Intranasal Administration of Recombinant Human Cartilage Glycoprotein-39. A Phase I Escalating Cohort Study in Patients with Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** To investigate safety and tolerability and pilot efficacy of repeated single doses of Org39141 in patients with active rheumatoid arthritis (RA). Org 39141 is recombinant human cartilage glycoprotein-39, intended to induce mucosal tolerance upon intranasal administration.

Methods. RA patients with moderate disease activity were treated for 4 weeks and followed for another 8 weeks. The trial had a sequential cohort design: RA patients in the first cohort received 4 intranasal doses (one per week) of either 25 μg Org 39141 or placebo; in subsequent cohorts, treatment with 125μg, 625 μg, or 3125 μg Org39141 was compared to placebo. Safety was evaluated by means of reporting adverse events, standard laboratory testing, and nose examination. The primary efficacy endpoint was RA disease activity as measured by the Disease Activity Score 28 (DAS28).

Results. A total of 36 patients were randomized. Org39141 was well tolerated, and no severe or serious adverse events (AE) were reported. In the pooled placebo group, a decrease in DAS28 was observed, but to a lesser extent than in the Org 39141 treatment groups. After 4 weeks of treatment, the mean decrease in DAS in the 625 μg Org 39141 treatment group (–24%) was statistically ($p = 0.02$) and clinically (EULAR criteria) significantly larger than in the pooled placebo group (–3%). Once-weekly intranasal treatment with Org39141 was well tolerated, and no serious or severe AE were reported. A trend towards efficacy was observed. Our results are encouraging for further clinical development of Org39141. (J Rheumatol 2006;33:1726–33)

Key Indexing Terms:
RHEUMATOID ARTHRITIS RECOMBINANT HUMAN CARTILAGE GLYCOPROTEIN-39
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Rheumatoid arthritis (RA), a disease affecting peripheral diarthrodial cartilaginous joints, is a chronic, potentially crippling rheumatic disease. The etiology of RA is complex and most likely multifactorial, involving multiple endogenous and exogenous factors in immunogenetically susceptible hosts. While the pathogenesis of the disease has only partly been elucidated, established rheumatoid synovitis is characterized by a number of features associated with autoimmunity. Separate lines of research support that T cells, including autoreactive T cells, play a key role in the initiation and propagation of RA[1]. In RA, T helper-1 type responses associated with inflammation are dominant[1,2]. These autoreactive T cells initiate and/or sustain the inflammation cascade in a joint upon recognition of a specific autoantigen.

Current disease modifying antirheumatic drugs (DMARD) are often non-targeted, general immunosuppressive drugs (e.g., methotrexate), have an unknown mode of action (e.g., gold), or generally suppress immune effector arms not only in inflamed joints, but throughout the body [e.g., anti-tumor necrosis factor-α (TNF-α) treatment]. The challenge for the newer therapies is to specifically inhibit the immune cells that initiate and perpetuate RA-related inflammation and tissue destruction in order to change the disease itself (instead of merely suppressing immune effector processes), thereby controlling the ultimate outcome of RA without causing severe side effects and/or general immunosuppression.

Autoantigen-specific immunotherapy by means of mucosal tolerance induction is an attractive option that potentially fulfils the above mentioned challenge. Tolerance induction by administration of an autoantigen on the mucosal surface may

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lead to the so-called bystander effect, resulting in the suppression of immune responses towards other autoantigens that are in close vicinity of the targeted autoantigen. The mechanism of mucosal, peripheral tolerance induction probably has evolved to protect the host from harmful inflammatory responses to predominantly non-dangerous ingested or inhaled antigens[3]. Oral tolerance induction targeting type II collagen as autoantigen has been tested both in animal models and in clinical settings, the clinical trials with RA patients showing mixed results[4]. The administration of antigens via the nasal mucosa is currently regarded as the most powerful route for induction of immunological tolerance.

