Fatal attraction: chemokines and rheumatoid arthritis
Haringman, J.J.
Chapter 8

A RANDOMIZED CONTROLLED TRIAL WITH AN ANTI-CCL2 (MCP-1) MONOCLONAL ANTIBODY IN PATIENTS WITH RHEUMATOID ARTHRITIS

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ABSTRACT

Objective. Chemokines such as CCL2/MCP-1 play a key role in leukocyte migration and are potential targets for treatment of chronic inflammatory disorders. The objective of this study was to evaluate the effects of human anti-CCL2/MCP-1 monoclonal antibody (ABN912) treatment in rheumatoid arthritis (RA).

Methods. Patients with active RA were enrolled in a randomized, placebo controlled dose escalation study of ABN912. Infusions at each dose level were administered on day 1 and day 15. In the dose escalation phase 4 cohorts of 8 patients (6 active : 2 placebo) underwent serial arthroscopic synovial tissue (ST) biopsy. Immunohistochemistry analysis and digital image analysis were used to characterize changes in ST. To assess clinical effects, an additional 21 patients were planned for inclusion at the highest tolerated dose.

Results. Forty-five patients were included to an interim analysis for futility: 33 patients received ABN912, and 12 received placebo. There was a dose related increase in ABN912 complexed total CCL2/MCP-1 levels in peripheral blood. There was no detectable clinical benefit compared to placebo. At the highest dose level, total CCL2/MCP-1 concentrations were associated with an increase in serum levels of CRP and with increased macrophage infiltration in the synovium.

Conclusion. ABN912 treatment was generally well tolerated, but did not result in detectable clinical or immunohistologic improvement, and may have been associated with worsening status in some patients treated with the highest dose. The results might be related to the fact that the total concentration of CCL2/MCP-1 in serum was highly elevated upon treatment with ABN912.

INTRODUCTION

Chemokines and chemokine receptors are key factors for leukocyte migration into inflamed synovium and they are potential targets for treatment of a variety of chronic inflammatory disorders including rheumatoid arthritis (RA) (1,2). Recently, it has been shown that chemokine blockade may result in relevant biological effects in RA patients (3). CCL2/MCP-1 has been suggested as a potential target in this disease. CCL2/MCP-1 levels are increased in the peripheral blood, synovial fluid and synovial tissue (ST) of RA patients compared to controls (2;4-6). Both synovial macrophages and fibroblast-like-synoviocytes are recognized to be capable of producing CCL2/MCP-1 within the synovium after stimulation with pro-inflammatory cytokines like IL-1 and TNF-α (6). CCL2/MCP-1 is a potent mediator of the migration of especially monocytes/macrophages and T cells (7-10). These cells have been shown to be directly involved in the induction and perpetuation of (chronic) synovitis and subsequent joint destruction in RA (11). In addition to these chemotactant capacities, CCL2/MCP-1 also has other relevant effects, for instance, stimulation of the production of (pro)-inflammatory cytokines and matrix metalloproteinases (MMPs), and CCL2/MCP-1 has been shown to modulate angiogenesis (12-14).

CCL2/MCP-1 blockade has been evaluated using neutralizing monoclonal antibodies (mAb) in several animal models. Anti-CCL2/MCP-1 mAb given before disease onset in an MRL-lpr mouse model of arthritis was shown to prevent the onset of arthritis (15). When given after disease onset the effect was less pronounced, although treatment still resulted in significant beneficial effects on arthritic symptoms and histology (15:16). In addition, CCL2/MCP-1 deficient MRL-fas(lpr) mice were in part protected from severe autoimmune disease (17). In RA patients successful treatment with disease-modifying antirheumatic drugs (DMARDs),
Blockade of CCL2/MCP-1 in Patients with Rheumatoid Arthritis

Prednisolone and anti-Tumor Necrosis Factor-α (TNF-α) antibodies was associated with decreased levels of CCL2/MCP-1 in serum and ST (5:18-20).
All together, the data suggest an important role for CCL2/MCP-1 in monocyte and lymphocyte migration and synovial inflammation. To test the hypothesis whether targeting this chemokine could be a safe and effective novel treatment approach for amelioration of arthritis in RA patients, we conducted a randomized, placebo-controlled dose escalation trial with a human monoclonal antibody directed against CCL2/MCP-1, including pharmacokinetic analysis and evaluation of synovial tissue biomarkers.

