Evaluation of Immunogenicity of the T Cell Costimulation Modulator Abatacept in Patients Treated for Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** The immunogenicity of abatacept, a selective costimulation modulator, administered intravenously, was assessed across Phase II and III trials in patients with rheumatoid arthritis (RA).

Methods. Two direct-format enzyme-linked immunosorbent assays evaluated antibody responses [whole abatacept molecule (CTLA-4 and Ig portion) and CTLA-4 portion only (Assay A)] in the Phase II trials. During the Phase III trials and 2-year open-label periods, a similar, but more sensitive, Assay B was employed. Serum samples collected prestudy, during treatment, and 56 and/or 85 days following the last dose were evaluated. Seropositive samples with anti-CTLA-4 reactivity and sufficiently low drug levels were further characterized for neutralizing activity (cell-based bioassay).

Results. A total of 2237 patients with both pre- and post-baseline serum samples were eligible for assessment. Of these, 62 (2.8%) patients demonstrated an anti-abatacept or anti-CTLA-4 response, determined using either Assay A or B. Using the more sensitive Assay B, 60 of 1990 patients (3.0%) demonstrated an antibody response to the whole abatacept molecule (n = 41, 2.1%) or the CTLA-4 portion (n = 19, 1.0%). Of the 1764 RA patients evaluated in the Phase III studies, 203 discontinued therapy and had sera collected 56 and/or 85 days after discontinuation. Patients who discontinued had a higher incidence of immunogenicity versus patients who did not discontinue (7.4% vs 2.6%, respectively). Of 20 patients positive for anti-CTLA-4 reactivity, 13 were eligible for assessment with the neutralization bioassay. Of these, 8 patients exhibited neutralizing activity. Seroconversion occurred with no adverse safety outcomes or effect on pharmacokinetic parameters. No consistent pattern was observed between antibody response and loss of efficacy (American College of Rheumatology 20 and Health Assessment Questionnaire responses).

Conclusion. Abatacept was associated with a low incidence of immunogenicity in patients with RA and lacked any adverse sequelae. (First Release Nov 15 2007; J Rheumatol 2007;34:2365–73)

Key Indexing Terms:
ABATACEPT IMMUNOGENICITY RHEUMATOID ARTHRITIS

In rheumatoid arthritis (RA), it is postulated that an unknown antigen is presented via the major histocompatibility complex and activates autoreactive T cells in the presence of a costimulatory signal. Subsequently, activated T cells recruit and activate downstream immune cells, orchestrating and perpetuating the cellular processes that lead to inflammation and joint destruction[1]. Abatacept is a soluble fusion protein that consists of the extracellular domain of human cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) linked to the modified Fc (hinge, CH2, and CH3 domains) portion of human immunoglobulin (Ig) G1 that targets and selectively modulates the CD80/CD86:CD28 costimulatory signal required for full T cell activation. Abatacept has demonstrated efficacy in patients with active RA and an inadequate response to methotrexate (MTX)[2-4] or to anti-tumor necrosis factor (TNF) therapy[5].

Therapeutic recombinant biologic agents, such as abatacept, can be immunogenic and therefore have the potential to elicit an antibody response. Immunogenicity against biologic agents can affect safety, efficacy and pharmacokinetics. Antibody-mediated clearance of a biologic therapy may reduce drug levels, or the antibody response may prevent the drug from binding to its pharmacologic target, both of which can lead to decreased efficacy and the need for dose escalation over time (i.e., "dose creep").

We examined the formation of anti-abatacept and anti-CTLA-4 antibodies, and their potential effect on the efficacy and safety of abatacept treatment. Since abatacept is likely to inhibit an immune response to itself, based on its activity as a selective costimulation modulator and as demonstrated by its

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Sponsored by Bristol-Myers Squibb, Princeton, NJ, USA.

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Accepted for publication August 22, 2007.

ability to suppress T cell-dependent antibody responses in clinical and nonclinical studies[6-8], we also examined the effect of missing 1 or 2 doses or discontinuing therapy on the level of immunogenicity. Finally, seropositive samples with anti-CTLA-4 reactivity were tested for neutralizing-antibody activity.

MATERIALS AND METHODS

Studies evaluated. To determine whether abatacept induces an immunogenic response in patients with RA, antibody response was assessed across multiple Phase II and III clinical trials, comprising 6 double-blind (DB) and 4 open-label (OL) study periods in 2237 RA patients, which included patients who had inadequate responses to MTX or anti-TNF therapy. One study (Phase IIa) also assessed abatacept for the treatment of RA as monotherapy (Table 1). Samples were generally collected pre-study, at multiple timepoints throughout treatment just prior to dosing when drug levels were at their lowest, and at 56 and/or 85 days following the last dose, to allow time for clearance of abatacept.

The studies included in this integrated analysis were approved by the institutional review boards and independent ethics committees at participating sites and were carried out in accord with the ethics principles of the Declaration of Helsinki (Clinical trial identifiers NCT00048581, NCT00162266, NCT00048568, NCT00048932, NCT00162279). All patients provided written informed consent before randomization.

