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ORIGINAL RESEARCH PAPER

Toreforant, an orally active histamine H_4-receptor antagonist, in patients with active rheumatoid arthritis despite methotrexate: mechanism of action results from a phase 2, multicenter, randomized, double-blind, placebo-controlled synovial biopsy study

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Abstract

Objective/design In a double-blind, placebo-controlled, multiple-dose study, we assessed the molecular mechanism of action of the selective histamine-4-receptor antagonist toreforant.

Patients/treatment Patients with active rheumatoid arthritis (RA) despite methotrexate were randomized (3:1) to toreforant 30 mg/day (weeks 0–52) or placebo (weeks 0–12) followed by toreforant 30 mg/day (weeks 12–52).

Methods Primary biomarker analyses comprised 39 different proteins/mRNA transcripts measured in synovial biopsy ($n = 39$) and/or time-matched serum ($n = 15$) samples collected at baseline and week 6. Clinical response was assessed using C-reactive protein-based 28-joint disease activity scores. Data were summarized using descriptive statistics.

Results Among 21 randomized, treated patients (toreforant-16, placebo-5), 18 (toreforant-13, placebo-5) completed the 12-week double-blind period (none completed open-label treatment) prior to the early study termination. Biomarker profiling indicated potential modest effects of toreforant on gene expression of histamine-1-receptor, tumor necrosis factor-alpha, and interleukin-8 in synovium. Potential trends between biomarkers and clinical response were observed with synovial monocyte chemoattractant protein-4 and phosphorylated extracellular-signal-regulated kinases and serum matrix metalloproteinase-3. Minimal synovial gene expression of interleukins-17A and 17F was detected.

Conclusions While clear biomarker signals associated with toreforant pharmacology in RA patients were not identified, modest associations between biomarkers and clinical response were noted. Synovial expression of interleukins-17A/17F was minimal. Limited sample size warrants cautious interpretation.

Keywords Histamine receptor antagonist · Pharmacodynamics · Rheumatoid arthritis · Biomarkers

Introduction

The histamine 4 receptor (H_4R) is a novel mediator of inflammatory diseases [1]. While mostly associated with allergic and atopic responses, histamine also has been implicated in immune-mediated diseases such as rheumatoid arthritis (RA) [2]. Histamine levels are elevated in patients with inflammatory joint disease [3], and the H_4R has been reported to be expressed on macrophage-like synoviocytes obtained from patients with RA [4, 5]. Preclinical data demonstrate a role for the H_4R in the pathogenesis of experimental arthritis [6], and a selective H_4R antagonist, toreforant, has been studied for the treatment of RA [7].

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The H_4R may be involved in several pathways important in RA, including the control of innate antigen presenting cell (APC) function and cytokine production, e.g., tumor necrosis factor (TNF) and interleukin (IL)-6 [6]. The receptor also mediates migration and cytokine release in mast cells, which might be highly activated in RA [8, 9]. A role for the H_4R in the development and function of T-helper (Th)17 cells has also been suggested [6, 10], although the role for Th17 and IL-17 in the pathogenesis of RA was controversial and still being evaluated at the time this biopsy study was planned [11, 12]. Thus, antagonism of the H_4R could be a target of RA therapy by acting on multiple pathways, but human mechanistic data are lacking.

Toreforant (JNJ-38518168), a selective H_4R antagonist, has been studied for the treatment of inflammatory diseases. While a previously conducted proof-of-concept study in RA suggested a therapeutic effect with 100 mg/day, further phase 2b assessments indicated lack of therapeutic effect at lower doses of toreforant (ClinicalTrials.gov Identifiers: NCT00941707 and NCT01679951) [7]. In prior studies with effective therapies in RA patients, biomarker analyses of synovial samples have yielded data useful in interpreting their mechanism of action (MOA) [13–16]. Therefore, concurrent with the toreforant phase 2b, double-blind, placebo-controlled clinical trial [7], a serial synovial biopsy study was initiated in patients with RA to elucidate the molecular MOA of toreforant.

Materials and methods

This study adheres to the CONSORT guidelines (http://www.consort-statement.org/).

Patients

Men and women 18–65 years of age, diagnosed with active RA [17] for ≥ 6 months and with persistent disease activity and knee involvement despite concomitant methotrexate (MTX) therapy (10–25 mg/week for ≥ 3 months), were eligible to participate. Patients were seropositive for either anti-cyclic citrullinated peptide antibody or rheumatoid factor. Oral corticosteroids were allowed if stable for ≥ 2 weeks at a dose ≤ 10 mg/day (prednisone or equipotent dose).

Study design

This randomized, double-blind, multicenter, placebo-controlled, parallel-group, multiple-dose study comprised a 12-week, placebo-controlled, double-blind period and a 40-week open-label extension period (ClinicalTrials.gov identifier: NCT01862224; registered May 24, 2013; Fig. 1). The authors confirm that related trials for this intervention are registered (ClinicalTrials.gov Identifiers: NCT00941707 and NCT01679951). Approximately 24 patients were to be randomly (3:1) assigned to receive toreforant 30 mg/day for 52 weeks or matching placebo for 12 weeks followed by toreforant 30 mg/day for 40 weeks. An interactive web response system was employed for randomization. After the 12-week placebo-controlled period, doses of MTX,