PATIENTS AND METHODS

Patients.
Over a period of 12 months male and female patients, aged 18-76, who were diagnosed with RA according to the criteria of the American College of Rheumatology (ACR) (21), were included in this multi-center study. We selected patients with a disease duration of at least 6 months prior to randomization, and active disease despite drug treatment. Active disease was defined by having at least 6 tender and 6 swollen joints (of 28 examined) (22) and at least one of the following: Westergren erythrocyte sedimentation rate (ESR) of at least 28 mm/hour, C-reactive protein (CRP) levels of at least 20 mg/L, or morning stiffness of at least 45 minutes.

All patients were taking methotrexate (MTX) therapy for at least 4 months with stable weekly doses of MTX (7.5-25 mg/week) for at least 1 month prior to randomization. No other DMARDs were allowed. Patients were allowed to use non-steroidal anti-inflammatory drugs (NSAIDs) and/or oral corticosteroids (≤10 mg/day of prednisolone or equivalent) provided that the doses had been stable for at least 1 month prior to randomization. MTX, NSAIDs and oral corticosteroids were kept stable in dose and frequency until at least day 43 of the study (primary efficacy endpoint). Patients were excluded if they had severe functional disability (class IV according to the ACR 1991 revised criteria (23)). Other exclusion criteria were active or latent bacterial, fungal or viral infections at the time of enrollment, including evidence of Human Immunodeficiency Virus (HIV) infection, Hepatitis B infection, Hepatitis C infection and/or a history of a positive purified protein derivative (PPD) of tuberculin skin test without prior BCG (Bacille Calmette Guerin) immunization; treatment with an investigational agent within 16 weeks prior to enrollment; presence of any other major chronic inflammatory disease: intra-articular or systemic corticosteroids injections within 4 weeks prior to randomization and clinically significant unstable concurrent diseases.

Patients who had received previous anti-TNF-α treatment and failed due to lack of efficacy were also excluded. Male and female patients of childbearing age used effective methods of birth control and women had negative pregnancy tests before entry. Pregnant or nursing mothers were excluded.

Written informed consent was obtained from every patient before screening and the study was approved by the institutional review boards of all participating centers.

Study protocol
This study was designed as a double-blind, randomized, placebo-controlled, sequential cohort dose escalation (0.3 mg/kg, 1 mg/kg, 3 mg/kg and 10 mg/kg) study of ABN912 (anti-CCL2/MCP-1 monoclonal antibody, Novartis Pharma AG, Basel, Switzerland) in RA patients, with an expansion phase of the highest tolerated dose. The primary objective was to assess safety and tolerability of ABN912 administered intravenously via a 2-hour infusion to patients with RA. Secondary objectives included the measurement of change on circulating CCL2/MCP-1 in serum, pharmacodynamic effects of ABN912 on peripheral blood
leukocytes, and the effect on synovial tissue at week 7 versus baseline. The overall study design is shown in Figure 1A.

Pharmacokinetic samples were taken periodically from each subject throughout the study to monitor serum concentration-time profiles of ABN912. Clinical, laboratory, and other safety parameters were assessed each visit during the study. Clinical assessments included tender and swollen joint count of 28 joints examined by a blinded assessor, subject’s global assessment of disease activity, investigator’s global assessment of disease activity, pain assessed by a visual analog scale, assessment of quality of life (Health Assessment Questionnaire), and measurement of ESR and serum CRP levels.

Site specific randomization sequences were generated by Novartis Pharma AG. Patients were randomized at least 48 hours before the first drug administration. The investigators and those responsible for administering the study drug were unaware of treatment allocation. Within every dose cohort of 8 patients, 2 were allocated placebo and 6 ABN912. In the expansion phase the placebo:ABN912 ratio was 1:2.

Patients who completed the study up to the end of week 7 were deemed fully evaluable. Patients who withdrew from the study before the second administration of the study drug were replaced.

Figure 1A. Design of trial schema.