Assessments. In addition to evaluating the antibody response to abatacept (in whole or in part), patients who developed a positive antibody response against abatacept were also assessed for both safety and efficacy. Safety evaluations included the incidence and type of prespecified peri-infusional adverse events (AE), overall AE, serious AE, and discontinuation. The effect of immunogenicity on efficacy was also examined in patients with a positive antibody response. Signs and symptoms were assessed using American College of Rheumatology (ACR20) responses[9]. Physical function was assessed using modified Health Assessment Questionnaire (HAQ) responses for the Phase II trials[10,11] and HAQ responses for Phase III trials. A HAQ response was defined as an improvement of ≥ 0.3 units in the HAQ Disability Index (DI)[10].

Immunogenicity assays

Basic assay formats. Because of high, preexisting crossreactivity directed against the Fc portion of abatacept in human serum, particularly in RA populations, 2 direct-format enzyme-linked immunosorbent assays (ELISA) were used to evaluate the antibody response. The anti-abatacept assay measured the antibody response to all portions of the molecule, but had lower sensitivity. The anti-CTLA-4 assay measured the antibody response to the CTLA-4 portion only, removing the Ig region (thereby conferring greater sensitivity). Both assays were used in either an endpoint-titer format (Assay A) or a screening format (Assay B).

Assay A format: Phase II double-blind clinical immunogenicity assay methods. The anti-abatacept assay and the anti-CTLA-4 assay used during Phase II RA trials were collectively referred to as Assay A. Here, abatacept or CTLA-4 was absorbed onto 96-well microtiter plates that were then incubated with test serum (3-fold serial dilutions starting at 1:10). Bound antibodies were detected using an alkaline-phosphatase-conjugated goat anti-human kappa and lambda light-chain-specific antibody cocktail (Southern Biotech, Birmingham, AL, USA) and visualized using a p-nitrophenyl phosphate substrate. Since no human anti-abatacept antibodies or positive control serum were available, these assays were validated using abatacept-specific antisera generated in a cynomolgus monkey. Results from each assay were expressed as endpoint-titer values. A patient was considered to have seroconverted when his/her endpoint titer increased by 2 or more serial dilutions (≥ 9-fold) relative to that individual's predose (Day 1) endpoint titer.

Assay B format: Phase III and Phase II open-label clinical immunogenicity assay methods. For the Phase III trials and 2-year Phase II OL periods, both the anti-abatacept and anti-CTLA-4 assays were modified to reduce non-specific background. Modifications included a reduction in the concentration of the coating reagent, use of a 10% milk-based buffer for sample dilutions, and (for the anti-abatacept assay) shaking sample dilutions overnight prior to analysis. These changes increased the signal-to-noise ratio in both the anti-abatacept and anti-CTLA-4 assays and resulted in improved sensitivity. The method to determine positivity was changed and was collectively referred to as Assay B. These assays were also validated using abatacept-specific antibodies purified from cynomolgus monkey antiserum. For each ELISA, 96-well microtiter plates coated with abatacept (0.25 μg/ml) or CTLA-4 (0.5 μg/ml) were incubated with test serum diluted 1:400 for 2 h at 22°C ± 5°C (anti-abatacept) or diluted 1:25 for 2 h at 32–40°C (anti-CTLA-4). After the primary incubation, bound antibodies were detected with a horseradish peroxidase (HRP)-conjugated goat anti-human antibody cocktail, kappa and lambda light-chain-specific for the anti-abatacept assay, or heavy-chain- and light-chain-specific for the anti-CTLA-4 assay, followed by tetramethylbenzidine substrate.

Results for the anti-abatacept assay were expressed as a pre-/post-ratio calculated by dividing post-dose sample optical density (OD) values by the corresponding pre-dose sample OD value analyzed on the same plate. Positivity was based on a cutoff value established by calculating the upper bound on a 95% prediction limit[12] for the mean post-/pre-ratio obtained from samples previously collected from placebo-treated RA patient samples. If the ratio value was less than the cutoff, the sample was considered negative and reported as a titer value < 400. Any value that exceeded this cutoff was considered conditionally positive. Since the anti-abatacept assay is dependent on the level of preexisting Ig reactivity of the sample, the estimated sensitivity ranged from 7.6 to 180.4 μg/ml (in neat serum).

Results for the anti-CTLA-4 assay were expressed as a "Ratio 1" value calculated by dividing the mean patient serum sample OD by the mean OD of the negative control on the same plate. Positivity was based on values established using pooled serum from placebo-treated RA patients as the negative control. If the value was less than the specified cutoff, the sample was considered negative and reported as a titer value < 25. Any value that exceeded this cutoff was considered conditionally positive. The cutoff was the upper bound on the 95% prediction limit for the mean "Ratio 1" determined from samples previously collected from placebo-treated RA patients. The estimated sensitivity of the anti-CTLA-4 assay was 260 ng/ml (in neat serum) based upon evaluation of the affinity-purified polyclonal monkey antibody in abatacept-naive RA patient sera.

