CLINICAL SCIENCE

Efficacy and safety of NI-0101, an anti-toll-like receptor 4 monoclonal antibody, in patients with rheumatoid arthritis after inadequate response to methotrexate: a phase II study

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ABSTRACT

Objectives Anti-citrullinated protein antibodies (ACPAs) form immune complexes with citrullinated proteins binding toll-like receptor (TLR) 4, which has been proposed as a mediator of rheumatoid arthritis (RA). NI-0101 is a first-in-class humanised monoclonal antibody blocking TLR4, as confirmed by inhibition of in vivo lipopolysaccharide-induced cytokine release in healthy volunteers. This study was design to confirm preclinical investigations supporting a biomarker-driven approach for treatment of patients with RA who present positive for these immune complexes.

Methods Placebo-controlled, double-blind, randomised (2:1) trial of the tolerability and efficacy of NI-0101 (5 mg/kg, every 2 weeks for 12 weeks) versus placebo in ACPA-positive RA patients with inadequate response to methotrexate. Efficacy measures included Disease Activity Score (28-joint count) with C reactive protein (DAS28-CRP), European League Against Rheumatism (EULAR) good and moderate responses, and American College of Rheumatology (ACR) 20, ACR50 and ACR70 responses. Subgroup analyses defined on biomarkers were conducted. Pharmacokinetics, pharmacodynamics and safety were reported.

Results 90 patients were randomised (NI-0101 (61) and placebo (29)); 86 completed the study. No significant between-group difference was observed for any of the efficacy endpoints. Subgroup analyses using baseline parameters as covariants did not reveal any population responding to NI-0101. Treatment-emergent adverse events occurred in 51.7% of patients who received placebo versus 52.5% for NI-0101.

Conclusions We demonstrate for the first time that in RA, a human immune-mediated inflammatory disease, blocking the TLR4 pathway alone does not improve disease parameters. Successful targeting of innate immune pathways in RA may require broader and/or earlier inhibitory approaches.

Key messages

What is already known about this subject?

- Citrullinated proteins and anti-citrullinated protein antibodies forming immune complexes belong to the damage-associated molecular pattern family, participating in innate immunity and are expressed in inflammatory conditions, such as in rheumatoid arthritis (RA).
- Immune and stromal cells are activated by these immune complexes via cellular receptors, including toll-like receptor (TLR) 4. NI-0101 is a humanised immunoglobulin G1κ monoclonal antibody engineered to bind to and block the activation of human TLR4, which has demonstrated a predictable pharmacokinetics, good safety profile and inhibition of in vivo lipopolysaccharide-induced cytokine production in healthy volunteers.

What does this study add?

- We assessed for the first time, in a placebo-controlled, double-blind, randomised study, the tolerability and efficacy of TLR4 blockade in RA patients with inadequate response to methotrexate (MTX). Study results indicated no significant differences between treatment arms for any of the clinical efficacy and pharmacodynamics endpoints included in prespecified subgroups positive for antibodies against selected citrullinated proteins.

How might this impact on clinical practice or future developments?

- This study demonstrated that the blockage of TLR4 is likely not a relevant target in RA patients with inadequate response to MTX and established disease, its role remains to be determined.
- Successful targeting of innate immune pathways in RA, and potentially also in other chronic inflammatory diseases, may require broader or earlier inhibitory approaches.

INTRODUCTION

Both innate and adaptive immune pathways are implicated in the pathogenesis of rheumatoid arthritis (RA).[1] Anti-citrullinated protein antibodies (ACPAs) are characteristic of RA and may be present prior to the emergence of clinical symptoms of the disease.[2 3] Citrullinated proteins and ACPAs form immune complexes[4 5] which belong to the damage-associated molecular pattern (DAMP) family.[6] DAMPs are important regulators of innate inflammatory responses. They drive pathogenic processes in RA by activating both immune and stromal cells by stimulating cellular receptors, including

toll-like receptor (TLR) 4.[7 8] This pattern recognition receptor can be activated by immune complexes formed by citrullinated proteins, including matrix-derived molecules (eg, citrullinated-fibrinogen) and their associated autoantibodies (ACPAs).[9–13] These molecules are upregulated in some patients with RA and are expressed in the synovium.[14] Numerous preclinical mechanistic studies have shown the potential role for TLR4 and its ligands in RA.[15–24]

Biological agents currently approved for the treatment of RA block the actions of tumour necrosis factor (TNF)-α or interleukin (IL)-6 receptor, directly interfere with the actions of T cells or deplete B cells.[25] T cell inhibition by abatacept and cytokine signalling reduction by Janus kinase inhibitors have also demonstrated efficacy for the treatment of RA.[26] Numerous targeted therapies are available, but unmet needs in the management of RA remain. Partial and loss of response are common and drug-free remission cannot be achieved in most patients.[27] Moreover, patients who fail one biological agent may receive even less benefit when switching to a second agent, even with a different mechanism of action.[28] This may in part reflect accrual of irreversible articular damage mediating chronicity in synovial pathology.[28] Some patients ultimately become resistant to all currently available therapeutics—so-called difficult-to-treat RA,[29] requiring new therapeutic solutions. Given the evidence supporting a role for TLR4 in RA pathogenesis, we explored inhibition of this pathway as a potential treatment target.

