Tolerance and immune regulation in rheumatoid arthritis

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Chapter 7

Possibilities for preventive treatment in Rheumatoid Arthritis? Lessons from Experimental Animal Models of Arthritis: a Systematic Literature Review and Meta-analysis

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ABSTRACT

Objective

Current research in rheumatoid arthritis focusses on preclinical disease phases as it is hypothesized that early preclinical treatment might prevent progression to full-blown disease. Since performance of studies in pre-arthritis phases in humans is challenging, animal models offer an opportunity to evaluate preventive treatments. We performed a systematic literature review and summarized treatment effects during different stages of arthritis development in animal models.

Methods

Eight medical literature databases were systematically searched. Studies were selected if they reported effects of synthetic or biological disease-modifying anti-rheumatic drugs in animal models of arthritis (collagen-induced arthritis and adjuvant-induced arthritis) on arthritis severity, as measured with arthritis severity scores, paw swelling or paw volume. Quality was assessed using an eleven item checklist. Study characteristics were extracted and effect sizes obtained in high-quality studies were summarized in meta-analyses. Studies were categorized in three groups; prophylactic (prior to generation of autoantibody response), pre-arthritis (after induction of autoantibody response) and therapeutic intervention (after arthritis development).

Results

Out of 1415 screened articles, 22 studies (including n=712 animals) were eligible, of good quality and included in meta-analyses. Prophylactic (16 experiments, n=312 animals) and pre-arthritis treatment (9 experiments, n=156 animals) both were associated with a reduction of arthritis severity ($p<0.001$ and $p=0.005$ respectively). Stratified analyses for different anti-rheumatic drugs initiated in the pre-arthritis phase suggested higher efficacy of methotrexate than of anti-TNF.

Conclusions

Data of experimental studies in animal models of arthritis suggest that prophylactic and pre-arthritis treatment strategies are effective and hint at differences in efficacy between anti-rheumatic drugs.

Introduction

During recent years, research in the field of rheumatoid arthritis (RA) has focused on the earliest stages of the disease. This has provided novel insights into the immunological processes that precede the transition from healthy to established disease. Currently, the field of RA is moving from disease suppressive treatments to prevention strategies, focussing on initiation of treatment in pre-arthritis phases. Within RA it has been shown that a prolonged symptom duration at treatment start is associated with a worse disease outcome, including a lower chance on achieving disease modifying anti-rheumatic drug (DMARD)-free sustained remission [1-4]. Therefore it is hypothesized that treatment initiation during pre-arthritis stages might result in an improved efficacy in preventing disease chronicity [5]. Within type I diabetes preventive trials have shown efficacy of very early intervention to prevent full blown disease [6-8].

To evaluate of very early treatment initiation can also prevent the development of RA, several placebo-controlled randomized controlled clinical trials have been initiated recently. These clinical trial studies will investigate the therapeutic potential of several immunomodulatory agents such as rituximab (PRAIRI study: NTR No. 1969), abatacept (APIPPRA study: ISRCTN No. 46017566 and ARIAA study: EudraCT No. 2014-000555-93), hydroxychloroquine (StopRA trial; NCT No. 02603146) and methotrexate (TREAT EARLIER; NTR No. 4853) in individuals at a risk of RA. A study evaluating the immunomodulatory effect of atorvastatin in seropositive arthralgia patients has also been initiated (STAPRA study: NTR No. 22389). Interestingly, design of these trials does not follow the normal development of phase 1,2 and subsequently phase 3 trials. Moreover, dosages used are from other indications targeting different biology and the length of treatment is relatively randomly defined. The performance of randomized clinical trials on preventative treatment strategies in individuals at risk for RA is therefore difficult to interpret. Early identification and recruitment of patients at risk for RA is difficult and execution of these studies is time consuming. Altogether, it will take several years before the majority of these currently ongoing clinical trials are completed and the results are known. Likewise, because of difference in study-design and patient selection it will be difficult to compare side-by-side which intervention will be most effective in reaching sustained symptom- and drug-free benefit.

Animal models of arthritis can function as innovative tools to study the potential of preventive therapies. Animal studies provide an opportunity to study the developing (auto)immune response at a very early disease phase and the translation of different stages of experimental arthritis development to the evolution of human disease might provide valuable information regarding possibilities of disease prevention. Murine models of arthritis are scientifically well defined, highly reproducible, genetic identical, represent different disease pathways relevant for RA and are readily available. While current animal

research mainly focusses on testing anti-rheumatic drugs in established disease, it is unknown whether preventive treatment in mouse models is also effective. In addition, it is unknown if the efficacy of prophylactic or pre-arthritis intervention depends on the type of anti-rheumatic drug used. These questions prompted us to perform a systematic literature review and summarize the knowledge on the efficacy of treatment initiated before arthritis was clinically evident. Our first aim was to evaluate if prophylactic or pre-arthritis treatment is effective in animal models of arthritis. The second aim was to evaluate the efficacy of different synthetic and biological DMARDs treatments initiated in pre-arthritis phases.

We focused on two widely accepted experimental models for RA; collagen-induced arthritis (CIA) and adjuvant-induced arthritis (AIA), both models are based on immunization-induced arthritis. In the CIA model, arthritis is induced by immunizations with cartilage proteins causing a break of tolerance and an immune-mediated inflammatory attack on the joints [9 10]. Animals receive type II collagen emulsified in complete Freund's adjuvant typically followed by a second injection three weeks later, leading to the development of chronic destructive arthritis. There are three developmental stages of arthritis in the CIA model; an induction phase, a pre-arthritis phase were auto-immunity is present in the absence of clinical symptoms, and established arthritis [11]. The AIA-model requires intradermal immunization with mycobacterial cell wall components suspended in mineral oil causing an acute and systemic inflammation. The development of arthritis in the AIA-model is thought to depend on a heat shock protein specific T-cell response. In contrast to CIA, AIA is self-limiting and contains three developmental stages of arthritis; incubation phase, pre-arthritis phase and a peak phase followed by a gradual regression of inflammation [12]. Thus, these experimental models of arthritis have clearly defined developmental stages that can be identified by immunological disease markers. According to these developmental stages of arthritis we discerned three types of treatment; prophylactic (prior to generation of autoantibodies), pre-arthritis (after induction of an autoantibody response) and therapeutic intervention (after arthritis development) (Figure 1). We performed a systematic literature review and summarized the effects of treatment initiated at these stages.