Confirmatory analyses. Conditionally-positive samples identified in each assay (anti-abatacept and anti-CTLA-4) and in each format (Assays A and B) were evaluated in an immunodepletion assay to determine specificity of the response. Anti-abatacept-positive samples were preincubated with approximately 40 μg/ml of abatacept (the CTLA-4 portion of the molecule), another unrelated Ig fusion protein (CD40Ig), or an unrelated protein (ovalbumin) to identify the region of the molecule against which the anti-abatacept reactivity might be directed (CTLA-4, Ig, or the junction region). Anti-CTLA-4-positive samples were similarly preincubated with abatacept, the CTLA-4 portion, or ovalbumin to confirm the specificity of the anti-CTLA-4 reactivity. Following preincubation, all samples were reanalyzed in the same original assay format described above. Samples where the preincubation resulted in ≥ 30% reduction in OD of the preincubated sample compared with the untreated sample were considered confirmed positives, resulting in a 5% overall false-positive rate. If confirmed, samples were titrated to identify the serum dilution that resulted in a ratio value equal to the cutoff of the particular assay (reported as the endpoint titer).

Neutralizing-antibody activity assessments. A bioassay was conducted to assess the ability of patient samples with drug-specific antibodies against CTLA-4 to inhibit or neutralize the activity of abatacept (inhibit binding to CD80/CD86), by preventing it from binding to CD80/CD86 on the T cell surface. Stable Jurkat T cell transfectants expressing a luciferase gene under the control of the interleukin 2 (IL-2) promoter were costimulated with Daudi B cells in the presence of anti-CD3 antibody. This costimulation, mediated through the interaction between CD28 on the Jurkat T cell and CD80/CD86 on the Daudi B cell in combination with anti-CD3 antibody, activates the IL-2

Table 1. Overview of studies included in this evaluation.

Phase	Study Design	Background Antirheumatic Therapy	No. of Patients in Comparator Group	No. of Patients Randomized and Treated with Abatacept 10 mg/kg or Fixed Dose	Other Doses, mg/kg	Total
Trials in RA patients with inadequate response to MTX						
Phase II	Randomized, dose-ranging, placebo-controlled DB trial in patients with active RA while on MTX	Days 1–180: MTX (10–30 mg/wk) Days 181–360: adjustment allowed (+1 other nonbiologic RA therapy)	119	115	105 (2.0†)	339
Phase III	Randomized, placebo-controlled DB in patients with active RA while on MTX	Days 1–169: MTX (10–30 mg/wk) Days 170–365: adjustment allowed (+1 other nonbiologic RA therapy)	219	433	0	652
Trials in RA patients with inadequate response to TNF-blocking agents						
Phase III	Randomized, placebo-controlled DB trial in patients with active RA on background DMARD who failed therapy with TNF-blocking agents due to lack of efficacy	Days 1–169: any nonbiologic RA therapy or anakinra	133	258	0	391
Safety study in RA						
Phase III	Randomized, placebo-controlled DB safety study in patients with RA (with or without preexisting comorbidities) on background DMARD and/or biologics	Days 1–85: stable doses: ± nonbiologic RA therapy ± biologic RA therapy Days 86–365: adjustment allowed: ± nonbiologic RA therapy ± biologic RA therapy	482	959	0	1441
Other supportive studies						
Phase II	Randomized, placebo-controlled DB trial in patients with active RA while on ETAN	Days 1–180: ETAN (25 mg 2×/wk) Days 181–360: adjustment allowed (± ETAN, +1 nonbiologic DMARD)	36	0	85 (2.0†)	121
Phase IIa	Randomized, dose-ranging, placebo-controlled DB trial in RA patients who failed at least 1 DMARD or ETAN; 85 days; followup through Day 169	None	32	33	26 (0.5†) 32 (2.0†)	122
Pharmacokinetic trials in healthy patients in RA program						
Phase II	OL, uncontrolled, single-dose pharmacokinetic study in healthy patients	None	0	30	0	30
Open-label extensions in RA						
Phase II	OL, uncontrolled trial; 84, 68, and 67 patients from previous DB 10 mg/kg, 2 mg/kg, and placebo arms, respectively	Day 361+: MTX (± 1 nonbiologic RA therapy)	0	219*	0	219
Phase II	OL, uncontrolled trial; 58 and 22 patients from previous DB 2 mg/kg and placebo arms, respectively	Day 361+: ± ETAN (± 1 nonbiologic RA therapy)	0	80*	0	80
Phase III	OL, uncontrolled trial; 218 and 99 patients from previous DB 10 mg/kg and placebo arms, respectively	Days 170+: any nonbiologic RA therapy or anakinra	0	317*	0	317
Phase III OL	OL, uncontrolled trial; 378 and 161 patients from previous DB 10 mg/kg and placebo arms, respectively	Day 360 + MTX (10–30 mg/wk) (+1 nonbiologic RA therapy)	0	539*	0	539

