CD4 Coating, But Not CD4 Depletion, Is a Predictor of Efficacy with Primatized™ Monoclonal Anti-CD4 Treatment of Active Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** Double blind studies were conducted with the anti-CD4 monoclonal antibody (Mab) keliximab in patients with active, stable rheumatoid arthritis (RA), to confirm preliminary evidence of efficacy and safety from open, uncontrolled studies.

Methods. We enrolled 136 and 186 patients into 2 consecutive, randomized, double blind trials, with similar populations [apart from inclusion of disease modifying antirheumatic drug (DMARD)-naïve patients in Study 2]. Patients received 4 weeks intravenous placebo or keliximab [40, 80, 120, or 140 mg twice weekly (bw), or 240 mg once weekly (ow)].The primary endpoint was the American College of Rheumatology (ACR) 20 response criteria, one week after the end of treatment.

Results. ACR 20 response rates in Study 1 were 19%, 42%, 51%*, and 69%* (*$p < 0.05$ compared to placebo), with placebo, 40, 80, or 140 mg keliximab bw, respectively. The response rates in Study 2 were 30%, 39%, 46% and 47% with placebo, 80 or 120 mg bw, or 240 mg keliximab ow, respectively. In the 2 studies, there was a dose dependent increase in peripheral blood CD4+ T cell coating with keliximab, but a different pattern of CD4 depletion was seen. While only 12% of keliximab treated patients in Study 1 had CD4 counts below 250 cells/mm^3 at the end of the treatment period, 47% fell below this level in Study 2. Clinical response was not correlated with CD4 depletion, but was correlated with CD4+ T cell coating with keliximab.

Conclusion. Coating of peripheral blood CD4+ T cells with keliximab, but not CD4 depletion, is a determinant of clinical response. (J Rheumatol 2002;29:220-9)

Key Indexing Terms:
RHEUMATOID ARTHRITIS
CD4 ANTIGEN
CD4-POSITIVE T LYMPHOCYTES
MONOCLONAL ANTIBODY

CD4+ T cells play an important role in the pathogenesis of rheumatoid arthritis (RA), although their contribution during the chronic stages of the disease remains uncertain. Various pieces of evidence have been cited, some of which have stood the test of time[1]. These include the predominance of CD4+ T cells in the inflamed synovium, their activation state and proximity to antigen presenting cells, and the association between RA and specific major histocompatibility complex (MHC) alleles. Other observations linking T cells with disease pathogenesis in RA are more controversial[1] and the experience with

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anti-CD4 monoclonal antibody (Mab) therapy has been disappointing.

Although early, uncontrolled studies with anti-CD4 Mab showed promising results[2-9], more recent data from 2 large, well controlled clinical trials with the chimeric Mab cM-T412 failed to show evidence of efficacy in 2 different RA populations, despite induction of CD4 depletion[10,11]. A controlled trial of the murine anti-CD4 Mab B-F5, used at the same dose that had previously been effective in an open label trial, also showed no difference from placebo[12]. The possible reasons for this discrepancy have been reviewed recently[13]. Despite these disappointing results, they have provided valuable insights into the biological effects of anti-CD4 Mab. Reduction in synovial cellularity (including T cells and monocyte/macrophages) and synovial adhesion molecule expression has been observed after 5 days of therapy with cM-T412, providing support for the notion that CD4+ T cells regulate the influx of other cell types into the joint[14]. Mab binding to the CD4 molecules expressed on T cells (CD4 coating) in synovial fluid has been shown to be facilitated by a longer period of dosing with cM-T412. Also, the percentage of synovial (but not peripheral blood) T cells coated correlated with clinical improvement in an open label study[9]. It was shown in a previous study that cM-T412 led to dose related CD4 coating in peripheral blood, which suggested that dose levels or dosing frequency used in anti-CD4 studies may have been inadequate[11]. Experience with the nondepleting anti-CD4 Mab OKTcdr4a has also indicated dose related CD4 coating[15]. Studies in preclinical models of autoimmunity showed that depletion of CD4+ T cells was not required for efficacy with anti-CD4 Mab[16], and the clinical studies confirmed at least that depletion of CD4+ T cells does not guarantee clinical response.

We report our experience with an anti-CD4 Mab, incorporating the lessons from these earlier studies. Keliximab (SB-210396, IDEC CE9.1) is a chimeric primate/human (Primatized™) anti-CD4 Mab with the antigen binding variable domains derived from a primate Mab, coupled to human constant domains of IgG1 isotype[17]. Keliximab binds to domain 1 on the CD4 molecule on human and chimpanzee T cells with high specificity and affinity, and mediates human effector functions *in vitro* and *in vivo*[18,19]. In open label, uncontrolled, single and repeat dose Phase I studies in patients with moderate to severe RA, keliximab was well tolerated and induced only transient CD4 depletion[20,21]. The results from 2 large double blind, randomized, placebo controlled, dose ranging studies of keliximab therapy in patients with RA are described below.

MATERIALS AND METHODS

Patients. Patients aged 18 to 80 years were recruited from 51 centers in the United States, Europe, and Australia. Patients met American College of Rheumatology (ACR) diagnostic criteria[22], with symptoms of at least 6 months' duration, and had active disease with at least 6 swollen and tender joints. Patients were allowed to receive nonsteroidal antiinflammatory drugs, corticosteroids (≤ 10 mg prednisolone or equivalent daily), and analgesics, at doses that had been stable for at least 30 days prior to study entry. In both studies, disease modifying antirheumatic drugs (DMARD) were withdrawn at least 4 weeks prior to randomization. Additionally, in Study 2, patients who had never received prior DMARD therapy (DMARD-naïve) were eligible to participate. All patients in Study 1 were rheumatoid factor (RF) positive according to protocol, whereas Study 2 enrolled RF negative patients as well.

