Functional aspects of the adaptive immune system in arthritis

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CHAPTER 4

CONVERSION TO SERONEGATIVE STATUS AFTER ABATACEPT TREATMENT IN PATIENTS WITH EARLY AND POOR PROGNOSTIC RHEUMATOID ARTHRITIS IS ASSOCIATED WITH BETTER RADIOGRAPHIC OUTCOMES AND SUSTAINED REMISSION

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ABSTRACT

Introduction This post hoc analysis evaluated the effects of the T cell co-stimulation blocker abatacept on anti-citrullinated protein antibodies (ACPA) and rheumatoid factor (RF) in early rheumatoid arthritis (RA), and the association between changes in serological status and clinical response.

Methods Data from a double-blind, randomised and controlled phase III study (AGREE) in methotrexate (MTX)-naive patients with early RA with poor prognostic factors were used in this analysis. Patients were randomised to abatacept (~10 mg/kg intravenously according to weight range) or placebo, plus MTX over 12 months followed by open-label abatacept plus MTX for a further 12 months. Autoantibody titres were determined by enzyme-linked immunosorbent assay at baseline and 6 and 12 months of the double-blind phase. Conversion to seronegative status was evaluated and its association with clinical response was assessed at months 6 and 12.

Results Patients receiving abatacept plus MTX showed a greater decrease in ACPA (but not RF) titres and higher rates of both ACPA and RF conversion to seronegative status than patients treated with MTX alone. A higher proportion of patients converting to ACPA seronegative status receiving abatacept plus MTX achieved remission according to Disease Activity Score in 28 joints (C-reactive protein) or Clinical Disease Activity Index than patients who remained ACPA seropositive. Patients who converted to ACPA seronegative status treated with abatacept plus MTX had a greater cumulative probability of achieving sustained remission and less radiographic progression than those receiving MTX alone or patients in either treatment arm who remained ACPA seropositive.

Conclusions Compared with MTX alone, treatment with abatacept plus MTX was more likely to induce conversion to ACPA/RF seronegative status in patients with early, erosive RA. Conversion to ACPA seronegative status was associated with better clinical and radiographic outcomes.

INTRODUCTION

Rheumatoid arthritis (RA) is characterised by the production of autoantibodies, in particular rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA)[1]. An estimated 50–70% of patients with RA present with detectable ACPA titres, which are mainly of the immunoglobulin (Ig)G isotype and directed against post-translationally modified proteins[1-3]. RF autoantibodies are primarily of the IgM isotype and directed against the Fc-portion of the IgG isotype[1]. RF and ACPA can be present without clinical symptoms for up to 10 years before the onset of RA[4-8], and as such make interesting early biomarkers for the disease. Both RF and ACPA are moderately correlated with markers of inflammation, although the correlation is greater for RF[9]. ACPA is particularly sensitive for diagnosis and is a better prognostic indicator than RF for more severe RA and more rapid disease progression[1,3]. In an early RA cohort, ACPA positivity was associated with a higher rate of joint destruction[10]. Hecht *et al.* demonstrated that both erosion number and size were highest in patients with concomitant ACPA and RF, and that their effects were additive[11]. On the other hand, the presence of RF compared with its absence is associated with higher disease activity in ACPA+ patients[12], in line with the amplifying role of RF[13]. In addition, RF- and ACPA-producing B cells are detectable at high levels in the synovial fluid of patients with RA, suggesting a direct contribution to synovial inflammation[14-17].

A recent report from Rombouts *et al.* provides evidence for a role of T cells in ACPA production. The authors reported that, unlike other autoantibodies or non-reactive IgG, ACPA IgG undergo N-linked glycosylation of the Fab variable domains[18]. The authors hypothesize that this glycosylation requires consensus sites not present in the germline Fab domain sequence, and that these sites are introduced by somatic hypermutation of the Ig variable region[18]. Somatic hypermutation occurs during the process of B-cell proliferation and differentiation that is regulated in part by activated T cells[3]. In addition, the strong association between ACPA and human leukocyte antigen class II genes suggests a role for antigen-specific CD4+ T cells in the immune response against citrullinated proteins[19].

Abatacept is a soluble fusion protein consisting of the extracellular domain of human cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) linked to the modified Fc portion of human IgG1. Abatacept binds to CD80/CD86 on antigen-presenting cells (APCs), thereby blocking the interaction between CD80/CD86 and CD28 on T cells and inhibiting T cell co-stimulation[20,21]. In addition to peptide–major histocompatibility complex recognition between APCs and T cells, co-stimulation is required for (naive) T cells to become fully activated[1]. Thus, if co-stimulation is blocked, B cell differentiation into antibody-producing cells will likely be inhibited and antibody production impaired. Treatment with abatacept, through inhibition of T cell co-stimulation, might therefore be expected to impact on antibody production by B cells.

