Abatacept"[tw] OR "BMS 188667"[tw] OR "BMS-188667"[tw] OR "BMS224818"[tw] OR "BMS-224818"[tw] OR "LEA29Y"[tw] OR "nulojix"[tw] OR "Orencia"[tw] OR "BELATACEPT"[tw] OR "CTLA-4-Ig"[tw] OR "Cytotoxic T lymphocyte-associated antigen 4-immunoglobulin"[tw] OR "CTLA4-Ig immunoconjugate"[tw] OR "CTLA4-Fc"[tw] OR "CTLA4-Ig"[tw] OR "Tocilizumab"[supplementary concept] OR "Tocilizumab"[tw] OR "MRA monoclonal antibody"[tw] OR "atlizumab"[tw] OR "Actemra"[tw] OR

"Rituximab"[supplementary concept] OR "rituximab"[tw] OR "Mabthera"[tw] OR "GP2013"[tw] OR "IDEC-C2B8"[tw] OR "Rituxan"[tw] OR "Prednisone"[Mesh] OR "prednisone"[tw] OR "Dehydrocortisone"[tw] OR "delta-Cortisone"[tw] OR "Prednison"[tw] OR "Sone"[tw] OR "Sterapred"[tw] OR "Ultracorten"[tw] OR "Winpred"[tw] OR "Apo-Prednisone"[tw] OR "Cortan"[tw] OR "Cortancyl"[tw] OR "Panafcort"[tw] OR "Cutason"[tw] OR "Decortin"[tw] OR "Dacortin"[tw] OR "Decortisyl"[tw] OR "Deltasone"[tw] OR "Encortone"[tw] OR "Encorton"[tw] OR "Enkortolon"[tw] OR "Kortancyl"[tw] OR "Liquid Pred"[tw] OR "Meticorten"[tw] OR "Orasone"[tw] OR "Panasol"[tw] OR "Predni Tablinen"[tw] OR "Prednidib"[tw] OR "Predniment"[tw] OR "Pronisone"[tw] OR "Rectodelt"[tw]) NOT (transgen*[ti] OR "Animals, Genetically Modified"[Majr])

Supplementary file 2 (S2). Inclusion criteria for experimental studies reporting:

1. prospective controlled experiments using small animals
2. treatment group with experimentally induced arthritis CIA or AIA model
3. matched control group of animals with induced arthritis which receive control treatment (placebo) or animals with arthritis without any intervention.
4. testing of anti-rheumatic drugs; synthetic DMARDs (*Methotrexate, Leflunomide, Cyclosporine, Sulfasalazine, Azathioprine, Hydroxychloroquine or Prednisolone/Dexamethasone*) or biological DMARDs (*anti-TNF, anti-IL-1, CTLA4-Ig, anti-IL-6 or anti-CD20 monoclonal antibodies)*
5. effects on clinical outcome defined as arthritis severity score, paw swelling or paw volume
6. effects on joint structural changes: histological- (synovial hyperplasia, cell infiltration, pannus formation, oedema, fibrosis, cartilage and bone destruction) or radiological scores (X-ray or microCT)

Supplementary file 3 (S3). Summary of study characteristics of the 22 studies that were included in the meta-analyses.

Study characteristic	Sub-groups	Number of studies	
Model of experimental arthritis	CIA	16	
	AIA	8	
Species	Rats	16	
	Mice	6	
Drugs tested	synthetic DMARDs		Dose mg/kg
	Methotrexate	*10*	*0.1-50mg/kg*
	Leflunomide	*2*	*3.75-10mg/kg*
	Cyclosporine	*1*	*2.5mg/kg*
	Sulfasalazine	*1*	*80mg/kg*
	Azathioprine	*1*	*5mg/kg*
	Hydroxychloroquine	*1*	*25mg/kg*
	Methylprednisolone	*1*	*2mg/kg*
	Dexamethasone	*1*	0.5mg/mg
	Biological DMARDs		Dose mg/kg
	anti-TNF	*5*	*0.75-2mg/kg*
	Anti-IL-1	*4*	*0.1-1mg/kg*
	CTLA4-Ig	*2*	*1-5mg/kg*
Route of administration	Oral	15	
	Subcutaneous	6	
	Intraperitoneal	9	
Treatment duration	1 week	6	
	2-4 weeks	8	