* Patients in the OL, uncontrolled periods are a subject of those who completed the DB, placebo-controlled study periods. †Abatacept dose, mg/kg. RA: rheumatoid arthritis; MTX: methotrexate; DB: double-blind; TNF: tumor necrosis factor; DMARD: disease modifying antirheumatic drug; ETAN: etanercpt; OL: open-label.

promoter, leading to increased transcription of the luciferase gene and increased luciferase protein expression. The luminescent signal is measured using a Luciferase Assay System. Since abatacept blocks the CD80/CD86:CD28 interaction, adding abatacept to the cell mixture blocks this IL-2 promoter activation and decreases luminescence. However, preincubation with a neutralizing antibody would restore the costimulation interactions and result in an increase in luminescence.

Abatacept-neutralizing antibody activity in patient samples was evaluat-

ed by determining the abatacept dose–response curve at concentrations of 0.1, 0.25, or 0.5 μg/ml in the bioassay, in the presence of 1:25 post-dose seropositive serum, and statistically comparing it to the dose–response curve in the presence of its corresponding Day 1 sample, in a similar bioassay[13]. An anti-human CTLA-4 murine monoclonal antibody (11D4) with abatacept-neutralizing activity in the bioassay was used as a positive control in each analytical run. Due to limitations inherent in the bioassay test method, only post-dose samples with existing levels of abatacept ≤ 1 μg/ml could be evaluated, since higher drug levels interfered with the neutralizing response, and further sample dilution would decrease assay sensitivity. The interassay precision was within 11% and the overall accuracy estimates were within 4% of their nominal levels.

Pharmacokinetic evaluation. Population pharmacokinetic analysis was performed on serum sample data from patients from the DB periods of the Phase II/III trials where a positive immune response was confirmed. Abatacept was quantified using a validated sandwich ELISA method with a lower limit of quantitation of 1 ng/ml and a range of reliable response of 1–30 ng/ml. To minimize any serum matrix effects on the assay, all study samples were diluted to 10% with phosphate-buffered saline containing 1% bovine serum albumin and 0.05% v/v Tween 20. In brief, polystyrene microtiter plates were coated with a monoclonal capture antibody raised against abatacept (clone 7F8). Samples were incubated with the capture antibody, and abatacept was detected using a biotinylated second monoclonal antibody (clone 11D4-biotin) also raised against abatacept. This was followed by incubation with streptavidin-HRP, then tetramethylbenzidine substrate. The validated population pharmacokinetic model was applied to individual patient serum concentration data, and maximum *a posteriori* Bayesian estimates of individual pharmacokinetic parameter values were obtained. The distribution of clearance, volume estimates, steady-state area under curve (AUC) values, and minimum concentration of the drug in the body after dosing (C_{min}) values for these patients were compared with the distribution of these values in a larger data set of patients from the same trials who did not develop an immune response.

RESULTS

Incidence of anti-abatacept and anti-CTLA-4 responses. A total of 2237 patients had both pre- and post-baseline serum samples and were eligible for assessment. Of these, 62 (2.8%) patients had evidence of an anti-abatacept or anti-CTLA-4 response, as determined using Assay A or B (Figure 1). All positive responses in the anti-abatacept assay were directed to the Fc or junction region; responses to the CTLA-4 regions were not detected. Three patients had a response to the junction region. No patient demonstrated an immune response to both the Fc and CTLA-4 domains of abatacept. When the more sensitive Assay B was used, an antibody response to abatacept was detected in 60 of 1990 patients (3.0%; Figure 1).

Of patients evaluated in the Phase III studies (n = 1764), 203 discontinued abatacept therapy during the DB or OL periods, or did not enter the subsequent OL period and had sera collected 56 and/or 85 days after discontinuation of therapy. Of the 203 patients, 15 (7.4%) had an immunopositive response to either abatacept (whole molecule; n = 5, 2.5%) or CTLA-4 (n = 10, 4.9%; Table 2). Of the remaining 1561 RA patients who completed the Phase III DB period and continued into OL treatment, 40 (2.6%) had a positive antibody response during the DB or OL periods, 33 (2.1%) to abatacept and 7 (0.4%) to CTLA-4. Interestingly, in the Phase IIa study of abatacept as monotherapy, no patient seroconverted for abatacept or the CTLA-4 portion of the molecule; however, the less sensitive Assay A format was employed in this study.

A total of 191 patients had a more than 30-day period without abatacept between their participation in the DB and OL periods. Of these, 3 (1.6%) patients had a positive antibody response to abatacept and 1 (0.5%) patient had a positive antibody response to CTLA-4 during the OL period (Table 2). Sera were also analyzed from 587 RA patients who missed 1–2 doses of study medication and restarted at any point during the study. Of these patients, 15 (2.6%) demonstrated a positive antibody response to abatacept and 7 (1.2%) had a positive antibody response to CTLA-4 (Table 2).