Abatacept is an effective treatment for both established[22,23] and early RA[24,25], and early treatment of RA has been shown to prevent disease progression and joint damage[24-27]. The

Abatacept trial to Gauge Remission and joint damage progression in methotrexate-naive patients with Early Erosive rheumatoid arthritis (AGREE) was a 2-year, phase III study with a 1-year, double-blind phase that assessed the efficacy, safety and tolerability of intravenous abatacept plus methotrexate (MTX) compared with placebo plus MTX, in MTX-naive patients with early erosive RA and poor prognostic indicators[28,29]. The primary results of the study demonstrated that treatment with abatacept plus MTX resulted in significantly greater and more sustained clinical and radiographic benefits than treatment with placebo plus MTX.
Since abatacept's mode of action includes inhibition of T cell co-stimulation, it was hypothesized that patients who converted to a seronegative status might have a better clinical response to abatacept treatment than those who remained seropositive. This post-hoc analysis of the AGREE study investigated the effects of abatacept in combination with MTX versus MTX alone on conversion to seronegative status in ACPA-seropositive and RF-seropositive patients, and the relationship between seroconversion and clinical response.

METHODS

Patient population and study design

This was a post hoc analysis performed using data from the previously published AGREE study (ClinicalTrials.gov identifier NCT00122382)[28,29]. Briefly, MTX-naive patients with early RA (≤2 years since diagnosis) who were positive for RF and/or ACPA antibodies and had evidence of erosion were randomised 1:1 to receive abatacept (~10 mg/kg intravenously according to weight range) plus MTX or placebo plus MTX (hereafter referred to as 'MTX alone') over a 12-month double-blind period followed by open-label abatacept plus MTX for an additional 12 months[28,29]. At baseline, all patients had high disease activity based on a tender joint count of ≥12, a swollen joint count of ≥10 and C-reactive protein (CRP) levels of ≥0.45 mg/dL.

Determination of autoantibody titres

Serum samples to assess levels of second-generation anti-cyclic citrullinated peptide-2 (a surrogate of ACPA) antibodies and RF were taken at screening and at 6 and 12 months of the double-blind period. Anti-cyclic citrullinated peptide-2 and RF antibody titres were determined by enzyme-linked immunosorbent assay (ELISA). The cut-off for ACPA positivity was 5 AU/mL and 15 IU/mL for RF positivity.

Outcome measures

ACPA and RF seroconversion was determined by comparing baseline antibody titres with titres at months 6 or 12 of the double-blind phase. All patients were positive for RF and/or ACPA at baseline. Those with antibody titres below the limit of detection by ELISA at months 6 or 12 were considered to have converted to a seronegative state.
Disease activity was measured using the Disease Activity Score in 28 joints (CRP) (DAS28 [CRP]) or the Clinical Disease Activity Index (CDAI). Remission was defined as DAS28 (CRP) <2.6 or CDAI ≤2.8. First remission was defined as the first visit at which a patient met the

requirements to achieve remission. Sustained first remission was defined as the first visit at which remission was reached and subsequently maintained for every visit up to month 12. First remission was determined after 6 and 12 months and sustained first remission was determined after 12 months of treatment.

Radiographs of the hands and feet were taken at screening, at 6 and 12 months and at the discontinuation visit. The Genant-modified Sharp scoring method was used to assess the mean change from baseline in total Sharp score (TSS), and erosion and joint space narrowing (JSN) scores at months 6 and 12.

Statistical analysis

In the original study, DAS28 (CRP)-defined remission was evaluated for the intent-to-treat population, with patients who discontinued considered to be non-responders. For the purpose of this report, analyses were based on patients with DAS28 (CRP) and CDAI data available at baseline and months 6 and 12. The proportions of patients achieving remission according to DAS28 (CRP) and CDAI were analysed as point estimates with 95% confidence intervals (CIs). Cumulative probability of time to achieve first remission and sustained first remission according to DAS28 (CRP)-defined and CDAI criteria were evaluated based on Kaplan–Meier estimates with 95% CIs. Patients who lost remission status were censored at the time of remission loss.

Mean changes from baseline in ACPA and RF titres were evaluated by analysis of covariance with treatment, baseline score and disease status as covariates. The adjusted mean change, treatment differences and corresponding 95% CIs were presented for months 6 and 12. In addition, the proportion of patients with conversion to ACPA and RF seronegative status at months 6 and 12 were analysed using point estimates with 95% CIs. The relationship between DAS28 (CRP) or CDAI remission and conversion to ACPA or RF seronegative status was investigated by determining the proportions (95% CIs) of patients in remission by seroconversion status at months 6 and 12, and between-group comparisons were made using the chi-square test. Mean changes from baseline in TSS, erosion and JSN scores were evaluated by analysis of covariance with treatment, baseline score and disease status as covariates. The adjusted mean change, treatment differences and corresponding 95% CIs were presented for months 6 and 12.

RESULTS

Patient population

In the original study, 509 patients were randomly assigned to receive abatacept plus MTX (n = 256) or MTX alone (n = 253)[22]. Of these, 459 patients completed year 1 and 433 completed year 2 [23]. Demographic data and baseline characteristics have been previously published[22,23]. Of the 434 patients who had ACPA status measures at baseline, month 6 and month 12, 21 (4.8%) were seronegative at month 6. Of the 461 patients who had RF status measures at baseline, month 6 and month 12, 61 (13.2%) were seronegative at month 6.