NI-0101 is a humanised immunoglobulin (Ig) G1κ monoclonal antibody engineered to bind to and block the activation of human TLR4. It interferes with TLR4 dimersation, preventing signal transduction through the TLR4 cytoplasmic pathway.[30] It has been demonstrated to inhibit the effects of lipopolysaccharide (LPS) administered to healthy volunteers, which is dependent on FcγRII.[31] The results from in vitro studies have demonstrated a correlation between levels of TLR4 ligands and blockade of innate inflammatory responses by NI-0101.[9]

METHODS

Study design

This was a phase II, proof-of-concept, randomised (2:1), placebo-controlled, double blind, international multicentre study in patients with moderate-to-severe ACPA-positive RA that previously responded inadequately to methotrexate (MTX). Patients received addition of NI-0101 (5 mg/kg administered every 2 weeks for 12 weeks) or placebo to ongoing MTX treatment for 12 weeks. Patients in both treatment arms were stratified on the basis of FcγRIIa genotype (RR/RH and HH) and C reactive protein (CRP) level (above and below 0.7 mg/dL, with a maximum of 25% below 0.7 mg/dL). Patients were followed up for 12 weeks after NI-0101 was stopped.

Patients

Male and female patients ≥18 years old and with body mass indices <30 and >18 kg/m^2 with a diagnosis of RA according to 2010 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) criteria, ACPA positive and disease duration ≥6 months since formal diagnosis were eligible for enrolment. Patients had active RA at screening, characterised by ≥6 of 66 swollen joints and ≥6 of 68 tender joints, confirmed synovitis in ≥1 of the six swollen joints, CRP >0.7 mg/dL or CRP level between 0.3 and 0.7 mg/dL if erythrocyte sedimentation rate (ESR) ≥30 mm/hour, and to have been receiving MTX for ≥3 months and a stable dose/regimen for ≥6 weeks prior to screening.

Patient participation was excluded by a history of autoimmune disease other than RA, prior receipt of a cytotoxic agent other than MTX or immunosuppressive drugs ≤3 months prior to screening (see online supplementary data for more details).

Patient and public involvement

It was not appropriate or possible to involve patients or the public in the design, or conduct, or reporting or dissemination of our research.

Assessments

Efficacy

Efficacy measures included OMERACT RA core outcome set and clinical study reported according to EULAR recommendations on conducting/reporting of clinical trials. Efficacy measures included mean values and changes from baseline in Disease Activity Score including 28-joint count using CRP or ESR (DAS28-CRP, DAS28-ESR); Simplified Disease Activity Index (SDAI) and Clinical Disease Activity Index (CDAI) scores; and proportions of patients achieving EULAR good, moderate and no response; or ACR20, ACR50 and ACR70 responses. Subgroup analyses included assessment of the effects of baseline (study day 0 prior to first treatment administration) patient characteristics and biomarkers (APCA, citrullinated peptide-specific APCA, circulating TLR4 ligands, rheumatoid factor (RF)) on clinical outcomes.

Pharmacokinetics and pharmacodynamics

NI-0101 concentrations was measured preinfusion, throughout the treatment and until the end of the follow-up period. Changes from baseline in CRP, IL-6, IL-1β, IL-8, TNF-α and C-X-C motif chemokine 10 (CXCL10) were evaluated.

Safety

Safety assessments consisted of recording of adverse events (AEs), clinical laboratory values and vital signs; and testing for the presence of antidrug antibodies (ADAs).

Statistical analysis

Study populations included the intent-to-treat-completer (c-ITT) analysis set, defined as all patients who were randomised and completed the treatment period; the per-protocol (PP) analysis set, defined as all patients in the c-ITT population without any major protocol deviations; and the safety (SAF) analysis set, defined as all patients who received at least part of the first infusion of NI-0101 or placebo. Patients were analysed according to the actual treatment received.

Efficacy endpoints were analysed by statistical models including treatment, score for each measure at baseline and randomisation stratification factors (FcγRIIa genotype and CRP level at baseline) as fixed effect covariates. Other covariates, including country, duration of RA, use of non-steroidal anti-inflammatory drugs and glucocorticoids at baseline, baseline joint counts, ESR values, VECTRA DA scores and ACPA level could also be investigated in analyses of DAS28-CRP and ACR50 results.

Calculation of sample size for the randomised treatment arms was based on the change in DAS28-CRP between the NI-0101 and the placebo groups for RR/RH population at week 12 compared with predose. It was estimated that 54 RR/RH patients (NI-0101:placebo; 36:18) gave a power of 80% at a two-sided significance level of 5% assuming a difference in DAS28-CRP of 1 point (SD=1.2) at 12 weeks between treatment and placebo (2:1 ratio). Considering that the population includes ≥66% of