	>1 month	8
Treatment strategy	Prophylactic	16
	Pre-arthritis	9
	Therapeutic	12
Clinical outcome	Arthritis severity score	14
	Paw swelling (mm)	8
	Paw volume (ml)	5
Joint structural changes	(Semi-)quantitative histological data scores:	
	synovial hyperplasia	*5*
	cell infiltration	*10*
	pannus formation	*3*
	oedema	*1*
	fibrosis	*2*
	cartilage destruction	*10*
	bone erosion	*10*
	Quantitative radiographic scores based on:	
	X-ray	8
	microCT	2

CIA = collagen induced arthritis, AIA = adjuvant induced arthritis, DMARDs = disease modifying anti-rheumatic drugs.

Supplementary file 4 (S4). Methodological quality assessment form for study quality and potential risk of bias as judged by the quality of reporting. Each item was scored as 1 if performed and 0 if not reported or not performed. Maximum score was 11 points.

Risk of Bias	Criteria	Explanation
Selection bias	1. Randomization	
	2. Allocation concealment	Concealing the allocation sequence from those assigning animals to experimental and control groups until moment of assessment.
Detection bias	3. Blinding	Keeping the persons who perform the experiment, collect data and assess outcome unaware of the treatment allocation
Other sources of bias	4. Evidence of proper arthritis induction	Histological, macroscopic, microscopic or X-ray evidence
	5. Sample size/power calculations before start of experiment	
	6. Statement regarding potential conflict of interest	
	7. Statement of compliance with animal welfare regulations	
	8. Standardized method data collection	Data collection at predefined time points

9. Validated scoring method for arthritis severity	Semi-quantitative clinical scoring system for each paw in a range from 0-4, caliper measurements of ankle joints, or use of a plethysmometer for measurements of small volume changes in paw volume.
10. Validated scoring method for joint damage	X-ray: modified Larsen scoring method. Histology: semi-quantitative for synovial and extra articular inflammation (in a range from 0-3) and bone erosions (in a range from 0-5).
11. Clear data presentation	Numbers of animals per group, data of both treatment and control group available.

Supplementary file S5 (S5). List of all 35 studies that met the inclusion criteria based on full paper assessment. Twenty-two studies [20-41] are included in the meta-analyses.

