Repeat-cycle study of high-dose intravenous 4162W94 anti-CD4 humanized monoclonal antibody in rheumatoid arthritis. A randomized placebo-controlled trial


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Abstract

Objective. Results of an earlier open-label pilot study showed that 4162W94 was a relatively non-depleting anti-CD4 monoclonal antibody that induced >80% down-modulation of CD4 molecules from the surface of T lymphocytes. This placebo-controlled repeat-cycle study was conducted in active rheumatoid arthritis (RA) patients to determine the duration of CD4 blockade required to achieve lasting clinical benefit.

Methods. Following DMARD washout, 48 patients (i.e. three cohorts of 16 patients) with ACR-defined RA were to be dosed with 1 (cohort 1), 2 (cohort 2) or 3 (cohort 3) cycles of 5×300 mg 4162W94 or placebo (12 and 4 patients per cohort respectively) at monthly intervals. There was at least 3 months of follow-up after dosing. Clinical outcome was assessed in evaluable patients (receiving at least 80% of each dose course) using ACR20 criteria (required on two consecutive visits). CD4 lymphocyte counts and adverse events were also monitored.

Results. Sixteen patients were dosed in each of the first two cohorts; however, the dose was reduced in cohort 3 after five patients had received up to two dose cycles due to accumulating evidence of a high frequency of skin rash. These patients were analysed according to the number of cycles received. A further eight patients received 5×100 mg for one to three cycles prior to stopping the study for administrative reasons. Four of 13 (P=0.119 vs placebo) and 7/13 (P=0.015 vs placebo) in cohorts 1 and 2 respectively achieved ACR20 response on at least two consecutive visits. CD4 lymphocyte counts and adverse events were also monitored.

Conclusion. 4162W94 demonstrated significant clinical efficacy in this study. However, because of unacceptable CD4 lymphopenia and rash, the original hypothesis that prolonged CD4 blockade would give lasting clinical benefit was not tested.

Key words: Rheumatoid arthritis, Monoclonal antibody, Anti-CD4, Non-depleting, Treatment.

Anti-CD4 monoclonal antibodies (mAbs) have been advocated as potential therapy in rheumatoid arthritis (RA) because they induce remissions that persist beyond the period of treatment in animal models of RA and other autoimmune diseases [1–3]. The mechanism of action of anti-CD4 antibodies in these models is not clear, but there is evidence to support two main hypotheses. First, functional blockade of CD4 co-receptor function during active disease results in long-term unresponsiveness of autoantigen-specific T cells. Secondly, treatment results in a shift from Th1 to Th2 predominance of the immune response. Results from a transgenic model of chronic arthritis suggested that both mechanisms may be invoked [2]. In clinical trials, depleting anti-CD4 mAb therapy in RA caused
prolonged and severe peripheral blood CD4 lymphocytopenia [4, 5]. Pharmacokinetic and pharmacodynamic studies have shown that CD4 blockade in synovial fluid rather than peripheral blood correlates with clinical response [6]. Therefore, it is difficult to achieve high synovial fluid antibody concentrations without severe peripheral blood CD4 lymphocytopenia. This limits the anti-CD4 mAb dose, although significant and sustained saturation of CD4-positive synovial lymphocytes appears critical to achieve long-term clinical benefit [7]. Consequently, depleting anti-CD4 mAbs have been abandoned as treatment for RA. In an animal model of RA, anti-CD4 mAbs that induced immunological tolerance did not require CD4 lymphocyte depletion [8]. Hence, we tested the safety and biological effect of a relatively non-depleting anti-CD4 mAb, 4162W94 (Glaxo Wellcome).