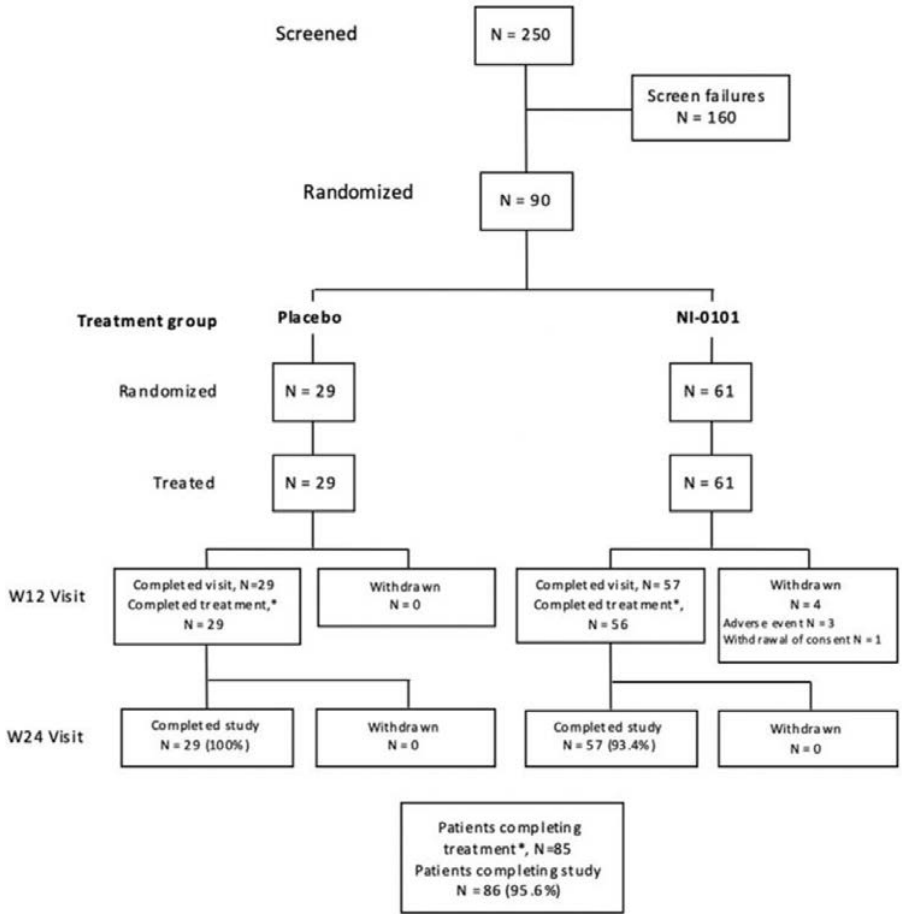

Figure 1 Patient disposition. Data in boxes represent numbers of patients. *Defined as patients who received at least five of the six scheduled infusions and had at least one evaluable efficacy data at week 12.

RR/RH, the total number of patients required to complete the treatment was calculated to be 81 (NI-0101:placebo; 54:27) to ensure at least 54 RR/RH patients completed treatment. Ninety patients were randomised to compensate for dropouts.

RESULTS

Patients and screening phase

Of 250 patients screened for eligibility, 90 were randomised (61 to NI-0101 and 29 to placebo group). All randomised patients received at least part of the first infusion of NI-0101 and 57 completed the week 12 visit along with 29 patients treated with placebo, all of these patients completed the follow-up phase to week 24 (figure 1). Baseline demographic and disease characteristics are summarised in table 1. There were no major imbalances between groups for most individual disease parameters. However, patients in the NI-0101 group had a longer duration of RA (8.5 years vs 5.4 years for placebo) and were younger at the time of RA diagnosis (45.7 years vs 51.2 years for placebo). The mean CRP level was also higher for patients allocated to receive NI-0101 (18.3 mg/L vs 13.4 mg/L for placebo) at baseline, whereas CRP levels at screening were slightly higher in the placebo group. CRP levels decreased between screening and baseline for most patients in each group, but the decline was greater for those who received placebo. Post hoc analysis demonstrated that the magnitude of the CRP decrease was dependent on the recruitment site of origin.

Efficacy

Both treatment groups demonstrated similar decreases from baseline to week 12 in DAS28-CRP with no significant between-group difference (figure 2A); a similar pattern was observed for DAS28-ESR (figure 2B). CDAI and SDAI scores decreased by approximately 40% from baseline to week 12, again with no significant differences between treatment groups (figure 2C,D). The proportion of patients achieving EULAR responses (good or moderate) increased with treatment. By week 12, 27.6% and 26.0% of patients in the placebo and NI-0101 groups, respectively, had achieved EULAR good responses; and 55.2% and 53.6% had achieved EULAR moderate responses (figure 3A). There were no significant between-group differences in ACR responses at week 12; 55.2% and 58.9% of patients in the placebo and NI-0101 groups, respectively, achieved ACR20 responses; 20.7% and 14.3% achieved ACR50 responses, and 10.3% and 10.7% achieved ACR70 responses (figure 3B–D). Swollen and tender joint counts also declined from baseline in both treatment groups. The changes in swollen joints from baseline to week 12 were –6.1 and –7.1 for the placebo and NI-0101 groups, respectively; and the respective values for tender joints were –6.3 and –8.1.

Subgroup analysis indicated no significant effects on stratification by CRP and FcγRIIa genotype for DAS28-CRP or ACR50 response. All subgroup analyses, based on levels of prespecified biomarkers (ACPA, RF, cFb-IC, anti-citrullinated protein/

Table 1 Baseline demographic and clinical characteristics

Baseline characteristic	Measure	Placebo, n (%) (n=29)	NI-0101, n (%) (n=61)
Sex, n (%)	Males	6 (20.7)	11 (18.0)
	Females	23 (79.3)	50 (82.0)
Race, n (%)	White	29 (100.0)	61 (100.0)
Age (years)	Mean (SD)	57.1 (13.07)	54.6 (11.10)
	Median (range)	59.1 (20–79)	56.3 (23–76)
Weight (kg)	Mean (SD)	68.8 (15.46)	71.4 (13.30)
	Median (range)	66.5 (47.0–103.9)	70.8 (45.6–98.9)
BMI (kg/m²)	Mean (SD)	25.2 (4.01)	26.3 (3.43)
	Median (range)	25.9 (18.0–29.8)	26.3 (18.4–32.0)
Duration of RA	Mean years (SD)	5.4 (4.82)	8.5 (7.86)
	Range	0.5–17.1	0.5–33.1
Age at RA diagnosis	Mean years (SD)	51.2 (13.62)	45.7 (11.56)
	Range	18–69	21–67
Steroid dose category	No steroid given	9 (31.0)	20 (32.8)
	1–5 mg	8 (27.6)	6 (9.8)
	5–10 mg	12 (41.4)	35 (57.4)
MTX dose category (mg/week)	3.5–10 mg	2 (6.9)	2 (3.3)
	10–20 mg	25 (86.2)	55 (90.2)
	20–25 mg	2 (6.9)	4 (6.6)
CRP (mg/L)	Mean (SD)	13.4 (14.03)	18.3 (26.63)
ESR (mm/hour)	Mean (SD)	43.1 (16.51)	45.3 (24.26)
RF (IU/mL)	Mean (SD)	127.6 (146.36)	149.3 (175.72)
ACPA (U/mL)	Mean (SD)	962.6 (1730.87)	676.2 (1072.80)
DAS28-CRP	Mean (SD)	5.8 (0.82)	5.9 (0.94)
DAS28-ESR	Mean (SD)	6.6 (0.88)	6.6 (0.91)
68-tender joint counts	Mean (SD)	28.9 (14.07)	27.5 (15.89)
66-swollen joint counts	Mean (SD)	16.3 (7.92)	16.8 (8.96)

ACPA, anti-citrullinated protein antibody; BMI, body mass index; CRP, C reactive protein; DAS28, Disease Activity Score, including a 28-joint count; ESR, erythrocyte sedimentation rate; MTX, methotrexate; RA, rheumatoid arthritis; RF, rheumatoid factor.

peptide antibodies, TLR4 ligands) measured at baseline and post hoc analyses using baseline disease-related parameters failed to demonstrate any significant treatment effects in any of the subgroups.

