Clinical Evidence for Utilization of the A3 Adenosine Receptor as a Target to Treat Rheumatoid Arthritis: Data from a Phase II Clinical Trial

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ABSTRACT. Objective. Adenosine exerts antiinflammatory effects via activation of the A3 adenosine receptor (A3AR), a Gi protein-associated cell-surface receptor, overexpressed in synovial tissue and peripheral blood mononuclear cells (PBMC) in patients with active rheumatoid arthritis (RA). CF101 is a highly specific orally bioavailable A3AR agonist.

Methods. This was a multicenter study, blinded to dose, designed to assess the clinical activity and safety of CF101 in active RA. Seventy-four patients were randomized to receive 0.1, 1.0, or 4.0 mg CF101 bid for 12 weeks. The primary efficacy endpoint was American College of Rheumatology 20% response (ACR20) at Week 12. A3AR expression levels were analyzed in PBMC from 18 patients.

Results. Maximal responses were observed with 1.0 mg bid, lower at 0.1 and 4.0 mg bid. At 12 weeks, 55.6%, 33.3%, and 11.5% of the patients receiving 1.0 mg CF101 achieved ACR20%, 50%, and 70% responses, respectively. CF101 was generally well tolerated, with mild headache (4.1%), nausea (2.7%), and rash (2.7%) being the most common treatment-related adverse events. Statistically significant correlations between A3AR overexpression at baseline and ACR50 and ACR70 responses were observed.

Conclusion. CF101 administered bid for 12 weeks resulted in improvement in signs and symptoms of RA that did not achieve statistical significance, and was safe and well tolerated. The expression level of A3AR was directly correlated with patient responses to CF101, suggesting its utilization as a biomarker for the pharmacodynamic and therapeutic effects of this novel agent. These findings require confirmation in a double-blind randomized placebo-controlled trial, currently under way. (J Rheumatol First Release Nov 15 2007)

Key Indexing Terms:
A3 ADENOSINE RECEPTOR                RHEUMATOID ARTHRITIS                CLINICAL TRIALS

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Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disorder presenting with pain, stiffness, and swelling of the joints resulting in impaired physical function and health related quality of life. Synovial inflammation ultimately leads to cartilage destruction, bone erosions, and subsequent joint deformities.1,2

Evidence supports a central role for tumor necrosis factor-α (TNF-α) in the induction and perpetuation of RA and its systemic manifestations3-5. An emphasis in recent years for early and aggressive treatment recommends that patients with newly diagnosed RA initiate disease modifying antirheumatic drug (DMARD) therapy within 3 months of diagnosis6. Methotrexate (MTX) is currently the most commonly used and consistently effective DMARD7. Combined treatment using MTX with biologics that target TNF-α have shown improved efficacy over monotherapy with either alone8-10. These newer RA therapeutics, although effective in many patients, are not without their risks. MTX, for example, requires careful monitoring and can cause serious hepatic and pulmonary toxicities4. TNF-α inhibitors are costly, require
parenteral administration, and have been associated with serious infections and other complications of long-term immunosuppression, including lymphomas11,12. As no cures have been identified, decades of treatment can be expected; and few patients maintain clinical responses with the same regimen for more than 2–5 years. Despite major recent advances in the treatment of RA, there remains a need for novel, safe, and effective therapies.

Adenosine, a purine nucleoside, is released into the extracellular environment by metabolically active cells and exerts antiinflammatory effects via activation of the A3 adenosine receptor (A3AR)13. The A3AR is a Gαi protein-associated cell-surface receptor, overexpressed in synovium, paw extracts, and peripheral blood mononuclear cells (PBMC) from rats with adjuvant-induced arthritis14. CF101 (also referred to as IB-MECA) is a highly specific orally bioavailable A3AR agonist. Recent studies have shown that it exerts antiinflammatory effects in adjuvant and collagen-induced arthritis. CF101 treatment suppressed clinical and pathological manifestations of the disease and prevented bony erosions.