Table 1. Patient demographic data and baseline disease characteristics by conversion to ACPA and RF seronegative status at month 6

	Conversion to ACPA seronegative status		Persistent ACPA seropositive		Conversion to RF seronegative status		Persistent RF seropositive	
	Abatacept + MTX (n = 15)	MTX alone (n = 6)	Abatacept + MTX (n = 212)	MTX alone (n = 202)	Abatacept + MTX (n = 39)	MTX alone (n = 22)	Abatacept + MTX (n = 191)	MTX alone (n = 209)
Age, years	50.7 (11.1)	61.2 (11.4)	49.8 (12.3)	48.8 (12.7)	51.6 (10.3)	49.5 (14.4)	49.6 (12.6)	49.7 (12.8)
Female, n (%)	13 (86.7)	6 (100)	157 (74.1)	159 (78.7)	29 (74.4)	18 (81.8)	145 (75.9)	170 (81.3)
Weight, kg	65.6 (17.0)	68.8 (16.6)	72.3 (17.8)	72.7 (17.9)	71.2 (17.2)	68.1 (16.1)	71.9 (18.3)	73.5 (18.1)
Race, White, n (%)	14 (93.3)	4 (66.7)	167 (78.8)	173 (85.6)	34 (87.2)	20 (90.9)	147 (77.0)	179 (85.6)
Region, n (%)								
N. America	2 (13.3)	0	40 (18.9)	27 (13.4)	9 (23.1)	3 (13.6)	32 (16.8)	34 (16.3)
S. America	5 (33.3)	0	83 (39.2)	87 (43.1)	7 (17.9)	9 (40.9)	88 (46.1)	88 (42.1)
Europe	7 (46.7)	4 (66.7)	72 (34.0)	75 (37.1)	20 (51.3)	8 (36.4)	56 (29.3)	74 (35.4)
ROW	1 (6.7)	2 (33.3)	17 (8.0)	13 (6.4)	3 (7.7)	2 (9.1)	15 (7.9)	13 (6.2)
Duration of RA, months	8.9 (8.8)	1.7 (1.5)	6.0 (7.4)	7.0 (7.1)	3.7 (5.0)	6.9 (8.0)	7.1 (8.0)	7.0 (7.1)
Tender joints	30.0 (16.2)	20.3 (6.9)	31.1 (14.9)	30.3 (13.7)	24.6 (14.3)	29.8 (15.0)	32.9 (15.1)	30.9 (14.0)
Swollen joints	23.2 (10.3)	15.8 (7.6)	22.9 (11.7)	22.4 (10.4)	20.9 (9.6)	20.4 (10.1)	23.7 (11.9)	22.4 (10.4)
Patient pain assessment	62.5 (25.5)	56.6 (27.4)	67.2 (22.2)	66.8 (22.5)	64.6 (24.9)	61.4 (22.3)	67.9 (22.4)	67.6 (22.8)
HAQ-DI	1.4 (0.7)	1.7 (0.6)	1.7 (0.7)	1.7 (0.7)	1.6 (0.5)	1.7 (0.6)	1.7 (0.7)	1.7 (0.7)
Patient global assessment, 100-mm VAS	61.7 (25.7)	50.3 (28.2)	66.3 (21.3)	64.3 (23.6)	67.5 (22.0)	61.5 (22.9)	65.4 (22.6)	63.7 (24.3)
Physician global assessment, 100-mm VAS	59.4 (16.3)	56.7 (17.4)	67.9 (18.2)	65.4 (19.1)	64.1 (18.5)	61.9 (16.0)	68.2 (18.3)	66.1 (19.4)
DAS28 (CRP)	6.2 (0.9)	5.9 (0.7)	6.3 (1.0)	6.3 (1.0)	6.1 (0.9)	6.0 (1.1)	6.4 (1.0)	6.3 (1.0)
DAS28 (ESR)	7.2 (0.6)	6.2 (0.7)	6.9 (1.0)	6.7 (1.1)	6.7 (0.8)	6.4 (1.3)	6.9 (1.0)	6.8 (1.1)
ESR, mm/h	44.4 (18.0)	55.5 (34.3)	49.5 (28.8)	49.8 (32.9)	48.5 (21.3)	41.2 (24.3)	49.4 (29.9)	51.1 (32.7)
CRP, mg/dL	2.4 (2.0)	4.7 (3.4)	3.3 (3.3)	3.8 (5.4)	3.0 (3.0)	2.6 (3.2)	3.2 (3.1)	3.8 (5.4)
Baseline RF positive, n (%)	14 (93.3)	6 (100)	204 (96.2)	197 (97.5)	39 (100)	22 (100)	191 (100)	209 (100)
Baseline ACPA positive, n (%)	15 (100)	6 (100)	212 (100)	202 (100)	34 (87.2)	15 (68.2)	179 (93.7)	185 (88.5)
Total Sharp score	7.1 (8.7)	15.4 (17.1)	7.7 (9.8)	6.7 (8.6)	6.6 (10.6)	5.7 (5.9)	7.6 (9.3)	6.5 (8.6)
JSN score	2.5 (4.6)	5.8 (9.7)	2.1 (4.1)	1.8 (3.9)	2.0 (4.8)	1.5 (2.5)	2.1 (3.9)	1.9 (4.1)
Erosion score	4.6 (5.0)	9.6 (8.1)	5.6 (6.3)	4.9 (5.5)	4.6 (6.2)	4.2 (3.9)	5.5 (6.1)	4.6 (5.2)