Study drug
ABN912 is a high affinity monoclonal human anti-human CCL2/MCP-1 antibody of the IgG4/κ isotype (molecular weight (MW) 145 kD, binding affinity measured by surface plasmon resonance 43.0 ± 2.9 pM). This antibody was derived from genetically engineered mice in which the IgG and κ immunoglobulin repertoire was functionally replaced by human IgG and κ immunoglobulin genes. The route of administration of ABN912 was by intravenous infusion and accordingly ABN912 was formulated in an aqueous solution at physiological conditions. The placebo solution was an isotonic phosphate buffered saline pH 6.0 containing polysorbate80. ABN912 and placebo were prepared in 250 mL infusion bags.
containing physiological saline solution. The characteristics of the ABN912 and placebo infusion bags were completely similar.

**Pharmacokinetic analysis**
The presence of total MCP-1 in serum was measured at screening, baseline, day 8, 15, 22, 29, 43 (primary efficacy endpoint), 57, 71, 85, 99 and 120 by using a sandwich enzyme immunoassay technique (ELISA, quantikine human MCP-1, DCP00, R&D systems) at Novartis Pharma AG. Details of the PK analysis will be published elsewhere (manuscript in preparation).

**Immunotypic analysis of peripheral blood mononuclear cells.**
To determine the effects on peripheral white blood cells, immuno-phenotyping for CD20-, CD3-, CD4-, CD8-, CD16-, CD14-, CD68-, CD45RO- and CD45RA positive cells was performed on screening, baseline, day 8, 15, 29, 43 (primary efficacy endpoint), 71, 99 and 120. In general, analysis was performed as 2 color flow cytometry with pairs of fluorescein isothiocyanate (FITC)- and phycoerythrin (PE)- labeled antibodies (all antibodies were commercially obtained from DAKO, Glostrup, Denmark) in combination with whole blood. Briefly each tube was thoroughly but gently vortexed for 10 seconds after adding 100 µl of blood to 10 µl of the monoclonal antibody combination. The tubes were then incubated for 20 minutes at room temperature in dark. After incubation, the erythrocytes were lysed by an automated lysing instrument (the Q-prep EPICS work station, Coulter Cytometry Hialeah, FL) that performs a three step procedure of preparing lymphocytes for analysis. After this step the samples were stored at 4°C until analysis. The samples were measured using a Coulter EPICS XL/MCL flow cytometer. The Coulter EPICS II software program was used for data analysis: the leucogate (CD45+CD14) fluorescent information, with forward and side scatter, was used to define and gate accurately the lymphoid population. For each sample 10,000 gated events were analyzed.

**Immunohistochemical synovial tissue analysis**
Arthroscopic synovial biopsy samples were obtained twice from the same actively inflamed wrist, knee or ankle joint using local anesthetics on baseline and day 43 (primary efficacy endpoint). During each procedure biopsies were taken from 6 or more sites of the joint to minimize sampling error (24:25). Arthroscopies, tissue sampling and storage were performed as described previously in detail (26). Patients who were included in the expansion phase were not subjected to the arthroscopy procedures.

All specimens were analyzed in the Academic Medical Center in Amsterdam. Serial sections were stained with the following monoclonal antibodies: anti-CD3 (SK7; Becton Dickinson), anti-CD22 (CLB-B-ly/1,6B11; Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands), anti-CD68 (EMB11; Dako, Glostrup, Denmark) and anti-CCL2/MCP-1 (MAB281, R&D systems Europe Ltd, Abingdon, UK). Sections with non-assessable tissue, defined by the absence of an intimal lining layer, were not analyzed. For control sections, the primary antibodies were omitted or irrelevant antibodies were applied. Staining for cellular markers was performed using a 3 step immunoperoxidase method as we have described previously (27). For the determination of chemokine expression, biotinylated tyramine was used for amplification, as we have described previously (28). After immunohistochemical staining, all coded sections were randomly analyzed by computer-assisted image analysis and the total number of positive cells (for CD3+ T-cells, CD22+ B-cells and CD68+ macrophages) or the mean integrated optical density for CCL2/MCP-1 per square mm was measured. For all markers, 18 high-power fields were
analyzed. CD68 expression was analyzed separately in the intimal lining layer and the synovial sublining. The images of the high-power fields were analyzed using the Qwin analysis system (Leica, Cambridge, UK), as described previously in detail (29;30).