Study design. Studies 1 and 2 were conducted consecutively and the majority of study variables were identical (Table 1). Both studies had a 4–8 week run-in period for confirmation of disease stability [< 30% change from baseline swollen joint count (SJC)] and withdrawal of DMARD therapy, if applicable, followed by randomization. Study 1 had a 4 week treatment period, with twice weekly (bw) dosing of 40, 80, or 140 mg keliximab or placebo, followed by 12 weeks' followup. We had planned to randomize 180 patients into the 4 treatment groups, but randomization to the 140 mg keliximab group was truncated following the development of biopsy proven leukocytoclastic vasculitis (LV). Patients in Study 2 were planned to have 21 weeks' treatment, consisting of a 4 week induction period using 80 or 120 mg keliximab or placebo biweekly (bw), or 240 mg keliximab once weekly (ow) followed by further doses as maintenance treatment, or as treatment for flare of disease. The doses chosen for Study 2 were based upon the efficacy data and the dose related cutaneous toxicity seen in Study 1. Enrolment of 480 patients was planned for Study 2 but, due to the development of CD4 depletion in an unexpected proportion of patients during the course of the study, recruitment was halted when only 186 patients had been randomized, and no further treatment was given to those already dosed. The timing of the primary efficacy endpoint was moved from the study end to one week after the end of induction, because of the very small number of patients who had completed the full study, and to facilitate comparison with Study 1. The primary efficacy endpoint for both studies was the achievement of a clinical response, as defined by ACR 20 criteria[23], one week after the end of treatment. All disease assessments including joint counts (66 swollen and 68 tender joints, although 28 joints only used for efficacy analyses[24]), pain scores, physician assessment of disease, duration of morning stiffness, modified Health Assessment Questionnaire[25], and erythrocyte sedimentation rate (ESR) were made by blinded assessors who had no

Table 1. Comparison of main study design and patient differences in Studies 1 and 2.

	Study 1	Study 2
No. of patients		
Planned	180	480
Enrolled	136	186
Patient population, n		
DMARD withdrawn	136	71
DMARD naïve		115
Keliximab induction doses, mg	40/80/140 bw	80/120 bw/240 ow
Intravenous infusion duration, h	1	1
CD4 count entry criterion, cells/mm^3	400	450
Predicted placebo ACR 20 response, %	20	15
Predicted keliximab ACR 20 response, %	55	40
Planned primary endpoint assessment, wks	5	22*
Statistical power, %	80	90
Study location	USA, The Netherlands	USA, France, Belgium, Australia, Switzerland, UK
No. of centers	17	38

* Moved to 5 weeks after the premature termination of the study.
bw: biweekly; ow: once weekly.

knowledge of the treatment assigned. Table 1 summarizes the main differences between the 2 studies.

Study treatment. Keliximab used for studies 1 and 2 was produced by different manufacturing processes, although both utilized similar Chinese hamster ovary (CHO) cell lines, with identical coding sequences inserted. The most notable molecular difference between keliximab made by the 2 processes was the level of aggregate (dimer) and nonglycosylated heavy chain; minor differences were also detected in C-terminal processing and in residual moisture of the finished, lyophilized product. Extensive comparisons in several *in vitro* and *in vivo* assays (using described methodologies[26-28]) did not identify significant immunological differences between the 2 materials. Lyophilized keliximab was dissolved in sterile water, and then added to sterile normal saline. Keliximab solution or placebo (saline only) was administered as an intravenous infusion over 1 h, using an in-line filter.

Immunological tests. Flow cytometry was performed on peripheral blood samples that were incubated with different fluorescence labelled antibody probes, and the red blood cells were lysed after a suitable incubation period, leaving the antibody-coated white cells intact. The absolute number of CD4+ T cells was determined using OKT4, which binds to an epitope on the CD4 molecule that is distinct from the epitope recognized by keliximab[26]. The lower limit of the normal range was 352 and 400 cells/mm^3, respectively, for the 2 cytometers used. CD4 antigen density was measured by OKT4 mean fluorescence intensity (MFI). The number of T cells coated with keliximab was determined by subtraction analysis after staining the CD4+ T cells with OKT4 and a second reagent, OKT4a, which binds to the same epitope as keliximab, and the percentage coating calculated. The majority of blood samples were trough samples, taken pre-dose on dosing days and at the same time of day on followup visits. In Study 1, the week 1 samples were taken post-dose.

Skin biopsies. Skin biopsies were taken in cases of significant rash, and examined by routine histology and immunohistology using central readers.

Statistical methods. The study sample size was based on the primary endpoint, the proportion of patients with ACR 20 responses. Study 1 was powered (80%) to detect a 35% difference (placebo response of 20%, keliximab response of 55%) at 5 weeks; Study 2 was powered (90%) to detect a 25% difference (placebo response of 15%, keliximab response of 40%) at 6 months. For both studies, the type I error level for hypothesis testing was 0.05 and was adjusted for multiple comparisons using a modified Bonferroni method[29]. All patients who were randomized and received at least one dose of study medication were to be included in the efficacy and safety analyses for Study 1 (intent to treat). Since Study 2 was terminated earlier, only patients who were randomized and completed induction treatment were included in the efficacy analysis. For ease of comparison, the data from Study 1 on patients who completed induction treatment are included in the Efficacy Results section. All randomized patients were included in the safety analysis. For the ACR index at the end of induction, Fisher's exact test was used to compare the active treatment groups with placebo. For individual ACR evaluations, percentage change was compared between active treatment groups and placebo at the end of induction using analysis of variance with treatment as the only factor in the model. Mantel-Haenszel general association statistics were used to compare the withdrawals due to insufficient therapeutic effect.