4162W94 is a fully humanized IgG1 monoclonal antibody which was selected because it lacks complement-mediated lysis activity and has very weak activity in antibody-dependent cytotoxicity. It mediates profound down-modulation of CD4 from the surface of both resting and activated T cells [9] and inhibits proliferative responses to antigens and mitogens in vitro [10]. In an earlier study of the same antibody construct produced in Chinese hamster ovary (CHO) cells in patients with severe psoriasis [11], profound down-modulation of CD4 and coating of the residual CD4 molecules was observed without significant CD4 cell depletion. In an open-label pilot study of patients with severe RA, after five daily treatments 4162W94, which is produced from mouse NS-0 myeloma cells, was non-immunogenic and did not reduce CD4 lymphocyte numbers significantly. Nevertheless, it caused dose-dependent CD4 blockade through down-modulation of the majority of CD4 from the lymphocyte cell surface and coating of the remaining molecules, and decreased inflammation in some patients [6]. A dose of 1500 mg (300 mg/day × 5 days) induced >80% coating/down-modulation of both blood and synovial fluid CD4 molecules that lasted >4 weeks. In some of these patients the presence of antibody in the synovial fluid was associated with a reduction in the concentrations of tumour necrosis factor α and interleukin 6 [6]. Studies in animal models of transplantation tolerance have shown that several weeks of anti-CD4 therapy are needed to allow a tolerant state to develop, suggesting that the duration of treatment may be a key variable in inducing prolonged benefit [12, 13]. Therefore, this dose of 4162W94 was administered in 4-week repeat cycles to RA patients in a multicentre, cohort-randomized, placebo-controlled, Phase II study to confirm its efficacy and evaluate the duration of CD4 blockade required to achieve lasting clinical benefit.

Methods

Patients

Patients satisfying the 1987 ACR diagnostic criteria for RA [14] were recruited from out-patient rheumatology clinics at London (UK), Leeds (UK), Erlangen (Germany) and Leiden (The Netherlands). Patients had to have clinically active disease as defined by the following: more than three swollen joints, more than six painful or tender joints and an ESR >29 mm/h. Pregnant women, nursing women and women of child-bearing potential who were not using an effective method of contraception were excluded. Patients were also excluded if they had had mAb therapy previously, a CD4 lymphocyte count ≤0.4 × 10^9/l, serious and uncontrolled medical illness or Steinbroker functional class IV. Written informed consent was obtained from each patient before enrolment. The study was approved by local ethics committees at King’s College Hospital, Guy’s Hospital, Leeds General Infirmary, St James University Hospital, Leiden University Hospital and Erlangen University Hospital.

Antibody

The production of 4162W94 has been described previously [11]. The placebo contained 10 ml of a matching liquid formulation without 4162W94. Both 4162W94 and placebo were administered by intravenous infusion over 2 h as a freshly prepared solution in 200 ml of 5% dextrose or normal saline.

Treatment protocol

DMARDs and intra-articular injections were stopped at least 4 weeks prior to dosing. Concomitant NSAIDs and oral prednisolone ≤10 mg/day were allowed. Patients were enrolled sequentially into three cohorts of 16 patients. In each cohort, 12 patients were randomized to receive 4162W94 and four patients received placebo. Patients in cohorts 1, 2 and 3 received one, two and three cycles of treatment respectively at monthly intervals. Each treatment course consisted of 300 mg of 4162W94 or placebo daily for 5 consecutive days. There was at least 3 months of follow-up period after dosing.

Clinical assessment

RA disease activity was assessed based on the ACR core data set [15]. A disability index for physical function in RA was measured using a modified Health Assessment Questionnaire (HAQ) [16]. Either ESR or CRP was acceptable in the assessment of the acute-phase response. ACR scores were assessed at screening and at weeks 1, 3, 5, 7, 10 and 14 in cohort 1, weeks 1, 3, 5, 7, 9, 11, 14 and 18 in cohort 2 and weeks 1, 3, 5, 7, 9, 11, 13, 15, 18 and 22 in cohort 3. A responder is defined as having ACR20 responses in at least two consecutive assessments. Haematology, biochemistry and adverse events were also assessed at each visit. Both patients and assessors were blinded to the nature of the treatment.
**Table 1. Patient accountability**

<table>
<thead>
<tr>
<th>Cohort</th>
<th>4162W94 Enrolled</th>
<th>Placebo</th>
<th>4162W94 No. of patients</th>
<th>Placebo</th>
<th>Inadequate response</th>
<th>Adverse experience</th>
<th>Consent withdrawn</th>
<th>Other*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15 (13)</td>
<td>5 (4)</td>
<td>4 (1)</td>
<td>6 (3)</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>13 (13)</td>
<td>4 (4)</td>
<td>3 (2)</td>
<td>8 (2)</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
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<tr>
<td>3</td>
<td>6 (3)</td>
<td>2 (0)</td>
<td>1 (0)</td>
<td>2 (1)</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

*Numbers enrolled include the five patients from the original (300 mg) cohort 3.