Human cartilage glycoprotein-39 (HC gp-39) has been identified as a potential key autoantigen in RA[5,6]. HC gp-39 is a major secretory product of articular chondrocytes and synovial cells[7]. The synthesis of HC gp-39 appears to be induced under inflammatory or degenerative conditions, and it was found that elevated serum levels of HC gp-39 reflect joint injury[8,9]. Epitopes of HC gp-39 are recognized by the immune system of patients with RA[5]. In addition, HC gp-39 protein was detected in RA synovial tissue, and epitopes of this protein are presented in the context of HLA class II molecules by antigen-presenting cells in inflamed joints of patients with RA[10,11]. The relevance of HC gp-39 in joint pathology was strongly supported by the demonstration that when mixed in adjuvant and subsequently injected into Balb/c mice, recombinant HC gp-39 induces arthritis. Recombinant HC gp-39 was shown to significantly reduce the clinical symptoms of arthritis when administered intranasally after disease developed in this model[5]. Recent findings demonstrate that recombinant HC gp39 is able to modulate arthritis in the collagen-induced arthritis model (DBA/1 mice) as well, thus showing cross-tolerance, which is presumably mediated by the mechanism of bystander suppression[6]. This animal model also showed that, to a large extent, the development of cartilage destruction and bone erosions could be prevented[6]. These findings indicate that intranasal treatment with HC gp-39 can potentially modify and possibly control arthritis and offers an opportunity for development as a therapy for RA.

Our Phase I trial is the first study investigating the intranasal route in RA. Org 39141 is a recombinant HC gp-39 produced by CHO cells. Instead of healthy volunteers, patients with RA were chosen as the trial population: RA patients were expected to benefit from mucosal tolerance induction by Org 39141, and occurrence of side effects of tolerance induction may depend on the presence of activated RA-related (autoreactive) T cells, thus limiting the predictive value of studies in healthy volunteers. The objectives of this Phase I trial were to investigate the safety and tolerability of, and to conduct a pilot study on, the efficacy of repeated single doses of Org 39141 in patients with RA.

MATERIALS AND METHODS

Patients. Patients were selected from the outpatient clinic of the Department of Rheumatic Diseases of the Radboud University Medical Center, Nijmegen, The Netherlands. Selection criteria included age between 17 and 66 years, meeting the revised 1987 American Rheumatism Association (ARA; American College of Rheumatology, ACR) criteria for RA classification, active polyarthritis defined as a modified Disease Activity Score (DAS28)[12] of over 3.2 at screening (i.e., at least moderate disease activity), RA history shorter than 10 years, no concomitant treatment with DMARD (other than sulfasalazine at a stable dose from 6 weeks prior to screening; other DMARD required a 6-week washout), no treatment with oral corticosteroids over 10 mg/day oral prednisone or equivalent (stable dose from 4 weeks prior to screening), stable dose of nonsteroidal antiinflammatory drugs (NSAID) from 4 weeks prior to screening, no intraarticular injections with corticosteroids from 4 weeks prior to screening, intact (nose draining) lymph nodes in the neck area, and no abnormal mucosa or other nose abnormalities that might accelerate or inhibit the uptake and/or processing of Org 39141 (e.g., polyps, mucosal atrophia) based on the opinion of the ear, nose and throat (ENT) specialist at screening. The trial protocol and informed consent were approved by the institutional ethics committee of the study center. All patients had given written consent for the study and for publication of the results.

Trial design. The trial was a randomized, double-blind, placebo-controlled, repeated single-dose, cohort sequential escalation trial performed in one study center. Four sequential cohorts of 10 patients each were to be studied. Following confirmation of eligibility, the patients of a cohort were to be randomized to receive treatment with nasal spray containing either Org 39141 (8 patients) or placebo (2 patients) over 4 weeks. Patients in the first cohort received the lowest dose (i.e., 25 μg Org 39141); patients in the subsequent 3 cohorts received doses of 125 μg, 625 μg, and 3125 μg Org 39141, respectively. After the treatment period, the patients were followed for 8 weeks. In the absence of prohibitive adverse events (AE), the next cohort started after 4 of the 8 weeks of this short-term followup of the patients of the previous cohort. The decision to escalate to the next higher dose cohort was taken by the principal investigator based on the results of the safety assessments.

Treatment. A dose was supplied as a nasal spraying system containing 1900 μl solution of a phosphate buffer with 0.3 M sodium chloride with 0 μg, 25 μg, 125 μg, 625 μg, or 1562.5 μg Org 39141 per 800 μl. Upon storage a few small particles consisting of protein might have been formed. Possible particles were removed by filtration (0.22 μm filter) just before the administration of the trial drug. Org 39141 was manufactured by NV Organon, Oss, The Netherlands. Org 39141 or placebo was administered intranasally once a week during 4 consecutive weeks by spraying 800 μl solution (i.e., 4 puffs of 100 μl per nostril) for the 25 μg, 125 μg, and 625 μg dose; and 1600μl (i.e., 8 puffs of 100 μl per nostril) for the 3125 μg dose. Each administration of trial medication was performed by the trial physician at the day clinic of the Radboud UMC. During 9 hours of visits to the day clinic, the various safety and efficacy assessments were performed by the same trial physician.