Data are mean (SD) unless stated otherwise. Conversion to ACPA or RF seronegative status at month 6 meant that patients who were ACPA or RF seropositive at baseline, respectively, became seronegative at month 6; persistent ACPA or RF seropositive meant that patients were ACPA or RF seropositive at both baseline and at month 6.

ACPA anti-citrullinated protein antibody, *CRP* C-reactive protein, *DAS28* Disease Activity Score in 28 joints, *ESR* erythrocyte sedimentation rate, *HAQ-DI* Health Assessment Questionnaire-Disability Index, *JSN* joint space narrowing, *MTX* methotrexate, *N. America* North America, *RA* rheumatoid arthritis, *RF* rheumatoid factor, *ROW* rest of world, *S. America* South America, *SD* standard deviation, *VAS* visual analogue scale

Patient demographic data and baseline disease characteristics by conversion to ACPA and RF seronegative status at month 6 are shown in Table 1. The baseline disease activity in the patients who seroconverted was DAS28-CRP 5.9 for the MTX treated patients compared to 6.2 in the Abatacept + MTX arm.

RF and ACPA titres following treatment with abatacept plus MTX or MTX alone

A decrease in autoantibody levels after 6 and 12 months, compared with baseline, was observed for all study groups. Mean ACPA and RF titres decreased from baseline following treatment with abatacept plus MTX and MTX alone (Figure 1). Whereas similar decreases in RF titres were observed in both treatment groups, treatment with abatacept plus MTX resulted in a larger decrease in ACPA titres versus MTX alone at both 6 and 12 months (the 95% CI of the estimate of difference did not cross 0; Figure 1).

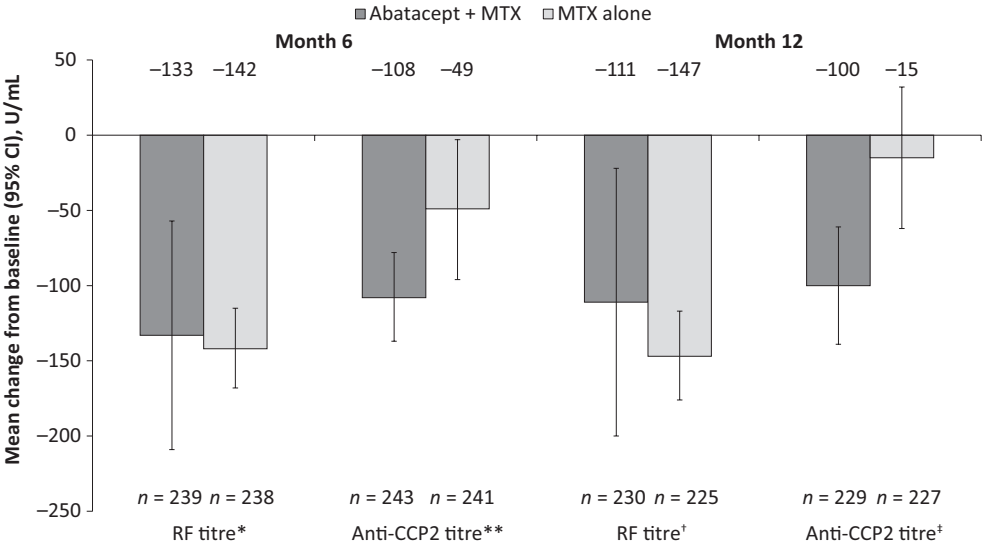

Figure 1. ACPA and RF titres in patients with early RA treated with abatacept + MTX compared with MTX alone. Antibody titres were determined by ELISA at baseline and month 6 and 12. Baseline to month 6 and baseline to month 12 were carried out as separate analyses. Baseline means (SD) for: *abatacept + MTX vs MTX alone were 305 (469) vs 273 (342); **abatacept + MTX vs placebo were 305 (534) vs 272 (514); †abatacept + MTX versus placebo were 297 (426) vs 272 (344); ‡abatacept + MTX versus placebo were 300 (537) vs 270 (524). *ACPA* anti-citrullinated protein antibody, *CI* confidence interval, *ELISA* enzyme-linked immunosorbent assay, *MTX* methotrexate, *RA* rheumatoid arthritis, *RF* rheumatoid factor, *SD* standard deviation

Conversion to RF and ACPA seronegative status following treatment with abatacept plus MTX or MTX alone