**Statistical analysis**

Descriptive summary statistics of the pharmacokinetic data were performed by dose group. The mean change from baseline at week 7 (primary endpoint) for each ACR20 response component (tender joint count, swollen joint count, subject’s global assessment of disease activity, investigator’s global assessment of disease activity, subject’s assessment of pain, HAQ and acute phase reactants) was compared between the dose cohorts and the pooled placebo-cohort by t-tests. Changes from baseline at week 7 in the number of peripheral blood mononuclear cells and in the number of ST CD3+ T-cells, CD22+ B-cells, CD68+ macrophages as well as the change in CCL2/MCP-1 staining between the ABN912 dose cohorts and the pooled placebo cohort were determined using t-tests.

**RESULTS**

Forty-five patients were randomized into the study from 4 centers in 3 countries in a 12 months period (Figure 1B). Of the 45 patients 25 were included in Amsterdam, 10 patients in Ghent, 6 in Dublin and 4 in Leiden. Thirty-three patients were included in the dose escalation phase and randomly allocated, including 1 replacement for a patient who was withdrawn. This patient, allocated ABN912 10 mg/kg, was withdrawn because of aggravated RA disease activity, which resulted in a dose increase of concomitant corticosteroids before the second dose of study drug was administered. This patient was followed up within the study for safety and tolerability, but was excluded from other analyses. Another 12 evaluable patients were randomized into the expansion phase of the highest tolerated dose (10 mg/kg). Of the patients in the expansion phase, 8 patients received ABN912 and 4 placebo treatment. Baseline patients’ demographics are summarized in Table 1. Patients were of similar sex, weight, age, state of disease activity and dose of concomitant MTX treatment.

Figure 1B. Trial profile.
ABN912 treatment was generally well tolerated.
Both infusions of ABN912 were well tolerated from 0.3 to 10 mg/kg. There were no hypersensitivity reactions. There was one subject in the 10 mg/kg dose group who experienced mild flushing, paraesthesia of the lower arm, nausea and elevated blood pressure after the first infusion, but the second infusion was unremarkable.
Overall 3 serious adverse events and 109 adverse events were reported in the study. Of the 3 serious adverse events 2 were not suspected to be related to the study drug (atrial fibrillation (placebo group) and bacterial infection due to splinters (ABN912 0.3 mg/kg group)). The third serious event was in a patient in the 1 mg/kg group who developed a viral bilateral pneumonia at the end of the study (day 120, 105 days after the last infusion).
Sixteen of the 109 (15%) adverse events were suspected to be possibly related to the study drug. These events were delayed menstruation, increased severity of arthritis, lymphadenopathy/lymphangitis, macular rash, tachycardia, bronchitis, pyrexia, diastolic hypertension, flushing (n=2), herpes simplex infection, nasopharyngitis, nausea, paraesthesia and pharyngolaryngeal pain. Of these 16 events, 7 occurred in the placebo group and all events were not unexpected in the patient population under study.
Consistent with the underlying disease the most frequently occurring adverse event was RA activity accounting for 16% (18/109) of all reported adverse events, followed by arthralgia (4%, 5/109), headache (4%, 4/109) and pharyngolaryngeal pain (4%, 4/109).

ABN912 treatment does not ameliorate rheumatoid arthritis.
Both in the ABN912 10 mg/kg group (n=14) and the placebo group (n=12) there was a response according to the ACR20 criteria (31) in 20% of the patients. On average there were no significant changes in the disease activity scores (DAS28) (22) in either the placebo or ABN912 treated groups (Table 1). The CRP levels tended to be increased after active treatment in the two highest dose groups (3 mg/kg (n=6) a mean increase (±sem) of 11 mg/L (±5), 10 mg/kg (n=12) a mean increase of 22 mg/L (±14)), but the changes did not reach statistical significance.

Table 1. Patient demographics, clinical characteristics, MTX dose at baseline and the change in DAS28 and CRP (mg/L) between baseline and day 43 (MTX = methotrexate, sem = standard error of mean).