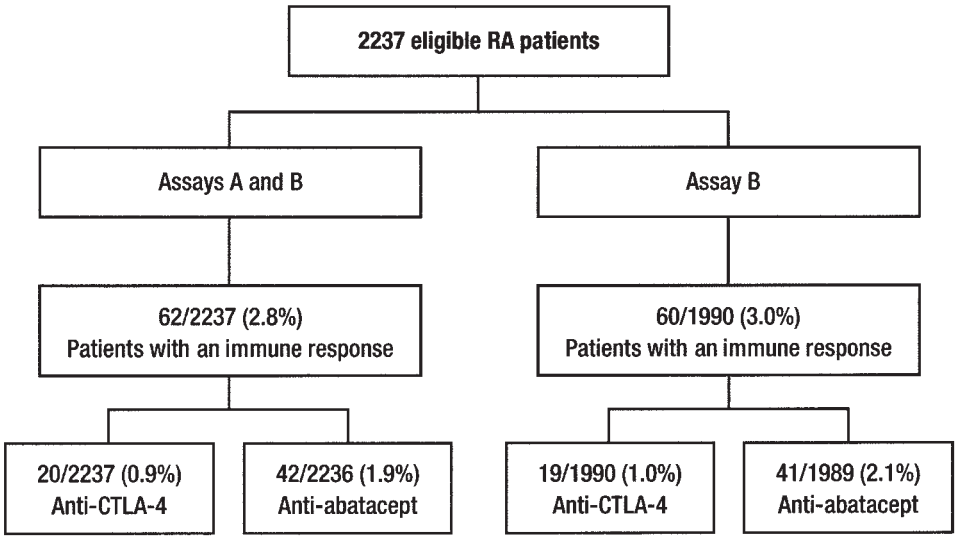

Figure 1. Total number of patients who tested positive for anti-abatacept or anti-CTLA-4 antibodies in any serum sample taken during or after therapy. Antibody response to the whole abatacept molecule (CTLA-4 and Ig portion) and the CTLA-4 portion only were determined using Assays A and B, as described in Materials and Methods.

Table 2. Number of seropositive patients (%) with abatacept use.

Scheduled Abatacept Use	No. of Positive Responses/No. Evaluated (%) Anti-Abatacept	Anti-CTLA-4	Total
Missed 1–2 doses and restarted use of abatacept	15/587 (2.6)	7/587 (1.2)	22/587 (3.7)
> 30 days without abatacept between DB and OL periods	3/191 (1.6)	1/191 (0.5)	4/191 (2.1)
Discontinued during Phase III (sera collected 56 and 85 days after dosing)	5/203 (2.5)	10/203 (4.9)	15/203 (7.4)
Completed Phase III DB and continued OL treatment	33/1561 (2.1)	7/1561 (0.4)	40/1561 (2.6)

DB: double-blind; OL: open-label.

Effect of concomitant MTX on immunogenicity. A total of 2451 patients received concomitant MTX and 493 did not. Overall, the percentage of patients with a positive antibody response to abatacept was generally similar whether they were receiving concomitant MTX or not (2.3% vs 1.4%, respectively; Table 3).

Neutralizing activity of anti-CTLA-4 antibodies. Twenty-four serum samples from 20 patients were confirmed positive for anti-CTLA-4 reactivity in the anti-CTLA-4 antibody screening assay. Fourteen of the 24 samples (collected from 13 patients) met the criteria (≤ 1 μg/ml abatacept) for evaluation in the neutralization bioassay, of which one was positive at Day 56 and 10 were positive at Day 85 post-dose. Nine of the 14 samples (taken from 8 patients) exhibited neutralizing-antibody activity. With the exception of septicemia in one patient, there were no medically significant AE reported in these patients at or near the time of seroconversion that were considered related to therapy in these 8 patients.

During the period following study discontinuation (56 and 85 days after), a period when the predominant number of samples was suitable for evaluation of neutralizing antibodies, efficacy data were not collected. Therefore, it was difficult to assess the influence of anti-CTLA-4-neutralizing antibodies on efficacy due to the small numbers of patients who had neutralizing activity and available efficacy data.

Pharmacokinetic evaluation. Pharmacokinetic parameters were estimated for 31 of the 32 patients who had a positive antibody response during the DB period of the Phase II/III trials. Sera samples for pharmacokinetic analyses were not necessarily collected on the day that a positive immune response was documented. Population pharmacokinetic modeling of patient data from the DB study periods suggested that the predicted pharmacokinetic parameters in the 31 immunopositive patients were comparable to those in a larger population of patients (n = 386) without a positive immune response. Trough serum concentrations on the study day during the DB period when seroconversion was documented were shown to range from 1.16 to 24.21 μg/ml, with the majority of serum concentrations between 5 and 20 μg/ml (Table 4). These concentrations fell within the range of trough concentrations predicted by the population pharmacokinetic model for seronegative patients (the predicted 5th to 95th percentiles ranged from 2 to 28 μg/ml), suggesting that seroconversion did not affect serum trough levels. Distribution of clearance and volume of central compartment by immunogenicity status is shown in Figures 2A and 2B, respectively.