**Mononuclear cell phenotype, 4162W94 plasma concentration and anti-4162W94 antibodies**

The mononuclear cell phenotypes CD3, CD3/CD8, CD3/CD4, CD3/CD25, CD4/HLA-DR, CD4/CD45Ro, CD4/CD45Ra, CD20, CD16 and CD14 were measured by immunofluorescence and flow cytometry as described previously [7]. The plasma concentration of 4162W94 was measured by a non-competitive time-resolved fluorescence immunoassay [6]. In brief, microtiter plates were coated with recombinant soluble human CD4, washed and then blocked with bovine serum albumin. Test plasma or spiked normal control plasma was diluted 1/10 in blocking buffer prior to addition to the plates. After incubation at room temperature for 2 h the plates were washed and biotinylated anti-4162W94 anti-idiotypic mAb (FC210) was added and the plates were incubated for 1 h at room temperature. FC210 was selected because it recognizes an idiotypic epitope exposed on 4162W94 when bound to CD4. After washing, europium-conjugated streptavidin was added and, following incubation at 37°C for 30 min, the plates were washed prior to addition of enhancement solution. This solution releases lanthanide ions, which are detected by time-resolved fluorescence. 4162W94 concentrations were determined against a standard curve plotted as log concentration vs log fluorescence. The limit of quantification in both plasma and synovial fluid for the assay was 0.5 μg/ml. Plasma anti-4162W94 antibodies were detected using the method described by Cobbold et al. [17]. Blood samples were taken at (i) weekly intervals until week 5 and at weeks 7, 10 and 14 in cohort 1, (ii) weekly until week 9 and at weeks 11, 14 and 18 in cohort 2, and (iii) weekly until week 13 and then at weeks 15, 18 and 22 in cohort 3. Clinical assessors were blinded to the results of the mononuclear cell phenotype, 4162W94 plasma concentration and anti-4162W94 antibodies.

**Statistical analysis**

Patients who were evaluable for efficacy analysis were defined as those who received at least 80% of their protocol dose plus at least one follow-up assessment. These data were analysed on an intention-to-treat basis according to protocol, i.e. excluding patients with major protocol violations. There were no major protocol violations in this study but seven patients were excluded from analysis as they did not receive at least 80% of the protocol dose. The numbers in this population are shown in Table 1 by cohort and treatment group.

**Results**

**Patient accountability**

A total of 45 patients were enrolled into the study, of whom 34 received 4162W94 and 11 placebo (Table 1). Cohorts 1 and 2 were completed by the enrolment of 16 patients into each. Sixteen patients were dosed in each of the first two cohorts. At the point when five patients had been recruited into cohort 3 and received up to two dose cycles, the dose of 4162W94 was reduced due to accumulating evidence of a high frequency of skin rash. Dosing was discontinued in these five patients and, for the purpose of the analysis, they were reallocated to cohort 1 or 2, depending on the number of dose cycles received. As a result, a further eight patients received 5 × 100 mg/day × 5 days for one (two patients), two (one patient) or three (three patients) dosing cycles.

**Demographic details**

The demographic details of the evaluable patients are shown in Table 2. There were more males in cohort 1 than the other cohorts but the difference was not statistically significant ($\chi^2$ test). A higher proportion of patients in cohort 2 received corticosteroids. The difference was not statistically significant ($\chi^2$ test).
Clinical efficacy

The number of responders (patients who satisfied the ACR20 response criteria on at least two consecutive occasions) was 4/14 ($P=0.094$ vs placebo) and 7/13 ($P=0.007$ vs placebo) in cohorts 1 and 2 respectively (Table 3). There were no placebo responders in this study. The median time to ACR20 response was 32 and 57 days in cohorts 1 and 2 respectively. Both ESR and CRP decreased in parallel with disease improvement (Fig. 1). Interestingly, the HAQ score also showed significant reduction (Fig. 1). Four 4162W94 patients (three in cohort 1 and one in cohort 2) maintained ACR20 response to the end of the study. An ACR50 response was achieved on two consecutive occasions in one 4162W94 patient in cohort 1 and two 4162W94 patients in cohort 2. Changes in individual components of the ACR core data set are shown in Fig. 1 and Table 4.