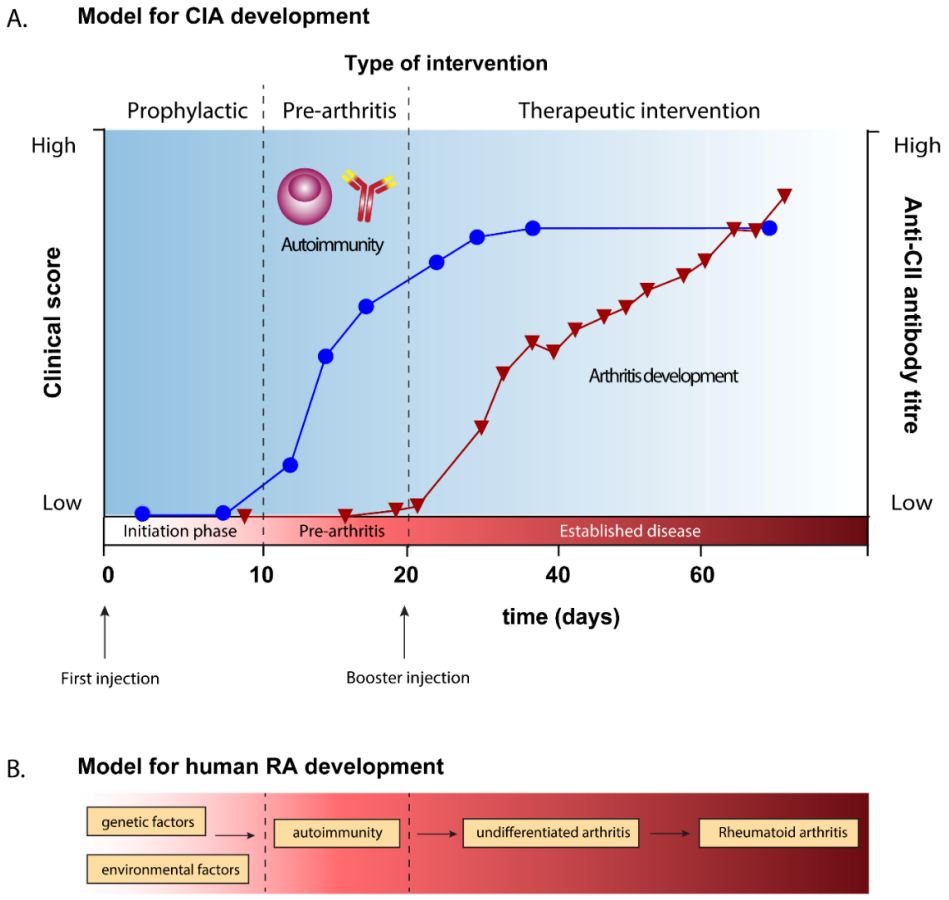


Figure 1. Schematic representation of developmental stages of collagen-induced arthritis (CIA) and human RA. Type of intervention during different developmental stages of experimental arthritis in the collagen-induced arthritis (CIA) model. Arthritis is generally induced by immunization with CII emulsified in complete Freund's adjuvant followed a booster injection three weeks later of CII in incomplete Freund's adjuvant. After immunization with type II collagen, during the initiation phase, auto-immunity towards collagen will develop (blue circles). Drug intervention during this time period is referred to as prophylactic treatment. This first stage is followed by a pre-arthritis phase which is characterized by the onset of autoimmunity and is marked by the development of autoantibodies against type II collagen which occurs around day 10. The time period in which auto-immunity is present and arthritis is still absent is referred to as the pre-arthritis period. Intervention during this pre-arthritis disease stage is described as pre-arthritis treatment. The time period of pre-arthritis arthritis is followed by the onset of arthritis (red triangles), which occurs around day 20 and leads to chronic destructive arthritis. Drug intervention at the established arthritis stage is described as therapeutic intervention (A). Schematic representation of human RA development. Genetic and environmental factors drive the onset of auto-immunity which subsequently leads to undifferentiated arthritis and finally full-blown disease (B).

Methods

Search strategy

Eight bibliographic databases (PubMed, Medline, Embase, Web of Science, Cochrane Library, CINAHL, Academic Search Premier and Science Direct), were searched to identify studies investigating treatment strategies in animal models for experimental arthritis (search conducted April 14, 2016). A systematic search strategy was developed for PubMed (see online supplementary file 1) and was subsequently applied in all other databases. Search terms were: rheumatoid arthritis, experimental models for arthritis, therapeutic intervention DMARDs (Methotrexate, Leflunomide, Cyclosporine, Sulfasalazine, Azathioprine or Hydroxychloroquine, Prednisolone) or biologicals (anti-TNF, Anti-IL-1, CTLA4-Ig, anti-IL-6 or anti-CD20) combined with the Boolean operators AND/OR.

Inclusion and exclusion criteria

The inclusion criteria are described in table 1. In short, we included published peer-reviewed studies reporting the effect of therapeutic intervention (synthetic or biological DMARDs) in the most commonly used in vivo models of experimental arthritis (CIA and AIA). Furthermore experiments should be prospectively controlled and information on clinical outcome and joint structural changes should be provided. Abstracts were assessed based on type of research (animal studies), drugs tested, outcome measures (arthritis severity) and duplicates. Full papers were assessed to identify experimental arthritis models, study design, treatment and control groups, therapeutic interventions and outcomes measurements used in the studies.

Table 1. 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)

Outcome measurements

Furthermore, studies were only selected if the following clinical outcomes were evaluated: arthritis severity scores (ordinal scale), paw swelling (mm) or paw volume (ml), or outcome measurements for joint structural changes by quantitative histological or radiographic scores measured with X-ray or microCT. An overview of the outcome measurements is presented in Table 2.

Table 2. Summary of study characteristics of the 22 studies included in the meta-analyses.

Study characteristic	Sub-groups	Number of studies	
Model of experimental arthritis	CIA	16	
	AIA	8	
Species	Rat	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.

Data extraction

We extracted individual study characteristics from each publication, and, where a publication reported more than 1 experiment, these data were also extracted and considered independent experiments. Extracted data included: experimental arthritis model, species, number of animals per group, drug and dose, route and time of drug administration, clinical-, histological- or radiological scores. Where arthritis severity measurements were performed serially, we only extracted the final time point.

Quality assessment of methodology

Study quality and risk of selection- and detection bias was assessed by a modified 11-point-item checklist, adapted from CAMARADES (Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental studies) [13]. The checklist comprises items of study methodology: randomisation (1); allocation concealment (2); blinding (3); evidence of induced arthritis (macroscopic, histological or radiological) (4), sample size/power calculations (5), statement of conflict of interest (6), statement of compliance with animal welfare regulations (7), standardized method for data collection (8), (semi)quantitative scoring method for disease activity (9), (semi)quantitative scoring method for joint damage (10) and clear data presentation (11). Each item was scored as 1 if the data were reported satisfactorily and 0 if not (unclear risk of bias) and maximum score was 11. The median quality score of six was considered to be sufficiently high quality for further analysis. An overview of the checklist is depicted in table 3**.**

Table 3. 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.

Categorisation of studies according to type of intervention

Studies were discerned into three groups according to the time point of treatment initiation: (1) Prophylactic treatment (day 0-9): therapy is initiated prior to injection of arthritis-stimulators or after injection of arthritis-stimulator but prior to the development of a systemic autoimmune response which is characterized by production of autoantibodies. (2) Pre-arthritis treatment (day 10-20): start of therapy after the development of auto-immunity but before the onset of clinically evident arthritis. (3) Therapeutic treatment (>20 day): initiation of treatment after the onset of arthritis, which occurs generally around day 21-22.

Meta-analysis

Only studies with high methodological quality (score ≥ 6) were summarized in meta-analyses. Furthermore; studies that did not provide standard deviations (SD) or standard errors of the mean (SEM) were excluded for the meta-analyses. To compare the severity of arthritis in the treatment group to an arthritic untreated control animal, we calculated the normalized mean difference (NMD) of arthritis clinical score, paw volume or paw swelling. The NMD effect size in the treated animals is calculated as a proportion of the mean in the control group [14]. Similarly, we calculated the NMD for each experimental comparison as the proportional improvement in the treated group compared with the control group, along with the standard error of the estimate. To account for anticipated heterogeneity we applied the DerSimonian and Laird random effects model [15] to calculate an overall treatment effect, this random-effects model for meta-analysis uses both within-the study variance and the between-study variance. We used Cochran's Q to calculate heterogeneity of the studies [16].

Results

Study selection and study quality

Our systematic search identified a total of 3486 titles from eight databases (Figure 2), 35 studies met the inclusion criteria based on full paper assessment. A summary overview of these 35 studies is presented in Table 1, for the complete overview see online supplementary Table S5. For further selection of studies for meta-analysis, 28 studies were considered to be of sufficient high methodological quality (score ≥ 6). Six of these studies did not report SDs or SEMs and therefore and could not be included in the meta-analyses [17-22]. Finally, the results of 22 studies, using a total of 712 animals (control animals, n =263 and treated animals, n = 449), were summarized in meta-analyses.

The most frequently used model of experimental arthritis was the CIA model (CIA 16 studies, AIA 8 studies). 16 studies used rats and 6 studies used mice (table 2). Thirteen out of 22 publications (59%) reported the blinded assessment of outcome, 15 (68%) reported randomization, and none reported performing a sample size calculation (table 4**)**. The outcome summarized in the meta-analysis was the proportion reduction of arthritis severity (expressed in NMD).

Histological- and radiographic scores were not consistently scored in uniform scoring scales. Therefore it was impossible to perform meta-analyses on these outcomes.