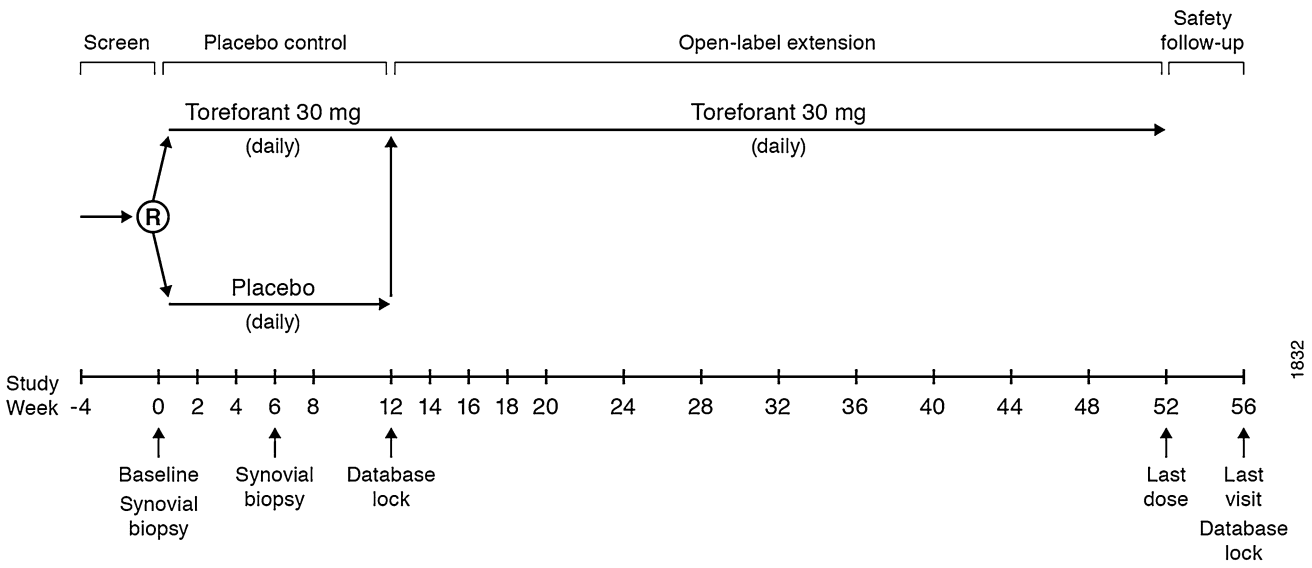

Fig. 1 Toreforant phase 2a synovial biopsy study design. *R* randomized

corticosteroids, or nonsteroidal anti-inflammatory drugs (NSAIDS)/other analgesics could be adjusted or discontinued, as appropriate, and treatment with corticosteroids or NSAIDS/other analgesics could be initiated.

Efficacy and safety evaluations

To evaluate relationships between week-12 efficacy and biomarkers, clinical response was assessed using the 28-joint disease activity score incorporating C-reactive protein (DAS28-CRP) [18]. DAS28-CRP responses were categorized as good, moderate, or none based on a combination of the actual DAS28-CRP score at week 12, as well as its change from baseline to week 12 [19, 20]. Safety assessments included adverse events (AEs), clinical laboratory determinations, and vital signs.

Synovial tissue and serum collection and analysis

Sample collection

To explore the toreforant MOA, molecular biomarker profiling of paired synovial biopsy samples collected at baseline and after 6 weeks of study treatment was undertaken. The 6-week post-baseline time biopsy point was selected, because histologic and biomarker changes are seen relatively early with molecules demonstrating efficacy in RA. Furthermore, focusing analyses on an early time point that precedes clinical improvement, in practice generally 4–8 weeks after treatment initiation [13, 14], minimizes secondary effects on the synovium that are not inherent to drug mechanism of action. Serum samples also were collected at the same visits to enable time-matched analysis of circulating biomarkers.

Synovial biopsy samples from a clinically involved knee joint were obtained using a small motorized shaver. Synovial tissue fragments (0.5–1.0 g depending on extent of synovial hyperproliferation) were collected arthroscopically using automated motorized shaver technology from multiple regions within the sampled joints (e.g., medial and lateral gutters and suprapatellar pouch for knees) [14]. Participating sites were provided study-specific training on this biopsy processing technique to ensure consistency.

Tissue fragments, pooled for each patient, were divided into aliquots for histological, messenger ribonucleic acid (mRNA), and protein lysate analyses. More than ten synovial fragments per tube or mold were collected for each of the three sample types. Synovial samples for immunohistochemistry (IHC) were embedded and frozen using optimal cutting temperature compound (ThermoFisher Scientific, Hampton, NH, USA). Protein and mRNA were to be analyzed in synovial tissues and/or blood for the determination of biomarker analyte levels. The mRNA samples were stored frozen in RNASTAT 60 (Tel-Test, Inc., Friendsville, TX, USA), and protein lysate samples were snap-frozen in dry ice/ethanol slurry.

Histologic evaluations

Histology was performed on 5-μM frozen sections. Synovial tissue quality and inflammation were assessed by scoring hematoxylin- and eosin-stained (Fisher Scientific International, Hampton, NH, USA) tissue sections. The quality of tissue fragments in each section was determined by the synovial biopsy analysis team at the University of California San Diego (San Diego, CA, USA), such that the presence of synovial lining (intima) and sublining (subintima) in a minimum of six tissue fragments defined an evaluable sample. Sections were then blindly scored by DLB for inflammation based on synovial intimal lining thickness (0 = 1–2 cells, 1 = 3–4 cells, 2 = 5–8 cells, 3 = > 8 cells), subintimal mononuclear cell infiltration (0 = 4% of field, 1 = 5–33%, 2 = 33–67%, 3 = > 67%), and lymphoid aggregates [0 = 0/low-power field (lpf), 1 = 2–4/lpf, 2 = 5–7/lpf, 3 = > 7/lpf] to yield a total score ranging from 0 to 9 [21–23]. Each tissue was read twice, and the mean of the two scores was used as the final inflammation score.

Employing evaluable synovial biopsy samples, IHC was used to quantify cell infiltration. Serial 5-μM sections were acetone-fixed, blocked, and probed using DAKO (Dako Denmark, Glostrup, Denmark) reagents as previously described [13]. The staining of markers for T-cell co-receptors (CD3, clone UCHT1), B-lymphocyte antigens (CD20, L26), macrophage cells (CD68, EMB11; sublining and total), mast/stem cell growth factor receptors (CD117, A4502), and plasma cells (CD138, CBL455) were quantified by digital image analysis using a Nikon E800 microscope and Image Pro software (AG Heinze, Lake Forest, CA, USA) [15]. Data were reported as average percent area stained in six fields.

Gene expression/biomarker evaluations

Utilizing paired synovial biopsy samples collected at baseline and week 6, synovial gene expression analysis was performed using quantitative polymerase chain reaction (qPCR) testing based on a standard curve method [24] as previously applied to serial synovial biopsy studies [14]. Synovial RNA was extracted from tissue fragments pooled by patient, and complementary DNA was synthesized. qPCR testing was performed using predeveloped reagents (Applied Biosystems, Foster City, CA, USA). Results were compared with a reference cellular standard curve and normalized for glyceraldehyde-3-phosphate dehydrogenase and reported as relative expression units [24].

