Targeted therapies in rheumatoid arthritis
Boumans, M.J.H.

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CHAPTER 9

A Phase IIA, Randomized, Double-Blind, Placebo-Controlled Trial of Apilimod Mesylate, an IL-12/IL-23 Inhibitor, in Patients with Rheumatoid Arthritis

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1Division of Clinical Immunology and Rheumatology, Academic Medical Center/University of Amsterdam, Amsterdam, the Netherlands; 2Synta Pharmaceuticals Corp., Lexington, MA, USA.

ABSTRACT

Objective: To investigate safety, tolerability, pharmacokinetics, and efficacy of apilimod mesylate, an oral interleukin (IL)-12/IL-23 inhibitor, in patients with rheumatoid arthritis (RA).

Methods: We performed a phase IIa, randomized, double-blind, placebo-controlled proof of principle study of apilimod, in combination with methotrexate, in 29 patients with active RA (3:1, apilimod to placebo) in three stages. Patients received apilimod 100 mg QD or placebo for 4 weeks (stage I) or 8 weeks (stage II). Stage III consisted of apilimod 100 mg BID or placebo for 8 weeks, with an optional extension of 4 weeks. Clinical response (DAS28 and ACR criteria) was assessed throughout; synovial tissue samples collected at baseline and day 29 (I/II) or 57 (III) were stained for cellular markers and cytokines by immunohistochemistry.

Results: While only mild adverse events were observed in stages I/II, all stage III patients experienced headache and/or nausea. Apilimod-treated patients (100 mg QD) showed a small, but significant, reduction in DAS28 at day 29 and 57 compared with baseline. ACR20 was reached in only 6% at day 29 and 25% at day 57, similar to responses in placebo group. Increasing the dosage (100 mg BID) did not improve clinical efficacy. Consistent with clinical results, there was no effect of apilimod on expression of synovial biomarkers. Of importance, there was also no effect of apilimod on synovial IL-12 and IL-23 expression.

Conclusion: Our results do not support the notion that IL-12/IL-23 inhibition by apilimod is able to induce robust clinical improvement of RA.
INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disorder, affecting synovial tissue. Activated immune cells are important in orchestrating synovial inflammation by producing several cytokines, including IL-12 and IL-23. IL-12 is a heterodimeric cytokine (p70) formed by 2 subunits, p40 and p35. The more recently discovered heterodimeric IL-23 is structurally closely related to IL-12 and shares the p40 subunit, but contains a unique p19 subunit. IL-12 acts in the initial inflammation phase by promoting differentiation of naïve CD4+ T cells to interferon (IFN)-γ producing T helper 1 (Th1) cells. IL-23 induces proliferation of effector memory T cells and plays a critical role in the pathogenesis of RA by inducing IL-17 producing T cells. Animal studies confirm this finding, as IL-23–deficient (p19−/−) mice and mice lacking IL-12 and IL-23 (p40−/− mice) was resistant to collagen-induced arthritis (CIA).

Apilimod mesylate (STA-5326), an oral, small-molecule, inhibits the production of IL-12 and IL-23. It selectively prevents nuclear translocation of c-Rel, a member of the Rel/nuclear factor (NF)-κB family of transcription factors thereby reducing both p35 and p40 promoter activities. However, the exact mechanism of action is unknown. Apilimod significantly reduces the production of IL-12, IL-23 and the IL12/23 p40 protein by human peripheral blood mononuclear cells stimulated with various stimuli. Therefore, we tested apilimod in a phase IIA proof of principle study in patients with active RA.

PATIENTS & METHODS

Patient selection. This study was conducted at the Academic Medical Center/University of Amsterdam (AMC/UvA). Patients with RA, who had a disease duration of ≥ six months, were included. All patients had active disease classified as ≥ 4 tender and swollen joints and 1 arthritis of a knee or ankle joint. Furthermore, study patients fulfilled one of the following criteria: C-reactive protein [CRP] ≥12 mg/L, erythrocyte sedimentation rate [ESR] ≥28 mm/hr or morning stiffness ≥45 minutes. Patients were on stable doses of MTX (at least 7.5 mg/week) and discontinued other DMARDs or biologicals, respectively 1 or 3 months prior to start of treatment. Oral glucocorticoids (£10 mg/day) and NSAIDs on a stable dose were allowed. The study was approved by the Medical Ethics Committee of the AMC/UvA and performed according to the Declaration of Helsinki. All participants gave written informed consent.