Pharmacokinetics

The NI-0101 pharmacokinetics (PK) profile showed expected concentrations with an elimination was consistent with simulations. Throughout the treatment period, NI-0101 concentrations were maintained above the targeted threshold of 10 000 ng/mL in the majority of patients. The half-life for the linear elimination phase was estimated to be approximately 6.4 days.

Pharmacodynamics

There were no significant differences between treatment groups for all biomarkers evaluated (table 2). Analysis of changes in CRP levels from baseline to week 12 showed small increases for both treatment groups (see online supplementary data).

Safety

NI-0101 infusions every 2 weeks elicited an acceptable safety and tolerability profile in patients with RA. The Data Monitoring Committee did not request for changes in the conduct of the study and no deaths were reported. Treatment-emergent adverse events (TEAEs) reported from baseline to week 24 occurred in similar proportions of patients in the placebo and NI-0101 groups; 51.7% and 52.5%, respectively (table 3). Five patients (5.6%) reported TEAEs considered to be related to NI-0101. One patient in the placebo group and three patients in the NI-0101 group discontinued treatment due to TEAEs; however, only one of these TEAEs (an infusion-related reaction (IRR)) was assessed as having a relationship with the administration of NI-0101. One patient in the placebo group experienced a serious adverse event (AE) (appendicitis and peritoneal abscess) as did three patients in the NI-0101 group (severe IRR, diagnosis of adenocarcinoma of the colon and diagnosis of ovarian cancer). In three other patients of the NI-0101 group, non-serious events (mild dermatitis, moderate urinary tract infection and alanine

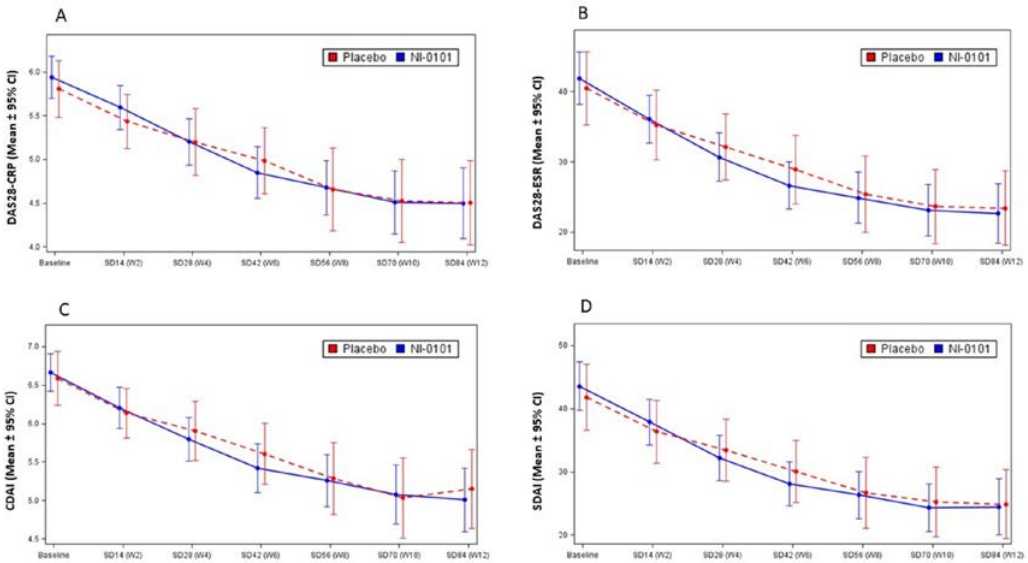

Figure 2 (A) DAS28-CRP scores. (B) DAS28-ESR scores. (C) CDAI scores. (D) SDAI scores. All values are means±95% CI. Placebo, n=28; NI-0101, n=54. CDAI, Clinical Disease Activity Index ; DAS28-CRP, Disease Activity Score (28-joint count) with C reactive protein; AS28-ESR, Disease Activity Score (28-joint count) with erythrocyte sedimentation rate; SDAI, Simplified Disease Activity Index.