Biomarker analyses were focused on 39 different proteins or mRNA transcripts selected based on implication in synovial inflammation and the pathogenesis of RA, or

relationship to histamine receptor biology. As shown in Table 1, 15 of the 39 analytes were assayed in both synovium and time-matched serum samples collected at baseline and week 6. The transcriptional analytes comprised several key regulatory cytokines and chemokines [e.g., IL-6, TNF, chemokine (C–X–C motif) ligand 13 (CXCL13), IL-17A, IL-17F, and interferon gamma (IFN_Y)], as well as factors involved in tissue remodeling [five different matrix metalloproteinases (MMPs)] and cell surface markers T-cell co-receptor CD3, B-lymphocyte antigen CD20, macrophage cell marker CD68 (sublining and total), mast/stem cell growth factor receptor CD117, and plasma cell marker CD138. In addition, the four histamine receptor family members (H_1R, H_2R, H_3R, and H_4R) were analyzed at the mRNA level, as were intracellular signaling proteins [e.g., monocyte chemotactic protein 4 (MCP4), phosphorylated

Table 1 Biomarkers measured at baseline and week 6

	Analyte name	Synovial biopsy tissue	Serum
1	Cluster of differentiation (CD) 117 mast/stem cell growth factor receptor	X	
2	CD138 plasma cell marker	X	
3	CD20 B-lymphocyte antigen	X	
4	CD3 T-cell co-receptor	X	
5	CD68 macrophage cell marker (sublining)	X	
6	CD68 macrophage cell marker (total)	X	
7	Chemokine (C-X-C motif) ligand 13 (CXCL13)	X	
8	Eotaxin (total)	X	X
9	Eotaxin-3	X	X
10	Forkhead box P3 (FOXP3)	X	
11	Histamine 1 receptor (H_1R)	X	
12	Histamine 2 receptor (H_2R)	X	
13	Histamine 3 receptor (H_3R)	X	
14	Histamine 4 receptor (H_4R)	X	
15	Synovial tissue H and E stain histology score	X	
16	Interferon gamma (IFN_Y)	X	
17	Interferon gamma-induced protein 10 (IP10)	X	X
18	Interleukin 17A (IL-17A)	X	
19	Interleukin 17F (IL-17F)	X	
20	Interleukin 1 Beta (IL-1B)	X	
21	Interleukin 4 (IL-4)	X	
22	Interleukin 6 (IL-6)	X	
23	Interleukin 8 (IL-8)	X	
24	Interleukin-8 (high abundance)		X
25	Monocyte chemotactic protein 1 (MCP1)	X	X
26	Monocyte chemotactic protein 4 (MCP4)	X	X
27	Macrophage-derived chemokine (MDC)	X	X
28	Macrophage inflammatory protein 1 alpha (MIP1a)	X	X
29	Macrophage inflammatory protein 1 beta (MIP1b)	X	X
30	Matrix metalloproteinase 1 (MMP-1)	X	X
31	Matrix metalloproteinase 10 (MMP-10)	X	X
32	Matrix metalloproteinase 2 (MMP-2)	X	X
33	Matrix metalloproteinase 3 (MMP-3)	X	X
34	Matrix metalloproteinase 9 (MMP-9)	X	X
35	Phosphorylate extracellular-signal-regulated kinase (pERK)	X	
36	Phosphorylated protein kinase B (pAKT)	X	
37	Stromal cell-derived growth factor 1 alpha (SDF1α)	X	
38	Thymus and activation regulated chemokine (TARC)	X	X
39	Tumor necrosis factor-alpha (TNFα)	X	

extracellular-signal-regulated kinase (pERK), phosphorylated protein kinase B (pAKT), and Forkhead box P3].

Measurement of post-translationally modified proteins was performed on synovial tissue extracts by enzyme-linked immunosorbent assay (ELISA). Pools of synovial tissue biopsy fragments were pulverized under liquid nitrogen and extracted with modified radioimmunoprecipitation assay buffer [25]. Measurement of pERK and pAKT was performed by ELISA (Cell Signaling, Danvers, MA, USA) per the manufacturer's instructions.

Data analyses

Two database locks were planned: the first after the last patient completed the week-12 visit and the second after the last patient completed the week-56 visit (not performed due to early study termination by the Sponsor). All RA-related evaluations/clinical response assessments and safety analyses were to be summarized for all randomized patients who received at least one dose of study drug. RA assessments made prior to the baseline synovial biopsy and first administration of study agent were to be considered baseline. No imputation was employed for missing response data in any analysis. AEs were coded using the current version of the Medical Dictionary for Regulatory Activities and summarized by preferred term, severity, and relationship to study agent.

The primary data set for biomarker analyses comprised the levels of 54 analytes measured in synovial biopsy samples ($n=39$) and/or time-matched serum samples ($n=15$). The synovial biopsy biomarker analyses were to be based on the pharmacodynamics-evaluable population, i.e., randomized patients with baseline and week-6 synovial biopsy samples of adequate quality. Baseline levels, reported as geometric means with 95% confidence intervals (CIs), and changes from baseline, reported as geometric means of percentage change with 95% CIs, in the levels of individual synovial tissue and serum analytes were summarized. Forest plots were generated to allow for visual assessment of any analytes displaying differences between comparison groups (i.e., 95% CI excludes 0 on the *y*-axis in one group but not the other).

To assess for potential associations between biomarkers and clinical response, biomarker data at baseline and as change from baseline to week 6 were compared between responder and nonresponder subgroups at week 12 (based on the primary efficacy endpoint of DAS28-CRP and the primary evaluation time point of week 12). Descriptive statistical methods were employed to summarize data. No formal sample-size determination was undertaken. Data from both arms were combined to increase the sample size in the nonresponder/responder groups.

Results

Patient disposition and baseline characteristics

Patients were screened at two centers, one in the Republic of Moldova and one in the United States of America, beginning June 4, 2013. The 21 randomized and treated patients (toreforant: 16, placebo: 5) were enrolled by the site in the Republic of Moldova. The first patient was treated on July 18, 2013; the last patient observation occurred on April 15, 2014; screening was put on hold on May 23, 2014; and the study was stopped on May 29, 2014. All randomized patients were included in clinical response, biomarker, and safety analyses (Fig. 2).

The Sponsor terminated the study early, when results from a concurrently running phase 2b toreforant efficacy study did not achieve its primary efficacy endpoint [7]. At the time of early termination, 18 patients (all from the Republic of Moldova; toreforant: 13, placebo: 5) had completed the double-blind period and entered the open-label period; no patient completed the open-label period (Fig. 2). Baseline demographic and patient characteristics were generally similar between the treatment groups (Table 2). As required by the trial protocol, all patients were receiving MTX and folic acid at baseline. Apart from these medications, the most common (reported in $\geq 10\%$ of patients) concomitant medications during the double-blind period were methylprednisolone (38.1%) and prednisolone (19.0%), such that 57.1% of all patients received concomitant systemic corticosteroids.