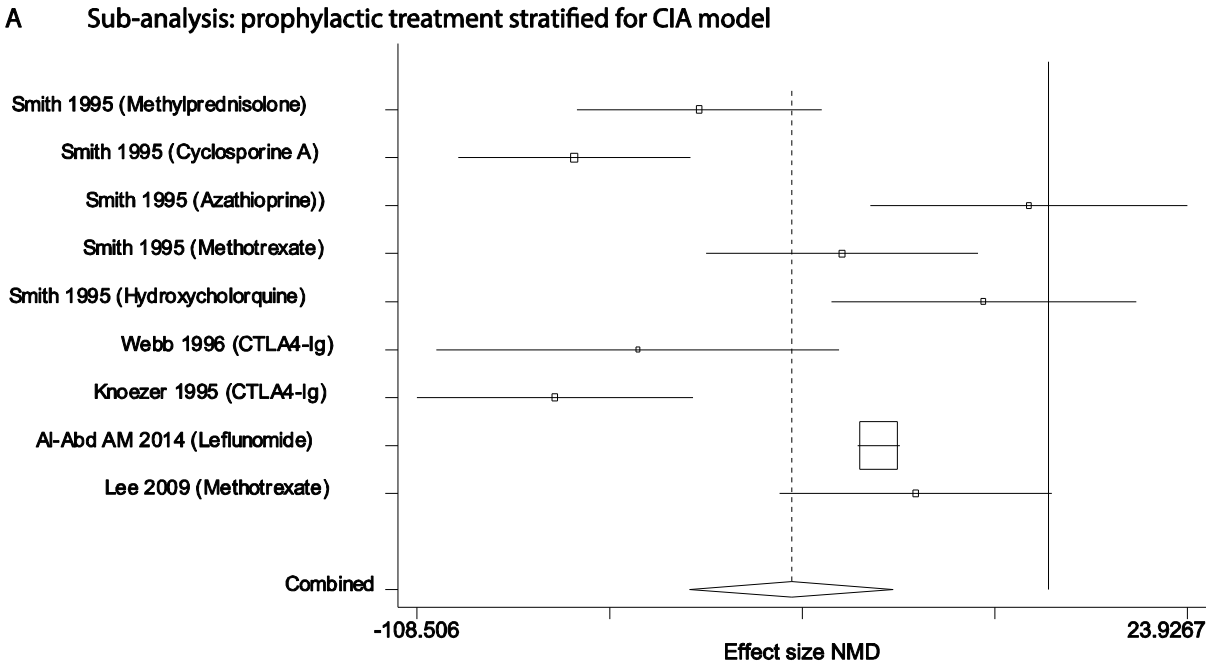
Author	Type of intervention	Model	Species	DMARD tested			Treatment duration				Outcome parameters	Quality score
Morgan 2001 [20]	prophylactic	AIA	rats	Methotrexate	0.3-10mg/kg/week	oral	6 weeks	9-20	yes	X-ray	paw swelling (mm) clinical score (0-16)	9
Lee 2009 [21]	prophylactic	CIA	mice	Methotrexate	50mg/kg/ week	oral	3 weeks	10	yes	no	clinical score (0-16), incidence (%)	8
Rovensky 2009 [22]	prophylactic	AIA	rats	Methotrexate	0,6mg/kg/week	oral	7 weeks	8	no	X-ray	paw volume (ml)	7
Rovensky 2003 [23]	prophylactic	AIA	rats	Cyclosporin A	2.5mg/kg/day	oral	7 weeks	10	no	X-ray	paw swelling (mm)	6
				Methotrexate	0.6mg/kg/week	oral	7 weeks	10				
Smith 1996 [24]	prophylactic	CIA	rats	methylprednisolone	2mg/kg daily	oral	4 weeks	10	yes	X-ray	clinical score (0-16), Δ paw volume	7
				Methotrexate	0.15mg/kg daily	oral	4 weeks	10				
				Azathioprine	5mg/kg daily	oral	4 weeks	10				
				Hydroxycholorquine	25mg/kg daily	oral	4 weeks	10				
Al-Abd AM 2014 [25]	prophylactic	CIA	mice	Leflunomide	3.75mg/kg/week	i.p.	5 weeks	10	yes	X-ray	clinical score (0-16)	6
Zuurmond 2011 [26]	prophylactic	AIA	rats	Anti-IL-1	0.1-2.8mg/kg/hour	s.c.	2 weeks	8	yes	no	clinical score (0-16), paw swelling (mm)	8
				Dexamethasone	0.5mg/kg/day	s.c.	2 weeks	8				
Webb 1996 [27]	prophylactic, therapeutic	CIA	mice	CTLA4-Ig	2 µg/kg 3x week	i.p.	2 weeks	10-15	yes	no	paw swelling (mm), clinical score (0-12)	7
Knoerzer 1995 [28]	prophylactic	CIA	rats	CTLA4-Ig	1mg/kg 3x week	i.p.	2 weeks	6	yes	no	clinical score (0-16)	8
Gowayed 2015 [29]	pre-arthritis	AIA	rats	Leflunomide	10mg/kg 3x week	oral	2 weeks	8	no	X-ray	paw swelling (mm)	8
Sakuma 2001 [30]	pre-arthritis	AIA	rats	Methotrexate	0.1-1mg/kg	oral	1 week	10	yes	no	paw volume (ml)	7
Le 2009 [31]	pre-arthritis	CIA	rats	Methotrexate	1mg/kg/ week	i.p.	once	8	yes	micro-CT	clinical score (0-8), paw swelling (mm)	6
Du 2008 [32]	pre-arthritis	CIA	rats	Methotrexate	1mg/kg 3x week	oral	3 weeks	7	no	micro-CT	clinical score (0-8)	7
Setoguchi 2010 [33]	pre-arthritis	CIA	rats	Etanercept	0.3mg/kg/day	s.c.	1 week	8-13	yes	no	paw volume (ml)	7
Bendele 2000 [34]	pre-arthritis	CIA AIA	rats	Anti-IL-1	20-100mg /kg/week	s.c.	1 week	8	yes	no	paw volume (ml)	7
Fener 1990 [35]	therapeutic	CIA	rats	Sulfazalazine	80mg/kg/ 5x week	oral	17 weeks	18	yes	X-ray	clinical score (0-12)	8