A numerically larger proportion of patients converted to become RF or ACPA seronegative in response to treatment with abatacept plus MTX versus MTX alone after 6 and 12 months of treatment. At 6 months, 17.0% (39/230) and 6.6% (15/227) of patients treated with abatacept plus MTX were RF and ACPA seronegative, respectively, compared with 9.5% (22/231) and 2.9% (6/208) of patients treated with MTX alone. At 12 months, 18.5% (41/222) and 7.1%

(15/212) of patients treated with abatacept plus MTX were RF and ACPA seronegative, respectively, compared with 14.6% (32/219) and 4.6% (9/198) of patients treated with MTX alone. The proportion of patients who converted to seronegative status was numerically higher in the abatacept plus MTX treatment group than in the MTX group. Estimated differences (95% CIs) between treatment groups for conversion to RF and ACPA seronegative status were, respectively, 7.4% (0.8–14.1) and 3.7% (–0.8 to 8.2) at 6 months, and 3.9% (–3.5 to 11.2) and 2.5% (–2.5 to 7.6) at 12 months; only the estimate of difference (95% CI) for RF seroconversion at month 6 did not cross 0 (Figure 2), indicating that abatacept plus MTX may have a particularly prominent effect on RF in the early treatment course.

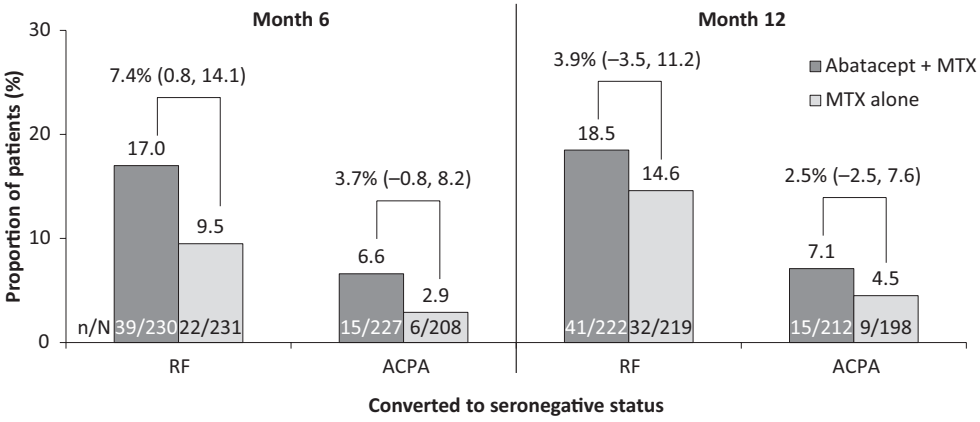

Figure 2. Conversion to ACPA and RF seronegative status in patients with early RA treated with abatacept + MTX compared with MTX alone. The proportion of patients with conversion to ACPA and RF seronegative status at months 6 and 12 and estimates of difference (95% CIs) between treatment groups are shown. Baseline to month 6 and baseline to month 12 were carried out as separate analyses. *ACPA* anti-citrullinated protein antibody, *CI* confidence interval, *ELISA* enzyme-linked immunosorbent assay, *MTX* methotrexate, *N* total number of patients in respective analysis, *n* number of patients that showed seroconversion, *RA* rheumatoid arthritis, *RF* rheumatoid factor

Clinical and radiographic responses by conversion to seronegative status

In the abatacept plus MTX arm, a higher proportion of patients who converted to ACPA seronegative status achieved DAS28 (CRP) and CDAI remission at 6 months compared with patients who were persistently ACPA seropositive (Figure 3); the estimate of difference (95% CI) between converters to seronegative status and those who were persistently ACPA seropositive did not cross 0 for DAS28 (CRP)-defined remission at month 6. The proportions (95% CIs) of patients who converted to ACPA seronegative status in the abatacept plus MTX arm and achieved DAS28 (CRP) and CDAI remission were 66.7% (42.8–90.5) and 46.7% (21.4–71.9) at 6 months, and 73.3% (51.0–95.7) and 46.7% (21.4–71.9) at 12 months, respectively. In comparison, the proportions (95% CIs) of patients who were persistently ACPA seropositive and achieved DAS28 (CRP) and CDAI remission were 32.6% (26.2–38.9) and 20.8% (15.3–26.2) at 6 months, and 48.7% (41.8–55.7) and 34.5% (27.9–41.2) at 12 months, respectively.

A higher proportion of patients treated with abatacept plus MTX achieved DAS28 (CRP) or CDAI remission at 6 and 12 months compared with patients treated with MTX alone, regardless of whether they converted to seronegative status or not. In the MTX alone arm, the proportions (95% CIs) of patients achieving DAS28 (CRP) and CDAI remission were 16.7% (0.0–46.5) and 16.7 (0.0–46.5) at 6 months, and 22.2% (0.0–49.4) and 11.1% (0.0–31.6) at 12 months, respectively, for patients who converted to ACPA seronegative status; and 21.8% (16.1–27.5) and 13.4% (8.7–18.1) at 6 months, and 31.8% (25.1–38.4) and 20.1% (14.4–25.8) at 12 months, respectively, for patients who were persistently ACPA seropositive.