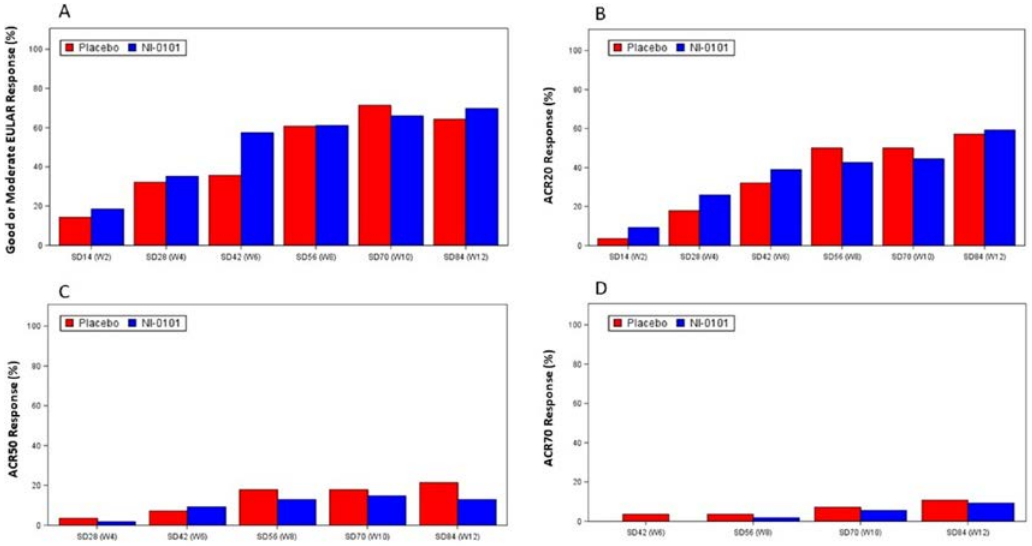

Figure 3 (A) Percentage of patients achieving EULAR good or moderate responses. (B–D) Percentages of patients achieving ACR20, 50 and 70 responses. ACR, American College of Rheumatology; EULAR, European League Against Rheumatism. Placebo, n=28; NI-0101, n=54. EULAR response at week 12: or 1.36, 95% CI (0.51; 3.67), p value 0.5381. ACR20 response at week 12: OR 1.07, 95% CI (0.42; 2.72), p value 0.8948. ACR50 response at week 12: OR 0.63, 95% CI (0.18; 2.18), p value 0.4665. ACR70 response at week 12: OR 0.94, 95% CI (0.20; 4.32), p value 0.9318.

aminotransferase grade 2 increase) were reported as related to NI-0101 but did not result in treatment discontinuation.

Infections were the most frequently reported AEs (11.5% and 13.8% in the NI-0101 and placebo groups, respectively). None of the infections reported in the NI-0101 group were rated as severe or serious. Most were respiratory tract infections commonly observed during autumn and winter. All were mild or moderate in intensity. Infections were not considered related to study treatment, except one moderate urinary tract infection.

No safety signals were identified for other safety parameters.

DISCUSSION

This is the first study to assess the efficacy of TLR4 inhibition in patients with RA or indeed with an immune-mediated inflammatory disease. The efficacy analysis showed consistent, but moderate, improvements for all endpoints evaluated for both treatment groups but no significant differences between addition of NI-0101 or placebo to MTX. Response level observed in the placebo group was higher than typically reported for clinical studies in this population, particularly for moderate response measured either by EULAR criteria or by ACR20 response. Good EULAR responses and achievement of ACR50 and ACR70 improvements in the placebo group were closer to values reported previously for patients with inadequate responses to MTX and continued on this treatment, although on the high end of such response rates.[32 33] In general, the NI-0101 treatment group showed similar or worse responses than the placebo group at week 12. Moreover, the improvements noted were lower than observed when other targeted DMARDs (biologics or small molecules) have been added to therapy in MTX-IR patients with RA.[34 35] Despite clinical improvement in both treatment groups, there was no significant reduction from baseline

Table 2 Assessments of inflammatory markers

		Change from baseline to W12, mean (SD)		P value	
Parameter, pg/mL	Baseline value, all patients, mean (SD)	Placebo (n=28)	Ni-0101 (n=54)	Treatment effect	Baseline value effect
CRP	15.6 (17.27)	−0.3 (2.83)	0.6 (2.11)	0.7688	–
IL-6	19.3 (59.2)	−5.3 (38.04)	−2.4 (18.22)	0.3978	<0.0001
GM-CSF*	9.4 (0)	0 (0)	0 (0)	–	–
IL-17A*	15.4 (0)	0 (0)	0 (0)	–	–
IL-10	0.8 (0.98)	0 (0.66)	0.3 (2.41)	0.5148	0.0319
IL-1β	1.2 (0.06)	0 (0)	0.1 (0.58)	–	<0.0001
IL-8	23.7 (18.87)	0.3 (12.24)	−3.0 (15.73)	0.2698	<0.0001
INF-γ	15.5 (30.05)	7.5 (31.50)	−0.2 (40.57)	0.7860	<0.0001
TNF-α	5.6 (11.99)	2.0 (11.49)	−0.1 (1.85)	0.5548	<0.0001
CXCL10	651.9 (542.8)	−17.4 (506.73)	−35.7 (338.77)	0.5624	<0.0001
MCP-1	422.9 (162.18)	13.4 (127.29)	−18.9 (124.58)	0.2667	0.0027

'Baseline value effect' assesses the effect of variability at baseline on the tested outcome. Here, baseline variability reported for the measured cytokines is higher than the tested treatment effect.

*Values were below limit of quantification.

CRP, C-reactive protein; CXCL10, C-X-C motif chemokine 10;GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; INF, interferon;IP-10, interferon gamma-induced protein 10; MCP, monocyte chemoattractant protein;TNF, tumour necrosis factor; W, week.

Table 3 TEAEs through 24 weeks

	Placebo, n (%) (n=29)	NI-0101, n (%) (n=61)
Pretreatment AEs	1 (3.4)	2 (3.3)
TEAEs to week 24	15 (51.7)	32 (52.5)
TEAEs related to administered treatment	0	5 (8.2)
Serious TEAEs	1 (3.4)	3 (4.9)
TEAEs leading to treatment discontinuation	1 (3.4)	3 (4.9)
TEAEs leading to death	0	0
TEAEs related to potential IRRs	3 (10.3)	9 (14.8)
TEAEs related to infections	5 (17.2)	17 (27.9)
TEAEs by highest severity		
Mild	6 (20.7)	12 (19.7)
Moderate	9 (31.0)	17 (27.9)
Severe	0	3 (4.9)
Life threatening	0	0
Fatal	0	0
Missing	0	0
TEAEs experienced by ≥5% of patients in either treatment group		
Nasopharyngitis	2 (10.3)	3 (4.9)
Upper respiratory tract infection	1 (3.4)	4 (6.6)
Condition aggravated	0	5 (8.2)

IRRs, infusion-related reactions; TEAE, treatment emergent adverse event.

in CRP, an objective measure of inflammation, for patients receiving either placebo or NI-0101 added to MTX. A potential therapeutic response to MTX background therapy during screening was observed based on CRP decrease, possibly driven by higher adherence to background treatment between screening and randomisation.