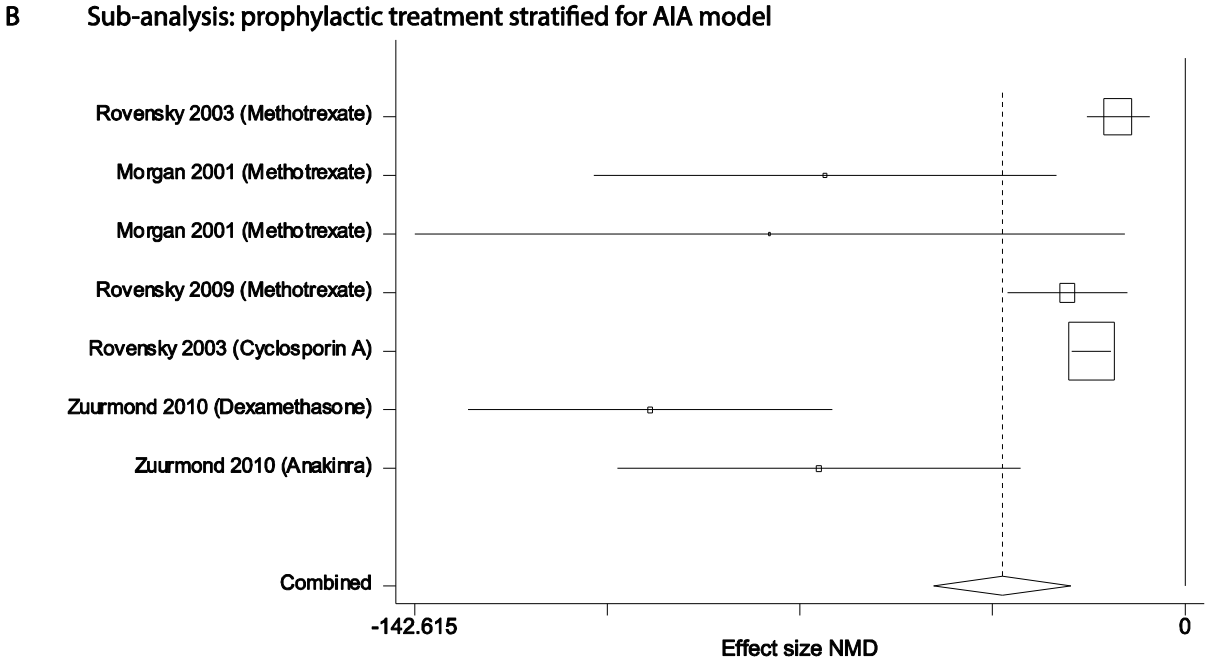
Zhang 2013 [36]	therapeutic	CIA	mice	Methotrexate	2mg/kg/ week	oral	6 weeks	10	yes	no	clinical score (0-16)	6
				Etanercept	4mg/kg 3x week	i.p.	6 weeks	10				
Saadat 2005 [37]	therapeutic	CIA	rats	Methotrexate	0,3mg/kg 3x week	i.p.	2 weeks	8	yes	X-ray	clinical score (0-16)	9
O'Valle 2015 [38]	therapeutic	CIA	mice	Etanercept	40µg/kg/ week	i.p.	4 weeks	20	yes	no	paw swelling (mm), clinical score (0-16)	8
Joosten 1996 [39]	therapeutic	CIA	mice	Anti-IL1	0.6mg/day	i.p.	1 week	10	yes	no	clinical score (0-8)	6
				Etanercept	75µg/day	i.p.	1 week	10				
Yang 2010 [40]	therapeutic	CIA	rats	Etanercept	1-9mg/kg week	s.c.	2 weeks	10	yes	no	paw swelling (mm)	8
Bendele 1999 [41]	therapeutic, pre-arthritis	CIA, AIA	rats	Anti-IL-1	100µl/hour	s.c.	1 week	7-8	yes	no	paw volume (ml)	6
Yi 2014 [14]	prophylactic	CIA	mice	Etanercept	0.5-8µg/kg 3x week	i.p.	5 weeks	-	yes	microCT	clinical score (0-16) and incidence (%)	6
Chen 2012 [15]	therapeutic	CIA	rats	Metrotrexate	0.1mg/kg/ week	oral	4 weeks	9-20	yes	no	clinical score (0-16)	7
Xinqiang 2010 [16]	therapeutic	CIA	rats	Metrotrexate	1.0mg/kg/ week	oral	4 weeks	10	yes	X-ray	clinical score (0-16)	7
Kliwinski 2005 [17]	prophylactic	CIA	rats	CTLA4-Ig	1mg/kg 3x week	i.p.	1 week	-	yes	microCT	paw volume (ml)	6