Study design. For all stages, patients were randomized (3:1, apilimod to placebo) on day 1. In stage I, 9 patients were treated with apilimod 100 mg once-daily (QD) and 3 with placebo for 4 weeks. In stage II 8 patients received apilimod 100 mg QD and 2 placebo for 8 weeks. The dosage of apilimod was increased to 100 mg twice-daily (BID) in stage III and 5 patients were treated with apilimod and 2 with placebo for 8 weeks, with an optional extension of 4 weeks. Apilimod was dispensed on clinic days for at-home oral administration (QD) for the next 2 weeks.

Clinical assessment, safety and pharmacokinetics. Routine safety and clinical assessments were performed at day 1, 15, 29; for stages II and III also at day 43 and 57; and at day 71 and 85 for the stage III extension. A follow up visit was performed 1 week after discontinuation of the study drug. Patients were evaluated according to the DAS28 and ACR response
criteria. In stage I, blood was drawn pre-dose and at 1, 2, 4, 6, and 8 hours post-dose for extensive pharmacokinetic determination of apilimod, apilimod metabolites, MTX, and 7-hydroxy-MTX at day 1, 15 and 29.

Synovial tissue collection and immunohistochemical analysis. An arthroscopy was performed under local anaesthesia at baseline and at day 29 (stages I/II) or 57 (stage III) to obtain synovial tissue samples from an actively inflamed knee or ankle, as previously described. Synovial tissue sections were stained with anti-CD68 antibody (Dako, Denmark) to detect macrophages, anti-IL-12p70 (R&D Systems Europe, UK) and anti-IL-23 (Biolegend, CA). See supplementary file for a detailed description and staining of the other cellular synovial markers. Expression of CD68 is presented as count/mm²; IL-12p70 and IL23 is presented as integrated optical density (IOD)/mm², an arbitrary unit representing the intensity of staining per mm².

Statistical analysis. Continuous data were described as mean and standard deviation (SD), if normally distributed, and as median and interquartile range (IQR), if not normally distributed. The unpaired Student’s t test or, where appropriate, Mann-Whitney U test were used for patient characteristics. Nominal data were represented as percentages and analyzed using the Fisher’s exact test. Effects of treatment were evaluated with a paired Student’s t test or Wilcoxon signed-rank test for non-normal distributions. All statistical analyses were performed with SPSS 17.0 software (SPSS, Chicago, IL).

RESULTS

Patient characteristics, safety and tolerability. Twenty-nine eligible patients were included. Disease characteristics were not significantly different between the apilimod and placebo treated groups; the weekly methotrexate dose was 21.1 (5.4) and 22.5 (5.2) milligrams respectively (mean (SD); supplementary table 1). As we observed a favorable safety profile in stage I (supplementary table 2), we continued with stage II and subsequently stage III. In stage I, 8 out of 9 apilimod-treated patients completed the study, while 1 patient, who developed side-effects (severe headache), withdrew from the study on day 29 and refused to undergo the second arthroscopy, but safety and clinical evaluations were completed for this patient. All patients treated in stage II completed the study. In stage III (100 mg BID), 3 out of 5 apilimod-treated patients continued until day 57 and 1 patient decided to extend the study until day 85. Two patients withdrew prior to day 57 due to side effects. While in stages I/II only mild, mainly gastro-intestinal, adverse events were observed in 15 patients treated with apilimod (88%), in stage III, all apilimod treated patients, but also placebo treated patients, experienced side-effects.

Apilimod treatment does not induce marked clinical improvement in RA patients. Apilimod-treated patients in stages I/II (100mg QD) showed a small but statistically significant reduction in DAS28 compared with baseline at day 29 (p = 0.03, n=17) and day 57 (p = 0.004, stage II only, n = 8; Table 1), while we did not observe a significant reduction in DAS28 in the placebo-treated patients. However, according to EULAR response criteria neither change could be classified as a response. An ACR20 response was seen in 1 of 17 patients (6%) after 4 weeks and in 2
Table 1. Clinical response in patients treated with apilimod or placebo