Influence of immunogenicity on the efficacy and safety of abatacept. To determine if the development of immunogenicity affected efficacy responses in abatacept-treated patients, ACR20 and HAQ responses were assessed in patients before and after the onset of a positive immune response. Figure 3 summarizes results from the efficacy analysis. Among patients who had a prior ACR20 or HAQ response, 17 and 23 patients, respectively, maintained their efficacy response after developing a positive immune response; 8 and 12 patients lost their ACR20 or HAQ response, respectively (Figure 3A). In patients who had no prior efficacy response, 4 and 5 patients achieved an ACR20 or HAQ response, respectively, after the onset of an immune response, while 3 and 6 patients, respectively, continued to lack efficacy (Figure 3B). Collectively, these data suggest that the development of immunogenicity did not appear to affect efficacy, but due to the small number of patients with a positive immune response, a definitive conclusion cannot be made.

To examine the influence of immunogenicity on safety, overall AE (serious and nonserious), acute peri-infusional AE, and discontinuations due to AE were examined in the patients who developed a positive antibody response to abatacept.

Table 3. Number of patients (%) with anti-abatacept or anti-CTLA-4 responses with or without receiving concomitant methotrexate (MTX).

Concomitant Treatment	No. of Positive Patients/No. Evaluated (%) Anti-Abatacept	Anti-CTLA-4	Total
MTX	40/2451 (1.6)	16/2451 (0.6)	56/2451 (2.3)
No MTX	2/493 (0.4)	5/493 (1.0)	7/493 (1.4)

Table 4. Abatacept concentration at time of seroconversion for patients with abatacept- or CTLA-4-specific antibody reactivity during the double-blind period.

Seropositive Patient	Visit/Sample, Study Day	Abatacept Trough Concentration at Seroconversion, μg/ml	Additional Information
Anti-abatacept antibodies			
1	720	NA	
2	720	NA	
3	169	18.14	
	365	Day 365 NA; Day 337 = 12.78	
4	169	Day 169 NA; Day 175 = 8.78	
5	365	10.24	
6	169	24.21	
7	169	12.14	
	365	NA	
8	169	12.17	
9	169	1.16	Missed previous dose
	365	8.78	
10	169	7.25	
	365	3.38	
11	169	8.70	
	365	8.28	
12	365	9.71	
13	169	13.95	
	365	9.49	
14	365	Day 365 NA; Day 281 = 6.93	
15	365	Day 365 NA; Day 291 = 7.83	
16	169	16.65	
	365	Day 365 NA; Day 278 = 18.29	
17	169	1.76	Missed previous dose
	365	2.22	Missed previous 3 doses
18	169	NA	
	365	NA	
Anti-CTLA-4 antibodies*			
1	180	0.003	2 mg/kg treatment group
2	1	< LLQ	
3	169	6.89	
	365	10.50	
4	169	4.5	
5	1	< LLQ	
6	281	14.2	

* An additional 6 patients discontinued from the study during the double-blind period and were positive for anti-CTLA-4 antibodies at 56 and 85 days after the last drug administration, and therefore had drug concentrations ≤ 1 μg/ml (data not shown). LLQ: lower level of quantitatoin (1 ng/ml); NA: not available.

Medical review of AE did not reveal any consistent pattern of events or relationship to the development of immunogenicity. One patient with a positive immune response had a serious AE of multiple sclerosis. This patient had a 10-year history of symptoms suggestive of demyelinating disease, but the diagnosis was not made earlier due to the slow development of the neurological disease and the masking of symptoms by RA. Another patient developed a nonserious AE that was reported as a nonspecific autoimmune disorder. Although initially diagnosed with RA at enrollment, at the final visit it was noted that the patient likely did not have RA and the symptoms were due to the patient's underlying medical condition.

DISCUSSION

In this population of more than 2000 patients with RA, abatacept was associated with a relatively low incidence of immunogenicity. Further, the incidence of immunogenicity was not increased in patients who missed 1–2 doses of abatacept, although patients who discontinued therapy were more frequently immunopositive (immune responses on Days 56 and/or 85 after the last dose) than patients who did not discontinue. Neutralizing-antibody activity was documented in 9 samples from 8 patients, but the effect of this finding could not be fully assessed, as neutralizing-antibody activity could only be evaluated once drug levels were < 1 μg/ml, and this