Exploration of CF101’s mechanism of action in preclinical studies revealed deregulation of the signaling pathway, resulting in inhibition of TNF-α. In addition, CF101 treatment prevented bone loss in animal models via downregulation of receptor activator of nuclear factor-κB (NF-κB) ligand, a known differentiation factor for osteoclasts14-16. Moreover, CF101 induced apoptosis of inflammatory cells and inhibited proliferation of autoreactive T cells14-18. CF101 may therefore act similarly to other anti-TNF-α therapies, but also via additional mechanistic pathways due to NF-κB inhibition.

In comparison to healthy subjects, A3AR upregulation was demonstrated in PBMC from patients with RA. High levels of A3AR expression were directly correlated with increased levels of NF-κB, known to act as a transcription factor of A3AR17. This may be attributed to high levels of TNF-α, which upregulates NF-κB14,16. Upregulation of A3AR suggested it as a therapeutic target to control inflammation in patients with active RA.

CF101 is in development for treatment of RA and other autoimmune disease indications. In single- and repeated-dose studies in healthy volunteers, pharmacokinetic measures were linear and proportional to dose, maximum plasma concentration of CF101 was achieved at 1–2 h, with an elimination half-life of approximately 9 h19; based on this half-life, a twice-daily dosing schedule was advanced into therapeutic trials.

This Phase II randomized trial examined the safety and clinical activity of CF101 in patients with active RA. In addition, correlations between A3AR expression in PBMC at baseline and response to treatment were assessed. A relatively large dose range was chosen, from 0.1 to 4.0 mg bid. The lowest dose was selected to be the approximate human equivalent dose, based on body surface area when compared to the effective dose-range in mouse models of 10 to 100 μg/kg, while the highest dose was the maximum tolerated in Phase I19. Thus, our trial explores the maximum feasible dose range in an attempt to establish a clinically relevant dose and, if possible, a dose-response relationship in human disease.

**MATERIALS AND METHODS**

*Patients.* Patients were required to be ≥ 18 years of age, meet American College of Rheumatology (ACR) criteria for the diagnosis of RA, be ACR Class I, II, or III20-22, with active disease: ≥ 6 swollen joints, ≥ 9 tender joints, and ≥ 1 of the following: (1) Westergren erythrocyte sedimentation rate (ESR) ≥ 28 mm/h, (2) C-reactive protein (CRP) > 2.0 mg/dl, or (3) morning stiffness ≥ 45 min. Documented intolerance or lack of efficacy to ≥ 1 DMARD was required, with a washout period of ≥ 4 weeks prior to protocol entry; prior exposure to biologic agents was prohibited. Those patients who were receiving baseline nonsteroidal antiinflammatory drugs and/or oral corticosteroids (≥ 10 mg/day prednisone equivalent) were allowed to continue them, provided the dose had been stable for at least 4 weeks prior to entry, and remained so throughout the study.

*Study protocol.* This was a 12-week, parallel-group, dose-finding Phase II study in which patients were randomized to 1 of 3 active-dose groups, which were double-blinded with respect to dose level through the use of identical-appearing capsules: 0.1 mg, 1.0 mg, or 4.0 mg oral CF101, administered twice daily. The above doses were established based on the multiple tolerated dose from Phase I study (4 mg) and a low dose (0.1 mg) to be the approximate human equivalent dose, based on body surface area, compared to the effective dose-range in mouse models of 10 to 100 μg/kg19. The clinical trial was conducted at 11 investigative sites in Israel in accord with the Declaration of Helsinki and Good Clinical Practice guidelines and regulations. An independent ethics committee at each participating site reviewed and approved the study protocol and informed consent, and all patients provided written informed consent prior to initiation of any study-related procedures.

*Efficacy assessments.* The primary efficacy endpoint was the percentage of patients with ACR ≥ 20% improvements at Week 12 (ACR20)22. Secondary efficacy endpoints included ACR50/70 responses and mean changes from baseline in the ACR response criteria, using a 28-joint count, visual analog scales (VAS) for assessments of pain and global disease activity, Health Assessment Questionnaire Disability Index (HAQ-DI)22, ESR, and CRP levels.

*Safety assessments.* Adverse events (AE), clinical laboratory measures, vital signs, and electrocardiograms (ECG) were assessed at Weeks 1, 2, 4, 6, 8, and 10 and endpoint.