The dosing schedule could not rely on previous experience with intranasal tolerance induction in humans. The dose range was chosen such that a range as wide as possible was covered. Animal studies with oral type II collagen showed efficacy in the range of 3–300 μg, while the effective dose range for nasal HC gp-39 treatment in mice appeared to be 3–100 μg, of which 25 and 50 μg appeared optimal. For an immunological treatment, the effective dose may not differ much between an animal and human being. For this reason, the dose effective in mice was chosen as the lowest dose. Subsequent dose levels were 5-fold of that dose up to a level maximized by the solubility of the protein in a small volume (i.e., 125, 625, and 3125 μg). It was reasoned that the total amount of administered protein that accumulated over time may relate to efficacy. As variation in dose and frequency would have led to too many different dosing cohorts, only the dose was varied. The fixed chosen frequency was based on the mouse studies: once a week appeared optimal.

Safety assessments. Safety data were obtained on each of the 4 treatment days and at followup assessments at 1, 2, 5, and 8 weeks after the last drug administration. Safety assessments included AE, examination of the application site by an ENT specialist, physical examination, vital signs, electrocardiogram (ECG), and routine laboratory testing (hematology, biochemistry, and urinal-

ysis). Serum levels of Org 39141 (i.e., endogenous HC gp-39[8,9] plus any Org 39141 systemically added via the nose, which would be indistinguishable) and possible serum and saliva antibodies against Org 39141 were determined.

Efficacy assessments. The primary efficacy endpoint was DAS28[12]. Secondary efficacy endpoints included the percentage of patients attaining ACR20 response[13] or "good response" according to the EULAR criterion[12], and the improvement of the individual measures of the ACR core set: tender and swollen joint counts (28 joints), patient assessment of pain (visual analog scale, VAS, 0–100 mm), patient global assessment of disease activity (VAS), physician global assessment of disease activity (VAS), Health Assessment Questionnaire (HAQ)[14], erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) level. All efficacy measures were assessed on each of the 4 treatment days (before trial drug administration) and at followup visits at 1, 2, 5, and 8 weeks after last treatment day.

Statistical analysis. Safety analyses were based on the all-subjects-treated population. AE were analyzed using patient incidence rates and were summarized by World Health Organization Adverse Reaction Dictionary (version 1997.2 using system organ classes and preferred terms) for each treatment group. In addition, for laboratory measures and vital signs, the number of values outside predefined safety limits was determined. Efficacy analyses were based on the per-protocol population (i.e., all randomized patients who had at least one post-baseline assessment for the primary efficacy endpoint and no major protocol violations as decided before unblinding). For all efficacy measures, the mean of the screening value and the value of the day of first trial drug administration was taken as the baseline value. Relative changes from baseline in DAS28 levels were compared between each of the 4 Org 39141 treatment groups and the placebo group (i.e., placebo treated patients pooled from all 4 cohorts). In order to correct for the effect of multiple comparisons versus the placebo group, Dunnett adjusted t tests and corresponding 90% confidence intervals were used.

RESULTS

Demographics and baseline characteristics. Between April and December 1999 a total of 36 patients were randomized into the 4 cohorts. All patients started treatment, i.e., 7 received placebo (all were pooled from the 4 cohorts), and 29 received Org 39141 (8, 8, 6, and 7 patients in the 25μg, 125 μg, 625 μg, and 3125 μg cohort, respectively). One patient in the 125 μg cohort discontinued treatment after 3 weeks but continued followup. No patient prematurely discontinued the trial. Efficacy data from one patient were excluded from the per-protocol analyses because of major protocol violations, resulting in N = 35 for the per-protocol analysis of the efficacy results. All patients in the per-protocol analyses met the ARA 1987 criteria for RA classification.

Most results given below are presented by comparing the group of pooled patients who received placebo with each of the 4 Org 39141 treatment groups (i.e., verum Org 39141-treated patients of a cohort). It should be noted that, due to the sequential cohort design of the trial, results might be subject to cohort effects: only within each cohort was the allocation to verum or placebo treatment randomized.

Patient demographics and disease characteristics are presented in Table 1. In the pooled placebo group, the percentage of women was lower (43%) than in the Org 39141 groups. Overall, 25 (69%) patients were female. All groups were comparable with respect to age, body weight, body height, and body mass index. All but 2 patients were Caucasian (94%). Compared to other treatment groups, patients in the 125 μg Org 39141 group had a longer history of RA (mean 5.9 yrs since diagnosis) and patients in the 625 μg Org 39141 group had a shorter duration of RA (mean 1.8 yrs since diagnosis). Baseline values of ACR core set measures and the DAS28 are shown in Table 2. In general, they are comparable among the 5 groups, except that the patients in the 125 μg Org 39141 group had a lower mean disease activity.