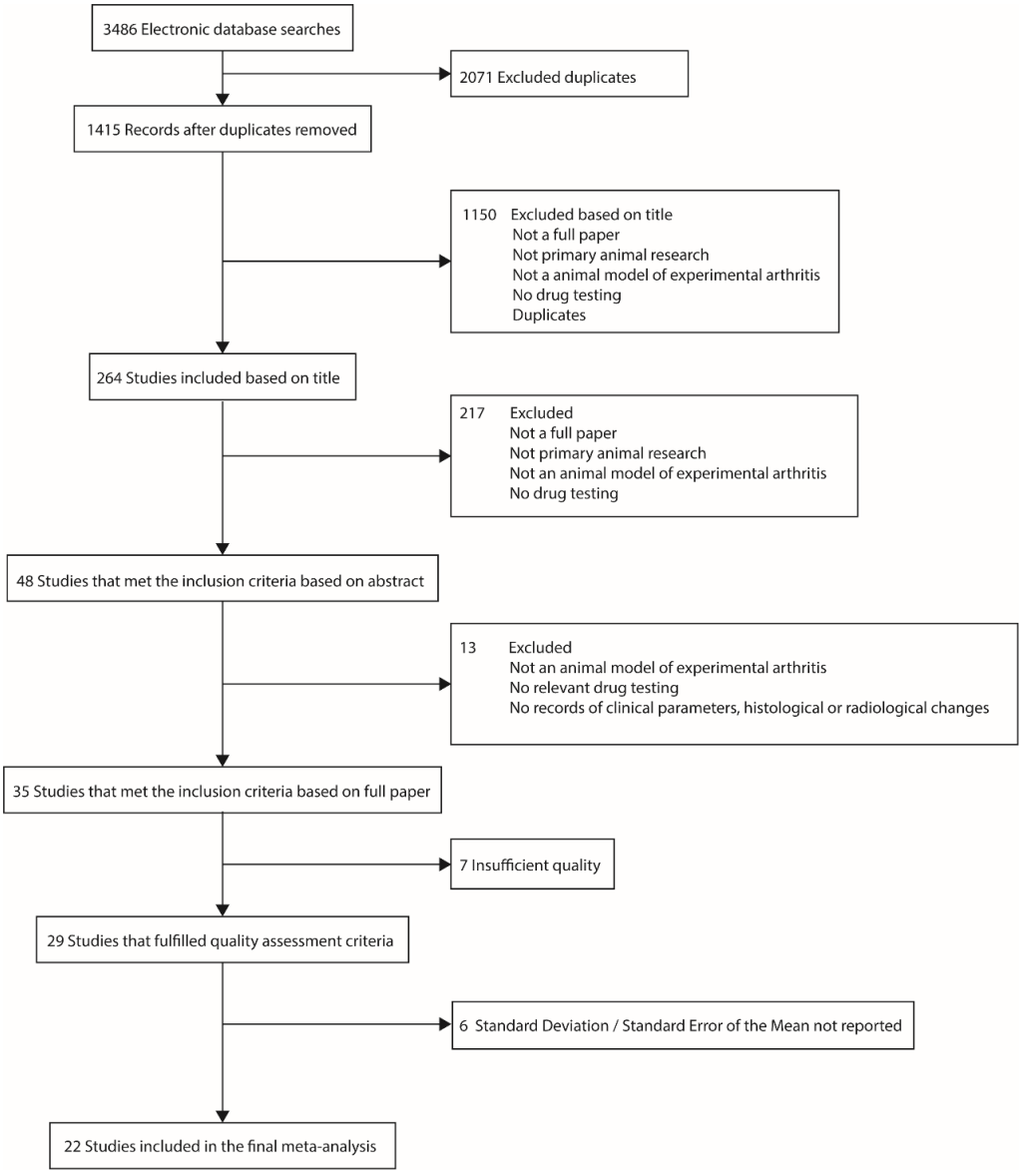


Figure 2. Flow diagram of study selection.

Table 4. 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 table 3.

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

Table 5. 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 [23]	prophylactic	AIA	rats	Methotrexate	6 weeks	paw swelling (mm) clinical score (0-16)	9
Lee 2009 [24]	prophylactic	CIA	mice	Methotrexate	3 weeks	clinical score (0-16), incidence (%)	8
Rovensky 2009 [25]	prophylactic	AIA	rats	Methotrexate	7 weeks	paw volume (ml)	7
Rovensky 2003 [26]	prophylactic	AIA	rats	Cyclosporin A Methotrexate	7 weeks 7 weeks	paw swelling (mm)	6
Smith 1996 [27]	prophylactic	CIA	rats	Methylpredni-solone Methotrexate Azathioprine Hydroxy-chloroquine	4 weeks 4 weeks 4 weeks 4 weeks	clinical score (0-16), Δ paw volume	7
Al-Abd AM 2014 [28]	prophylactic	CIA	mice	Leflunomide	5 weeks	clinical score (0-16)	6
Zuurmond 2011 [29]	prophylactic	AIA	rats	Anti-IL-1 Dexametha-sone	2 weeks 2 weeks	clinical score (0-16), paw swelling (mm)	8
Webb 1996 [30]	prophylactic, therapeutic	CIA	mice	CTLA4-Ig	2 weeks	paw swelling (mm), clinical score (0-12)	7
Knoerzer 1995 [31]	prophylactic	CIA	rats	CTLA4-Ig	2 weeks	clinical score (0-16)	8
Gowayed 2015 [32]	pre-arthritis	AIA	rats	Leflunomide	2 weeks	paw swelling (mm)	8
Sakuma 2001 [33]	pre-arthritis	AIA	rats	Methotrexate	1 week	paw volume (ml)	7

Le 2009 [34]	pre-arthritis	CIA	rats	Methotrexate	once	clinical score (0-8), paw swelling (mm)	6
Du 2008 [35]	pre-arthritis	CIA	rats	Methotrexate	3 weeks	clinical score (0-8)	7
Setoguchi 2010 [36]	pre-arthritis	CIA	rats	Etanercept	1 week	paw volume (ml)	7
Bendele 2000 [37]	pre-arthritis	CIA AIA	rats	Anti-IL-1	1 week	paw volume (ml)	7
Fener 1990 [38]	therapeutic	CIA	rats	Sulfazalazine	17 weeks	clinical score (0-12)	8
Zhang 2013 [39]	therapeutic	CIA	mice	Methotrexate Etanercept	6 weeks 6 weeks	clinical score (0-16)	6
Saadat 2005 [40]	therapeutic	CIA	rats	Methotrexate	2 weeks	clinical score (0-16)	9
O'Valle 2015 [41]	therapeutic	CIA	mice	Etanercept	4 weeks	paw swelling (mm), clinical score (0-16)	8
Joosten 1996 [42]	therapeutic	CIA	mice	Anti-IL1 Etanercept	1 week 1 week	clinical score (0-8)	6
Yang 2010 [43]	therapeutic	CIA	rats	Etanercept	2 weeks	paw swelling (mm)	8
Bendele 1999 [44]	therapeutic, pre-arthritis	CIA, AIA	rats	Anti-IL-1	1 week	paw volume (ml)	6
Yi 2014 [17]	prophylactic	CIA	mice	Etanercept	5 weeks	clinical score (0-16) and incidence (%)	6
Chen 2012 [18]	therapeutic	CIA	rats	Metrotrexate	4 weeks	clinical score (0-16)	7
Xinqiang 2010 [19]	therapeutic	CIA	rats	Metrotrexate	4 weeks	clinical score (0-16)	7
Kliwinski 2005 [20]	prophylactic	CIA	rats	CTLA4-Ig	1 week	paw volume (ml)	6

Hsu 2010 [21]	pre-arthritis	CIA	rats	Etanercept	4 weeks	clinical score (0-8), paw swelling (mm)	7
Stolina 2009 [22]	prophylactic	CIA, AIA	rats	Anti-IL-1	1 week	paw volume (ml), paw swelling (mm)	8
Kim YH 2015 [45]	pre-arthritis	CIA	rats	Methotrexate	1 week	only histology and radiological outcome	5
Yao 2013 [46]	therapeutic	CIA	rats	Leflunomide, Metrotrexate	3 weeks 3 weeks	clinical score (0-16)	5
Teramachi 2011 [47]	prophylactic	AIA	rats	Metrotrexate	3 weeks	paw volume (ml)	4
Baggott 2007 [48]	prophylactic	AIA	rats	Metrotrexate	4 weeks	only radiological outcome	5
Brauer 1994 [49]	prophylactic	AIA	rats	Cyclosporin A	2-4 weeks	paw swelling (mm)	2
Wooley 1993 [50]	pre-arthritis	CIA	mice	Anti-IL-1	2 weeks	clinical score (0-12), paw swelling (mm)	5
Brahn 1991 [51]	prophylactic	CIA	rats	Cyclosporin A Methotrexate	3 weeks 3 weeks	clinical score (0-16) and incidence (%)	5

Effect of intervention on clinical outcome

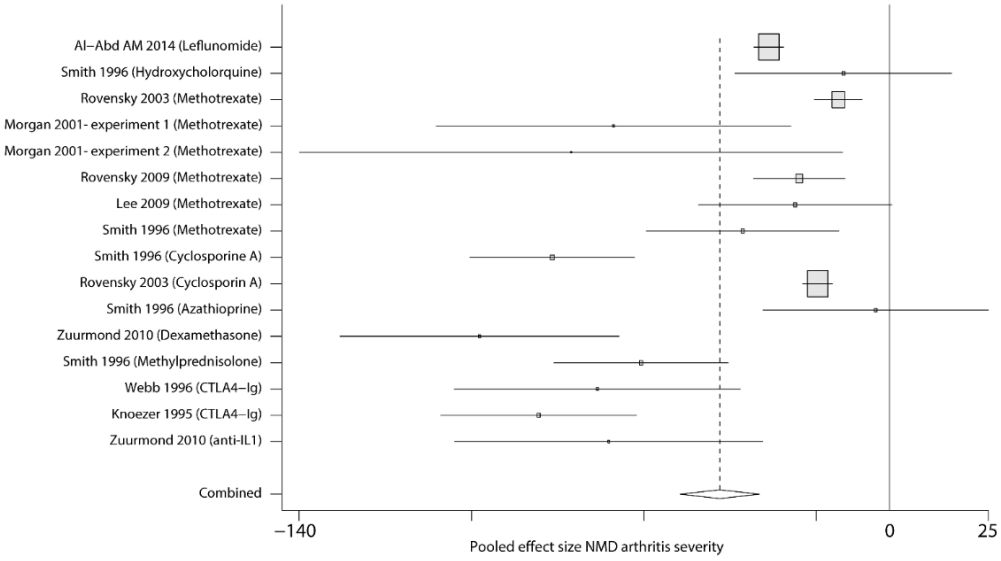
The treatment efficacy on arthritis severity was studied per disease stage.