*A3AR expression level analysis.* For quantification of A3AR levels, heparinized peripheral blood samples were taken from 18 subjects at 8 of the 11 investigative sites at baseline prior to dosing with CF101. PBMC were separated and protein extracts analyzed by Western blot. Receptor levels were quantified by calculation of the ratio of the optical density of each patient sample to that obtained from healthy controls (PBMC pooled from 5 healthy volunteers). Correlations between A3AR levels and ACR50 responses and other disease characteristics were evaluated in an exploratory fashion.

*Statistical considerations.* Eighty-four patients were to be enrolled in this trial. This sample size takes into account 28 enrolled patients in each arm, which accounts for an anticipated dropout rate of 10%. Therefore, a total of 75 evaluable patients, 25 in each arm, were to be enrolled.

Sample size was determined on the basis of a comparison of response rates for CF101 4.0 mg versus CF101 0.1 mg. The response rate in the 0.1 mg group was estimated to be 20%. A sample size of 25 patients per group allowed for a power of 0.80 to detect a response rate of 62% in the 4.0 mg group in a corrected chi-square test performed at a level of 0.05. This sample size was expected to be sufficient to estimate the slope of the dose-response curve.

All patients randomized who received at least one dose of study medication were included in efficacy and safety analyses. Efficacy analyses were calculated using the intent to treat population and “last observation carried forward” statistical approach. P values for baseline comparisons were generated
RESULTS

Patient demographics, baseline disease characteristics, and disposition. Eighty-five were screened and 74 patients enrolled at 11 investigative sites in Israel, randomized to receive 0.1 mg (n = 22), 1.0 mg (n = 27), or 4.0 mg (n = 25) CF101 bid (Figure 1). Demographic and disease characteristics did not differ statistically between dose groups (p > 0.13), summarized in Table 1. Most were female (82.4%) and Caucasian (97.3%). About two-thirds of the patient population had previously received at least 1 DMARD, and half had received MTX; the mean number of previous DMARD failed was 2.0. Patients who were admitted had complied with the protocol drug washout provisions, and no patient had been receiving an agent that required 4 weeks’ washout.

Fifty patients (67.6%) completed 12 weeks of treatment (16, 19, and 15 patients in the 0.1 mg, 1.0 mg, and 4.0 mg dose groups, respectively). Twenty-four patients (32.4%) discontinued study treatment early due to lack of efficacy (n = 16), noncompliance (n = 2), AE (n = 3), or withdrawal of consent (n = 3) (Figure 1). No statistically significant differences among dose groups were found for demographic and baseline characteristics (p > 0.13).

ACR responses. Following 12 weeks of treatment, ACR20 responses were achieved in 42.9%, 55.6%, and 41.7% of patients enrolled in the 0.1 mg, 1.0 mg, and 4.0 mg CF101 dose groups, respectively. ACR50 response rates were reported for 28.6%, 33.3%, and 12.5% of patients, and ACR70 response rates were reported for 9.5%, 11.1%, and 4.2% patients after 12 weeks of treatment with 0.1 mg, 1.0 mg, and 4.0 mg CF101, respectively. Although not statistically significant (p > 0.05), the highest proportion of responders were in the 1.0 mg CF101 dose group (Figure 2A). ACR20 and ACR50 responses were observed as early as 2 weeks after initiation of CF101 treatment (Figures 2B and 2C).

Tender and swollen joint counts. Decreases in tender and swollen joint counts were evident as early as 2 weeks after treatment initiation in all 3 dose groups, with patients in the 1.0 mg CF101 dose group showing the most robust responses. Mean reductions in tender and swollen joint count compared to baseline measurements are shown by treatment in Figures 3A and 3B, respectively. Mean changes from baseline to Week 12 in all components of the ACR response criteria are displayed in Table 2.