The absence of a significant effect of adding NI-0101 to MTX was further confirmed by the lack of treatment-associated changes in levels of cytokines downstream from TLR4 and known to be involved in the inflammation characteristic of RA.[36] The lack of effect of NI-0101 versus placebo on levels of inflammatory molecules evaluated in this study extended to IL-6, TNF-α, IL-8 and IL-1β, all of which have been shown to be elevated in monocytes from synovial fluid through TLR4 signalling and blocked by exposure to NI-0101 in vitro.[9 37]

During the follow-up period, when the patient and treating physician knew that NI-0101 was no longer being administered (while remaining blinded to prior treatment allocation), the results for all efficacy endpoints remained stable or decreased by similar amounts in both treatment arms. As the elimination half-life of NI-0101 is 6.4 days, it would have been reasonable to expect some continued benefit after treatment withdrawal, if it had significant efficacy.

Preplanned subgroup analyses using baseline levels of TLR4-related biomarkers were conducted to test the hypothesis that RA patients with elevated levels of TLR4 ligands (eg, citrullinated protein immune complexes) would have an increased response to the addition of NI-0101 to MTX. However, patient segmentation on the basis of the selected biomarkers failed to demonstrate any benefit of NI-0101 versus placebo. Furthermore, post hoc subgroup analyses using baseline disease and demographic parameters, including, but not limited to, baseline CRP levels and variations during screening, country of origin and disease duration, were conducted to potentially identify confounding parameters, but none showed a statistically significant effect on any between-treatment differences. The PK results from this study and PK/pharmacodynamic analysis from a prior study[31] suggest that the levels of NI-0101 achieved in the patients in this trial were sufficient to achieve TLR4 pathway blockade between two dosing intervals, regardless of the FcγRIIa polymorphism. Thus, it is unlikely that insufficient levels of NI-0101 contributed to the observed lack of clinical effect.

Given that NI-0101 has been shown to be a potent inhibitor of TLR4, as demonstrated by the lack of induction of inflammatory cytokines after in vivo LPS administration in healthy volunteers after having received NI-0101 and that literature on pathogenic processes in RA reports the involvement of the stimulation of this receptor,[7–12 31] the lack of significant clinical and pharmacodynamic effects in this study are surprising. It is possible that redundancy in TLR signalling may underlie the lack of effect of TLR4 blockade in this trial. In fact, TLR2, TLR4, TLR5 and TLR7 have all been considered to be potentially involved in the pathology of RA.[38] It cannot be excluded that NI-0101 may provide clinical benefit when combined with other targeted agents. Indeed, the preclinical hypothesis tested in this study was supported by the observed correlation in vitro between NI-0101 response and the presence of specific immune complexes against citrullinated proteins.[9] The presence of antibodies against citrullinated proteins has been reported even before the first clinical manifestation of RA. It is conceivable, perhaps that immune complexes signalling through TLR4 could play a significant pathogenic role in early RA, whereas other inflammatory processes are predominant when RA is already established and therefore blocking TLR4 may not provide any benefit.

We demonstrate satisfactory safety and tolerability of TLR4 inhibition with NI-0101. There were no significant differences between treatment groups in the frequency of AEs. The type and intensity of AEs reported in this study were similar to those observed in prior clinical trials in similar patient cohorts,[39 40] and of the three serious AEs (IRR, adenocarcinoma of the colon and ovarian cancer) reported in the NI-0101 group, only the IRR was related to NI-0101 administration.

TLR4 has been shown to play an important role in immune response to Gram-negative bacteria.[37] However, the results suggest no increased risk for infections with NI-0101 and are consistent with findings from healthy volunteers who received NI-0101, as well as those obtained with other molecules targeting the same pathways.[31 41 42] No systemic Gram-negative infections were reported. The incidence of urinary tract infections (6.6%), all in female patients, appeared no greater than that reported for postmenopausal women who constitute the majority of the RA population.[43 44]

This study demonstrated that the blockage of TLR4 is likely not a relevant target in RA patients with inadequate response to MTX, as shown by the absence of NI-0101 effect versus placebo on clinical endpoints or on changes in levels of inflammatory cytokines or chemokines. In addition, none of the subgroup analyses identified a subset of patients that received benefit from NI-0101. The results showed an expected PK profile, desired concentrations and no safety concerns for NI-0101. The lack of significant effect of NI-0101 in this well-controlled prospective clinical trial indicates that blocking the TLR4 pathway alone is unlikely to benefit patients with established RA. The role of TLR4 and of anti-citrullinated antibodies forming immune complexes in prior diagnosis and in early RA remains to be established. The good NI-0101 safety and PK profiles support further exploration in other diseases, in particular when microbial products are involved in inflammatory diseases or when high microbial translocation is observed (eg, HIV).

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Contributors EM, EHC, IMcI, KdG, PJ, GL and CdM participated in the design of the study. EM, KdG, GL and TK participated in data collection. EM, KdG and GL participated in data analysis. EM, EHC, IMcI, KdG, PJ, GL and CdM participated in interpreting the data, in writing and in critically reviewing the manuscript. All authors approved the final version. EHC and IMcI contributed equally to the study design and data interpretation.