The relationship between clinical response and various pharmacodynamic parameters was explored, although the study was not powered to detect differences. Area under the curve (AUC) of percentage of coating was calculated using the trapezoid method and was used to explore the relationship with change from baseline SJC. The SJC was chosen because it is the most objective component of the ACR index. Linear regression was used and the correlation coefficient calculated. For Study 1, patients who received at least 2 infusions and had baseline and end induction SJC values were included in the analysis; for Study 2, the analysis was performed separately for both all patients and keliximab treated patients who completed induction. Only the results on the keliximab treated patients are presented, as results from the 2 patient populations were similar. As analysis of the data shows that the percentage of coated T cells tends to drop rapidly from 100% to zero, it is possible that the AUC calculated using the trapezoid rule might underestimate the true AUC for the low dose treatment groups. As a confirmatory analysis, the relationship between the SJC and the rank of % coating AUC was also analyzed.

In Study 2, logistic regression was used to assess the relationship between the number of patients with CD4 depletion and patient characteristics. Since there was no depletion in the placebo group, only patients who received active drug were included.

RESULTS

Patient populations. A total of 136 and 186 patients were enrolled into Studies 1 and 2, respectively. The majority of patients in Study 1 were enrolled in the US, but Study 2 enrolled patients from the US, Europe, and Australia (see Table 1 and Acknowledgment). Table 2 shows that the patient populations in the 2 studies were very similar, the main differences being the inclusion of rheumatoid factor (RF) nega-

Table 2. Comparison of baseline characteristics for patients completing induction therapy in Studies 1 and 2.

	Study 1					Study 2				
	All Patients	Placebo	KMab 40 mg	KMab 80 mg	KMab 140 mg	All Patients	Placebo	KMab 80 mg	KMab 120 mg	KMab 240 mg
Randomized, n	136	40	41	38	17*	186	46	46	50	44
Completed induction, n	122	36	38	35	13	142**	31	36	40	35
Female patients, %	73.5	72.5	68.3	81.6	70.6	80.6	78.3	73.9	84.0	86.4
RF +, n (%)	122 (100)	40	41	38	17	106 (75)	23	26	28	29
Mean duration of RA, yrs	10.5	9.9	11.0	11.0	9.7	11.4	14.0	10.6	9.8	11.8
Mean SJC (28 joints)	15.6	15.3	16.8	14.8	14.2	14.0	14.5	13.8	14.4	13.4
Mean TJC (28 joints)	14.6	14.6	16.4	14.1	10.6	15.0	15.6	14.8	15.6	13.8
DMARD naïve pts., n (%)	0 (0)	0	0	0	0	56 (39)	13	14	15	14
MTX in last 3 mo, n (%)	108 (79)	31	32	33	12	35 (25)	7	10	9	9
Corticosteroids in last 3 mo, n (%)	110 (81)	32	34	32	12	104 (73)	24	25	31	24
Mean baseline CD4 count, cells/mm^3	1002	896	1038	1098	972	1035	1081	1010	985	1068

* One patient randomized to 140 mg group received 7 × 80 mg, and 1 × 20 mg keliximab.
** 137 patients reached week 5. KMab: keliximab.

tive patients (25%) and DMARD-naïve patients (39%) in Study 2, but not in Study 1. Fewer patients in Study 2 had received methotrexate within the 3 months prior to study entry. Overall, the demographics and clinical characteristics of patients in both studies were similar: in Studies 1 and 2, respectively, patients had a mean duration of RA of 10.5 and 11.4 years, a mean SJC of 15.6 and 14.0 joints, a mean ESR of 40.1 and 42.6 mm/h, and mean modified Health Assessment Questionnaire (mHAQ) scores of 1.0 and 1.1, respectively, consistent with a diagnosis of established, active RA. In both studies, the treatment groups were well matched, although there were fewer patients randomized to the 140 mg keliximab group in Study 1, and both studies enrolled fewer patients than originally planned (see below).

Efficacy results

Clinical responses. Figures 1A and 1B show the ACR 20 response data from the studies for those patients who completed the 4 week induction treatment (Study 1: 122 patients, Study 2: 142 patients). In Study 1, there was a statistically significant difference in clinical response at the end of induction for both the 80 mg and 140 mg keliximab groups, compared to placebo, with a clear dose-response relationship (Figure 1A). In Study 2, although there was a dose-response trend in efficacy, the keliximab ACR 20 response rates were lower than in Study 1, with a higher placebo response, and the differences failed to reach statistical significance. DMARD-naïve patients had higher ACR 20 responses than DMARD-withdrawn patients, with more than a third of placebo treated patients achieving an ACR 20 response, although none of these differences were statistically significant (Figure 1B). Comparing the DMARD withdrawn patients in both studies, it was clear that there was a similar placebo response, but the keliximab response rates in Study 2 were lower than those in Study 1. Analysis of the secondary efficacy data in Study 2 revealed a statistically significant improvement ($p < 0.05$) in the 120 mg bw keliximab group compared to placebo for percentage of change in the SJC, the physician and patient global assessment of disease, and the number of patients withdrawing for insufficient therapeutic effect (Table 3). The percentage of change in SJC and the number of patients withdrawing for insufficient therapeutic effect was also statistically significantly better than placebo in the 240 mg ow keliximab group ($p < 0.05$).