Safety. Thirty (40.5%) patients reported AE, 9 (40.9%), 7 (25.9%), and 14 (56.0%), respectively, in the 0.1 mg, 1.0 mg, and 4.0 mg CF101 dose groups. AE reported by 2 or more patients are summarized in Table 3, those considered by the investigator to be related to study drug administration included headache, rash, nausea, and hot flush.
Three patients discontinued study treatment due to AE, all in the 4.0 mg CF101 dose group. One patient reported headache, nausea and vomiting, mild, about 3 weeks following the first dose of study medication; a second patient experienced atrial tachycardia about 8 weeks following the first dose of study medication; and a third patient, a 79-year-old man with a history of mild Parkinson’s disease, was hospitalized due to a disease exacerbation. This was classified as a serious AE, “possibly” related to CF101.

In 3 patients, 2 receiving 1.0 mg and 1 receiving 4.0 mg CF101, ECG abnormalities were observed, including mild sinus tachycardia (1.0 mg, judged “possibly” CF101-related by the investigator), mild sinus bradycardia (1.0 mg, “probably” CF101-related), and moderate paroxysmal atrial tachycardia (4.0 mg, “probably” related). There was no evidence of clinically significant QT/QTc interval prolongations in any patient.

No significant changes in laboratory values from baseline were noted in any dose group.

A3AR expression level at baseline. Logistic regression analyses in 18 patients with RA revealed statistically significant correlations between baseline A3AR expression levels and ACR50 and ACR70 responses (p = 0.036) (Figure 4).

To further investigate this relationship, Spearman correlations between A3AR expression level and 8 individual ACR efficacy variables (tender and swollen joint counts, physician and patient global assessments of disease activity, patient...
pain, ESR, CRP, and HAQ-DI) were computed. Significant relationships were demonstrated between baseline A3AR levels and patient global assessments of disease activity (p = 0.007), pain (p = 0.007), and physician global assessment of disease activity (p = 0.013).

**DISCUSSION**

Results of our study demonstrate that CF101, a novel, orally bioavailable drug targeting the A3AR, shows evidence of potential clinical benefit in patients with active RA, with acceptable tolerability. Additionally, statistically significant correlations between A3AR expression levels at baseline and ACR50/70 responses to treatment were observed.

In this trial, CF101 was administered as monotherapy, without background DMARD treatment, to patients with RA who had failed a mean of 2.0 DMARD. Clinical responses to CF101 were noted as early as 2 weeks after treatment initiation and sustained over 12 weeks, indicating that the antirheumatic activity is relatively rapid in onset as well as persistent. The safety profile of CF101 was excellent, with no
Figure 3. Mean (SD) reduction from baseline to Week 12 among RA patients during treatment with 0.1 mg, 1.0 mg, or 4.0 mg CF101 twice daily for 12 weeks, in tender joint count (A), and swollen joint count (B); 28-joint counts were assessed at 2, 4, 6, 8, 10, and 12 weeks after initiation of treatment.

Table 2. Mean (SD) change in ACR components from Week 0 to Week 12.

<table>
<thead>
<tr>
<th></th>
<th>0.1 mg CF101, n = 22</th>
<th>1.0 mg CF101, n = 27</th>
<th>4.0 mg CF101, n = 25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tender joint count (0–28)</td>
<td>−9.4 (8.4)</td>
<td>−9.9 (7.5)</td>
<td>−7.9 (8.8)</td>
</tr>
<tr>
<td>Swollen joint count (0–28)</td>
<td>−7.5 (7.9)</td>
<td>−8.1 (5.5)</td>
<td>−6.4 (8.0)</td>
</tr>
<tr>
<td>Physician global (1–100 mm VAS)</td>
<td>−17.2 (26.9)</td>
<td>−19.6 (27.1)</td>
<td>−16.8 (24.8)</td>
</tr>
<tr>
<td>Patient global (1–100 mm VAS)</td>
<td>−12.3 (32.2)</td>
<td>−21.2 (30.4)</td>
<td>−12.4 (28.0)</td>
</tr>
<tr>
<td>Patient pain score (1–100 mm VAS)</td>
<td>−12.3 (30.3)</td>
<td>−23.7 (32.3)</td>
<td>−18.5 (25.5)</td>
</tr>
<tr>
<td>HAQ Disability Index</td>
<td>−0.2 (0.6)</td>
<td>−0.2 (0.4)</td>
<td>−0.2 (0.7)</td>
</tr>
<tr>
<td>CRP, mg/dl</td>
<td>8.52 (26.17)</td>
<td>−0.76 (11.15)</td>
<td>3.27 (14.90)</td>
</tr>
</tbody>
</table>