<table>
<thead>
<tr>
<th>Stage I (n = 9) apilimod 100 mg QD</th>
<th>Baseline</th>
<th>Day 29</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAS28, mean (SD)</td>
<td>5.4 (0.7)</td>
<td>5.2 (0.8)</td>
</tr>
<tr>
<td>ΔDAS28, mean (p-value)</td>
<td>-</td>
<td>-0.2 (0.59)</td>
</tr>
<tr>
<td>ACR20, n (%)</td>
<td>-</td>
<td>1 (11.1%)</td>
</tr>
<tr>
<td>ACR50, n (%)</td>
<td>-</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>ACR70, n (%)</td>
<td>-</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stage II (n = 8) apilimod 100 mg QD</th>
<th>Baseline</th>
<th>Day 29</th>
<th>Day 57</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAS28, mean (SD)</td>
<td>5.5 (1.2)</td>
<td>4.8 (1.3)</td>
<td>4.9 (1.2)</td>
</tr>
<tr>
<td>ΔDAS28, mean (p-value)</td>
<td>-</td>
<td>-0.7 (0.02)</td>
<td>-0.6 (0.004)</td>
</tr>
<tr>
<td>ACR20, n (%)</td>
<td>-</td>
<td>0 (0%)</td>
<td>2 (25%)</td>
</tr>
<tr>
<td>ACR50, n (%)</td>
<td>-</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>ACR70, n (%)</td>
<td>-</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stages I/II, pooled (n = 17) apilimod 100 mg QD</th>
<th>Baseline</th>
<th>Day 29</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAS28, mean (SD)</td>
<td>5.4 (0.9)</td>
<td>5.0 (1.0)</td>
</tr>
<tr>
<td>ΔDAS28, mean (p-value)</td>
<td>-</td>
<td>-0.4 (0.03)</td>
</tr>
<tr>
<td>ACR20, n (%)</td>
<td>-</td>
<td>1 (6%)</td>
</tr>
<tr>
<td>ACR50, n (%)</td>
<td>-</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>ACR70, n (%)</td>
<td>-</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stage III (n = 5) apilimod 100 mg BID</th>
<th>Baseline</th>
<th>Day 29</th>
<th>Day 57</th>
<th>Day 85</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAS28, median (IQR)</td>
<td>5.4 (4.8-6.9)</td>
<td>5.2 (5.1-5.9)</td>
<td>5.1 (5.0-7.0)</td>
<td>4.9 (NA)</td>
</tr>
<tr>
<td>ΔDAS28, mean (p-value)*</td>
<td>-</td>
<td>-0.2*</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>ACR20, n (%)</td>
<td>-</td>
<td>0 (0%)</td>
<td>1 (33%)</td>
<td>1 (33%)</td>
</tr>
<tr>
<td>ACR50, n (%)</td>
<td>-</td>
<td>1 (25%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>ACR70, n (%)</td>
<td>-</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Placebo, pooled (n = 7)</th>
<th>Baseline</th>
<th>Day 29</th>
<th>Day 57</th>
<th>Day 85</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAS28, median (SD)</td>
<td>5.3 (4.8-5.7)</td>
<td>4.8 (4.5-5.6)</td>
<td>4.6 (2.7-5.3)</td>
<td>3.3 (NA)</td>
</tr>
<tr>
<td>ΔDAS28, mean (p-value)*</td>
<td>-</td>
<td>-0.5 (0.46)</td>
<td>-0.7*</td>
<td>NA</td>
</tr>
<tr>
<td>ACR20, n (%)</td>
<td>-</td>
<td>0 (0%)</td>
<td>1 (25%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>ACR50, n (%)</td>
<td>-</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>ACR70, n (%)</td>
<td>-</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

In stage I, 1 patient on apilimod withdrew from the study on day 29 due to side effects (severe headache); safety and clinical evaluations were completed, but not the second arthroscopy. In stage III, 1 apilimod-treated patient withdrew on day 15 and 1 apilimod-treated patient on day 29 due to side-effects, and therefore, had no second arthroscopy at day 57. Only 1 of 5 patients in the apilimod and 1 patient in the placebo group decided to extend the study until day 85. Data are represented as n (%), mean (SD) or median (IQR=interquartile range), as appropriate. Placebo samples were pooled from all stages. Treatment effects were compared using a paired Student’s t test.