Safety. Org 39141 was well tolerated, and no serious or severe AE were reported during the trial. No patients withdrew from the study due to AE. Twenty-five of the 36 patients (69%) experienced at least one AE during the trial period: 3 (43%) in the placebo group, 7 (88%) in the 25 μg group, 6 (75%) in the 125 μg group, 4 (67%) in the 625 μg group, and 5 (71%) in the 3125 μg group. The most frequently reported AE were dizziness (experienced by 14%, 13%, 13%, 33%, and 29% of the patients in the placebo, 25 μg, 125 μg, 625 μg, and the 3125 μg Org 39141 groups, respectively), and headache (reported by 0%, 0%, 38%, 17%, and 57% of the patients). Four AE, i.e., 2 exacerbations of RA (both in the 25 μg group), one hyperemia of the nasal mucosa (625 μg group), and one dry mouth (25 μg group) were considered possibly related to the trial medication by the investigator. For 4 patients (11%), application site reactions were reported: 1 (13%) in the 25 μg group, 2 (33%) in the 625 μg group, and 1 (14%) in the 3125 μg group. All application site reactions were mild and remitted before the end of the trial. Symptoms related to the nasal mucosa and inflamed or swollen lymph nodes in the neck were observed in patients treated with Org 39141 but not in placebo-treated patients. Comparison of the 5 groups is complicated because of the sequential dose-escalating design of the trial (i.e., cohorts separated in time are compared) and small group sizes, but in general the observed number of AE was somewhat higher in the Org 39141 treatment groups compared to the pooled placebo group.

Only 4 patients experienced clinically relevant (in the opinion of the investigator) increased laboratory values, i.e., eosinophils (625 μg group), non-fasting glucose level (25 μg group), triglyceride level (3125 μg group), and potassium concentration (3125 μg group). All increased levels returned to normal before the end of the trial. No clinically relevant changes from baseline were reported for urinalysis, physical examination, ECG, vital signs, or body weight.

Org 39141 serum levels and antibodies against Org 39141. No serum IgG, IgM, or IgE antibodies against Org 39141 were found in blood samples taken before the first trial drug administration or at 1 or 8 weeks after the last trial drug administration. In addition, no Org 39141-specific antibodies of the IgA subclass were found in the saliva taken at the same assessments. At baseline, prior to the first trial drug administration, the mean serum HC gp-39 concentration ranged between 23.3 ng/ml in the 3125 μg group and 42.0 ng/ml in the placebo group. At 1 and at 8 weeks after the 4-week Org 39141 administration, no increase or only minor increases in the serum HC gp-39 concentrations were observed in all

Table 1. Patient demographics and disease characteristics at baseline.

	Placebo	Org 39141 Treatment Group, μg/week				
	N = 7	28 μg, N = 8	125 μg, N = 8	625 μg, N = 6	3125 μg, N = 7	Total, N = 36
Female, N (%)	3 (43)	5 (63)	6 (75)	5 (83)	6 (86)	25 (69)
Male, N (%)	4 (57)	3 (38)	2 (25)	1 (17)	1 (14)	11 (31)
Age, yrs, mean ± SD	50.7 ± 9.0	52.1 ± 12.6	46.1 ± 10.6	49.5 ± 6.6	49.4 ± 10.7	49.6 ± 9.9
Body weight, kg, mean ± SD	73.9 ± 8.5	81.2 ± 16.5	64.5 ± 12.2	71.9 ± 21.8	67.8 ± 4.3	71.9 ± 14.3
Body mass index, kg/m^2, mean ± SD	25.3 ± 4.1	27.6 ± 3.6	22.7 ± 4.3	25.5 ± 7.0	24.7 ± 1.8	25.2 ± 4.4
RA duration since onset, yrs, mean ± SD	5.0 ± 3.4	4.3 ± 2.7	7.3 ± 4.9	2.4 ± 1.3	6.4 ± 3.0	5.2 ± 3.6
RA duration since diagnosis, yrs, mean ± SD	4.2 ± 3.6	3.5 ± 2.3	5.9 ± 3.5	1.8 ± 1.5	3.9 ± 2.7	4.0 ± 3.0
Concomitant sulfasalazine, N (%)	5 (71)	6 (75)	6 (75)	5 (83)	7 (100)	29 (81)
Concomitant oral corticosteroids, N (%)	0 (0)	1 (13)	0 (0)	1 (17)	0 (0)	2 (6)
Concomitant NSAID, N (%)	6 (86)	8 (100)	5 (63)	6 (100)	7 (100)	32 (89)
Presence of IgM RF, N (%)	6* (100)*	7* (100)*	6 (75)	6 (100)	7 (100)	32 (94)

* For 2 patients (one in the placebo group and one in the 25 μg Org 39141 group), rheumatoid factor (RF) was not assessed.