DAS28-CRP

At week 12, a numerically greater mean decrease from baseline in the DAS28-CRP score was observed in the toreforant (1.82) than placebo (0.90) group (Fig. 3). Consistently, a numerically greater proportion of toreforant- (84.6%) than placebo- (40.0%) treated patients achieved DAS28-CRP response of good/moderate at week 12 (Table 2).

Gene expression and biomarker findings

Baseline synovial biopsy samples were available for all 21 randomized patients. Paired synovial samples (baseline and week 6) were available for 19 of the 21 randomized patients.

Changes from baseline to week 6 were qualitatively compared between the placebo and toreforant arms. Results for the entire list of analytes are shown in Fig. 4; potential differences of note were observed in H_1R mRNA and, to a more modest extent, macrophage-derived chemokine protein levels in serum (as defined by the 95% CI range not crossing

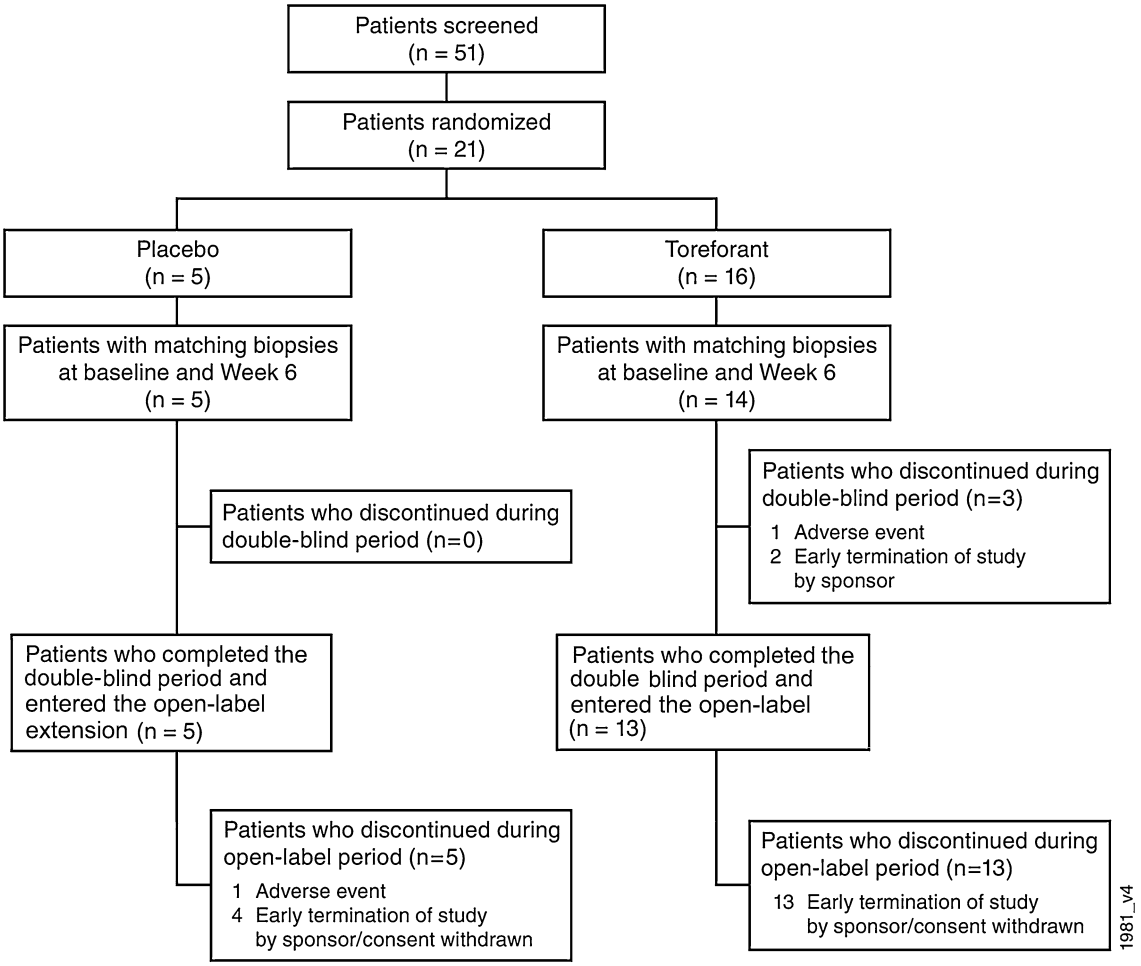

Fig. 2 Study flow

the *y*-axis at 0 in one group but not the other). However, the ability to further assess the robustness of these differences is limited by the small sample size (with paired biopsy data available only from 19 patients overall, and only five in the placebo arm). Nonetheless, within the limitations of the data set, major differences between placebo and toreforant arms were not apparent. Possible exceptions, in addition to the aforementioned analytes, were synovial IL-8, stromal cell-derived factor 1 alpha (SDF-1α), and TNFα mRNA, which displayed patterns suggesting the trends of increase (IL-8) and reduction (SDF-1α and TNFα) in the toreforant arm. However, the upper limit of the 95% CI crossed the *y*-axis at 0. When assessed in the context of all patients (both arms combined), a trend for reduction from baseline in levels of H_1R (synovium) and serum MMP-9 was observed, with the upper limit of the 95% CI not crossing the *y*-axis at 0 only in the case of H_1R.

IL-17 mRNA was measured as a potential H_4R regulatory mediator, as described for psoriasis [26]. For IL-17A, signals were detected in only 5 of the 21 baseline samples and in 3 of the 19 week-6 samples. Paired data from matched pre- and post-treatment samples were only available in two cases. For IL-17F, none of the samples had a detectable signal. Cytokines and chemokines with established roles in RA pathogenesis such as TNF, IL-6, and CXLC13 were detectable in all samples, which, consistent with the modest therapeutic benefit of IL-17 targeted approaches in RA, suggests that IL-17A and IL-17F may be expressed to a much lesser extent in synovium.