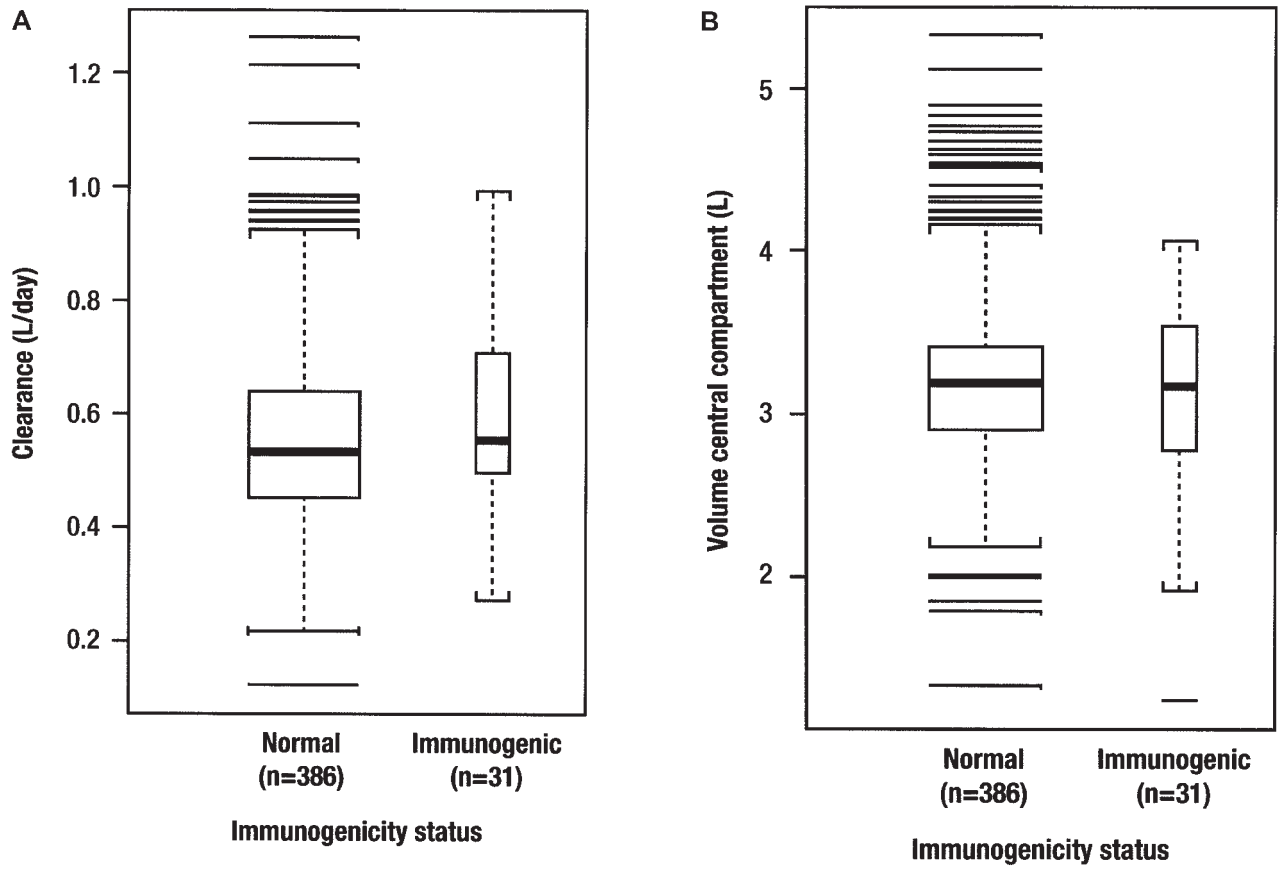

Figure 2. (A) Distribution of clearance; (B) volume of central compartment by immunogenicity status.