Prophylactic treatment

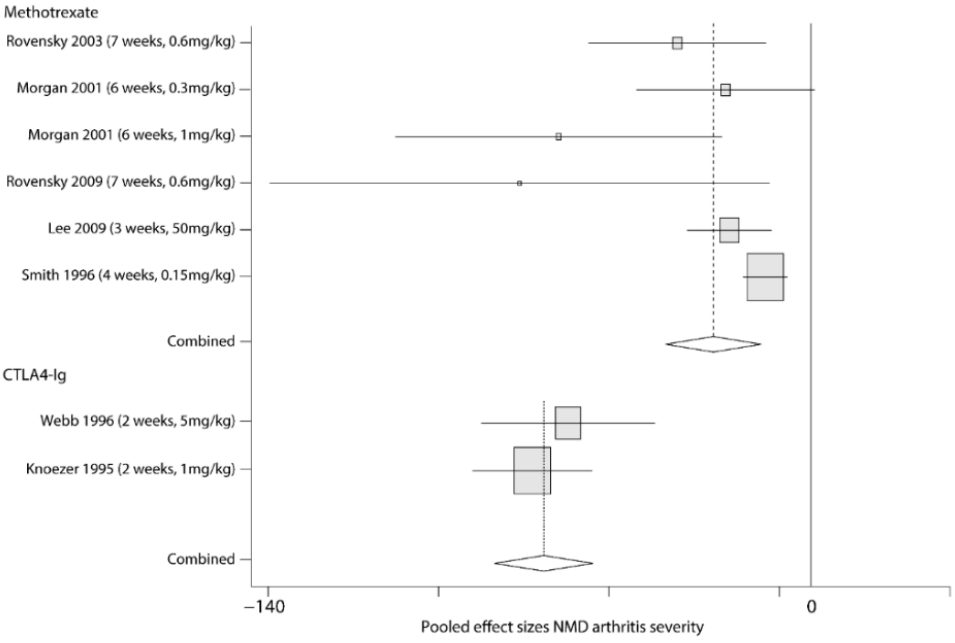
Sixteen experiments (including 312 animals) studied the effect of prophylactic intervention on the severity of arthritis (Figure 3A). Several DMARDs were tested in a prophylactic setting (*methotrexate* [6 experiments] [23-27]*, leflunomide* [1 experiment] [28]*, cyclosporine A* [2 experiments] [26 27]*, azathioprine* [1 experiment] [27]*, hydroxycholoroquine* [1 experiment] [27]*,* and *methylprednisolone/dexamethasone* [2 experiments] [27 29], *anti-IL1* [1 experiments] [29] and *CTLA4-Ig* [2 experiments] [30 31]. The combined effect size of the different studies indicated that prophylactic intervention is associated with a reduction of arthritis severity in animal models of arthritis ($p<0.001$). Prophylactic treatment with both methotrexate ($p< 0.001$) and CTLA4-Ig ($p<0.001$) was significantly associated with a reduction in arthritis severity (Figure 3B).

Figure 3. Prophylactic intervention in experimental models of arthritis. Effect of prophylactic intervention on arthritis severity reported in 16 individual experiments using synthetic or biological DMARDs. The pooled effect size of normalized mean difference (NMD) in arthritis severity is -40.1 (95CI=-50.6 to -31.4, z-value=-8.2, $p<0.001$) (A). Stratified meta-analysis of the different experiments investigating the effects of different anti-rheumatic drugs further specified for methotrexate, pooled estimate -26.0 (95CI=-38.6 to -13.5, $p<0.001$) and CTLA4-Ig, pooled estimate -80.2 (95CI=-100.0 to -60.7, $p<0.001$). Treatment length was on average 5.5 weeks for methotrexate and 2 weeks for CTLA4-Ig (B).

A Prophylactic treatment with DMARDs



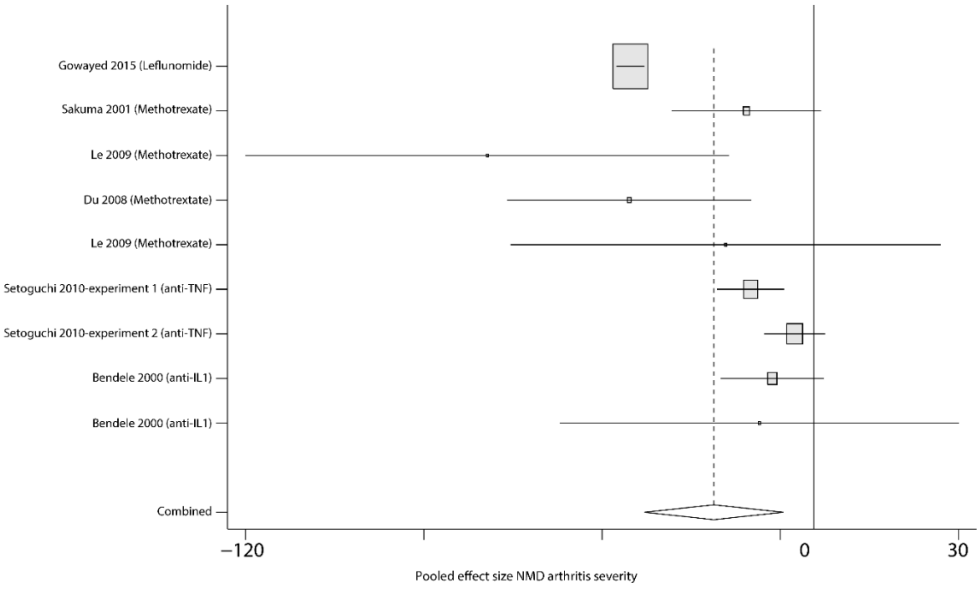
B Prophylactic treatment stratified for DMARD



Pre-arthritis treatment

Nine experiments (including 156 animals) studied the effect of early pre-arthritis treatment on arthritis severity (Figure 4A). DMARDs tested were *leflunomide* [1 experiments] [32], *methotrexate* [4 experiments] [33-35]*, anti-TNF* [2 experiments] [36] *, anti-IL1* [2 experiments] [37]. A meta-analysis of these studies demonstrated that pre-arthritis intervention is associated with a reduction of arthritis severity in animal models of arthritis (p=0.005). Stratified analysis for most commonly used DMARDs revealed that treatment with methotrexate was significantly associated with less arthritis severity (p<0.01), while no statistically significant results were obtained for anti-TNF (p=0.065) and anti-IL1 (p=0.098) (Figure 4B). None of the animal studies performed in a pre-arthritis stage evaluated a reduction of arthritis incidence or a delay in arthritis onset after short-term treatment.