Association between biomarkers and clinical response

At baseline, comparisons of geometric means of absolute or $\log_2$-transformed biomarker levels between responder and nonresponder subgroups were significant for one analyte only without adjustment for multiple comparisons (none were significant when adjusted for multiple comparisons). Baseline pERK levels were higher in nonresponders than responders (Fig. 5a), with $\log_2$-transformed geometric

Table 2 Summary of baseline characteristics and DAS28-CRP response through week 12

	Placebo	Toreforant	All patients
Randomized patients	5	16	21
Baseline patient characteristics			
White (non-latino/hispanic), *n* (%)	5 (100.0)	16 (100.0)	21 (100.0)
Female, *n* (%)	4 (80.0)	14 (87.5)	18 (85.7)
Age (years), mean (SD)	54.4 (6.19)	49.8 (9.39)	50.9 (8.83)
Weight (kg), mean (SD)	69.8 (15.74)	74.1 (12.58)	73.0 (13.10)
Body mass index (kg/m^2), mean (SD)	24.6 (5.42)	27.6 (4.64)	26.9 (4.87)
CRP (mg/L), mean (SD)	1.2 (0.84)	2.1 (2.52)	1.9 (2.25)
Number of swollen joints (0–28), mean (SD)	6.8 (4.71)	10.9 (5.52)	10.0 (5.53)
Number of tender joints (0–28), mean (SD)	18.8 (5.63)	18.4 (7.77)	18.5 (7.19)
DAS28-CRP, mean (SD)	5.0 (0.84)	5.2 (0.89)	5.1 (0.86)
HAQ-DI (0–3), mean (SD)	1.7 (0.58)	1.7 (0.59)	1.7 (0.58)
DAS28-CRP response, n (%)			
Pts with DAS28-CRP good/moderate response, *n*	5	13	18
Week 2	2 (40.0)	10 (76.9)	12 (66.7)
Week 4	4 (80.0)	12 (92.3)	16 (88.9)
Week 6	3 (60.0)	10 (76.9)	13 (72.2)
Week 8	4 (80.0)	8 (61.5)	12 (66.7)
Week 12	2 (40.0)	11 (84.6)	13 (72.2)

CRP C-reactive protein, *DAS28-CRP* 28-joint disease activity score employing C-reactive protein, *HAQ-DI* health assessment questionnaire disability index, *SD* standard deviation

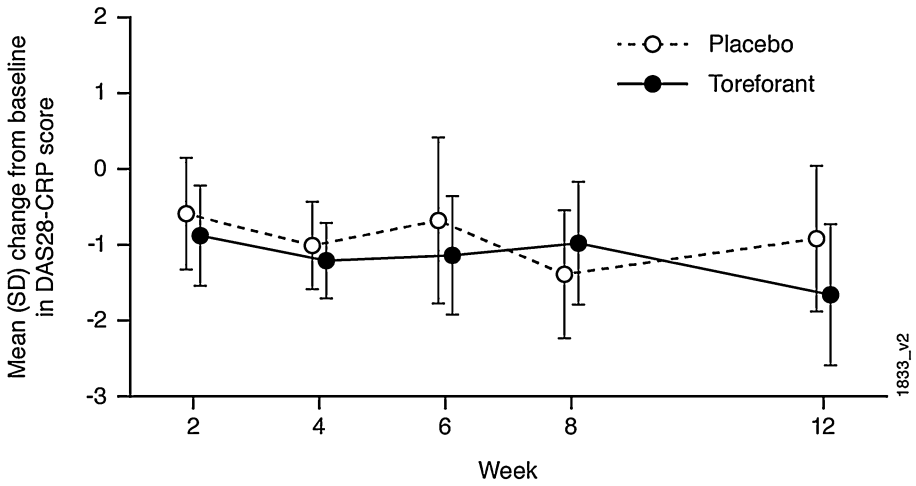

Fig. 3 Change in DAS28-CRP levels over time through week 12 of the double-blind treatment period [placebo (open circles) $n=5$ at each time point. Toreforant (solid circles) $n=16$ at week 2; $n=14$ for weeks 4, 6, and 8; and $n=16$ for week 12]. *DAS28-CRP* 28-joint disease activity score employing C-reactive protein, *SD* standard deviation

means (95% CIs) of 2.38 (0.23, 24.91) vs. 0.12 (0.03, 0.42) ($p=0.03$, unadjusted for multiple comparisons). A similar trend in pERK levels in biopsy samples was not observed at week 6; whether this difference is due to effects on pERK levels related to study participation over time is not known.

For associations with clinical response and changes from baseline in biomarker levels, analytes of interest, i.e., MMP-3 (serum), MCP4 (tissue), H_1R (tissue), and IFN_γ (tissue), were selected based on 95% CIs excluding 0, as shown in Fig. 6. Plots of the individual changes in each group are shown in Fig. 5 for serum MMP-3 (panel B) and tissue MCP4 (panel C), the two analytes with the least amount of overlap between the data points in each responder category. Overall, more marked decreases in serum MMP-3 levels, and increases in tissue MCP4 levels, were observed in responders than nonresponders. More marked decreases in tissue H_1R levels were observed in responders than nonresponders, while tissue IFN_γ levels increased more in nonresponders than responders.

Safety results

Through week 12, AEs were reported by 43.8% (7/16) and 20.0% (1/5) of toreforant- and placebo-treated patients,