Adverse events

Overall, adverse events were reported for 97% of patients who received 4162W94 vs 73% who received
Tender joint count 16
Swollen joint count 17 ± 5
Pain score (mm) 55 ± 20

4162W94, infusion-related reaction occurred on the first day of dosing in eight and 11 patients in cohorts 1 and 2 respectively. In one patient who received three cycles of 4162W94 was withdrawn in six patients. Five cases were biopsied and in one there was cellular infiltrate centred on blood vessels that was suggestive of drug-induced vasculitis. None was reported as a serious adverse event.

Serious adverse events were reported for five patients (three on 4162W94 and two on placebo). Specifically events for the 4162W94 group were: syncope/vasovagal event (two episodes in one patient); back pain (one patient) and abdominal pain/rectal bleeding (one patient). Events in the placebo group were RA flare (one patient) and anaemia (one patient).

Skin rash (generally pruritic and mild or moderate in intensity) was reported in 21 (62%) patients who received 4162W94 vs one (9%) patient on placebo. In all patients, these did not recur on subsequent days of dosing during the 5-day treatment cycle. However, in cohort 2, seven patients developed infusion-related events on the first day of the second 5-day treatment cycle. In one patient who received three cycles of 4162W94, infusion-related reaction occurred on the first day of each treatment cycle.

Discussion

Previous clinical trials of depleting anti-CD4 mAbs produced conflicting results. We have argued that severe peripheral blood CD4 lymphopenia limited the doses of mAbs used. Hence, the concentration of mAb in the synovial joint was insufficient for therapy. In our previous open study, 4162W94 was relatively non-depleting and a pharmacodynamic study showed that

### Table 4. Individual items of the ACR core data set before and after treatment (mean ± s.d.) based on last-observation-carried-forward analysis

<table>
<thead>
<tr>
<th></th>
<th>Cohort 1</th>
<th>Cohort 2</th>
<th>Cohort 3</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>14</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>Tender joint count</td>
<td>16 ± 9</td>
<td>10 ± 8</td>
<td>12 ± 4</td>
<td>17 ± 7</td>
</tr>
<tr>
<td>Swollen joint count</td>
<td>17 ± 5</td>
<td>13 ± 9</td>
<td>15 ± 5</td>
<td>11 ± 5</td>
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<tr>
<td>Pain score (mm)*</td>
<td>55 ± 20</td>
<td>39 ± 29</td>
<td>61 ± 25</td>
<td>58 ± 21</td>
</tr>
<tr>
<td>Patient global assessment (mm)*</td>
<td>54 ± 22</td>
<td>38 ± 24</td>
<td>65 ± 25</td>
<td>50 ± 23</td>
</tr>
<tr>
<td>Assessor global assessment (mm)*</td>
<td>60 ± 17</td>
<td>32 ± 29</td>
<td>65 ± 17</td>
<td>55 ± 22</td>
</tr>
<tr>
<td>HAQ (range 0–3)</td>
<td>1.8 ± 0.6</td>
<td>1.4 ± 0.7</td>
<td>2 ± 0.7</td>
<td>1.3 ± 0.6</td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>37 ± 16</td>
<td>39 ± 18</td>
<td>47 ± 31</td>
<td>38 ± 28</td>
</tr>
</tbody>
</table>

*Measured on a 100-mm visual analogue scale.