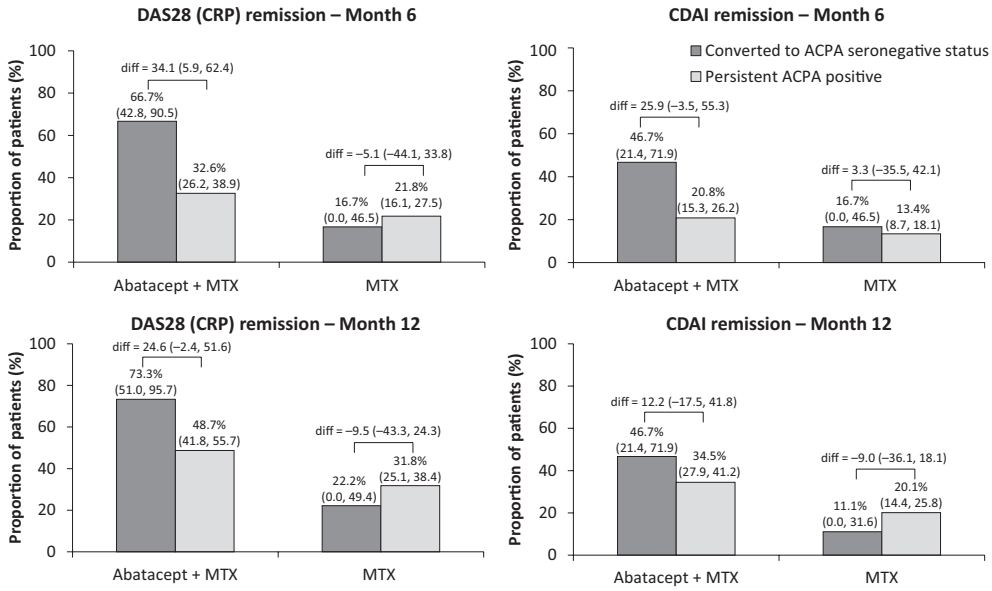

Figure 3. Percentage of patients achieving remission by conversion to ACPA seronegative status. Antibody titres were determined by ELISA at baseline and months 6 and 12. Baseline to month 6 and baseline to month 12 were carried out as separate analyses. *ACPA* anti-citrullinated protein antibody, *CDAI* Clinical Disease Activity Index, *CRP* C-reactive protein, *DAS28* Disease Activity Score in 28 joints, *ELISA* enzyme-linked immunosorbent assay, *MTX* methotrexate

In the abatacept plus MTX treatment arm, numerically, there was a higher cumulative probability of reaching sustained first DAS28 (CRP)-defined remission among patients who converted to seronegative status compared with those who remained ACPA seropositive (Figure 4). This difference was not observed among patients who received MTX alone. In patients who remained ACPA seropositive, there was a statistically significant benefit in the abatacept plus MTX group compared with MTX alone ($p = 0.001$; log-rank test). The proportion of patients who achieved sustained remission was consistently higher in the abatacept plus MTX treatment group versus MTX alone.

In both treatment groups, patients who underwent conversion to ACPA seronegative status showed less radiographic progression, as indicated by a smaller mean change from baseline

in Genant-modified TSS, erosion and JSN scores at both 6 and 12 months, than patients who were persistently ACPA seropositive (Figure 5). The estimate of difference (95% CI) between those who converted to seronegative status and those who remained ACPA seropositive did not cross 0 only for TSS and erosion score in the abatacept plus MTX group at month 12. Differences in TSS and erosions scores, but not JSN scores, between converters to ACPA seronegative status and patients who were persistently ACPA seropositive were larger among patients treated with abatacept plus MTX compared with those who received MTX alone.