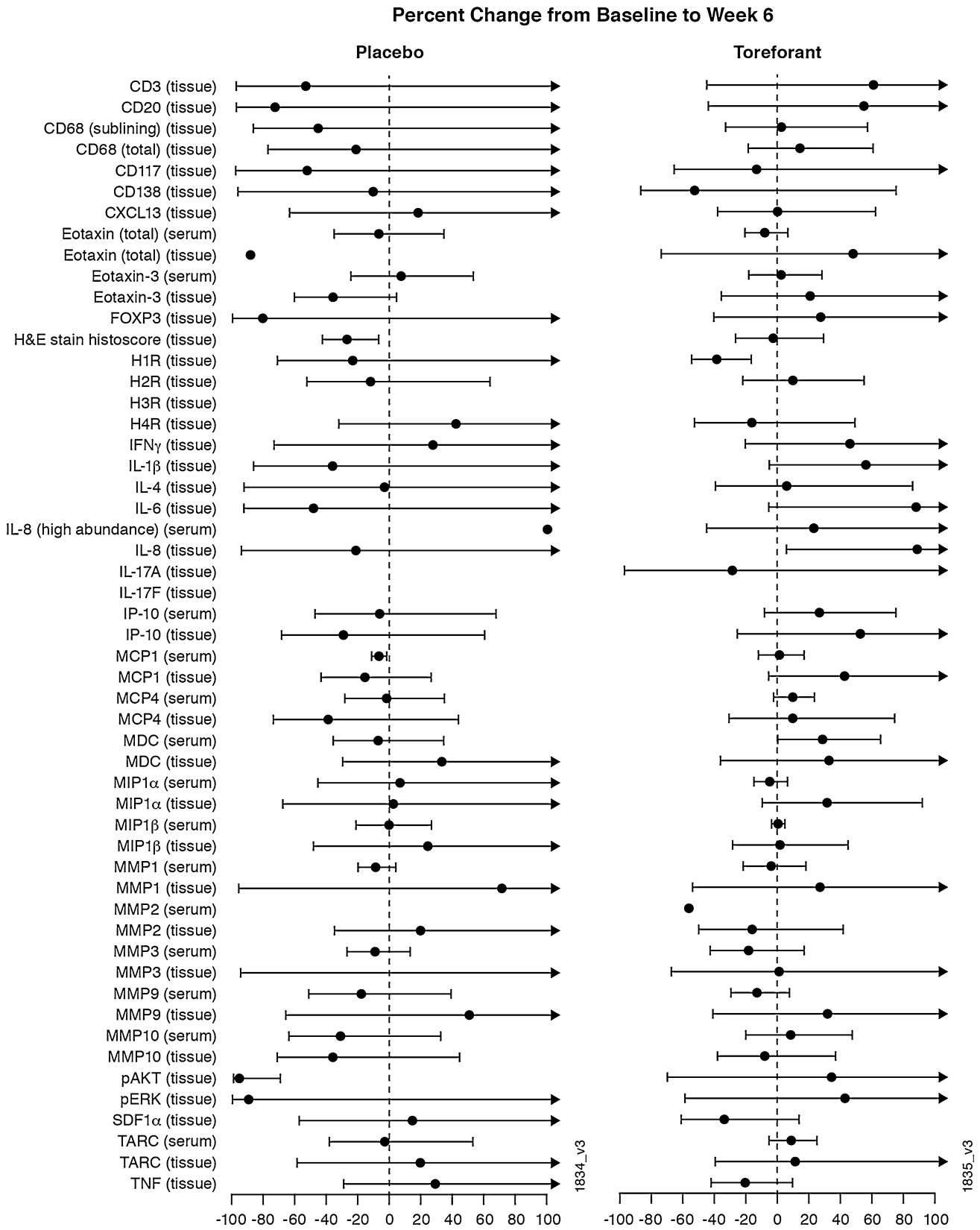

Fig. 4 Percent change in synovial biomarkers from baseline to week 6. Data shown among all PD-evaluable patients are geometric mean (solid circle) and surrounding 95% CI (arrow to the right indicates upper bound extends beyond the length of the *x*-axis). *CI* confidence interval, *PD* pharmacodynamics

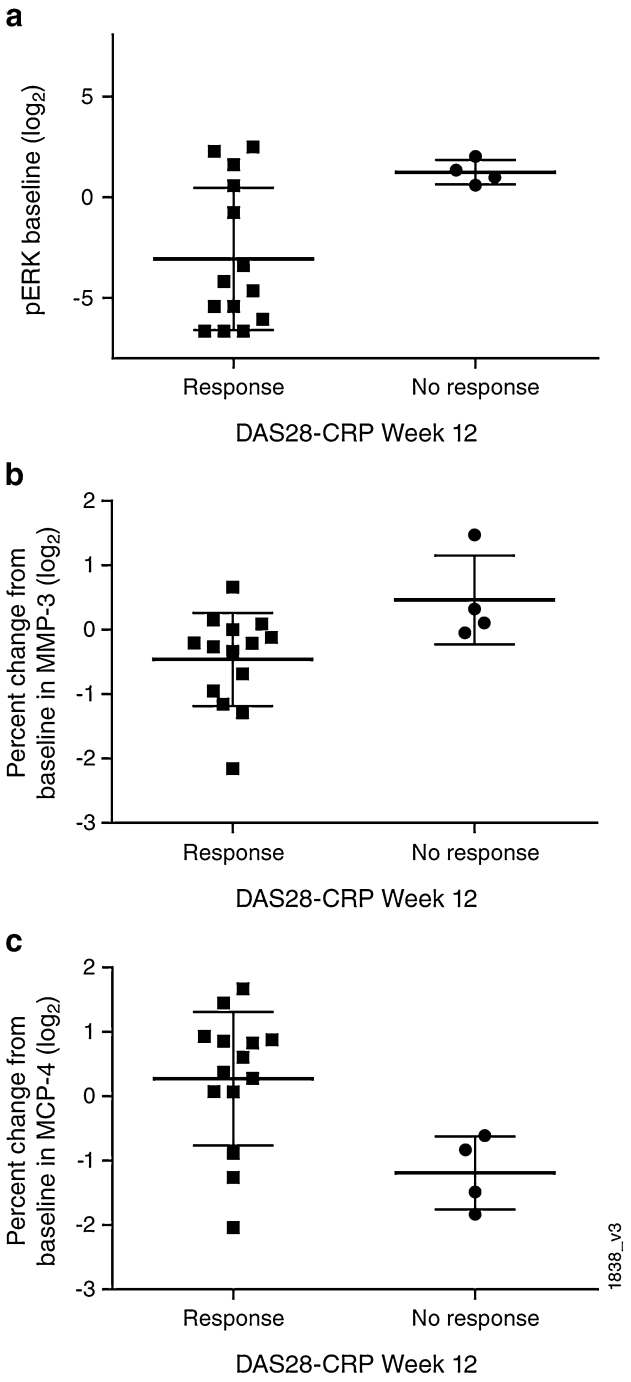

Fig. 5 Associations between biomarker levels and DAS28-CRP response at week 12. Biomarker levels of individuals achieving DAS28-CRP response are depicted via solid square and those with no response by solid circle; summary data are geometric mean (95% CI). **a** pERK at baseline, **b** MMP-3 (serum) changes from baseline to week 6, **c** MCP4 changes from baseline to week 6 in PD-evaluable patients. *CI* confidence interval, *DAS28-CRP* 28-joint disease activity score employing C-reactive protein, *MCP4* monocyte chemotactic protein 4, *MMP-3* matrix metalloproteinase 3, *PD* pharmacodynamics, *pERK* phosphorylated extracellular-signal-regulated kinase

respectively. The most common AEs for toreforant-treated patients were urinary tract infections (25.0%; four patients, three of whom received concomitant systemic corticosteroids) and iron deficiency anemia (18.8%, three patients). After week 12, AEs were reported by 38.5% (5/13) and 20.0% (1/5) of patients initially randomized to toreforant and placebo, respectively, who continued into the open-label period. Urinary tract infections and increased aspartate aminotransferase (AST) were the most common AEs among toreforant-randomized patients during this phase of the study. One placebo-randomized patient had AEs reported after week 12 (angina pectoris, atrial fibrillation, and cardiac failure); these AEs led to discontinuation of study agent during the open-label period (Table 3).