A Pre-arthritis treatment with DMARDs



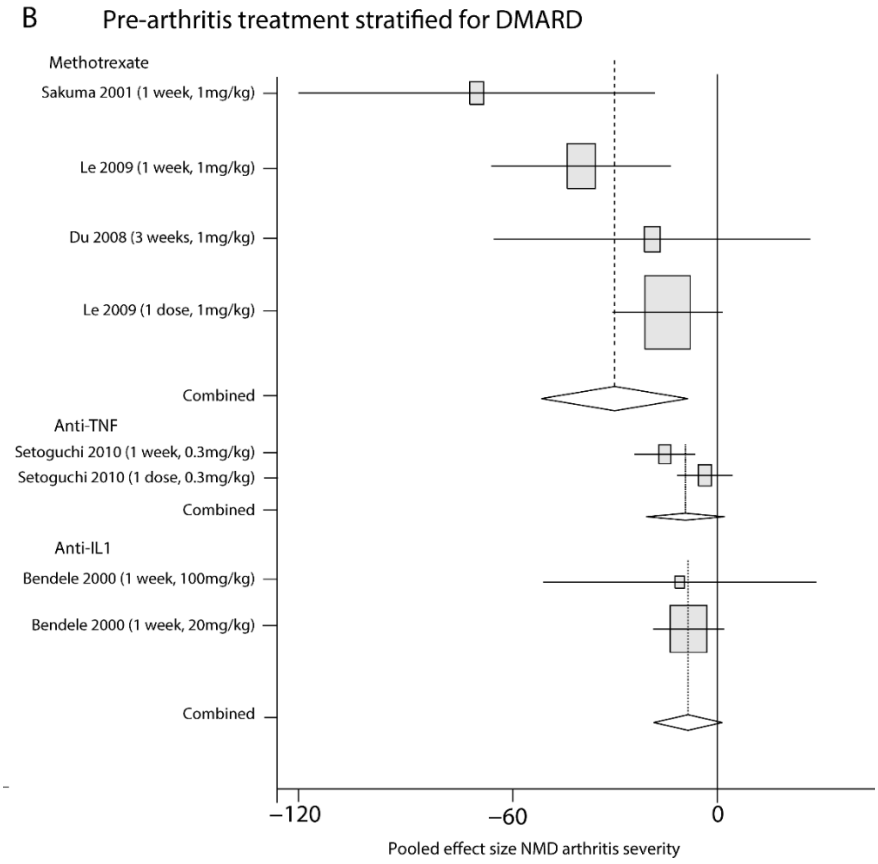
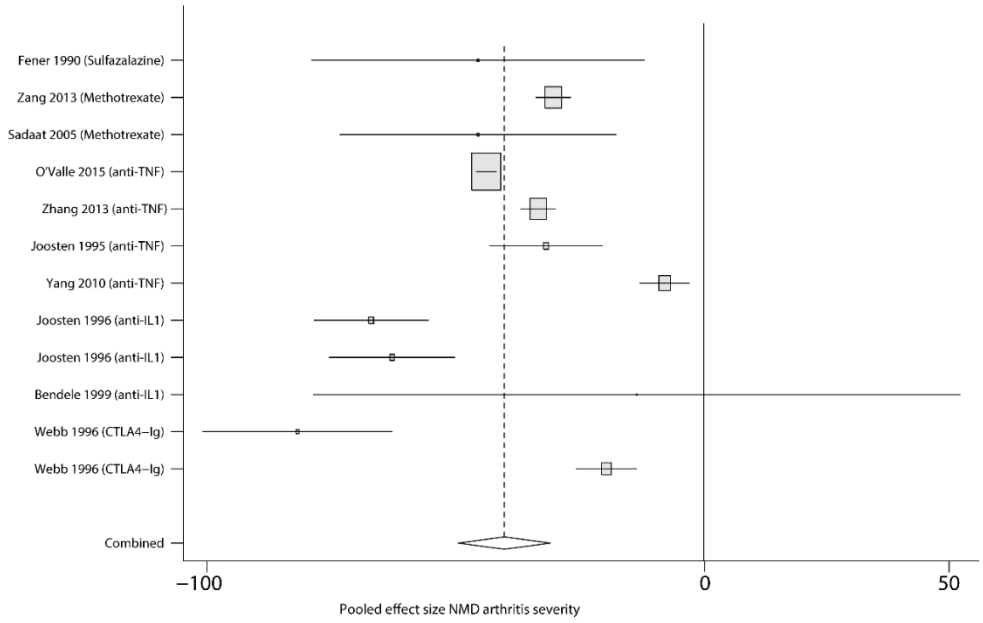


Figure 4. Pre-arthritis intervention in experimental models of arthritis. Effect of pre-arthritis intervention on arthritis severity reported in 9 individual experiments using synthetic or biological DMARDs in a pre-arthritis phase of arthritis. The pooled effect size of normalized mean difference (NMD) in arthritis severity is -21.2 (95CI=-35.9 to -6.5, z-value=-2.8, p=0.005) (A). Stratified meta-analysis of the different experiments investigating the effects of different anti-rheumatic drugs further specified for methotrexate pooled estimate -29.5 (95CI=-50.6 to -8.4, p=0.006), anti-TNF pooled estimate -8.6 (95CI=-17.7 to 0.5, p=0.065), and anti-IL1 pooled estimate -9.0 (95CI=-19.6 to 1.6, p=0.098). Treatment length was on average 1 week for methotrexate, anti-TNF and anti-IL1 (B).

Therapeutic treatment

Twelve experiments (including 244 animals) studied the effect of therapeutic treatment in established arthritis (Figure 5A). Anti-rheumatic drugs tested in established disease were *sulfasalazine* [1 experiment] [38], *methotrexate* [2 experiments] [39 40] *, anti-TNF* [4 experiments] [39 41-43]*, anti-IL1* [3 experiments] [42 44] and *CTLA4-Ig* [2 experiments] [30]. Therapeutic treatment with methotrexate ($p<0.001$), anti-TNF ($p<0.001$) anti-IL1 ($p<0.001$) and CTLA4-Ig ($p<0.001$) in established disease were all significantly associated with a reduced arthritis severity (Figure 5B).

A Therapeutic intervention with DMARDs in established disease



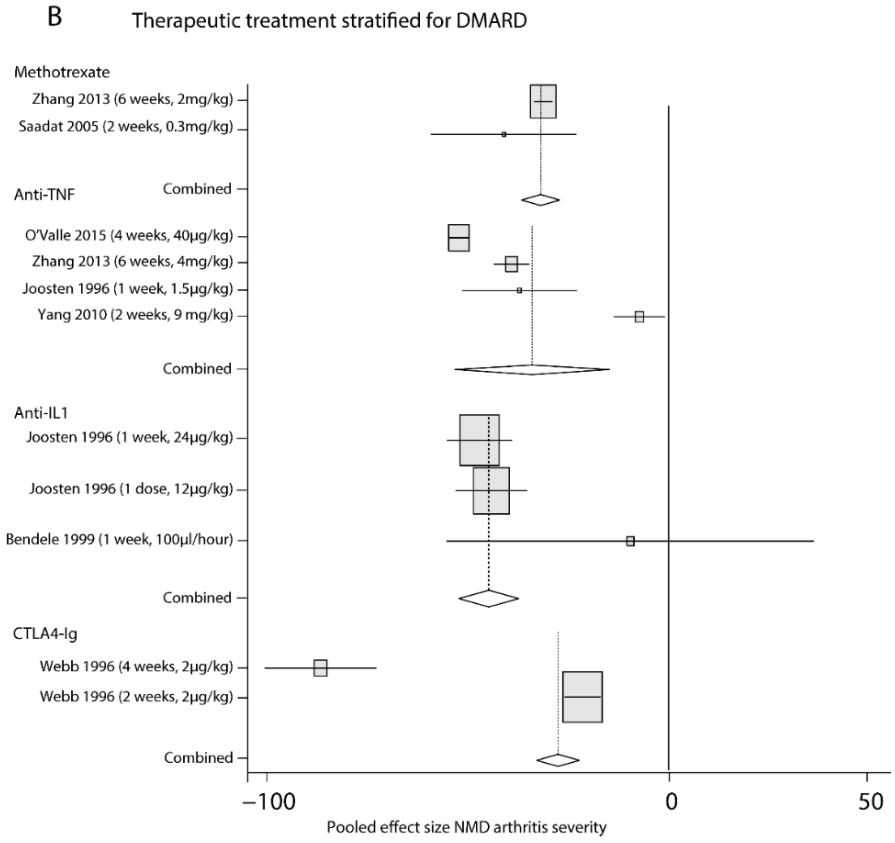


Figure 5. Therapeutic intervention in experimental models of arthritis. Effect of therapeutic intervention on arthritis severity reported in 12 individual experiments using synthetic or biological DMARDs in established arthritis. The pooled effect size of normalized mean difference (NMD) in arthritis severity is -44.2 (95CI=-54.4 to -34.0, z-value=-8.5, p<0.001) (A). Stratified meta-analysis of the different experiments investigating the effects of different anti-rheumatic drugs further specified for methotrexate -34.4 (95CI=-42.5 to -26.3, p<0.001), anti-TNF -32.2 (95CI=-49.2 to -15.1, p<0.001), anti-IL1 -70.6 (95CI=-79.9 to -61.3, p<0.001)and CTLA4-Ig -27.9 (95CI=-34.3 to -21.5, p<0.001). Treatment length was on average 4 weeks for methotrexate, 3.3 weeks for anti-TNF, 1 week for anti-IL1 and 3 weeks for CTLA4-Ig (B).

Sub-analyses

Since CIA and AIA somewhat differ in disease pathology we have performed separate sub-analyses for these models which showed similar results as that of the total group (Fig. 6, 7).