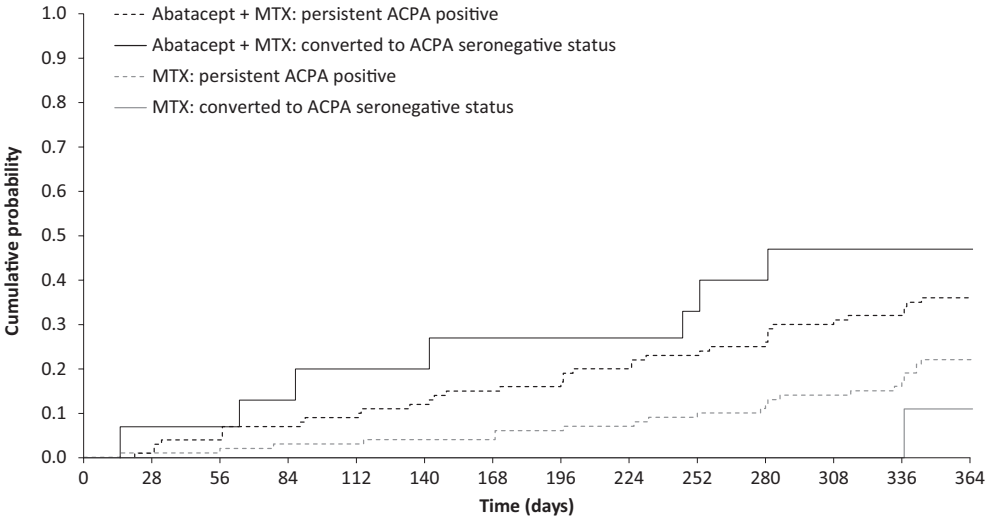

Figure 4. Cumulative probability of time to achieve first sustained DAS28 (CRP) remission by conversion to ACPA seronegative status. The cumulative probability of the time to achieve sustained first DAS28 (CRP) remission over 12 months in all patients treated with abatacept + MTX or MTX alone who underwent conversion to ACPA seronegative status compared with those who remained ACPA seropositive was evaluated based on estimated Kaplan–Meier curves with corresponding 95% CIs. In patients who remained ACPA seropositive, there was a statistically significant benefit in the abatacept plus MTX group compared with MTX alone (p = 0.001; log-rank test). There were no significant differences between the abatacept plus MTX versus MTX alone treatment groups in patients who underwent conversion to ACPA seronegative status, or within treatment groups between converters to ACPA seronegative status compared with those who remained ACPA seropositive. Antibody titres were determined by ELISA at baseline and months 6 and 12. Baseline to month 6 and baseline to month 12 were carried out as separate analyses. *ACPA* anti-citrullinated protein antibody, *CI* confidence interval, *CRP* C-reactive protein, *DAS28* Disease Activity Score in 28 joints, *ELISA* enzyme-linked immunosorbent assay, *MTX* methotrexate

DISCUSSION

In the AGREE study, patients with early, poor prognostic RA (erosions, highly active disease and seropositivity; 96.5% and 89.0% of patients were RF or ACPA seropositive), who were treated with abatacept plus MTX for 12 months achieved sustainable clinical, functional and radiographic benefits compared with patients treated with MTX alone[28-30]. The present posthoc analysis investigated the effect of abatacept in combination with MTX on RF and ACPA titres and the potential association between ACPA titres and clinical response. Combined

treatment with abatacept and MTX led to a decrease in both RF and ACPA titres over 6 and 12 months, and conversion to RF and ACPA seronegative status in 17.0–18.5% and 6.6–7.1% of patients, respectively. In those patients who converted to an autoantibody negative status

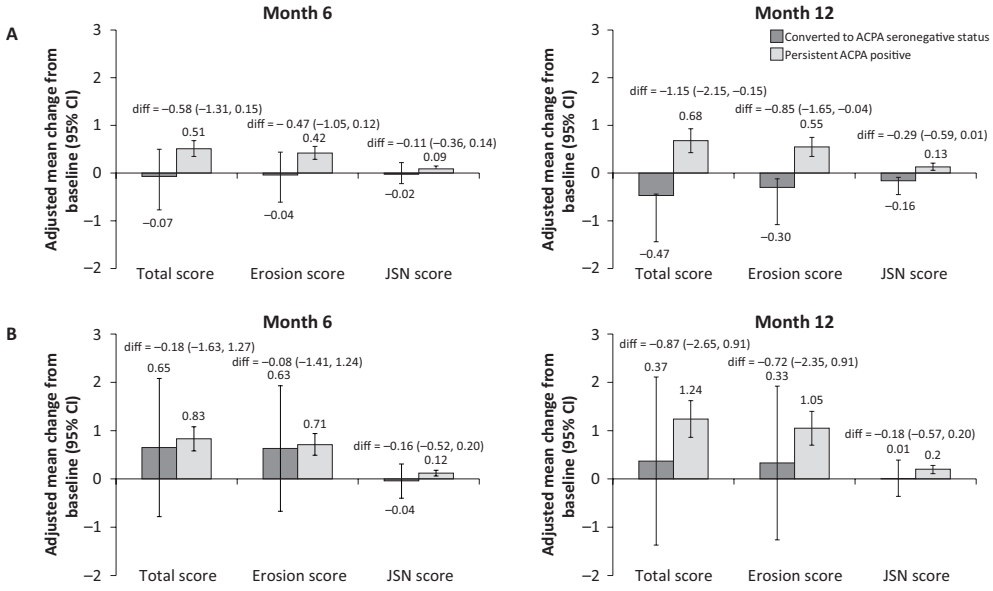

Figure 5. Radiographic outcomes in patients with early RA treated with (A) abatacept + MTX or (B) MTX alone by conversion to ACPA seronegative status. Antibody titres were determined by ELISA at baseline and months 6 and 12. Baseline to month 6 and baseline to month 12 were carried out as separate analyses. Error bars represent 95% CIs. *ACPA* anti-citrullinated protein antibody, *CI* confidence interval, *ELISA* enzyme-linked immunosorbent assay, *JSN* joint space narrowing, *MTX* methotrexate, *RA* rheumatoid arthritis, *TSS* total Sharp score

the remission rates were higher than in those patients who did not seroconvert.

Abatacept inhibits T cell co-stimulation by binding to CD80 and CD86 on APCs and blocking the binding of CD28 to CD80/86 [20]. B cells proliferate and differentiate into antibody-producing cells and switch from production of IgM to IgG antibodies in response to stimuli from activated CD4+ T cells, e.g. increased cytokine production[3]. Thus, abatacept has the potential to indirectly impact IgG isotype switching by inhibiting the co-stimulation and activation of T cells.