* No statistics performed due to small group size. † Non-parametric Wilcoxon signed-rank test performed to calculate statistical significant differences compared with baseline due to small patient number; ΔDAS28 = mean changes in disease activity score in 28 joints (DAS28) compared with baseline; NA = not applicable—patient number too small; ns = not significant; ACR20 = 20% American College of Rheumatology improvement response; ACR50 = 50% response; ACR70 = 70% response.
of 8 patients (25%) after 8 weeks of treatment. In stage III, we did not observe a significant reduction in DAS28 during the study, although the number of patients was small. Only 1 of 3 apilimod-treated patients achieved an ACR20 response after 8 weeks (day 57) and 12 weeks (day 85) treatment. One patient reached ACR50 after 4 weeks, but this clinical response had disappeared after 8 weeks. Patients in the placebo group showed no significant change in DAS28 at day 29; patient numbers during later visits were too small to draw any meaningful conclusions. Taken together, apilimod treatment did not induce robust clinical improvement.

**Apilimod treatment does not significantly affect synovial biomarkers.** There was no significant difference in the number of CD68+ synovial macrophages at baseline compared with day 29 (Figure 1). Additionally, no significant changes were observed in the expression of the other synovial markers (supplementary figure). In stage III, the number of patients who underwent an arthroscopy at day 57 was too small to draw any conclusions. Of importance, apilimod treatment did not result in a decrease of IL-12 and IL-23 expression in the synovial tissue after 4 weeks of treatment (Figure 2).

![Figure 1. Effect of apilimod or placebo treatment on CD68+ cells in synovial tissue.](image)

Expression (count/mm²) of CD68+ macrophages in the sublining (A,B) and lining (C,D) layer of synovial tissue of rheumatoid arthritis patients treated with apilimod 100 mg QD (A,C) or placebo (B,D). To obtain synovial tissue samples, an arthroscopy was performed at baseline and at day 29 (stages I/II). Placebo samples were pooled from all stages. A non-parametric Mann-Whitney test was used to compare expression between baseline and day 29; there were no significant changes.
In the present trial, we evaluated the effects of treatment with apilimod mesylate. Overall, apilimod 100 mg QD was well tolerated and did not interfere with MTX treatment. However, nearly all patients treated with the double dosage experienced side effects. We observed a small, but statistically significant, reduction of DAS28 after 4 and 8 weeks of treatment with apilimod 100 mg QD, but patients did not fulfil the EULAR response criteria. Moreover, the change in the number of CD68+ synovial sublining macrophages, which has previously been proven to be a

**DISCUSSION**

Figure 2. IL-12p70 and IL-23 expression in the synovial tissue. Representative pictures of IL-12p70 and IL-23 staining from biopsies taken before (A) and after treatment (B) with apilimod. Original magnification 20x. In picture C-F, the change in either IL-12p70 or IL-23 staining of patients treated with apilimod or placebo is depicted. Expression (IOD) of IL-12p70 (C,D) and IL-23 (E,F) in the synovial tissue of patients treated with apilimod 100 mg QD (C,E) or placebo (D,F). A non-parametric Mann-Whitney test was used to compare expression between baseline and day 29; there were no significant changes.
sensitive biomarker for clinical response \(^{10}\), did not reach statistical significance after 4 weeks. In addition, apilimod treatment did not result in its presumed biological effect: no decrease was observed of IL-12 and IL-23 expression in the synovial tissue after 4 weeks of treatment.

There are several possible reasons why treatment with apilimod did not lead to clinical improvement in RA patients. First, there is the possibility that there was a false-negative result due to the relatively small number of patients in this phase IIa study. However, previous studies with a similar design \(^{10-12}\) have indicated that the number of patients included in our study should be sufficient to detect relevant, robust changes in the synovium after effective treatment. A second explanation for lack of efficacy in our study could be the short duration of treatment, although all effective anti-rheumatic treatments that we tested did induce early changes in the synovium preceding clinical improvement. Moreover, there was no (trend towards) clear cut clinical improvement after 8 weeks of treatment in patients participating in stage III. Third, we need to take into account the possibility that IL-12/IL-23 is perhaps not a good target for the treatment of RA.

The data of our trial with apilimod are consistent with a recently published clinical trial in 220 patients with Crohn’s disease: treatment with apilimod (50 mg or 100 mg QID) was not more effective than placebo \(^{13}\), on the other hand this disease responded well to treatment with monoclonal antibodies against the p40 subunit of IL-12/IL-23 \(^{14}\). Our analyses suggest that the failure of apilimod to influence RA and Crohn’s disease may in fact be due to the possibility that insufficient levels of the compound were reached at the site of inflammation to mediate a potential biological effect. Consistent with our clinical findings, there was no reduction in the expression levels of IL-12 and IL-23 in the inflamed synovium, in contrast to the effects of apilimod \textit{in vitro}. Thus, the results of our study do not support further drug development in larger clinical trials for RA. It is too early, however, to conclude that IL-12/IL-23 or its receptor could not be good therapeutic targets for the treatment of RA.