Pharmacodynamic data. Both studies showed a dose-dependent decrease in CD4 antigen density, as measured by the CD4 MFI (Figures 2A, 2B). Downmodulation of the CD4 antigen persisted for up to 3 weeks after the end of dosing in the high dose groups. In both studies, there was a dose-dependent increase in the mean percentage of CD4+ T cell coating with keliximab (Figures 3A, 3B), and a dose-dependent increase in the duration of coating in Study 1 (Figure 3A). The CD4 cells remained coated with keliximab during induction and for up to 3 weeks after the end of dosing in the high dose groups. The "shoulder" pattern observed in Figure 3A was due to the week 1 sample in Study 1 being taken post-dose, while all others were pre-dose samples.

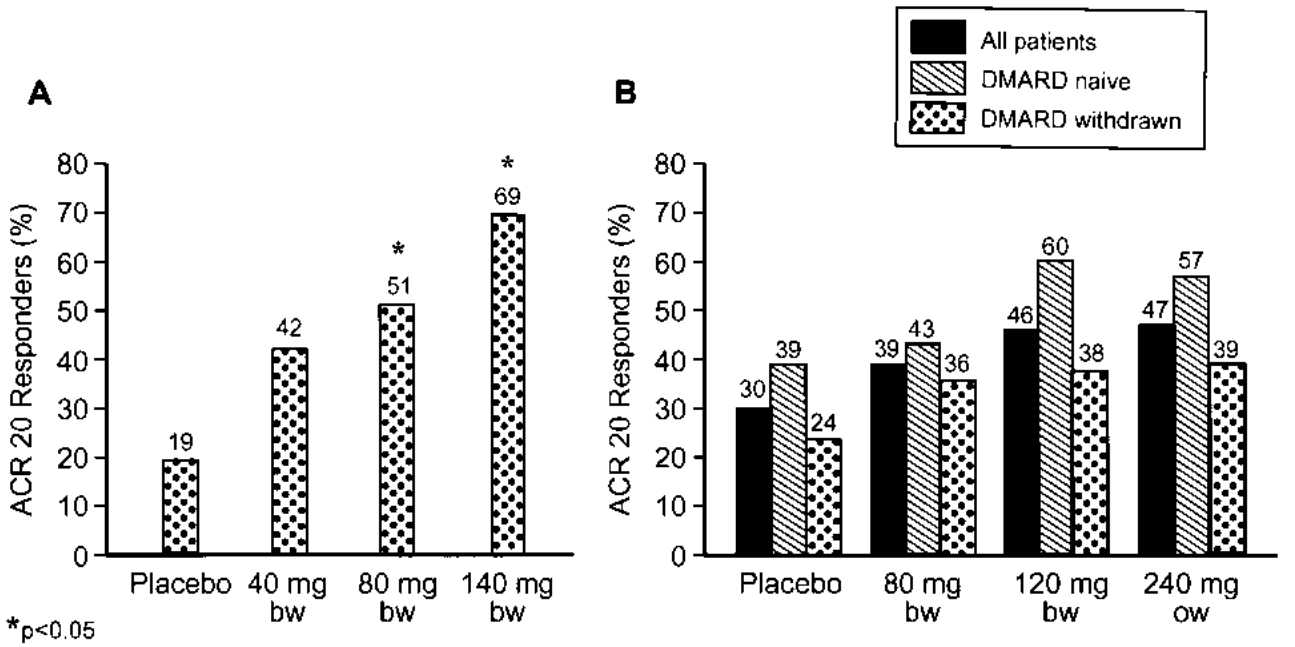

Figure 1. Primary efficacy response at the end of induction for all patients completing induction treatment. A. Study 1: DMARD-withdrawn patients only. A statistically significant difference in clinical response was observed for 80 mg and 140 mg bw keliximab groups, compared to placebo, with a clear dose-response. B. Study 2. There was no statistically significant difference in clinical response for any keliximab group due to lower keliximab and higher placebo ACR 20 response rates, compared to Study 1. DMARD-naïve patients had higher responses than DMARD-withdrawn patients.

Table 3. Selected secondary efficacy results from Study 2.

	Placebo	80 mg	120 mg	240 mg
Change in swollen joint count, %	–17	–33	–43*	–40*
Change in physician global assessment of disease, %	–14	–24	–37*	–31
Change in patient global assessment of disease, %	–17	–26	–41*	–24
Patients withdrawing for insufficient therapeutic effect, n	12	6*	4*	4*

* Significant vs placebo at $p < 0.05$, adjusted for multiple comparisons.