In the present study, after 6 and 12 months, a greater decrease in ACPA titres was observed with treatment with abatacept plus MTX compared with MTX alone, whereas mean decreases from baseline in RF titres were similar for the two treatment arms. However, in observational studies independent of the use of biological agents reductions in RF as well as ACPA levels have been observed and, indeed in line with the present study, more frequent RF seroconversion than ACPA seroconversion was observed. Reductions of both autoantibodies were linked to a reduction of disease activity and associated with reductions in disease activity[31]. RF autoantibodies are primarily of the IgM isotype whereas ACPA are primarily of

the IgG isotype[1]. B cells do not require T cell help to produce IgM isotype antibodies, whereas switching from IgM to IgG isotypes is a feature of B-cell somatic hypermutation, which occurs during proliferation and differentiation of B cells – in part regulated by activated T cells[3]. Thus, the difference in effect of abatacept plus MTX compared with MTX alone on RF versus ACPA titres might be explained by this difference in autoantibody isotype. In contrast, treatment with abatacept plus MTX led to higher rates of conversion to RF or ACPA seronegative status compared with treatment with MTX alone. Although abatacept inhibits T cell activation, it also exerts anti-inflammatory effects in a T cell independent way[32], potentially through direct effects on B cells[33] and macrophages[34].

Current treatment strategies for RA employ a targeted approach aimed at reaching remission or low disease activity[35,36]. The present analysis showed that in the abatacept plus MTX treatment arm the proportion of patients who achieved DAS28 (CRP)- or CDAI-defined remission was higher among those who converted to seronegative status than those who remained persistently ACPA seropositive. Furthermore, the cumulative probability of achieving sustained first remission according to DAS28 (CRP)-defined criteria was higher among patients who converted to ACPA seronegative status treated with abatacept plus MTX than in those who remained ACPA seropositive. The small proportion of patients who were converters to ACPA seronegative status showed less radiographic progression over 12 months than patients who remained ACPA seropositive, regardless of treatment.

These findings are in line with previous studies of abatacept in patients with early RA. In the ADJUST trial[25], patients with undifferentiated arthritis or very early RA treated with abatacept for 6 months had delayed disease progression and prolonged inhibition of radiographic progression after cessation of treatment versus placebo, with a decrease from baseline in RF and ACPA titres[25]. In the AVERT study[24], compared with patients treated with MTX alone, patients treated with abatacept plus MTX showed significantly higher rates of remission and a higher number of patients achieved sustained drug-free remission after withdrawal of all therapy, as well as reduced inflammation and structural damage progression as assessed by changes in MRI scores (synovitis, osteitis and bone erosions)[37]. Furthermore, in a post hoc analysis of the AVERT study (MTX-naïve patients with early RA and highly active and erosive disease; 100% and 95.2% of patients were ACPA and RF positive, respectively), a higher proportion of patients receiving abatacept plus MTX underwent conversion to ACPA seronegative status compared with those receiving MTX[38]. In addition, a numerically higher proportion of patients treated with abatacept plus MTX who became seronegative (ACPA IgM isotype) achieved clinical remission at 12 months compared with those who did not seroconvert, differences that were not seen for patients treated with MTX alone[38].

On the other hand, a post hoc analysis of the AMPLE trial suggested that, despite a similar clinical response over 2 years between the two treatment groups, only abatacept plus MTX produced a continuous decline in the median levels of most ACPAs beyond 1 year of treatment; an effect that was not sustained with adalimumab plus MTX[39].

In the abatacept plus MTX group, a link between conversion to seronegative status and remission/inhibition of structural damage was noticeable, while this link was less obvious in the MTX group. Taken together, these data demonstrate that abatacept is an effective treatment in patients with early RA and that, by modulating T-cell responses at very early stages of the disease, it might be possible to alter underlying autoimmune processes; i.e. slowing or halting disease progression with the potential for sustained drug-free remission.

There are limitations to post hoc analyses, which should be considered when interpreting the data presented here. The present post hoc analysis was a completers-only analysis, carried out on a subset of patients included in the original AGREE study who had complete data sets. The study was not designed or powered to detect differences between the treatment groups based on seroconversion status, thus statistical testing in this analysis should be interpreted with caution. Moreover there are no formal corrections for multiple testing. Finally, this post hoc analysis was carried out in a relatively small population; as such, only some of the findings reached 'significance', particularly in larger subgroups of patients. The findings would benefit from validation in a larger patient population.

In conclusion, the present post hoc analysis demonstrated that treatment with abatacept in combination with MTX led to a decrease in autoantibody titres, resulting in some patients undergoing conversion to RF and ACPA seronegative status. Conversion to ACPA seronegative status was associated with higher rates of remission, an increased likelihood of achieving sustained remission and less radiographic progression.

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PART III

CYTOKINE PRODUCING CD4+ T CELLS IN DISEASE AND HEALTH