No deaths were reported during the study, and no serious AEs were reported by the investigator through week 12. However, one patient in the toreforant group, a 42-year-old female (with a history of transient elevated transaminases and of toxic hepatitis suspected to be related to leflunomide), had severe events of increased AST/alanine aminotransferase/alkaline phosphatase that were upgraded to serious by the Sponsor. This patient discontinued study agent due to these AEs, which resolved without treatment. This patient received concomitant systemic corticosteroids. After week 12, one toreforant-randomized patient, a 51-year-old female, had a serious AE (femoral neck fracture following a fall on ice). The patient had surgery and continued study participation.

No clinically important and/or consistent changes from baseline in hematology, chemistry, urinalysis, or vital sign parameters were observed in either treatment group. A summary of AEs reported during the truncated open-label period is also provided in Table 3.

Discussion

This report summarizes biomarker, safety, and efficacy results from an MOA study of the H_4R-antagonist toreforant in RA patients. The biomarker findings do not reveal robust pharmacodynamics effects associated with toreforant treatment either in synovial or serum samples, although some trends are apparent. The previous studies of H_4R-antagonist pharmacology and target modulation have only shown the inhibition of histamine-induced eosinophil shape change in blood samples [27]. However, biological effects in other cell and sample types have not been identified. The results of this study, which evaluated 39 analytes, suggest that the modulation of these readouts was modest at best, at least within the context of relatively short-term treatment (6 weeks) and limited sample size.

Patients receiving toreforant during the double-blind period had a numerically higher median decrease from

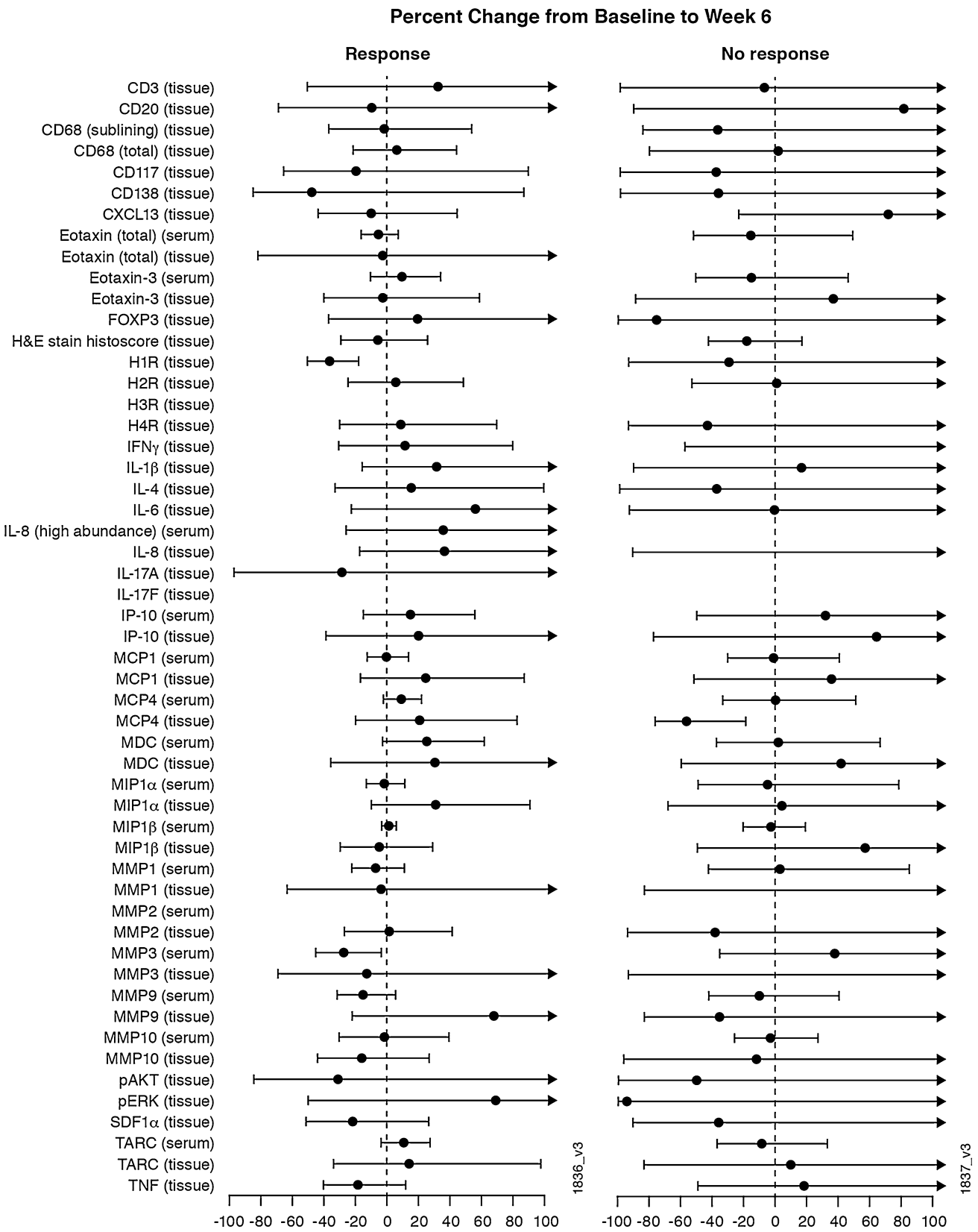

Fig. 6 Percent change in synovial biomarkers from baseline to week 6 by week 12 DAS28-CRP response. Data shown among all PD-evaluable patients are geometric (solid circle) and surrounding 95% CI (arrow to the right indicates upper bound extends beyond the length of the *x*-axis). *CI* confidence interval, *DAS28-CRP* 28-joint disease activity score employing C-reactive protein, *PD* pharmacodynamics