### Reference List

9. Kraan MC, Reece RJ, Smeets TJ, Veale DJ, Emery P, Tak PP. Comparison of synovial tissues from the


Supplementary figure. Change in synovial inflammatory cells of patients treated with apilimod or placebo. Expression (count/mm²) of CD3⁺ T-lymphocytes (A,B), CD22⁺ B-lymfocytes (C,D), CD55⁺ fibroblast-like synoviocytes (E,F) and expression (IOD) of IL-1β (G,H) in the synovial tissue of patients treated with apilimod 100 mg QD (A,C,E,G) or placebo (B,D,F,H). A non-parametric Mann-Whitney test was used to compare expression between baseline and day 29; there were no significant changes.
### Supplementary table 1. Baseline disease characteristics

<table>
<thead>
<tr>
<th></th>
<th>Apilimod (n=22)</th>
<th>Placebo (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female, n (%)</td>
<td>19 (86)</td>
<td>4 (57)</td>
</tr>
<tr>
<td>Caucasian, n (%)</td>
<td>15 (68)</td>
<td>5 (71)</td>
</tr>
<tr>
<td>Age, years*</td>
<td>55 (14)</td>
<td>55 (17)</td>
</tr>
<tr>
<td>Disease duration (months)*</td>
<td>83 (72)</td>
<td>64 (75)</td>
</tr>
<tr>
<td>Disease severity class II, n (%)</td>
<td>17 (77)</td>
<td>6 (86)</td>
</tr>
<tr>
<td>RF and/or anti-CCP positive, n(%)</td>
<td>18 (82)</td>
<td>6 (86)</td>
</tr>
<tr>
<td>DAS28*</td>
<td>5.5 (1.0)</td>
<td>5.2 (0.6)</td>
</tr>
<tr>
<td>IgM-RF (IU/mL)*</td>
<td>115 (59-640)*</td>
<td>76 (70-104)*</td>
</tr>
<tr>
<td>Anti-CCP (IU/mL)*</td>
<td>338 (114-2805)*</td>
<td>551 (122-799)*</td>
</tr>
<tr>
<td>ESR (mm/hr)*</td>
<td>31.6 (20.0)*</td>
<td>24.7 (12.9)</td>
</tr>
<tr>
<td>MTX dosage (mg)*</td>
<td>21.1 (5.4)</td>
<td>22.5 (5.2)</td>
</tr>
<tr>
<td>Concomitant NSAIDs, n (%)</td>
<td>16 (73%)</td>
<td>5 (71%)</td>
</tr>
<tr>
<td>Concomitant corticosteroids, n (%)</td>
<td>6 (27%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

Data are represented as n (%), mean (SD)* or median (IQR)* as appropriate. Baseline characteristics were compared between patients randomized to receive apilimod or placebo (* unpaired Student’s t test or † Mann-Whitney U test). For nominal data, a Fisher’s exact test was used. Positive IgM-RF was defined as serum levels ≥12.5 IU/mL, positive anti-CCP was defined as serum levels ≥25 IU/mL, DAS28 = disease activity score in 28 joints; RF = rheumatoid factor; anti-CCP = anti-cyclic citrullinated peptide antibodies; ESR = erythrocyte sedimentation rate; MTX = methotrexate; NSAIDs = non-steroidal anti-inflammatory drugs. a = 13; b = 3; c = 18; d = 4
### Supplementary table 2. Adverse events