Table 2. RA disease activity at baseline. Values are mean ± SD.

	Placebo	Org 39141 Treatment Group, μg/week			
	Placebo, N = 7	25 μg, N = 8	125 μg, N = 7	625 μg, N = 6	3125 μg, N = 7
DAS28	4.4 ± 0.7	4.7 ± 1.1	3.4 ± 0.8	4.3 ± 0.9	3.9 ± 0.7
Tender joint counts, based on 28 joints	4.7 ± 2.3	7.4 ± 5.2	4.2 ± 2.0	4.8 ± 1.8	6.2 ± 3.6
Swollen joint counts, based on 28 joints	8.5 ± 2.6	1.7 ± 3.7	7.4 ± 2.6	7.4 ± 1.2	7.9 ± 2.4
Patient assessment of pain*	34.5 ± 15.4	31.8 ± 22.6	26.2 ± 12.4	42.3 ± 20.3	38.9 ± 24.7
Patient global assessment of disease activity*	41.8 ± 15.1	31.8 ± 19.9	26.1 ± 12.8	45.4 ± 20.4	37.8 ± 26.0
Physician global assessment of disease activity*	35.9 ± 14.2	38.6 ± 15.3	28.4 ± 9.6	36.8 ± 17.0	37.9 ± 11.4
HAQ scores	0.53 ± 0.28	0.54 ± 0.44	0.22 ± 0.31	0.62 ± 0.60	0.56 ± 0.47
ESR, mm/h	17.6 ± 12.1	19.9 ± 13.2	6.6 ± 7.2	15.4 ± 10.6	6.8 ± 3.3
CRP, mg/l	27.1 ± 23.4	18.3 ± 12.4	3.9 ± 1.7	13.4 ± 11.4	7.2 ± 5.9
Duration of morning stiffness, hh:mm	0:54 ± 1:04	1:02 ± 1:25	1:01 ± 1:03	1:17 ± 1:54	0:39 ± 0:38

* VAS scores of 0–100. HAQ: Health Assessment Questionnaire; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein.

groups. There was no relationship between treatment dose and increases in serum levels.

Efficacy. DAS28. On hospital visit Days 1, 8, 15, and 22, the trial drug was administered as a nasal spray; Days 29, 36, 57, and 78 were followup hospital visit days. In Figure 1, the mean DAS28 is plotted per group against the hospital visit day. In the period between screening and Day 1, DAS28 values decreased in all treatment groups, with the largest decrease seen in the 125μg Org 39141 group. The mean of the screening value and the Day 1 value is used as the DAS28 baseline value. During the 4 weeks of treatment and the 8 weeks of short-term followup, the mean RA disease activity as measured by the DAS28 decreased compared to baseline value in the Org 39141 groups. In the placebo group a decrease was also observed in comparison with the baseline value, but to a lesser extent. The average DAS28 over the first and second week after the last trial drug administration (i.e., the mean of Day 29 and 36) showed the largest decrease in the 625 μg Org 39141 group (–24%), while the DAS28 for the placebo group had decreased 3% (see Table 3). The difference of –21% between the 625 μg Org 39141 group and the placebo group was statistically significant, with a p value of 0.02, and a 90% confidence interval from 37% to 5% (see Table 3). At Days 57–78, the highest mean decrease in DAS28 was again observed in the 625μg Org 39141 group: –23% versus –5% in the placebo group (not statistically significant, p = 0.25). For the other Org 39141 groups, no statistically significant differences with the placebo group were found.

EULAR response. At Day 29 (1 week after the last trial drug administration) moderate EULAR responders were observed in all Org 39141 groups, and good responders (i.e., decrease of over 1.2 DAS28 points plus end status below 3.2) were observed in the 25 μg and 625μg Org 39141 groups, while in the placebo group only nonresponders were observed (see Figure 2).