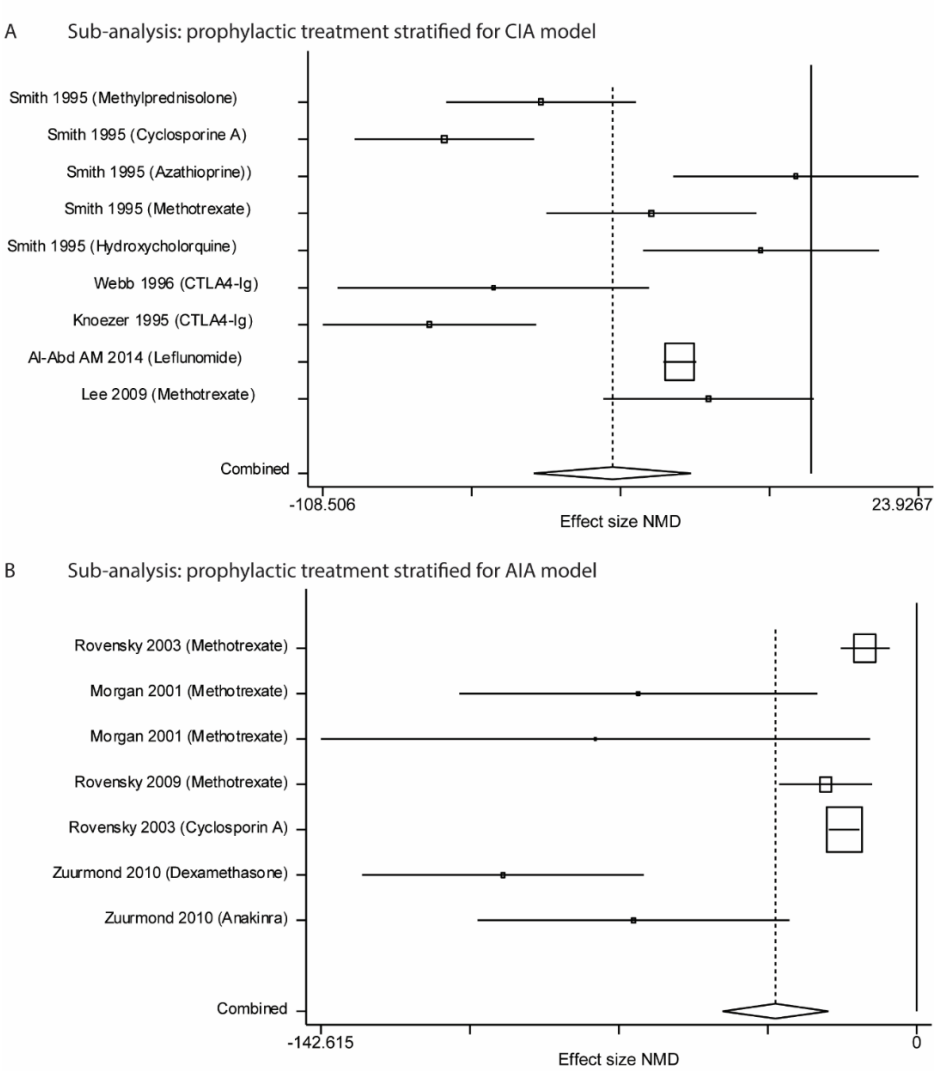


Figure 6. Sub-analysis of prophylactic intervention stratified for CIA and AIA. Effect of prophylactic intervention on arthritis severity stratified for the CIA model (9 studies). The pooled effect size of normalized mean difference (NMD) in arthritis severity is -44.1 (95CI=-61.6 to -26.6, z-value=-4.9, 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).

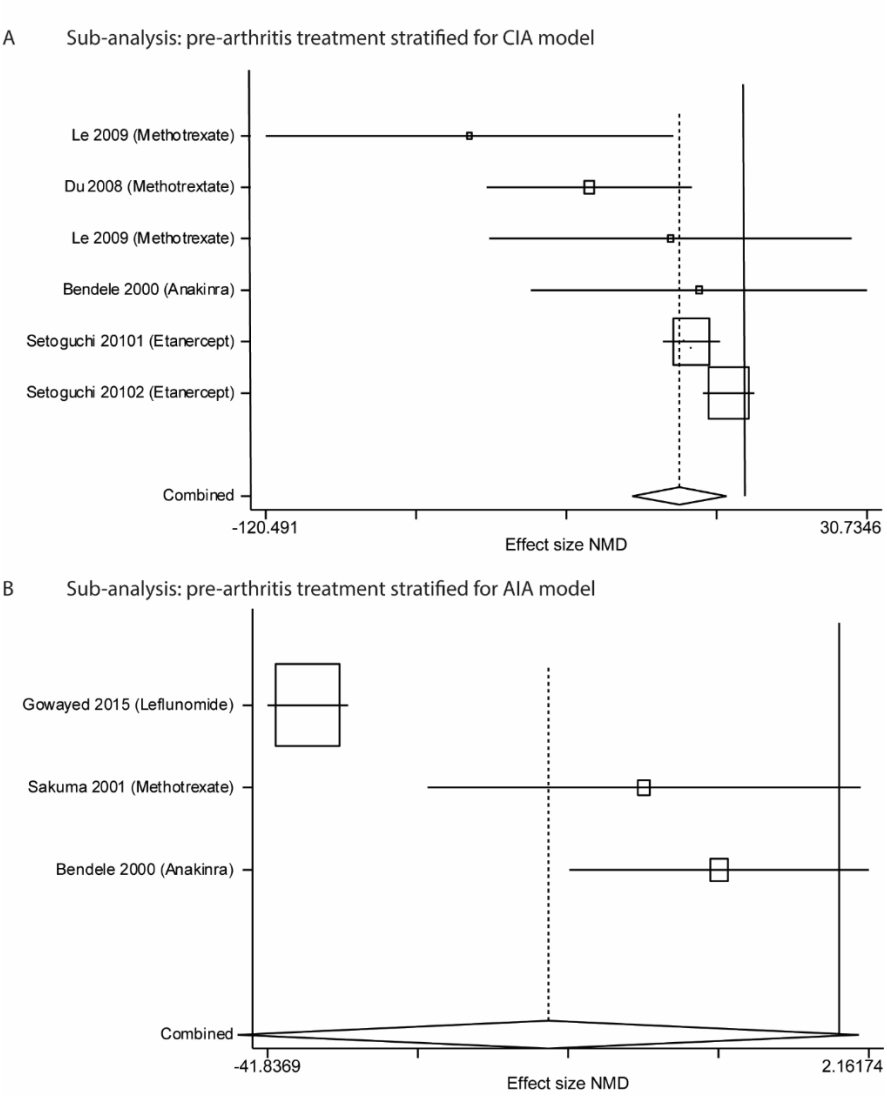


Figure 7. Sub-analysis of pre-arthritis intervention stratified for CIA and AIA. Effect of prophylactic intervention on arthritis severity stratified for the CIA model (6 studies). The pooled effect size of normalized mean difference (NMD) in arthritis severity is -16.5 (95CI=-16.5 to -28.3, z-value=-4.6, 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).

Discussion

The present systematic literature review and the meta-analyses of data on treatment in animal models of experimental arthritis reveal that 'prophylactic and pre-arthritis treatment strategies' are effective and result in less severe disease. Currently research emphasises on early identification of individuals at risk for developing RA with as ultimate goal the reduction of disease severity or even to prevent clinically manifest disease. Although it will take years before the results of current clinical trials will be at the stage of publishing, results of the present study in animals support the concept that very early treatment may be effective. Observational studies in RA patients have provided evidence that support the concept of a therapeutic 'window of opportunity'. The period in which the disease is most susceptible to treatment is presumed to consist of the first three months after symptom onset [52 53]. Although some studies have treated patients rapidly after arthritis has become clinically evident [54-57], studies that initiate treatment already in pre-arthritis phases are more challenging to perform, because of the difficulty to identify patients with arthralgia and a high predicted risk for developing RA. Some of the preventive studies in human RA aim to target autoantibody positive subjects with arthralgia and we hypothesize that this phase corresponds to intervention in the pre-arthritis phase of the CIA model. But, although the CIA mouse model is widely used to mimic the antibody-dependent process of RA pathogenesis, anti-collagen antibodies are not the most prominent antibodies in human RA and conflicting results on ACPA induction in CIA exist [58]. Thus, while CIA and AIA are informative, these models only partially resemble the situation in human RA and reflect only some of the basic disease mechanisms and molecular pathways involved in RA development. Despite the disparities between animal models of arthritis and RA, animal models of arthritis are of interest, and the summarized data suggest that very early treatment is effective.