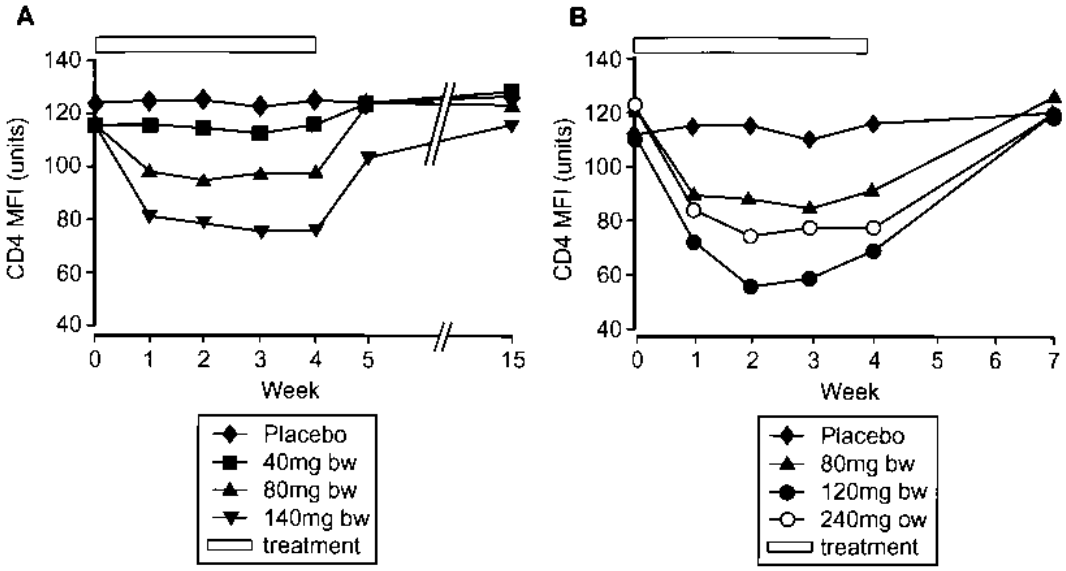

Figure 2. Mean CD4 antigen density, as measured by OKT4 mean fluorescence intensity (MFI) for all patients in Studies 1 (A) and 2 (B). Keliximab dose dependent downmodulation of CD4 antigen, persisting for up to 3 weeks post-dosing in the high dose groups. The clinical activity of keliximab may, in part, be explained by downmodulation of the CD4 antigen on T cells.

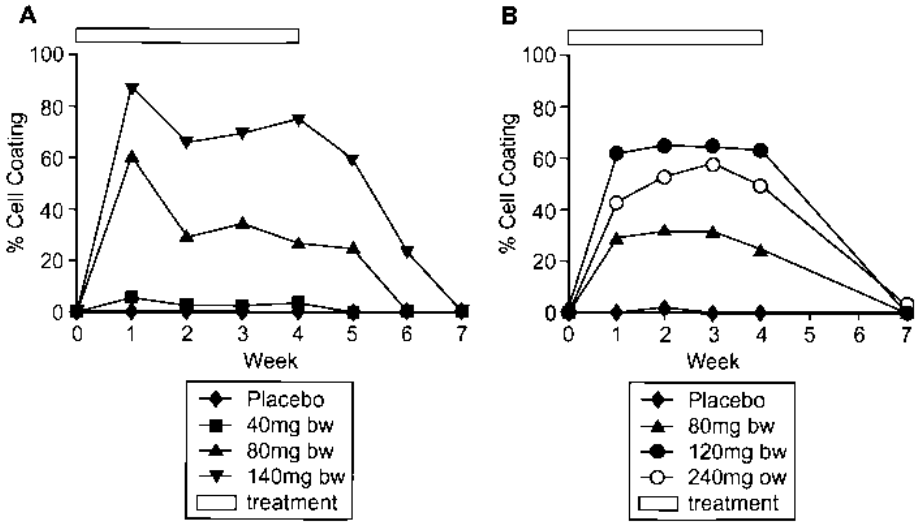

Figure 3. Mean CD4+ T cell coating, as measured by OKT4a binding, for all patients in Studies 1 (A) and 2 (B). A. Keliximab dose dependent percentage of CD4 coating and duration of coating, coating persisting for up to 3 weeks post-dosing. B. Keliximab dose dependent percentage of CD4 coating, coating persisting for up to 3 weeks post-dosing. The clinical activity of keliximab may, in part, be explained by sustained coating of CD4+ T cells, preventing interaction of CD4 with MHC II on antigen-presenting cells.

Correlation between T cell coating and clinical response. Analysis of the scatter plots of the AUC for percentage of CD4 coating over time, and the change from baseline SJC at the end of induction showed that the coating AUC was a statistically significant predictor of clinical response in both studies ($r = -0.288$, $p < 0.001$; $r = -0.275$, $p < 0.005$) (Figures 4A, 4B). Similar results were also found with the rank analysis, indicating no underapproximation in the low dose groups. A higher degree of coated T cells and a longer duration of coating of the T cells with keliximab predicted an improved clinical response. There was no such correlation between CD4 antigen density and clinical response (data not shown).

Correlation between CD4 count and clinical response. No analysis of CD4 count and clinical response was performed in Study 1, in view of the small number of patients with CD4 depletion. In Study 2, there was no apparent correlation ($r = -0.062$, $p = 0.53$) between reduction in CD4 count and subsequent clinical response at the end of induction in keliximab treated patients, as assessed by change in SJC (Figure 5).