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Combined placebo group (n=12)</th>
<th>ABN912 0.3 mg/kg (n=6)</th>
<th>ABN912 1 mg/kg (n=6)</th>
<th>ABN912 3 mg/kg (n=6)</th>
<th>ABN912 10 mg/kg (n=15)</th>
<th>Total study population (n=45)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years, mean, range)</td>
<td>51 (37-74)</td>
<td>50 (38-65)</td>
<td>52 (46-73)</td>
<td>54 (34-68)</td>
<td>56 (35-76)</td>
<td>53 (34-76)</td>
</tr>
<tr>
<td>Male/Female</td>
<td>7/5</td>
<td>4/2</td>
<td>3/3</td>
<td>1/5</td>
<td>5/10</td>
<td>20/25</td>
</tr>
<tr>
<td>Disease Status at baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rheumatoid factor (number of patients positive/negative)</td>
<td>9/3</td>
<td>6/0</td>
<td>4/2</td>
<td>5/1</td>
<td>14/1</td>
<td>38/7</td>
</tr>
<tr>
<td>DAS28 (mean ± sem)</td>
<td>5.66 (0.23)</td>
<td>6.46 (0.29)</td>
<td>5.96 (0.43)</td>
<td>5.34 (0.42)</td>
<td>5.76 (0.26)</td>
<td>5.80 (0.14)</td>
</tr>
<tr>
<td>Concomitant MTX treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MTX dose (mg/week mean ± sem)</td>
<td>16 (1)</td>
<td>18 (3)</td>
<td>18 (2)</td>
<td>16 (2)</td>
<td>18 (1)</td>
<td>17 (1)</td>
</tr>
<tr>
<td>Change in DAS28 and CRP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DAS28 (mean ± sem)</td>
<td>-0.79 (0.36)</td>
<td>0.49 (0.18)</td>
<td>-0.67 (0.70)</td>
<td>-0.12 (0.39)</td>
<td>-0.12 (0.37)</td>
<td></td>
</tr>
<tr>
<td>CRP (mean ± sem)</td>
<td>-6 (6)</td>
<td>-3 (3)</td>
<td>-4 (5)</td>
<td>11 (5)</td>
<td>22 (14)</td>
<td></td>
</tr>
</tbody>
</table>
ABN912 increases the concentration of total CCL2/MCP-1 in the circulation.
ABN912 manifested the typical pharmacokinetics of monoclonal antibodies, including dose linearity, half life of approximately 2 weeks and distribution volume consistent with predominantly intravascular distribution. ABN912 rapidly lowered free CCL2/MCP-1 in serum upon administration (Table 2). Thirty minutes after the first ABN912 infusion, total (ABN912 bound plus free) CCL2/MCP-1 concentrations began to rise precipitously. These effects are consistent with a rapid binding of free CCL2/MCP-1 in the intravascular space and mobilization of CCL2/MCP-1 from the extravascular compartment (Table 2, Figure 2). Of interest, this increase was dose-dependent within the dose range tested, and in the 10 mg/kg dose group the total CCL2/MCP-1 levels were over 2000-fold higher than the free CCL2/MCP-1 levels at baseline.

Table 2. Summary of CCL2/MCP-1 free and total concentrations (pg/mL) during and after an intravenous (2h-infusion) dose of 0.3 and 3 mg/kg ABN912 in two subjects.

<table>
<thead>
<tr>
<th>Serum Samples</th>
<th>Subject 1, 0.3 mg/kg</th>
<th>Subject 2, 3 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Free</td>
<td>Total</td>
</tr>
<tr>
<td>Day 1, Pre</td>
<td>411</td>
<td>517</td>
</tr>
<tr>
<td>Day 1, 0.5h</td>
<td>0</td>
<td>2622</td>
</tr>
<tr>
<td>Day 1, 1h</td>
<td>0</td>
<td>3945</td>
</tr>
<tr>
<td>Day 1, 1.5h</td>
<td>0</td>
<td>4702</td>
</tr>
<tr>
<td>Day 1, 2h</td>
<td>0</td>
<td>8773</td>
</tr>
<tr>
<td>Day 1, 3h</td>
<td>0</td>
<td>11086</td>
</tr>
<tr>
<td>Day 1, 4h</td>
<td>35</td>
<td>15481</td>
</tr>
<tr>
<td>Day 1, 6h</td>
<td>41</td>
<td>23603</td>
</tr>
</tbody>
</table>

ABN912 treatment does not affect peripheral white blood cell subsets.
There were no changes in the total number of white blood cells measured by flow cytometry in either the ABN912 treated groups or the placebo group. On average no statistically significant changes compared to baseline were observed for the number of CD14+ monocytes, CD20+ B-lymphocytes, CD3+, CD4+ and CD8+ T-lymphocytes, and the number of CD16+ cells after treatment (data not shown).