The second aim was to evaluate the effect of different medications in animal models. The most frequently studied DMARDs were methotrexate and anti-TNF. In humans several other drugs (abatacept, hydroxychloroquine, atorvastatin) are now also being investigate in pre-arthritis phases. Studies investigating the effect of hydroxychloroquine on animal models are limited; in our meta-analysis we included only one study that evaluated hydroxychloroquine on CIA. Our literature search was limited to studies that tested DMARDs, statins were not included. Nevertheless, the effects of statins have been studied, with contrasting results; some studies reported anti-inflammatory effects [44, 45] while others pointed to an accelerated onset of CIA in mice [46]. In our meta-analysis we observed a higher effect for metrotrexate then for anti-TNF, this may suggest that methotrexate is more disease-modifying in this very early disease phase. Though, formal conclusions on the difference in efficacy or treatment dose cannot be made and translation to the human setting is limited. None of the pre-arthritis studies compared the different medications

head-to-head. We distinguished treatment started in the initiation phase (prophylactic intervention) and in the phase autoantibodies had developed (pre-arthritis intervention). Meta-analyses suggested that treatment started in both phases was effective. However, none of the studies performed side-by-side comparisons of prophylactic, pre-arthritis and therapeutic interventions. Therefore we cannot conclude if the first two strategies are more effective than treatment initiated in the established disease phase. Similarly, the results obtained on prophylactic strategies cannot be compared to those of pre-arthritis strategies. There are more limitations. Surprisingly, the majority of studies that aimed to test the efficacy of prophylactic treatment used an extended treatment period (average of 4.6 weeks, thus continuing within the phase of established disease). Principally, treatment is preventive if given in pre-clinical phases only. Studies that initiated treatment at a pre-arthritis stage did treat animals with a short course of (at average) 1 week and still observed less severe arthritis in the clinical phase of the disease [33-37]. Furthermore none of the studies evaluated the occurrence of clinical arthritis. Hence there are no data from animal models to conclude if clinical disease can be prevented. For human translation, it would be most interesting to determine whether preventive treatment can actually reverse autoimmunity and prevent RA.

The studies that were evaluated were heterogeneous in several aspects. The use of standard operating procedures for validation of results is crucial to reduce study heterogeneity. Using a 11-point-item quality checklist we aimed to select studies that had a reliable study design; despite the funnel used, still considerable differences were present in the experiments that were included in the meta-analysis. Mice as well as rats were studied. In addition, results of two different animal models (CIA, AIA) were evaluated. Sub-analyses stratified for CIA and AIA, however, showed similar results as that of the total group. Furthermore, arthritis severity was assessed in different ways. Although a validated method was use to compared these outcomes (normalized mean difference), this adds to the heterogeneity. For these different reasons, the NMDs –including those resulting from the meta-analyses performed for different disease phases and for the different treatments- should not be compared in their effect size. Thus although the efficacy of different treatment strategies cannot be compared, this study provides an overview of all available data on animal models and provides an evaluation if treatment initiated in very early disease phases is effective.

Based on the present evaluation of the available literature we conclude that the ideal experiment on animals for this research question should still be performed. This study should test interventions side-by-side in different disease phases and with a similar treatment schedule to be able to compare efficacy. In addition, a head-to-head comparison of DMARDs like methotrexate, abatacept, rituximab and anti-TNF with a defined duration could answer the question whether pre-arthritis treatment can reverse autoimmunity and prevent arthritis in mouse models. Proper controls are sham-treated mice that develop a

natural course of experimental-induced arthritis. The presence and evolution of systemic autoimmunity in CIA, defined by autoantibody production against CII, should be determined and linked to clinical outcome. Ideally, the effect of (a short course of) pre-arthritis treatment should be evaluated over an extended period of time to determine whether there is long-term arthritis-free-'benefit' or a delayed onset of arthritis.

In conclusion, this study systematically evaluated the results of animal studies and suggested that both prophylactic and pre-arthritis treatment strategies lead to a significant reduction of arthritis severity scores and hits at a possibility for preventive therapy in RA. However, larger studies are needed to confirm this.

References

1. van der Linden MP, le Cessie S, Raza K, et al. Long-term impact of delay in assessment of patients with early arthritis. Arthritis and rheumatism 2010;**62**(12):3537-46.
2. Anderson JJ, Wells G, Verhoeven AC, et al. Factors predicting response to treatment in rheumatoid arthritis: the importance of disease duration. Arthritis and rheumatism 2000;**43**(1):22-9 .
3. Feldman DE, Bernatsky S, Houde M, et al. Early consultation with a rheumatologist for RA: does it reduce subsequent use of orthopaedic surgery? Rheumatology 2013;**52**(3):452-9.
4. Symmons DP, Jones MA, Scott DL, et al. Longterm mortality outcome in patients with rheumatoid arthritis: early presenters continue to do well. The Journal of rheumatology 1998;**25**(6):1072-7
5. van Vollenhoven RF, Nagy G, Tak PP. Early start and stop of biologics: has the time come? BMC medicine 2014;**12**:25.
6. Knip M, Virtanen SM, Seppa K, et al. Dietary intervention in infancy and later signs of beta-cell autoimmunity. The New England journal of medicine 2010;**363**(20):1900-8.
7. Keymeulen B, Vandemeulebroucke E, Ziegler AG, et al. Insulin needs after CD3-antibody therapy in new-onset type 1 diabetes. The New England journal of medicine 2005;**352**(25):2598-608.
8. Orban T, Bundy B, Becker DJ, et al. Costimulation modulation with abatacept in patients with recent-onset type 1 diabetes: follow-up 1 year after cessation of treatment. Diabetes care 2014;**37**(4):1069-75.
9. Holmdahl R, Andersson M, Goldschmidt TJ, et al. Type II collagen autoimmunity in animals and provocations leading to arthritis. Immunological reviews 1990;**118**:193-232
10. Pearson CM. Development of arthritis, periarthritis and periostitis in rats given adjuvants. Proceedings of the Society for Experimental Biology and Medicine Society for Experimental Biology and Medicine 1956;**91**(1):95-101
11. Batsalova T, Lindh I, Backlund J, et al. Comparative analysis of collagen type II-specific immune responses during development of collagen-induced arthritis in two B10 mouse strains. Arthritis research & therapy 2012;**14**(6):R237.
12. Kim EY, Chi HH, Bouziane M, et al. Regulation of autoimmune arthritis by the pro-inflammatory cytokine interferon-gamma. Clinical immunology 2008;**127**(1):98-106.
13. Macleod MR, O'Collins T, Howells DW, et al. Pooling of animal experimental data reveals influence of study design and publication bias. Stroke; a journal of cerebral circulation 2004;**35**(5):1203-8.
14. Vesterinen HM, Sena ES, Egan KJ, et al. Meta-analysis of data from animal studies: a practical guide. Journal of neuroscience methods 2014;**221**:92-102 doi: 10.1016/j.jneumeth.2013.09.010[published Online First: Epub Date]|.
15. DerSimonian R, Laird N. Meta-analysis in clinical trials. Controlled clinical trials 1986;**7**(3):177-88
16. Cochran WG. The Combination of Estimates from Different Experiments. Biometrics 1954;**10**(1):101-29.