**Mononuclear cell phenotype and 4162W94 plasma concentration**

Sustained CD4 lymphocytopenia was observed following treatment with 4162W94, particularly after the second treatment cycle in cohort 2 patients. CD4 lymphocyte suppression (<0.2×10^3/l on at least two successive occasions) occurred in 11/34 patients who received 4162W94 vs none on placebo. In all the patients, CD4 lymphocyte counts remained <0.4×10^3/l a year after treatment. Figure 2 shows mean CD4 lymphocyte counts during the study for 4162W94 cohorts 1 and 2 and the placebo group. Data from cohort 3 patients receiving 4162W94 are too limited for presentation. There were no significant changes in the numbers of other mononuclear cell subtypes.

Although data on coating and down-modulation are not available, plasma 4162W94 levels were maintained between cycles. Detectable levels of free 4162W94 in plasma were maintained between cycles in 7/12 patients receiving two courses of 4162W94 300 mg/day×5 days. No anti-4162W94 antibody developed in any patient at any time during the study.
high doses could be given to achieve significant down-modulation of CD4 and coating of lymphocytes in the synovial fluid. In this randomized, placebo-controlled trial, we show that the humanized anti-CD4 mAb 4162W94 suppressed synovitis and produced statistically significant clinical improvement in RA patients. However, both the magnitude and the duration of the clinical response were limited. The main limitation of 4162W94 was the adverse events, especially CD4 lymphopenia and skin rashes. When administered in successive courses, 4162W94 induced significant and protracted CD4 lymphocytopenia, and consequently the 4162W94 dose had to be reduced. Therefore, the original hypothesis that sustained CD4 blockade would induce tolerance and prolonged clinical benefit was not fully tested in this study due to dose-limiting rash and CD4 lymphocyte depletion. A high incidence of pruritic rash (62%) was noted across all dose groups and plasma concentrations and was the primary reason for premature discontinuation in four patients. The mechanism leading to the development of skin rash is not known. It may be speculated that 4162W94 did indeed induce alteration in the host immune response, with a swing to a Th2 profile. Future studies with anti-CD4 mAbs must investigate *ex vivo* cytokine production by peripheral and joint mononuclear cells before and after infusion.

The efficacy data of this randomized placebo control trial confirm the result of our previous open study [6]. It is similar to the result of a trial of a primatized non-depleting anti-CD4 mAb (IDEC-CE9.1, also known as SB-210396) in RA [18], in which treated patients showed statistically significant clinical improvement. Data from these randomized placebo-controlled trials confirmed that anti-CD4 mAbs, when given in sufficient doses, could suppress synovitis and improve symptoms in chronic established RA. It lends support to the T-cell hypothesis in chronic established RA.

The role of CD4 lymphocytes in chronic established disease, as opposed to the initiation of RA, has been controversial. Currently, there are two opposing hypotheses on the mechanism that sustains inflammation in chronic RA: the mesenchymal and T-cell hypotheses. The former proposes that, in chronic RA, T cells are suppressed in the rheumatoid synovium and inflammation is perpetuated by an autocrine and paracrine feedback loop involving monocytes, synoviocytes and various monokines [19]. By contrast, the T-cell hypothesis argues that T cells remain important in perpetuating synovitis and that they exert their effects through cell-to-cell contact as well as paracrine effects via secreted lymphokines [20]. The efficacy of non-depleting anti-CD4 mAbs strongly suggests that CD4-positive T cells continue to perpetuate inflammation in chronic established RA, especially as in this particular situation 4162W94 has no known effect on the function of CD4-positive monocytes [21].

Interestingly, there was no immune response to 4162W94 itself in this population, suggesting that immune tolerance to the humanized mAb may have been induced. It may be concluded that strategies aiming to induce sustained functional blockade of CD4 by the use of completely non-depleting anti-CD4 mAbs may have therapeutic potential in RA. Although 4162W94 caused less severe CD4 lymphopenia than depleting anti-CD4 mAbs, it causes significant CD4 depletion after repeated high-dose treatment. Hence, it could not be used to block CD4 function chronically. Truly non-depleting anti-CD4 mAbs should be assessed as a treatment for RA that has the potential to induce long-term disease improvement.

**References**


21. Newman I, Connolly DA, Choy EHS et al. Therapeutic effective humanised non-depleting anti-CD4 monoclonal antibody 4162w94 has no effect on monocytoid cell lines. Immunology 1997;92(Suppl.):117.