Figure 2. Serum concentration-time profile of total (antibody-bound and free) CCL2/MCP-1 ± standard error of the mean in humans following two intravenous (2h-infusion) doses of placebo or ABN912 (0.3 mg/kg, 1 mg/kg, 3 mg/kg, 10 mg/kg) at days 1 and 15.
The synovial cell infiltrate is not reduced after ABN912 treatment.
All patients in the dose escalation phase underwent a synovial biopsy procedure in an actively
inflamed joint at baseline (n=33). One patient was excluded from further pharmacodynamic
analyses, including the second arthroscopy, after a medication change due to increased RA
activity. Staining was negative in control ST sections. Sections with non-assessable tissue,
deﬁned by the absence of an intimal lining layer, were omitted before analysis (n=5). None of
the changes in the number of lymphocytes, macrophages and the expression of CCL2/MCP-1
reached statistical signiﬁcance when compared to the combined placebo group (Table 3,
Figure 3A,B). In the ABN912 10 mg/kg group the number of CD68+ sublining macrophages
tended to be increased after treatment, but the difference did not reach statistical signiﬁcance.

Table 3. Changes from baseline in synovial tissue parameters in all analyzable subjects participating in the dose
escalation phase.
The data represent the mean change at day 43 from baseline (± standard error of the mean (sem)) in the total
number of CD3+ T-lymphocytes, CD22+ B-lymphocytes, CD68+ macrophages in the intimal lining layer,
CD68+ macrophages in the sublining per mm² ST and the mean integrated optical density (IOD) CCL2/MCP-1
per mm².

<table>
<thead>
<tr>
<th>(Mean ± sem)</th>
<th>Combined</th>
<th>ABN912</th>
<th>ABN912</th>
<th>ABN912</th>
<th>ABN912</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo group</td>
<td>0.3 mg/kg</td>
<td>1 mg/kg</td>
<td>3 mg/kg</td>
<td>10 mg/kg</td>
</tr>
<tr>
<td>CD3+ lymphocytes</td>
<td>45 (38)</td>
<td>34 (173)</td>
<td>-103 (74)</td>
<td>0 (16)</td>
<td>127 (102)</td>
</tr>
<tr>
<td>CD22+ lymphocytes</td>
<td>20 (14)</td>
<td>63 (46)</td>
<td>-18 (70)</td>
<td>9 (42)</td>
<td>-26 (23)</td>
</tr>
<tr>
<td>CD68+ lining macrophages</td>
<td>-7 (15)</td>
<td>80 (69)</td>
<td>-5 (32)</td>
<td>1 (53)</td>
<td>37 (18)</td>
</tr>
<tr>
<td>CD68+ sublining macrophages</td>
<td>-82 (32)</td>
<td>96 (100)</td>
<td>-38 (120)</td>
<td>-55 (70)</td>
<td>201 (163)</td>
</tr>
<tr>
<td>CCL2/MCP-1</td>
<td>-132 (86)</td>
<td>-128 (120)</td>
<td>7 (25)</td>
<td>197 (148)</td>
<td>41 (258)</td>
</tr>
</tbody>
</table>

DISCUSSION
This study shows that treatment with a human monoclonal anti-CCL2/MCP-1 antibody
(ABN912) was well tolerated, but did not result in beneﬁcial clinical or immunohistologic
effects. Treatment with 2 intravenous infusions of the study drug resulted in a dose dependent
increase of serum total CCL2/MCP-1. In addition, there was, on average a dose dependent
rise in CRP levels in the two highest dose groups (ABN912 3 mg/kg and 10 mg/kg) and a
trend toward increased numbers of sublining macrophages in ST in the 10 mg/kg group.
The so far unprecedented rise in total CCL2/MCP-1 levels upon treatment with a mAb may
explain the lack of efﬁcacy of the treatment. Formation of circulating complexes during mAb
treatment concomitant with a rise of total protein levels in serum has been described before:
treatment of patients with multiple myeloma with mAb directed at IL-6 is known to lead to
the formation of complexed IL-6 in human plasma or serum at high concentrations (32;33).
Binding of IL-6 to anti-IL-6 mAb seems to affect biochemical and pharmacologic properties
of IL-6 in vivo (32;34;35). In patients with RA it has also been reported that treatment with
neutralizing IL-6 mAb resulted in increased serum IL-6 levels (36). Similarly, treatment with
neutralizing IL-4 mAb is known to enhance and prolong in vivo IL-4 activity (37). Consistent
with the serum and ST data, there was also a dose dependent increase in bound CCL2/MCP-1
in the synovial ﬂuid of 4 analyzed patients after active treatment (data not shown).
In our study there was a dose dependent increase in total serum CCL2/MCP-1 levels, which could be related to an increase in CRP levels and synovial sublining macrophages in the 10 mg/kg dose group. These outcomes are in line with a recent study, which showed that changes in the number of ST sublining macrophages are associated with the clinical response to treatment (38;39). In analogy to the results obtained with anti-IL-6 or anti-IL-4 treatment, one can assume that the bioactivity of MCP-1 was not effectively neutralized in these patients. Conceivably, the release of bioactive CCL2/MCP-1 from the ABN912:CCL2/MCP-1 complex plays a role in persistent or perhaps increased disease activity in patients treated with the highest dosages of ABN912. Given the high levels of ABN912:CCL2/MCP-1 complex, one can calculate that, according to the mass action law, the expected concentration of free, and presumably bioactive, CCL2/MCP-1 in serum is about 25 pg/ml at day 21 in the 10mg/kg dose group. This calculated concentration of free CCL2/MCP-1 is clearly lower than steady state concentration in patients at baseline or placebocontrols (data not shown).