17. Yi H, Kim J, Jung H, et al. Induced production of anti-etanercept antibody in collagen-induced arthritis. Mol Med Rep 2014;**9**(6):2301-08.
18. Chen Z, Tu S, Hu Y, et al. Prediction of response of collagen-induced arthritis rats to methotrexate: an (1)H-NMR-based urine metabolomic analysis. J Huazhong Univ Sci Technolog Med Sci 2012;**32**(3):438-43.
19. Xinqiang S, Fei L, Nan L, et al. Therapeutic efficacy of experimental rheumatoid arthritis with low-dose methotrexate by increasing partially CD4+CD25+ Treg cells and inducing Th1 to Th2 shift in both cells and cytokines. Biomed Pharmacother 2010;**64**(7):463-71.
20. Kliwinski C, Kukral D, Postelnek J, et al. Prophylactic administration of abatacept prevents disease and bone destruction in a rat model of collagen-induced arthritis. J Autoimmun 2005;**25**(3):165-71.
21. Hsu YH, Chang MS. Interleukin-20 antibody is a potential therapeutic agent for experimental arthritis. Arthritis and rheumatism 2010;**62**(11):3311-21.
22. Stolina M, Schett G, Dwyer D, et al. RANKL inhibition by osteoprotegerin prevents bone loss without affecting local or systemic inflammation parameters in two rat arthritis models: comparison with anti-TNFalpha or anti-IL-1 therapies. Arthritis research & therapy 2009;**11**(6):R187.
23. Morgan SL, Baggott JE, Bernreuter WK, et al. MTX affects inflammation and tissue destruction differently in the rat AA model. The Journal of rheumatology 2001;**28**(7):1476-81
24. Lee JD, Huh JE, Baek YH, et al. The efficacy and mechanism action of RvCSd, a new herbal agent, on immune suppression and cartilage protection in a mouse model of rheumatoid arthritis. J Pharmacol Sci 2009;**109**(2):211-21.
25. Rovensky J, Stancikova M, Rovenska E, et al. Treatment of rat adjuvant arthritis with flavonoid (Detralex), methotrexate, and their combination. Ann N Y Acad Sci 2009;**1173**:798-804.
26. Rovensky J, Svik K, Stancikova M, et al. Effect of immunostimulatory ribomunyl on the preventive treatment of rat adjuvant arthritis with cyclosporine and methotrexate. The Journal of rheumatology 2003;**30**(9):2027-32.
27. Smith RJ, Sly LM. Type II collagen-induced arthritis in the diabetic-resistant biobreeding rat: inflammatory and histopathological features of joint pathology and effects of antiinflammatory and antirheumatic drugs on this chronic arthritic process. J Pharmacol Exp Ther 1996;**277**(3):1801-13
28. Al-Abd AM, Al-Abbasi FA, Nofal SM, et al. Nimesulide improves the symptomatic and disease modifying effects of leflunomide in collagen induced arthritis. PLoS One 2014;**9**(11):e111843.
29. Zuurmond AM, Koudijs A, van EB, et al. Integration of efficacy, pharmacokinetic and safety assessment of interleukin-1 receptor antagonist in a preclinical model of arthritis. Regul Toxicol Pharmacol 2011;**59**(3):461-70.
30. Webb LM, Walmsley MJ, Feldmann M. Prevention and amelioration of collagen-induced arthritis by blockade of the CD28 co-stimulatory pathway: requirement for both B7-1 and B7-2. Eur J Immunol 1996;**26**(10):2320-28.

31. Knoerzer DB, Karr RW, Schwartz BD, et al. Collagen-induced arthritis in the BB rat. Prevention of disease by treatment with CTLA-4-Ig. J Clin Invest 1995;**96**(2):987-93.
32. Gowayed MA, Refaat R, Ahmed WM, et al. Effect of galantamine on adjuvant-induced arthritis in Rats. Eur J Pharmacol 2015.
33. Sakuma S, Nishigaki F, Magari K, et al. FK506 is superior to methotrexate in therapeutic effects on advanced stage of rat adjuvant-induced arthritis. Inflamm Res 2001;**50**(10):509-14
34. Le GB, Soltner E, Charrier C, et al. A combination of methotrexate and zoledronic acid prevents bone erosions and systemic bone mass loss in collagen induced arthritis. Arthritis research & therapy 2009;**11**(6):R185.
35. Du F, Lu LJ, Fu Q, et al. T-614, a novel immunomodulator, attenuates joint inflammation and articular damage in collagen-induced arthritis. Arthritis research & therapy 2008;**10**(6):R136.
36. Setoguchi C, Tsuji F, Katsuta O, et al. Combined effects of bucillamine and etanercept on a rat type II collagen-induced arthritis model. Mod Rheumatol 2010;**20**(4):381-88.
37. Bendele AM, Chlipala ES, Scherrer J, et al. Combination benefit of treatment with the cytokine inhibitors interleukin-1 receptor antagonist and PEGylated soluble tumor necrosis factor receptor type I in animal models of rheumatoid arthritis. Arthritis and rheumatism 2000;**43**(12):2648-59.
38. Fener P, Bannwarth B, Gillet P, et al. Influence of sulfasalazine on established collagen arthritis in rats. Clinical and experimental rheumatology 1990;**8**(2):167-70
39. Zhang L, Li P, Song S, et al. Comparative efficacy of TACI-Ig with TNF-alpha inhibitor and methotrexate in DBA/1 mice with collagen-induced arthritis. Eur J Pharmacol 2013;**708**(1-3):113-23.
40. Saadat F, Cuzzocrea S, Di PR, et al. Effect of pyrimethamine in experimental rheumatoid arthritis. Med Sci Monit 2005;**11**(8):BR293-BR99 doi: 7042 [pii][published Online First: Epub Date]|.
41. O'Valle F, Peregrina M, Crespo-Lora V, et al. Osteoarticular Expression of Musashi-1 in an Experimental Model of Arthritis. Biomed Res Int 2015;**2015**:681456 doi: 10.1155/2015/681456 [doi][published Online First: Epub Date]|.
42. Joosten LA, Helsen MM, van de Loo FA, et al. Anticytokine treatment of established type II collagen-induced arthritis in DBA/1 mice. A comparative study using anti-TNF alpha, anti-IL-1 alpha/beta, and IL-1Ra. Arthritis and rheumatism 1996;**39**(5):797-809
43. Yang T, Wang Z, Wu F, et al. A variant of TNFR2-Fc fusion protein exhibits improved efficacy in treating experimental rheumatoid arthritis. PLoS computational biology 2010;**6**(2):e1000669.
44. Bendele A, McAbee T, Sennello G, et al. Efficacy of sustained blood levels of interleukin-1 receptor antagonist in animal models of arthritis: comparison of efficacy in animal models with human clinical data. Arthritis and rheumatism 1999;**42**(3):498-506.
45. Kim YH, Kang JS. Effect of methotrexate on collagen-induced arthritis assessed by micro-computed tomography and histopathological examination in female rats. Biomol Ther (Seoul) 2015;**23**(2):195-200.

46. Yao Y, Ding CZ, Fang Y. Combination of MTX and LEF attenuates inflammatory bone erosion by down-regulation of receptor activator of NF-kB ligand and interleukin-17 in type II collagen-induced arthritis rats. Rheumatol Int 2013;**33**(7):1845-53
47. Teramachi J, Kukita A, Li YJ, et al. Adenosine abolishes MTX-induced suppression of osteoclastogenesis and inflammatory bone destruction in adjuvant-induced arthritis. Lab Invest 2011;**91**(5):719-31.
48. Baggott JE, Morgan SL. Methotrexate and erythro-9-(2-hydroxynon-3-yl) adenine therapy for rat adjuvant arthritis and the effect of methotrexate on in vivo purine metabolism. Eur J Pharm Sci 2007;**31**(2):95-101 |.
49. Brauer R, Kette H, Henzgen S, et al. Influence of cyclosporin A on cytokine levels in synovial fluid and serum of rats with antigen-induced arthritis. Agents Actions 1994;**41**(1-2):96-98
50. Wooley PH, Whalen JD, Chapman DL, et al. The effect of an interleukin-1 receptor antagonist protein on type II collagen-induced arthritis and antigen-induced arthritis in mice. Arthritis and rheumatism 1993;**36**(9):1305-14
51. Brahn E, Peacock DJ, Banquerigo ML. Suppression of collagen-induced arthritis by combination cyclosporin A and methotrexate therapy. Arthritis and rheumatism 1991;**34**(10):1282-88
52. Quinn MA, Emery P. Window of opportunity in early rheumatoid arthritis: possibility of altering the disease process with early intervention. Clinical and experimental rheumatology 2003;**21**(5 Suppl 31):S154-7
53. van Nies JA, Krabben A, Schoones JW, et al. What is the evidence for the presence of a therapeutic window of opportunity in rheumatoid arthritis? A systematic literature review. Ann Rheum Dis 2014;**73**(5):861-70.
54. van Dongen H, van Aken J, Lard LR, et al. Efficacy of methotrexate treatment in patients with probable rheumatoid arthritis: a double-blind, randomized, placebo-controlled trial. Arthritis and rheumatism 2007;**56**(5):1424-32.
55. Verstappen SM, McCoy MJ, Roberts C, et al. Beneficial effects of a 3-week course of intramuscular glucocorticoid injections in patients with very early inflammatory polyarthritis: results of the STIVEA trial. Ann Rheum Dis 2010;**69**(3):503-9
56. Machold KP, Landewe R, Smolen JS, et al. The Stop Arthritis Very Early (SAVE) trial, an international multicentre, randomised, double-blind, placebo-controlled trial on glucocorticoids in very early arthritis. Ann Rheum Dis 2010;**69**(3):495-502.
57. Emery P, Durez P, Dougados M, et al. Impact of T-cell costimulation modulation in patients with undifferentiated inflammatory arthritis or very early rheumatoid arthritis: a clinical and imaging study of abatacept (the ADJUST trial). Ann Rheum Dis 2010;**69**(3):510-6.
58. Vossenaar ER, Nijenhuis S, Helsen MM, et al. Citrullination of synovial proteins in murine models of rheumatoid arthritis. Arthritis and rheumatism 2003;**48**(9):2489-500.