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Competing interests EM, KdG, GL and CdM were employees and stock options holders of Novimmune SA. PJ was consultant of Novimmune SA. EHC and IMcI were consultants of Novimmune SA: EHC received consultancy fees or grants from UCB, Pfizer, BioCancer, Biogen, Novartis, Roche, Amgen, Chugai, Eli Lilly, Sanofi, Abbvie, Janssen, Gilead and Bristol Myer Squibbs. IMcI received consultancy fees and grants from Celgen, Janssen, Novartis, Beorhinger Ingelheim, Abbvie, Eli Lilly, Bristol Myer Squibbs, GlaxoSmithKline and Pfizer. TK received Investigator fees from Novimmune SA to conduct the study. Novimmune SA and Genentech entered into a collaboration agreement for the development of NI-0101, under this agreement Novimmune SA received funding from Genentech.

Patient consent for publication Not required.

Ethics approval All relevant study documentation and amendments were approved by Independent Ethics Committees. The study was conducted in accordance with the principles set forth in the Declaration of Helsinki, the Guidelines of the International Council for Harmonisation (ICH) on Good Clinical Practice (GCP) Guideline E6 (R2) (EMA/CPMP/ICH/135/95), European Union (EU) Directive 95/46/EC, and other applicable regulatory requirements. Patients provided informed written consent prior to any study procedures.

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REFERENCES

1 McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. *N Engl J Med Overseas Ed* 2011;365:2205–19.
2 Rantapää-Dahlqvist S, de Jong BAW, Berglin E, *et al*. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis Rheum* 2003;48:2741–9.
3 Nielen MMJ, van Schaardenburg D, Reesink HW, *et al*. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. *Arthritis Rheum* 2004;50:380–6.
4 Kurowska W, Kuca-Warnawin EH, Radzikowska A, *et al*. The role of anti-citrullinated protein antibodies (AcpA) in the pathogenesis of rheumatoid arthritis. *Cejoi* 2017;42:390–8.
5 Sohn DH, Rhodes C, Onuma K, *et al*. Local joint inflammation and histone citrullination in a murine model of the transition from preclinical autoimmunity to inflammatory arthritis. *Arthritis Rheumatol* 2015;67:2877–87.
6 Stolberg-Stolberg JA, Furman BD, Garrigues NW, *et al*. Effects of cartilage impact with and without fracture on chondrocyte viability and the release of inflammatory markers. *J Orthop Res* 2013;31:1283–92.
7 Huang Q-Q, Pope RM. The role of toll-like receptors in rheumatoid arthritis. *Curr Rheumatol Rep* 2009;11:357–64.
8 Goh FG, Midwood KS. Intrinsic danger: activation of Toll-like receptors in rheumatoid arthritis. *Rheumatology* 2012;51:7–23.
9 Hatterer E, Shang L, Simonet P, *et al*. A specific anti-citrullinated protein antibody profile identifies a group of rheumatoid arthritis patients with a toll-like receptor 4-mediated disease. *Arthritis Res Ther* 2016;18.
10 Smiley ST, King JA, Hancock WW. Fibrinogen stimulates macrophage chemokine secretion through toll-like receptor 4. *J Immunol* 2001;167:2887–94.
11 Wenink MH, Santegoets KCM, Roelofs MF, *et al*. The inhibitory Fc gamma IIb receptor dampens TLR4-mediated immune responses and is selectively up-regulated on dendritic cells from rheumatoid arthritis patients with quiescent disease. *J Immunol* 2009;183:4509–20.
12 Anquetil F, Clavel C, Offer G, *et al*. IgM and IgA rheumatoid factors purified from rheumatoid arthritis sera boost the Fc receptor- and complement-dependent effector functions of the disease-specific Anti–Citrullinated protein autoantibodies. *J Immunol* 2015;194:3664–74.
13 Manivel VA, Sohrabian A, Rönnelid J. Granulocyte-augmented chemokine production induced by type II collagen containing immune complexes is mediated via TLR4 in rheumatoid arthritis patients. *Eur J Immunol* 2016;46:2822–34.
14 Ospelt C, Brentano F, Rengel Y, *et al*. Overexpression of toll-like receptors 3 and 4 in synovial tissue from patients with early rheumatoid arthritis: toll-like receptor expression in early and longstanding arthritis. *Arthritis Rheum* 2008;58:3684–92.
15 Achek A, Shah M, Seo JY, *et al*. Linear and rationally designed stapled peptides abrogate TLR4 pathway and relieve inflammatory symptoms in rheumatoid arthritis rat model. *J Med Chem* 2019;62:6495–511.
16 Li D, Zhou Q, Hu G, *et al*. MiRNA-506 inhibits rheumatoid arthritis fibroblast-like synoviocytes proliferation and induces apoptosis by targetting TLR4. *Biosci Rep* 2019;39.
17 Umekita K, Miyauchi S, Nomura H, *et al*. Neutrophil-derived lactoferrin induces the inflammatory responses of rheumatoid arthritis synovial fibroblasts via Toll-like receptor 4. *Clin Exp Rheumatol* 2019;37:834–41.
18 Roome T, Aziz S, Razzak A, *et al*. Opuntioside, opuntiol and its metallic nanoparticles attenuate adjuvant-induced arthritis: novel suppressors of Toll-like receptors -2 and -4. *Biomed Pharmacother* 2019;112.
19 Alsousi AA, Igwe OJ. Redox-active trace metal-induced release of high mobility group box 1(HMGB1) and inflammatory cytokines in fibroblast-like synovial cells is Toll-like receptor 4 (TLR4) dependent. *Biochim Biophys Acta Mol Basis Dis* 2018;1864:3847–58.
20 Abed El-Gaphar OAM, Abo-Youssef AM, Halal GK. Levetiracetam mitigates lipopolysaccharide-induced JAK2/STAT3 and TLR4/MAPK signaling pathways activation in a rat model of adjuvant- induced arthritis. *Eur J Pharmacol* 2018;826:85–95.
21 Lee J-H, Kim B, Jin WJ, *et al*. Pathogenic roles of CXCL10 signaling through CXCR3 and TLR4 in macrophages and T cells: relevance for arthritis. *Arthritis Res Ther* 2017;19.
22 Tripathy A, Khanna S, Padhan P, *et al*. Direct recognition of LPS drive TLR4 expressing CD8+ T cell activation in patients with rheumatoid arthritis. *Sci Rep* 2017;7:933.
23 Kiyeko GW, Hatterer E, Herren S, *et al*. Spatiotemporal expression of endogenous TLR4 ligands leads to inflammation and bone erosion in mouse collagen-induced arthritis. *Eur J Immunol* 2016;46:2629–38.
24 Watanabe T, Takahashi N, Hirabara S, *et al*. Hyaluronan inhibits Tlr-4-Dependent RANKL expression in human rheumatoid arthritis synovial fibroblasts. *PLoS One* 2016;11:e0153142.
25 Blair HA, Deeks ED. Abatacept: a review in rheumatoid arthritis. *Drugs* 2017;77:1221–33.
26 Lee YH, Bae S-C. Comparative efficacy and safety of tocilizumab, rituximab, abatacept and tofacitinib in patients with active rheumatoid arthritis that inadequately responds to tumor necrosis factor inhibitors: a Bayesian network meta-analysis of randomized controlled tri. *Int J Rheum Dis* 2016;19:1103–11.
27 Kuijper TM, Lamers-Karnebeek FBG, Jacobs JWG, *et al*. Flare rate in patients with rheumatoid arthritis in low disease activity or remission when tapering or stopping synthetic or biologic DMARD: a systematic review. *J Rheumatol* 2015;42:2012–22.
28 Brown S, Everett CC, Naraghi K, *et al*. Alternative tumour necrosis factor inhibitors (TNFi) or abatacept or rituximab following failure of initial TNFi in rheumatoid arthritis: the switch RCT. *Health Technol Assess* 2018;22:1–280.
29 de Hair MJH, Jacobs JWG, Schoneveld JLM, *et al*. Difficult-To-Treat rheumatoid arthritis: an area of unmet clinical need. *Rheumatology* 2017. doi:10.1093/rheumatology/kex349. [Epub ahead of print: 04 Oct 2017].
30 Dunn-Siegrist I, Leger O, Daubeuf B, *et al*. Pivotal involvement of Fcgamma receptor IIA in the neutralization of lipopolysaccharide signaling via a potent novel anti-TLR4 monoclonal antibody 15C1. *J Biol Chem* 2007;282:34817–27.
31 Monnet E, Lapeyre G, Poelgeest Evan, *et al*. Evidence of NI-0101 pharmacological activity, an anti-TLR4 antibody, in a randomized phase I dose escalation study in healthy volunteers receiving LPS. *Clin. Pharmacol. Ther.* 2017;101:200–8.
32 Kremer JM, Cohen S, Wilkinson BE, *et al*. A phase IIb dose-ranging study of the oral JAK inhibitor tofacitinib (CP-690,550) versus placebo in combination with background methotrexate in patients with active rheumatoid arthritis and an inadequate response to methotrexate alone. *Arthritis Rheum* 2012;64:970–81.
33 Genovese MC, Bojin S, Biagini IM, *et al*. Tabalumab in rheumatoid arthritis patients with an inadequate response to methotrexate and naive to biologic therapy: a phase II, randomized, placebo-controlled trial. *Arthritis Rheum* 2013;65:880–9.
34 Camean-Castillo M, Gimeno-Ballester V, Rios-Sanchez E, *et al*. Network meta-analysis of tofacitinib versus biologic treatments in moderate-to-severe rheumatoid arthritis patients. *J Clin Pharm Ther* 2019;44:384–96.