Hsu 2010 [18]	pre-arthritis	CIA	rats	Etanercept	6mg/kg 3x week	s.c.	4 weeks	9	yes	microCT	clinical score (0-8), paw swelling (mm)	7
Stolina 2009 [19]	prophylactic	CIA, AIA	rats	Anti-IL-1	100mg/kg/ day	s.c.	1 week	8	yes	X-ray	paw volume (ml), paw swelling (mm)	8
Kim YH 2015 [42]	pre-arthritis	CIA	rats	Methotrexate	1.5mg/kg/ day	oral	1 week	-	yes	X-ray, microCT	only histology and radiological outcome	5
Yao 2013 [43]	therapeutic	CIA	rats	Leflunomide,	130mg/kg/day	oral	3 weeks	10	yes	X-ray	clinical score (0-16)	5
				Metrotrexate	3.8mg/kg/ week	oral	3 weeks	10				
Teramachi 2011 [44]	prophylactic	AIA	rats	Metrotrexate	1mg/kg/ week	i.p..	3 weeks	-	yes	X-ray	paw volume (ml)	4
Baggott 2007 [45]	prophylactic	AIA	rats	Metrotrexate	0.3-3mg/kg/ week	i.p.	4 weeks	8-18	yes	x-ray	only radiological outcome	5
Brauer 1994 [46]	prophylactic	AIA	rats	Cyclosporin A	5mg/kg/ day	i.p.	2-4 weeks	10-12	yes	no	paw swelling (mm)	2
Wooley 1993 [47]	pre-arthritis	CIA	mice	Anti-IL-1	2µg/kg/ day	i.p.	2 weeks	-	yes	no	clinical score (0-12), paw swelling (mm)	5
Brahn 1991 [48]	prophylactic	CIA	rats	Cyclosporin A	4-10mg/kg/ day	i.p.	3 weeks	18	yes	X-ray	clinical score (0-16) and incidence (%)	5
				Methotrexate	30-80µg/kg/ day	i.p.	3 weeks	18				