VAS: visual analog scale; HAQ: Health Assessment Questionnaire; CRP: C-reactive protein.
specific emergent safety concerns. Although tachycardia has been observed in normal subjects at relatively high doses of CF101, the ECG changes reported in this trial appeared to be sporadic, self-limited, and probably incidental. Until more information is available, adenosine A3 agonists such as CF101 should be used with caution in patients with known arrhythmias.

These data must be viewed with caution. First, despite the 40-fold dose range of CF101 utilized in this trial, the clinical effects were not dose-dependent. Our experience with this drug shows that in vitro and in vivo studies it exerts a bell-shape dose-response curve (unpublished data). It is also well established that G protein-coupled receptors, at some point, when agonist concentration goes up, most probably develop tachyphylaxis, which results in less response. In addition, the lack of dose-dependent response in our study could be a function of the relatively small sample size, or an indication that all 3 dose levels were on a flat portion of the dose-response curve, which must be further investigated in future trials. Further, no placebo-treated group was included in our study, making it difficult to calibrate the absolute effects of CF101 therapy. Therefore, the results require confirmation in a placebo-controlled, double-blind, randomized trial, and such a trial is currently under way. Nonetheless, correlations between ACR responses and A3AR expression suggest that a measure of the pharmacodynamic effect of CF101 is available.

The most intriguing finding in our trial was the significant

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**Table 3. Treatment-emergent adverse events reported by at least 2 patients.**

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>No. (% Patients Reporting Adverse Event*)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1 mg CF101, n = 22</td>
</tr>
<tr>
<td>Nausea</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Fever</td>
<td>2 (9.1)</td>
</tr>
<tr>
<td>Bronchitis</td>
<td>1 (4.5)</td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>1 (4.5)</td>
</tr>
<tr>
<td>Viral infection</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Fall</td>
<td>2 (9.1)</td>
</tr>
<tr>
<td>Headache</td>
<td>2 (9.1)</td>
</tr>
<tr>
<td>Cough</td>
<td>1 (4.5)</td>
</tr>
<tr>
<td>Hot flush</td>
<td>1 (4.5)</td>
</tr>
<tr>
<td>Rash</td>
<td>1 (4.5)</td>
</tr>
</tbody>
</table>

* Number of patients within a category may not sum to total number of patients reporting adverse events as 1 patient may have reported more than 1 adverse event.

**Figure 4.** Baseline PBMC A3AR levels in patients with RA, expressed as units, were calculated as a ratio of the optical density measurement of each sample compared to the optical density obtained from a pooled sample of PBMC collected from 5 healthy volunteers. The baseline PBMC A3AR level of each of 18 patients is shown relative to that patient’s ACR50 response following 12 weeks of treatment with CF101. Median A3AR expression levels are indicated by a bar.
correlation between baseline A3AR expression level in PBMC and the individual ACR50/70 responses following CF101 treatment at Week 12. Interestingly, significant correlations were also observed between baseline A3AR levels and improvement in 3 components of the ACR criteria: patient global assessment of disease activity, pain, and physician global assessment of disease activity. Improvements in RA symptoms have been demonstrated to be best indicated by patient-reported outcome measures, as they appear to be less susceptible to a placebo effect.\(^\text{25-27}\)

CF101 administered orally twice daily for up to 12 weeks resulted in improvement in the clinical signs and symptoms of RA that was clinically notable but did not reach statistical significance as compared to baseline. CF101 was safe and well tolerated. The expression level of A3AR was shown to be directly correlated with patient responses to CF101, suggesting its utilization as a biomarker for the pharmacodynamic effects of this novel agent, and perhaps a predictor of clinical response. These findings require confirmation and are therefore being explored at present in a larger double-blind, randomized, placebo-controlled clinical trial.

REFERENCES