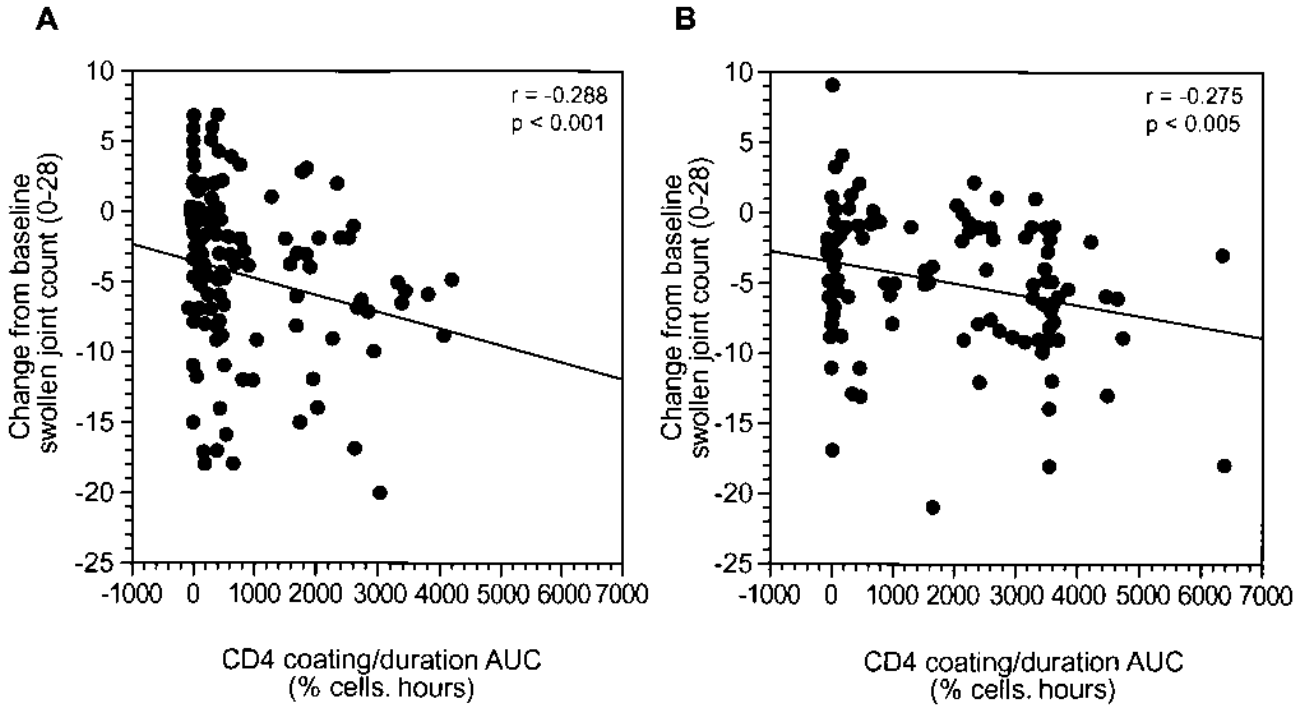

Figure 4. Correlation between CD4+ T cell coating and clinical response in keliximab treated patients in Studies 1 (A) and 2 (B). Statistically significant correlation between percentage of CD4 coating area under the curve (AUC) and change from baseline swollen joint count at the end of induction. CD4 coating predicted clinical response.

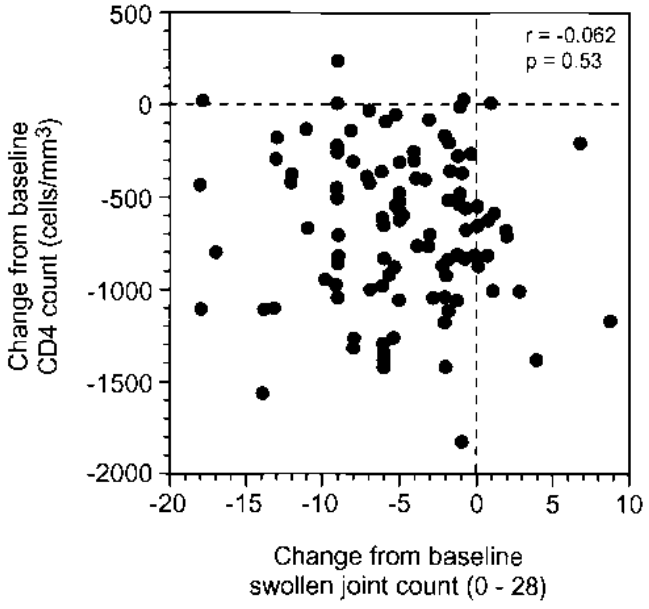

Figure 5. Lack of correlation of CD4 count with clinical response in Study 2. No correlation between change from baseline CD4 count and change from baseline swollen joint count at end of induction. Depletion did not predict clinical response.

Safety results

CD4 data. Figures 6A and 6B show the change in mean CD4 count with time for Studies 1 and 2, respectively, during the course of induction. In Study 1, 10 of 86 (12%) of all keliximab treated patients who completed treatment had CD4 counts below 250 cells/mm^3 at the end of treatment. In the 80 mg keliximab group, the one common dosage group between the 2 studies, only 2 of 34 patients (6%) had a CD4 count below 250 cells/mm^3. CD4 depletion was generally short-lived, with no patients persisting with CD4 counts below 250 cells/mm^3 23 months after the end of the study. In contrast, in

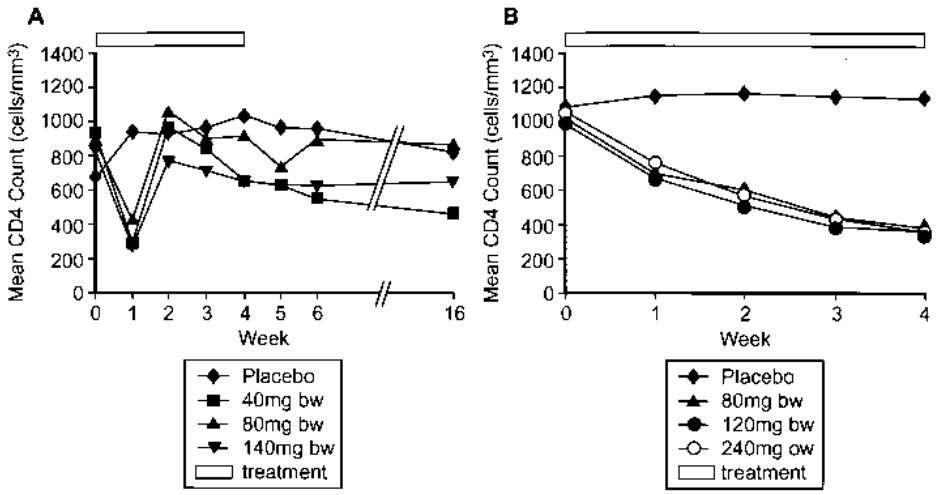

Figure 6. Mean CD4+ T cell counts for all patients in Studies 1 (A) and 2 (B). A. Only transient reduction in CD4 counts in the majority of keliximab treated patients. B. Sustained reduction in CD4 counts in the majority of keliximab treated patients.