Supplementary file 6 (S6). Number and percentage of publications reporting individual components of the study quality checklist for the 22 studies that were included in the meta-analyses. The quality checklist is depicted in **S4**.

Quality criteria	Total studies (n=22)	%
Randomisation	15	69
Allocation concealment	9	40
Blinding	13	59
Evidence of proper arthritis induction	20	90
Sample size/power calculations	0	0
Statement regarding potential conflict of interest	7	32
Statement of compliance with animal welfare	17	77
Standardized method for data collection	18	81
Validated scoring method for arthritis severity	21	100
Validated scoring method for joint damage	19	86
Clear data presentation (group size, treatment and control groups)	14	63

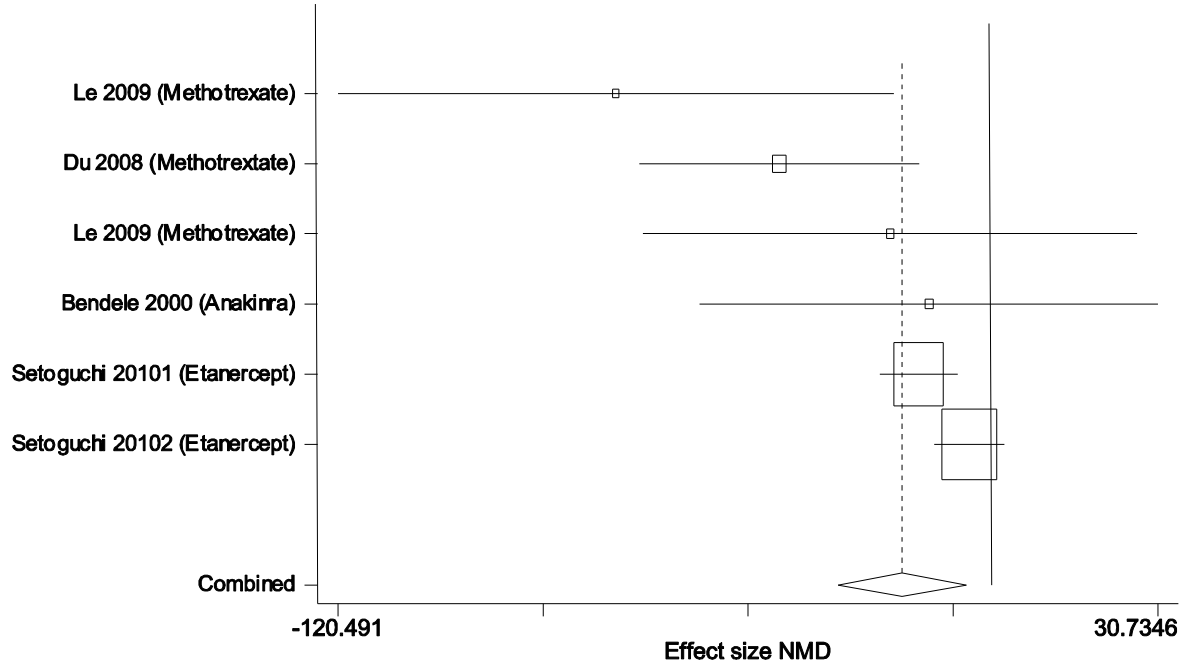
Supplementary file 7 (S7). Sub-analysis of prophylactic intervention stratified for CIA and AIA. Effect of prophylactic intervention on arthritis severity stratified for the CIA model (9 studies). The pooled effect size of normalized mean difference (NMD) in arthritis severity is -44.1 (95CI=-61.6 to -26.6, z-value=-4.9, p<0.001) (A). Stratified meta-analysis of the different experiments investigating the effects of different anti-rheumatic drugs further specified for the AIA model (7 studies), pooled estimate -33.9 (95CI=-46.6 to -21.2, z-value = -5.2, p<0.001) (B).





Supplementary file 8 (S8). Sub-analysis of pre-arthritis intervention stratified for CIA and AIA. Effect of prophylactic intervention on arthritis severity stratified for the CIA model (6 studies). The pooled effect size of normalized mean difference (NMD) in arthritis severity is -16.5 (95CI=-16.5 to -28.3, z-value=-4.6, p=0.006) (A). Stratified meta-analysis of the different experiments investigating the effects of different anti-rheumatic drugs further specified for the AIA model (3 studies), pooled estimate -21.3 (95CI=-44.0 to 1.5, p=0.07).

A Sub-analysis: pre-arthritis treatment stratified for CIA model



B Sub-analysis: pre-arthritis treatment stratified for AIA model