However, this calculation does not take into account binding of CCL2/MCP-1 to extracellular
matrix or the Duffy receptor on erythrocytes, which could delay the disappearance of CCL2/MCP-1 from the circulation (40). Thus, free CCL2/MCP-1 levels after ABN912 treatment may in fact be higher than expected, although this remains to be shown. One solution to enhance clearance of the circulating immune complexes could be the application of cocktails with several monomeric antibodies against different epitopes of the target protein, which was shown to result in rapid uptake and elimination of these trimeric complexes for anti-IL-6 treatment (41).

Besides the formation and apparent slow clearance of immune complexes, there may be other explanations for the absence of efficacy. Recent data show there might be an ambiguous role for CCL2/MCP-1 and its only receptor CCR2 in inflammation. Targeting CCR2 with mAb in collagen-induced arthritis in mice resulted in improved clinical and histologic signs during disease initiation, but such treatment aggravated disease during the progression phase (42). Interestingly, there was a clear increase (~5fold) in the number of CD25+CCR2+ (regulatory) T-lymphocytes in the animals treated with the anti-CCR2 mAb during the disease course. The observation that interference with CCR2 can aggravate disease has also been described in collagen-induced arthritis CCR2 knockout mice, which presented a phenotype comparable to severe human RA (43). In addition to these studies, there are other reports suggesting that absence of CCR2 can actually worsen disease, although most CCR2 knockout models have impaired monocyte/macrophage migration and a better outcome of inflammatory diseases (43-49). In our study, neutralizing the most potent ligand of CCR2, CCL2/MCP-1, did not result in decreased cell recruitment, which might be explained in part by the suggested regulatory role of CCR2 on T cells in arthritis models.

In addition to the possible regulatory role of CCR2-mediated cell migration, there is also evidence for a possible regulatory role of CCL2/MCP-1 itself, which may be the reason that blocking this protein does not result in clinical and/or biological efficacy. Several reports have shown the ability of MMPs to cleave certain chemokines like CCL7/MCP-3, CCL8/MCP-2 and CCL2/MCP-1 (50-52). In all cases, MMP cleavage resulted in conversion of agonist activity into antagonist activity, although for CCL2/MCP-1 this was not complete with approximately 20% agonist activity retained (53). MMP-1 and MMP-3 have been described to cleave CCL2/MCP-1 and are both present at high concentrations in ST and co-localize with CCL2/MCP-1 expression in the intimal lining layer (13:20). As binding of these cleaved chemokines to their receptor, CCR2, does not stimulate chemotactic activity, blockade of CCL2/MCP-1 in vivo may not necessarily result in the desired therapeutic effects.

In conclusion, we did not observe a beneficial effect on disease activity or biomarkers after treatment with the ABN912 anti-CCL2/MCP-1 mAb. This might be explained by immune complex formation with a concomitant rise in the concentration of total serum CCL2/MCP-1 levels. However, we cannot exclude the possibility that CCL2/MCP-1 is not a good therapeutic target in RA. Studies using other approaches to block the interaction between CCL2/MCP-1 and its receptor may further clarify this controversy in the near future.

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