Study 2, after the end of induction treatment, 49 of 104 (47%) of all keliximab treated patients who completed induction had CD4 counts below 250 cells/mm^3. The highest proportion of patients with CD4 depletion was in the keliximab 80 mg bw group (19 of 34 patients, 56%). In some cases, CD4 recovery has been slow, but by September 2000 (39 months after the cessation of the study), only 4 of the 104 (4%) keliximab treated patients still had CD4 counts below 250 cells/mm^3 (Table 4).

The data from Study 2 were examined to determine which, if any, of the patient characteristics (age, duration of RA, prior DMARD or corticosteroid use, baseline CD4 count, RF seropositivity, HLA phenotype) might predict subsequent CD4 depletion (at least 2 CD4 counts below 450 cells/mm^3, the entry criterion for the study). The only statistically significant predictors were the baseline CD4 count: each decrease of 100 cells/mm^3 increasing the risk (odds) of subsequent depletion by 12% ($p = 0.0244$); and the duration of RA: each year increase in disease duration decreasing the risk (odds) of subsequent depletion by 5% ($p = 0.0401$).

Cutaneous reactions. Table 5 lists the patients with rash in the 2 studies, those requiring biopsy, and those with confirmed leukocytoclastic vasculitis. Ten percent and 14% of patients in Study 1 and 2, respectively, had rash, with 2% and 6% requiring biopsy. As 2 of the 3 patients biopsied in Study 1 (all randomized to the 140 mg keliximab group) had a histological diagnosis consistent with LV, this dose was discontinued after 17 of a planned 45 patients had been randomized, and no further retreatment was administered to those already treated. One patient in Study 2 had a clinical diagnosis of LV, with cutaneous immunofluorescence results consistent with this diagnosis. However, LV could not be confirmed by light microscopy. In all cases, the vasculitis was limited to the skin and rapidly responded to treatment.

Table 4. Comparison of CD4 depletion by dose group in Studies 1 and 2 (CD4 count < 250 cells/ mm^3).

	Study 1					Study 2				
	All Patients	Placebo	KMab 40 mg	KMab 80 mg	KMab 140 mg	All Patients	Placebo	KMab 80 mg	KMab 120 mg	KMab 240 mg
Mean end induction CD4 count, cells/mm^3	—	961	629	752	644	—	1142	381	346	374
No. of patients with CD4 depletion at end induction (%)	10/122 (8)	0/36 (0)	7/39 (18)	2/34 (6)	1/13 (8)	49/134 (37)	0/30 (0)	19/34 (56)	16/39 (41)	14/31 (45)
No. of patients with CD4 depletion in September 2000 (%)	0	0	0	0	0	4/134 (3)	0/30 (0)	4/34 (12)	0/39 (0)	0/31 (0)

KMab: keliximab.

Table 5. Comparison of other safety data in Studies 1 and 2.

	Study 1					Study 2				
	All Patients	Placebo	KMab 40 mg	KMab 80 mg	KMab 140 mg	All Patients	Placebo	KMab 80 mg	KMab 120 mg	KMab 240 mg
No. of pts. reporting rash (%)	14 (10)	1	1	9	3	26 (14)	1	10	11	4
No. of patients requiring biopsy (%)	3 (2)	0	0	0	3	11 (6)	0	5	4	2
No. of patients with LV	2	0	0	0	2	1	0	1	0	0
No. of patients with infusion reactions (%)	20 (15)	4	5	8	3	75 (40)	10	22	25	18

KMab: keliximab. LV: leukocytoclastic vasculitis.

One patient in Study 1, with a history of intermittent, low grade lymphopenia and previous treatment with immunosuppressive drugs, noted skin lesions that were subsequently diagnosed as Kaposi's sarcoma (KS) about 9 months after treatment with keliximab and subsequent prolonged CD4 depletion. The patient received local radiotherapy to the lesions, with improvement. The ethnic background of the patient (Hispanic), the lack of visceral involvement, and the relatively indolent course were all characteristic of classic-type KS. Overall, the clinical safety experience with these patients experiencing longterm CD4 lymphopenia has been good, with no serious opportunistic infections or other AIDS-defining illnesses reported.

Other safety results. Infusion related events (such as rigors, chills, and fever) were relatively uncommon in Study 1, reported in 16/96 (17%) of keliximab treated patients with no serious events. In contrast, during Study 2, infusion related events occurred in 65/140 (46%) of keliximab treated patients (Table 5), rigors and fever being the most commonly reported events. The events were considered serious in 6 patients, due to prolongation of the hospitalization period postinfusion, but no event resulted in overnight stay.

DISCUSSION

Although immunogenetic, histological, and animal model data strongly support a central role for CD4+ T cells in the initiation of RA, their importance in established disease has remained uncertain. This probably reflects the failure of earlier controlled trials with anti-CD4 Mab. Mounting evidence suggests that the epitope recognized by a CD4 Mab has a profound influence on the biological effects[30]. Differences in the Fab portions of different Mab tested in RA may explain why earlier studies failed to show efficacy in controlled trials.

The data presented here show that anti-CD4 Mab therapy can be effective in established RA, and also support previous preclinical studies reporting that depletion of CD4+ T cells is not required for clinical benefit and that the clinical response correlates with coating of the CD4 molecule.