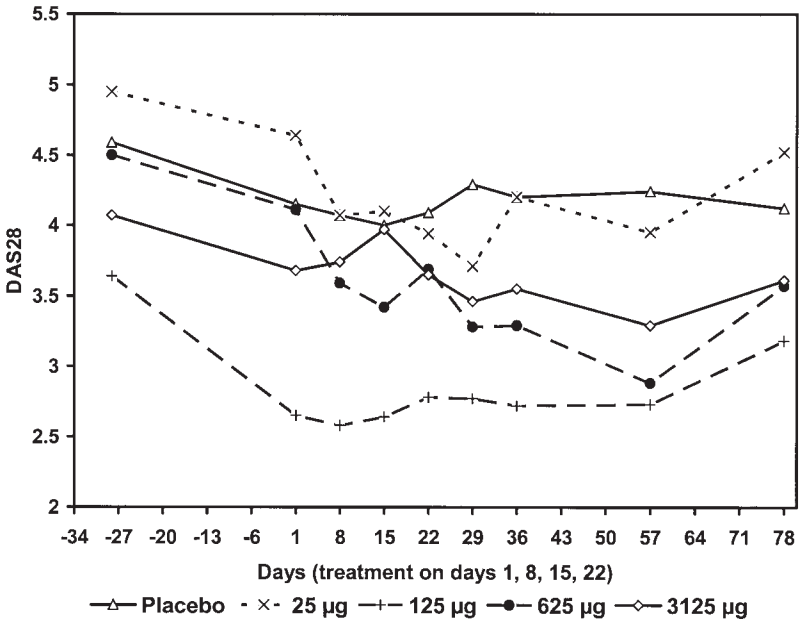

Figure 1. Mean DAS28 per treatment group over time.

Table 3. DAS28, relative change from baseline at Days 29–36 and Days 57–78.

	Placebo N = 7	Org 39141 Treatment Group, µg/week 25 µg, N = 8	125 µg, N = 7	625 µg, N = 6	3125 µg, N = 7
At days 29–36					
Mean % change ± SD	–3.0 ± 13.0	–14.2 ± 11.6	–7.0 ± 12.8	–24.1 ± 11.3	–8.2 ± 15.8
Estimated difference vs placebo, % (90% CI)		–11.1 (–26.3, 4.1)	–3.9 (–19.6, 11.8)	–21.1 (–37.4, –4.8)	–5.2 (–20.9, 10.5
p value		0.306	0.946	0.023	0.869
At days 57–78					
Mean % change ± SD	–5.2 ± 13.8	–9.2 ± 10.6	1.7 ± 23.8	–23.3 ± 14.1	–12.2 ± 24.9
Estimated difference vs placebo, % (90% CI)		–3.9 (–25.3, 17.4)	7.0 (–15.1, 29.0)	–18.1 (–41.1, 4.9)	–7.0 (–29.0, 15.1)
p value		0.982	0.885	0.247	0.886

CI: confidence interval.

ACR20 response. The highest ACR20 response rates were found in the 625 µg Org 39141 group. At Day 29, response rates ranged between 14% (one responder in the placebo group) and 50% (3 responders in the 625 µg Org 39141 group). At Day 78, ACR20 response rates ranged between 0% (in both the 25 µg and the 125 µg Org 39141 groups) and 50% in the 625 µg Org 39141 group.

ACR core set measures. Baseline values for these measures are shown in Table 2. In general, changes in the individual core set measures showed large variation compared to the effect sizes, probably caused by the small group sizes. In the 25 µg, 125 µg, and 625 µg Org 39141 groups, the tender joint count showed a decrease from baseline both at Day 29 and at Day 78. For the placebo group and the 3125 µg Org 39141 group, the number of tender joints had slightly increased (see Table 4). For swollen joint counts, a decrease was seen at all assessments for all treatment groups. No notable dose relationship was observed. The patient's assessment of pain was measured by VAS scores. In the 2 highest Org 39141 groups, the mean pain assessment scores showed a tendency to decrease during the trial, while the patients in the 3 lowest dose groups (placebo, 25 µg, and 125 µg Org 39141 groups) recorded similar or higher mean VAS scores. Patients in the placebo, 625 µg, and 3125 µg Org 39141 groups tended to consider their disease activity (i.e., VAS for patient global assessment of disease activity) as reduced, whereas the mean patient global VAS scores increased for patients treated with 25 µg or 125 µg Org 39141. However, the variation in the results was high, precluding firm conclusions (see also Table 2). Disease activity as assessed by the investigator using the