35 Singh JA, Hossain A, Mudano AS, *et al*. Biologics or tofacitinib for people with rheumatoid arthritis naive to methotrexate: a systematic review and network meta-analysis. *Cochrane Database Syst Rev* 2017;59.
36 Guijarro-Muñoz I, Compte M, Álvarez-Cienfuegos A, *et al*. Lipopolysaccharide activates Toll-like receptor 4 (TLR4)-mediated NF-κB signaling pathway and proinflammatory response in human pericytes. *J Biol Chem* 2014;289:2457–68.
37 Shahrara S, Park CC, Temkin V, *et al*. Rantes modulates TLR4-Induced cytokine secretion in human peripheral blood monocytes. *J Immunol* 2006;177:5077–87.
38 Elshabrawy HA, Essani AE, Szekanecz Z, *et al*. TIrs, future potential therapeutic targets for RA. *Autoimmun Rev* 2017;16:103–13.
39 Smolen JS, Burmester G-R, Combe B, *et al*. Head-to-head comparison of certolizumab pegol versus adalimumab in rheumatoid arthritis: 2-year efficacy and safety results from the randomised EXXELERATE study. *The Lancet* 2016;388:2763–74.
40 Genovese MC, Braun DK, Erickson JS, *et al*. Safety and efficacy of open-label subcutaneous ixekizumab treatment for 48 weeks in a phase II study in biologic-naive and TNF-IR patients with rheumatoid arthritis. *J Rheumatol* 2016;43:289–97.
41 Wong YN, Rossignol D, Rose JR, *et al*. Safety, pharmacokinetics, and pharmacodynamics of E5564, a lipid A antagonist, during an ascending single-dose clinical study. *J Clin Pharmacol* 2003;43:735–42.
42 Rice TW, Wheeler AP, Bernard GR, *et al*. A randomized, double-blind, placebo-controlled trial of TAK-242 for the treatment of severe sepsis*. *Crit Care Med* 2010;38:1685–94.
43 Nicolle L. Best pharmacological practice: urinary tract infections. *Expert Opin Pharmacother* 2003;4:693–704.
44 Raz R. Urinary tract infection in postmenopausal women. *Korean J Urol* 2011;52:801–8.