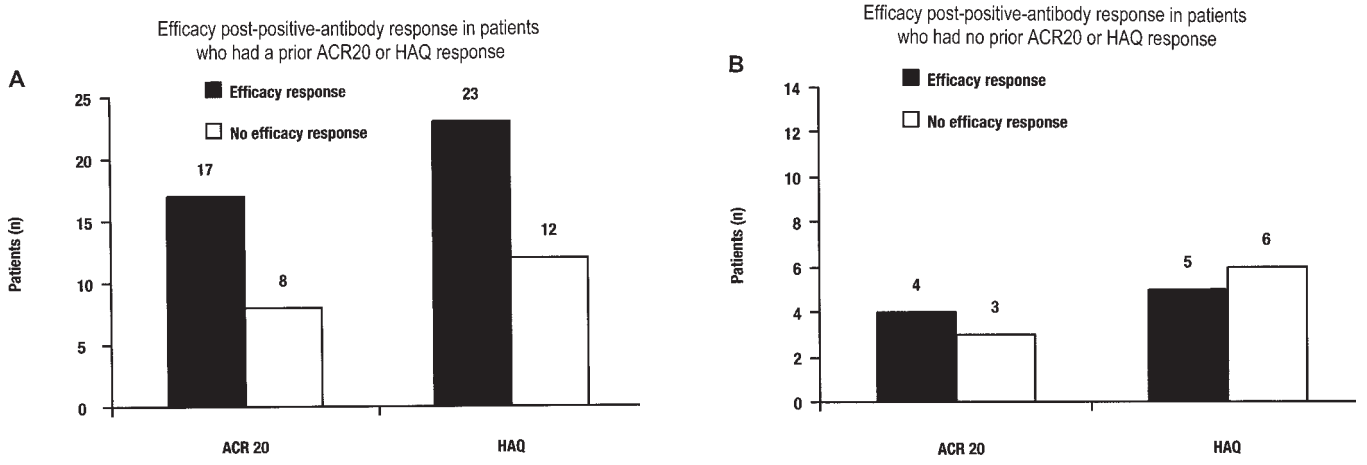

Figure 3. ACR20 and HAQ responses in patients who had (A) an efficacy response or (B) no efficacy response, prior to developing anti-abatacept antibodies (to the molecule, in whole or in part), across the Phase II and Phase III open-label studies. Excludes patients who had missing assessments pre-/post-positive immune response (ACR20, n = 8; HAQ, n = 16). The modified HAQ was used for the Phase II studies.

generally occurred once a patient discontinued drug treatment. Therefore, it is possible that the number of patients with neutralizing antibodies is understated. Estimates of pharmacokinetic measures (clearance, central compartment volume) were comparable in seropositive patients and the larger population of patients who did not seroconvert.

Immunogenicity is expected to be limited in patients receiving background immunosuppressant therapy. However, no patient receiving abatacept as monotherapy developed antibody responses to either abatacept or the CTLA-4 portion of the molecule, suggesting that background immunosuppression is not responsible for the low incidence of immunogenicity seen in this large patient population. Also, patients who were receiving concomitant MTX did not have a greater inci-

dence of immunogenicity, compared with those who were not receiving MTX. Rather, because of its activity as a selective costimulation modulator, abatacept is likely to inhibit an immune response to itself. The reason for a greater incidence of immunogenicity in patients who discontinued is unknown, but may be explained by this same mechanism (during active treatment, abatacept may prevent the development of an antibody response to itself), or could be due to an increase in the detection of an antibody when the drug is not present.

Since the advent of biologic therapy, there have been reports of patients developing serologic evidence of potential autoimmunity, e.g., antinuclear antibodies or anti-double-stranded DNA antibodies, following treatment with anti-TNF agents[14-18] and γ-interferon[19]. Also, autoimmune conditions, such as lupus-like syndrome, have been reported in patients undergoing biologic therapy, although it is not clear if the development of autoimmunity is related to immunogenicity. While these biologic therapies have a different mechanistic effect than abatacept in the treatment of RA, there is a potential mechanism by which an antibody response to abatacept could elicit autoimmunity. Antibodies targeting the CTLA-4 portion of abatacept may have the potential to bind to endogenous CTLA-4, and therefore block its endogenous regulatory activity. This, in turn, could result in an increase in autoimmunity, as is seen with anti-CTLA-4 treatments currently being investigated for tumor regression[20,21]. No significant autoimmune disorders were reported in the individual trials of abatacept in patients with an inadequate response to MTX[3] and in those patients with an inadequate response to anti-TNF therapy[5], suggesting no increased risk of autoimmunity with abatacept.

Overall, no definitive patterns of serious AE or AE emerged in patients who developed a positive antibody response. There were few reports of medically significant AE, serious AE, or prespecified peri-infusional events during the DB or OL periods in the immunopositive patients. Importantly, clinical data suggest that hypersensitivity reactions do not occur following missed or interrupted dosing. In addition, no consistent pattern in AE was observed, nor was a relationship to immunogenicity detected. Therefore, the development of immunogenicity in patients did not appear to be associated with adverse safety outcomes. However, due to the small number of immunopositive individuals, conclusions concerning the effect of immunogenicity on safety cannot be made at this time.

Immunogenicity has also been linked to a reduction in clinical efficacy, leading to a need for dose escalation in some patients with some protein therapeutics[22]. The effect of immunogenicity on efficacy was examined by evaluating the ACR20 and HAQ responses before and after a positive antibody response. No consistent pattern was observed between antibody response and efficacy as measured by ACR20 and HAQ responses. While the number of patients with a positive antibody response to abatacept was too low to determine the influence of immunogenicity on the efficacy of abatacept, the clinical benefit seen with abatacept in the treatment of RA patients with an inadequate response to MTX has been shown to be maintained over 3 years without the need for dose alteration[23,24]. It was not possible to fully examine the effect of neutralizing antibodies on efficacy in this study due to the small number of patients with reactivity; however, the limited amount of data suggest no significant effect.

Development of antibodies directed against abatacept (in whole or part) occurred at a low incidence in this large population of patients with RA (including those who missed 1–2 doses). Although comprehensive efficacy and safety evaluations were not feasible due to the small number of patients who developed reactivity to anti-abatacept or the CTLA-4 portion, this evaluation suggests that the development of immunogenicity did not appear to be associated with adverse safety or efficacy outcomes.

ACKNOWLEDGMENT

The authors thank Elizabeth Burtally, MSc, for her editorial assistance.

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