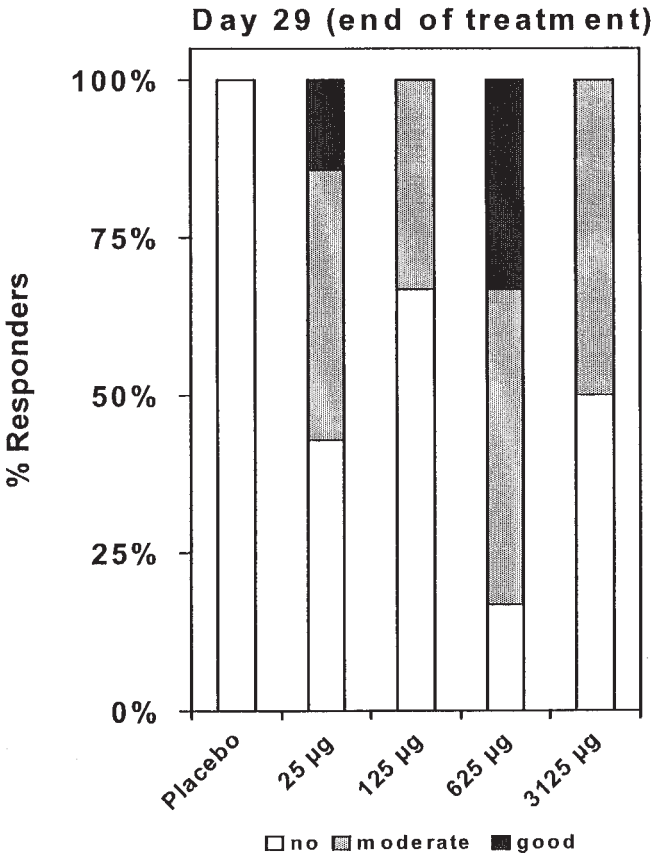

Figure 2. Response according to EULAR criteria.

VAS decreased in all groups. The decrease was most pronounced in the 625 μg and 3125 μg Org 39141 groups. No clear trends in the HAQ scores for physical function were observed in any of the treatment groups. In general, changes from baseline were negligible compared to the standard deviation (SD). Although there were some minor changes in the ESR and CRP values, no clear trends were visible. Moreover, the CRP levels at baseline of the Org 39141 treatment groups were already low for RA patients (see Table 2). The duration of morning stiffness compared to baseline decreased for patients treated with placebo, 125μg, and 625 μg Org 39141, whereas the mean duration of morning stiffness for the 25 μg and 3125 μg Org 39141 group tended to increase compared to baseline.

DISCUSSION

In this first human study, 4 weeks of intranasal administration of a once weekly dose of Org 39141 (recombinant human cartilage glycoprotein-39) or placebo (i.e., buffer solution) was tolerated well by the 36 (29 verum, 7 placebo) patients with RA treated in the 25 μg, 125 μg, 625 μg, or 3125 μg dose cohort. No serious or severe AE were reported. The most frequently reported AE were dizziness, which was reported in all treatment groups including the placebo group and was in all cases mild; and headache, which was reported in the 3 highest Org 39141 treatment groups and was mild to moderate. Indeed, AE related to the protein itself were not expected, since HC gp-39 is naturally present in serum, both in healthy persons and at elevated levels in patients with RA[8,9], and since systemic accumulation upon intranasal administration is neither intended nor anticipated. After treatment in this study, no dose related increase in serum HC gp-39 levels was observed, nor were antibodies against Org 39141 detected.

That animal models support the concept of tolerance induction is based on the following proposed mechanism of action[5,6]: upon intranasal administration of the RA-associated autoantigen HC gp-39 (Org 39141), antigen-presenting cells of the nasal mucosa become activated and migrate to the nose, draining lymph nodes in the neck area[15]. Here, these antigen-presenting cells activate T regulator cells that subsequently migrate to the inflamed joints and become activated for a second time as the result of high local expression of the relevant autoantigen (HC gp-39). This second antigen encounter is thought to result in the excretion of antiinflammatory cytokines, leading to the local suppression of immune cell activities. In our Phase I trial, some patients experienced a swollen nasal mucosa and/or swollen lymph nodes in the neck, which might be indicative of the proposed mechanism of action. Further, no AE associated with general immunosuppression, such as fever or increased infection rate, were reported. After the study, the patients were followed for another 2 years, to evaluate the longterm effects of Org 39141. No AE were reported that indicate longterm safety concerns. The HC gp-39 antigen-specific intervention using Org 39141 is considered relatively safe since general immunosuppression is not expected, in contrast to therapies with a general immunosuppressive action including anti-TNF-α treatment[16,17].