The results of the 2 randomized, double blind trials differed, with statistically significant differences in the ACR 20 response from placebo at the 2 highest doses in the first but not the second study. A number of factors may account for the inability to detect a significant difference between keliximab and placebo in the second trial. A major contributing factor was the high placebo response rate (30%) in Study 2. In Study 2, DMARD-naïve patients were included in addition to DMARD-withdrawn patients. The placebo response rates in the DMARD-naïve patients in Study 2 were higher (39%) than those in the DMARD-withdrawn patients in either study (19 and 24% for Studies 1 and 2, respectively). The reason for the higher placebo rate in Study 2 is unclear, although it might reflect expectation bias on the part of the investigators/assessors, given the positive results from the first study[31]. However, a second factor contributing to the negative second study was the lower ACR response rates for keliximab in Study 2, suggesting some other elements had changed between the 2 studies.

Another potential source of difference in outcome is the change in the process of manufacture for keliximab used for the 2 studies. A manufacturing change was required for Study 2 in order to achieve the necessary scale-up for a large development program, and to remove from the production process products of bovine and human origin. Although the inserted coding construct remained the same, a new CHO cell line adapted to grow in serum-free conditions was used, and changes in culture conditions and purification steps were made. There is no data to support the notion that a structural change in the product resulted that influenced efficacy; however, process modifications may have led to undetected changes in the Mab structure/function and thereby provide another possible explanation for the less impressive clinical results in Study 2.

It may be significant that keliximab used in Study 1 did not behave as a typical IgG1 Mab. Cell-directed Mab of that isotype would be expected to have substantial complement and Fc binding activities and are consequently expected to mediate effector functions[32], resulting in a reduction in target cell numbers *in vivo*. Keliximab does not efficiently bind to or fix complement, but does bind Fcγ receptors and mediates FcγR dependent effector functions *in vitro*[18]. Although there was a

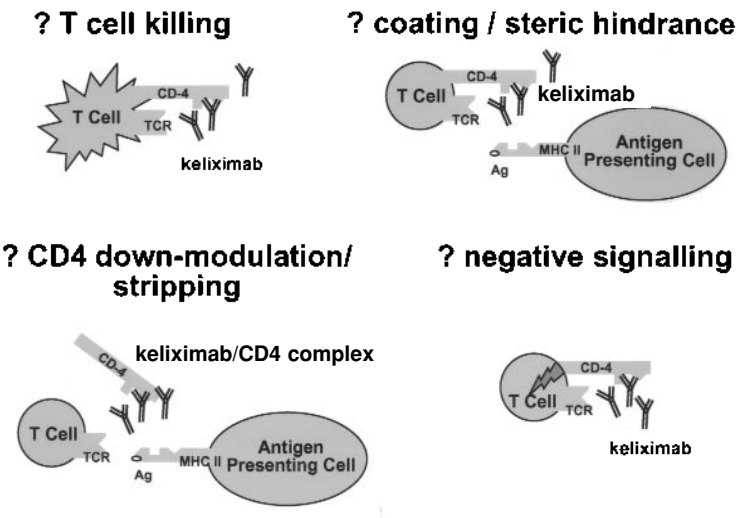

Figure 7. Proposed mechanisms of action for keliximab.

transient reduction of CD4+ T cell numbers at week 1 in Study 1, most patients saw recovery of counts by week 2, suggesting that the initial fall was a result of redistribution, rather than true cell depletion. Only 12% of patients in this study went on to show longer term CD4+ T cell depletion, with recovery for all patients within 23 months. In contrast, in Study 2, keliximab caused sustained CD4+ T cell depletion in nearly half the patients treated, with only slow recovery, although the mean baseline CD4 count for patients from both studies was not dissimilar. This is a more typical depletion pattern expected for an IgG1 Mab.

Symptoms associated with cytokine release, such as fever, chills, and rigors, are also known to be Fc function dependent, and to be highly isotype-specific[33]. In Study 2, more than one-third of patients experienced infusion related adverse experiences, in contrast to Study 1. Interestingly, however, there was no correlation between those patients experiencing CD4 depletion and those reporting symptoms associated with cytokine release.

The principal determinant of subsequent CD4 depletion in Study 2 was lower baseline CD4 count; surprisingly, shorter disease duration was also a risk factor for subsequent depletion, although this may be a reflection of other factors such as more aggressive early disease.

Biologic determinants of clinical efficacy were also evaluated in both studies. An analysis of change from baseline in CD4 count versus change in an efficacy variable (SJC) revealed no correlation in Study 2. In contrast, we saw a clear relationship between keliximab coating of CD4 molecules on peripheral blood T cells and efficacy in both studies, CD4 coating being dose dependent, as also observed in animal studies[28]. It seems reasonable to speculate that effective and sustained interference with CD4/MHC interactions, either by coating CD4 with Mab or possibly by reducing CD4 density, is the mechanism of action of anti-CD4 in RA. However, other possible mechanisms include the functional modification of CD4+ T cells, resulting in suppression of TH1 cytokine production and/or induction of TH2 cytokines[16]. Putative mechanisms of action for keliximab are shown in Figure 7.

Our data support the use of anti-CD4 Mab in the treatment of RA. Nondepleting anti-CD4 Mab may need to be administered at sufficient dose and frequency to sustain CD4 coating and/or downmodulation in order to be effective. Although no serious consequences of longterm CD4+ T cell depletion have been observed, reversible interference with CD4/MHC II interactions without cell killing with nondepleting anti-CD4 Mab may be more appropriate from a safety point of view.

Overall, our findings support the notion that CD4+ T cells retain an important pathogenetic role in established RA and that use of appropriately constructed anti-CD4 Mab may be expected to result in clinical improvement in this disease.

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