<table>
<thead>
<tr>
<th></th>
<th>Apilimod 100 mg QD (n = 17)</th>
<th>Apilimod 100 mg BID (n = 5)</th>
<th>Placebo (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with any events</td>
<td>15 (88%)</td>
<td>5 (100%)</td>
<td>4 (57%)</td>
</tr>
<tr>
<td>Dizziness</td>
<td>7 (41%)</td>
<td>0</td>
<td>1 (14%)</td>
</tr>
<tr>
<td>Gastro-intestinal complaints*</td>
<td>6 (35%)</td>
<td>5 (100%)</td>
<td>3 (43%)</td>
</tr>
<tr>
<td>Headache</td>
<td>4 (24%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other neurological symptoms†</td>
<td>3 (18 %)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cardiac palpitations</td>
<td>1 (6%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>URTI</td>
<td>2 (12%)</td>
<td>3 (60%)</td>
<td>2 (29%)</td>
</tr>
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<td>Dysuria or UTI</td>
<td>1 (6%)</td>
<td>1 (20%)</td>
<td>0</td>
</tr>
<tr>
<td>Aggravation RA*</td>
<td>2 (12%)</td>
<td>2 (40%)</td>
<td>0</td>
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<tr>
<td>Musculoskeletal complaints</td>
<td>2 (12%)</td>
<td>0</td>
<td>0</td>
</tr>
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<td>Dyspnoea</td>
<td>1 (6%)</td>
<td>0</td>
<td>0</td>
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<tr>
<td>HSV reactivation</td>
<td>1 (6%)</td>
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<td>0</td>
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<tr>
<td>Anorexia</td>
<td>1 (6%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Leukocytosis</td>
<td>1 (6%)</td>
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<tr>
<td>CPK increase</td>
<td>1(6%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sleeping disorder</td>
<td>1 (6%)</td>
<td>1 (20%)</td>
<td>0</td>
</tr>
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</table>

All reported adverse events by patients receiving apilimod 100 mg QD or placebo for 4–8 weeks (stages I/II), and apilimod 100 mg BID or placebo for 2–12 weeks (stage III). Data are represented as n (%). Placebo samples were pooled from all stages. One patient from stage I with headache complaints chose not to undergo a second arthroscopy at day 2, but safety and clinical evaluations were completed. In stage III, all patients treated with apilimod 100 mg BID experienced debilitating side-effects and 2 of 5 patients withdrew prior to day 57. After 8 weeks of treatment, only one patient chose to enter the extension part of the study (4 weeks of additional treatment, until day 85). And only one placebo-treated completed the entire study.

HSV = herpes simplex virus; URTI= upper respiratory tract infection; RA = rheumatoid arthritis; UTI = urinary tract infection; CPK = creatine phosphokinase. * Gastro-intestinal complaints consisted of nausea, vomiting, dyspepsia, abdominal pain, diarrhea. † Aggravation RA: arthralgia, arthritis, tendinitis. ‡ Other neurological symptoms: paresthesia, tremor, anosmia, etc.
SUPPLEMENTARY FILE

Immunohistochemistry. Minimally six tissue specimens were embedded en bloc in optimal cutting temperature (OCT) compound (Tissue-Tek, Sakura Finetek Europe BV, the Netherlands) and stored in liquid nitrogen. Serial cryostat sections (5 μm) were cut and mounted on Star Frost adhesive glass slides (Knittelgläser, Germany). Next to CD68, IL-12p70 and IL23, synovial tissue sections were stained using the following monoclonal antibodies: anti-CD3 (Becton Dickinson, CA) for T cells, anti-CD22 (Bioconnect B.V., The Netherlands) for B cells, anti-CD55 (Bioconnect B.V., The Netherlands) to detect fibroblast-like synoviocytes (FLS), anti-IL-1β (Acris Antibodies GmbH, Germany). Primary antibodies were incubated for 1 hour (or overnight for the IL-1β staining). After fixation of the sections with acetone for 10 minutes at room temperature, endogenous peroxidase activity was inhibited using 0.1% sodium azide and 0.3% hydrogen peroxide in PBS for 20 minutes. Primary antibodies were incubated for 60 minutes. Bound antibody was detected with a polymer-HRP anti-mouse IgG Envision+ System (Dako, Glostrup, Denmark) for CD3, CD68, CD22 and CD55 and PowerVision poly-HRP ready-to-use (Immunologic, ImmunoVision Tech. Co., CA) for the IL12p70 and IL-23. The IL-1β staining was performed using a 3-step immunoperoxidase method. Antibody was finally detected using aminoethylcarbazole (AEC, Dako) as dye. Sections were counterstained with Gill’s hematoxylin. Stained sections were analyzed blinded for study treatment and time point. Expression of the various markers was quantified using digital image analysis, as previously described. Expression of CD3, CD22, CD55, and CD68 is presented as count/mm2; IL-1β, IL-12p70 and IL23 is presented as integrated optical density (IOD)/mm2, an arbitrary unit representing the intensity of staining per mm2.

SUPPLEMENTARY REFERENCE