Efficacy of Org 39141 treatment was assessed by analyzing the change of a composite measure for disease activity, the DAS28. Although the 4 different dose cohorts were treated sequentially, and no randomization was performed between the different dose cohorts, for convenience the trial results of the placebo patients as pooled from all 4 cohorts and each of the 4 groups of verum Org 39141-treated patients were compared. Further, because the trial was a Phase I study, the groups were rather small (6–8 patients). During the trial, disease activity decreased in all groups. A notable reduction in disease activity, as measured by DAS28, was already observed during the screening period (i.e., before first administration of the study drug). This decrease may be partially explained by regression to the mean, but may also be due to patients' expectation of receiving a promising new drug as well as to the extra care they received during the screening period. After the 4 weeks of treatment, the additional decrease in disease activity was more pronounced in the verum Org 39141 treatment groups. The difference in the decrease of DAS28 between the 625 μg group and the placebo group (–24% vs –3%) reached statistical ($p = 0.02$) and clinical (i.e.,

Table 4. Tender and swollen joints and visual analog scale (VAS) patient global assessment and erythrocyte sedimentation rate (ESR), change from baseline at Days 29 and Days 78. Values are mean ± SD.

	Placebo	Org 39141 Treatment Group, μg/week			
	Placebo, N = 7	25 μg, N = 8	125 μg, N = 7	625 μg, N = 6	3125 μg, N = 7
Change in tender joint count, based on 28 joints					
Day 29	0.0 ± 3.0	–2.3 ± 2.3	–1.8 ± 0.7	–3.5 ± 1.6	0.5 ± 2.8
Day 78	2.1 ± 7.0	–1.8 ± 1.7	–1.3 ± 0.8	–3.2 ± 1.1	1.8 ± 5.4
Change in swollen joint count, based on 28 joints					
Day 29	–1.2 ± 3.4	–4.7 ± 2.5	–3.9 ± 1.2	–4.3 ± 1.0	–3.8 ± 2.0
Day 78	–1.4 ± 4.9	–1.8 ± 1.7	–3.8 ± 1.8	–3.9 ± 1.0	–2.2 ± 1.9
Change in VAS (0–100 mm), patient global assessment of disease activity					
Day 29	–8.2 ± 15.3	2.8 ± 13.4	5.6 ± 9.0	–14.1 ± 10.6	–16.8 ± 18.9
Day 78	–6.9 ± 13.6	12.8 ± 17.0	13.1 ± 14.9	–7.4 ± 14.6	0.5 ± 21.9
Change in ESR					
Day 29	2.8 ± 7.9	0.6 ± 5.8	1.4 ± 2.8	0.8 ± 1.8	0.2 ± 3.9
Day 78	–1.2 ± 8.7	–3.2 ± 6.0	5.6 ± 10.2	1.8 ± 6.4	–1.1 ± 2.4

EULAR response) significance. This decrease can mainly be attributed to improvement of the tender and swollen joint count scores. It is not clear whether the stable, concomitant treatment with sulfasalazine (81% of the patients), NSAID (89%), and oral corticosteroids (6%) may have influenced the Org 39141 treatment efficacy by interference with its mechanism of action.

Induction of tolerance in RA using a complexed epitope of HC gp-39 has been studied in a Phase I setting[18]. Instead of employing the mucosal route of tolerance induction, that study evaluated the intravenous administration of HLA-DR-complexed antigen without costimulatory signals [i.e., an immunodominant epitope of HC gp-39 complexed to HLA-DR4 (B*0401)], which has been demonstrated in animal models to induce immunological tolerance. Over 6 weeks, 31 HLA-DRB1*0401 (i.e., HLA-DR4) positive patients with persistent RA disease activity received 7 intravenous infusions of the soluble complex of native HLA-DRB1*0401 complexed with (i.e., binding to) the synthetic 263-275 13-mer peptide of HC gp-39 or placebo. Treatment was well tolerated, with injection site reaction the most common adverse event. There was no loss of reactivity to recall antigens, change in cell counts, or antibodies to HLA-DR. Some evidence of clinical response was seen; responses were more common among patients receiving the highest doses and among those with baseline T cell reactivity to the HC gp-39 peptide.

Our Phase I trial studying intranasal administration of Org 39141 (recombinant HC gp-39) revealed no safety concerns related to 4 weeks dosing from 25 to 3125 μg of this protein. A trend towards efficacy was observed upon Org 39141 treatment compared to placebo. The results are encouraging for further clinical development of Org 39141 as a new modality for treatment of rheumatoid arthritis, aiming at antigen-based, specific intervention in the immunological propagation of the inflammatory process.

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