Table 3 Summary of AEs reported during the double-blind and open-label treatment periods

	Double-blind treatment (weeks 0–12)	
	Placebo	Toreforant 30 mg
Treated patients	5	16
Mean weeks of follow-up	11.86	10.84
Patients with ≥ 1 AE[a], *n* (%)	1 (20.0)	7 (43.8)
Urinary tract infection	0	4 (25.0)[b]
Iron deficiency anemia	0	3 (18.8)[c]
Alanine aminotransferase increased	1 (20.0)	1 (6.3)[d]
Alkaline phosphatase increased	1 (20.0)	1 (6.3)[d]
Arthritis	0	1 (6.3)
Aspartate aminotransferase increased	1 (20.0)	1 (6.3)[d]
Creatine phosphokinase increased	0	1 (6.3)
Hypoalbuminemia	0	1 (6.3)[e]
Bilirubin increased	1 (20.0)	0

	Open-label treatment (after week 12 through last follow-up)		
	Placebo→ toreforant 30 mg	Toreforant 30 mg	All toreforant-treated patients
Treated patients	5	13	18
Mean weeks of follow-up	12.69	14.95	14.32
Patients with ≥ 1 AE[a], *n* (%)	1 (20.0)	5 (38.5)	6 (33.3)
Aspartate aminotransferase increased	0	3 (23.1)	3 (16.7)
Urinary tract infection	0	3 (23.1)	3 (16.7)
Alanine aminotransferase increased	0	2 (15.4)	2 (11.1)
Angina pectoris	1 (20.0)	0	1 (5.6)
Atrial fibrillation	1 (20.0)	0	1 (5.6)
Cardiac failure	1 (20.0)	0	1 (5.6)
Femoral neck fracture	0	1 (7.7)	1 (5.6)
Hypoalbuminemia	0	1 (7.7)	1 (5.6)
Iron deficiency anemia	0	1 (7.7)	1 (5.6)

AE adverse event, *MedDRA* Medical Dictionary for Regulatory Activities

[a]AEs are summarized by MedDRA preferred term in descending order of incidence among toreforant-treated patients

[b]Three patients received concomitant systemic corticosteroids

[c]One patient received concomitant systemic corticosteroids

[d]The increased alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase were reported by a single toreforant-treated patient who received concomitant systemic corticosteroids

[e]Patient received concomitant systemic corticosteroids

baseline in DAS28-CRP and numerically higher rates of DAS28-CRP response relative to placebo. However, given the small sample size, no statistical analysis was performed for clinical assessments, limiting interpretation of these results. Two other clinical studies with toreforant in patients with RA (one previous and one concurrent with this study) yielded discordant findings [7]. Some improvement in RA signs/symptoms were observed with toreforant 100 mg in a phase 2a study, but no efficacy was observed with toreforant at lower doses in phase 2b evaluations. The changes in DAS28-CRP level in this study were similar to those reported in the phase 2a study. Of note, although no efficacy was observed overall in the phase 2b study, results from patients enrolled in Europe indicated numerically greater improvement for toreforant 30 mg versus placebo and the mean changes in DAS28-CRP scores from baseline to week 12 (i.e., − 1.5 for toreforant versus − 0.9 for placebo) were very similar to those reported in this study. In general, the toreforant safety profile was consistent between this study and the concurrent phase 2b study, with urinary tract infections being more common in the toreforant- than placebo-treated patients. However, no

anemia was observed in the larger phase 2b study or other toreforant studies [7].

Trends indicating a possible association between a small number of the biomarker analytes and clinical responses at week 12 (independent of treatment arm) were observed for baseline pERK level in tissue and for the change from baseline to week 6 in tissue MCP4 and serum MMP-3 levels. MCP4 (also known as CCL13) has been reported to be elevated at both the mRNA and protein levels in articular cartilage specimens from joints of RA patients [28]. In addition, reductions in serum MMP-3 levels have been reported to occur with TNF blockade in RA patient studies [29], and MMP-3 is one of the analytes in a multi-biomarker disease activity test that is available for use in the clinic for assessing disease severity [30]. This study's limited sample size should be considered when interpreting these findings. Investigations in studies with larger populations may further inform whether any of these molecules may have utility as correlates of clinical response in RA. However, the previous studies by our group have demonstrated robust effects in serial biopsy studies and suggest that the pharmacologic action of the H_4R antagonist is most likely modest [13, 14, 31].

When evaluated in preclinical in vivo (mouse collagen-induced arthritis) and in vitro (mouse and human blood) models, treatment with an H_4R antagonist reduced IL-17 production and arthritis disease severity [6]. In the current clinical trial, IL-17A and IL-17F gene expression in synovial samples was either not detected or present in very low amounts. The previous reports in the literature on IL-17 expression in RA synovium have measured protein levels, mostly by IHC [32], rather than mRNA, methods and/or have made measurements in ex vivo explant cultures rather than freshly preserved tissue [33]. Information on IL-17F expression is even more limited. The very low levels of RNA transcripts for these two cytokines are consistent with findings that blockade of IL-17 or IL-23 (which drives IL-17 expression) has only modest clinical efficacy in RA patients compared with psoriasis and psoriatic arthritis. As there is some evidence suggesting a potential role for H_4R in Th17 biology, such activity would not likely be a driver of clinical efficacy in the RA setting.

Conclusions

Results of this study conducted to investigate the MOA of the H_4R-antagonist toreforant in RA, including molecular correlative analysis in synovium, provide information on the levels of expression of several proteins and transcripts in synovial tissue samples and in circulation. The mRNA levels of IL-17A and IL-17F were very low in synovium. Potential trends indicating the correlation of specific analytes with clinical response are described. Toreforant treatment appeared to be well tolerated, with no new safety findings reported. The study was terminated early, when results from a concurrently running phase 2b efficacy study did not demonstrate a toreforant treatment effect in RA.

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Data availability The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest SE DePrimo, C Calderon, D Chen, PJ Dunford*, W Barchuk*, and RL Thurmond are/were employees of Janssen, a Johnson and Johnson (J&J) Pharmaceutical Company, and own stock in J&J. DL Boyle and GS Firestein have each received research grant funding from Janssen. *These authors were employed by Janssen at the time this study was conducted.

Ethical approval All procedures performed in studies involving human participants were in accordance with ethical standards of the institutional review board and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained for all individual participants included in the study.

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